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Evaluación agro-ambiental del olivar basada en técnicas espectroscópicas

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fuera coronas, porque este perro solo es siervo de ese rey que es su persona,
adiós al yugo, de esta nación que lo que no gana en el norte lo usa aquí como verdugo,
no más caretas, ni la patética visión de un pueblo inculto de pandereta,
abajo el manto, los san benitos y las burlas se despeñen de una vez por el barranco
un hombre queda, tan solo un hombre un andaluz que cada día lo que venga lo desafía,
que por febrero, vive cantando pa salvarse del naufragio universal de un año entero
esta montaña no es sierra morena, es la piedra más grande del camino
si no la apartas eso es lo que dejas a tus hijos y sus hijos
vengo que muerdo, dios vengo que muerdo, si piensas como yo dame tu mano
subamos hasta el filo de los sueños que oigan como les gritamos
hermano, ladra, luego cabalgamos
Antonio Martínez Ares, El perro andalú, 2018

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Abstract / Resumen

Abstract

In the present PhD, the study of the agro-environmental system of the olive grove is approached, paying special attention to the problem of soil degradation. In addition, other related aspects have been considered such as the nutritional status of the olive tree and the use of organic amendments to improve soil fertility. For this type of studies, a large number of laboratory analyses are usually required. As an alternative to traditional wet chemical analysis, the possibility of using infrared spectroscopy techniques, in both middle (FTIR) and near infrared regions (FT-NIR), has been evaluated. In general, infrared spectra provide a large amount of information on the mineralogical composition of the samples and, to a lesser extent, on the organic matter. The strong overlapping between bands of inorganic compounds makes usually difficult the interpretation of the bands of organic compounds, especially in cases like this, where soils are poor in organic matter. Both techniques provide similar information, which may be in some way complementary. The use of FTIR and FT-NIR is presented as a good alternative for the characterization of olive groves, as well as the possible effects derived from soil management and lithological material.

The use of chemometrics tools is mandatory to get useful information from a high number of spectra. First, a principal component analysis was used to study possible groupings among soil samples based on their infrared spectra. The PCA analysis together with discriminant analysis (PLS-DA) have been proved very useful to find patterns of grouping, as well as their source. In addition, these tools are able to differentiate between soil types and, to a lesser extent, between the soils management. The combined use of both regions of the infrared spectrum is the best option for the discrimination of soils according to their quality. On the one hand, it has been demonstrated, the ability of infrared spectroscopy to classify the soil samples according to their geological material or according to their management, and, on the other hand, that the effects derived from soil management are reflected in a remarkable way in both regions of the spectrum infrared.

The importance of the systems of soil management and the lithological material in the soils quality has been also demonstrated. In this way, more respectful management of the agro-system such as organic management gives rise to soils with better properties, compared to the soils under conventional management. In addition, the effects of irrigation over soil properties have been studied, demonstrating that the continued use of low quality water causes salinization and sodification of the soil, with the consequent loss of fertility. However, the effect of irrigation and management depends on the geological material, since more favorable substratum such as colluvial limestones better support degradation processes compared to marls, while carbonated soils are better able to withstand sodification processes than siliceous soils.

Furthermore, we used the spectra to predict some soil properties by using a partial least squares regression (PLS). We confirmed that infrared spectroscopy has a great ability to predict physical-chemical parameters of soils, with excellent results especially for those parameters that have a direct reflection in the spectra as OC, N and $CaCO_3$, but also for parameters in certain point correlated with some of its bands, such as pH and CEC, closely related to the amount of carbonates and clays respectively, or P and K related in part to organic matter. However, the prediction of textural or biological parameters was more difficult because there is no direct influence in the spectra. We also found the great capacity of the infrared spectroscopy to predict a global biological soil quality index (GMea), that could be useful for a quickly characterization of the soil. Although both regions show predictive capacity, the FT-NIR spectra produce better results. For a prediction as accurate as possible, the ideal would be using both regions together, especially with the application of mid level data fusion strategies. Hence, these techniques are a great alternative to typical laboratory analysis, being much faster and cheaper. However, it is very important to take into account that not all parameters were predicted with the same accuracy. Furthermore, the calibrations that have been developed are only applicable to similar samples, and that the use of this type of models to other different soil samples have to be explored. Therefore, for its use as a substitute for the laboratory, it would be necessary to increase the number of samples as well as to use wider concentration ranges or even to develop individual prediction models depending on the origin of the sample.

For a complete assessment of olive agro-system, we also need to characterize the plant nutritional status. This is currently made by leave nutrient analysis, which is usually expensive and time consuming. We predicted these nutrients by using two spectroscopic techniques, FT-NIR and energy dispersive X-ray fluorescence (EDXRF). To use both spectra working together, we also applied two different data fusion strategies, low and mid level. FT-NIR and EDXRF are capable of predicting the majority of essential nutrients for the olive tree in the leaf, and in this way, they could partially replace the traditional laboratory analysis, although these models are not totally accurate for some micronutrients. On the other hand, the data fusion strategy seems to be useful to combine spectroscopic techniques, especially at the mid level, that built the best possible models, although with slight improvement comparing to individual models.

Finally, we studied the possibility of using organic amendments to restore soil organic matter level, as a possible solution for the degradation of the olive agro-system. First, the effect of the addition of spent coffee grounds (SCG) over soils was studied. SCG has a clear effect on the increase of soil organic matter, in all its fractions, which in the short term generate a clear improvement in soil fertility, especially in more clayey soils like the Vega soil, that is able to stabilize more amount of organic matter. However, the analysis of humic acids shows that the functionality of organic matter is reduced, due to a decrease of its complexity and stability, so a continued input of this amendment could negatively affect the ability of the organic matter to provide nutrients to the plant in the long term. To solve this problem, we tried the mixing of different amendments as an alternative, mixing SCG with olive mill pomace compost (COMP), in this way correcting the problem of organic matter quality, but also the acid pH of SCG and the high C/N of this particular COMP.

By another side, we studied characteristics of different olive mill pomace with and without composting process. We conclude that the composting of olive mill pomace is a totally necessary process to improve this amendment, although even after this, it continues to present problems such as high water repellency. This problem was found in all the amendments studied and in order to eliminate this harmful effect, a thermal treatment was applied. We found that thermal treatment, especially at 275ºC, is effective to eliminate negative aspects such as toxicity and water repellency, while generating an amendment with a higher quality organic matter, with a greater proportion of more stable organic material. On the other hand, the mixture of amendments of SCG and COMP is demonstrated as a viable alternative, since its mixture manages to correct some non-optimal parameters such as pH or C/N ratio, resulting in amendments of quality improved with intermediate parameters between both. Furthermore, this amendment after thermal treatment becomes as an alternative of equal or higher quality in all aspects than the COMP and SCG amendments separately.

Resumen

En la presente tesis doctoral se aborda el estudio del sistema agro-ambiental del olivar con especial atención a la problemática de la degradación del suelo sobre el que se desarrolla el mismo. Además, se han contemplado otros aspectos como el estado nutricional del olivo a través del análisis foliar y la utilización de enmiendas orgánicas para mejorar la fertilidad del suelo. Para este tipo de estudios se requieren habitualmente un gran número de análisis de laboratorio. Como alternativa a los análisis tradicionales, se ha evaluado la posibilidad de utilizar técnicas de espectroscopía infrarroja, tanto espectroscopía de infrarrojo medio (FTIR) como espectroscopía de infrarrojo cercano (FT-NIR), para realizar un diagnóstico de la calidad del suelo de una manera más económica y rápida. Además, al no necesitar de reactivos químicos, la espectroscopía infrarroja puede calificarse como una tecnología analítica "verde", compatible con los principios básicos de la sostenibilidad ambiental. En general, los espectros de infrarrojo proporcionan gran cantidad de información sobre la composición mineralógica de las muestras y, en menor medida, sobre la materia orgánica, ya que las bandas de compuestos inorgánicos a menudo se superponen y dificultan la interpretación de las bandas de compuestos orgánicos, especialmente en suelos pobres en materia orgánica como los que se tratan en estos trabajos. Ambas técnicas proporcionan información similar, que puede ser de alguna manera complementaria. La utilización de FTIR y FT-NIR se presenta como una buena alternativa para la caracterización de los suelos de olivar, y los posibles efectos derivados de su manejo y material litológico.

Para la gestión del alto número de espectros y la extracción de la máxima cantidad de información, se ha recurrido al empleo de diferentes herramientas quimiométricas. En primer lugar, se utilizó un análisis de componentes principales para estudiar posibles agrupaciones entre las muestras de suelos en función de sus espectros infrarrojos. El análisis PCA junto con análisis discriminantes como PLS-DA demostraron ser muy útiles para encontrar patrones de agrupación, así como el origen de éstos. Además, con estas herramientas se pudo diferenciar entre tipos de suelo y, en menor medida, entre el manejo de estos suelos. El uso combinado de ambas regiones del espectro infrarrojo demuestra ser la mejor opción para la discriminación de los suelos en función de su calidad. Se demuestra, por un lado, la capacidad de la espectroscopia infrarroja para clasificar las muestras según su material geológico o su manejo, y, por otro lado, que los efectos derivados del manejo del suelo se reflejan de forma notable en ambas regiones del espectro infrarrojo.

Se ha puesto de manifiesto la importancia del sistema de manejo del suelo, y el material litológico sobre el que se desarrolla en la calidad de los mismos. Así, el estudio realizado demuestra que un manejo más respetuoso del agro-sistema, como el manejo ecológico, da lugar a suelos con mejores propiedades, en comparación con los suelos bajo manejo convencional. También se demuestra la influencia de la irrigación, ya que el uso continuado de aguas de mala calidad provoca la salinización y la sodificación del suelo, con la consiguiente pérdida de fertilidad. Sin embargo, el efecto de estos factores depende fuertemente del material geológico, ya que un sustrato más favorable, como las calizas coluviales, resiste mejor los procesos de degradación en comparación con las margas, mientras los suelos carbonatados presentan mayor capacidad para resistir los procesos de sodificación que los silíceos.

Asimismo, se utilizaron los espectros infrarrojos para predecir algunas propiedades del suelo mediante regresión de mínimos cuadrados parciales (PLS). Confirmamos que la espectroscopia infrarroja tiene una gran capacidad para predecir parámetros físico-químicos de los suelos, con excelentes resultados, especialmente para aquellos parámetros que tienen un reflejo directo en el espectro como OC, N y $CaCO_3$, pero también para parámetros en cierto punto correlacionados con algunas de sus bandas, tales como pH y CEC, estrechamente relacionados con la cantidad de carbonatos y arcillas, respectivamente, o P y K relacionadas en parte con la materia orgánica. Sin embargo, los resultados para la predicción de parámetros texturales y biológicos fueron inferiores, debido a que estos no tienen reflejo directo en el espectro. Sin embargo, se obtuvieron buenos resultados a la hora de predecir un índice de calidad biológica global del suelo (GMea), que puede ser de utilidad para caracterizar rápidamente el agro-sistema. Aunque ambas regiones muestran capacidad predictiva, los espectros FT-NIR producen mejores resultados. Para obtener una predicción lo más precisa posible, lo ideal sería utilizar ambas regiones juntas, especialmente con la aplicación de *mid level data fusion*. Por lo tanto, estas técnicas son una excelente alternativa al análisis de laboratorio tradicional, ya que son mucho más rápidas y económicas. No obstante, es muy importante tener en cuenta que no se predicen con la misma precisión todos los parámetros, y que las calibraciones realizadas solo se aplican a muestras similares, produciendo la extrapolación de este tipo de modelos a muestras de suelo diferentes pobres resultados. Por lo tanto, para su uso como sustituto del laboratorio, sería necesario tener un mayor rango de muestras o modelos de predicción individuales según el tipo de muestra.

Para una evaluación completa del agro-sistema de olivar también se caracterizó el estado nutricional de la planta. Esto se hace actualmente mediante el análisis de nutrientes en hoja, proceso que suele ser costoso y que requiere mucho tiempo. Se crearon modelos predictivos para estos nutrientes mediante el uso de dos técnicas espectroscópicas, FT-NIR y fluorescencia de rayos X por energía dispersiva (EDXRF). Para utilizar ambos espectros en conjunto, aplicamos dos estrategias diferentes de fusión de datos, *low level* y *mid level*. FT-NIR y EDXRF son capaces de predecir la mayoría de los nutrientes esenciales en la hoja de olivo, pudiendo de esta manera reemplazar parcialmente el análisis de laboratorio tradicional, aunque teniendo en cuenta que los modelos para algunos micronutrientes no son totalmente confiables. Por otro lado, la estrategia de fusión de datos parece ser útil para combinar técnicas espectroscópicas, especialmente el *mid level*, con el que se consiguieron los modelos más precisos, aunque con una leve mejora en comparación con los modelos individuales.

Finalmente, se ha estudiado la posibilidad de utilizar enmiendas orgánicas para restaurar el nivel de materia orgánica del suelo, como una posible solución para la degradación del agro-sistema de olivar. Primero, se estudió el efecto de la adición de los posos de café (SCG) sobre los suelos. Los SCG producen un claro aumento de la materia orgánica del suelo, en todas sus fracciones. A corto plazo esto generaría una clara mejora en la fertilidad del suelo, especialmente en suelos más arcillosos como el suelo de Vega, que puede estabilizar más cantidad de materia orgánica. Sin embargo, el análisis de los ácidos húmicos muestra que la funcionalidad de esta materia orgánica se ve reducida, debido a una disminución de su complejidad y estabilidad, por lo que una adición continuada de esta enmienda podría afectar negativamente la capacidad de la materia orgánica para proporcionar nutrientes a la planta a largo plazo.

Para resolver este problema, se mezclaron los SCG con otra enmienda orgánica, el compost de alperujo (COMP), corrigiendo así el problema de la calidad de la materia orgánica, pero también el pH ácido de los SCG y el alto C/N de este COMP. Por otro lado, se estudiaron las características de diferentes alperujos con y sin proceso de compostaje. El compostaje del alperujo se demuestra como un proceso totalmente necesario para mejorar esta enmienda, aunque incluso después de esto, continúa presentando problemas como la elevada repelencia al agua. Este problema se encontró en todas las enmiendas estudiadas. Para eliminar este efecto pernicioso, se aplicó un tratamiento térmico. Se puede concluir que este tratamiento, especialmente a 275ºC, es eficaz para eliminar aspectos negativos como la toxicidad y la repelencia al agua, a la vez que genera una enmienda con una fracción orgánica de mayor calidad, con un aumento de la proporción de material orgánico más estable. Por otro lado, la mezcla de enmiendas de SCG y COMP se demuestra como una alternativa viable, ya que su mezcla logra corregir algunos parámetros no óptimos como el pH o la relación C/N, dando como resultado enmiendas de una calidad mejorada, con parámetros intermedios entre ambos. Además, esta enmienda después del tratamiento térmico se convierte en una alternativa de igual o mayor calidad en todos los aspectos en comparación con las enmiendas COMP y SCG por separado.

Capítulo 1
Introducción

1. El agro-sistema del olivar mediterráneo

El olivo (*Olea europeae L.)*, es una especie perteneciente el género *Olea* dentro de la familia botánica *Oleaceae*. *Olea europeae L.* (Figura 1) se considera una de las plantas cultivadas más antiguas, cuyo origen se remonta 4000 - 3000 años a.C. en la zona de Palestina (Civantos, 2008). Su distribución principal es entre las latitudes 30°- 45° tanto en el hemisferio norte como en el hemisferio sur, en regiones climáticas consideradas de tipo mediterráneo, que se caracterizan por su bajo nivel de precipitaciones y sus veranos secos y calurosos, ocupando un total de 11.3 millones de hectáreas. La mayor parte de este cultivo, aproximadamente el 80%, se concentra en los países de la cuenca mediterránea (Vilar Hernández y Cárdenas García, 2016), principalmente en la parte europea (principalmente Grecia, Italia y España), con alrededor de 6.7 millones de hectáreas, siendo su presencia en otras regiones tales como Oceanía o Sudamérica meramente anecdótica. De la proporción de olivar europea, alrededor del 39% de esta superficie se concentra en España, siendo el país con mayor superficie de este cultivo de la Unión Europea (2.6 millones de hectáreas). Dentro de España, la comunidad autónoma andaluza acumula 1.6 millones de hectáreas, lo que representa más del 50% de este cultivo en el país (MAPAMA, 2017). La provincia de Jaén, con 577.754 hectáreas, destaca como la provincia con mayor superficie de olivar (Consejería de Agricultura Pesca y Desarrollo Rural, 2017). Cabe reseñar que este cultivo se ha convertido en el paisaje característico y representativo de la región, así como una seña de identidad adoptada por los giennenses en ámbitos muy diversos.

Figura 1. Olivo centenario (Castillo de Locubín, Jaén).

El principal recurso obtenido del olivar es su fruto, la aceituna, que se puede consumir tras un proceso de endulzado o de cocido con álcalis, aunque su utilización más común es para la producción de aceite de oliva virgen, que se extrae en las almazaras, mediante medios únicamente mecánicos o físicos. El aceite de oliva es un producto utilizado en diferentes ámbitos, principalmente como alimento, usándose también para la producción de productos medicinales, de cosmética y de higiene. El consumo de este producto se ha incrementado en la última década debido a sus propiedades nutricionales y su gran cantidad de antioxidantes (Schwingshackl y Hoffmann, 2014; Zeb y Murkovic, 2011). Del total de la producción de aceite de oliva mundial, la cual se estima, de acuerdo con los datos publicados por el Consejo Oleícola Internacional (COI), entre 2.5 y 3 millones de toneladas según la campaña, España destaca como el principal país productor a nivel mundial, con una media de 1.2 millones de toneladas. Dentro del estado español, Jaén es la principal provincia productora, con producciones que oscilan entre las 360 – 400 mil toneladas anuales. Si tenemos en cuenta que esto supone el 15% de la producción de aceite de oliva a nivel mundial, parece claro que la provincia de Jaén es una región clave en este sector. Todos estos datos indican que el sector olivarero tiene gran peso en la economía europea, especialmente en los países Mediterráneos, y se convierte en una actividad económica fundamental para el desarrollo de la región andaluza, y en especial para la provincia de Jaén.

Figura 2. Aceite de oliva.

1.1 El suelo del olivar

El olivo es una planta terrestre, por lo que necesita un sustrato adecuado para su crecimiento y desarrollo. Dicho sustrato es el suelo, normalmente considerado como la fracción de tierra que cubre la superficie como resultado de la meteorización *in situ* de las rocas o la acumulación de materia mineral transportada por agua, viento o hielo. La característica distintiva del suelo es que, al material mineral alterado física y químicamente, simultáneamente se le va incorporando un material orgánico, que puede ser tanto vivo, incluyendo plantas, fauna y microfauna propia del suelo, así como una amplísima variedad de microorganismos (virus, bacterias y hongos), así como material orgánico muerto, que incluiría los restos de organismos, así como los diferentes compuestos generados en su degradación y polimerización (Nortcliff et al., 2000). El suelo debe ser capaz de proporcionar un contexto físico, químico y biológico adecuado para la supervivencia y desarrollo de la planta, así como un depósito de agua y nutrientes, además de ser capaz de hacer que estos estén disponibles para la planta.

Existen tipologías de suelos muy diferentes, que dependen de diversos factores edafoclimáticos, tales como el material parental sobre el que se forma, la altitud, la pendiente, la temperatura y las precipitaciones, entre otros. Las principales tipologías de suelos de la provincia de Jaén, ordenadas de más a menos comunes (FAO, 2006; Junta de Andalucía, 2005), son las siguientes:

- Regosoles: suelos abundantes en el centro y el sur de la provincia, desarrollados sobre materiales no excesivamente consolidados y que presentan una escasa evolución, generalmente debido a su reciente formación sobre aportes recientes no aluviales, o al desarrollarse en zonas con fuertes procesos erosivos que provocan un continuo rejuvenecimiento de los suelos. Se pueden distinguir entre Regosoles calcáreos, éutricos y dístricos.
- Calcisoles: se desarrollan generalmente sobre margas, areniscas o mezcla de ambas, aunque a veces también pueden desarrollarse sobre costras calizas, arcillas triásicas y conglomerados. El carácter diferencial de estos suelos es la presencia de un horizonte cálcico con más del 40% de $CaCO_3$, con dominancia de la ilita en la fracción arcilla. En las superficies más antiguas presentan fuertes acumulaciones de carbonato cálcico que en muchas ocasiones están cementadas y forman horizontes petrocálcicos.
- Cambisoles: suelos con un cierto grado de evolución, con amplia representación dentro de la provincia; se desarrollan sobre distintas litologías y en relieves relativamente suaves o protegidos de los procesos erosivos por la cobertura vegetal. Pueden diferenciarse cinco tipos todos ellos caracterizados por presentar un horizonte de diagnóstico cámbico. El carbonato cálcico se distribuye de forma homogénea en el perfil, pero no da lugar acumulaciones.
- Luvisoles: son suelos muy antiguos, que dan al paisaje una policromía variada y peculiar. Presentan un horizonte árgico, debajo de un horizonte ócrico superficial. No presentan un contenido muy alto en materia orgánica, pero sí una gran capacidad de cambio catiónico debido al alto contenido en arcilla, principalmente ilita, y en mucha menor cantidad, montmorillonita.
- Vertisoles: también denominados “tierras negras andaluzas”. Son muy frecuentes en la Depresión del Guadalquivir, en la comarca agrícola de La Loma, aunque pueden aparecer en otros puntos de la provincia. Presentan una escasa diferenciación de sus horizontes, debido a movimientos internos de materiales y a la formación de grandes grietas en los períodos estivales, que tienen su origen en un alto contenido en arcillas expansivas del tipo esmectita. Aparecen en las vaguadas o depresiones, sobre coluvios de margas de gran potencia enriquecidos en arcillas. La pedregosidad superficial es nula.
- Fluvisoles: suelos profundos y formados sobre depósitos aluviales que presentan un escaso grado de evolución. Dentro de éstos se pueden distinguir los Fluvisoles calcáreos y los Fluvisoles eútricos. Son suelos muy fértiles, dedicados generalmente al cultivo de productos hortícolas y algodón. En la fracción arcilla, la ilita y la montmorillonita son las más abundantes.

- Leptosoles: están escasamente representados en la provincia, sólo en posiciones resguardadas de la erosión y con microambientes generalmente más húmedos que los de las zonas circundantes. Suelos de perfil A-R, limitados en profundidad por una roca continua y dura, a menos de 15 cm de la superficie. Son característicos de zonas escarpadas sometidas a una fuerte erosión que rejuvenece constantemente el suelo.

1.2 Materia orgánica y su papel clave

La materia orgánica es uno de los factores más importantes a la hora de determinar la productividad del suelo, ya que esta es la principal fuente de aporte de nutrientes a largo plazo. Además, la materia orgánica es la principal fuente de carbono orgánico del medio edáfico, cuya cuantificación parece ser un gran indicador para detectar cambios en su salud y calidad (Wiesmeier et al., 2019). Sin embargo, la materia orgánica del suelo (SOM) es una fase de composición muy heterogénea que hoy día sigue requiriendo estudios más completos. Esta fase se genera en el suelo, principalmente por el aporte de restos vegetales, y en menor medida animales que se incorporan en superficie. Estos restos pueden estar en un estado más fresco, descomponerse mediante el proceso de mineralización a compuestos más simples, o con el paso del tiempo dar lugar a compuestos más complejos mediante reacciones de polimerización. Este proceso recibe el nombre de humificación, y da lugar a las sustancias húmicas, que representan generalmente alrededor del 60 - 80% del total de materia orgánica. Están constituidas por compuestos orgánicos que han sido transformados por la acción de ciertos microorganismos e influenciados por procesos abióticos. Las sustancias húmicas son, por tanto, grupos heterogéneos, que no pueden clasificarse claramente por su composición, sino que tradicionalmente se clasifican de acuerdo con su comportamiento frente a diferentes reactivos, principalmente su solubilidad en medio ácido/básico, lo cual está asociado a una serie de características comunes para cada una de las fracciones. Las sustancias húmicas se clasifican en ácidos fúlvicos (FA), ácidos húmicos (HA) y huminas (Figura 3).

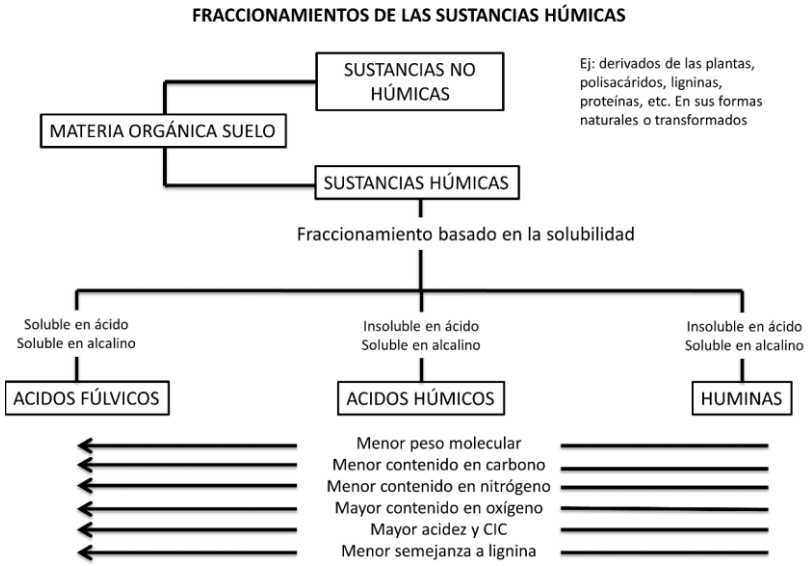

Figura 3. Clasificación y características de las sustancias húmicas (elaboración propia).

Actualmente persiste una gran controversia sobre la naturaleza de la materia orgánica, con estudios que ponen en duda los conceptos convencionales de humificación (visión tradicional), limitados a observaciones de la materia orgánica solubilizada en extractos alcalinos. La visión emergente diferencia las fracciones de la materia orgánica según la solubilidad en agua y la accesibilidad a esta de los diferentes microorganismos, tal y como se muestra en la Figura 4 (Lehmann y Kleber, 2015).

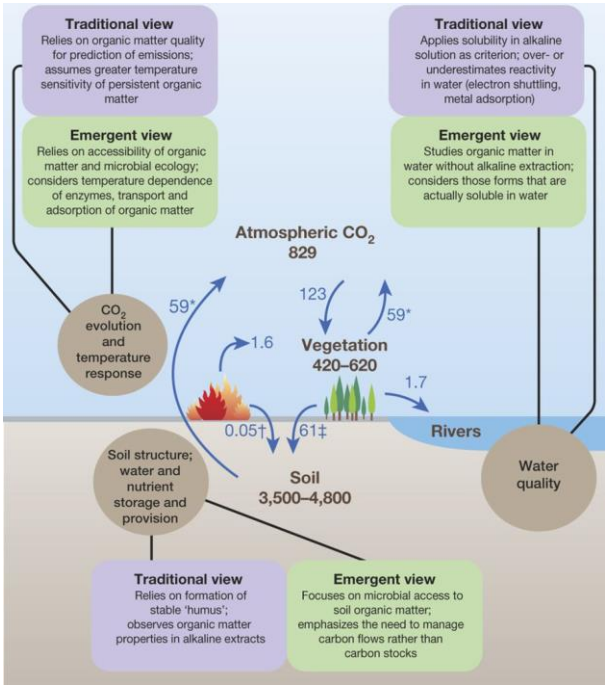

Figura 4. Visión tradicional y emergente de la naturaleza de la materia orgánica del suelo (Lehmann y Kleber, 2015)

Ya sea desde el punto de vista de la visión tradicional, o de la visión emergente, la cantidad y tipo de materia orgánica se ve afectada por diversos factores en el ambiente mediterráneo, tales como el material parental, las condiciones climáticas o la vegetación, que también deben tenerse en cuenta (Ruiz Sinoga et al., 2012). Cabe destacar que el factor que afecta de manera más determinante a la cantidad y calidad de la materia orgánica es el manejo del suelo (Viscarra Rossel et al., 2014), ya que prácticas agrícolas como el laboreo cambian el contenido y las características de la materia orgánica en general, y de las sustancias húmicas en particular (Ding et al., 2002). Resulta clave estudiar y comprender la materia orgánica del suelo, dado que esta tiene un papel relevante en la estabilización de los agregados del suelo, la capacidad de infiltración, el ciclado de nutrientes, la conservación del suelo y su resistencia frente a la erosión. Concentraciones bajas de materia orgánica en los ambientes semiáridos puede conllevar una progresiva degradación de la calidad del suelo y su productividad (Caravaca et al., 2002). Sin embargo, una cantidad elevada de materia orgánica no garantiza la fertilidad del suelo, la calidad de esta materia orgánica es incluso de mayor relevancia (Almendros et al., 2005), siendo especialmente importante el estudio de la fracción de ácidos húmicos, que es el mejor reflejo del estado de la materia orgánica del suelo (Aranda et al., 2011).

Cabe destacar, además, que el estudio de los niveles de materia orgánica, y en especial de carbono orgánico, ha cobrado gran importancia en los últimos años. Esto es debido a que, la capacidad de los suelos para almacenarlo puede representar una función decisiva para paliar el cambio climático. Comprender esta relación entre los cambios en el clima y el carbono orgánico resulta imprescindible para edafólogos, agrónomos y agricultores. De este modo, se podrían encontrar las mejores prácticas de manejo agrícola para mantener o aumentar el carbono orgánico del suelo, y mejorar la resistencia de los agro-sistemas contra posibles condiciones climáticas más extremas.

1.3 Sistemas de manejo del suelo

1.3.1 Sistemas de manejo convencionales

Tradicionalmente en el olivar mediterráneo, podemos encontrar diferentes sistemas de cultivo que buscan obtener la máxima producción, y por tanto intentan que el suelo, independientemente de su tipología, sea lo más fértil posible. Según Pastor (2008) estos manejos son:

- Laboreo: sistema más ampliamente utilizado en olivicultura. Consiste en el uso de aperos de labranza que penetran entre 15 - 20 cm en el suelo con la intención de prepararlo para maximizar su capacidad de infiltración, eliminando también la vegetación espontánea. También se realiza un laboreo más profundo (25 cm) en los meses de primavera pera eliminar totalmente la vegetación espontánea, mientras en verano el laboreo es más superficial con la intención de "pulverizar el suelo" y evitar la pérdida de agua por evaporación.
- No laboreo con suelo desnudo: este sistema sustituye el laboreo, por el uso de diferentes herbicidas que mantienen el suelo con ausencia total de vegetación durante todo el año. Ha probado ser más productivo, ya que se disminuye en gran medida la competencia por los nutrientes y el agua del olivo con otras plantas.
- Semilaboreo: sistema mixto que solo realiza laboreo en las calles que quedan entre los olivos, mientras se utiliza un herbicida residual en la zona bajo la copa del árbol para eliminar la vegetación espontánea.
- Laboreo mínimo: se trata de un sistema mixto en el que tan solo se realizan dos laboreos superficiales (0-5 cm) para mejorar la infiltración, mientras que se utiliza herbicida para eliminar la vegetación espontánea.

Figura 5. Olivar tradicional (Fuensanta de Martos, Jaén)

Los sistemas de laboreo y suelo desnudo han sido los más empleados tradicionalmente, debido a su elevado rendimiento. Sin embargo, el sistema de laboreo provoca graves perjuicios en el medio edáfico, ya que a medio-largo plazo acaba generando una elevada tasa de erosión del suelo (Rodríguez Martín et al., 2016). Un problema similar ocurre cuando utilizamos un sistema de no laboreo con suelo desnudo, donde el mantener el suelo descubierto durante todo el año lo hace muy vulnerable a la erosión hídrica, principal mecanismo erosivo en el ambiente mediterráneo (Castro et al., 2008). Estos problemas se acrecientan en zonas con desnivel elevado, debido a la erosión laminar originada por los flujos de agua a favor de la pendiente. La intensificación de la degradación debida a procesos erosivos puede llegar a provocar graves daños, pudiendo incluso llegar a originar procesos de desertificación (Hill et al., 2008). Uno de los problemas principales generados por los procesos erosivos es la eliminación de la capa más superficial del suelo. En esta capa se acumula la materia orgánica, responsable del aporte a largo plazo de nutrientes al suelo, mediante su mineralización. Otro perjuicio del manejo tradicional del cultivo de olivar, es la pérdida de biodiversidad, debido a la acción biocida de los herbicidas y pesticidas que afectan a la flora y a la microfauna típica del olivar, así como a los microorganismos. Esto provoca el desequilibrio de los agro-sistemas y da como resultado una disminución en el control natural de plagas y enfermedades (Sánchez-Moreno et al., 2015). A este problema hay que sumarle una pérdida en el reciclaje de nutrientes y una alteración en los ciclos biogeoquímicos, y por tanto una disminución de la capacidad del suelo para suministrar el alimento necesario a la planta (Sastre et al., 2016).

Figura 6. Olivar degradado por erosión hídrica laminar. Exposición de pie de olivo (Fuente la Peña, Jaén)

1.3.2 Sistemas de manejo alternativos en el olivar

En los últimos años se han extendido en olivicultura diversos manejos alternativos. Se caracterizan por disminuir el laboreo y el uso de compuestos químicos de síntesis, intentando además mantener cubiertas vegetales o inertes para proteger el suelo frente a la erosión hídrica. El objetivo de estos manejos es minimizar o corregir los procesos de degradación asociados a manejos tradicionales. Una descripción de estos manejos alternativos según Pastor (2008) se muestra a continuación:

- Cultivo con cubierta inerte: consiste en añadir o permitir la existencia de una cubierta inerte sobre el suelo, ya sea a partir de materiales sintéticos, rocas o restos de plantas.
- Cultivo en no laboreo con cubierta vegetal espontanea durante el invierno: consiste en permitir el crecimiento de la vegetación espontánea durante los meses de invierno. De esta manera se consigue mejorar la infiltración y la protección del suelo. Esta vegetación se elimina en primavera mediante una siega química o mecánica, pudiendo dejar los restos vegetales sobre el suelo para servir de fuente de nutrientes y protección.
- Cultivo con cubierta viva: consiste en sembrar en las calles del cultivo una o varias especies vegetales, de manera que, al efecto protector de esta capa, se le sumen los posibles efectos beneficiosos que esta puede tener sobre el suelo y la planta. En este manejo se realiza una siega en primavera, bien sea química o física, pudiendo dejar los restos vegetales sobre el suelo para servir de fuente de nutrientes y protección.
- Cultivo orgánico o ecológico: surge como un modelo alternativo de cultivo, que intenta producir cultivos con alto valor nutricional, manteniendo y mejorando la fertilidad del suelo, contribuyendo a su conservación (Milgroom et al., 2007), respetando los ciclos naturales del agro-ecosistema, sin utilizar ningún producto sintetizado químicamente. Este manejo permite el desarrollo de la vegetación espontánea, que puede no eliminarse, o en todo caso se elimina en primavera mediante un laboreo superficial (5-10 cm).

Figura 7. Olivar ecológico (Valle del Atanor)

Todas estas alternativas han demostrado ser útiles para reducir la erosión y la degradación del suelo. Además, el uso de cubiertas vegetales aumenta el contenido en materia orgánica y diferentes macronutrientes en el suelo (Aranda et al., 2011), con ciertos efectos positivos sobre la fracción de ácidos húmicos (Slepetiene et al., 2010), mejorando además algunas propiedades físicas del suelo e incidiendo de manera positiva en el estado fisiológico de la planta y en la calidad del fruto que produce (Calero et al., 2013). Además, en el caso del manejo ecológico, se suman los múltiples beneficios medioambientales de este, dado que conlleva una reducción del uso de fitosanitarios, evitando así una posible contaminación del suelo y las aguas subterráneas. Asimismo, este manejo contribuye de manera muy eficiente al secuestro de carbono (Vicente-Vicente et al., 2017), paliando de algún modo los efectos del calentamiento global. Este tipo de manejo está cada vez más extendido en el olivar mediterráneo, y se calcula que alrededor del 30% del cultivo ecológico de la región de Andalucía corresponde a olivar ecológico. Sin embargo, para una buena conservación del suelo, hay que añadir al manejo ecológico otras prácticas como el no laboreo o el uso de enmiendas para mantener un nivel de nutrientes adecuado, ya que este manejo por sí solo parece no ser suficiente para recuperar y mantener la salud del suelo (Sánchez-Moreno et al., 2018).

1.4 Irrigación y abonado en el olivar

Otro de los factores determinantes en el manejo del cultivo de olivar es la gestión del agua. En la mayoría de las zonas olivareras mediterráneas la lluvia es el único aporte hídrico para el olivar, lo que convierte al agua en el principal factor limitante de la producción (Pastor, 2008). El olivar se ha cultivado tradicionalmente como un cultivo de secano, pues está bien adaptado a los ambientes semiáridos. Sin embargo, desde hace tiempo se ha demostrado que la introducción de sistemas de regadío aumenta considerablemente el rendimiento de las plantaciones, incluso con aportaciones muy pequeñas de agua (Orgaz y Fereres, 2008). Es por esto que la práctica del regadío en el olivar está cada vez más extendida. Se calcula que en España existen al menos 784859 hectáreas de olivar de regadío, lo que supone alrededor del 30% de la superficie total de olivar, existiendo además una tendencia al aumento de la superficie en regadío en los últimos años (MAPAMA, 2017).

Sin embargo, no son pocos los casos en que este riego se hace sin tener en cuenta las necesidades reales de la planta, ya que no se realizan estudios previos. Asimismo, el agua utilizada presenta diferente composición según el lugar del que se tome, por lo que resulta de gran importancia caracterizar las aguas aportadas mediante el riego, para poder así conocer cuáles pueden ser sus efectos sobre el cultivo (Melgar et al., 2009). El uso de aguas de riego de mala calidad con alta concentración de sales/sodio puede provocar una salinización y/o sodificación de los suelos (Jalali et al., 2008; Qadir y Oster, 2004), problema que se acentúa en regiones de clima árido o semiárido (Gorji et al., 2015) donde la lixiviación y transporte de sales solubles no es tan eficiente como en regiones húmedas. Elevados valores de salinidad provocan un desequilibrio en la cantidad de nutrientes, con déficit en la mayoría de los casos (Grattan y Grieve, 1998), así como disminución de la actividad biológica y ciclos biogeoquímicos desbalanceados (Rietz y Haynes, 2003; Zeng et al., 2013). Por otro lado, los altos niveles de sodio generan graves problemas, como la dispersión de arcillas, al introducirse el ion sodio en la interlámina de los filosilicatos (Sumner, 1993) y la rápida descomposición de la materia orgánica, disminuyendo sus niveles en el suelo (Nelson y Oades, 1998).

Ambos procesos generan una destrucción de la estructura del suelo, que desemboca en una disminución de la tasa de infiltración, que a su vez puede generar encostramiento y sellado del suelo (Amezketa et al., 2003), impidiendo totalmente la entrada de agua. La salinidad de los suelos tiene además graves efectos sobre las plantas, ya que provoca estrés hídrico, al reducir el potencial osmótico del suelo, lo que termina afectando al crecimiento vegetativo (Setia et al., 2013; Shannon y Grieve, 1998), pudiendo llegar incluso a provocar la muerte de algunas plantas (Aragüés et al., 2005). En el caso del olivar, la salinidad del suelo puede provocar una disminución en la calidad del fruto del olivo (Zeleke et al., 2012), así como problemas en su crecimiento vegetativo, pudiendo en los casos extremos llegar a provocar la muerte de plantas jóvenes (Aragüés et al., 2005; Benlloch et al., 1991). En definitiva, la salinidad de los suelos, es un problema creciente, que afecta a suelos de todo el mundo (Daliakopoulos et al., 2016). Este problema está cada vez más extendido en el mundo agrícola, en el año 2000 la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO) calculaba que 831 millones de hectáreas, repartidas entre los 5 continentes, estaban afectadas por alguno de estos dos problemas (Rengasamy, 2006), cantidad que ha ido en aumento debido a la intensificación de los cultivos a nivel mundial, por lo que este problema es ya considerado la segunda causa más importante de pérdida de la calidad del suelo a nivel mundial.

Por último, otra de las prácticas habituales en el olivar es el abonado, que se utiliza para poder satisfacer las necesidades nutritivas de la planta. Para poder alcanzar el máximo rendimiento del cultivo, reduciendo al mínimo los costes económicos y medioambientales, es necesario realizar una fertilización adecuada al estado actual del cultivo, dado que para cada uno de los nutrientes existen niveles óptimos para la planta (Fernández-Escobar et al., 2009) en los cuales se alcanza la máxima producción. Una fertilización excesiva, sin embargo, puede provocar desbalances en la planta, contaminar tanto el medio edáfico como las aguas subterráneas (Fernandez-Escobar, 2011), e incluso si se alcanzan niveles muy elevados de algunos nutrientes pueden aparecer casos de toxicidad. Para realizar un programa de fertilización adecuada, es indispensable un buen diagnóstico del estado nutricional de la planta, el cual suele realizarse a través del análisis foliar, ya que no siempre el tener un suelo con nivel de nutrientes y de fertilidad adecuados garantiza un cultivo sano y productivo.

2. Enmiendas orgánicas como alternativa para mejorar la fertilidad del suelo

En el agro-sistema de olivar es común encontrar niveles bajos de materia orgánica, lo que provoca importantes perjuicios, dado que tal y como se ha comentado en el apartado "1.2 Materia orgánica y su papel clave", niveles bajos de esta fracción disminuyen la fertilidad del suelo. Una posible solución para esta problemática puede ser la recuperación de niveles de materia orgánica adecuados en el suelo, siendo la utilización de enmiendas orgánicas de diversos orígenes y naturaleza, una de las mejores opciones para este propósito (Pérez-Lomas et al., 2010). La incorporación de este tipo de materiales al suelo, puede minimizar el impacto de las prácticas agrícolas inadecuadas, a la vez que mantener y mejorar la productividad, especialmente en ecosistemas con ambientes tan extremos como el mediterráneo (Aguilera et al., 2013). Esto genera a su vez un impacto positivo en las diferentes propiedades del suelo, ya que el uso de enmiendas orgánicas ha demostrado mejorar parámetros físicos, químicos y biológicos del suelo, así como su calidad general (Reichstein et al., 2013).

2.1 Compost de alperujo y otros materiales orgánicos poco evolucionados

La utilización de subproductos del olivar como enmiendas orgánicas, es una posibilidad muy interesante en la provincia de Jaén. Es el caso del alperujo, que se obtiene como resultado del proceso de extracción del aceite de oliva en las almazaras. Se calcula que este subproducto se genera a nivel español en cantidades que rondan las seis millones de toneladas anuales (Lozano-García et al., 2011). Se trata de un material semisólido, con alto contenido en polifenoles, lípidos y materia orgánica, lo que lo convierte en un material altamente contaminante, fitotóxico y con efecto antimicrobiano (Serramiá et al., 2010). Su alto contenido en agua (50-70%), lo convierte en un subproducto de muy difícil manejo, y un problema considerable para la industria olivarera (López-Piñeiro et al., 2007). Su aplicación directa en campo está prohibida, pero si este material se composta, se puede conseguir mejorar sus propiedades, consiguiendo una alternativa realmente interesante para su reutilización como enmienda orgánica para el suelo (Fernández-Hernández et al., 2014). Estaríamos realizando un ciclo de reciclado, donde los nutrientes extraídos del agro-sistema de olivar acaban volviendo a él, generando a su vez un importante ahorro en costes al sustituir al abonado tradicional, haciendo este cultivo mucho más sostenible. Para el compostaje del alperujo se suele utilizar, como material compactante, otro subproducto propio del olivar, los restos de poda. Este residuo agrícola lignocelulósico del que se generan alrededor de 3000 kg / ha (Tomás-Pejó et al., 2008), cuya eliminación es necesaria para mantener los campos limpios y evitar la propagación de enfermedades vegetales (Romero-García et al., 2014). Por estas razones, su reutilización para el compostaje del alperujo parece una alternativa ideal.

Figura 8. Producción de alperujo en una almazara (Olivarero, 2015).

La utilización de compost de alperujo como enmienda orgánica genera un claro incremento en la fertilidad y la calidad del suelo, ya que produce un aumento a largo plazo (entre 1.5 y 3.5 veces) en la cantidad de materia orgánica, nitrógeno total, fosforo disponible, potasio disponible y estabilidad de los agregados, así como un incremento de la actividad biológica, medida a partir de actividades enzimáticas de entre el 180 y el 420% (García-Ruiz et al., 2012). La aplicación del compost de alperujo suele tener efectos positivos sobre la planta, incrementando el rendimiento del cultivo y mejorando su salud (Toscano et al., 2013). Esta enmienda también tiene una serie de características que podríamos considerar negativas, y que deben tenerse en cuenta al aplicarla al suelo. El compost de alperujo presenta por lo general pH elevado, normalmente superior a 8. Su aplicación constante sobre el suelo puede producir alcalinización, que daría lugar a un posible bloqueo de nutrientes y disminución de la actividad biológica a largo plazo. A esto se une su elevada conductividad eléctrica, con el peligro que esto puede conllevar de salinizar el suelo, y una relación C/N elevada cuando no está suficientemente maduro, que puede alterar y/o perjudicar los ciclos biogeoquímicos y la tasa de mineralización de la materia orgánica del suelo.

Figura 9. Compost de alperujo (García-Ruiz et al., 2012).

Además de estas enmiendas basadas en subproductos de olivar, otra posible alternativa sería la utilización de otros materiales frescos sin compostar. Un caso interesante son los posos o granzas del café. Este material, del que se calculan que a nivel mundial se producen más de siete millones de toneladas anuales en la actualidad (Hardgrove y Livesley, 2016) no recibe ningún uso específico, por lo que comúnmente acaba siendo depositado en vertederos. Diferentes autores proponen la utilización de este subproducto como enmienda orgánica en el suelo (Eshetu, 2013), y aunque este uso no ha sido completamente estudiado (Cruz y Marques dos Santos Cordovil, 2015; Kourmentza et al., 2018), ya existen algunos trabajos que han demostrado su utilidad como enmienda orgánica para suelos agrícolas mediterráneos, estando su utilización en la implantación de semilleros en viveros o suelos de invernadero aún por explotar. Entre sus principales beneficios se ha descrito la mejora de las propiedades físicas y fisicoquímicas del suelo, incrementando la cantidad de algunos nutrientes tales como nitrógeno, fósforo, potasio y carbono orgánico (Cervera-Mata et al., 2017; Yamane et al., 2014). Por otro lado, este subproducto tiene sobre el suelo una gran capacidad quelante, debido a la presencia de melanoidinas, estructuras poliméricas heterogéneas de elevado peso molecular, generadas al someter determinados alimentos a altas temperaturas, semejantes a sustancias húmicas, que se pueden usar para sintetizar quelatos férricos y de zinc, entre otros, que más tarde se degradarían quedando libres los nutrientes para que las plantas puedan incorporarlos en su metabolismo (Takenaka et al., 2005). Sin embargo, la aplicación de esta enmienda orgánica también puede generar ciertos perjuicios sobre el agro-sistema. La presencia de sustancias como la cafeína, taninos y polifenoles en los posos de café, puede tener efecto tóxico para las plantas y los microorganismos del suelo (Campos-Vega et al., 2015). Además, presentan un pH acido, por lo que la aplicación repetida de esta enmienda puede provocar una acidificación del suelo, induciendo problemas de toxicidad y bloqueo de nutrientes. De hecho, algunos estudios han puesto de manifiesto ciertos efectos negativos sobre la actividad biológica con la aplicación de esta enmienda (Hardgrove y Livesley, 2016).

Figura 10. Posos de café (Quintana, 2013).

En definitiva, puede decirse que estas enmiendas pueden presentar efectos beneficiosos para el agro-sistema, siendo el más importante el incremento del contenido en carbono orgánico y materia orgánica. No obstante, antes de aplicarlas en suelos agrícolas, resulta de gran importancia caracterizar como este nuevo aporte de materia orgánica influye en la composición de la materia orgánica total del suelo, ya que puede modificar en gran medida la composición de las diferentes fases de esta, sobre todo sobre los ácidos húmicos (Senesi y Plaza, 2007). Estos cambios pueden influir en la funcionalidad de la materia orgánica (Pérez-

Lomas et al., 2010), afectando a la reserva de carbono orgánico del suelo, la disponibilidad de nutrientes en el corto, medio y largo plazo, así como a la resistencia de esta materia orgánica a la degradación (Djukic et al., 2010). También es importante tener en cuenta la influencia que las características del suelo pueden tener en la respuesta a la adición de estas enmiendas, ya que factores como la granulometría, la naturaleza mineralógica de las partículas del suelo, así como la arquitectura de la matriz del suelo (estructura) intervienen decisivamente en la descomposición, evolución y estabilización de los compuestos orgánicos (Baldock y Skjemstad, 2000).

El factor más crítico a considerar en este tipo de enmiendas orgánicas es su elevada repelencia al agua (Aranda et al., 2016). Esta repelencia se genera en estos materiales debido a la acumulación de diferentes compuestos hidrofóbicos, principalmente compuestos orgánicos con un alto carácter alifático, presentes de manera natural como producto de la descomposición del material fresco y poco evolucionado. La repelencia al agua afecta al régimen hídrico del suelo, reduciendo en gran medida la capacidad de infiltración del suelo (Mohawesh et al., 2014), así como la conductividad hidráulica y el agua disponible para la planta (Doerr y Thomas, 2000). A esto se le une el aumento del riesgo de erosión, en forma de erosión hídrica superficial, debido a la acumulación de agua en superficie que no puede ser filtrada, y que arrastra todo el material que encuentra a su paso (Diamantopoulos et al., 2013), especialmente al inicio de la estación lluviosa. Este efecto se amplificaría aún más en zonas de elevada pendiente (Ahn et al., 2013), generando o agravando este problema medioambiental, que ya está bastante extendido en el olivar mediterráneo. Además, el aumento de la erosión derivada de la repelencia al agua, podría generar un desajuste en la cantidad de nutrientes disponibles para la planta, e incluso provocar una contaminación de las aguas subterráneas (Arye et al., 2011).

Para reducir los efectos perjudiciales de las enmiendas previamente descritos, existen diferentes alternativas. Una posible solución, que se explora por primera vez en la presente tesis doctoral, es la mezcla de diferentes enmiendas orgánicas antes de su aplicación, de manera que los efectos positivos se sumen, pudiendo contrarrestar algunos de sus efectos negativos. Un ejemplo sería la mezcla de compost de alperujo con los posos de café, ya que se podrían corregir los elevados niveles de relación C/N de los compost de alperujo inmaduros, así como el pH, cuyo valor podría compensarse y acercarse a un valor neutro. Además, la mezcla de estas dos enmiendas orgánicas daría lugar al desarrollo de compost más funcionales y de mayor calidad (Emmanuel et al., 2017). Sin embargo, la mezcla de enmiendas orgánicas no soluciona todos los efectos perjudiciales que estás pueden tener para el agro-sistema tras su aplicación, como es la generación de repelencia al agua una vez añadidas al suelo. Existen diferentes estudios que han demostrado que cuando los suelos son sometidos a elevadas temperaturas, debido a eventos incendiarios, la repelencia al agua mostrada por este tipo de suelos tiende a desaparecer, resultados que han sido replicados en laboratorio con estudios sobre la materia orgánica (García-Corona et al., 2004; Mataix-Solera y Doerr, 2004). Las altas temperaturas disminuyen la repelencia al agua debido a la degradación térmica o la vaporización de las sustancias orgánicas responsables de la hidrofobicidad, principalmente los hidrocarburos con un carácter alifático (Simkovic et al., 2008). Estos hallazgos sugieren que los tratamientos térmicos sobre las fases orgánicas consiguen eliminar la repelencia al agua, por lo que este tipo de metodología podría ser aplicable

a las diferentes enmiendas orgánicas con efectos similares. Además se ha demostrado que el tratamiento a altas temperatura sobre fracciones orgánicas cambia en gran medida su composición, aumentando la fracción de materia orgánica más estable a la degradación química y biológica (González-Pérez et al., 2004). Puesto que el tratamiento térmico sobre las enmiendas orgánicas ha sido poco investigado, se requiere de un estudio en profundidad, no solo para determinar si se consigue realmente eliminar la repelencia al agua, sino también para estudiar los efectos y los cambios en la funcionalidad de la materia orgánica de estas enmiendas, así como en la materia orgánica del suelo tras su aplicación (Pérez-Lomas et al., 2010).

3. Caracterización tradicional del agro-sistema de olivar

La caracterización del agro-sistema de olivar, requiere información sobre todos los aspectos que influyen en su salud, esto es el medio edáfico, el estado nutricional de la planta, y los diferentes insumos que se vayan a añadir, ya sean enmiendas orgánicas, abonos, agua de riego, etc.

3.1 Análisis del suelo

El análisis del suelo, realizado con cierta periodicidad, resulta de gran utilidad para conocer variaciones en la cantidad de nutrientes disponibles, e imprescindible para el diagnóstico de deficiencias y/o posibles toxicidades por exceso de algún elemento (Fernández-Escobar, 2008). Los procedimientos típicos para la caracterización y evaluación de suelos son complejos, especialmente cuando se trabaja con un gran número de muestras (He et al., 2007). El análisis de los caracteres morfológicos *in situ* está muy condicionado por la meteorología, que puede impedir realizar el muestreo o incluso alterar los resultados de las variables. Mientras, para conseguir una evaluación completa de las características del suelo estudiado, es necesario un análisis más completo en el laboratorio. Este análisis normalmente incluye gran cantidad de parámetros, que se clasifican en parámetros físicos, químicos y biológicos. Los principales análisis realizados en suelos se describen a continuación:

Parámetros físicos; son aquellos que hacen referencia al estado físico del suelo, su estructura y la capacidad para contener aire y agua. Los parámetros físicos más comunes son:

- Textura: este parámetro clasifica el suelo según % en peso de cada tamaño granulométrico, arcilla (< 2 μm diámetro), limo (2 - 50 μm diámetro) y arena (0.05 - 1 mm diámetro). Este parámetro aporta mucha información sobre el estado físico y físico-químico del suelo.
- Fragmentos gruesos: se refiere a la fracción granulométrica mayor de 2 mm de diámetro. Esta fracción es indicativa de la pedregosidad del suelo, y por lo general, valores altos son indicativos de suelos poco evolucionados o muy degradados.
- Densidad real y densidad aparente: la densidad real, que es la densidad media de las partículas sólidas que componen el suelo, y la densidad aparente, que es aquella que tiene en cuenta el volumen de suelo ocupados por los poros. Mientras la densidad nos da una idea de qué fracciones pueden estar predominando en el suelo, la densidad aparente nos habla sobre el grado de aireación, porosidad y compactación del suelo.

- Capacidad de campo (CC), punto de marchitamiento permanente (PMP) y agua útil: se define como capacidad de campo a la máxima cantidad de agua que un suelo es capaz de retener. Por su parte el punto de marchitamiento permanente es la cantidad de agua mínima, por debajo de la cual la planta es incapaz de seguir extrayendo agua del suelo. La cantidad de agua por encima del punto de marchitamiento permanente, hasta el nivel de la capacidad de campo, es lo que se considera agua útil. Estos parámetros son de gran importancia ya que nos indica la reserva de agua de la que planta puede disponer, y a partir de que cantidad esta será inaccesible.

- Porosidad total, macroporosidad y microporosidad: la porosidad del suelo es el sistema de cavidades y espacios vacíos presentes en él. Estos pueden ser de diferentes tamaños, diferenciándose principalmente entre macroscópicos (> 8 μm diámetro), generalmente llenos de aire (macroporosidad), y microscópicos (< 8 μm diámetro), de menores proporciones, en gran parte ocupados por agua retenida por las fuerzas capilares (microporosidad). Una correcta distribución de aire y agua requiere una buena distribución entre macro y microporosidad. Un exceso de microporosidad genera un ambiente asfixiante con escaso suministro de oxígeno a las raíces, mientras un exceso de macroporosidad supone una incapacidad del suelo para suministrar el agua necesaria a la planta.

Parámetros químicos: son aquellos que hacen referencia a la concentración en el suelo de determinados elementos o compuestos. Los parámetros medidos comúnmente son:

- pH: medida del catión hidrogenión (H^+), definido como el logaritmo negativo en base 10 de la concentración de este catión en moles L^{-1}. Valores bajos de pH indica gran concentración de este ion, y por tanto acidez, mientras que valores altos indican alcalinidad. El valor de pH nos indica la disponibilidad y posible bloqueo de algunos nutrientes.
- Conductividad eléctrica (CE): este parámetro mide la capacidad del suelo para conducir la corriente eléctrica, que depende de la cantidad de sales disueltas presentes, por lo que un valor excesivo indicaría una salinización del suelo.
- Materia orgánica y carbono orgánico: estos dos parámetros están correlacionados, y suelen calcularse mediante el uso del Factor de Bremmelen (1.724). Su elevada importancia ha sido previamente discutida en el aparatado 1.2 Materia orgánica y su papel clave.
- Nitrógeno total: este parámetro es uno de los principales macronutrientes para todos los cultivos. Se encuentra en el suelo en diferentes formas, por lo que también resulta a veces importante conocer la cantidad de cada una de ellas.
- Fosforo extraíble: este elemento, que aparece en diferentes formas en el suelo (orgánico e inorgánico), es un macronutriente para la mayoría de las plantas, por lo que su presencia y disponibilidad en el suelo es indispensable.
- Cationes: los principales cationes presentes en el suelo son Ca, Mg, K y Na. Estos cationes están presentes tanto en la solución del suelo, como formando parte de algunos minerales. Todos ellos son nutrientes necesarios para el correcto desarrollo de la planta.

- Micronutrientes: los principales micronutrientes son B, Cl, Cu, Fe, Mn, Mo y Zn. Aunque son necesarios en cantidades muy pequeñas para la planta, son totalmente esenciales para su correcto desarrollo.
- Capacidad de intercambio catiónico (CIC) y cationes en el complejo de cambio: la capacidad de intercambio catiónico es la capacidad que tiene el complejo de cambio del suelo para retener y liberar cationes. Un CIC elevado indica una gran capacidad del suelo para aportar los cationes necesarios a la planta. Los cationes que se retienen en el complejo de cambio son Ca, Mg, K y Na. La proporción de estos indica que nutrientes están más biodisponibles, así como posibles déficits.

Parámetros biológicos; estos parámetros están relacionados con la cantidad y variedad de organismos vivos presentes en el suelo, así como su actividad. Aunque no suelen incluirse en las analíticas típicas de laboratorio, son parámetros que aportan gran cantidad de información. Algunos ejemplos de parámetros biológicos son:

- Biomasa microbiana y su composición elemental: la biomasa microbiana constituye el componente vivo de la materia orgánica del suelo, siendo una fracción muy lábil, que responde rápidamente a cualquier cambio producido en el suelo. Se suelen cuantificar sus diferentes fracciones, carbono microbiano (Cmic), nitrógeno microbiano (Nmic), fósforo microbiano (Pmic) y azufre microbiano (Smic), pudiendo asociar estos parámetros al tipo de población microbiana y su abundancia.
- Respiración basal: es una medida del dióxido de carbono generado por toda la materia orgánica viva del suelo, y nos da una idea del nivel de actividad biológica presente en el suelo.
- Actividades enzimáticas: son medidas más específicas, normalmente relacionadas con alguna transformación que sucede en los diferentes ciclos biogeoquímicos del suelo. Algunos ejemplos son la actividad deshidrogenasa (actividad global en el suelo), actividad B-glucosidasa (ciclo del carbono), actividad arilsulfatasa (ciclo del azufre), actividad fosfatasa (ciclo del fosforo) o el potencial de nitrificación (ciclo del nitrógeno).

Los procedimientos típicos para la determinación de estos parámetros en suelos, implican metodologías que requieren preparaciones de muestra largas y tediosas así como de la utilización de distintos instrumentos de medida, algunos relativamente sencillos y asequibles, como pHmetros, conductímetros, espectrómetros UV-vis y otros de mayor coste de adquisición, mantenimiento y funcionamiento como pueden ser espectrómetros de absorción atómica, espectrómetros de emisión atómica de plasma acoplado por inducción o cromatógrafos iónicos (Fernández-Escobar et al., 2009; Shenk et al., 1979). En el caso de los parámetros biológicos hay que añadir además el requerimiento de períodos de incubación que pueden llegar a ser incluso de varias semanas. Por tanto, un análisis completo del suelo es un proceso complejo y costoso, lo que impide en muchos casos, la realización de una evaluación tan completa como sería deseable del estado de degradación de los suelos. Se trata, además, de metodologías de análisis que necesitan del empleo de reactivos químicos tóxicos (Benton Jones, 2001), que generan residuos contaminantes tanto a la hora de utilizarlos, como en sus procesos fabricación, convirtiéndose en general en metodologías analíticas poco sostenibles.

Una opción alternativa a la determinación de todos los parámetros del suelo para caracterizarlo, podría ser la estimación global de la calidad del suelo. Por desgracia, ninguna variable por sí sola, o combinación de ellas ha demostrado ser capaz de reflejar la gran variedad de procesos que influyen en la calidad del suelo (Puglisi et al., 2006). Frente a esto, diferentes investigaciones han demostrado que lo más útil para la medida de la calidad del suelo sería la utilización de índices sintéticos de calidad del suelo (Bastida et al., 2008), que integren un determinado número de variables, las cuales sean capaces de dar como resultado un solo índice numérico, que nos permita asociar a cada muestra de suelo un número indicador de su calidad total. Pero la elaboración de estos índices no resulta sencilla, y sigue siendo uno de los principales retos hoy día para la ciencia del suelo (Paz-Ferreiro y Fu, 2016; Zornoza et al., 2008), estando todavía en discusión que algoritmos son los más eficientes para la creación de estos índices, que tipo de variables resultan de mayor utilidad para su creación, que número de variables es el ideal para crear un índice efectivo, entre otros muchos factores a tener en cuenta. La elaboración de estos índices, unido a la creación de una metodología que permita calcularlos de manera rápida, supondría una gran herramienta que permitiría caracterizar el suelo de manera mucho más efectiva que los análisis tradicionales de laboratorio.

3.2 Análisis foliar

El análisis foliar, es el mejor método de diagnóstico del estado nutricional de la plantación, debido a que la hoja es el principal reflejo del metabolismo de la planta. Existe una clara relación entre los niveles de los diferentes elementos en hoja y su efecto sobre el rendimiento y las afecciones del cultivo. Resulta especialmente útil para detectar desordenes nutritivos, bien sea por niveles bajos de nutrientes o incluso por deficiencias que pueden ser perjudiciales para la salud de la planta (Fernández-Escobar, 2008). Los principales elementos nutritivos del olivo son dieciséis, que se clasifican según su disponibilidad para la planta y la cantidad necesaria en:

- Elementos no minerales: carbono (C), hidrógeno (H) y oxígeno (O), son nutrientes que no necesitan fertilización ya que la planta los coge del aire y el agua, ya sea a través de los estomas o de las raíces.
- Macronutrientes minerales: nitrógeno (N), fósforo (P), potasio (K), magnesio (Mg), calcio (Ca) y azufre (S), son los nutrientes que la planta necesita en mayor cantidad.
- Micronutrientes minerales: manganeso (Mn), zinc (Zn), boro (B), hierro (Fe), cobre (Cu), molibdeno (Mo) y cloro (Cl), elementos necesarios en menor cantidad, pero igualmente indispensables para el correcto desarrollo de la planta.

ELEMENTO	Niveles nutritivos estándar sobre peso seco			
	Deficiente (MB)	Bajo (B)	Normal (N)	Alto (A)
N (%)	< 1,40	1,41 - 1,50	1,51 - 2,00	> 2,00
P (%)	< 0,05	0,06 - 0,09	0,10 - 0,30	-
K (%)	< 0,40	0,40 - 0,79	0,80 - 1,00	> 1,00
Ca (%)	< 0,30	0,30 - 1,00	> 1,00	-
Mg (%)	< 0,08	0,08 - 0,10	> 0,10	-
Mn (p.p.m.)	-	-	> 20	-
Zn (p.p.m.)	-	-	> 10	-
Cu (p.p.m.)	-	-	> 4	-
B (p.p.m.)	<14	14 -19	20 -150	-

Figura 11. Tabla de niveles nutritivos en la hoja de olivo (Junta de Andalucía, 2008)

Estos análisis nos permiten determinar rápidamente que elementos están en mayor o menor cantidad en la planta, y junto con el análisis del medio edáfico, se convierten en la forma ideal de caracterizar el agro-sistema del olivo y conocer en profundidad el estado del cultivo, permitiéndonos tomar las decisiones adecuadas a la hora de intentar alcanzar la máxima producción, a la vez que mantenemos una buena salud de suelo y planta. Sin embargo, para la medida de estos parámetros foliares, como sucedía con los análisis de laboratorio en el caso de los suelos, la mayoría de métodos incluyen valoraciones, digestiones por vía húmeda y otros tratamientos de muestra, que utilizan reactivos con mayor o menor peligrosidad, así como el uso de equipos de espectroscopía UV-Vis o de absorción atómica o fotometría de llama (Fernández-Escobar et al., 2009; Shenk et al., 1979). Todos ellos suelen resultar tediosos, a la par de costosos en tiempo y dinero.

4. Metodologías analíticas basadas en la espectroscopía

4.1 Espectroscopía infrarroja

Puesto que las metodologías tradicionales de análisis del agro-sistema de olivar presentan los inconvenientes comentados anteriormente, se hace necesaria la implementación de nuevas metodologías que permitan una caracterización más rápida y económica que estos métodos convencionales. Una posible alternativa es la utilización de la espectroscopia infrarroja (Rotbart et al., 2013; Viscarra Rossel et al., 2006). Esta técnica presenta numerosas ventajas, destacando el mínimo tratamiento de muestra necesario (ninguno en algunos casos), su capacidad de determinar diferentes parámetros de interés de modo simultáneo, la posibilidad de implementación *in situ*, y su rapidez de análisis y precisión.

La espectroscopia infrarroja es una técnica de espectroscopia molecular, en la cual se utiliza la radiación correspondiente a la franja electromagnética del infrarrojo, que va desde los números de onda de 12800 hasta los 10 cm^{-1}, correspondiente a las longitudes de onda de 0.78 a 1000 µm. Desde el punto de vista aplicado, la espectroscopía infrarroja se divide, dependiendo de la longitud de onda utilizada, entre:

- Espectroscopía de infrarrojo medio (MIR), que se corresponde con la franja comprendida entre los 4000 y los 400 cm^{-1} (2.5 - 25 μm).
- Espectroscopía de infrarrojo cercano (NIR) que se corresponde a la franja con números de onda entre los 12500 y los 4000 cm^{-1} (800 - 2500 nm).

Para obtener un espectro se hace incidir un haz de radiación infrarroja sobre la muestra, de manera que cuando coincide la frecuencia del haz con la frecuencia de vibración natural de algunos de los grupos funcionales, se produce un fenómeno de absorción de la radiación, sirviendo esta energía para aumentar la amplitud de los movimientos vibracionales de la molécula. Las vibraciones moleculares fundamentales (Figura 12) pueden ser de tensión, cuando se produce un cambio en la distancia interatómica a lo largo del eje del enlace entre dos átomos, pudiendo ser simétricas o asimétricas, o vibraciones de flexión, cuando cambia el ángulo entre dos enlaces, pudiendo estas ser de cuatro tipos (tijereteo, balanceo, aleteo o torsión). Para que una vibración molecular sea activa en IR debe producir un cambio en el momento dipolar de la molécula. Dado que la energía de la radiación absorbida (directamente proporcional a la frecuencia e inversamente proporcional a la longitud de onda) corresponde a la diferencia entre dos estados energéticos de la molécula absorbente , el espectro de absorción resultante presenta una serie de bandas características que pueden usarse con fines analíticos (Stenberg et al., 2010).

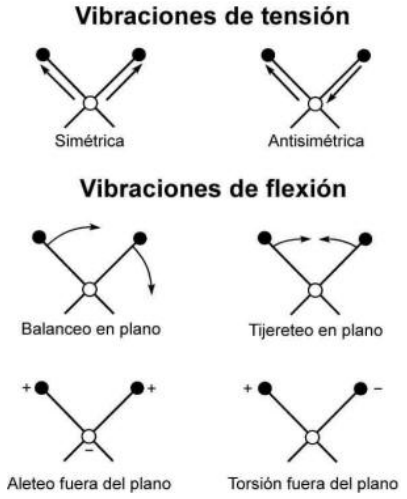

Figura 12. Tipos de vibraciones moleculares fundamentales (Skoog, 2001)

Los instrumentos para el registro de espectros infrarrojos utilizan frecuentemente tecnología para aplicar la transformada de Fourier. A estas técnicas se las refiere en literatura añadiendo el sufijo FT para indicar su utilización, quedando como FT-NIR para el caso de infrarrojo cercano, o FTIR para infrarrojo medio, región en la que el uso de esta tecnología está muy generalizado. En este tipo de instrumentos, la radiación emitida por la fuente se introduce en un interferómetro, donde es modulada, y dirigida hacia la muestra, para después llegar al detector que registra el interferograma. En el interferómetro la radiación de la fuente se divide en dos haces, una de las cuales se refleja en un espejo móvil y otro en un espejo fijo, de manera que al combinarse de nuevo dan lugar a una serie de fenómenos de interferencia constructiva y destructiva al seguir ambos haces caminos diferentes (Figura 13). El retardo óptico de la señal registrada da lugar al

interferograma. El interferograma es la medida de la variación de la energía en función del retardo óptico, es decir, en función del tiempo. Toda la información sobre la radiación emitida por la fuente y su interacción con la muestra está contenida en el interferograma, y mediante la aplicación del algoritmo de la transformada de Fourier esta información en el dominio tiempo se convierte al dominio de la frecuencia, dando lugar al espectro infrarrojo típico, con la representación de la radiación respecto a la frecuencia o el número de onda.

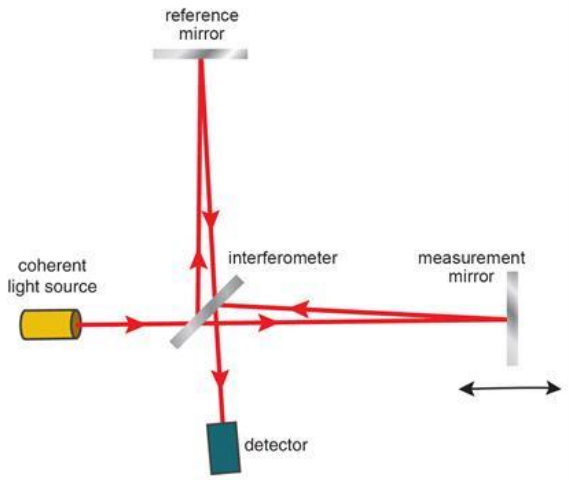

Figura 13. Esquema del funcionamiento de un interferómetro (Renishaw plc.)

La espectroscopía infrarroja se ha utilizado ampliamente en el estudio del suelo, al ser sensible a ambas fases del mismo, orgánica e inorgánica, (Du y Zhou, 2009). La aplicación más extendida es la creación de modelos predictivos de diferentes parámetros de interés, especialmente la cantidad de materia orgánica o carbono orgánico, así como otros nutrientes tales como nitrógeno, fósforo o potasio (He et al., 2007; Soriano-Disla et al., 2014), siendo los resultado generalmente satisfactorios, especialmente en el caso de la predicción de la materia orgánica. También se han predicho de manera bastante fiable otras variables tales como el pH, o CIC (Chodak et al., 2004). En el caso de las variables físicas, los resultados son más diversos. Algunos estudios han conseguido predecir variables tales como la cantidad de arcilla, el estado de agregación del suelo, o la capacidad de campo (Cozzolino y Morón, 2003; Soriano-Disla et al., 2014), aunque muchas de ellas con una precisión reducida. Por otro lado, existen también estudios acerca de la viabilidad de predecir variables biológicas, con resultados contradictorios, ya que mientras algunos autores son capaces de predecir con buenos resultados variables como la respiración, la biomasa microbiana o algunas actividades enzimáticas específicas, en la mayoría de los casos los resultados suelen ser relativamente pobres, dando lugar a modelos predictivos poco robustos (Bellino et al., 2016; Dick et al., 2013; Heinze et al., 2013; Reeves et al., 2000). La espectroscopía infrarroja también puede usarse como método para clasificar y discriminar entre diferentes tipologías de suelo (D'Acqui et al., 2010), siendo además posible estudiar con esta tecnología los efectos del uso del suelo, así como sus niveles de degradación y la necesidad de llevar a cabo actividades de restauración. En este sentido, existen pocos estudios disponibles en regiones mediterráneas semiáridas (Fontán et al., 2011; Zornoza et al., 2008).

En lo referente al análisis foliar también existen estudios que han demostrado la aplicabilidad de la espectroscopia infrarroja para la predicción de los niveles de algunos de los nutrientes más importantes para las plantas. La mayoría de estudios se han centrado en la determinación de nitrógeno en hojas, con ejemplos de aplicación como el manzano, algodón o pepino (Ji-yong et al., 2011; Riley y Cánaves, 2002; Zhang et al., 2012). Existen también algunos estudios para otros macronutrientes, como el fósforo y el potasio, con buenos resultados en plantas como la caña de azúcar (Chen et al., 2002), si bien en otros casos como, la planta de té o la mano de buda, la técnica presenta ciertas limitaciones, debido a las bajas concentraciones de estos nutrientes (Liao et al., 2012; Yanfang et al., 2013). La determinación de micronutrientes con esta técnica está menos extendida, y, por lo general, arroja resultados más discretos (Galvez-Sola et al., 2015; Petisco et al., 2008; Shao y He, 2013; Yarce y Rojas, 2012). Por tanto, la espectroscopía infrarroja se demuestra como eficaz para el diagnóstico nutricional, aunque con ciertas limitaciones y resultados muy diversos según la planta sobre la que se realice (Liao et al., 2012). En el caso de la hoja de olivo, son escasos los estudios, únicamente se ha descrito su uso para determinar umbrales de contenido en nitrógeno (Rotbart et al., 2013) o clasificar muestras entre deficientes y no deficientes en nitrógeno y potasio (Gómez-Casero et al., 2007).

En el caso de las enmiendas orgánicas, la espectroscopia infrarroja se ha utilizado como una técnica complementaria de análisis, que puede aportar información sobre los componentes tanto orgánicos como inorgánicos de las diferentes enmiendas. Asimismo, la información sobre el predominio de diferentes grupos funcionales, puede relacionarse con diferentes efectos, tales como la toxicidad, o las propiedades antioxidantes (Magalhães et al., 2016; Páscoa et al., 2013), así como para estimar la calidad y grado de evolución de su fracción orgánica (Tomati et al., 2001).

Por tanto, a la luz de la bibliografía existente parece claro que la espectroscopia infrarroja puede ser una alternativa ventajosa para estudiar las diferentes matrices del agro-sistema de olivar, como el suelo o la hoja. Así, algunos autores han cifrado el posible ahorro económico de su utilización en un contexto agrícola en hasta un 80% (Nduwamungu et al., 2009), proporcionando además mayor reproducibilidad que las técnicas de laboratorio tradicionales (Genot et al., 2011). Por otro lado, la existencia y cada vez más amplio desarrollo de equipos portátiles de tamaño reducido y con unas prestaciones muy satisfactorias para realizar este tipo de medidas, podría permitir en un futuro el análisis del conjunto de las propiedades *in situ* (Reeves, 2010). Este enfoque sería especialmente útil en la denominada agricultura de precisión, consistente en un monitoreo espacio-temporal del suelo para conocer su composición utilizando tecnología GPS, lo que permite adaptar las prácticas agrícolas a las necesidades reales del cultivo, optimizando parámetros tales como el riego o el abonado (D'Acqui et al., 2010).

4.2 Fluorescencia de rayos X por energía dispersiva

Otra alternativa al análisis convencional es el uso de la fluorescencia de rayos X (XRF). Esta técnica se basa en la medida de la radiación emitida, en forma de emisión secundaria o fluorescencia, en la región de longitud de onda correspondiente a la radiación X. Para ello la muestra es excitada mediante el uso de una fuente emisora de rayos X. La radiación X incidente o primaria provoca un movimiento de los electrones situados en las capas interiores del átomo excitado. Tras esto, aquellos electrones situados en las capas más externas ocupan los lugares vacantes, mientras el exceso energético resultante de esta transición se emite en forma de radiación X fluorescente o secundaria. Esta radiación de fluorescencia es característica para cada elemento, y según el cambio de orbital que se produzca, para cada elemento existen diferentes transiciones, que se denominan:

- Kα: transición de orbital L a orbital K
- Kβ: transición de orbital M a orbital K
- Lα: transición de orbital M a orbital L
- Lβ: transición de orbital M a orbital L

Conociendo las energías características de cada elemento (Figura 14), se puede detectar su presencia, así como cuantificarlos mediante el uso de calibraciones de parámetros fundamentales. Un aspecto particularmente interesante de esta técnica y que la hace atractiva en aplicaciones de tipo agro-ambiental es la existencia de equipos portátiles de fluorescencia de rayos X por energía dispersiva (EDXRF). Se trata de equipos extremadamente compactos y robustos, muy útiles en aplicaciones de campo (Kalnicky y Singhvi, 2001).

Z		Element	$K\alpha_1$	$K\beta_1$	$L\alpha_1$	$L\beta_1$	Z		Element	$K\alpha_1$	$K\beta_1$	$L\alpha_1$	$L\beta_1$
3	Li	Lithium					34	Se	Selenium	11.224	12.497	1.379	1.419
4	Be	Beryllium	0.108				35	Br	Bromine	11.924	13.292	1.481	1.526
5	B	Boron	0.183				36	Kr	Krypton	12.648	14.112	1.585	1.636
6	C	Carbon	0.277				37	Rb	Rubidium	13.396	14.961	1.692	1.751
7	N	Nitrogen	0.392				38	Sr	Strontium	14.165	15.835	1.806	1.871
8	O	Oxygen	0.525				39	Y	Yttrium	14.958	16.739	1.924	1.998
9	F	Fluorine	0.677				40	Zr	Zirconium	15.775	17.668	2.044	2.126
10	Ne	Neon	0.849				41	Nb	Niobium	16.615	18.625	2.169	2.260
11	Na	Sodium	1.040				42	Mo	Molybdenum	17.480	19.606	2.292	2.394
12	Mg	Magnesium	1.254	1.302			43	Tc	Technetium	18.367	20.626	2.423	2.535
13	Al	Aluminium	1.486	1.557			44	Ru	Ruthenium	19.279	21.656	2.558	2.683
14	Si	Silicon	1.740	1.837			45	Rh	Rhodium	20.216	22.724	2.697	2.834
15	P	Phosphorus	2.010	2.139			46	Pd	Palladium	21.177	23.818	2.838	2.990
16	S	Sulfur	2.309	2.465			47	Ag	Silver	22.163	24.941	2.983	3.150
17	Cl	Chlorine	2.622	2.812			48	Cd	Cadmium	23.173	26.093	3.133	3.315
18	Ar	Argon	2.958	3.190			49	In	Indium	24.210	27.275	3.286	3.487
19	K	Potassium	3.314	3.590			50	Sn	Tin	25.271	28.485	3.444	3.663
20	Ca	Calcium	3.692	4.013	0.341	0.345	51	Sb	Antimony	26.359	29.725	3.604	3.842
21	Sc	Scandium	4.093	4.464	0.395	0.400	52	Te	Tellurium	27.473	30.993	3.768	4.029
22	Ti	Titanium	4.512	4.933	0.452	0.458	53	I	Iodine	28.612	32.294	3.938	4.221
23	V	Vanadium	4.953	5.428	0.510	0.518	54	Xe	Xenon	29.775	33.620	4.110	4.418
24	Cr	Chromium	5.415	5.947	0.572	0.582	55	Cs	Cesium	30.973	34.982	4.285	4.619
25	Mn	Manganese	5.900	6.492	0.637	0.648	56	Ba	Barium	32.194	36.378	4.466	4.828
26	Fe	Iron	6.405	7.059	0.705	0.718	57	La	Lanthanum	33.442	37.797	4.647	5.038
27	Co	Cobalt	6.931	7.649	0.775	0.790	58	Ce	Cerium	34.720	39.256	4.839	5.262
28	Ni	Nickel	7.480	8.267	0.849	0.866	59	Pr	Praseodymium	36.027	40.749	5.035	5.492
29	Cu	Copper	8.046	8.904	0.928	0.947	60	Nd	Neodymium	37.361	42.272	5.228	5.719
30	Zn	Zinc	8.637	9.570	1.012	1.035	61	Pm	Promethium	38.725	43.827	5.432	5.961
31	Ga	Gallium	9.251	10.267	1.098	1.125	62	Sm	Samarium	40.118	45.414	5.633	6.201
32	Ge	Germanium	9.886	10.982	1.188	1.218	63	Eu	Europium	41.542	47.038	5.849	6.458
33	As	Arsenic	10.543	11.726	1.282	1.317	64	Gd	Gadolinium	42.996	48.695	6.053	6.708

Figura 14. Extracto de la tabla de emisión de fluorescencia de rayos X. Bruker Corp.

La fluorescencia de rayos X ha sido ampliamente utilizada para la determinación de elementos químicos en campos muy diversos como la minería, el patrimonio artístico o la joyería (Omote et al., 1995). Su uso en muestras orgánicas está mucho menos extendido, posiblemente debido a los menores niveles de concentración de los elementos detectables y a la mayor complejidad de estas matrices, lo que dificulta la aplicación de los modelos de calibración basados en parámetros fundamentales, al ser más probable la existencia de interferencias de la matriz, a través de fenómenos como la absorción de rayos X. Sin embargo, en los últimos años se ha iniciado algunos estudios sobre la aplicación de esta técnica en análisis foliar. Así, se han determinado desde algunos macronutrientes hasta elementos traza en diferentes tipos de plantas, como la caña de azúcar o el té (Khuder et al., 2009; Obiajunwa et al., 2002; Pessanha et al., 2010; Xie et al., 1998). Por lo general, con esta técnica los metales pesados tales como manganeso, hierro, cobre, níquel y zinc pueden ser detectados y estimados, incluso con límites de cuantificación del orden de las pocas partes por millón (ppm). Sin embargo, se ha demostrado que son necesarias mayores concentraciones para la determinación de elementos más ligeros como el azufre, el fosforo, el potasio, el magnesio o el calcio. En el caso de la hoja de olivo, tan solo se puede encontrar en bibliografía un estudio sobre la determinación de niveles de hierro en plantas enfermas de fumagina (Aragão et al., 2001). También se ha empleado esta técnica para la determinación de la composición elemental de los restos de biomasa, lo que incluye hoja de olivo, pero también otros residuos procedentes del olivar (García-Maraver et al., 2014), sin estar esta aplicación enfocada a conocer el estado nutricional de la planta.

4.3 Quimiometría

Tanto la espectroscopia infrarroja como la fluorescencia de rayos X permiten realizar un gran número de medidas en un tiempo razonable, pero la gestión y tratamiento de estos datos, que en la mayoría de los casos incluyen entre 1000 y 2000 variables para cada espectro, requiere el uso de herramientas quimiométricas para obtener información relevante. La International Chemometrics Society define la quimiometría como la ciencia que permite relacionar las mediciones realizadas en un sistema químico o proceso con el estado del mismo, mediante la aplicación de métodos matemáticos o estadísticos (Miller y Miller, 2002). Las técnicas quimiométricas se pueden clasificar en técnicas supervisadas y técnicas no supervisadas.

Se llaman técnicas no supervisadas a aquellas en que se modela directamente la información, tratada como variables aleatorias, sin incluir ningún conocimiento a priori, es decir sin incluir ninguna variable numérica o categórica distinta a las observaciones (el espectro en nuestro caso). Estas técnicas se utilizan para la detección de patrones, para la comprensión de los datos y/o para realizar agrupaciones. Con estos objetivos, a lo largo de la presente tesis doctoral se utilizaron diferentes técnicas quimiométricas no supervisadas:

- Análisis de componentes principales (PCA): técnica utilizada para realizar una reducción de variables sin pérdida de información relevante. Las nuevas variables obtenidas, llamadas componentes principales, se descomponen en puntuaciones (scores) y cargas (loadings). El análisis en conjunto de puntuaciones y cargas nos permite conocer similitudes y/o diferencias entre muestras gracias a las puntuaciones, y a que se deben mediante el análisis de las cargas (Ríos Castro et al., 2013).

- Análisis jerárquico de conglomerados (HCA): en esta técnica cada muestra se representa como un punto en un espacio multidimensional, y se miden las similitudes entre ellas usando diferentes métodos, siendo el más común la distancia euclídea. Para la formación de los agrupamientos se utilizan diferentes algoritmos, como el método de Ward, en el cual se unen las muestras con el criterio de que al sumarse la heterogeneidad debe crecer lo mínimo posible. Independientemente del algoritmo utilizado, se genera un dendrograma bidimensional (Ríos Castro et al., 2013).

Por otro lado, las técnicas supervisadas son aquellas que generan un modelo empírico que relaciona el valor de un atributo, llamado etiqueta, que puede ser categórico o numérico, con las observaciones (los espectros). Si la etiqueta es categórica el modelo será de clasificación mientras que si es numérica tendremos un modelo de regresión. Con estos modelos podemos estimar variables asociadas a las muestras, calibrando modelos multivariable a partir de las variables de interés. Para la modelización de espectros se han utilizado técnicas multivariantes muy diferentes, que van desde una regresión de componentes principales (PCR), *support vector machines* (SVM) o *random forest* (RF), hasta técnicas mucho más complejas, que requieren elevados tiempos de computación tales como las redes neuronales artificiales (ANN). No existe acuerdo sobre que técnicas es la más potente, pudiendo variar los resultados dentro de un mismo estudio dependiendo del parámetro predicho (Gholizadeh et al., 2013; Rossel y Behrens, 2010). De entre todas estas técnicas, posiblemente, la regresión por mínimos cuadrados parciales (PLSR) es la más ampliamente utilizada, con resultados muy satisfactorios en la mayorías de estudios (Brown et al., 2006).
Este método es una regresión bilineal mediante la cual se obtienen un número de factores o variables latentes, construidas como combinación de los datos espectrales originales, y que son utilizados como predictores en una ecuación de predicción. Las variables latentes se crean de manera parecida a los componentes principales de un PCA, pero la descomposición está guiada por la variabilidad de la etiqueta o etiquetas introducidas, maximizando la covarianza entre las observaciones y la variable de interés.

Asimismo, existen técnicas quimiométricas que permiten fusionar diferentes fuentes de información espectral, para de esta manera conseguir modelos más robustos y resultados más precisos. Este tipo de estrategia ha demostrado ser de utilidad a la hora de fusionar espectros Raman, infrarrojos y de fluorescencia de rayos X, entre otros (Li et al., 2018; Márquez et al., 2016; Ramos et al., 2008; Wang et al., 2015). Para este tipo de metodología, dependiendo del tratamiento matemático que se realice de los datos, existen tres niveles de fusión, que se clasifican en (Figura 15):

- *Low level data fusion*: este nivel es el más básico, y consiste en tomar los datos obtenidos de ambas técnicas, y unirlos como si fueran uno solo, normalmente tras realizar algún tratamiento matemático de escalado para hacer que la magnitud de los datos sea parecida. Una vez se tiene la nueva matriz con los datos unidos, sobre esta se aplican los métodos quimiométricos.
- *Mid level data fusion*: este nivel es considerado el intermedio, y consiste en una reducción de las variables iniciales previa a la concatenación de estas. Esta reducción y/o selección de variables se suele hacer con técnicas quimiométricas, y busca conseguir una matriz más pequeña, pero que siga recogiendo toda la información relevante. Una vez realizada la reducción de variables, se unen las variables obtenidas para cada técnica, y sobre esta matriz se realizan los cálculos quimiométricos.

- *High-level data fusion*: este es el nivel más avanzado, y consiste en unir los resultados obtenidos para cada uno de los datos disponibles, para así generar un resultado más preciso. Aunque este nivel no suele utilizarse en quimiometría para predecir variables cuantitativas, si resulta muy útil para modelos de toma de decisión o discriminantes.

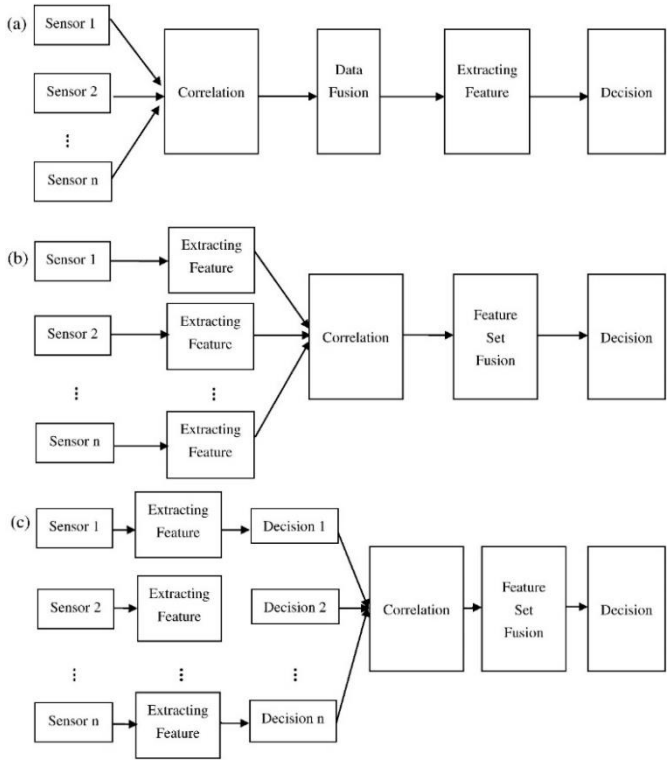

Figura 15. Niveles de fusión de datos (Sun et al., 2017)

Por otro lado, antes de construir un modelo quimiométrico, es indispensable realizar una selección de muestras representativas para la calibración y validación interna, es decir las muestras que se van a utilizar para construir y entrenar el modelo. Asimismo, también es necesario seleccionar una serie de muestras para la validación externa o independiente, que se utilizan para evaluar el rendimiento del modelo. Las muestras de calibración deben cubrir la variabilidad esperada en el conjunto completo de muestras actuales y futuras, tanto en lo referente a espectros como a datos analíticos (Dardenne et al., 2000), mientras el conjunto de validación debe ser independiente del conjunto de calibración, para evitar una evaluación optimista del rendimiento del modelo. El número de muestras disponibles para la calibración y la validación suele verse influido en gran medida por el costo y la complejidad de la adquisición de los datos de referencia, es por esto que en muchas ocasiones se utiliza la validación cruzada como método de entrenamiento y validación. Este método es especialmente adecuado cuando se dispone de pocos datos, en un intento por utilizar la mayor cantidad posible de valores. Se usa comúnmente para validar la calibración durante el entrenamiento del modelo y de esta manera ajustarlo, siendo cada muestra (validación cruzada leave one out), o grupo de muestras, eliminada cada vez del grupo de calibración, y utilizada únicamente para validar el modelo, repitiendo este proceso generalmente para el total de las muestras o conjuntos de muestras. Para la creación de estos modelos, en el caso de los suelos algunos autores han llegado a desarrollar una base de datos espectral, que recoge espectros representativos de todos los tipos de suelos del mundo (Viscarra

Rossel et al., 2016). Dicha herramienta puede ser de una gran utilidad, ya que puede permitir conseguir modelos empíricos aplicables a cualquier tipo de suelo del planeta, aunque el trabajar con dicha cantidad de muestras hace la modelización más compleja. Sin embargo, estimaciones realizadas por algunos autores cifran en alrededor de 100 el número de muestras necesarias para la creación de modelos robustos, lo que resulta en un trabajo más manejable que puede aportar resultados similares (Brown et al., 2006).

El preprocesado de los datos espectrales es el paso más importante antes del modelado quimiométrico y clave para obtener modelos robustos y precisos (Rinnan et al., 2009). El preprocesado consiste en una serie de tratamientos matemáticos que se realizan sobre el espectro, de manera que se optimiza y se hace más accesible la información, reduciendo además el ruido (Du y Zhou, 2009). Los métodos de preprocesado de los espectros se emplean para eliminar cualquier información inapropiada, que, en caso de ser introducida, no puede ser manejada correctamente por las técnicas de modelado. Algunos métodos de preprocesado utilizados con frecuencia, son la *multiplicative scatter correction* (MSC), el *smoothing* y derivada de Savitzky-Golay, *continuum removal* (CR), así como derivadas, correcciones de línea base y técnicas de centrado y escalado de los datos (Gholizadeh et al., 2015).

Capítulo 2
Hipótesis de trabajo y objetivos

Hipótesis de trabajo

La degradación de los suelos con la consiguiente pérdida de fertilidad, ha sido identificada en los últimos años como una de las principales amenazas para la sostenibilidad del olivar, un cultivo clave en Andalucía y, en particular, en la provincia de Jaén. En este contexto, se hace necesario un mejor conocimiento de los efectos que los distintos sistemas de manejo del olivar tienen sobre la calidad de los suelos, ya que problemas como la erosión, la pérdida de nutrientes (en particular de materia orgánica) o la salinización pueden estar ligados, en muchos casos, a ciertas prácticas agrícolas (laboreo, eliminación de cubiertas vegetales, riego, etc.) características del manejo convencional. Por el contario, sistemas de manejo más sostenibles podrían mantener o incluso mejorar la calidad del suelo y su fertilidad. En este sentido, el compostaje de residuos del propio olivar (como restos de poda y alperujo), o el reciclaje de otros subproductos como los posos de café en un estado más bruto, para su uso como enmiendas orgánicas es un aspecto interesante, pero de nuevo, requiere un buen conocimiento de los distintos efectos de dichas enmiendas sobre el suelo.

Los métodos tradicionales de análisis de suelo, hoja y otros insumos del agro-sistema, suelen incluir procedimientos experimentales largos y costosos que implican en ocasiones la utilización de reactivos contaminantes. Frente a ellos, el empleo de técnicas espectroscópicas como la espectroscopía infrarroja y la fluorescencia de rayos X, combinadas cuando sea necesario con herramientas quimiométricas, puede ofrecer una alternativa rápida y eficaz para la caracterización de todos los aspectos del agro-sistema del olivar.

Objetivos

I. Caracterizar el agro-sistema del olivar, especialmente el medio edáfico, estudiando los posibles efectos sobre el mismo de factores como la litología del suelo o los sistemas de manejo.
II. Evaluar el potencial de la espectroscopia infrarroja como técnica para discriminar entre diferentes tipologías de suelos de olivar, así como entre suelos en diferente estado de degradación.
III. Valorar el potencial de la espectroscopia infrarroja como técnica para evaluar el estado agroambiental del suelo, analizando los efectos derivados del manejo (cultivo convencional vs ecológico, fertirrigación y utilización de enmiendas orgánicas), y el diferente comportamiento asociado a la litología del suelo.
IV. Comprobar el potencial de la espectroscopia infrarroja, tanto FTIR como FT-NIR, por separado y trabajando en conjunto, para predecir propiedades físicas, físico-químicas y biológicas en suelos de olivar, así como índices sintéticos derivados de estas propiedades.
V. Analizar la composición de diferentes enmiendas orgánicas (compost de alperujo y posos de café), para entender mejor sus efectos sobre el suelo, y desarrollar estrategias para corregir algunos de sus inconvenientes mejorando su calidad.
VI. Evaluar la capacidad de diferentes técnicas espectroscópicas, tanto por separado como trabajando en conjunto, para determinar el estado nutricional del olivo mediante el análisis de la hoja.

Capítulo 3
Resumen de materiales y métodos

En este apartado se describen las muestras empleadas y se resumen las principales características de los procedimientos experimentales desarrollados en esta Tesis doctoral, así como la instrumentación empleada y los parámetros instrumentales relevantes. También se describen brevemente las herramientas quimiométricas utilizadas para el análisis de datos. La información completa específica de cada trabajo de investigación realizado, puede encontrarse en los capítulos correspondientes a las publicaciones.

1. Obtención y preparación de muestra

En esta tesis doctoral se han considerado muestras de suelo, de hoja de olivo y de enmiendas orgánicas. La Figura 16 presenta las localizaciones de las muestras de suelo. Estas se tomaron en el Valle del Atanor (Parque Natural de Sierra Mágina, Jaén, 81 muestras), en diferentes zonas de Sierra Mágina (Cambil y Pegalajar, 97 muestras) y de la Comarca de Jaén (Puente Tablas y Puente de la Sierra, 60 muestras), así como una parcela de olivar sometida a irrigación (Guarromán, 20 muestras). Además, se contó con muestras pertenecientes a la empresa Agronutrientes Jaén S.C.A. procedentes de más de 20 municipios de toda la provincia de Jaén (71 muestras), así como con 90 muestras, obtenidas tras un período de incubación, a partir de suelo de Vega y suelo Rojo, adquiridos en Pinos Puente y Padul respectivamente, en la provincia de Granada. Todas estas muestras se recogieron en los primeros centímetros del perfil del suelo, que según el caso fue desde los 5 hasta los 30 centímetros. Posteriormente fueron secadas al aire y después tamizadas con una luz de malla de 2 mm para obtener la fracción denominada tierra fina. Estas muestras se analizaron para los trabajos descritos en los capítulos 6, 7, 8 y 11.

Alrededor de 2500 muestras de hoja de olivo fueron suministradas por el laboratorio Agronutrientes Jaén S.C.A. y procedían de fincas de olivar de más de 30 municipios diferentes. Se recogieron durante los meses de verano e invierno de los años 2013 – 2016, se prepararon lavándolas con un jabón libre de fosfatos y agua destilada para eliminar el polvo y otros contaminantes. Después se secaron a 60 °C durante 24 h y se molturaron con un molinillo eléctrico. Las muestras se conservaron en oscuridad a 4 °C. Estas muestras se utilizaron en el trabajo del capítulo 9.

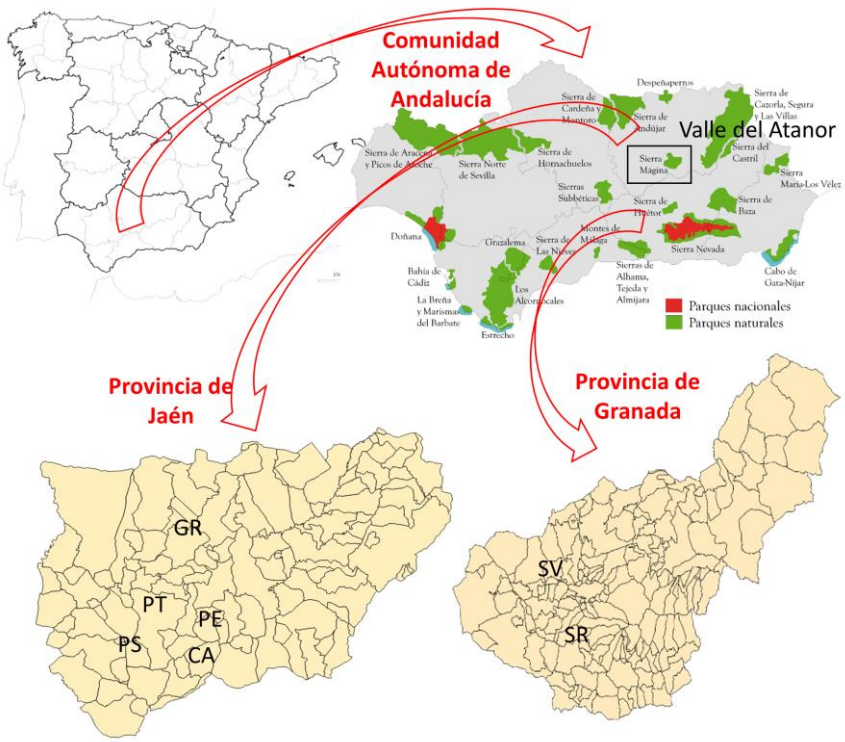

Figura 16. Mapa representativo de las diferentes localizaciones donde se tomaron muestras de suelo. El valle del Atanor en Sierra Mágina y las localizaciones dentro de las provincias de Jaén: Guarromán (GR), Cambil (CA), Pegalajar (PE), Puente Tablas (PT), Puente de la Sierra (PS); y Granada: Suelos de Vega en Pinos Puente (SV) y Suelos Rojos en Padul (SR).

Las muestras de enmiendas orgánicas se obtuvieron de diferentes entidades públicas y privadas. Así las muestras de compost de alperujo procedían de Alcanova S.L. (Alcaudete, Jaén), Cooperativa Nuestra Señora de los Remedios (Noguerones, Jaén), Instituto Andaluz de Investigación y Formación Agraria y Pesquera (Mengíbar, Jaén) y Olivarera Hermejor de la Reina (Villanueva de la Reina, Jaén, 2 muestras). Esta última empresa suministró también una muestra de alperujo sin compostar. Las muestras de posos de café se proceden de la cafetería de la Universidad de Jaén y la empresa Café Cumbal, S.A. Los trabajos dedicados a estas enmiendas orgánicas se recogen en los capítulos 10, 11 y 12.

2. Determinaciones para la caracterización físico-química y biológica

El análisis de suelos se realizó siguiendo las normas de la Society of Agronomy y la Soil Science Society of America (Klute, 1986; Page, 1982). Se determinaron los parámetros físico-químicos que se recogen en la tabla. Estos análisis fueron en parte llevados a cabo por las empresas Ensayos y Validaciones. S.L.L. y Agronutrientes Jaén S.C.A.

Además, se realizó un fraccionamiento de la materia orgánica siguiendo el procedimiento descrito por la IHSS (Swift, 1996), con medida del carbono de las fracciones obtenidas (huminas, carbono extraíble total, ácidos húmicos y ácidos fúlvicos) mediante digestión con $K_2Cr_2O_7$ and H_2SO_4 a 155°C durante 30 min y posterior determinación en un espectrómetro UV-visible (Mingorance et al., 2007). Por otro lado, se estimaron variables biológicas como la actividad enzimáticas para fosfatasa ácida, fosfatasa alcalina, β-glucosidasa, arilsulfatasa, deshidrogenasa (García-Ruiz et al., 2008), así como el potencial de nitrificación y la actividad respiratoria *in vitro* mediante una trampa de NaOH midiendo el CO_2 liberado por las muestras al 60% de su capacidad de campo, por un período de 40 días.

Los nutrientes seleccionados para su estudio en el caso de las muestras de hoja de olivo fueron nitrógeno (por el método de Kjeldahl), fósforo y boro (colorimetría), potasio (espectroscopia de emisión atómica de llama), calcio, magnesio, manganeso y zinc (espectroscopia de absorción atómica de llama). En todos los casos, excepto el del nitrógeno, se realizó una preparación de las muestras consistente en una calcinación en horno de mufla durante 8 horas. Estos análisis se realizaron en la empresa Agronutrientes Jaén S.C.A.

Tabla 1. Parámetros físico-químicos analizados y método utilizado.

Parámetro	Método
pH	Potenciometría
Conductividad eléctrica	Potenciometría
Carbono orgánico	Walkley–Black
Nitrógeno total	Kjeldahl
Fósforo total	Olsen
Potasio total	Fotometría de llama
Capacidad de intercambio catiónico	Solución saturada con 0.4N NaOAc-0.1N NaCl
Cationes en el complejo de cambio	Extracción con acetato de amonio + Espectroscopía de absorción atómica
Carbonato y bicarbonato	Calcímetro de Bernard
Textura	Pipeta de Robinson
Cloruros	Valoración con $AgNO_3$ y K_2CrO_4
Sulfatos	Precipitación con acetona y valoración con E.D.T.A (sobre el extracto de saturación)
Densidad aparente	Bourger
Conductividad hidráulica y estabilidad estructural	Tamizado en húmedo mediante un permeámetro de cabeza constante
Agua retenida, punto de marchitamiento permanente y agua útil	Membrana de Richards
Dispersión mecánica y espontánea de arcillas	Suspensión en agua destilada, agitación y calentamiento durante 4 h, y medida en $MgSO_4$

En el caso de las enmiendas orgánicas, los parámetros físico-químicos analizados fueron: pH y conductividad eléctrica por métodos similares a los empleados en suelo, carbono orgánico, nitrógeno y cenizas, empleando un analizador elemental LECO TruSpec CHN 620-100-400; fosforo, potasio, calcio y magnesio mediante digestión y posterior análisis elemental en un ICP-OES VARIAN 715-ES. Estos análisis se realizaron en Ensayos y Validaciones. S.L.L., Instituto Andaluz de Investigación y Formación Agraria y Pesquera (Mengíbar, Jaén) y Laboratorio TCAL.

En el caso de las muestras de enmiendas orgánicas y de suelo mezclado con enmiendas, se realizó un análisis termogravimétrico y análisis térmico diferencial utilizando un TGA/SDTA 851e Mettler Toledo, la medida de la repelencia al agua mediante el test de la gota o *water drop penetration time test* (WDPT) (Wessel, 1988); y el test de fitotoxicidad mediante el método de Zucconi (Zucconi et al., 1981).

3. Técnicas espectroscópicas

En esta tesis doctoral, uno de los principales objetivos es evaluar el potencial de las técnicas espectroscópicas, especialmente la espectroscopía infrarroja, como alternativa al análisis tradicional de laboratorio. Además, como técnicas complementarias en algunos trabajos se utilizó la espectroscopia UV-visible y la fluorescencia de rayos X. A continuación de indican brevemente las condiciones habituales de medida y los parámetros instrumentales correspondientes.

Espectroscopia de infrarrojo medio (FTIR) e infrarrojo cercano (FT-NIR): la espectroscopia infrarroja, proporciona información molecular acerca de las muestras por estar basada en las vibraciones de los enlaces de los grupos funcionales. Para las medidas en infrarrojo, las muestras de tierra fina, enmiendas orgánicas y hojas de olivo se molieron y homogeneizaron con un mortero de ágata. Los espectros de infrarrojo medio se registraron con un espectrómetro Varian 660 FTIR (Varian, Inc.), equipado con un accesorio de reflexión total atenuada (ATR, Pike Technologies) con un cristal de diamante de tres reflexiones (Figura 17).

Figura 17. Espectrómetro Varian 660 FTIR (Varian, Inc.) y accesorio ATR.

La medida en reflexión total atenuada se basa en la formación de una onda evanescente en el límite entre dos superficies con diferente índice de refracción. Cuando el haz infrarrojo incide con un ángulo mayor que un valor crítico, la radiación electromagnética no puede propagarse al medio de menor índice de refracción y se refleja totalmente en la cara interna del cristal de ATR. Sin embargo, la onda evanescente es capaz de ceder su energía al otro medio a una distancia inferior a unos micrómetros. Al colocar la muestra en el lado exterior en contacto directo con el cristal de ATR, la radiación se verá atenuada por su interacción con esta, registrándose un espectro similar al de absorción. Las muestras molidas se colocaban directamente sobre el cristal, y se aplicaba una presión controlada para asegurar máximo contacto entre muestra y cristal. Los espectros se registraron en un rango de 600 a 4000 cm^{-1}, con una resolución de 2 o 4 cm^{-1}, siendo cada espectro resultante la media de 128 repeticiones o acumulaciones. Como fondo se utilizó el espectro del aire en el cristal del ATR limpio.

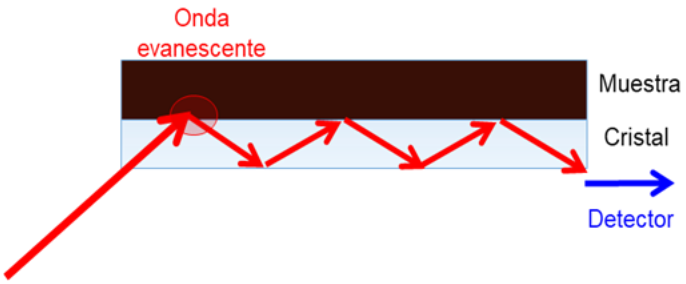

Figura 18. Medida por reflexión total atenuada.

Para la medida en infrarrojo cercano, se utilizó un espectrómetro Antaris FT-NIR analyzer (Thermo Nicolet Corporation) con accesorio de reflectancia difusa. La muestra se irradia con un haz de infrarrojo, produciéndose el fenómeno de reflexión difusa, en el que la radiación penetra unos mm dentro de la muestra, donde se producen múltiples fenómenos de absorción, reflexión y dispersión en todas las direcciones. La esfera integradora permite recoger eficazmente la radiación dirigiéndola al detector (Figura 19). El equipo dispone de una cápsula de tres centímetros que se llena con la muestra, y se coloca en la parte superior de la ventana de zafiro de la esfera integradora de oro. La cápsula de muestra se gira durante la medida para incrementar la superficie de muestra de medida. El equipo cierra automáticamente la ventana de medida y utiliza la referencia de la esfera de oro como fondo antes de la adquisición de cada espectro. Las medidas se realizaron en un rango de 4000 - 10000 cm^{-1}, con una resolución de 8 cm^{-1} y siendo cada espectro resultante la media de 128 repeticiones o acumulaciones.

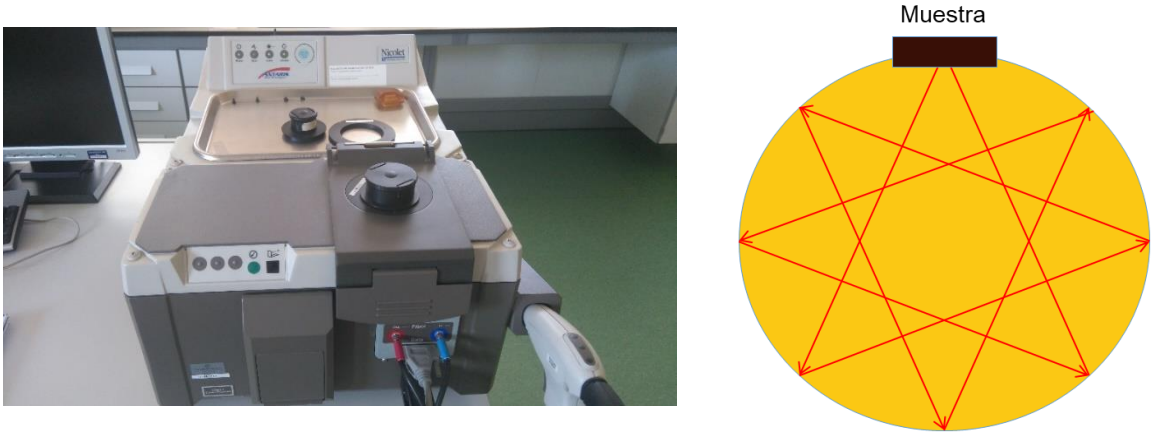

Figura 19. Espectrómetro Antaris FT-NIR analyzer (Thermo Nicolet Corporation) y esquema de la medida por reflectancia difusa.

Espectroscopia ultravioleta-visible (UV-Vis). Utilizada en estos trabajos para obtener información sobre la aromaticidad y complejidad de las moléculas orgánicas del suelo. Se utilizó un espectrómetro UV-Vis Varian Cary 50 (Varian, Inc.) y las medidas se realizaron sobre extractos de suelo o enmiendas orgánicas. Estos extractos se prepararon con 0.1 g de muestra a la que se añadieron 25 ml de NaOH 0.5 M y se agitaron 2 h. Tras un reposo de 12 h se centrifugaron a 3000 rpm durante 25 min y se midieron directamente, o mediante diluciones, en el rango de longitudes de onda 200-800 nm.

La fluorescencia de rayos X permite obtener información acerca de la composición elemental de las muestras. Las muestras, hojas de olivo en este caso, se midieron utilizando un equipo portátil Niton XL3t GOLDD+ (Thermo Fisher Scientific Inc.) montado sobre un soporte de plomo, Shielded SmartStand (Thermo Fisher Scientific Inc.) para evitar cualquier exposición del operador a la radiación. El instrumento está equipado con un ánodo de Rh de 45 kV y 40 µA de emisión máxima, y un detector "Geometrically Optimized Large Drift Detector" (GOLDD). Dispone de múltiples filtros de excitación, que están optimizados para diferentes rangos de energía, *main* (40 kV), *low* (20 kV) y *light range* (10 kV).
Las medidas se realizaron rellenando una capsula de polietileno de 32x24 mm (26 mm de diámetro interno) con unos 3 g de muestra que se cubría con una lámina de polipropileno. Para la medida se asumía geometría infinitamente gruesa (*infinitely thick geometry*). El método de medida del equipo utilizado fue "soils and mineral" con tiempos de medida de 45 s para el *main range*, y 30 s tanto para *low* como *light range*.

Figura 20. Capsula de polietileno para medida en EDXRF

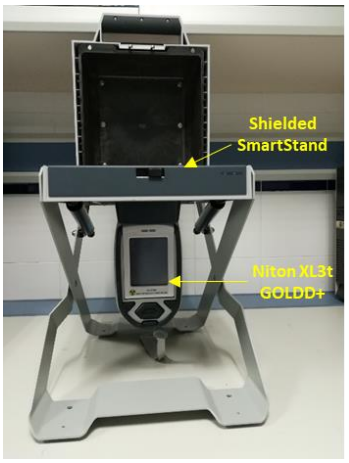

Figura 21. Niton XL3t GOLDD+ y shielded SmartStand (Thermo Fisher Scientific Inc.)

4. Estadística y Quimiometría

Para el análisis de los resultados se emplearon técnicas de estadística descriptiva (medias aritméticas y desviación estándar), análisis de normalidad (test Saphiro-Wilk, test de Kolmogorov-Smirnov), análisis de la varianza (ANOVA de una vía, Kruskal-Wallis ANOVA), test de significación (t-student, test de Levene, test de mínima diferencia significativa (LSD) de Fisher), y análisis de correlaciones entre variables (test de correlación de Pearson). Este análisis se realizó empleando el paquete de software Statgraphics Centurion XVI software (StatPoint Technologies, Inc.)

Además, para el tratamiento y análisis de los espectros, especialmente los espectros de infrarrojo, y encaminado a obtener la máxima información de los mismos se utilizaron diferentes herramientas quimiométricas con el paquete de software MATLAB 2008R (MathWorks, Natik, USA) con la PLS-toolbox de Eigenvector Research Inc. (Wenatchee, USA).

En todos los casos fue necesario un preprocesado de los espectros. Una descripción detallada se encuentra en cada caso particular. Para la exploración preliminar de los datos se empleó siempre el análisis de componentes principales (PCA) que, de una forma sencilla, muestra las relaciones y tendencias entre las muestras y también entre las variables de estudio. Esta técnica utiliza una transformación ortogonal para convertir un conjunto de observaciones de variables posiblemente correlacionadas, en un conjunto de valores de variables no correlacionadas linealmente, llamadas componentes principales. Esta transformación se define de tal manera que el primer componente principal tiene la mayor varianza posible (es decir, representa la mayor variabilidad posible en los datos), y cada componente subsiguiente a su vez tiene la mayor varianza posible bajo la restricción que es ortogonal a los componentes anteriores. Los vectores resultantes son un conjunto de bases ortogonales no correlacionadas, que pueden utilizarse en lugar de las variables originales suponiendo una reducción de las dimensiones de estudio.

En los trabajos que se presentan en esta memoria, se han utilizado las herramientas de quimiometría con dos fines principalmente, realizar una discriminación entre distintos grupos o clases de muestras (capítulos 6 y 7), para lo que se ha empleado análisis discriminante de mínimos cuadrados parciales (PLS-DA); y/o predecir el valor de un determinado parámetro para lo que se ha utilizado regresión mediante mínimos cuadrados parciales o "partial least squares" (PLS) (capítulos 7 y 9). Ambas herramientas se basan en la construcción de una serie de variables latentes que se relacionan con la clase o el valor del parámetro a definir. Un modelo PLS intentará encontrar la dirección multidimensional en el espacio X que explica la dirección de varianza multidimensional máxima en el espacio Y. En el primer caso será una variable categórica y en el segundo, numérica.

Sin embargo, para la creación de modelos efectivos no solo es importante el tratamiento del espectro y el método de clasificación o regresión a utilizar, sino que también resulta un factor clave la selección de un conjunto de muestras adecuado, tanto para la construcción del modelo como para su validación. Para ello en los casos en los que se disponía de gran cantidad de muestras, se aplicó un análisis de conglomerados jerárquico (HCA) para seleccionar un set de muestras representativo. Se empleó el método de la mínima varianza de Ward que crea un dendrograma agrupando las muestras similares.

Para evaluar la eficacia de los modelos quimiométricos propuestos, es necesario realizar una validación de los mismos. La validación más segura es aquella que se realiza con muestras que no se han incluido en el modelo de entrenamiento, pero no siempre es posible utilizarla debido a la limitación del número de muestras. En los casos en que una validación independiente no es posible, se ha empleado la validación cruzada, generalmente de los tipos "leave one out", y "random subsets". El primer tipo deja una muestra fuera de la calibración y la predice, repitiendo el proceso para todas las muestras; el segundo construye bloques de muestras escogidas aleatoriamente, dejando cada vez fuera del modelo de calibración un solo bloque para su uso como validación.

Por último, para cuantificar la capacidad de los modelos para predecir las diferentes variables se calculó la ratio de desviación predictiva (RPD):

$$\text{RPD} = \frac{\text{SDv}}{(\text{RMSEv} \times \sqrt{(n/(n-1))})}$$

Siendo, SDv la desviación estándar de la variable predicha; RMSEv, la desviación de la predicción en el modelo; y n, el número de muestras

El RPD es útil para comparar el rendimiento de los diferentes modelos, ya que estandariza el error cuadrático medio de la predicción (RMSE) de los modelos respecto a la dispersión de la población. Sin embargo, no existe una base estadística para determinar el umbral con el fin de clasificar los modelos según su confiabilidad y, por lo tanto, no hay acuerdo en la literatura sobre la importancia de un determinado valor de RPD. Algunos autores estiman umbrales muy altos, considerando buenos rendimientos de modelos con valores de RPD de 5 (Malley et al., 2000; Williams y Sobering, 1993). Sin embargo, otros autores consideran que los valores de RPD alrededor de 2 ya corresponden a modelos con buena capacidad predictiva (Chang et al., 2001; Dunn et al., 2002; Viscarra Rossel et al., 2006). Por lo tanto, en esta tesis doctoral, hemos considerado este parámetro en términos de comparación entre el rendimiento de los diferentes modelos probados.

Por último, se describe aquí la posibilidad de emplear técnicas de fusión de datos o "data fusion" (Márquez et al., 2016) para la integración o fusión de la información aportada por diferentes técnicas espectroscópicas, para de esta manera aprovechar la posible sinergia o complementariedad entre ellas. En el desarrollo de esta tesis se aplicaron diferentes estrategias de fusión de datos *low level* y *mid level*, para trabajar en conjunto con los espectros FTIR y FT-NIR, así como FT-NIR y EDXRF. El nivel *low level* consiste en concatenar los espectros de las diferentes técnicas utilizadas, aplicando un preprocesado adecuado para minimizar la diferencia entre las escalas; mientras el nivel *mid level* implica una extracción previa de la información relevante de cada una de las técnicas, para después juntar las nuevas matrices obtenidas.

Capítulo 4
Discusión conjunta de resultados

Esta Tesis doctoral se presenta por la modalidad de compendio de publicaciones y según la normativa debe incluir este capítulo dedicado a la discusión conjunta de los resultados obtenidos. Para facilitar la lectura del mismo y la comprensión de los resultados que se exponen, se presenta a continuación un esquema (ver Figura 22) en el que se reflejan las temáticas desarrolladas a lo largo de los siete trabajos, junto con una breve descripción de las mismas.

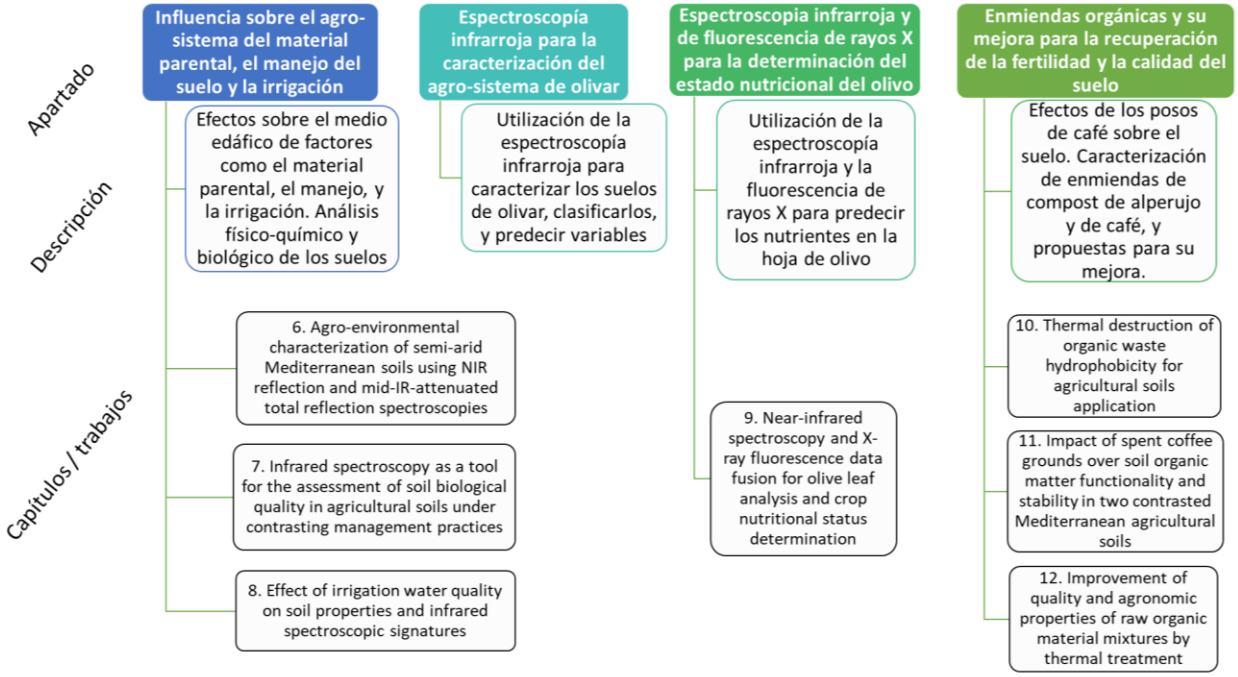

Figura 22. Esquema-resumen sobre las temáticas que se desarrollan en la discusión conjunta de resultados junto con los trabajos derivados de las mismas.

1. Influencia sobre el agro-sistema del material parental, el manejo del suelo y la irrigación

Los trabajos realizados sobre caracterización de distintos suelos de olivar (capítulos 6, 7 y 8) buscaban estudiar los efectos del material geológico, así como los sistemas de manejo sobre la calidad de los mismos. Un resultado interesante es que la acumulación de carbono orgánico en el suelo parece tener una clara dependencia del material litológico sobre el que se desarrolla. Así, se observó cómo materiales de origen coluvial muestran unas mejores condiciones para la acumulación de carbono en comparación con litologías de tipo margas. Las muestras del Valle del Atanor (Tabla 2), y las de Sierra Magina (Cambil y Pegalajar) con sustrato coluvial, muestran valores mayores que las de Puente Tablas y Puente Sierra (Tabla 3), ubicadas

en la Comarca Metropolitana de Jaén y cuyo sustrato es una mezcla de material coluvial y margas. Por otro lado, comparando material carbonatado y silíceo, se observa una tendencia del primero a una mayor acumulación de carbono orgánico. El material litológico por tanto es un factor clave que determina la acumulación, la estabilización y la evolución de la materia orgánica, pero, además, el sistema de manejo se revela asimismo como de gran importancia. Se puede observar como los diferentes manejos también provocan cambios en la cantidad de carbono orgánico (OC) en el suelo (Tabla 2 y Tabla 3). Así, manejos más respetuosos con el ecosistema como el manejo ecológico, que incluye la incorporación de materia orgánica de la vegetación espontánea, presentan siempre una mayor cantidad de OC en comparación a un manejo convencional. Cabe destacar, que en ningún caso se alcanzan valores similares a los de un suelo natural, por lo que un manejo agrícola, en este caso un cultivo de olivar, produce niveles inferiores de OC respecto a entornos no antropizados. Por tanto, el manejo también se convierte en un factor clave, que produce importantes cambios en la cantidad y calidad de la materia orgánica en los suelos (Aranda et al., 2011).

El manejo y el material litológico afectan, pues, de manera determinante a la fase orgánica del suelo, por lo que es de gran interés ver cómo afectan al resto de parámetros. Las muestras sobre material coluvial (Tabla 2) presentan mayor cantidad de nitrógeno total, aunque el mayor efecto se observa con el manejo, ya que las muestras de suelo con manejo ecológico presentan mayor cantidad de la mayoría de nutrientes, si bien en ningún caso alcanzan valores de OC, nutrientes, CIC y capacidad de campo tan elevados como el suelo natural, que sería el más fértil de la serie. Un efecto similar se obtiene en las muestras asociadas a localidades de la provincia de Jaén (Tabla 3), con una mayor cantidad de nutrientes en las muestras sobre materiales de tipo coluvial (Pegalajar y Cambil), pero también un claro aumento de nutrientes en los manejos ecológicos. Estas diferencias también se ven reflejadas en las variables biológicas (Tabla 4) y en el índice GMea que sirve como indicador de la calidad biológica global del suelo (García-Ruiz et al., 2008) y que se define como la raíz sexta del producto de las seis actividades enzimáticas medidas. El aumento de la actividad enzimática en los manejos ecológicos es especialmente significativo para la fosfatasa ácida y la deshidrogenasa, así como para el índice GMea. Es destacable que la única excepción es el potencial de nitrificación, mayor en el manejo convencional, lo que puede atribuirse al excesivo abonado. En general, se puede afirmar que el manejo ecológico mejora la calidad biológica del suelo, gracias a la influencia positiva de las enmiendas orgánicas y la vegetación espontánea. Comparando entre localizaciones con este manejo, vuelve a destacar la influencia del material litológico, así las muestras de Pegalajar, ubicadas sobre material coluvial, presentan las mejores propiedades biológicas, por encima de las muestras de Puente Tablas y Puente Sierra, desarrolladas sobre mezcla de coluvios y margas. Las muestras de Cambil, tienen una calidad biológica menor, lo que parece deberse a sus menores niveles de materia orgánica, que pueden estar asociados a que parte de estas muestras también se desarrollan sobre margas. Además, en este caso deben tenerse en cuenta los niveles más elevados de sodio. Estos pueden afectar a la actividad biológica del suelo y provocar una mayor descomposición de la materia orgánica.

Tabla 2. Caracterización físico-química de las muestras del Valle del Atanor (Sierra Mágina).

Manejo	Material	n		OC (%)	N (%)	pH	Arcilla (%)	Limo (%)	Arena (%)	CIC (meq/ 100g)	$CaCO_3$ (%)	Densidad aparente (g/cc)	K^+ (ppm)	P (ppm)	FG (%)	Capacidad de Campo (%)
Conv	Coluvial	18	Rango	0.8-2.6	0.08-0.21	7.8-8.3	24-51	26-42	19-42	23-29	22-70	1.2-1.4	4.2-43	124-640	33-53	32-43
			Media ± SD	1.5 ± 0.4	0.12 ± 0.03	8.1 ± 0.1	36 ± 7	33 ± 5	31 ± 6	26 ± 2	44 ± 13	1.3 ± 0.1	13.2 ± 8.8	272 ± 145	43 ± 6	37 ± 3
Conv	Margas	21	Rango	0.4-1.2	0.04-0.1	8-8.6	19-45	24-45	11-51	16-30	34-74	1.2-1.6	3-23	83-173	7-41	23-41
			Media ± SD	0.8 ± 0.2	0.07 ± 0.02	8.2 ± 0.1	30 ± 8	35 ± 6	35 ± 11	20 ± 4	61 ± 9	1.4 ± 0.1	12.5 ± 6.9	119 ± 25	24 ± 9	32 ± 5
Eco	Coluvial	19	Rango	1.8-5.3	0.11-0.47	7.8-8.2	26-55	27-38	17-40	18-39	10-61	1.1-1.5	2-65	140-995	20-48	29-43
			Media ± SD	3.3 ± 1	0.22 ± 0.09	7.9 ± 0.1	38 ± 7	33 ± 3	29 ± 7	30 ± 6	34 ± 15	1.3 ± 0.1	17.9 ± 17.4	433 ± 206	35 ± 7	37 ± 4
Eco	Margas	18	Rango	0.5-3.6	0.06-0.28	7.7-8.2	25-46	30-47	15-45	19-30	35-63	1.1-1.4	2-23.5	36-323	7-44	34-41
			Media ± SD	1.9 ± 0.8	0.14 ± 0.06	8 ± 0.1	35 ± 5	40 ± 5	25 ± 8	24 ± 3	49 ± 7	1.3 ± 0.1	7.9 ± 5.5	149 ± 82	24 ± 8	37 ± 2
SN	Coluvial	4	Rango	6.1-7.5	0.3-0.46	7.7-7.8	28-41	30-35	25-43	42-49	2-4	1-1.1	4-7	526-824	31-64	39-41
			Media ± SD	6.7 ± 0.5	0.37 ± 0.07	7.7 ± 0	36 ± 5	32 ± 2	32 ± 7	47 ± 3	3 ± 1	1 ± 0.1	5.5 ± 1.5	662 ± 115	49 ± 12	40 ± 1

Conv: convencional; Eco: ecológico; SN: suelo natural.

Tabla 3. Caracterización físico-química de las muestras de la Provincia de Jaén. Media ± SD.

Localización	Manejo	n	OC (%)	pH	Arcilla (%)	Limo (%)	Arena (%)	CIC (cmol kg^{-1})	$CaCO_3$ (%)	Ca^{2+} (meq/l)	Mg^{2+} (meq/l)	K^{+} (ppm)	P (ppm)	Capacidad de Campo (%)	Na^{+} (ppm)
PT	Conv	14	2.6 ± 0.9	7.6 ± 0.1	46 ± 2	37 ± 2	17 ± 1	12 ± 1	31 ± 1	60 ± 3	2.3 ± 0.6	2.6 ± 0.6	47 ± 27	0.26 ± 0.02	0.09 ± 0.01
PT	Eco	19	4 ± 1.9	7.6± 0.1	27 ± 3	51 ± 3	22 ± 1	16 ± 3	33 ± 5	122 ± 24	5.4 ± 2.6	2.5 ± 0.3	105 ± 29	0.27± 0.01	1.30 ± 1.40
PS	Conv	15	2.3 ± 0.6	7.6 ± 0.3	27 ± 3	59 ± 4	14 ± 1	8 ± 1	70 ± 1	65 ± 2	0.9 ± 0.2	0.6 ± 0.3	10 ± 2	0.31 ± 0.02	0.17 ± 0.05
PS	Eco	12	3.1 ± 0.9	7.8 ± 0.1	16 ± 9	67 ± 11	18 ± 3	10 ± 1	70 ± 3	61 ± 3	2.0 ± 0.3	1.0 ± 0.1	15 ± 3	0.32 ± 0.02	0.46 ± 0.20
PE	Conv	12	4 ± 1.7	7.9 ± 0.1	15 ± 10	70 ± 9	15 ± 1	17 ± 5	57 ± 19	78 ± 10	1.2 ± 0.3	1.6 ± 0.6	19 ± 16	0.32 ± 0.02	0.07 ± 0.01
PE	Eco	12	5.4 ± 1	7.8 ± 0.1	32 ± 3	49 ± 3	18 ± 2	20 ± 1	55 ± 1	76 ± 5	8.2 ± 1.3	3.1 ± 1.7	61 ± 12	0.33 ± 0.01	0.07 ± 0.01
CA	Conv	38	3.3 ± 0.6	7.9 ± 0.3	27 ± 6	34 ± 5	40 ± 8	17 ± 4	28 ± 13	13 ± 3	2.3 ± 0.7	3.4 ± 0.6	23 ± 12	0.30 ± 0.04	0.36 ± 0.32
CA	Eco	35	3.5 ± 0.8	7.8 ± 0.2	28 ± 6	31 ± 5	42 ± 9	16 ± 3	31 ± 12	11 ± 4	3.1 ± 1.4	1.4 ± 0.4	26 ± 22	0.31 ± 0.10	0.40 ± 0.08

PT: Puente Tablas; PS: Puente Sierra; PE: Pegalajar; CA: Cambil; Conv: convencional; Eco: ecológico.

Tabla 4. Resultados de la medida de las actividades enzimáticas y el índice GMea según el lugar de muestreo y el manejo del cultivo. Rango, media y desviación estándar (SD). Las letras indican diferencias significativas (P < 0.05; test de Kruskal-Wallis).

Manejo			Deshidrogenasa	β-Glucosidasa	Arilsulfatasa	Fosfatasa alcalina	Fosfatasa ácida	Potencial de nitrificación	GMea
PT	Conv	Rango	3 - 13	140 - 331	2 - 114	97 - 301	44 - 200	0.2 - 1.0	12 - 40
		Media ± SD	8 ± 4 de	196 ± 49 b	36 ± 37 ab	195 ± 49 b	91 ± 43 a	0.6 - 0.2 bcd	25 ± 8 c
PT	Eco	Rango	3 - 14	42 - 336	3 - 226	43 - 341	30 - 458	0.2 - 0.4	10 - 40
		Media ± SD	7 ± 3 d	197 ± 115 b	43 ± 53 b	183 ± 119 b	166 ± 98 b	0.3 ± 0.1 ab	25 ± 9 c
PS	Conv	Rango	1 - 6	86 - 408	1 - 327	45 - 349	29 - 213	0 - 0.5	10 - 46
		Media ± SD	3 ± 1 a	234 ± 114 b	56 ± 86 b	214 ± 82 b	101 ± 48 a	0.2 ± 0.1 a	19 ± 9 b
PS	Eco	Rango	3 - 15	115 - 614	9 - 249	64 - 421	64 - 347	0.1 - 0.5	9 - 62
		Media ± SD	9 ± 3 e	426 ± 177 c	65 ± 67 b	287 ± 106 c	151 ± 85 b	0.3 ± 0.1 ab	34 ± 14 d
PE	Conv	Rango	2 - 11	123 - 1070	45 - 287	154 - 762	112 - 444	0.2 - 0.7	31 - 69
		Media ± SD	6 ± 3 cd	556 ± 289 d	156 ± 81 c	432 - 152 d	209 ± 83 bc	0.5 ± 0.2 abc	49 ± 12 e
PE	Eco	Rango	3 - 18	181 - 945	293 - 46	149 - 657	164 - 370	0.4 - 1.5	47 - 92
		Media ± SD	9 ± 5 e	681 ± 198 e	171 ± 76 c	472 ± 141 d	260 ± 75 c	0.8 ± 0.3 d	65 ± 15 f
CA	Conv	Rango	2 - 8	15 - 58	2 - 24	9 - 44	42 - 207	0.2 - 3.0	6 - 18
		Media ± SD	4 ± 1 ab	35 ± 10 a	10 ± 5 a	26 ± 8 a	110 ± 37 a	1.1 ± 0.7 e	12 ± 3 a
CA	Eco	Rango	2 - 8	27 - 66	4 - 21	16 - 54	68 - 443	0.1 - 1.8	7 - 19
		Media ± SD	5 ± 2 bc	43 ± 10 a	14 ± 4 a	37 ± 9 a	184 ± 78 b	0.6 ± 0.4 cd	14 ± 2 ab
Significación estadística		Manejo	O > C	O = C	O = C	O = C	O > C	C > O	O > C
		Localización	PE = PT > PS > CA	PE > PT > PS > CA	PE > PT = PS > CA	PE > PS > PT > CA	PE > PS = PT = CA	CA > PE ≥ PT ≥ PS	PE > PT = PS > CA

PT: Puente Tablas; PS: Puente Sierra; PE: Pegalajar; CA: Cambil; Conv: convencional; Eco: ecológico. Deshidrogenasa en µg TPF g-1 h-1; β-Glucosidasa, Arilsulfatasa, Fosfatasa alcalina y Fosfatasa ácida en µg pNP g-1 h-1, y Potencial de nitrificación en µg N-NO2 g-1 h-1.

Otro aspecto interesante es la influencia de la irrigación sobre la calidad de los suelos. Para ello, nos fijamos en la finca de la localidad de Guarromán en la que se aplicó riego durante más de 15 años. En esta finca se obtuvieron incrementos de producción durante los primeros años de regadío, pero a partir del quinto año esta comenzó a reducirse, hasta que en la actualidad los niveles son muy bajos y preocupantes. Si observamos los resultados del análisis físico y físico-químico de las muestras de suelo de esta finca (Tabla 5), destacan valores elevados de pH, especialmente en las muestras expuestas al bulbo de irrigación. Los posibles problemas de salinidad se descartan por la baja conductividad; sin embargo, estos valores de pH podrían estar indicando problemas de sodicidad (Daliakopoulos et al., 2016), dado también las concentraciones elevadas de sodio. El sodio es el catión en mayor concentración en los suelos silíceos, y el segundo en los carbonatados, con una proporción lejos de la óptima (Navarro Blaya y Navarro García, 2003). El incremento en las muestras de zonas expuestas al bulbo de irrigación, apunta al riego como causante de este efecto. Los valores elevados de porcentaje de sodio intercambiable (PSI) indican también un problema de sodicidad, especialmente importante en las muestras silíceas. Como consecuencia de esto, observamos valores preocupantes de infiltración (ks), llegando a clasificar las muestras silíceas próximas al bulbo de irrigación como impermeables (Landon, 1984). Además, la baja estabilidad de los agregados en todas las muestras, y la elevada densidad aparente, indican que existe una elevada compactación en estos suelos, y que su estructura se ha visto claramente afectada tras la exposición al regadío. La analítica del agua de riego parece confirmar este hecho, ya que, aunque presenta valores normales de pH (7.9) y CE (0.6 dS/m), sus elevados valores de sodio (66.7 mg/L), cloruros (29.6 mg/L) y bicarbonatos (297.5 mg/L) deben tenerse en cuenta para explicar esta posible reducción de la producción.

En definitiva, los resultados obtenidos muestran como, el agua de riego utilizada, aunque a priori no debería suponer ningún riesgo para el suelo, con su adición continuada ha provocado problemas debido a la gran cantidad de sodio que se añade al suelo a través de ella. Si comparamos el efecto según la tipología de suelo, observamos la gran importancia de ésta a la hora de responder a la aplicación de aguas de riego. Vemos como los suelos carbonatados, mantienen mejor sus propiedades físicas, indicando el importante papel del calcio a la hora de mejorar y mantener la estructura, en contraposición con el sodio, en mayor concentración en las muestras silíceas, que provoca una destrucción de la estructura, haciendo el suelo más susceptible a la compactación (Sumner, 1993). Esta mayor cantidad de sodio en las muestras silíceas, provoca un incremento en la dispersión de las arcillas, generando una destrucción de la estructura en estas muestras. Esta dispersión provoca asimismo una mayor descomposición de la materia orgánica (Nelson y Oades, 1998), que se va perdiendo con el tiempo, provocando una pérdida de fertilidad en estos suelos. Esto se observa en las propiedades químicas con una clara tendencia a menores valores de materia orgánica y nitrógeno. Además, las elevadas concentraciones de sodio dan lugar a valores de sodio intercambiable muy elevadas, especialmente preocupante en los suelos silíceos, donde alcanza valores superiores al 20%, que son indicativos de problemas para la salud de la planta (Benlloch et al., 1991).

El efecto del bulbo de irrigación no se traduce en diferencias significativas en los parámetros físicos, indicando que la estructura está igualmente afectada a lo largo de toda la parcela. Sin embargo, los problemas de infiltración se concentran en mayor medida en las zonas de calle, debido a que es por estas por donde se produce el paso de la maquinaria agrícola, compactando el suelo, lo que provoca tanto el aumento de su densidad, como un proceso de sellado, que disminuye la infiltración.

En resumen, se observa como el manejo del suelo afecta de manera determinante a la mayoría de propiedades del suelo, así, manejos más respetuosos con el agro-sistema como el manejo ecológico dan lugar a suelos con mejores propiedades físicas, químicas y biológicas, en comparación con los suelos bajo manejo convencional. La irrigación también afecta a la salud del suelo, ya que el uso continuado de aguas con altas concentraciones de sales o sodio provocan salinización y sodificación, con la consecuente pérdida de fertilidad. Sin embargo, el efecto de estos manejos depende en gran medida del material geológico, ya que sustratos más favorables como los coluvios soportan mejor los procesos de degradación en comparación con las margas, mientras los suelos carbonatados son capaces de resistir mejor los procesos de sodificación que suelos de tipo silíceo.

Tabla 5. Caracterización físico-química de las muestras de Guarromán con sistema de irrigación.

Zona	Material	n		OC (%)	N (%)	pH	Arcilla (%)	Limo (%)	Arena (%)	CIC (cmol kg^{-1})	$CaCO_3$ (%)	Ca (meq/l)	Mg (meq/l)	K^+ (meq/l)	Densidad aparente (g/cc)
Bulbo	Sil	5	Media ± SD	0.76 ± 0.24	0.05 ± 0.01	9 ± 0.4	23 ± 12	13 ± 4	64 ± 12	17.6 ± 5	0.17 ± 0.3	1.44 ± 0.86	1.11 ± 0.46	0.23 ± 0.05	1.6 ± 0.27
Calle	Carb	5	Media ± SD	0.65 ± 0.25	0.05 ± 0.01	8.1 ± 0.4	31 ± 6	11 ± 8	57 ± 11	18.4 ± 4.5	0.04 ± 0.08	1.28 ± 0.31	0.67 ± 0.13	0.64 ± 0.98	1.63 ± 0.08
Bulbo	Sil	5	Media ± SD	1.05 ± 0.21	0.08 ± 0.01	8.6 ± 0.1	21 ± 9	13 ± 5	67 ± 9	24.4 ± 1.5	5.89 ± 3.83	2.07 ± 0.81	1.4 ± 0.52	0.24 ± 0.07	1.28 ± 0.12
Calle	Carb	5	Media ± SD	0.72 ± 0.15	0.05 ± 0.01	8.5 ± 0.1	23 ± 2	13 ± 4	64 ± 4	24.4 ± 0.9	8.1 ± 5.34	2.6 ± 0.3	0.57 ± 0.06	0.18 ± 0.04	1.43 ± 0.15
Archivo Agronutrientes Jaén S.C.A.		71	Rango	0.12 - 1.51	-	6.8 - 9.5	12-60	3-25	30 - 78	4.1 - 34.5	0.2 - 83.8	1.1 - 55.7	0.4 - 17	1 - 15.4	
			Media ± SD	0.52 ± 0.41	-	8.3 ± 0.4	37.6 ± 9.6	12.2 ± 3.8	50.1 ± 10.7	17.4 ± 6.8	29.2 ± 18.8	7.3 ± 9.8	2 ± 2.5	3.2 ± 2.9	

Zona	Material	n		SB (%)	CE (dS/m)	Na^+ (meq/L)	SAR	PSI (%)	EA	ks (cm/h)	HCO^{3-} (meq/L)	Cloruros (meq/l)	Sulfatos (meq/l)	DEA (%)	DMA (%)
Bulbo	Sil	5	Media ± SD	52.6 ± 4.3	0.91 ± 0.32	5.61 ± 2.13	5.01 ± 1.31	24.1 ± 3.4	0.44 ± 0.25	0.093 ± 0.087	0.67 ± 0.49	0.44 ± 0.25	1 ± 2.2	0.82 ± 0.66	0.232 ± 0.112
Calle	Carb	5	Media ± SD	50.6 ± 4	0.44 ± 0.1	1.99 ± 0.8	2 ± 0.72	19.6 ± 3.2	0.55 ± 0.08	0.064 ± 0.039	0.53 ± 0.22	0.55 ± 0.08	0 ± 0	1.89 ± 1.12	0.136 ± 0.023
Bulbo	Sil	5	Media ± SD	52.2 ± 1.8	0.84 ± 0.17	4.33 ± 1.62	3.54 ± 1.83	15.5 ± 0.9	0.4 ± 0.14	0.051 ± 0.031	1.1 ± 1.32	0.4 ± 0.14	0.4 ± 0.5	2.37 ± 2.06	0.152 ± 0.016
Calle	Carb	5	Media ± SD	49.4 ± 1.8	0.53 ± 0.04	1.73 ± 0.22	1.38 ± 0.18	13.8 ± 1.3	0.43 ± 0.13	0.103 ± 0.059	0.57 ± 0.1	0.43 ± 0.13	0 ± 0	0.49 ± 0.34	0.184 ± 0.018
Archivo Agronutrientes Jaén S.C.A.		71	Rango		0.4 - 3.9	0 - 6.2	0.3 - 8.3	-57.8			0.2 - 29.8				
			Media ± SD		1.1 ± 0.8	0.4 ± 1	1.9 ± 1.5	4.3 ± 9.3			6 ± 7.9				

Bulbo: muestras afectadas por el bulbo de irrigación; calle: muestras no afectadas por el bulbo de irrigación; Sil: silíceo; Carb: carbonatado. SB: saturación de bases; EA: estabilidad de los agregados; DEA: dispersión espontánea de las arcillas; DMA: dispersión mecánica de las arcillas.

2. Espectroscopía infrarroja para la caracterización del agro-sistema de olivar

La utilización de la espectroscopía infrarroja como alternativa para la caracterización de todos los aspectos del agro-sistema de olivar puede agilizar los procesos de decisión, puesto que permitiría la obtención de información más rápida y eficiente que en los casos expuestos de caracterización tradicional. Veamos algunos ejemplos de los trabajos realizados que se describen en detalle en los capítulos 6, 7 y 8.

Si comparamos los espectros FTIR de las muestras del Valle del Atanor (Figura 23), vemos que en los cuatro tipos de suelos cultivados las bandas más intensas corresponden a la banda de arcillas y polisacáridos (alrededor de 1000 cm^{-1}) y carbonatos (1396, 872 y 711 cm^{-1}). Las bandas de carbonatos están más marcadas en los suelos desarrollados sobre margas, mientras en el suelo natural son casi inexistentes, hecho que puede atribuirse a los procesos de decarbonatación, probablemente favorecidos por la fuerte pendiente de la zona en que se desarrolla el suelo natural. Las bandas asociadas al estiramiento OH de los grupos hidroxilo de la interlámina de minerales de tipo ilita (3697 cm^{-1}) y al estiramiento OH de kaolinita, ilita y montmorillonita (3619 cm^{-1}) tienen gran peso en el espectro. La materia orgánica del suelo también presenta bandas de absorción características, sin embargo, debido a su superposición con las bandas de componentes minerales y su baja concentración en las muestras de suelo resultan difícil de identificar y comparar de forma clara. Un ejemplo son las bandas de 2920 y 2850 cm^{-1}, que son difíciles de apreciar. Estas bandas indican la presencia de compuestos alifáticos, presentando mayor intensidad en los espectros de muestras de suelo con mayores concentraciones de carbono orgánico total, como es el caso de las muestras de suelo natural y de manejo ecológico.

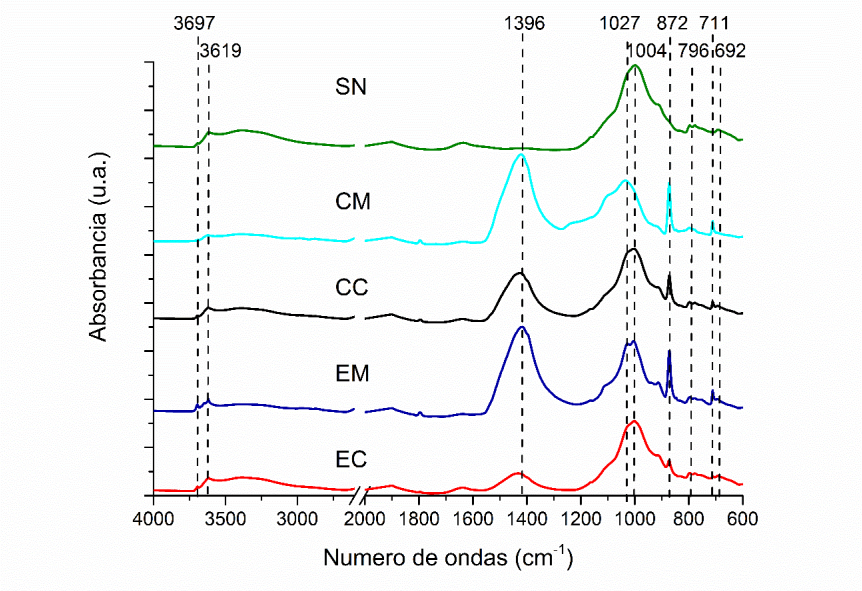

Figura 23. Espectros FTIR medios de las muestras del Valle del ATANOR. CC: convencional de coluvios; CM: convencional de margas; EC: ecológico de coluvios; EM: ecológico de margas; SN: suelo natural.

En general, el espectro FTIR parece aportar gran cantidad de información sobre la composición mineralógica de las muestras, y en menor medida sobre la composición orgánica, ya que las bandas de compuestos inorgánicos suelen solapar e impedir la interpretación sencilla de las bandas orgánicas. Por tanto, la interpretación del espectro FTIR puede ser de gran utilidad para caracterizar las muestras de suelos, y poder establecer comparaciones entre ellas, sobre todo en lo que respecta a la tipología de suelo, aunque también en menor medida en lo referente al manejo.

Por otro lado, en el caso de los espectros FT-NIR, la interpretación de los espectros es más complicada porque la mayoría de las bandas se deben a combinaciones y solapamientos de vibraciones fundamentales de compuestos orgánicos e inorgánicos, además de que el espectro se ve afectado por parámetros como el tamaño de partícula. En el caso de las muestras del Valle del Atanor (Figura 24), la absorción cerca de 7070 cm^{-1} está relacionada con el segundo sobretono de la vibración de estiramiento O-H en minerales de tipo arcilla como la kaolinita. Esta banda aparece más marcada en las muestras sobre margas. Además, cambia ligeramente dependiendo de la molécula o mineral al que esté unido el O-H, pero resulta difícil asociar los cambios en esta banda a minerales específicos. La banda 5220 cm^{-1} está relacionada con una combinación de la vibración del OH de la interlámina de filosilicatos y moléculas de agua, y del 2º sobretono del grupo funcional C=O asociado a diferentes compuestos orgánicos de naturaleza recalcitrante como las ligninas o los ácidos húmicos. Mientras la banda a 4530 cm^{-1} es una combinación de vibración de –O-H metálico y estiramiento de O-H asociados a minerales de arcilla, como la esmectita y la ilita. Las bandas de vibración de los enlaces NH y OH de la materia orgánica también pueden contribuir en esta región. En este caso, la banda de carbonatos tiene una señal muy débil en comparación con FTIR (4265 cm^{-1}). Para el caso de las absorciones asociadas a materia orgánica, de nuevo resulta muy difícil asociar las bandas referentes a este tipo de compuestos, y en este caso especialmente debido a la gran cantidad de solapamiento entre bandas.

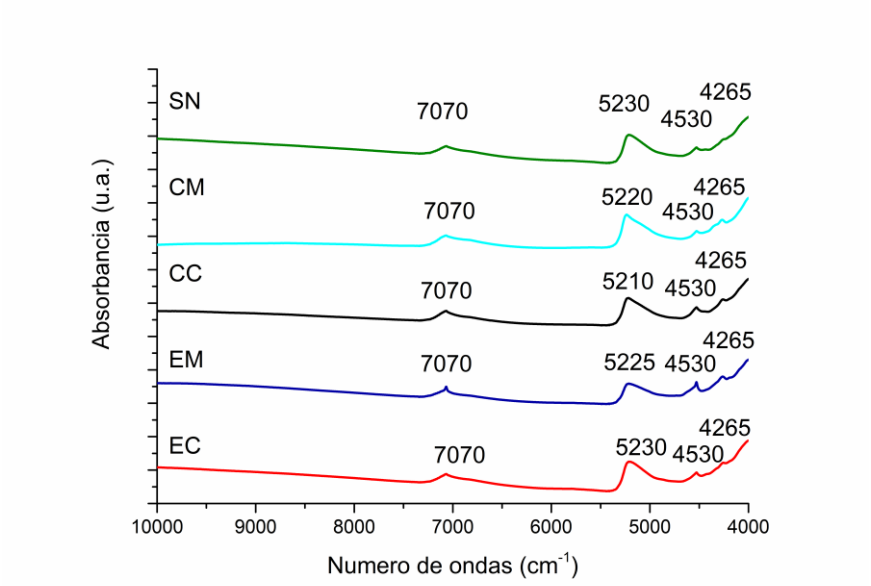

Figura 24. Espectros FT-NIR medios de las muestras del Valle del ATANOR. CC: convencional de coluvios; CM: convencional de margas; EC: ecológico de coluvios; EM: ecológico de margas; SN: suelo natural.

En general podemos comprobar como los espectros FT-NIR aportan información sobre las fases inorgánica y orgánica del suelo, aunque especialmente de este primera, tras el análisis de los espectros a simple vista. Por tanto, podemos decir que es una técnica que aporta información muy similar a los espectros FTIR, pudiendo ser hasta cierto punto complementarias, aunque en ambos casos parecen ser técnicas de utilidad para caracterizar los suelos de olivar y posibles efectos derivados de su manejo y material parental. Sin embargo, la interpretación en el infrarrojo cercano resulta mucho más difícil, lo que unido a un menor número de bandas en total nos lleva a pensar que esta región del espectro infrarrojo puede estar aportando menos información, o que esta está mucho menos disponible que en el caso de la espectroscopía de infrarrojo medio.

Debido al solapamiento de bandas en ambas regiones del espectro infrarrojo, así como a la dificultad para extraer información relevante del espectro con un simple análisis visual, resulta muy común la utilización de diferentes análisis quimiométricos, que nos ayuden a sacar toda la información que los espectros infrarrojos aportan. Se incluyen aquí también parte de los resultados obtenidos durante la estancia de investigación en CSIRO Land and Water en la que se aplicaron diferentes herramientas quimiómetricas y de fusión de datos a los espectros infrarrojos con el objetivo de desarrollar modelos predictivos robustos.

En primer lugar, se aplicó un análisis de componentes principales (PCA) a los espectros infrarrojos, previamente preprocesados con la aplicación de una línea base. Si observamos los resultados obtenidos al aplicar el PCA sobre los espectros FTIR de las muestras del Valle del Atanor (Figura 25), más del 95% de la varianza total es capturada por los 3 primeros PCs, lo que indica que la mayor parte del espectro está reflejada en estos primeros componentes. Podemos ver una clara diferenciación de las muestras de suelo natural en el PC1, seguidas de las muestras sobre coluvios, y al final de este las muestras de margas. Sin embargo, también se aprecia que son las muestras de olivar ecológico coluvial las que aparecen más cerca del suelo natural, lo que podría estar indicando un gradiente en este PC de fertilidad del suelo, siendo el suelo natural el más fértil seguido del olivar ecológico sobre coluvios, con los otros tres grupos mezclados entre sí. Si observamos las principales bandas responsables de este PC vemos como el agrupamiento se realiza respecto a factores litológicos, como la cantidad de carbonatos, pero también debido a ciertas bandas de origen orgánico, lo que explica esta clasificación respecto a la calidad del suelo. Por otro lado, el PC2 claramente discrimina los materiales, teniendo los materiales coluviales valores próximos a 0 en este componente, mientras las muestras de margas se diferencian en valores positivos y negativos, dependiendo de su manejo. De nuevo en este PC las bandas de carbonatos tienen el mayor peso, con ciertas bandas orgánicas aportando también cierta información, lo que explica el claro aislamiento de las muestras de olivar convencional de margas, las muestras más degradadas de las estudiadas en el valle del Atanor.

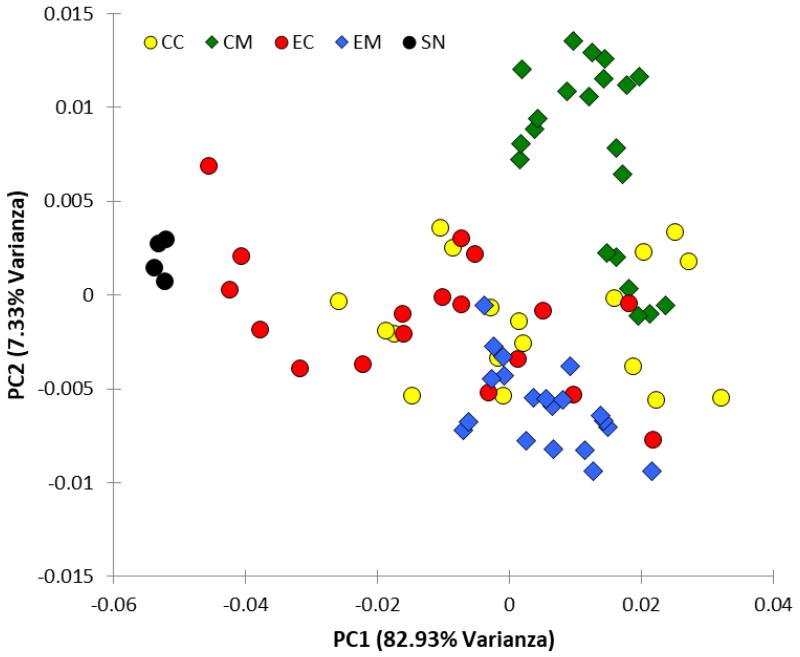

Figura 25. Gráfico de puntuaciones del análisis de componentes principales (PCA) de los espectros FTIR de las muestras del Valle del Atanor. Muestras agrupadas por manejo y material parental. CC: convencional de coluvios; CM: convencional de margas; EC: ecológico de coluvios; EM: ecológico de margas; SN: suelo natural.

Por otro lado, si observamos el PCA aplicado sobre los espectros FT-NIR (Figura 26), de nuevo 3 PCs son capaces de capturar casi el 95% de la varianza total. Pero en este caso, el agrupamiento de las muestras resulta muy evidente, dado que el PC2 claramente está separando las muestras de margas en la parte superior, de las muestras de coluvios en la parte inferior. El PC1 de nuevo parece agrupar las muestras por el nivel de fertilidad, tal y como sucedía con los espectros FTIR. Además, entre el PC1 y el PC2 parecen crear un plano que posiciona a las muestras según sus niveles de fertilidad, dejando aisladas a las muestras de olivar convencional de margas, muestras con un nivel de degradación muy elevado y una fertilidad bastante reducida. Este agrupamiento sucede debido a una combinación de diferentes bandas, cuya interpretación resulta complicada debido al elevado solapamiento de las bandas en esta región.

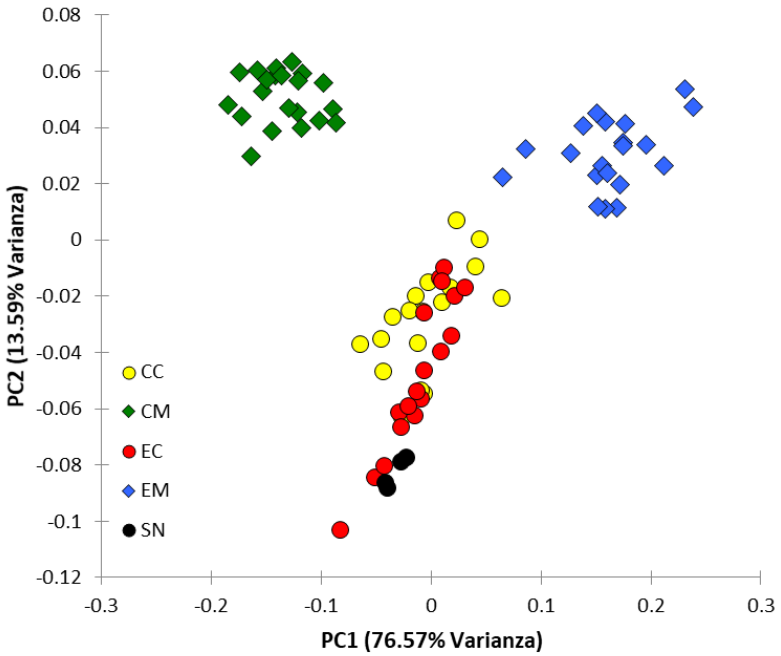

Figura 26. Gráfico de puntuaciones del análisis de componentes principales (PCA) de los espectros FT-NIR de las muestras del Valle del Atanor. Muestras agrupadas por manejo y material parental. CC: convencional de coluvios; CM: convencional de margas; EC: ecológico de coluvios; EM: ecológico de margas; SN: suelo natural.

En el caso del set compuesto por las muestras de Sierra Mágina y la Comarca Metropolitana de Jaén, tan solo se midió el espectro de infrarrojo cercano (FT-NIR). Si realizamos un análisis PCA (Figura 27), podemos ver un agrupamiento muy claro según la localización de las muestras, siendo las muestras de Cambil las únicas que se entremezclan un poco con el resto de grupos. En este caso los 3 primeros PCs solo son capaces de capturar el 75% de la varianza, lo que indica una mayor variabilidad de estos respecto al FTIR y otros FT-NIR. El PC1 claramente separa las muestras de acuerdo a su material litológico, colocando las muestras de coluvios (Pegalajar y Cambil) con valores negativos, mientras las mezclas de margas más coluvios tienen valores positivos en este componente. Algunas muestras de Cambil aparecen con valores negativos, debido a que parte de las muestras de esta localización también presentaban una mezcla de margas y coluvios como material parental. Esta separación litológica también está relacionada con la calidad biológica de los diferentes suelos, como se ha expuesto anteriormente. Por otro lado, el PC2 separa los suelos con un mayor contenido en carbonatos (Puente Sierra), de aquellos con mayor contenido en materia orgánica (Pegalajar y Puente Tablas). De nuevo las muestras de Cambil muestran un comportamiento mixto, al ser un conjunto de muestras mayor, con diferentes características, similares a los otros grupos. La influencia del manejo no se aprecia claramente en el PCA (Figura 28), y aunque se puede intuir una ligera separación dentro de cada grupo, no hay una clara evidencia en el PCA de este agrupamiento dependiendo del manejo de los suelos.

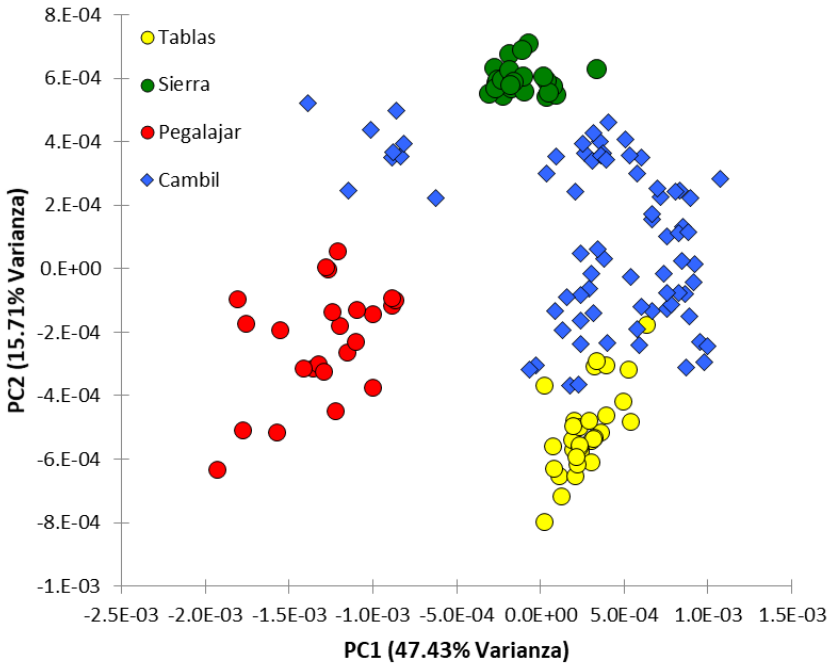

Figura 27. Gráfico de puntuaciones del análisis de componentes principales (PCA) de los espectros FT-NIR de las muestras de la provincia de Jaén. Muestras agrupadas por localización.

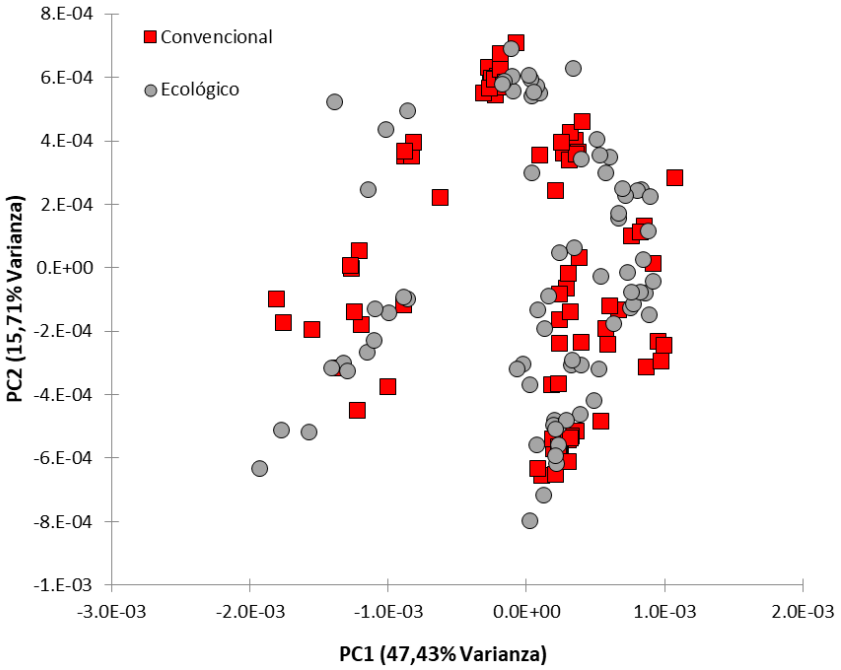

Figura 28. Gráfico de puntuaciones del análisis de componentes principales (PCA) de los espectros FT-NIR de las muestras de la provincia de Jaén. Muestras agrupadas por manejo.

Con el objetivo de comprobar la capacidad de la espectroscopía infrarroja, para discernir entre suelos degradados debido a problemas de salinidad, con suelos no afectados, se han utilizado las muestras del archivo de suelos de la entidad Agronutrientes Jaén S.C.A. para su comparación con las muestras afectadas por sodicidad de Guarromán. Un resumen de las características de las muestras del archivo de suelos se mostraba en la Tabla 5, donde se puede ver que entre las muestras incluidas predomina el pH básico, aunque existe una amplia variedad para la mayoría de parámetros, con niveles de materia orgánica desde muy bajos a aceptables y niveles de iones y cationes desde prácticamente 0 hasta niveles muy altos. En general, en estos suelos no hay problemas de salinidad ni sodicidad, si bien algunos presentan valores elevados para algunos de los parámetros indicadores, tales como el RAS, PSI y CE. La capacidad de la espectroscopía infrarroja para discernir entre estos suelos, y aquellos afectados por salinidad de la localidad de Guarromán, se evaluó con un PCA aplicado a los espectros FTRI y FT-NIR de todas las muestras en conjunto. En el gráfico de puntuaciones de los componentes PC1 y PC2 (Figura 29) podemos ver una clara agrupación de las muestras de Guarromán a lo largo del PC2 (28.08% Varianza), indicando una clara diferencia en el espectro entre las muestras estudiadas, con problemas de salinidad, y el resto de muestras de la base de datos. Según las cargas de este PC, esta discriminación se basa en las bandas de materia orgánica e impurezas de silicatos (1000 cm^{-1}) y carbonatos (711, 871 y 1423 cm^{-1}), así como en menor medida bandas asociadas a materiales orgánicos más evolucionados (OH interlámina a 5227 cm^{-1}), ilita (3620, 4000 y 4265 cm^{-1}) y kaolinita (4440 y 7000 cm^{-1}), lo que parece indicar una discriminación basándose en la calidad general del suelo.

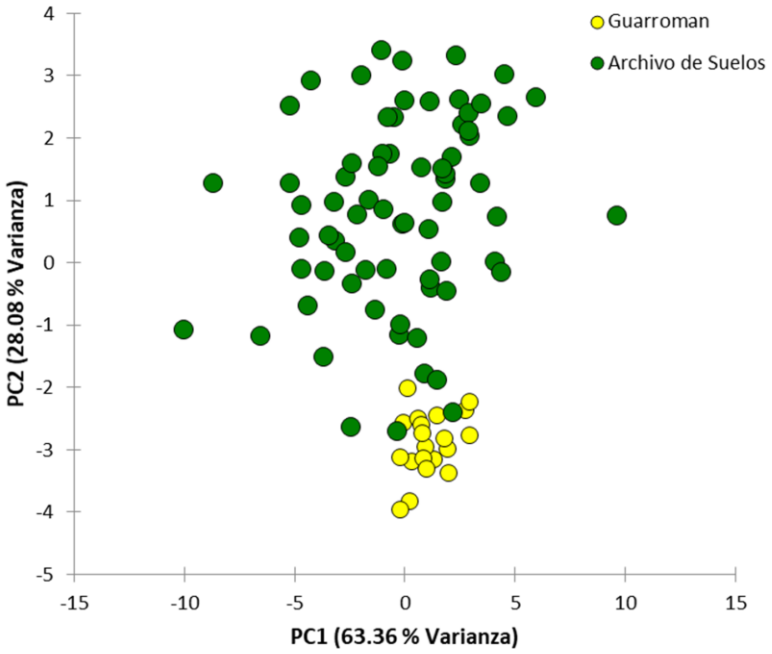

Figura 29. Análisis de componentes principales (PCA) de los espectros FT-NIR y FTIR de las muestras de Guarromán en comparación con el archivo de suelos de Agronutrientes Jaén S.C.A.

Por tanto, el análisis PCA aplicado a los espectros infrarrojos resulta muy útil para diferenciar entre tipologías de suelo, y en menor medida entre los manejos aplicados a dichos suelos. Además, parece ser bastante sensible a la calidad del suelo, siendo capaz de encontrar tendencias en las muestras dependiendo

de su fertilidad. Si comparamos FTIR y FT-NIR, este último parece presentar una mayor capacidad para agrupar las muestras según tipología, y especialmente según su manejo, aunque la interpretación de la razón de estos agrupamientos resulta más compleja en esta región del espectro infrarrojo.
El análisis PCA ha demostrado la capacidad de la espectroscopía infrarroja para diferenciar muestras de suelo. Para comprobar la capacidad real de los espectros FTIR y FT-NIR para clasificar muestras de suelo, se realizó un análisis discriminante de mínimos cuadrados parciales (PLS-DA), en el cual, además de los espectros infrarrojos, se introduce la información de las clases, y se crea un modelo que predice la clase de cada muestra. A la hora de clasificar las muestras en función de las tipologías de suelo, observamos como ambas regiones espectrales arrojan resultados muy satisfactorios (Tabla 6 y Tabla 7), mostrando solamente FTIR ciertas dificultades a la hora de clasificar entre suelos bajo manejo ecológico con material coluvial y suelos bajo manejo convencional con material coluvial, en las muestras pertenecientes al valle del Atanor. Si intentamos discriminar las muestras solo por manejo, el rendimiento del modelo disminuye, pero aun así genera resultados muy positivos, demostrando por un lado, la capacidad de la espectroscopía infrarroja para clasificar muestras ya sea bien acorde a su material geológico o a su manejo, y por otro lado que efectivamente los efectos derivados del manejo del suelo tienen su reflejo en ambas regiones del espectro infrarrojo, aunque estas sean difíciles de detectar con un simple análisis visual.

La espectroscopía infrarroja demuestra ser sensible a la calidad y fertilidad de los suelos, siendo capaz de clasificarlos dependiendo de sus características. Esto se debe a la gran cantidad de información sobre la composición de los suelos recogida en el espectro. La posibilidad de utilizar el espectro infrarrojo para predecir ciertos parámetros del suelo se plantea como una estrategia eficaz para reducir la cantidad de análisis precisos en el análisis de laboratorio tradicional. En este caso utilizaremos la regresión de mínimo cuadrados parciales (PLS). Para la creación de modelos predictivos robustos, en el caso de los suelos, el número de muestras consideradas es vital. En este sentido, se han propuesto bases de datos espectrales o librerías espectrales, que recogen espectros representativos de todos los tipos de suelos del mundo (Viscarra Rossel et al., 2016), si bien esta herramienta puede ser de una gran utilidad, ya que puede permitir conseguir modelos empíricos aplicables a todo tipo de suelo, el trabajar con dicha cantidad de muestras hace la modelización más compleja. Otros autores estiman que un número de muestras cercano a 100 podrían ser suficientes para creación de modelos robustos, lo que resulta en un trabajo más manejable que puede aportar resultados similares (Brown et al., 2006). En esta última línea, el número de muestras disponibles en nuestros trabajos podía ser adecuado para crear modelos predictivos de los diferentes parámetros medidos, modelos que podrían ser aplicables en un futuro para la predicción de parámetros en muestras de características similares. En primer lugar, se necesita preprocesar el espectro, para conseguir que la información importante esté más accesible y reducir el ruido. Un ejemplo del preprocesado en el caso de los espectros FTIR se puede ver en la Figura 30.

Tabla 6. Clasificación de muestras de suelos según su material geológico y/o manejo, mediante PLS-DA de los espectros infrarrojos de las muestras de Sierra Mágina y la Comarca Metropolitana de Jaén.

		Sensibilidad				Especificidad			
	Espectro	CA	PE	PS	PT	CA	PE	PS	PT
Calibración	FT-NIR	0.973	1	1	1	0.962	1	1	0.994
CV Bloques Aleatorios	FT-NIR	1	0.977	1	0.992	0.995	0.98	0.991	0.99
	Espectro	Convencional		Ecológico		Convencional		Ecológico	
Calibración	FT-NIR	0.962		0.962		0.962		0.962	
CV Bloques Aleatorios	FT-NIR	0.846		0.751		0.751		0.846	

PT: Puente Tablas; PS: Puente Sierra; PE: Pegalajar; CA: Cambil.

Tabla 7. Clasificación de muestras de suelos según su material geológico y/o manejo, mediante PLS-DA de los espectros infrarrojos de las muestras del Valle del Atanor.

		Sensibilidad					Especificidad				
	Espectro	EC	EM	CC	CM	SN	EC	EM	CC	CM	SN
Calibración	FTIR	1	1	0.926	1	1	0.853	0.99	0.892	1	1
	FT-NIR	1	1	1	1	1	1	1	1	1	1
	FTIR + FT-NIR	1	1	1	1	1	1	1	1	1	1
CV Bloques Aleatorios	FTIR	0.993	0.978	0.889	1	1	0.849	0.982	0.89	1	1
	FT-NIR	0.741	1	0.97	1	0.9	0.92	1	0.998	1	0.99
	FTIR + FT-NIR	0.933	1	0.993	1	0.983	0.98	1	0.994	1	1

EC: ecológico de coluvios; EM: ecológico de margas; CC: convencional de coluvios; CM: convencional de margas; SN: suelo natural.

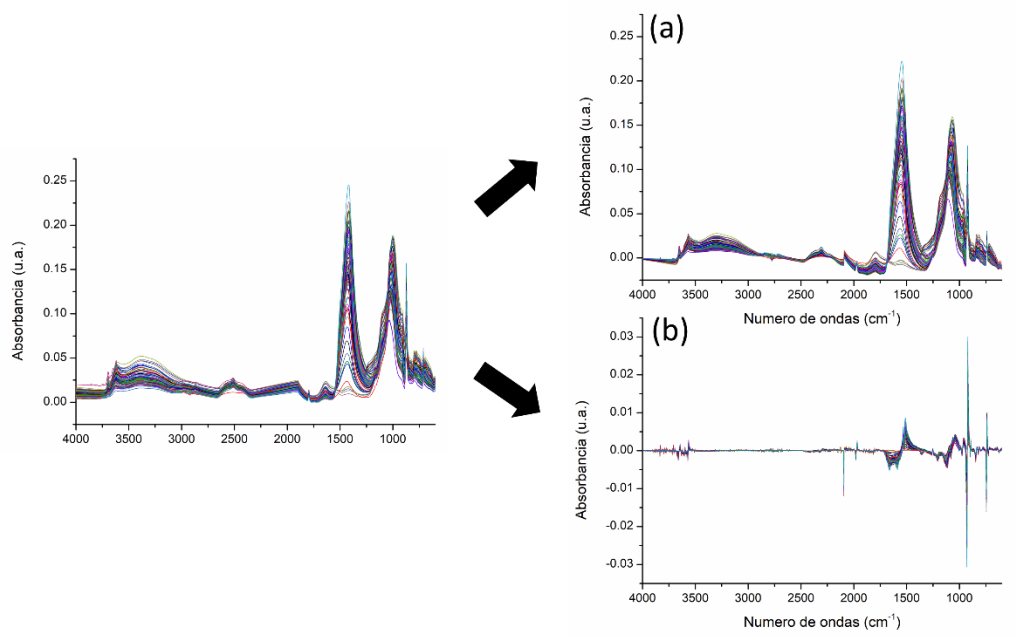

Figura 30. Espectros FTIR antes y después de aplicar preprocesado. (a) centrado medio, (b) 2ª derivada.

Se construyeron modelos predictivos para algunos de los principales parámetros del suelo (OC, N, P, K, $CaCO_3$, arcilla, limo, arena, pH y CIC), utilizando las muestras del Valle del Atanor. La Tabla 8 muestra los parámetros estadísticos obtenidos para los mejores modelos en cada caso. En el caso del FTIR, se obtuvieron modelos predictivos con RPD mayor que 2, para OC, N, P y $CaCO_3$, así como modelos predictivos con un buen ajuste para CIC y pH. Sin embargo, la capacidad predictiva de estos modelos para parámetros texturales y K bajaba a valores de RPD cercanos a 1, lo que indica que estos modelos solo serían válidos para comparar entre cantidades elevadas o bajas del parámetro (Viscarra Rossel et al., 2006). Este ajuste de los modelos también puede apreciarse en la Figura 32.

En el caso de los espectros FT-NIR, para los cuales se puede ver un ejemplo de preprocesado en la Figura 31, los modelos predictivos obtenidos tienen una capacidad predictiva similar a los de FTIR (Tabla 8), aunque para la mayoría de los casos las predicciones son mejores, con un RPD superior excepto en el caso del $CaCO_3$. Destaca especialmente la excelente predicción para el OC con RPD > 4, y como en este caso el pH tiene un RPD > 2, pudiendo considerársele un modelo más preciso y robusto. El ajuste de estos modelos puede comprobarse en la Figura 33.

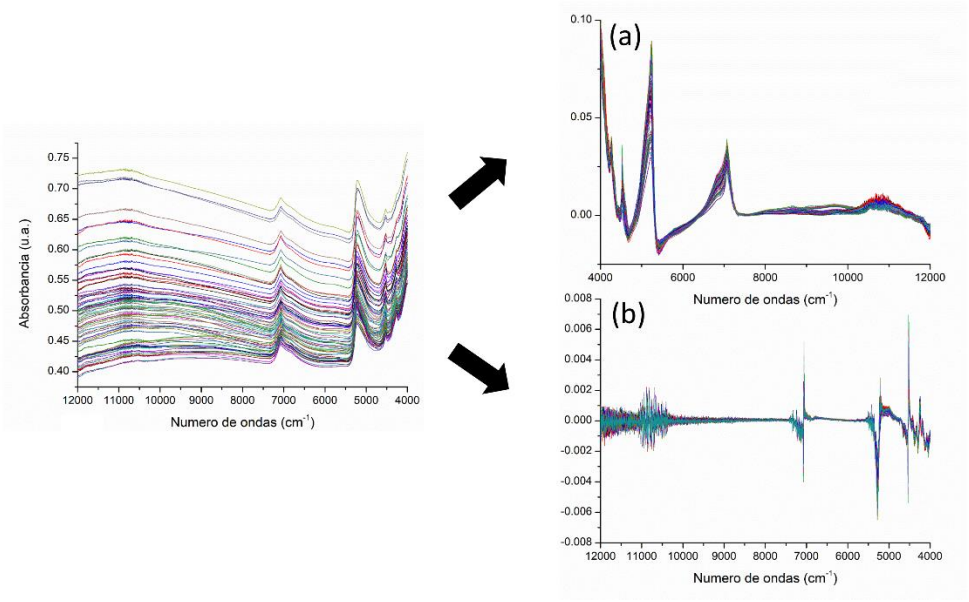

Figura 31. Espectros FT-NIR antes y después de aplicar preprocesado. (a) centrado medio, (b) 2ª derivada.

Por tanto, ambas regiones del espectro infrarrojo presentan una gran capacidad para predecir variables físico-químicas por lo que se plantea la posibilidad de explorar su capacidad de predicción conjunta. Para ello se construyeron modelos predictivos utilizando ambas regiones del espectro unidas mediante estrategias de *data fusion, low y mid level*. En el primer caso, concatenando los espectros normalizados y en el segundo fusionando las puntuaciones (scores) obtenidas de los modelos PLS individuales de FTIR y FT-NIR, a los que se les aplicó un escalado como paso previo a la construcción de los modelos. La combinación de ambas regiones genera resultados similares al mejor modelo de cada uno de ellos individualmente en el caso del *low level* (Tabla 8), aunque en algunos casos con un pequeño descenso en su capacidad predictiva al presentar un RPD menor. El ajuste de estos modelos puede verse en la Figura 34. Sin embargo, el mid level genera un claro incremento en la predicción de la mayoría de las variables, con RPD muy superiores para todos los casos excepto limo y arena, demostrando que la fusión de ambas regiones espectrales previa aplicación de técnicas quimiométricas regresivas da lugar a los mejores resultados, y parece ser la alternativa más potente para crear modelos predictivos.

Se puede comprobar por tanto como la espectroscopía infrarroja tiene una gran capacidad para predecir parámetros físico-químicos de los suelos, con resultados excelente especialmente para aquellos parámetros que tienen un reflejo directo en el espectro como OC, N y $CaCO_3$, pero también para parámetros en cierto punto correlacionados con algunas de sus bandas, como podría ser pH y CIC, íntimamente relacionados con la cantidad de carbonatos y arcillas respectivamente, o P y K relacionados en parte con la materia orgánica. Sin embargo, la predicción de parámetros texturales resulta más difícil al no tener reflejo directo en el espectro, excepto en el caso de la arcilla que si tiene un reflejo claro en el espectro. Aunque ambas regiones muestran capacidad predictiva, los espectros FT-NIR demuestran contener más información y mayor capacidad predictiva, aunque para una predicción lo más precisa posible lo ideal sería utilizar ambas regiones unidas mediante técnicas de fusion de datos.

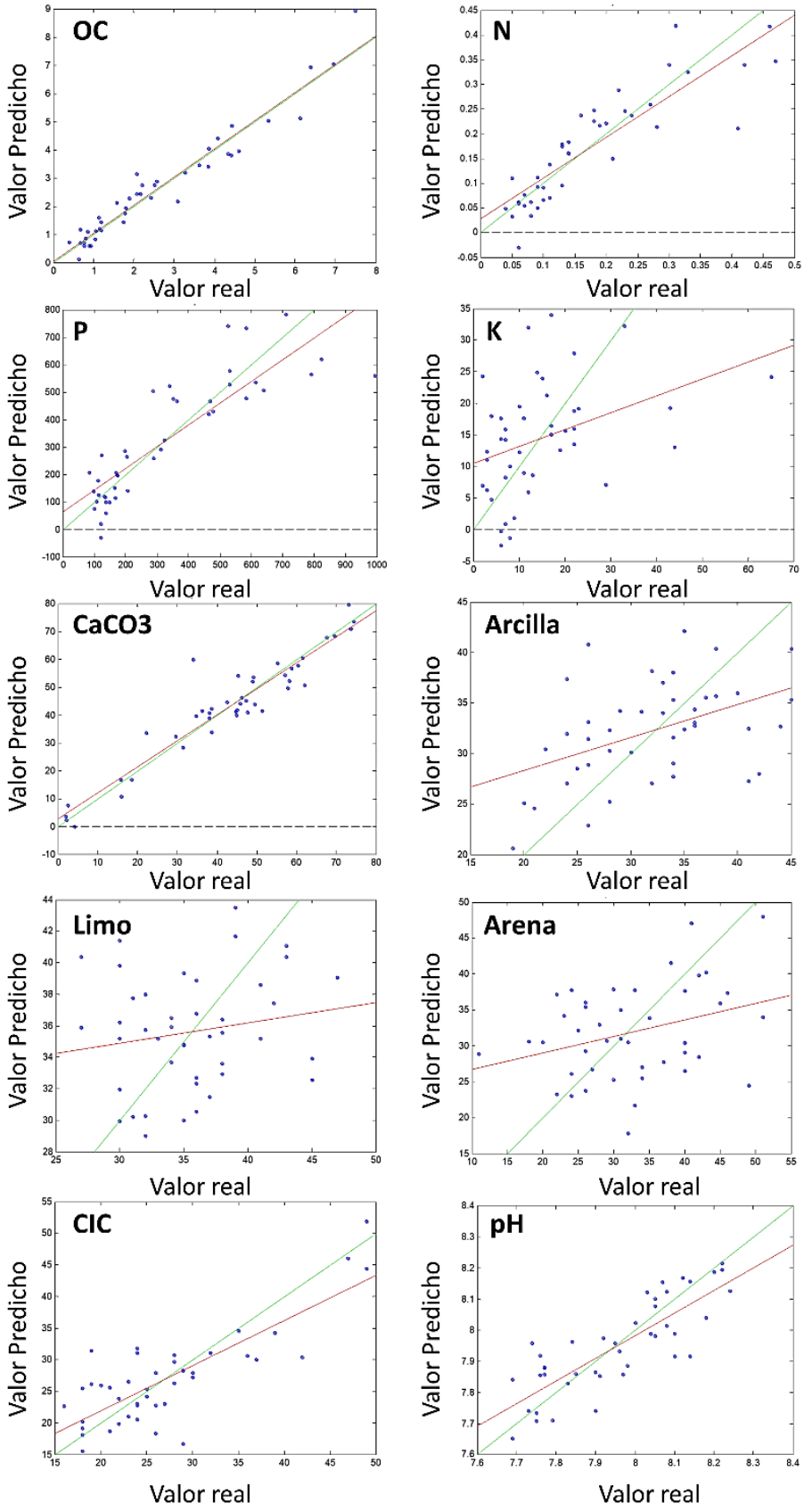

Figura 32. Valor predicho vs valor real de los modelos construidos utilizando regresión PLS con los espectros FTIR de las muestras del Valle del Atanor. La línea verde representa un ajuste perfecto, mientras la línea roja es el ajuste del modelo.

Tabla 8. Resultados de la predicción de variables de suelo en las muestras del Valle del Atanor mediante regresión PLS utilizando los espectros infrarrojos. Validación cruzada (CV) de 5 bloques y 5 iteraciones.

Espectro	Parámetro	OC	N	P	K	$CaCO_3$	Arcilla	Limo	Arena	CIC	pH
FTIR	Preprocesado	MC	SM, 2ºD, MC	MC	MC	SM, 2ºD, MC	SM, 2ºD, MC	SM, 2ºD, MC	MC	MC	MC
	LVs	8	9	6	6	7	7	7	7	6	10
	RMSEC	0.33	0.04	89	9.51	4.38	4	3.59	6.89	3.88	0.05
	RMSECV	0.51	0.06	115.6	12.36	5.89	6.18	5.39	9.68	5.02	0.09
	R^2 Cal	0.97	0.9	0.85	0.43	0.95	0.66	0.45	0.43	0.78	0.92
	R^2 CV	0.93	0.77	0.76	0.15	0.91	0.25	0.02	0.09	0.66	0.72
	RPD	3.74	2.12	2.07	1.04	3.36	1.13	0.92	0.97	1.69	1.8
FT-NIR	Preprocesado	2ºD, MC	2ºD, MC	MC	MC	MC	BL, 2ºD, MC	2ºD, MC	2ºD, MC	MC	2ºD, MC
	LVs	7	4	10	6	8	7	9	7	8	6
	RMSEC	0.11	0.03	35.51	7.85	4.55	2.07	0.93	2.99	3.38	0.03
	RMSECV	0.4	0.05	81.62	11.26	7.2	7.21	5.32	10.78	5.16	0.08
	R^2 Cal	1	0.91	0.98	0.61	0.94	0.91	0.96	0.89	0.83	0.96
	R^2 CV	0.96	0.81	0.9	0.32	0.88	0.11	0.08	0.02	0.65	0.77
	RPD	4.84	2.29	2.93	1.14	2.75	0.97	0.93	0.87	1.65	2.07
FTIR + FTNIR (Low level Fusion)	Preprocesado	2ºD, MC	2ºD, MC	2ºD, MC	MC	2ºD, MC	2ºD, MC	BL, 2ºD, MC	2ºD, MC	BL, 2ºD, MC	BL, 2ºD, MC
	LVs	10	8	7	8	8	7	6	7	5	9
	RMSEC	0.07	0.02	52.05	7.48	3.62	3.23	3.26	4.67	4.06	0.02
	RMSECV	0.4	0.05	108.38	11.42	6.21	6.3	5.51	10.03	5.54	0.08
	R^2 Cal	1	0.96	0.95	0.65	0.96	0.78	0.55	0.74	0.76	0.98
	R^2 CV	0.96	0.81	0.8	0.32	0.91	0.27	0.05	0.08	0.59	0.78
	RPD	4.84	2.24	2.2	1.13	3.19	1.11	0.9	0.93	1.53	2.02
FTIR + FTNIR (Mid level Fusion)	Preprocesado	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC
	LVs	11	16	12	12	13	13	6	5	20	17
	RMSEC	0.06	0.01	34.22	3.82	2.62	1.93	2.48	4.02	1.55	0.02
	RMSECV	0.22	0.02	42.11	5.91	4.25	3.05	4.66	7.52	2.7	0.05
	R^2 Cal	1	0.98	0.98	0.78	0.97	0.9	0.58	0.74	0.88	0.98
	R^2 CV	0.98	0.95	0.96	0.69	0.93	0.84	0.36	0.42	0.84	0.94
	RPD	8.76	4.68	5.66	2.18	4.66	2.29	1.06	1.24	3.14	3.19

LVs: número de variables latentes, RMSEC: root mean square error en la calibración, RMSECV: root mean square error en la validación cruzada. MC: centrado medio; 2ºD: 2ª derivada; SM: suavizado; BL: línea base.

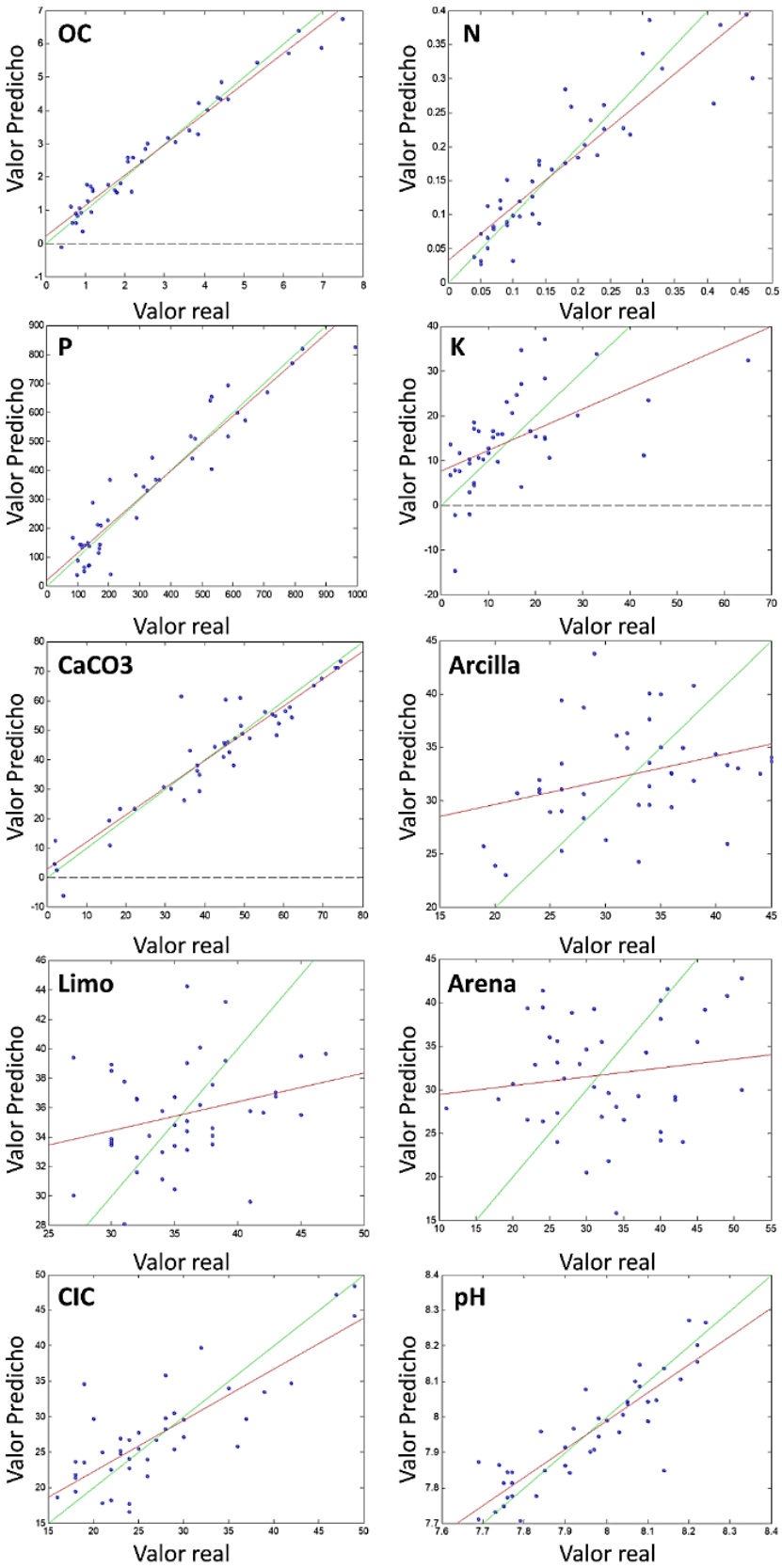

Figura 33. Valor predicho vs valor real de los modelos construidos utilizando regresión PLS con los espectros FT-NIR de las muestras del Valle del Atanor. La línea verde representa un ajuste perfecto, mientras la línea roja es el ajuste del modelo.

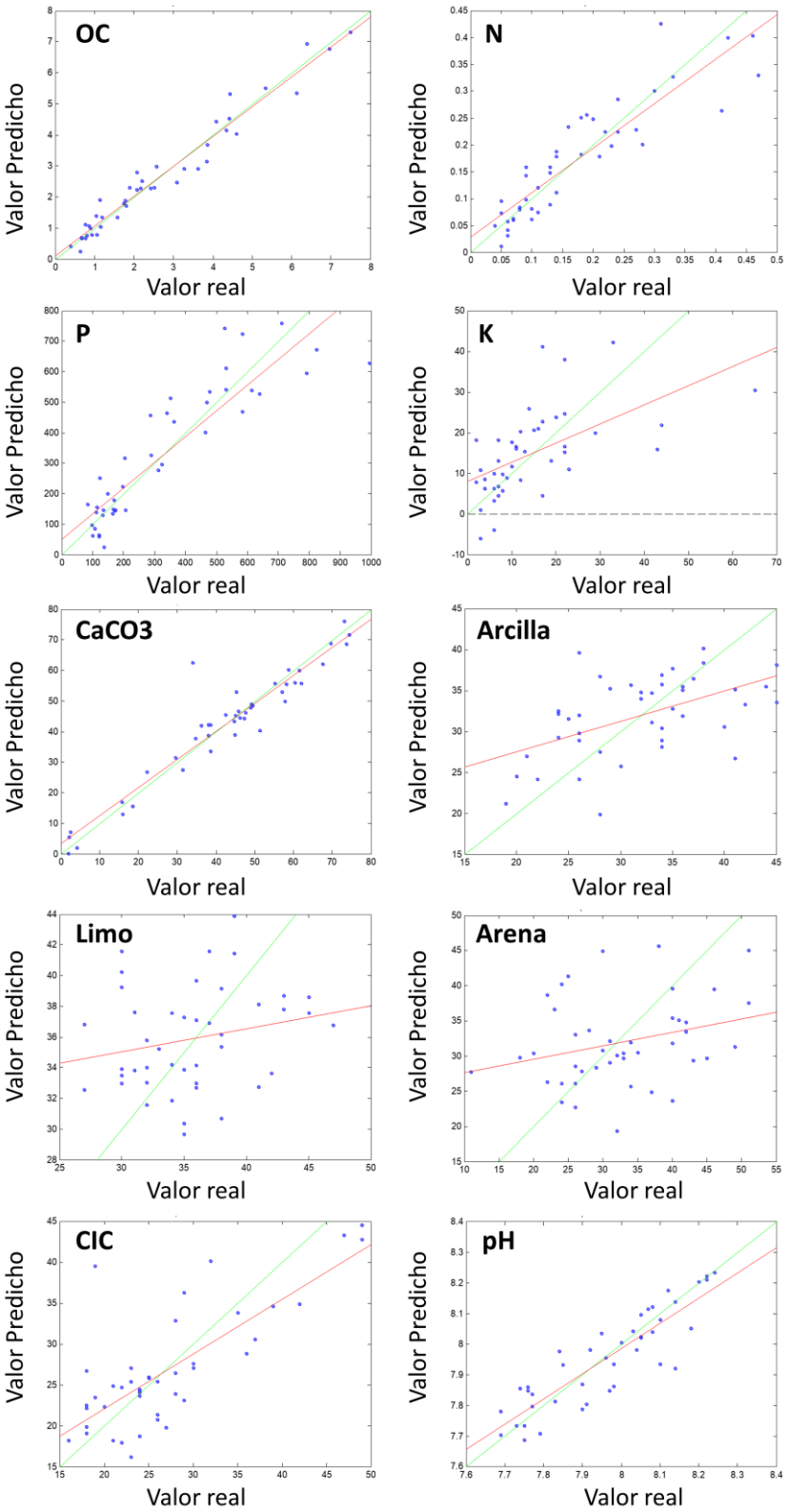

Figura 34. Valor predicho vs valor real de los modelos construidos utilizando regresión PLS con los espectros FTIR + FT-NIR de las muestras del Valle del Atanor. La línea verde representa un ajuste perfecto, mientras la línea roja es el ajuste del modelo.

Vista la capacidad de la espectroscopía infrarroja para predecir variables físico-químicas, se comprobó su capacidad para predecir variables biológicas del suelo. Para ello se intentó predecir las diferentes actividades enzimáticas, así como el índice GMea, medidos para las muestras asociadas a diferentes localizaciones de la provincia de Jaén. Para este caso se utilizó el espectro FT-NIR, cuyos mejores modelos se muestran en la Tabla 9. En todos ellos podemos observar como es necesaria una gran cantidad de variables latentes, probablemente debido a que la información más relacionada con la parte orgánica y biológica del espectro no tiene tanto peso como la mineralógica. La capacidad predictiva para las actividades enzimáticas en general es intermedia, como se puede ver en la Figura 35, con buenos resultados para la β-glucosidasa y la fosfatasa alcalina, con RPD cercano a 1.6, mientras para el resto de actividades se obtienen RPD cercanos a 1.2, indicando menor precisión en la predicción. Estos resultados parecen indicar una mayor dificultad en general para predecir actividades enzimáticas específicas, posiblemente debido a la falta de relación directa entre estas y la materia orgánica del suelo (Soriano-Disla et al., 2014). Sin embargo, una predicción bastante más precisa se obtuvo para el índice GMea, con RPD = 1.81. Esto parece indicar una mayor capacidad de la espectroscopía infrarroja para predecir la calidad biológica global del suelo, que parámetros específicos, lo que puede ser de mayor utilidad ya que el uso de índices de calidad nos permite caracterizar las condiciones del agro-sistema de una manera rápida y precisa (Bastida et al., 2008), y en este caso nos permitiría conocer la calidad biológica del suelo estudiado.

Tabla 9. Resultados de la predicción de variables biológicas en las muestras de la provincia de Jaén mediante regresión PLS utilizando los espectros FT-NIR. Espectros preprocesados mediante detrend y centrado medio. Validación cruzada (CV) de 5 bloques y 5 iteraciones.

Parámetro	Deshidrogenasa	β-Glucosidasa	Arilsulfatasa	Fosfatasa alcalina	Fosfatasa ácida	Potencial de nitrificación	GMea
LVs	6	10	10	10	10	10	10
RMSEC	2.67	115.08	48.94	89.13	62.2	0.27	8.57
RMSECV	2.86	154.53	52.72	104.72	69.31	0.44	9.69
R^2 Cal	0.32	0.77	0.52	0.72	0.44	0.75	0.76
R^2 CV	0.23	0.6	0.46	0.63	0.33	0.36	0.7
RPD	1.14	1.56	1.34	1.63	1.21	1.23	1.81

LVs: número de variables latentes, RMSEC: root mean square error en la calibración, RMSECV: root mean square error en la validación cruzada.

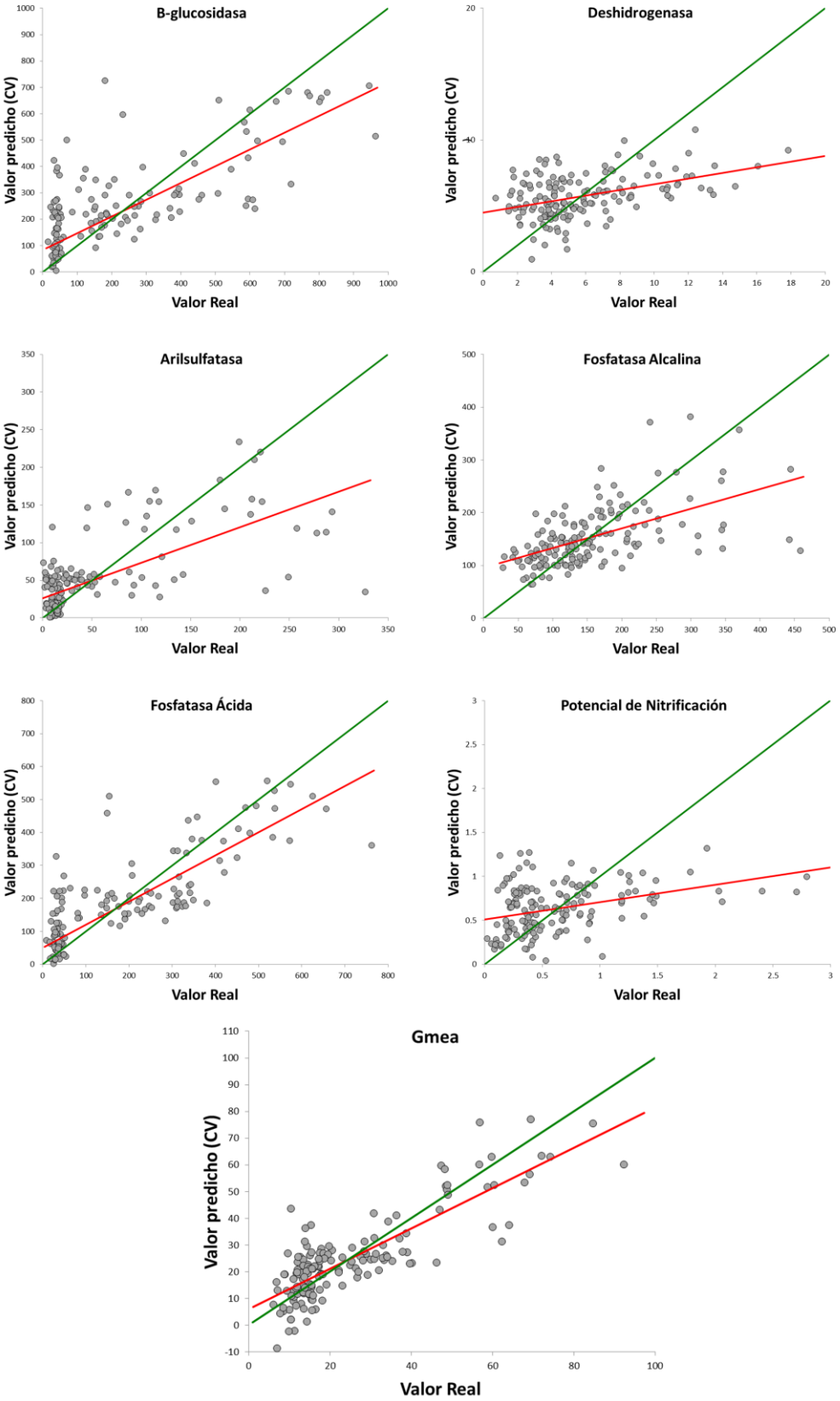

Figura 35. Valor predicho vs valor real de los modelos construidos utilizando regresión PLS con los espectros FT-NIR de las muestras de la provincia de Jaén. La línea verde representa un ajuste perfecto, mientras la línea roja es el ajuste del modelo.

En resumen, hemos podido comprobar como la espectroscopía infrarroja tiene un gran potencial como técnica para predecir diferentes parámetros del suelo, especialmente aquellos con clara representación en el espectro, aunque también demuestra una gran capacidad para predecir parámetros correlacionados en parte con estos parámetros, así como índices de calidad del suelo. Esto convierte a esta técnica en una gran alternativa a los análisis tradicionales de laboratorio, al ser mucho más rápida y económica. Sin embargo, resulta de gran importancia tener en cuenta que no todos los parámetros se predicen con igual precisión usando esta técnica, así como que las calibraciones realizadas solo son aplicables a muestras de características similares, y que extrapolar este tipo de modelos a muestras de suelo muy diferentes arrojaría pobres resultados. Por tanto, para su utilización como sustituto del laboratorio sería necesario contar con un mayor rango de muestras, que cubran la mayoría de tipologías de suelo, o bien contar con modelos de predicción individuales dependiendo del origen de la muestra. Aun así, los análisis de laboratorio seguirían siendo necesarios, aunque en menor medida ya que solo serían necesarios para recalibrar los modelos para mantener la precisión, o para ampliar el rango de muestras contenidas en el modelo y hacerlo aplicable a una región mayor.

3. Espectroscopía infrarroja y de fluorescencia de rayos X para la determinación del estado nutricional del olivo

Como se comentó en la introducción, para una completa caracterización del agro-sistema de olivar no basta con estudiar el suelo, sino que también resulta de gran importancia conocer el estado nutricional de la planta. Esto se hace generalmente a partir del análisis foliar que igual que en el caso del suelo, resulta costoso en tiempo y dinero. Teniendo en cuenta la capacidad de la espectroscopía infrarroja para la caracterización del suelo, se propone el uso de la espectroscopía FT-NIR para la predicción de parámetros en hoja de olivo. Además, y como técnica complementaria se plantea el uso de la fluorescencia de rayos X por energía dispersiva (EDXRF), que permite obtener información elemental que es muy interesante en análisis foliar. Para utilizar ambas técnicas en conjunto, se probaron diferentes estrategias de fusión de datos, para comprobar la posible sinergia y complementariedad de ambas técnicas espectroscópicas. Un esquema de las estrategias de fusión de datos utilizadas en este estudio puede verse en la Figura 36. Los resultados aquí expuestos son un resumen de los mostrados en el capítulo 9.

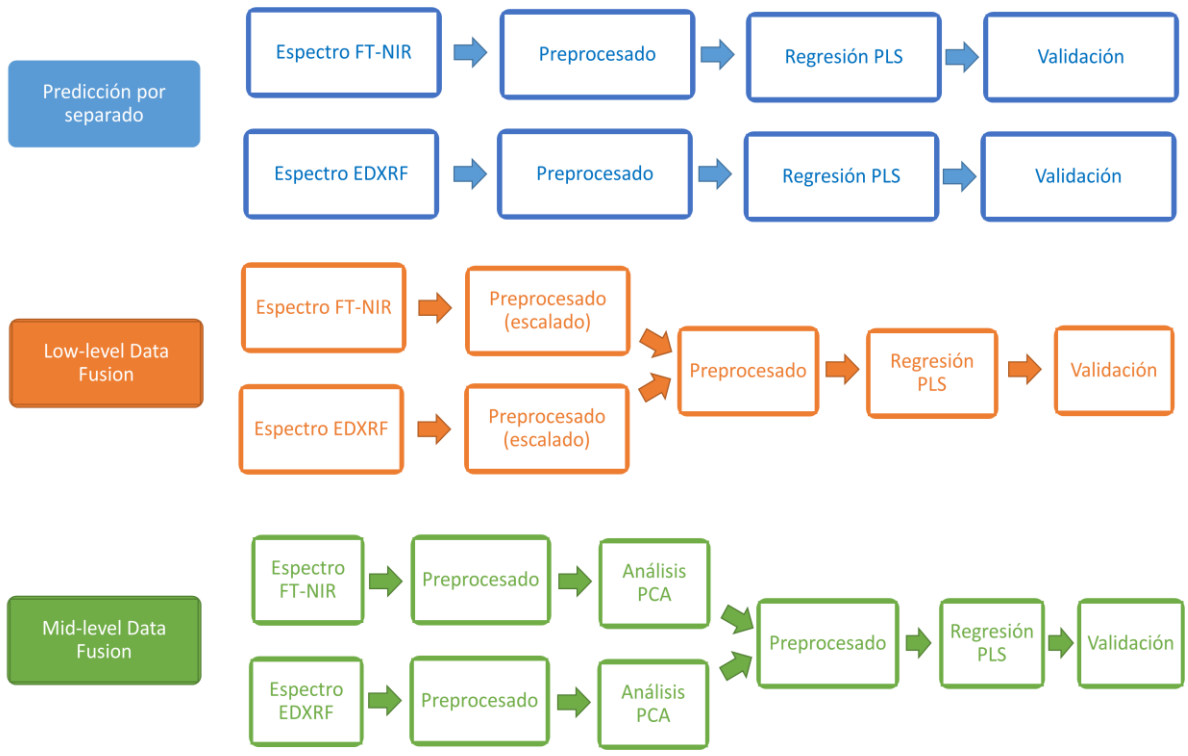

Figura 36. Esquema de los diferentes niveles de fusión de datos utilizados para la predicción de nutrientes en hoja de olivo con FT-NIR y EDXRF.

En este caso, se contaban con más de 2500 muestras de hoja, secadas y molidas, con la analítica completa, proporcionadas por el laboratorio Agronutrientes Jaén S.C.A. Estas muestras fueron tomadas durante 4 años (2013-2016), en dos épocas de muestreo (invierno y verano). Dado que tal cantidad de muestras podría ser difícil de manejar, se realizó una selección de muestras para establecer un set más reducido pero que cubriese el máximo rango posible de concentraciones para cada nutriente. Se utilizó análisis de conglomerados jerárquicos (Figura 37) sobre una matriz construida con las analíticas completas para cada muestra. Se seleccionó una distancia que generase 12 - 18 grupos cada año y se eligieron 1 - 3 muestras por grupo dependiendo del tamaño, generándose un set de entrenamiento/calibración de 133 muestras (Tabla 10). Por otro lado, se seleccionaron 60 muestras totalmente al azar como grupo de validación.

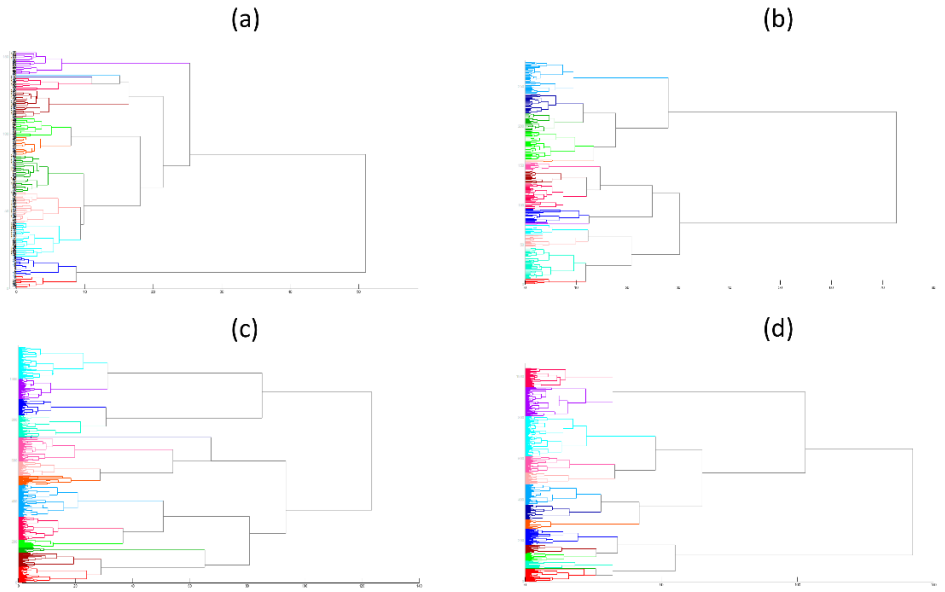

Figura 37. Análisis jerárquico de conglomerados de la matriz con los resultados analíticos para los diferentes años a) 2013, b) 2014, c) 2015 y d) 2016.

Tabla 10. Rango (mínimo - máximo) de cada uno de los nutrientes en los diferentes grupos de muestras.

Conjunto de muestras	n	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)	Zn (ppm)	B (ppm)
2013	152	0.94 - 1.75	0.047 - 0.198	0.42 - 1.42	0.61 - 2.52	0.067 - 0.21	11 - 71	7 - 57	13 - 55
2014	281	0.86 - 1.78	0.046 - 0.174	0.33 - 1.31	0.63 - 2.62	0.048 - 0.224	11 - 69	6 - 36	13 - 39
2015	1156	0.71 - 1.99	0.036 - 0.171	0.27 - 1.22	0.59 - 2.73	0.051 - 0.232	10 - 70	6 - 53	11 - 52
2016	1042	0.67 - 1.99	0.037 - 0.175	0.28 - 1.44	0.63 - 2.73	0.056 - 0.234	9 - 75	5 - 56	11 - 49
Total	2530	0.67 - 1.99	0.036 - 0.198	0.27 - 1.44	0.59 - 2.73	0.048 - 0.234	9 - 75	5 - 57	11 - 55
Calibración	133	0.71 - 1.98	0.047 - 0.198	0.3 - 1.42	0.63 - 2.71	0.048 - 0.211	11 - 69	6 - 57	13 - 47
Validación	60	1.15 - 1.99	0.044 - 0.163	0.3 - 1.26	0.66 - 2.72	0.05 - 0.228	6 - 61	6 - 22	11 - 50

Los mejores modelos predictivos para cada nivel de fusión de datos se muestran en la Tabla 11. Para la predicción de nutrientes usando ambas técnicas de manera individual se probaron diferentes preprocesados, obteniendo los mejores resultados para FT-NIR con *detrend* y centrado medio. Se obtuvieron predicciones muy precisas para Ca, N y K, aunque en el caso de los nutrientes presentes en menores cantidades en las muestras (ppm) los resultados fueron más pobres, especialmente en el caso del Zn. Destaca la buena predicción del B, que puede deberse a la asociación de este con varios compuestos muy importantes en el metabolismo de la planta (Liakopoulos y Karabourniotis, 2005). Para el caso de EDXRF, se obtuvieron excelentes modelos para Ca y K, pero también buenos modelos para P, mientras para Mg, Mn y Zn los resultados fueron más discretos. En general los modelos para la predicción de micronutrientes fueron mejores utilizando esta técnica que en el caso de FT-NIR. Para el caso del N y el B, las predicciones

son más imprecisas, dado que estos solo pueden ser calculados utilizando el espectro XRF mediante correlación con otros elementos.

Los objetivos de utilizar los niveles de fusión de datos es aprovechar la posible sinergia entre las diferentes técnicas de manera que se obtengan predicciones más precisas. El nivel de fusión *low* se realizó concatenando los espectros, previo normalizado respecto al área y respecto a la intensidad máxima, en ambos casos. Para la fusión de espectros se juntó FT-NIR con cada uno de los rangos de EDXRF por separado, así como todos ellos en conjunto, obteniéndose los mejores resultados en la fusión con el *low range*, excepto para el P que fue mejor el *light range*, tal y como ocurría para los modelos individuales, ya que el P tiene mayor reflejo en este rango de energía. Comparando los mejores modelos en la fusión de datos *low level* con las técnicas de manera individual (Tabla 11), la mayoría de los modelos tienen un rendimiento similar o inferior, lo que parece indicar que este nivel de fusión de datos no es la mejor opción. Por otro lado, el *mid level*, que se aplicó fusionando la información relevante de ambos espectros, lo cual se extrajo mediante un PCA, arrojó resultados similares para la mayoría de nutrientes, pero con un claro incremento de la precisión en la predicción del Mn (Tabla 11), y más leve para Mg. Este nivel de fusión produce una mejora de los resultados y parece ser una buena alternativa. Con estos modelos (Figura 38) podríamos predecir de manera efectiva nutrientes como Ca, K y Mn, mientras otros nutrientes como N y P podrían ser predichos, aunque existe margen para mejorar la precisión de estos modelos. Por último, para Mg y B los modelos serían útiles para diferenciar entre niveles elevados y bajos de nutrientes, lo que podría ser útil a la hora de diagnosticar deficiencias.

Tabla 11. Resultados de la predicción de los diferentes nutrientes en hoja de oliva mediante FT-NIR y EDXRF de manera individual, y mediante diferentes estrategias de fusión de datos. Validación cruzada (CV) de 5 bloques y 5 iteraciones.

FT-NIR. Preprocesado: detrend y centrado medio.								
Elemento	**N (%)**	**P (%)**	**K (%)**	**Ca (%)**	**Mg (%)**	**Mn (ppm)**	**Zn (ppm)**	**B (ppm)**
LVs	9	8	13	8	12	12	3	14
RMSEC	0.086	0.011	0.016	0.151	0.011	4.145	1.957	0.807
RMSECV	0.111	0.016	0.103	0.191	0.029	11.372	6.241	4.511
RMSEP	0.091	0.025	0.120	0.198	0.030	9.075	5.660	7.358
R2 Cal	0.82	0.8	0.99	0.9	0.91	0.89	0.88	0.99
R2 CV	0.71	0.62	0.73	0.85	0.39	0.26	0	0.63
R2 Pred	0.73	0.22	0.65	0.78	0.3	0.41	0	0.33
RPD CV	1.8	1.6	1.9	2.5	1.3	1.1	0.9	1.6
RPD Pred	1.8	0.9	1.5	2.1	1.2	1.3	0.6	1.1
EDXRF. Preprocesado: centrado medio								
Rango	**Light**	**Light**	**Low**	**Low**	**Light + Low + Main**	**Light + Low + Main**	**Light + Low + Main**	**Light + Low + Main**
Elemento	**N (%)**	**P (%)**	**K (%)**	**Ca (%)**	**Mg (%)**	**Mn (ppm)**	**Zn (ppm)**	**B (ppm)**
LVs	6	8	3	3	5	8	12	5
RMSEC	0.119	0.007	0.060	0.158	0.030	5.492	0.747	4.767
RMSECV	0.135	0.012	0.064	0.161	0.032	8.817	5.575	5.199
RMSEP	0.166	0.015	0.111	0.182	0.033	7.041	3.333	6.747
R2 Cal	0.65	0.93	0.9	0.89	0.35	0.81	0.98	0.56
R2 CV	0.56	0.78	0.89	0.89	0.28	0.54	0.09	0.5
R2 Pred	0.28	0.59	0.62	0.8	0.11	0.65	0.21	0.28
RPD CV	1.5	2.1	3.1	3.0	1.2	1.4	1.0	1.4
RPD Pred	1.0	1.6	1.6	2.2	1.1	1.6	0.9	1.2

LVs: número de variables latentes, RMSEC: root mean square error en la calibración, RMSECV: root mean square error en la validación cruzada.

Tabla 12 (continuación). Resultados de la predicción de los diferentes nutrientes en hoja de oliva mediante FT-NIR y EDXRF de manera individual, y mediante diferentes estrategias de fusión de datos. Validación cruzada (CV) de 5 bloques y 5 iteraciones.

Low Level Data Fusion. Preprocesado: normalizado								
Elemento	**N (%)**	**P (%)**	**K (%)**	**Ca (%)**	**Mg (%)**	**Mn (ppm)**	**Zn (ppm)**	**B (ppm)**
LVs	5	8	3	3	5	4	11	8
RMSEC	0.115	0.006	0.062	0.16	0.027	7.626	1.613	2.305
RMSECV	0.159	0.014	0.113	0.177	0.039	7.797	3.998	7.092
RMSEP	0.67	0.94	0.9	0.89	0.46	0.63	0.92	0.9
R2 Cal	0.32	0.58	0.61	0.81	0.05	0.54	0.04	0.25
R2 CV	1	1.6	1.6	2.3	0.9	1.5	0.8	1.1
R2 Pred	0.32	0.58	0.61	0.81	0.05	0.54	0.04	0.25
RPD CV	1.6	2.1	3	2.9	1.2	1.2	1.1	1.5
RPD Pred	1	1.6	1.6	2.3	0.9	1.5	0.8	1.1
Mid Level Data Fusion. Preprocesado: escalado								
Elemento	**N (%)**	**P (%)**	**K (%)**	**Ca (%)**	**Mg (%)**	**Mn (ppm)**	**Zn (ppm)**	**B (ppm)**
LVs	3	6	7	2	11	7	6	2
RMSEC	0.087	0.010	0.051	0.135	0.021	2.834	4.876	4.035
RMSECV	0.110	0.013	0.067	0.162	0.027	4.588	6.376	4.769
RMSEP	0.094	0.014	0.110	0.183	0.036	6.867	5.201	6.473
R2 Cal	0.81	0.84	0.93	0.92	0.67	0.94	0.23	0.69
R2 CV	0.71	0.74	0.89	0.89	0.5	0.85	0	0.58
R2 Pred	0.68	0.6	0.63	0.8	0.25	0.71	0	0.34
RPD CV	1.8	1.9	2.9	3.0	1.4	2.8	0.9	1.5
RPD Pred	1.7	1.6	1.6	2.2	1.0	1.7	0.6	1.2

LVs: número de variables latentes, RMSEC: root mean square error en la calibración, RMSECV: root mean square error en la validación cruzada.

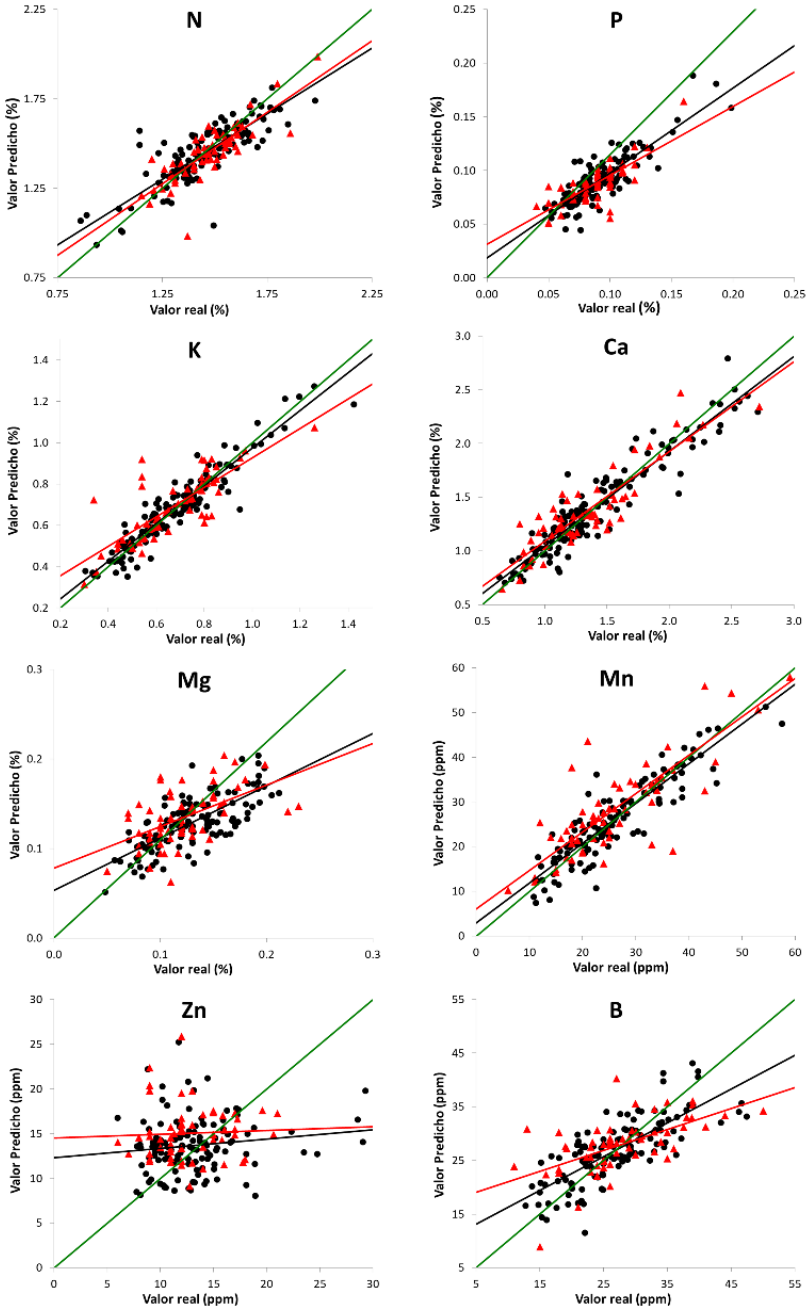

Figura 38. Valor predicho vs valor real de los modelos construidos utilizando regresión PLS con los espectros FT-NIR y EDXRF con mid level data fusión. La línea verde representa un ajuste perfecto, mientras la línea roja es el ajuste del modelo en la validación cruzada y la línea negra el ajuste del modelo en la validación.

Por tanto, comprobamos que FT-NIR y EDXRF son capaces de predecir la mayoría de nutrientes esenciales para el olivo en la hoja, de manera que en parte podrían sustituir al análisis tradicional de laboratorio, aunque tal y como ocurría con suelos, sería necesaria una recalibración de los modelos con el tiempo, y tener en cuenta que estos modelos no son totalmente precisos para algunos de los nutrientes. Por otro lado, la estrategia de fusión de datos parece ser de utilidad para combinar técnicas espectroscópicas, especialmente el *mid level*, y aunque la mejora es ligera, su uso sería la mejor opción para obtener los modelos más precisos posibles.

4. Enmiendas orgánicas y su mejora para la recuperación de la fertilidad

Se ha comprobado el claro efecto que tiene el manejo del suelo sobre su calidad, así como algunos problemas de degradación asociados a manejos del suelo más convencionales. Como posible solución a esta problemática proponemos el uso de enmiendas orgánicas, para aumentar los niveles de materia orgánica en el suelo, y así poder mejorar la fertilidad y calidad del agro-sistema. En este apartado se van a comentar los resultados obtenidos al analizar los efectos de la adición de ciertas enmiendas sobre el suelo, así como el estudio de algunos de los principales inconvenientes de las enmiendas orgánicas, y posibles estrategias para corregirlos, que se describen con mayor profundidad en los capítulos 10, 11 y 12.

En primer lugar, se van a discutir los efectos que tiene la aplicación de enmiendas orgánicas con un escaso grado de transformación sobre el suelo, en este caso los posos de café, haciendo hincapié en los posibles cambios que su uso puede generar sobre la cantidad y calidad de la materia orgánica. Se estudió el efecto de la adición de posos de café sobre dos tipologías de suelo diferentes procedentes de Granada (Tabla 13), para analizar posibles diferencias en la evolución y estabilización de la materia orgánica asociadas al tipo de material geológico. La mayor diferencia entre estos suelos es el mayor pH del suelo de vega, asociado a la mayor cantidad de carbonatos, además de una mayor cantidad de carbono orgánico, así como de la mayoría de elementos, aunque con una peor relación C/N, y una textura más arcillosa frente a la textura más franca del suelo rojo.

Sobre ambos suelos se añadió un 2.5 y un 10% en peso de posos de café (SCG) cuya analítica completa aparece en la Tabla 15. Estas mezclas, así como los suelos sin enmienda, se incubaron durante 0, 30 y 60 días. A todas las muestras se les aplicó un fraccionamiento de la materia orgánica (Swift, 1996) y se caracterizó la cantidad de cada una de sus fracciones (Tabla 14). Los resultados muestran un claro aumento de todas las fracciones de la materia orgánica, pero especialmente importante en las fracciones más lábiles (TEC, HWSC). El aumento en la fracción de huminas solo es significativo para las muestras con 10% de adición de posos de café, indicando un menor aumento de las fracciones más complejas y recalcitrantes de materia orgánica. Si observamos el índice de humificación, vemos diferencias entre las tipologías de suelo, con mayores valores de este en el suelo rojo, lo que muestra una menor presencia inicial de compuestos menos complejos en estos suelos, tendencia que se mantiene con el añadido de SCG. Estos resultados se reafirman con los resultados del análisis termogravimétrico (Figura 39), para el cual se calcularon las áreas correspondientes a compuestos lábiles y recalcitrantes en el análisis térmico diferencial simultáneo (SDTA) siguiendo lo establecido por (Lopez-Capel et al., 2005). Podemos ver como aumentan ambas fracciones, lábil y recalcitrante, aunque esta última lo hace en mucha menor medida, lo que provoca una reducción de la termoestabilidad de la materia orgánica de las muestras. De nuevo comparando suelos observamos una mayor cantidad de compuestos lábiles en los suelos de vega, así como un mayor aumento de estos con la adición de SCG, lo que puede deberse en gran parte a factores mineralógicos y granulométricos (Rakhsh et al., 2017), dado que la mayor cantidad de arcilla en el suelo de vega, le proporciona una mayor capacidad de retener y estabilizar materia orgánica.

Tabla 13. Caracterización físico-química de las muestras de suelos de vega y suelo rojo (Granada). Media ± SD.

	OC (%)	N (%)	C/N	pH	Arcilla (%)	Limo (%)	Arena (%)	$CaCO_3$ (%)	CE (dS/m)
Suelo Rojo	1.16 ± 0.11	0.11 ± 0.03	11 ± 2	7.2 ± 0.2	43.2 ± 0.9	18.0 ± 1.2	38.8 ± 2.1	1.6 ± 0.5	0.6 ± 0.1
Suelo Vega	1.36 ± 0.08	0.11 ± 0.05	13 ± 2	8.2 ± 0.1	58.0 ± 1.7	29.9 ± 0.9	12 ± 2.6	39 ± 3.2	1.3 ± 0.2

Tabla 14. Caracterización de la materia orgánica en los suelos tras la adición de los posos de café. La parte inferior de la tabla muestra la significación estadística al comparar las muestras agrupadas según el tipo de suelo, el % de SCG y los días de maduración.

Suelo	SCG %	n	TEC (mg C / Kg)	HWSC (mg C / Kg)	Huminas (mg C / Kg)	HA (mg C / Kg)	FA (mg C / Kg)	Ratio HA/FA	Índice de Humificación	Absorbancia en UV-Vis		
										A4 (472nm)	A6 (680nm)	A4/A6
Rojo	0	5	5198 ± 1154 a	228 ± 132 ab	819 ± 348 a	2640 ± 658 a	229 ± 33 a	11.6 ± 3.1 a	0.84 ± 0.25 ab	0.57 ± 0.04 a	0.13 ± 0.01 a	4.5 ± 0.2 b
Rojo	2.5	5	8571 ± 1492 b	702 ± 124 c	1722 ± 800 a	4326 ± 1176 b	374 ± 108 b	11.7 ± 1.9 a	0.86 ± 0.18 ab	0.53 ± 0.03 a	0.1 ± 0.01 a	5.2 ± 0.2 c
Rojo	10	5	18382 ± 2831 c	1574 ± 499 d	14247 ± 3244 b	8515 ± 2176 c	754 ± 107 c	11.5 ± 3.3 a	1.03 ± 0.32 b	0.51 ± 0.04 a	0.1 ± 0.01 a	5.1 ± 0.3 c
Vega	0	5	5342 ± 510 a	173 ± 88 a	1943 ± 1110 a	3222 ± 851 ab	274 ± 43 ab	11.8 ± 2.8 a	0.58 ± 0.29 a	0.88 ± 0.29 b	0.22 ± 0.08 b	4 ± 0.2 a
Vega	2.5	5	8224 ± 1093 b	511 ± 68 bc	2918 ± 787 a	4334 ± 599 b	370 ± 81 b	12.1 ± 2.4 a	0.75 ± 0.12 ab	0.92 ± 0.16 b	0.23 ± 0.04 b	4.1 ± 0.1 a
Vega	10	5	16964 ± 1756 c	1394 ± 249 d	15084 ± 3488 b	8846 ± 1071 c	680 ± 159 c	13.5 ± 3.1 a	0.79 ± 0.13 ab	0.82 ± 0.08 b	0.18 ± 0.01 b	4.4 ± 0.2 b
Tipo de Suelo			SR=SV	SR=SV	SR=SV	SR=SV	SR=SV	SR=SV	SR>SV	SV>SR	SV>SR	SR>SV
SCG %			10>2.5>0	10>2.5>0	10>2.5=0	10>2.5>0	10>2.5>0	0=2.5=10	0=2.5=10	0=2.5=10	0=2.5=10	10≥2.5≥0
Días de maduración			0=30=60	0=30=60	0=30=60	0=30=60	0=30=60	0=30=60	0=30=60	0=30=60	0=30=60	0=30=60

TEC: carbono total extraíble; HWSC: carbono soluble en agua caliente; HA: ácidos húmicos; FA: ácidos fúlvicos

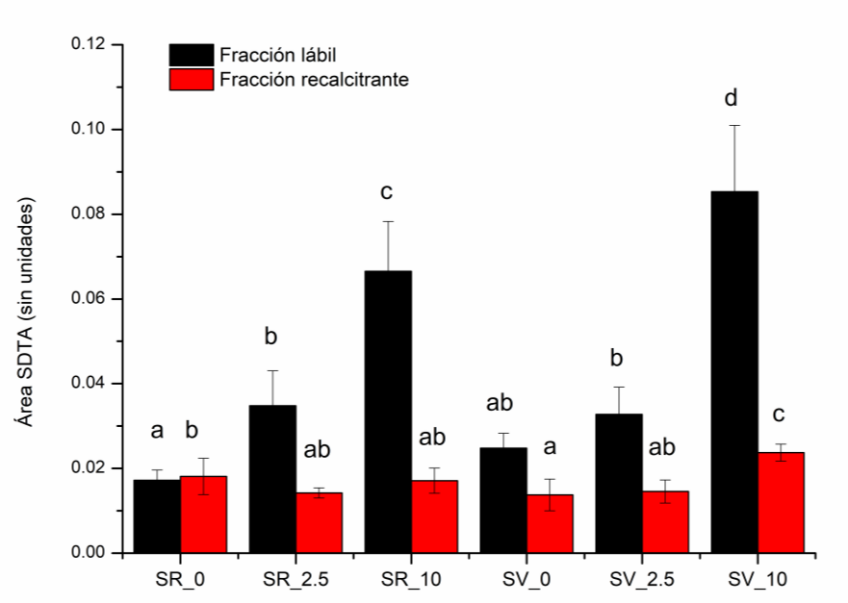

Figura 39. Áreas correspondientes a la fracción lábil y recalcitrante calculadas en SDTA en las diferentes mezclas de suelo y SCG. Las letras indican diferencias significativas en comparación con las muestras del mismo estudio (P < 0.05; test de Kruskal-Wallis).

Por tanto, la adición de SCG al suelo genera un aumento de todas las fracciones de la materia orgánica del suelo, tanto de aquellas más simples y fácilmente descomponibles por los microorganismos, como de aquellas más complejas y recalcitrantes. Esto generaría una buena reserva de materia orgánica. Sin embargo, el aumento de los compuestos más lábiles es mucho mayor, lo que podría afectar a la funcionalidad de la materia orgánica. Para ahondar en esta cuestión, se estudiaron los ácidos húmicos extraídos de las diferentes muestras, fracción que es la que mejor representa el estado de humificación y evolución de la materia orgánica (Buurman et al., 2009). Se midió el espectro UV-Vis, así como el espectro FTIR de los ácidos húmicos de las diferentes muestras. Por un lado, la absorbancia del espectro UV-Vis, que depende del tamaño molecular y el peso de las moléculas orgánicas, no arrojó diferencias significativas con la adición del café, aunque si mostró diferencias entre las diferentes tipologías de suelo, con mayor A4 y A6 en el caso del suelo de vega, mostrando más cantidad de material parcialmente y totalmente humificado, lo que puede deberse a la mayor cantidad de arcillas de estos suelos. Por otro lado, en el caso de los espectros FTIR se calculó el área relativa de las principales bandas. Las principales diferencias se encontraron en las bandas asociadas al estiramiento antisimétrico C-H del metileno y los grupos terminales metil (2920 y 2850 cm^{-1}), asociados a compuestos alifáticos, así como en las bandas de amida I y amida II (1651 y 1543 cm^{-1} respectivamente), asociadas a formas poco evolucionadas de nitrógeno. Todas estas bandas aparecieron con mayor área en el suelo rojo, a la vez que también aumentaba su señal con la adición de SCG. Por otro lado, con la adición de SCG se aprecia un descenso relativo en las bandas asociadas a grupos funcionales de compuestos más recalcitrantes (1412 y 1260 cm^{-1}), indicando una pérdida complejidad en los ácidos húmicos. Estas diferencias se aprecian claramente en el PCA que se realizó sobre todos los datos obtenidos para cada una de las muestras, incluido el análisis de los ácidos húmicos, donde además se representan las cargas de cada una de las variables en los principales componentes (Figura 40). En este podemos ver como las fracciones de la materia orgánica se sitúan cercanas entre sí, con valores altos en el PC2, cerca de las muestras con 10% de SCG, mostrando una fuerte correlación entre la adición de SCG y la cantidad de todas

las fracciones de SOM. Además, se encontraron correlaciones altas en el test de correlación de Pearson (> 0.9) entre la cantidad de SCG añadida y las fracciones de SOM individuales (TEC, HWSC, Huminas, HA y FA) con $p < 0.001$. Las muestras con adición de SCG también aparecen cerca del área lábil y recalcitrante del SDTA, mostrando una correlación positiva con el área total de compuestos lábiles (0.918), y en menor medida con el área recalcitrante (0.5171). Sin embargo, el índice de termoestabilidad calculado dividiendo la cantidad de recalcitrantes entre la cantidad de lábiles más recalcitrantes, parece estar más cerca de las muestras sin enmienda, lo que indica una mayor termoestabilidad en las muestras sin adición de SCG. Las fracciones de SOM también muestran una correlación alta con las áreas de SDTA, lo que parece indicar que el factor principal en el análisis es el aumento de la SOM total y todas sus fracciones con la incorporación de SCG, y que los cambios en la funcionalidad de la materia orgánica no pueden apreciarse claramente mediante una cuantificación de las fracciones. Sin embargo, las técnicas espectroscópicas y especialmente el espectro FTIR parecen ser más sensibles a los cambios de funcionalidad de SOM. Las bandas de 1412 y 1260 cm^{-1} aparecen cerca de las muestras sin adición de SCG, y el % de SCG muestra una correlación inversa con ellas (-0.8401 y -0.6886, respectivamente), que indican una disminución de estos grupos funcionales en HA cuando SCG es incorporado a los suelos. Estas bandas también mostraron una fuerte correlación con el índice de termoestabilidad, lo que indica que estos grupos funcionales son los mejores para reflejar la estabilidad y la complejidad de la materia orgánica. Por otro lado, cerca de las muestras con 10% de SCG, aparece la ratio A4 / A6, que muestra una gran correlación con las bandas de FTIR asociadas a compuestos más lábiles (2920, 2850, 1651, 1543 y 1454 cm^{-1}), mostrando que en estas domina los grupos funcionales y las moléculas menos estables. Además, se pueden apreciar las diferencias entre los suelos, especialmente una mayor concentración de todas las formas de SOM en SV, así como una mayor complejidad macromolecular en sus HA. Los resultados obtenidos demuestran que los espectros de infrarrojo medio de HA contienen información valiosa y que las características de la funcionalidad SOM pueden representarse comprimiendo el espectro infrarrojo en unos pocos vectores que son suficientes para describir sus características.

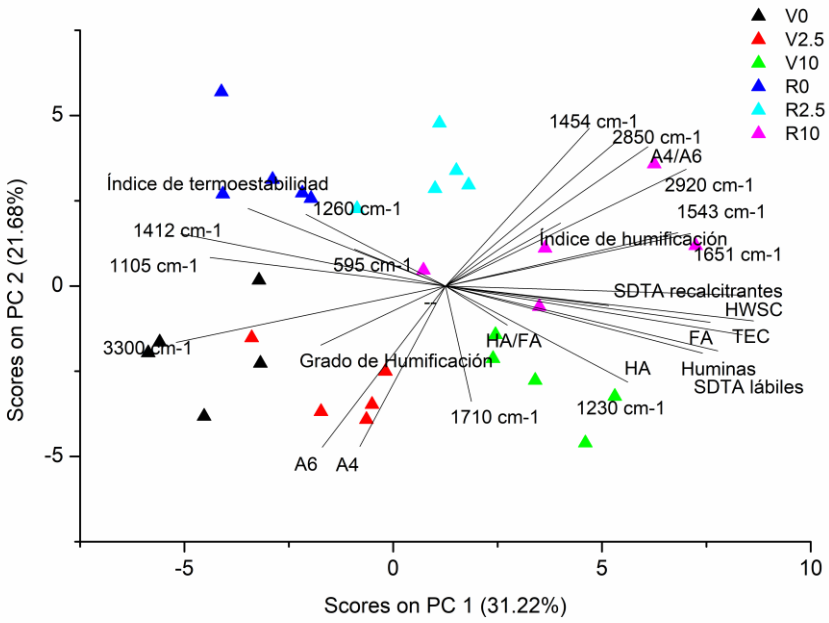

Figura 40. Superposición de los scores de las muestras en el PCA, con las cargas de los diferentes parámetros en los mismos componentes. V0: suelo de vega con 0% SCG; R0: suelo rojo con 0% SCG; V2.5: suelo de vega con 2.5% SCG; R2.5: suelo rojo con 2.5% SCG; V10: suelo de vega con 10% SCG; R10: suelo rojo con 10% SCG.

Por tanto, podemos comprobar como la adición de SCG sobre el suelo tiene un claro efecto sobre el aumento de la materia orgánica, en todas sus fracciones, lo que a corto plazo generaría una clara mejora de la fertilidad de los suelos, especialmente en aquellos más arcillosos como el suelo de vega que es capaz de retener y estabilizar más cantidad de materia orgánica. Esto, además, tendría un efecto de sumidero de carbono en el suelo, y ayudaría a paliar los efectos del cambio climático. Sin embargo, un aporte continuado de esta enmienda afecta de manera negativa a la funcionalidad de la materia orgánica, ya que como se comprueba en el análisis de los ácidos húmicos, la complejidad y estabilidad de la materia orgánica se ve reducida tras la aplicación de esta enmienda.

Los posos de café como enmienda demuestran tener ciertos inconvenientes a la hora de aplicarlos al suelo, al poder afectar a la funcionalidad de la materia orgánica. Además esta enmienda orgánica presenta otras desventajas como su pH ácido, y la presencia de compuestos como los taninos, la cafeína y los polifenoles, que son tóxicos en altas concentraciones para la microbiota y las plantas (Campos-Vega et al., 2015). Una posible solución para esta problemática podría ser la mezcla con otras enmiendas, como el compost de alperujo (COMP). Esta opción podría corregir el pH ácido, dado el elevado pH de los compost de alperujo, corrigiendo a su vez la elevada relación C / N típicos de este tipo de enmienda (Lozano-García et al., 2011).

Tabla 15. Caracterización química de las diferentes enmiendas orgánicas. Media ± desviación estándar.

Muestra	Origen	Composición	pH	CE (dS/m)	Cenizas (%)	OC (%)	N (%)	C/N	P (%)	K (%)	Ca (%)	Mg (%)
COMP1	Hermejor de la Reina	50% Alperujo 25% Restos de poda 25% Gallinaza	8.8 ± 0.2	1.2 ± 0.2	11.8 ± 1	27.9 ± 0.3	1.7 ± 0.1	16.5 ± 0.9	0.5 ± 0.07	1.52 ± 0.15	5.58 ± 0.25	0.71 ± 0.08
COMP2	Hermejor de la Reina	50% Alperujo 25% Restos de poda 25% Gallinaza	8.1 ± 0.2	1.5 ± 0.4	11.8 ± 0.6	31 ± 1.2	1.8 ± 0.1	17.2 ± 0.5	0.57 ± 0.03	1.64 ± 0.11	6.23 ± 0.32	0.67 ± 0.09
COMP3	Cooperativa N.S. Remedios	80% Alperujo 10% Restos de poda 10% Gallinaza	9 ± 0.2	1.1 ± 0.2	16.6 ± 1	22.6 ± 0.5	1.8 ± 0.1	12.9 ± 0.9	0.51 ± 0.09	1.3 ± 0.19	6.45 ± 0.19	0.26 ± 0.05
COMP4	Alcanova S.L.	80% Alperujo 10% Restos de poda 10% Gallinaza	8.9 ± 0.2	0.9 ± 0.1	9.8 ± 0.8	28.8 ± 1	1.7 ± 0.1	16.9 ± 1.3	0.8 ± 0.12	1.95 ± 0.09	8.41 ± 0.19	0.65 ± 0.04
COMP5	IFAPA	30% Alperujo 70% Restos de poda	6.1 ± 0.4	3.5 ± 0.5	7 ± 0.4	51.9 ± 0.5	1.5 ± 0	34.6 ± 1.3	0.94 ± 0.07	1.33 ± 0.21	3.23 ± 0.15	0.89 ± 0.04
OMP	Hermejor de la Reina	100% Alperujo no compostado	5.8 ± 0.3	0.9 ± 0.2	0.8 ± 0.2	54.6 ± 0.8	1.5 ± 0.1	36.4 ± 1.8	0.28 ± 0.07	1.03 ± 0.12	0.66 ± 0.14	0.1 ± 0.02
CF	Cafetería Universidad Jaén	100% posos de café	5 ± 0.2	2.1 ± 0.2	1.6 ± 0.2	52.9 ± 0.5	2.3 ± 0.1	23 ± 1.1	1.06 ± 0.15	0.3 ± 0.1	0.51 ± 0.03	0 ± 0.01
COMP5-CF1	Mezcla de 80% COMP5 y 20% CF		7 ± 0.3	0.8 ± 0.1	2.2 ± 0.2	54.2 ± 0.6	1.7 ± 0.1	32.5 ± 1.2	0.27 ± 0.05	1.15 ± 0.18	2.04 ± 0.13	0.27 ± 0.03
COMP5-CF2	Mezcla de 60% COMP5 y 40% CF		6.9 ± 0.2	0.7 ± 0.1	1.6 ± 0.2	54.2 ± 1	1.7 ± 0.1	32.5 ± 1.2	0.27 ± 0.06	1.05 ± 0.08	1.73 ± 0.15	0.2 ± 0.03
COMP5-CF3	Mezcla de 40% COMP5 y 60% CF		6.7 ± 0.2	0.6 ± 0.1	0.8 ± 0.1	53.8 ± 0.9	1.7 ± 0.1	31.1 ± 1	0.26 ± 0.04	0.73 ± 0.1	1.23 ± 0.11	0.2 ± 0.06
COMP5-CF4	Mezcla de 20% COMP5 y 80% CF		6.3 ± 0.2	0.5 ± 0.1	2.8 ± 0.5	52.6 ± 1.4	1.8 ± 0.1	28.9 ± 0.8	0.27 ± 0.04	0.51 ± 0.1	0.78 ± 0.14	0.22 ± 0.05
SCG	Café Cumbal S.L.	100% posos de café	5.76	4.5	-	59.38	1.867	32	0.23	3.07	-	-

Pero además estas enmiendas presentan otro grave problema, la repelencia al agua, que puede afectar de manera muy negativa al agro-sistema, ya que provoca una reducción de la infiltración, que podría disparar las tasas de erosión. Para corregir este problema se propone la aplicación de elevadas temperaturas, metodología que ha demostrado ser capaz de eliminar la hidrofobicidad en las fases orgánicas de los suelos (García-Corona et al., 2004). Las muestras utilizadas para estudiar el efecto de la mezcla de enmiendas, la importancia del proceso de compostaje en el caso del alperujo, así como los cambios que se pueden producir en estas enmiendas con la aplicación de un tratamiento térmico, se describen en la Tabla 15. Podemos ver que el pH de los compost de alperujo es bastante elevado como era de esperar, y que este aumento se debe al proceso de compostaje, ya que el alperujo sin compostar muestra pH ácido. Este pH es aún mayor en los casos donde se añade gallinaza, debido a la gran cantidad de carbonato cálcico en este estiércol. El compostaje también disminuye la concentración de carbono orgánico, con la consiguiente disminución en la relación C / N, provocando además un aumento en P, K, Ca y Mg. Por otro lado, comparando las muestras CF y COMP5 que se utilizaron para las mezclas, la muestra de COMP5 destaca por su alta conductividad eléctrica, en parte asociada a una mayor concentración de algunos cationes, y su bajo pH (6.1). Este valor inusualmente bajo de pH podría indicar un proceso de compostaje inadecuado, al tener valores más cercanos a un alperujo sin compostar que al resto de COMP. Las mezclas de COMP5 y CF, muestran valores asociados con % de cada muestra que incluyen, quedando como una muy buena opción como enmienda, ya que presentan una cantidad bastante equilibrada de los nutrientes principales y un pH cercano a la neutralidad. Pero además vemos como tras realizar las mezclas se aprecia una reducción de la CE, lo que parece ser una mejora y nos lleva a pensar que las mezclas tienen mejores propiedades que las enmiendas por separado.

Para una mayor caracterización de estas muestras, y especialmente de su fase orgánica, se aplicaron una serie de análisis que incluyen espectroscopía FTIR y UV-Vis, así como un análisis termogravimétrico, de fitotoxicidad y de repelencia al agua, cuyos resultados se muestran en la Tabla 16. Observamos que todas las enmiendas presentan una elevada repelencia al agua (WDPT), que se ve reducida con el compostaje, pero que aun así continúa siendo muy elevada. Esta repelencia está claramente correlacionada con el índice de hidrofobicidad, calculado utilizando el área de las bandas a 2920 y 2850 cm^{-1} del espectro FTIR (Matějková y Šimon, 2012), asociadas al grupo funcional C-H de ácidos alifáticos con carácter hidrófobo. Además, se observa como con el compostaje aumenta la ratio 1606/1740 cm^{-1}, que indica la presencia de compuestos aromáticos más estables. Lo mismo ocurre con el índice de termoestabilidad y el índice de germinación, lo que indica que el proceso de compostaje mejora la estabilidad de la materia orgánica y reduce su toxicidad, lo que lo convierte en un proceso totalmente indispensable para el alperujo. Los posos de café presentan bajos A4 y A6, así como bajo índice de termoestabilidad y ratio 1606/1740 cm^{-1}, lo que vuelve a poner de manifiesto el carácter poco complejo de la materia orgánica de esta enmienda. Sin embargo, observamos como la mezcla de CF y COMP genera enmiendas con valores intermedios en la mayoría de parámetros, aunque destaca la mejora en el índice de germinación donde los valores son superiores a las enmiendas por separado.

Podemos argumentar por tanto que el compostaje del alperujo es un proceso totalmente necesario para mejorar la calidad esta enmienda, aunque aún tras este, sigue presentando problemas como la repelencia al agua. Asimismo, la mezcla de COMP y CF, genera una enmienda con parámetros intermedios, con una clara mejora en algunos de ellos respecto a las enmiendas individuales, pero mantiene la problemática de la repelencia al agua, así como una materia orgánica en general no muy evolucionada. Como posible solución a estos problemas se sometió a todas las muestras a temperaturas de 175, 225 y 275ºC (teniendo en cuenta que las muestras iniciales habían sido sometidas a 65ºC para eliminar la humedad). Para estas nuevas muestras se repitieron los análisis previamente descritos. El análisis de los espectros FTIR a las diferentes temperaturas (Figura 41), muestra una clara reducción de los grupos alifáticos responsables de la hidrofobicidad (2920 y 2850 cm^{-1}), especialmente a partir de 225ºC, así como una clara variación en la forma del espectro a partir de estas temperaturas, lo que estaría indicando una modificación de la composición de las enmiendas, principalmente de su fase orgánica.

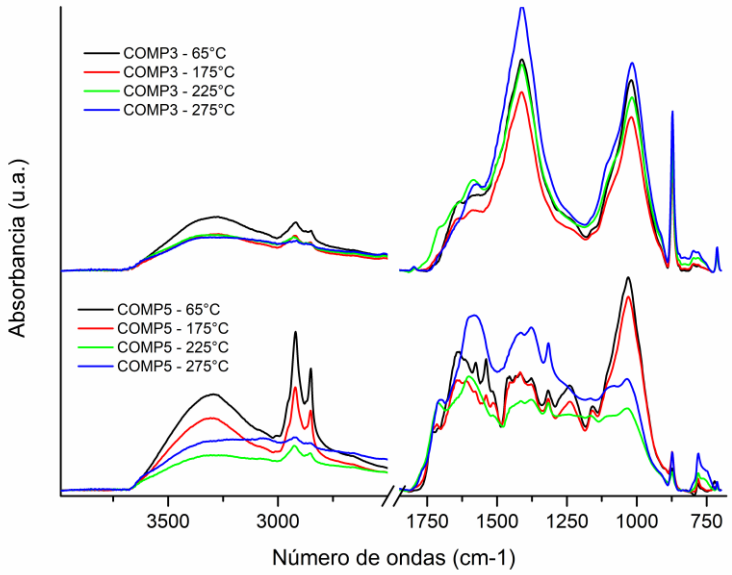

Figura 41. Espectros FTIR de las enmiendas orgánicas a las diferentes temperaturas.

Tabla 16. Caracterización de la fase orgánica de las enmiendas mediante espectroscopía UV-Vis, espectroscopía IR, análisis termogravimétrico, medida de la fitotoxicidad y test del tiempo de penetración de la gota de agua (WDPT).

Muestra	A4 (472nm)	A6 (680nm)	A4/A6 Ratio	Índice de Hidrofobicidad	1606/1740 cm^{-1} Ratio	Índice de Termoestabilidad	Índice de Germinación (%)	WDPT (s)
COMP1	0.38 ± 0.03	0.11 ± 0.01	3.53 ± 0.12	0.068 ± 0.005	1.22 ± 0.06	0.754 ± 0.017	165.6 ± 6.3	345 ± 14
COMP2	0.39 ± 0.06	0.11 ± 0.01	3.4 ± 0.55	0.068 ± 0.004	1.24 ± 0.03	0.755 ± 0.011	151.5 ± 4.5	550 ± 13
COMP3	0.45 ± 0.05	0.12 ± 0.01	3.7 ± 0.36	0.172 ± 0.013	1.21 ± 0.03	0.809 ± 0.011	168.9 ± 4.4	65 ± 6
COMP4	0.34 ± 0.04	0.11 ± 0.01	3.14 ± 0.18	0.179 ± 0.008	1.17 ± 0.02	0.754 ± 0.012	110.4 ± 3.3	150 ± 16
COMP5	0.31 ± 0.04	0.12 ± 0.01	2.49 ± 0.16	0.424 ± 0.017	1.12 ± 0.02	0.687 ± 0.019	93 ± 4.5	930 ± 29
OMP	0.2 ± 0.02	0.08 ± 0.01	2.37 ± 0.15	0.576 ± 0.023	0.91 ± 0.07	0.58 ± 0.012	43.7 ± 2.2	2580 ± 52
CF	0.22 ± 0.04	0.09 ± 0	2.62 ± 0.4	0.53 ± 0.012	0.95 ± 0.04	0.358 ± 0.015	77.4 ± 2.6	3180 ± 62
COMP5-CF1	0.28 ± 0.03	0.11 ± 0.01	2.47 ± 0.21	0.48 ± 0.016	1.07 ± 0.03	0.628 ± 0.018	113.7 ± 4.4	2010 ± 33
COMP5-CF2	0.26 ± 0.02	0.1 ± 0.01	2.59 ± 0.1	0.552 ± 0.018	1 ± 0.03	0.635 ± 0.015	111.1 ± 3.5	2280 ± 48
COMP5-CF3	0.27 ± 0.03	0.1 ± 0.01	2.66 ± 0.07	0.579 ± 0.013	0.99 ± 0.04	0.607 ± 0.014	116.7 ± 5.7	2580 ± 55
COMP5-CF4	0.23 ± 0.02	0.09 ± 0.01	2.63 ± 0.13	0.583 ± 0.017	0.95 ± 0.04	0.523 ± 0.011	118.5 ± 4	3120 ± 53

Los valores medios de cada uno de los parámetros medidos para las diferentes enmiendas estudiadas se muestra en la Figura 42. Podemos ver como la repelencia al agua se mantiene, o incluso aumenta con los tratamientos a 175 y 225ºC, mientras el índice de hidrofobicidad disminuye. Esto podría estar ocurriendo porque a pesar de reducir la cantidad de compuestos alifáticos hidrófobos, los que se mantienen en la muestra se evaporaron parcialmente, depositándose en la superficie, lo que provoca que una mayor cantidad de estos compuestos estén situados en la superficie de la muestra, aumentando por tanto el efecto de repelencia al agua. Por otro lado, vemos como a 275ºC la repelencia al agua desaparece, lo que indica que esta temperatura es idónea para eliminar este problema. Además, observamos como con el tratamiento térmico aumenta la termoestabilidad y la cantidad de compuestos aromáticos más estables en las enmiendas orgánicas (ratio 1606/1740 cm^{-1}), lo que indica que el tratamiento térmico además provoca una disminución de la materia orgánica más lábil, así como una generación y/o acumulación de la materia orgánica más recalcitrante, dando lugar a enmiendas orgánicas que aportarían al suelo una materia orgánica con mejor calidad y funcionalidad. Este efecto es mayor conforme aumenta la temperatura, por lo que parece que el tratamiento a 275ºC se demuestra como el mejor de las temperaturas ensayadas para eliminar la repelencia al agua, a la vez que mejora la calidad de las enmiendas orgánicas.

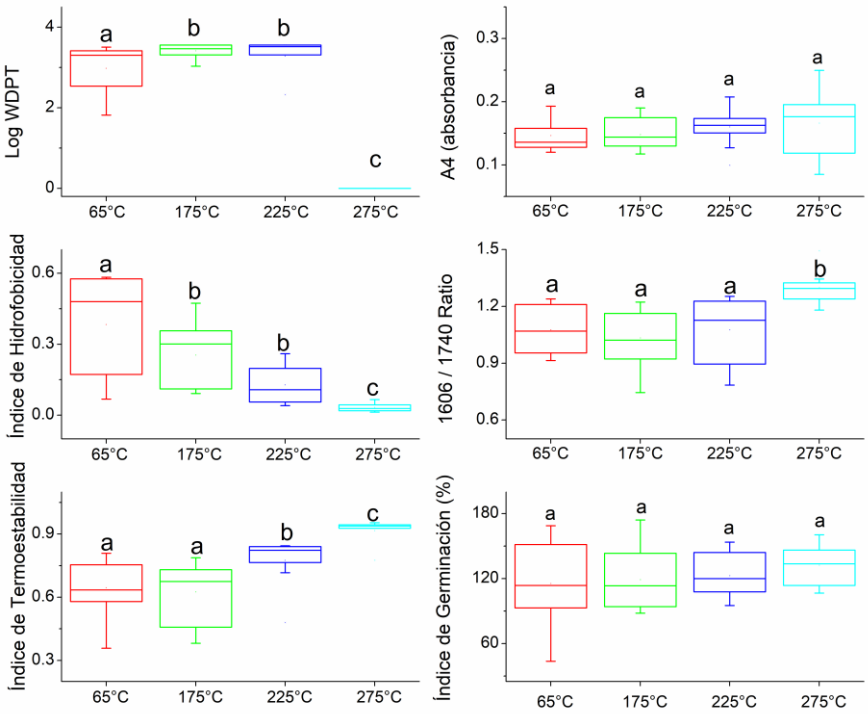

Figura 42. Gráfico de cajas y bigotes de la media de las diferentes variables medidas, para las muestras de enmiendas orgánicas a las diferentes temperaturas.

Esta mejora tambien se refleja en las mezclas de COMP y CF, tal y como puede verse en la Figura 43, donde además podemos observar como la calidad de las enmiendas que son mezcla, especialmente la mezcla COMP5-CF3, obtienen mayores valores en parámetros como el índice de germinación respecto a las enmiendas de partida, obteniendo además valores muy similares para la mayoría de parámetros respecto a la mejor enmienda, en este caso el COMP5.

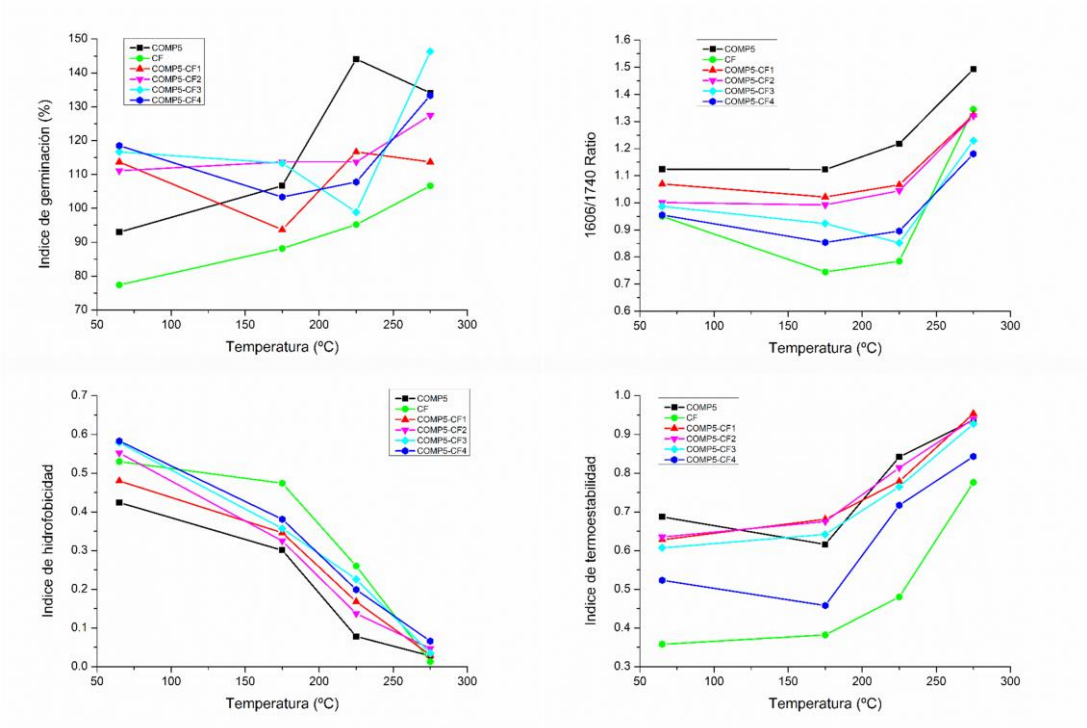

Figura 43. Valores para los diferentes parámetros medidos en las muestras de enmiendas orgánicas COMP5, CF y las mezclas entre ellas.

En resumen, podemos decir que el tratamiento térmico, especialmente a 275ºC, es eficaz para mejorar las enmiendas orgánicas, eliminando aspectos negativos como la toxicidad y la repelencia al agua, a la vez que se genera una enmienda con una fracción orgánica de mayor calidad, con mayor proporción de material orgánico más estable. Por otro lado, la mezcla de enmiendas de posos de café y compost de alperujo se demuestra como una alternativa viable, dado que su mezcla consigue corregir algunos parámetros no óptimos como el pH, dando lugar a enmiendas de una calidad mejorada con parámetros intermedios entre ambas, enmienda que con tratamiento térmico se convierte en una alternativa de igual o mayor calidad en todos los aspectos que el compost de alperujo y los posos de café por separado.

A continuación, se presentan los trabajos de investigación desarrollados en la presente tesis doctoral, cada uno de ellos en un capítulo individual, desde el capítulo 6 hasta el capítulo 12, siguiendo el orden establecido en la Figura 22.

Capítulo 5
Referencias bibliográficas

En este capítulo se recoge la bibliografía utilizada en los capítulos de introducción, resumen de materiales y métodos, así como discusión conjunta de resultados. La bibliografía específica de cada uno de los capítulos asociados a trabajos de investigación (capítulos 6-12) se encuentra al final de los mismos.

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Capítulo 6
Agro-environmental characterization of semi-arid Mediterranean soils using NIR reflection and mid-IR-attenuated total reflection spectroscopies

Este capítulo se corresponde con el artículo "Agro-environmental characterization of semi-arid Mediterranean soils using NIR reflection and mid-IR-attenuated total reflection spectroscopies", publicado en la revista Vibrational Spectroscopy. En este artículo se hace un estudio sobre la aplicabilidad de la espectroscopía infrarroja FTIR y FT-NIR para caracterizar suelos mediterráneos.

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* Con las muestras incluidas en este estudio se desarrollaron modelos predictivos de varibles, mediante la aplicación de diferentes herramientas quimiómetricas y de fusión de datos durante la estancia de investigación en CSIRO Land and Water. El trabajo sobre los resultados obtenidos se encuentra aún en redacción. Un resumen de parte de esos resultados se expuso en el capítulo 4.

Abstract

The use of infrared spectroscopy to perform an agro-environmental soil characterization in areas with high degradation risks, like the Mediterranean semi-arid environments, has been evaluated using diffuse reflection near infrared spectroscopy (NIR) and attenuated total reflection middle infrared spectroscopy (ATR-FTIR). Soils from calcareous substratum, beneath three management types (organic, conventional and natural vegetation) have been studied. Factors like the parent material, the depth of sampling and the slope position have been considered. Although the parent material seems to be the factor most influencing the infrared spectral pattern, especially in the mid-IR region, soils under different management practices can also be differentiated on the basis of their spectral characteristics. It is interesting to note that the differences between the soils under conventional practices and after more than twenty years of organic management are much more evident for soils developed on marls than for those developed on colluvial limestones. This can be attributed to less degradation of the latter type of soils even under conventional management due to their more evolved nature and resilience. Considering the results, a combination of NIR and mid-IR spectroscopy could improve soil classification performance, being especially useful for the fast identification of those soils suffering severe degradation.

1. Introduction

The type of management exerts a decisive influence on the characteristics of Mediterranean soils, especially in the case of longer cultivation or deforesting. The generalized use of conventional soil management for decades, with an intense use of the moldboard plow, mineral fertilization and herbicides, decreases soil quality with important economic and environmental consequences difficult to evaluate. On the other hand, the purpose of organic management (and other environmentally sustainable agriculture practices) is to grow the highest-quality food, while conserving and improving the fertility of the soil (1) and quality (2), and it could contribute to soil conservation by increasing soil protection and lowering erosion risk (3). These improvements are crucial for sustaining and enhancing crop productivity in a context where climatic conditions become more extreme (4). In this sense, the characterization of the environmental status (discriminating between soil types, management, and by extension, land degradation) is an essential step for assessing the fragility of the Mediterranean agro-agro-systems that are prone to erosion (5). This makes necessary to evaluate the soil degradation processes related mainly to the common use of unsuitable farming techniques, as well as the effectiveness of more sustainable practices.

The use of rapid techniques that provide complete information in a fast way would be very interesting in these areas to select the better management according to the type of soils and other agroenvironmental conditions (e.g., semi-arid Mediterranean soils are very conditioned by the geological material). In addition, this information could help in the future to improve the objectives of proper management planning and sustainable land use. Consequently, this type of studies, whose main goal is a rapid soil characterization based on its major properties, could play an important role in a more sustainable and precision agriculture. Spectroscopic techniques, as middle infrared with attenuated total reflection (ATR-FTIR) and near infrared (NIR) diffuse reflection, can constitute a valuable solution when they are used in combination with chemometrics tools. During the last years a number of articles devoted to characterization of soils by using NIR have demonstrated its applicability mainly focused on the determination of organic matter and other parameters as pH and N, P and K contents (6–10), very important in the soil chemical fertility and maintenance of the crops. Although less employed, near infrared spectroscopy it is also useful to classify soil typologies (11,12), soil horizons (13) and to assess the effects of land use and land management on soil condition (14).

On the other hand, mid-IR spectroscopy, has been applied to soil science much more recently as a predictive tool, although its use is being increased due to the specificity of the absorbance bands in comparison to those in near infrared (15–19). Also, mid-IR attenuated total reflection (ATR) spectroscopy could be used to identify the main types of agricultural soils based on essential absorbance bands associated with soil constituents, such as carbonates, clay minerals and organic compounds (20).

However, the potential of IR reflection spectroscopy has to be further investigated (21,22). The fundamental vibrations of most soil materials can be detected in the middle IR region while overtones and combinations can be found in the NIR region. Hence, the combination of both techniques could provide valuable additional information in the agricultural and environmental sciences.

Despite the wide and increasing application of IR spectroscopy to agricultural soils characterization, very limited information is available from semi-arid regions (23), from Mediterranean climate (7,17), or specifically under semi-arid Mediterranean climate conditions (24). It is noteworthy that, for these same climatic conditions, the interaction between management practices and soil type (or specifically the parent material soil factor forming) is also poorly studied (16).

The purpose of this study is the agro-environmental characterization of soils from olive groves under contrasting management conditions (organic, conventional and natural vegetation). Soils from colluvial limestones and marls located in different slope positions and two sampling depths have been considered. The results will inform whether the methodology based on IR spectroscopy is sensitive enough to discriminate between soil types, management type and, by extension, soil degradation. This feasibility study (with a relatively small number of samples), was carried out in a zone of special ecological interest (the Sierra Mágina Natural Park, Southern Spain) located in a semi-arid Mediterranean area and, therefore, especially sensitive to desertification.

2. Materials and methods

2.1. Study site and soil sampling

The study area was located in the Atanor valley, Sierra Mágina Natural Park (Jaén, Southern Spain, Figure 1). The area is geologically placed on the Subbetic Domain (Betic Mountain Range), with a Jurassic-age and two contrasting lithologies: limestones and marls (25). With a semi-arid Mediterranean climate, it presents a mean annual temperature of 17.3 ◦C and annual rainfall around 450 mm.

Following the criteria of dominant geological substrates (colluvial limestones and marls) and the maximum possible geographic proximity, four olive grove plots were selected. Two of them correspond to organic farms that were under this practice for more than 22 years (the oldest organic olive grove farm in the south of Spain; certified by CAAE: certification institution for organic production for the implementation of the EU regulations 2092/91 and 1806/99 in the Andalusian region), with no mineral fertilization or pesticides, no-tilled soils, in which the vegetative cover is kept under control by mowing from early to late spring, and animal manure is incorporated about every 4 years. Furthermore, conventional management is considered with two grove plots and they are characterized by three-five chisel passes a year to a depth of 15–20 cm from early spring to early autumn, and weed control with residual herbicides. These olive grove plots are not experimental but lay out on currently productive private farms. In addition, another plot under natural vegetation (mainly Quercus sp.) over slope deposits from limestones, was also included in the study.

To consider the slope effect in the soils sampling, each agronomic plot was divided in three parts according with its position: upper-slope, middle-slope and lower-slope. Furthermore, two soil layers were considered for this study, a surface layer consisted in 0–5 cm depth and a wider one of 0–30 cm depth corresponding to the main roots zone of the olive tree. Under natural vegetation only 0–5 cm depth was sampled, because Leptosols in this mountainous area very shallow soils, highly gravelly and stony.

Three composite samples were obtained in each case by the mixture of five subsamples of randomly taken within a 5 m radius. In this way 80 soil samples were obtained that represent the high variability of the studied area.

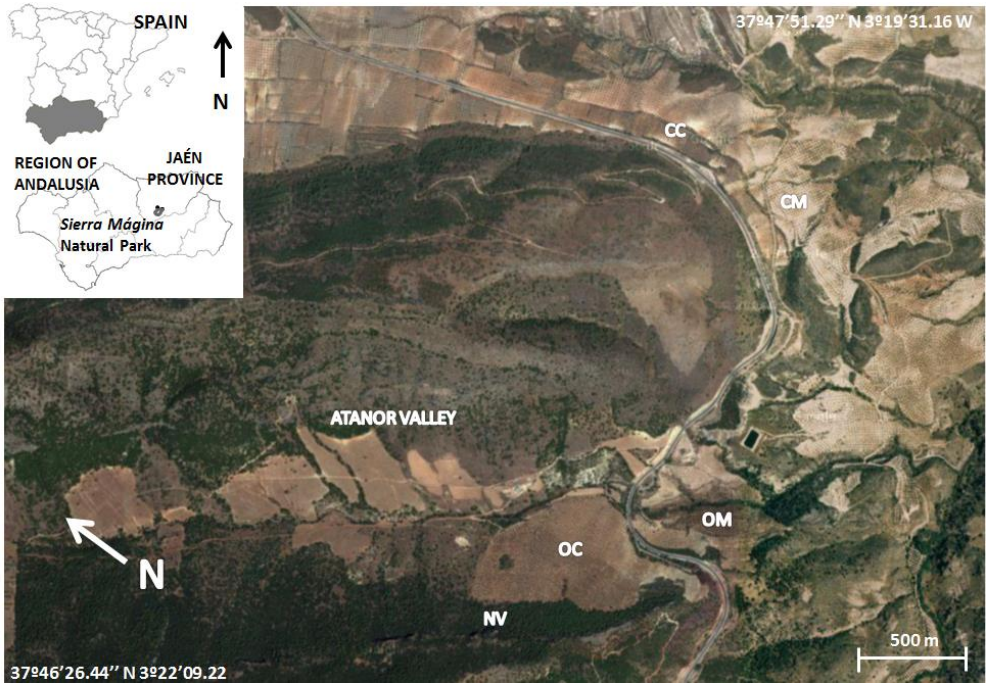

Figure 1. Location of the study area (Atanor Valley, Sierra Mágina Natural Park). NV (natural vegetation), OC (organic olive groves over colluvia), OM (organic olive groves over marls), CC (conventional olive groves over colluvia), CM (conventional olive groves over marls).

2.2. Analytical procedures

Soil samples were air-dried at room temperature and passed through a 2-mm sieve to obtain the fine-earth fraction (<2 mm). Typical analytical parameters of soils were studied under the procedures outlined by American Society of Agronomy and Soil Science Society of America (26,27). Briefly, pH (1:2.5 soil suspension in distilled water) was measured by potentiometry; organic carbon content (OC), total nitrogen (N) and extractable phosphorus (P) with Walkley–Black, Kjeldhal and Olsen methods, respectively; and available potassium (K) was determined by flame photometry.

With fine-earth soil samples, milling to powder, diffuse reflection NIR spectra between 4000 and 12 000 cm−1 were registered using an Antaris FT-NIR analyzer (Thermo Nicolet Corp.) with gold integrating sphere with internal reference, 8 cm−1 resolution, and 64 accumulations. The sample cup was placed on top of the integrating sphere optics and rotated during measurement.

Mid-IR spectra were registered between 650 and 4000 cm−1 with a Varian 660 FTIR spectrometer with an attenuated total reflection (ATR, Pike Technologies) accessory with a threereflection diamond crystal and a torque-limited pressure applicator to keep the sample in contact with the crystal. Samples were finely ground in an agate mortar and placed directly on the ATR crystal and then pressure was applied to ensure good contact between the sample and the ATR element. Resolution was set to 2 cm−1 and 128 accumulations were recorded for each spectrum. The spectra of air on the clean dry ATR crystal was used as background.

2.3. Data analysis

All statistical treatments were performed by using MATLAB 2008R (MathWorks, Natik, USA) employing the PLS-toolbox from Eigenvector Research Inc. (Wenatchee, USA).

Principal component analysis (PCA) is an unsupervised technique that has been used to investigate the grouping between the samples. PCA was performed on the covariance matrix of the data for mid-IR and NIR spectra independently. Following the Kaiser criterion (28), only those PCs with eigenvalues greater than unity were retained.

Partial least squares discriminant analysis (PLS-DA) consists of a classical PLS regression in which the response variable is a categorical variable (replaced by the set of dummy variables describing the categories) expressing the class membership of the statistical units. PLS-DA increases the separation between groups by rotating PCA components such that a maximum separation among classes is obtained, and to understand which variables carry the class-separating information. Therefore, PLS-DA is a supervised classification technique that requires a training set of spectra with known class membership and a test set. Leave-one-out cross validation was used to assess the optimal complexity number of latent variables.

Different approaches based on cross-validation were used to estimate the expected performance of the models when applied to unknown data. All of them involved a series of steps called sub-validation steps in which a subset of objects from the dataset are removed, the model is built using the remaining objects (the training set), and then the resulting model is applied to the removed objects (test set). This way, each sub-validation experiment involves testing a model with objects that were not used to build the model. The four different cross-validation methods employed vary with respect to how the different sample subsets are selected for these sub-validation steps. For Leave-one-out each single object in the data set was used as a test set. For Contiguous Blocks each test set was determined by selecting contiguous blocks of three objects in the data set, starting at object number 1. For Venetian Blinds each test set was determined by selecting every 6th object in the data set, starting at object numbered 1 through 6. For Random Subsets 6 different test sets were determined through random selection of 21 objects in the data set, such that no single object was in more than one test set. This procedure was repeated 5 times. Prior to applying PCA and PLS-DA, spectra were baseline corrected, second derived and mean-centered.

3. Results and discussion

3.1. Physicochemical characterization of soils: main fertility parameters

Table 1 shows the results of the physicochemical analysis of the soils. It must be noted that averaged soil properties studied for sampled surface layers shows agreement with that of the soil profiles studied previously (29).

Coarse fragments content was higher in samples from colluvial limestones (M1 and SM), and showed a marked tendency to accumulate in surface (0–5 cm depth), responding to the dynamic nature of the soil parent materials. The mean values for all macronutrients, except for pH, were higher in samples from 0 to 5 cm depth. The values for OC and N, as well as the C/N ratio, were markedly higher in the organic management and samples from natural vegetation, and significantly lower in the conventional management on marls. As expected, an opposite tendency was observed for pH, because of its negative relationship with soil OC.

Scattered values were observed in the extractable P variable, without a clear relationship with the geological parent material or the management practices, although the highest value was reached in the samples with organic management on colluvial material. Finally, the available K was found closely related positively to the management, being high at natural and organic management, and with the parent material, coinciding with previous studies (30) where colluvial soils of the study area (Sierra Mágina N.P.) were found to be rich in mica, a mineral that has been related to higher K levels in soils (31).

3.2. Infrared spectra of the soil samples

Typical spectra of each type of soil management and parent material in the near and middle infrared regions are depicted in Figure 2. All soil samples show very similar infrared spectra with slight differences in the intensity and position of certain bands.

The mid-IR spectrum shows more distinctive spectral features than the NIR spectrum and they are easier to interpret in terms of chemical composition. For the four types of cultivated soils the most intense bands in the mid-IR spectra correspond to clays (32) (around 1000 cm−1) and calcite (a broad band centered at 1415–1420 cm−1) and two narrow bands at 872 and 711 cm−1. It is interesting to note that in the natural soil the bands of calcite are nearly absent. This fact can be attributed to dissolution/leaching of calcium carbonate. These geochemical processes in the soil have been probably favored by the steep slope of the area where the natural soil plot is located, which allowed weathering products to be washed away by rains. The greater amount of rain, typical of higher altitudes, probably intensifies this process.

Soil organic matter also has characteristic absorption bands in the mid-IR range, however, due to their overlapping with the bands of mineral components and its low concentration in the soil samples (total organic carbon ≤ 74.9 g kg−1), they could not readily be identified by simple visual evaluation in spectra of bulk soil samples. Only extremely weak bands around 2942–2916 and 2850 cm−1 can be observed, which indicate the presence of aliphatic C–H stretchings. These bands are more evident in the spectra of soil samples with higher concentrations of total organic carbon, as is the case of the organic management.

Table 1. Range and mean values (SD) of main soil parameters to characterize the study area.

	OC		**OM**		**CC**		**CM**		**NV**			
Soil depth	0-5 cm (n=9)	0-30 cm (n=9)	0-5 cm (n=9)	0-30 cm (n=10)	0-5 cm (n=9)	0-30 cm (n=9)	0-5 cm (n=12)	0-30 cm (n=9)	0-5 cm (n=4)	Range Mean (SD)	Skew	Kurtosis
CF (%)	26.2-47.5 37 (7.78)	19.8-42.3 33 (7.11)	14.6-44 25 (8.35)	7.1-32.6 23 (8.57)	34.6-53.4 44 (6.77)	23.7-48.3 41 (4.84)	14.5-39 25 (7.95)	7.3-40.9 23 (11.43)	31.1-64.4 49 (13.77)	7.1-64.4 32 (11.73)	0.38	-0.61
pH (1:2.5)	7.7-7.9 7.8 (0.06)	8.0-8.2 8.1 (0.07)	7.7-8.0 7.9 (0.12)	8.0-8.2 8.1 (0.07)	7.8-8.1 8 (0.1)	8.1-8.3 8.2 (0.04)	8.0-8.2 8.1 (0.08)	8.2-8.6 8.4 (0.1)	7.7-7.8 7.7 (0.05)	7.7-8.6 8.1 (0.19)	-0.54	-0.51
TOC (g kg^{-1})	22-53.4 41.3 (8.5)	18.2-31.4 25.3 (5)	11.4-36.3 24.3 (8)	5.2-21.9 14.4 (5.1)	10.5-25.7 16.9 (5)	8-17.2 13.1 (2.5)	4-11.9 8.1 (2)	3.5-11.5 8 (2.5)	61.2-74.9 67.4 (6.1)	3.5-74.9 20.9 (15.61)	5.83	4.33
N (g kg^{-1})	1.8-4.7 2.8 (1)	1.1-2.2 1.7 (0.43)	0.8-2.8 1.7 (0.71)	0.6-1.9 1.2 (0.39)	0.8-2.1 1.3 (0.38)	0.9-1.4 1.1 (0.16)	0.4-1 0.7 (0.19)	0.4-0.9 0.7 (0.15)	3-4.6 3.7 (0.8)	0.4-4.7 1.5 (0.94)	6.01	5.05
C/N	11-26 15 (5.23)	13-17 15 (1.31)	13-22 14 (3.28)	9-15 12 (1.62)	12-16 13 (1.55)	9-13 12 (1.47)	8-19 13 (2.9)	7-13 11 (1.84)	15-22 18 (3.41)	7-26 13 (3.38)	4.60	4.37
P (mg kg^{-1})	2-65 25 (20)	4-43 10 (12)	2-22 9 (6)	2-23 8 (6)	6-43 16 (11)	6-22 10 (5)	3-23 13 (7)	3-22 11 (7)	4-7 5 (2)	2-65 12 (10.97)	8.52	5.70
K (mg kg^{-1})	340-995 579 (198)	140-408 287 (89)	84-323 201 (88)	36-209 111 (52)	148-640 327 (179)	124-419 217 (91)	97-173 127 (25)	83-159 109 (23)	526-824 661 (133)	36-995 259 (199.74)	5.70	3.99

OC: olive cultivation (22 years ago organic management) with herbaceous plants. Well drained. Slope deposits from limestones. OM: olive cultivation (22 years ago organic management) with herbaceous plants. Moderately well drained. Marls and some slope deposits from limestones. CC: olive cultivation (conventional tillage). Well drained. Slope deposits from limestones. CM: olive cultivation (conventional tillage). Moderately well drained. Marls and some slope deposits from limestones. NV: natural vegetation (Quercus sp.). Well drained. Slope deposits from limestones. Range: min–max. SD: standard deviation; CF: coarse fragments; TOC: total organic carbon; N: total nitrogen; P: extractable phosphorus; K: available potassium.

Interpretation of NIR spectra is more complicated because the bands are due to combinations and overtones of fundamental vibrations of organic and inorganic compounds and the spectral response is usually more affected by other factors like particle size. The absorption near 7070 cm−1 (1400 nm) is related to the first overtone of the O–H stretch vibration in metal–O–H (33). These will shift slightly depending on the molecule or mineral to which the O–H is attached. The feature near 5220 cm−1 (1900 nm) is related to combination vibrations of H–O–H bend and O–H stretch of water (34). The combination metal–O–H bend and O–H stretch vibrations which appear near 4530 cm−1 (2200 nm) is diagnostic of clay minerals, like smectite and illite (35). Interestingly, the contribution of calcite to the soil spectrum in the NIR region (a weak band that can be seen at 4265 cm−1) is much weaker than in the mid-IR. Finally, the absorptions due to organic matter in the NIR region, as also occurred in the case of mid-IR, are weak and difficult to identify by visual inspection of NIR spectra.

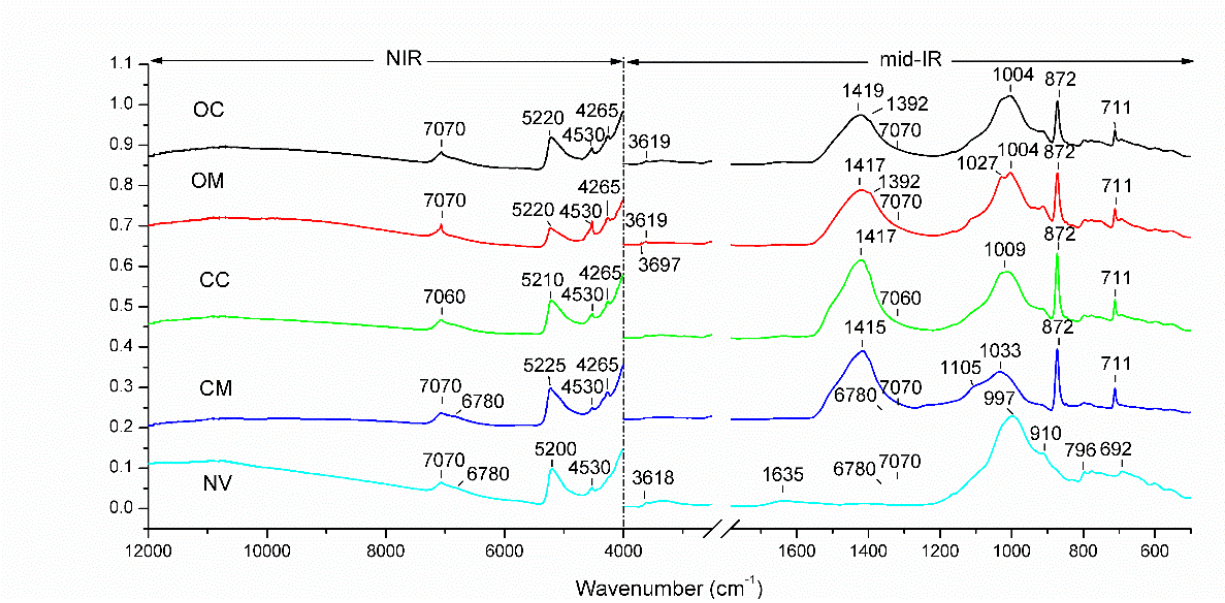

Figure 2. Typical IR spectra of soils studied in the mid-IR and NIR regions. OC (colluvial soils under organic management), OM (marly soils under organic management), CC (colluvial soils under conventional management), CM (marly soils under conventional management), and NV (soils with natural vegetation). Spectral traces have been offset for clarity.

3.3. Exploratory analysis of infrared spectra of soils

A preliminary exploration of the data using principal component analysis (PCA) was carried out in order to evaluate the influence of the parent material and the type of management on the natural groupings of the samples according to their spectral characteristics in the mid-IR and NIR regions. This preliminary exploration was carried out separately for the samples taken from the surface layer (0–5 cm depth) and the wider one (0–30 cm).

In the case of the surface layer, the samples of the natural soil were included in the study in order to investigate the possible spectral similarities and differences with respect to cultivated soils; thereby, assess the degree of recovery of olive-grove soils under organic management.

Three PCs captured 95% of the variance in the mid-IR region for samples of the surface layer, whereas in the NIR region ten PCs were necessary to account for only the 70% of the spectral variance. These results probably reflect a major complexity of the variance sources in the NIR spectral region.

In Figure 3 the score values of the samples in the space defined by PC1 and PC3 for the mid-IR region are shown. With the exception of the natural soil samples that formed a tight cluster, no clear groupings can be observed regarding either the type of soil or the management condition. However, certain trends can be found, especially regarding the type of soil. Thus, soils developed over marls are located farther than colluvial limestone soils with respect to the natural soil. The first principal component for mid-IR was mainly related to the CaCO3 content, as can be seen in Figure 4, where the eigenvector plot of this component is compared with the second derivative of the spectrum of CaCO3. Thus, samples with low content of CaCO3 (like the natural soil) have high positive values for the scores of this component.

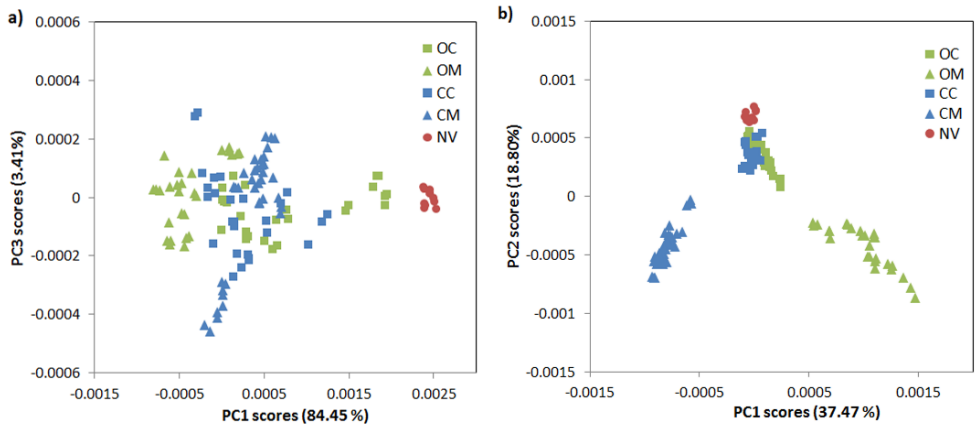

Figure 3. PCA scatter plot of soil samples (sampling depth 0–5 cm) for (a) PC1 and PC3 for mid-IR region and (b) PC1 and PC2 for NIR region.

Considering NIR spectra the groupings are much more pronounced, as can be seen in Figure 3 PC2 clearly separate the soils according to the type of substrate. Soils developed on colluvial limestones are more related to the natural soil (that has the same parent material) and appear separated from marly soils. On the other hand, PC1 establishes a substantial difference between marly soils under organic management (with high positive score values) and the same type of soil under conventional management (with negative score values). However, the soils developed on colluvial limestones do not show a clear separation regarding type of management.

When dealing with the samples taken from 0–30 cm depth, similar results were obtained with three PCs explaining 94.15% of the spectral variance in the mid-IR region and ten to reach 70.67% in the NIR region. Again, clearer groupings were observed considering the NIR region than for the mid-IR region. In both regions, differences due to soil management were less evident for these samples. These results are reasonable considering that the main difference between the organic and the conventional management is the tillage, which affects predominantly the first centimeters of soil.

No clear tendencies regarding the slope position of the sampling locations could be identified in this exploratory analysis.

The obtained results are in agreement with the edaphic characteristics of the two types of soil. In the Mediterranean region, marly soils mainly Regosols and Calcisols (36), severely degraded by erosion after more than one century of intensive agricultural use (37), show poorer profile evolution than colluvial soils (e.g., Luvisols (38)). In this context, the soils developed on marly geological parent material (Regosols) in the study area are poorly developed soils, barely evolved. Decades of conventional olive-grove agricultural practices have led to soil loss by erosion producing a severe degradation and a drastic decrease in fertility. Here, it is demonstrated that the introduction of organic management during 22 years has improved the condition of the soil and this is reflected in the significant spectral differences between these soils under the two types of agricultural practices.

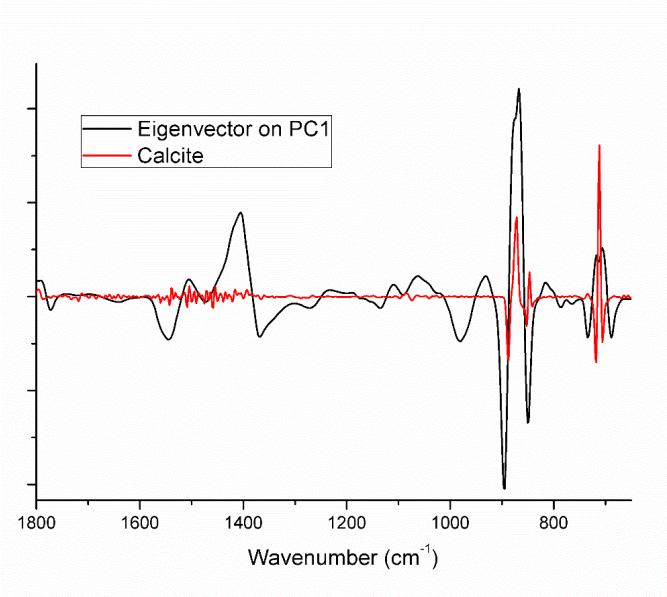

Figure 4. Comparison of the eigenvectors for PC1 for the mid-IR region (sampling depth 0–5 cm) and the second derivative ATR-FTIR spectrum of calcite standard.

On the other hand, the soils developed on colluvial limestones (Luvisols) have a higher degree of evolution and resilience. This evolution was mainly related to higher phyllosilicates content, higher cation exchange capacity, higher humification degree, as well as richer porosity and structural development (29). These are very fertile soils, which are usually present in the Mediterranean area, and are traditionally very suitable for a wide range of crops. For these soils, the differences between conventional and organic management are less evident, probably because these more developed soils are more resistant to the degradation caused by the conventional practices retaining some of the characteristics of the original colluvial soil (like C and N contents).

Regarding the type of soil Leptosol, although it is not a very advanced type of soil, the lower amount of calcite and the presence of natural vegetation induces very significant spectral changes that clearly differentiate it with respect to the other soils. It is remarkable the significantly greater contribution of organic matter to this soil, as shown in Table 1.
Therefore, from a pedogenetic viewpoint, clear differences were found between soil typologies that could explain much of the results.

3.4. Spectral classification of soils according to agro-environmental soil conditions

Preliminary exploratory analysis using PCA revealed the potential for a classification of soils using infrared spectroscopy. A PLS-DA algorithm was then employed to build a classification model for both soil typology and soil management conditions identification on the basis of the information contained in mid-IR spectra and NIR spectra of samples taken from 0–5 cm depth. The samples were then labeled as belonging to one of the five classes: OC (colluvial soils under organic management), OM (marly soils under organic management), CC (colluvial soils under conventional management), CM (marly soils under conventional management), and NV (soils with natural vegetation).

PLS-DA is a supervised classification tool, which relies on sample class information of the training set to generate models by maximizing separation between groups of observations. This is achieved by rotating PCA components and selecting the variables that carry the class-separating information such that a maximum separation among classes is obtained. Before statistical analysis, all data was subjected to preprocessing as described in Section 2.3. The optimal complexity of the models was established using leave-one-out cross-validation, resulting in four and five latent variables for the models in the NIR and mid-IR spectral regions, respectively. Calibration results (without cross-validation) resulted in sensitivity (rate of true positives) and specificity (rate of true negatives) of 100% for all classes using the NIR region. For the mid-IR model, sensitivity and specificity were lower for the colluvial soils. This seems to be in agreement with the results of the exploratory analysis. Taking into account the limited number of samples and the difficulties of the sampling, it was not possible to have a different data set of enough size to carry out an external validation. For this reason, different approaches of cross-validation were considered in order to avoid too over-optimistic validation results that can be obtained if only the classical leave-one-out validation approach is considered.

As described in the Section 2.3, the four different approaches vary with respect to how the different sample subsets are selected for the sub-validation steps. Table 2 shows a comparison of the performance of the models including the data corresponding to the validation scheme for each approach. Similar tendencies were observed for all the cross-validation approaches indicating the robustness of the models. The most realistic prediction data can be considered those with higher amount of samples in the test set (39) namely Venetian Blinds and Random subsets. The results using the leave-one-out approach were slightly over-optimistic, whereas the use of Continuous Blocks led to the lowest prediction performance, probably because the data were not randomly ordered and this cross-validation approach removed too much information of the same class at once.

Regarding the ability to classify the soils samples correctly, the quality of the predictions using the mid-IR region was very good for the classes CM, OM and NV in all cases. For classes CC and OC, cross-validation reduced the quality of the predictions but not significantly. In the case of the NIR models, the best predictions were obtained for classes CC, CM and OM. However, the sensitivity of the predictions for class OC (organic soils developed on colluvial limestones) was drastically lower using cross-validation in comparison with the calibration results. This could be indicative of certain overfitting of the NIR models for this class. In any case, it seems that the classification of these soils (organic soils developed on colluvial limestones) is complicated for both spectral regions. A deeper inspection of the results for the classes with lower prediction rates for both mid-IR and NIR models can be seen in Figure 5.

In the case of mid-IR these results clearly show a certain overlap of the two classes of colluvial soils either under conventional or organic management (classes CC and OC). Thus, specificity for class OC is low because some samples of CC (which shows high dispersion in the prediction results) are classified as belonging to class OC. Considering the NIR region the low specificity for class OC is due to the wrong classification of some samples of soil with natural vegetation (class NV) as belonging to this class. Looking at the results of the different validation approaches, the main problems are related to the overlapping with the natural soil class, although some samples of other classes are also wrongly classified as belonging to class OC. It seems that, in general, the NIR models have difficulties to differentiate class OC from the rest of the classes and several samples of this class are not recognized correctly, resulting in low sensitivity.

These results can be understood on the basis of the observations already discussed in the exploratory analysis. The lower degradation suffered by the colluvial soils, even under conventional management, makes more difficult to differentiate the type of management. Thus, for these soils the spectral characteristics of the parent material have much more influence on the classification than those related to the form of soil management. Thus, classes CC, OC and NV, all developed on the same parent material (colluvial limestones), are difficult to distinguish. What it is interesting is that the source or errors seems to be different for the two spectral regions. Thus, whereas using the mid-IR region the main difficulties are found to differentiate between colluvial soils under organic or conventional management, for the NIR region the major problem is the distinction between colluvial soils under organic management and the same type

of soil with natural vegetation. Considering these results, a combination of both spectral regions could provide improved classification performance.

Table 2. Classification performance of PLS-DA using mid-IR and NIR spectral regions separately.

					Sensitivity					Specificity				
		Nº samples in test set	Nº sub-validation experiments	Class	1OC	2OM	3CC	4CM	5NV	1OC	2OM	3CC	4CM	5NV
Calibration				Mid IR	1.000	1.000	0.926	1.000	1.000	0.853	0.990	0.892	1.000	1.000
				NIR	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Cross validation	Leave one out	1	128	Mid IR	1.000	0.963	0.889	1.000	1.000	0.853	0.980	0.892	1.000	1.000
				NIR	0.778	1.000	1.000	1.000	0.917	0.922	1.000	1.000	1.000	0.991
	Contiguous Block	6	21	Mid IR	0.926	0.963	0.815	0.972	1.000	0.725	0.980	0.892	0.968	1.000
				NIR	0.556	1.000	1.000	1.000	0.833	0.873	1.000	0.971	1.000	0.991
	Venetian blinds	21	6	Mid IR	1.000	0.963	0.889	1.000	1.000	0.843	0.980	0.892	1.000	1.000
				NIR	0.852	1.000	1.000	1.000	1.000	0.941	1.000	1.000	1.000	0.991
	Random subsets	21	30	Mid IR	0.993	0.978	0.889	1.000	1.000	0.849	0.982	0.890	1.000	1.000
				NIR	0.741	1.000	0.970	1.000	0.900	0.920	1.000	0.998	1.000	0.990

OC: olive cultivation (22 years ago organic management) with herbaceous plants. Slope deposits from limestones. OM: olive cultivation (22 years ago organic management) with herbaceous plants. Marls and some slope deposits from limestones. CC: olive cultivation (conventional tillage). Slope deposits from limestones. CM: olive cultivation (conventional tillage). Marls and some slope deposits from limestones. NV: natural vegetation (Quercus sp.). Slope deposits from limestones.

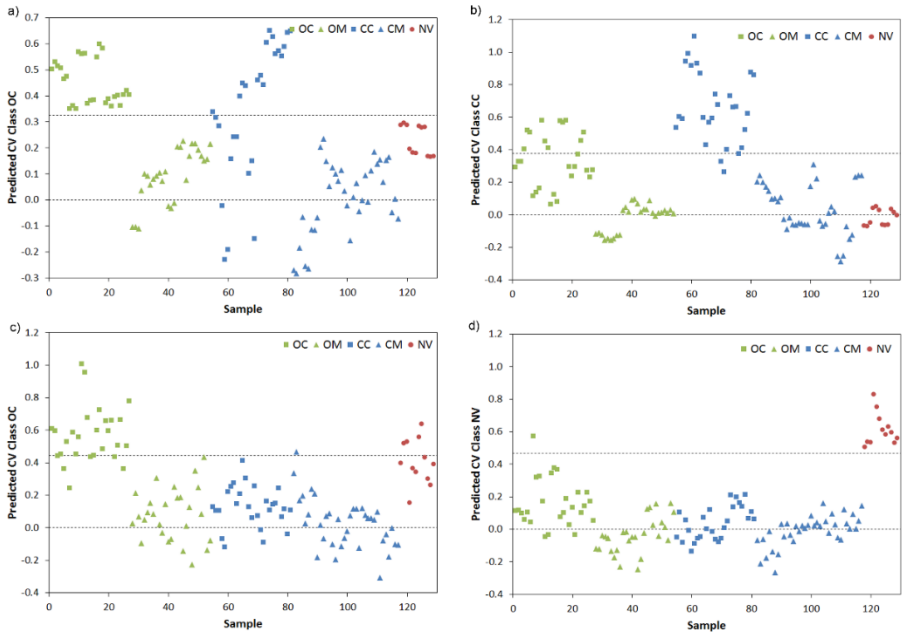

Figure 5. PLS-DA predicted values for (a) OC class using mid-IR region, (b) CC class using mid-IR region, (c) OC class using NIR region and (d) NV class using NIR region. In all cases cross-validation was performed using venetian blinds.

The model developed using the combined information of both mid-IR and NIR regions was constructed with seven latent variables. The results of the classification are shown in Figure 6. As can be seen, the prediction performance is better than when using the spectral regions separately with sensitivity and specificity higher than 98% for the all classes except for class OC. In any case, also the results for this class are much better than with the separated models, showing a classification performance higher than 93%. The inspection of the PLSDA eigenvectors can provide information about the statistical impact of each spectral region on the chemometric model. As can be seen in Figure 7, for the first latent variable (LV) the mid-IR region has higher influence than the NIR. In fact, the information contained in this LV in mid-IR resembles the second derivative spectrum of calcite already shown in Figure 3, with the strongest features located at 714 and 869 cm−1. Interestingly, in the NIR region, despite its weakness, one of the remarkable features is located at 4280 cm−1, characteristic of carbonates. On the contrary, for the rest of the LVs the contribution of both spectral regions is similar, with the NIR region being slightly more intense in most of them. In Figure 7 the eigenvector of the second LV is shown as an example. In this eigenvector, it is especially remarkable the contribution of clays with strong features around 1000 cm−1 in the mid-IR region and 4525 and 7079 cm−1 in the NIR. Together with the strong contributions attributed to mineral matter weaker spectral features characteristic of organic matter can be observed. For the second LV it is remarkable the contribution of aliphatics with CH stretching overtones in the region from 4000 to 4400 cm−1. This is consistent with recent studies that have attributed the main NIR spectral features of soil organic matter to aliphatic carbons, particularly in carbohydrates (40). The corresponding first order spectral features can be seen between 2850 and 2910 cm−1 although very weak due to the short penetration of the evanescent wave in the 3000 cm−1 region when using the ATR technique.

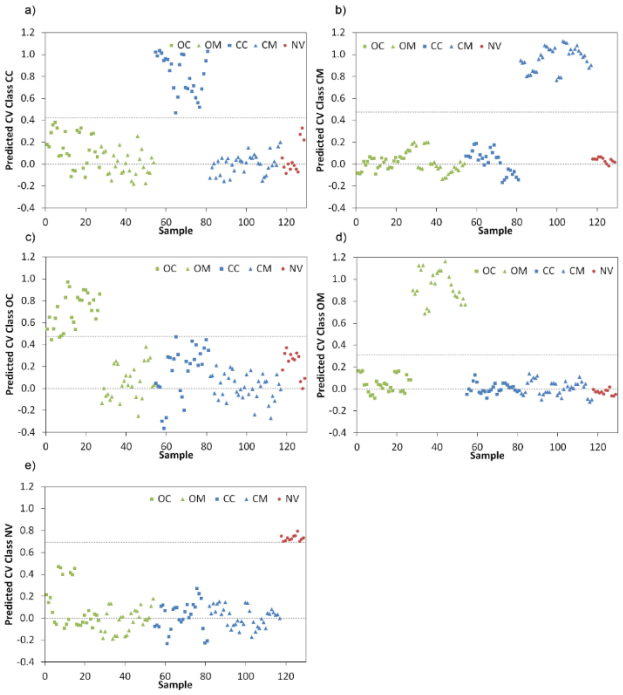

Figure 6. PLS-DA predicted values using the information combined form NIR and mid-IR regions for the different classes of soils: (a) OC, (b) OM, (c) CC, (d) CM and (e) NV. In all cases cross-validation was performed using random subsets.

Table 3. Classification performance of PLS-DA using mid-IR and NIR spectral regions combined.

				Sensitivity					Specificity				
		Nº samples in test set	Nº sub-validation experiments	1CC	2CM	3EC	4EM	5SN	1CC	2CM	3EC	4EM	5SN
Calibration				1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CV	Venetian blinds	21	6	1.000	1.000	0.963	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Random subsets	21	30	0.993	1.000	0.933	1.000	0.983	0.994	1.000	0.980	1.000	1.000

OC: olive cultivation (22 years ago organic management) with herbaceous plants. Slope deposits from limestones. OM: olive cultivation (22 years ago organic management) with herbaceous plants. Marls and some slope deposits from limestones. CC: olive cultivation (conventional tillage). Slope deposits from limestones. CM: olive cultivation (conventional tillage). Marls and some slope deposits from limestones. NV: natural vegetation (Quercus sp.). Slope deposits from limestones.

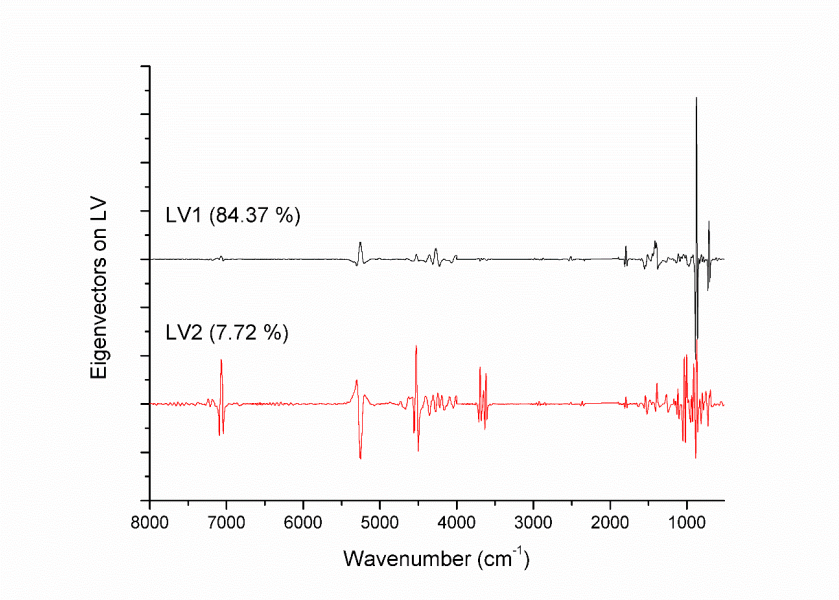

Figure 7. Eigenvectors for the first and second latent variables of the discriminant PLSDA model using the combined information from the two spectral regions mid-IR and NIR.

4. Conclusions

The results demonstrate that mid-IR and NIR spectra of soil samples contain valuable information and that the characteristics of a soil may be represented by compressing mid-IR and NIR spectra into a few vectors that are sufficient to describe its characteristics. Furthermore, the information acquired from the spectra is similar to that depicted by classical soil classifications. Thus, a clear distinction is observed between the colluvial and the marly soils. The latter have poor fertility and are less resistant against degradation. These soils under conventional management are clearly identified by their spectral characteristics, with either mid-IR or NIR or by combining both techniques. This is a very interesting aspect because it demonstrates the usefulness of these techniques to identify severely degraded areas, an important issue for agro-environmental evaluation of agricultural soils. Furthermore, these results also give evidence of the beneficial effect of organic management on severely degraded soils. The quality of marly soils after more than twenty years under organic management has clearly improved in comparison with those soils under conventional management, as indicate the higher values of OC and N, parameters very relevant for soil fertility and quality (41,42), and this is also reflected in the spectral characteristics of the soil.

Finally, it is remarkable the essential role of the soil typology, as it has been shown in the radically different behavior of the colluvial soils and the marly soils. The soils developed on colluvial limestones were described as more evolved soils in a previous study (29), and related with better physical and physical–chemical soil properties. These characteristics are determinant in a higher resilience against degradation, which is reflected in the higher similarity between these soils under organic and conventional management. Consequently, the same form of management will have a very different effect depending on the soil type. This is important with a view to developing more efficient agro-environmental plans in the studied cultivation area and can contribute to improving sustainability of agricultural systems. As long as the

influence of the soil type and geological substrate material in each zone is clearly established, it should be possible to define long-term soil conservation strategies.

The conclusions found may also be of general interest since many other parts of the world with semi-arid climate conditions are intensely cultivated and have serious desertification problems.

Acknowledgments

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Capítulo 7
Infrared spectroscopy as a tool for the assessment of soil biological quality in agricultural soils under contrasting management practices

Este capítulo se corresponde con el artículo "Infrared spectroscopy as a tool for the assessment of soil biological quality in agricultural soils under contrasting management practices", publicado en la revista Ecological Indicators. En este artículo se pone a prueba la capacidad de la espectroscopía FT-NIR para determinar la calidad biológica del suelo.

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Abstract

Soil quality estimation is one of the most important means of evaluating changes in soil resulting from management. However, there is still no unanimity about what parameters should be included in soil quality assessment. Although there is a consensus on the inclusion of biological variables, mainly because they are robust and tend to show a short-term response after disturbances, their measurement is usually expensive and time consuming. This study explored the feasibility of using infrared spectroscopy for estimating biological parameters in olive grove soils under organic and conventional management. Four types of soils in two areas with contrasting parent material (marls and colluvial limestones) in southern Spain were selected. Biological status was established by measuring soil enzyme activities related to nutrient cycling (acid phosphatase, alkaline phosphatase, β-glucosidase, arylsulfatase and dehydrogenase) and potential nitrification rate, and calculating their geometric mean as the GMea index.

The infrared spectroscopic signature of the soil proved to be sensitive to biological status, and discriminated the different types of soil, which had quite different biological activity, relatively well (sensitivity and specificity over 97%), but did not discriminate different soil management practices quite as well (sensitivity and specificity over 75%). In addition, the results acceptably predicted the biological GMea index (RPD ≈ 2) from near infrared (NIR) spectra by means of partial least squares (PLS) regression. These results demonstrate the applicability of infrared spectroscopy as a fast and efficient technique for estimating biological soil quality, as well as for discriminating between soils with differing biological quality.

1. Introduction

It is well known that one of the most significant human alterations of the global environment is the intensification of agriculture (Matson et al., 1997; Vitousek, 1994). Sensitive to these issues, the European Commission has been a motor of environmentally-friendly agricultural policies (EU Common Agricultural Policy, 1992), promoting crop management practices, such as organic farming, that optimize yields while preserving soil health and protecting the environment. One explicit goal of organic farming is to maintain and eventually improve soil quality (often used synonymously with soil fertility or soil health) through management practices that preserve the soil's functionality and biological potential. Around 30% of organic farming in the region of Andalusia (southern Spain) is in olive groves. Olive oil production is of enormous economic and social importance in this region, and olive groves are the dominant landscape in more than two million hectares. Therefore, the demand for suitable soil quality indicators for evaluating and monitoring the impact and measuring the success of specific agricultural practices has increased. Indeed, the development of monitoring programs is of fundamental importance for the precise definition of policy-relevant end points.

This makes research in soil science focus increasingly on the search for soil quality quantification indicators, even though the concept itself is not well-established (Bastida et al., 2008), since no single or combined biological or physicochemical variable is able to reflect the many interacting processes responsible for soil quality (Puglisi et al., 2006). Physical and chemical properties have been used extensively to measure soil quality (Miralles et al., 2007). However, these properties usually change on too long a time scale (decades) to be practicable for management. On the contrary, soil properties based on biological and biochemical activities, such as soil enzymes, have been shown to respond to small changes in soil conditions, thus providing information sensitive to subtle alterations in soil quality (García-Ruiz et al., 2008; Pascual et al., 2000). However, determination of these soil quality indicators is usually quite time consuming. To be of practical use in assessing soil quality, bioindicators should be easily measured by standardized methods, and reflect changes in management within a relevant time frame, e.g., by providing early warning of potential threats to soil quality. Infrared spectroscopy has been proposed as an alternative method for determining soil quality (Paz-Ferreiro and Fu, 2016). Over the past decade, this technique, particularly in the form of reflectance measurements in the near infrared region (NIR), has rapidly developed into a fast robust analytical method for many agricultural, and food products (Blanco and Villarroya, 2002). In soil science, infrared spectroscopy has been used for assessing properties related to moisture and organic matter content, including carbon and nitrogen, and cation exchange capacity (Chodak et al., 2004), as well as for predicting physical and physicochemical variables (Bellino et al., 2016; Soriano-Disla et al., 2014) The effectiveness of NIR reflectance spectroscopy has also been shown for estimating soil nutrients (Cozzolino and Morón, 2003; Islam et al., 2003), physical characteristics (Sørensen et al., 2005) and some biochemical properties (Cohen et al., 2005; Reeves et al., 2000). Total and particulate organic carbon and charcoal carbon were appropriately estimated by infrared spectroscopy (Janik et al., 2007). Soil organic carbon content was unbiased predicted by reflectance spectroscopy of 20000 soil samples from 23 countries (Stevens et al., 2013).

The advantages of using NIR reflectance spectroscopy include simple sample pretreatment (sieving), lack of chemical reagents and its non-destructive nature, and analysis is rapid, inexpensive and accurate.

For soil quality, infrared spectroscopy has already proved to be effective in discriminating between types of soils and management (Aranda et al., 2014). However, the few attempts made at predicting biological variables have shown rather discrepant results (Dick et al., 2013; Heinze et al., 2013).

The aim of this study was to assess the capability of NIR infrared spectroscopy to discriminate among soils under contrasting management practices (conventional versus organic) and to predict both individual (specific soil enzymes) and combined (GMea) soil biological indicators.

2. Materials and methods

2.1. Study site and soil sampling

Two pedoclimatic areas, Sierra Mágina and Jaén were studied. Both areas have a semi-arid Mediterranean climate, with low precipitation and mean temperatures of around 16ºC. Soils at the sites, which were chosen to include different soil characteristics, were Cambil (CA) and Pegalajar (PE) in Sierra Mágina, and Puente Tablas (PT) and Puente de la Sierra (PS) in Jaén. The approximate location of sampling points may be seen in Figure . According to the Andalusian soil map (Junta de Andalucía, 2005) and the Food and Agriculture Organization (FAO, 2006), soils in Sierra Mágina are Cutanic Luvisols (PE) over colluvial limestones, and Haplic Cambisols (CA) over a mix of marls and colluvial limestones, whereas soils in the Jaén area are classified as Haplic Regosols (PS) and Haplic Cambisols (PT) over marls. Further information about each site's geological and climate characteristics may be found in
Table *1*.

Table 1. Climatic, soil type and some landscape features of the sites.

Location	Management	n	Altitude (m.a.s.l.)	Slope (%)	Orientation	MAR (mm)	MAT (˚C)	Geological substrate	Soil type*
PT	Conventional	14	923	9	SW	559	16.9	marls	Haplic cambisol
PT	Organic	19	923	7	SW	559	16.9	marls	Haplic cambisol
PS	Conventional	15	1012	11	SE	559	16.9	marls	Haplic regosoll
PS	Organic	12	1012	13	SE	559	16.9	marls	Haplic regosol
PE	Conventional	12	825	5	N	553	15.3	Colluvial limestones	Cutanic Luvisol
PE	Organic	12	825	7	N	553	15.3	Colluvial limestones	Cutanic Luvisol
CA	Conventional	38	874	12	SW	553	15.3	Mix of marls and colluvial limestones	Haplic cambisol
CA	Organic	35	874	14	SW	553	15.3	Mix of marls and colluvial limestones	Haplic cambisol

*PT: Puente Tablas; PS: Puente Sierra; PE: Pegalajar; CA: Cambil. MAR: mean annual rainfall; MAT: mean annual temperature; *FAO (2006)*

Two olive groves, one under organic and the other under conventional management, but comparable in terms of tree age, density and landscape features, were selected at each site. Groves under organic management are fertilized with sheep, chicken or cow manure or a commercial organic fertilizer, and weed control is carried out by mechanical tillage or mowing. Conventional farms are characterized by plowing 3-5 times a year to a depth of 15-20 cm from early spring to early autumn, and weeds are controlled with herbicides with an annual application of ammonium sulfate or urea as the fertilizer. In addition, preventive pest control treatments are applied using any of several different commercial pesticides and copper sulfate. A total of 157 samples were collected in the eight groves. Five top 10 cm soil samples, each composed of four subsamples, were randomly collected in the inter-row area of the olive groves at approximately fifty meters away from each other.

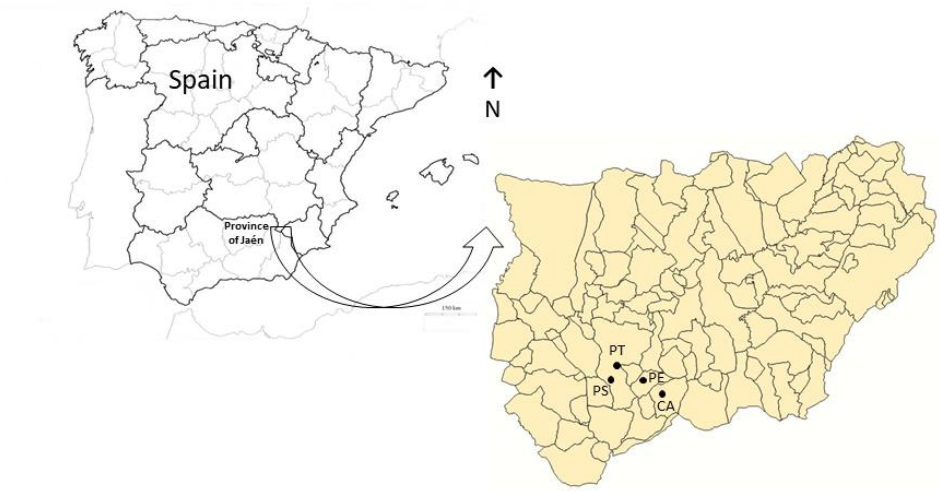

Figure 1. Location of the study area: Cambil (CA) and Pegalajar (PE) in Sierra Mágina; Puente Tablas (PT) and Puente de la Sierra (PS) in Jaén.

2.2. Soil analysis

All analytical soil data refer to the fine-earth fraction (<2 mm). Chemical analyses followed the standard procedures, and were as outlined by the American Society of Agronomy and Soil Science Society of America (Klute, 1982; Page, 1982). Organic carbon (OC) content was determined by the Walkley-Black's method with dichromate oxidation (1N K2Cr2O7 and 0.5N Fe(NH4)2(SO4)2 as the titrant for excess Cr2O72-). pH was measured by potentiometry in distilled water (1:2.5, w/v). Electrical conductivity (EC) was measured at 25°C in water extracts (1:5, w/v). Equivalent CaCO3 was determined by volumetry with a Bernard calcimeter, reacting carbonates with hydrochloric acid (10% HCl, w/w). Total phosphorus was extracted with 0.5N NaHCO3 (pH 8.5), and quantified using a mixed reagent of (NH4)6Mo7O24 and K(SbO)C4H4O6, and ascorbic acid as a color-developing reagent, and finally, phosphorus was determined by colorimetry (Olsen's method). The cation exchange capacity (CEC) was determined using a saturation solution (0.4N

NaOAc-0.1N NaCl; pH 8.2) and an extracting solution (0.5N MgNO3). The exchangeable Na extracted, which is equivalent to the CEC, was determined by flame photometry. Exchangeable basic cations were extracted by the ammonium acetate method (1N NH4OAc; pH7), and the concentrations determined by atomic absorption spectrometry. Clay and sand contents were determined using the Robinson pipette method after eliminating carbonates and soluble salts with 1M NaOAc (pH 5), the organic matter with H2O2 (30%), and iron oxides with Na2S2O4 and saturated NaCl, and finally, dispersion with Na-hexametaphosphate (50 g/L solution). Enzyme activities were measured using 1 g air-dried soil. Acid phosphatase (EC 3.1.3.2, orthophosphoric-monoester phosphohydrolase, acid optimum), alkaline phosphatase (EC 3.1.3.1, orthophosphoric-monoester phosphohydrolase, alkaline optimum), arylsulfatase (EC 3.1.6.1, arylsulfate sulfohydrolase) and β-glucosidase (EC 3.2.1.21, β-D-glucosidase glucohydrolase) activities were determined according to Tabatabai (1982) and reported as μg p-nitrophenol (pNP) g^{-1} h^{-1}. Controls were performed in all cases by adding the substrate after the reaction had stopped, and before filtration of the soil suspension. Dehydrogenase was determined as described by Casida et al. (1964), with the following modification: 1 g soil mixed with 0.01 g CaCO3 was incubated in the presence of 1 ml 3% 2,3,5 triphenyl tetrazolium chloride (TTC) and 3 ml water at 37ºC in the dark. After 6 h, 10 ml methanol were added, and the suspension was homogenized, filtered and washed with methanol until the reddish color (caused by the reduction of TTC to triphenyl formazan) had disappeared from the soil suspension. Absorbance at 485 nm was compared to triphenyl formazan standards. The potential rate of soil ammonium oxidation (i.e., potential nitrification rate) was analyzed (Kandeler, 1995). Activity values and rates are reported on an oven-dry soil weight basis.

The geometric mean of the different enzyme activities measured (GMea) was used as a soil biological quality index. This index has proved to be a good integrative indicator of soil quality and has been successfully applied to soil quality assessment (García-Ruiz et al., 2009). It was calculated following (García-Ruiz et al., 2008):

Eq 1. $$GMea = \sqrt{(6\&AcP \times AlP \times Glu \times Ary \times Dehy \times PN)}$$

where, AcP is acid phosphatase, AlP is alkaline phosphatase, Glu is β-glucosidase, Ary is arylsulfatase, Dehy is dehydrogenase and PN is the potential nitrification rate.

2.3. Infrared spectroscopy

Soil samples were first finely ground in an agate mortar. Diffuse reflectance NIR spectra between 4000 and 10000 cm^{-1} were recorded using an Antaris FT-NIR analyzer (Thermo Nicolet Corporation) equipped with a solid sampling system with internal gold-coated integrating sphere module., Three centimetre sampling cups filled with soil from the samples were placed on top of the sapphire window of the integrating sphere and rotated for measurement. A computer-controlled gold reference reflector, was automatically positioned in front of the window to acquire the background spectrum before each sample measurement.. Samples were measured in triplicate with 8 cm^{-1} resolution, and 128 accumulations. The mean of the three spectra was used as representative of each soil sample.

2.4. Statistical analysis

Data analysis included correlations between variables (Pearson's correlation coefficient) and two-way ANOVA. Assumptions of normality were tested using the Saphiro-Wilk test. The ANOVA F-test was applied for variables fitting a normal distribution. The non-parametric test (Kruskal-Wallis ANOVA) was used for non-normal variables. The Fisher LSD test was used to check significant differences between means. These statistical procedures were carried out using Statgraphics Centurion XVI software (StatPoint Technologies, Inc.).

2.5. Chemometric analysis

A preliminary exploration of NIR spectra of the samples was performed using principal component analysis (PCA). Partial least squares discriminant analysis (PLS-DA) was employed to classify soil samples by management and type of soil. Partial least squares (PLS) regression was used to build predictive models for individual enzyme activities and the GMea biological index. Random subsets cross-validation was used to estimate the expected performance of the models. This involved five sub-validation experiments in which one fifth of the samples were randomly removed from the calibration set and used only for prediction.

Prior to applying PCA and PLS, different spectra preprocesses were tested, including derivation, smoothing, detrending, baseline correction and mean centering in order to reduce noise and baseline influence, and make relevant data more accessible and improve model construction.

Accuracy of the predictive models was estimated using the ratio of predictive deviation (RPD) which is calculated as:

Eq 2. $$RPD = SDv/(RMSEv \times \sqrt{(n/(n-1))})$$

where SDv is the standard deviation of the variable entered, RMSEv is the deviation in the model prediction and n is the number of samples. According to Chang et al., (2001) RPD above 2.0 is associated with accurate predictive models, between 1.4 and 2.0 indicates fair models with room for improvement, and RPD below 1.4 indicates poor predictive capacity.

All chemometric treatments were performed using MATLAB 2008R (MathWorks, Natik, USA) with the PLS-toolbox from Eigenvector Research Inc. (Wenatchee, USA).

3. Results and discussion

3.1. Effects of management on soil physico-chemical properties

The physico-chemical properties of the soil samples are shown in Table 2. All the soils studied were characterized by basic pH and relatively high Ca2+ levels. Soil pH and cation exchange capacity showed no significant differences between organic and conventional management, whereas water holding capacity was slightly higher in soils under organic management although the difference was not significant at some of the sites. However, exchangeable Mg2+, labile P and organic matter content were significantly higher ($P<0.05$) in soils under organic management than under conventional at all the sites, and the highest soil organic matter content was found in the organic groves at PE, while soil exchangeable Ca2+, Mg2+ and K+ were higher in PT organic groves, showing site influence. These results indicate generally higher fertility of

soils under organic management. This was not unexpected, as organic management in olive groves includes the application of manure and the presence of a cover crop which increases the organic matter content, and thereby the direct and indirect increase in other indicators of soil fertility (García-Ruiz et al., 2012). Moreover, soils at the two sites in Sierra Mágina (CA and PE) had higher organic matter content and cation exchangeable capacity than the soils at the two sites in Jaén (PT and PS), regardless of management type. As reported by Aranda et al. (2011), this could be explained by the overlapping of current soil management and its biogenic background, representative of the original soil in this area, and hence, maintaining higher organic matter content and biological soil quality. It could also be due to the combination of higher sodium and relatively low calcium contents in the latter, even when the levels of these cations are not problematic. It has previously been shown that sodium generates clay dispersion by entering the phyllosilicate interlayer (Sumner, 1993) and generating faster organic matter decomposition (Nelson and Oades, 1998).

3.2. Soil enzyme activities

Individual soil enzyme activities and the GMea index are shown by site in Table 3. A clear trend toward higher enzyme activity was observed in organic groves, but only in acid phosphatase and dehydrogenase (a general indicator of biological activity) the difference between organic and conventional management was statistically significant at all the sites. The trend toward higher soil enzyme and dehydrogenase activities under an organic management agrees well with the finding of others (e.g. Mäder et al. (2002) and Benitez et al. (2006). For instance, dehydrogenase, an indicator of microbiological activity involved in oxidative phosphorylation (Alef and Nannipieri, 1995), has been found to decrease in soils treated with herbicide (Reinecke et al., 2002) and/or in tilled soils: routine practices at the conventional farms in this study. Our results using hydrolytic enzymes suggest that soil functionality (in this context the capacity to cleave organic compounds) was enhanced under organic management (Mäder et al., 2002). This shows that organic management clearly improves soil biological quality, mainly due to presence of plant cover (Moreno et al., 2009), which protects the soil from erosion and promotes organic matter. The use of organic amendments (Macci et al., 2016), also increases nutrient and organic carbon availability and soil biodiversity. Conventionally managed groves, on the other hand, showed significantly higher potential nitrification. This is consistent with the long-term application of ammonium or urea as the main fertilizer, and suggests the presence of an important community of autotrophic nitrifying bacteria.

Lowest individual soil enzyme and dehydrogenase activities were observed in the CA samples. As already mentioned, this site had low soil organic matter and the highest soil sodium content. Therefore, other physico-chemical soil characteristics might be strong determinants of the effects of soil management on soil quality. Soil enzyme activities at PE were generally the highest, indicating the relatively high biological quality of soils at this site. The presence of a surrounding natural forest might be an influence, through increased landscape complexity and biodiversity.

Table 2. Physico-chemical properties (mean ± standard deviation) of the soil samples from sites under different management. Different letters between management within a site denote significant differences (P< 0.05 probability level; LSD test).

Location	Management	N	O.M. (%)	Clay (%)	Lime (%)	Sand (%)	Ca^{2+} (cmol kg^{-1})	Mg^{2+} (cmol kg^{-1})	K^{+} (cmol kg^{-1})	Na^{+} (cmol kg^{-1})	P (cmol kg^{-1})	Carbonates (%)	CEC (cmol kg^{-1})	pH	WHC (ml ml^{-1})
PT	Conventional	14	4.5 ± 1.6 ab	46 ± 2 d	37 ± 2 ab	17 ± 1 b	60 ± 3 b	2.3 ± 0.6 b	2.6 ± 0.6 c	0.09 ± 0.01 a	47 ± 27 cd	31 ± 1 a	12 ± 1 b	7.6 ± 0.1 a	0.26 ± 0.02 a
PT	Organic	19	6.8 ± 3.2 d	27 ± 3 b	51 ± 3 c	22 ± 1 c	122 ± 24 d	5.4 ± 2.6 c	2.5 ± 0.3 c	1.30 ± 1.40 abcd	105 ± 29 e	33 ± 5 a	16 ± 3 b	7.6± 0.1 a	0.27± 0.01 a
PS	Conventional	15	3.9 ± 1.1 a	27 ± 3 b	59 ± 4 cd	14 ± 1 a	65 ± 2 b	0.9 ± 0.2 a	0.6 ± 0.3 a	0.17 ± 0.05 b	10 ± 2 a	70 ± 1 c	8 ± 1 a	7.6 ± 0.3 a	0.31 ± 0.02 a
PS	Organic	12	5.4 ± 1.5 bcd	16 ± 9 a	67 ± 11 de	18 ± 3 b	61 ± 3 b	2.0 ± 0.3 b	1.0 ± 0.1 b	0.46 ± 0.20 c	15 ± 3 b	70 ± 3 c	10 ± 1 a	7.8 ± 0.1 a	0.32 ± 0.02 a
PE	Conventional	12	6.9 ± 2.9 d	15 ± 10 a	70 ± 9 e	15 ± 1 a	78 ± 10 bc	1.2 ± 0.3 a	1.6 ± 0.6 b	0.07 ± 0.01 a	19 ± 16 abc	57 ± 19 bc	17 ± 5 b	7.9 ± 0.1 a	0.32 ± 0.02 a
PE	Organic	12	9.3 ± 1.7 e	32 ± 3 bc	49 ± 3 c	18 ± 2 b	76 ± 5 bc	8.2 ± 1.3 d	3.1 ± 1.7 cd	0.07 ± 0.01 a	61 ± 12 d	55 ± 1 b	20 ± 1 b	7.8 ± 0.1 a	0.33 ± 0.01 a
CA	Conventional	38	5.6 ± 1.1 bc	27 ± 6 b	34 ± 5 a	40 ± 8 d	13 ± 3 a	2.3 ± 0.7 b	3.4 ± 0.6 d	0.36 ± 0.32 abc	23 ± 12 bc	28 ± 13 a	17 ± 4 b	7.9 ± 0.3 a	0.30 ± 0.04 a
CA	Organic	35	6.0 ± 1.4 cd	28 ± 6 b	31 ± 5 a	42 ± 9 d	11 ± 4 a	3.1 ± 1.4 b	1.4 ± 0.4 b	0.40 ± 0.08 c	26 ± 22 bc	31 ± 12 a	16 ± 3 b	7.8 ± 0.2 a	0.31 ± 0.10 a

PT: Puente Tablas; PS: Puente Sierra; PE: Pegalajar; CA: Cambil. O.M.: organic matter; Ca2+, Mg2+, Na+ and K+ (exchangeable bases); P: available phosphorus; CEC: cation exchange capacity; WHC: water holding capacity.

The fact that differences in some of the soil enzyme activities between organic and conventionally managed olive oil groves were not significant, suggests that the use of activity of a single soil enzyme is insufficient to define soil biological quality properly. This is shown, in the case of PT, where dehydrogenase was the highest, but individual soil enzyme activities were relatively low. GMea which is an index resulting from the combination of soil enzymes related to C, P and S cycling, dehydrogenase activity, and potential nitrification rate, was significantly higher in soils of organically managed olive groves. Therefore, GMea was able to adequately show the increase in soil biological activity under organic management. This is because this index is the result of the product of the single soil enzyme, dehydrogenase activities and the potential nitrification rate. Even though some of the soil enzymes showed no significant differences with management, values tended to be higher under organic management, and small differences, although not significant, were amplified in the GMea.

3.3. Exploratory analysis of near infrared spectra of soils

Mean NIR diffuse reflectance spectra of soil samples are shown in Figure 2 for each of the four study sites in both organic and conventional management.

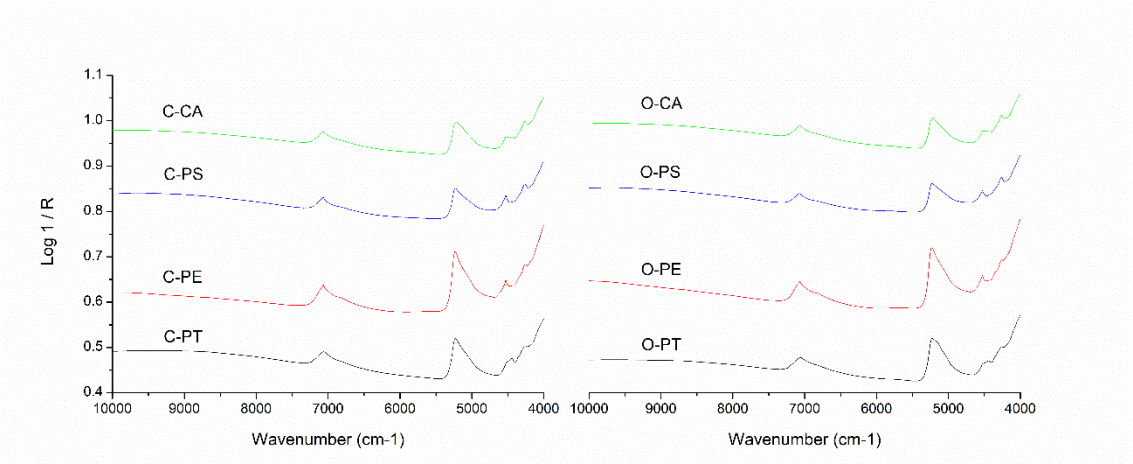

Figure 2. NIR spectra of the different study sites. Mean spectra are shown for each site for both organic and conventional management. C and O stand for conventional and organic, respectively.

NIR spectra provide information about various soil constituents, including both organic and inorganic components. Layer silicates are characterized by the presence of water molecules that interact with each other, with surface oxygen atoms, and with exchangeable cations. Thus, absorptions due to vibration modes (overtones and combination bands) of water molecules and lattice hydroxyl groups are clearly observable (Cariati et al., 1981). The band located near 7060 cm^{-1} is related to overlapping of the first overtone of the O-H stretching vibration of water and hydroxyl groups in clay. The strongest absorption band in the NIR spectra of soils, near 5200cm^{-1}, is related to H-O-H bend and O-H stretch combination bands in structural water. The absorptions due to metal–OH bend plus O–H stretch combination appear around 4520 cm^{-1} for clay minerals. The band at around 4260 cm^{-1} is attributed to the second overtone of the antisymmetric stretching of the CO32- ion. Soil organic matter (SOM) also leads to absorption bands in the NIR mainly related to the overtones and combination bands of O–H, C–H, and N–H groups but these absorptions cannot be clearly identified by visual inspection of the spectra.

Table 3. Soil enzyme activities and GMae index of soil samples for different sites and types of management. Values are the range (min - max) and mean ± standard deviation. Maximum and minimum, mean and standard deviation are also shown for the whole dataset. Letters denote significant differences (P < 0.05; Kruskal-Wallis test).

Site	Management		Dehydrogenase	β-Glucosidase	Arylsulphatase	Alkaline phosphatase	Acid phosphatase	Potential nitrification	GMea
PT	Conventional	Range	3 - 13	140 - 331	2 - 114	97 - 301	44 - 200	0.2 - 1.0	12 - 40
		Mean ± SD	8 ± 4 de	196 ± 49 b	36 ± 37 ab	195 ± 49 b	91 ± 43 a	0.6 - 0.2 bcd	25 ± 8 c
PT	Organic	Range	3 - 14	42 - 336	3 - 226	43 - 341	30 - 458	0.2 - 0.4	10 - 40
		Mean ± SD	7 ± 3 d	197 ± 115 b	43 ± 53 b	183 ± 119 b	166 ± 98 b	0.3 ± 0.1 ab	25 ± 9 c
PS	Conventional	Range	1 - 6	86 - 408	1 - 327	45 - 349	29 - 213	0 - 0.5	10 - 46
		Mean ± SD	3 ± 1 a	234 ± 114 b	56 ± 86 b	214 ± 82 b	101 ± 48 a	0.2 ± 0.1 a	19 ± 9 b
PS	Organic	Range	3 - 15	115 - 614	9 - 249	64 - 421	64 - 347	0.1 - 0.5	9 - 62
		Mean ± SD	9 ± 3 e	426 ± 177 c	65 ± 67 b	287 ± 106 c	151 ± 85 b	0.3 ± 0.1 ab	34 ± 14 d
PE	Conventional	Range	2 - 11	123 - 1070	45 - 287	154 - 762	112 - 444	0.2 - 0.7	31 - 69
		Mean ± SD	6 ± 3 cd	556 ± 289 d	156 ± 81 c	432 - 152 d	209 ± 83 bc	0.5 ± 0.2 abc	49 ± 12 e
PE	Organic	Range	3 - 18	181 - 945	293 - 46	149 - 657	164 - 370	0.4 - 1.5	47 - 92
		Mean ± SD	9 ± 5 e	681 ± 198 e	171 ± 76 c	472 ± 141 d	260 ± 75 c	0.8 ± 0.3 d	65 ± 15 f
CA	Conventional	Range	2 - 8	15 - 58	2 - 24	9 - 44	42 - 207	0.2 - 3.0	6 - 18
		Mean ± SD	4 ± 1 ab	35 ± 10 a	10 ± 5 a	26 ± 8 a	110 ± 37 a	1.1 ± 0.7 e	12 ± 3 a
CA	Organic	Range	2 - 8	27 - 66	4 - 21	16 - 54	68 - 443	0.1 - 1.8	7 - 19
		Mean ± SD	5 ± 2 bc	43 ± 10 a	14 ± 4 a	37 ± 9 a	184 ± 78 b	0.6 ± 0.4 cd	14 ± 2 ab
	Management		O > C	O = C	O = C	O = C	O > C	C > O	O > C
	Site		PE = PT > PS > CA	PE > PT > PS > CA	PE > PT = PS > CA	PE > PS > PT > CA	PE > PS = PT = CA	CA > PE ≥ PT ≥ PS	PE > PT = PS > CA
	Overall	Range	1 - 18	15 - 1070	1 - 327	9 - 762	28 - 458	0 - 3.0	6 - 92
		Mean ± SD	6 ± 3	211 ± 240	49 ± 71	167 ± 170	153 ± 84	0.6 ± 0.5	25 ± 18

PT: Puente Tablas; PS: Puente Sierra; PE: Pegalajar; CA: Cambil. Dehydrogenase in µg TPF g-1 h-1; β-Glucosidase, Arylsulphatase, Alkaline phosphatase and Acid phosphatase in µg pNP g-1 h-1, and Potential nitrification in µg N-NO2 g-1 h-1.

Thus, small changes in intensity and band position in the NIR spectra, as observed in Figure 2, reflect differences in soil composition. However, bands in NIR spectra are broad due to overlapping absorptions of soil constituents and also weak due to low concentration, particularly in the case of organic compounds. Therefore, in order to find correlations between NIR spectra and soil characteristics, the information needs to be mathematically extracted from the spectra using chemometric techniques. Due to the high correlation of some biological properties with variables like TOC or SOM whose changes are reflected in NIR spectral features, reflectance spectra could be useful as predictors of soil biological quality.

First, a PCA analysis was performed to explore the data and examine its structure and the existence of any natural clusters. As can be seen in Figure 3, in the space defined by the two first principal components, samples from PE, PT and PS formed clearly separate groups whereas samples from CA were more heterogeneous and overlapped with other sites. The influence of the parent material was mainly reflected in PC1 (47.43% variance), which clearly discriminated between soils developed over colluvial limestones (PE, with negative scores) and soils developed over marls (PS and PT, with positive scores). Most of the samples from CA showed positive scores for PC1, although there was a subgroup with negative scores. This is not surprising, because soils are developed over a mix of marls and colluvial limestones at this site. These observations are in agreement with previous studies (Aranda et al., 2011), which concluded that the main qualitative changes affecting organic matter in the study area were induced by the geological substrate (soil type). In particular, the soil over colluvial limestones, where organic matter was transformed into more stable and evolved forms of humus, had improved physicochemical and biological properties. On the other hand, PC2 established a certain separation between highly carbonated soils (PS) with low SOM content and soils with less carbonate and richer in organic matter (PE, PT). CA samples again showed more heterogeneity and fell in between these categories, because they are both less carbonated and not very rich in organic matter.

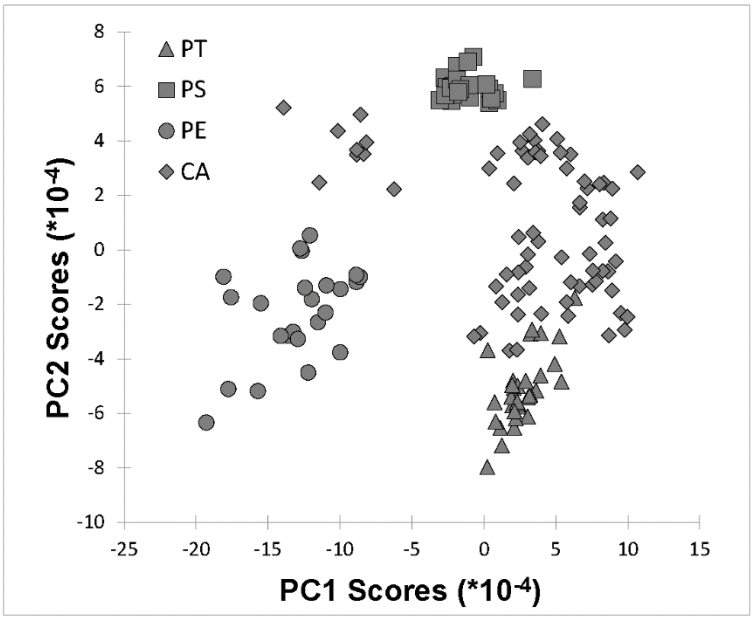

Figure 3. PCA scatter plot of NIR spectra for PC1 (47.43% variance) and PC2 (15.71%) of soil samples. Mean center and second derivative preprocessing are applied. Squares show PS, circles PE, triangles PT and diamonds CA.

Mean values of scores on PC1 and PC2 differed significantly among sites but differences between conventional and organic managementwere non-significant. These results suggest that the main sources of spectral variance are related to differences in soil types, and thus, PCA is useful in discriminating sites, but not type of management. Soil management is probably reflected in differences in soil organic matter. The SOC pool is composed of a complex, heterogeneous and very diverse array of organic compounds, such as microbial carbon, root exudate, clay and silt protected organic carbon, humus, etc., in different stages of decomposition and stabilization. These compounds are in soil in minor proportions, and therefore, their contribution to the NIR spectra is not as strong as that of the mineral fraction.

To further explore the feasibility of discriminating between samples with different biological quality and soil properties, a PLS-DA analysis was performed. PLS-DA is a supervised method which uses the information on the sample category (site or management) to extract those latent variables (LVs) that maximize interclass variance. This is a particularly useful strategy when the main source of variance in the data is not related to the classification problem to be solved, as is the case here. The results in

Table 4 show almost perfect discrimination among the different sites using 8 LVs, with sensitivity (true positives) and specificity (true negatives) close to 100%. Discrimination between management practices was also good, but both sensitivity and specificity decreased, revealing that this discrimination was more difficult. The lowest cross-validation values were in sensitivity for organic management and in specificity for conventionally managed samples. In fact, 24% of the samples of soil under organic management were not correctly recognized, and were classified as conventionally managed. Most of the incorrectly classified samples were from CA. The particular characteristics of the soils at that site, which have a predominately sandy texture and low exchangeable calcium content, could be responsible for the poor soil fertility and generally low soil biological activity observed despite organic management. This could also be the reason why these organic samples were similar to samples from other sites under conventional management. Difficulties in management discrimination could also be due to the fact that all these olive groves were under conventional management until relatively recently, and time under organic management has been insufficient for soil conditions to change completely. Summarizing, due to stronger influence of the type of parent material in the NIR spectra it is more difficult to discriminate between management practices, but the technique has proved to be sensitive to both location and management. This is in agreement with Aranda et al. (2014) who already showed that infrared spectra were able to detect differences in soil samples from land under different management practices.

Table 4. Classification of PLS-DA using NIR spectral region of mean sample spectra. Spectra were preprocessed using detrending and mean center in both cases.

		Sensitivity				Specificity			
	Number of LVs	CA	PE	PS	PT	CA	PE	PS	PT
Calibration	8	0.973	1.000	1.000	1.000	0.962	1.000	1.000	0.994
CV Random Subsets	8	1.000	0.977	1.000	0.992	0.995	0.980	0.991	0.990

PT: Puente Tablas. PS: Puente Sierra. PE: Pegalajar. CA: Cambil.

		Sensitivity		Specificity	
	Number of LVs	Conventional	Organic	Conventional	Organic
Calibration	11	0.962	0.962	0.962	0.962
CV Random Subsets	11	0.846	0.751	0.751	0.846

3.4. Prediction of biological activity by Partial Least Squares Regression

Considering that some of the enzymes assayed are based on organic compounds with functional groups able to absorb radiation in the NIR region and provoke direct changes in the reflectance characteristics of the samples, partial least squares regression models were built to predict individual enzyme activities, potential nitrification rate and GMea index.

The best predictive models and their statistics are summarized in Table 5. In all cases, a large number of latent variables were necessary, probably because the most important sources of spectral variability are related to soil mineralogy as discussed above. The predictive ability for individual enzyme activity was generally medium, with good calibration and validation results for β-glucosidase and alkaline phosphatase (R2 Cal higher than 0.7 and R2 CV higher than 0.6)) medium for arylsulphatase (R2 Cal higher than 0.5 and R2 CV higher than 0.4) and low for acid phosphate and dehydrogenase activitie and potential nitrification rate.

Figure 4 shows the relationship between the soil enzyme activities measured and predicted by the PLS model (cross-validation data). The regression coefficient between them was mostly over 0.60 ($P< 0.01$), except for acid phosphate, dehydrogenase activities and potential nitrification rates. This could be due to the lack of a direct relationship between those enzymatic activities and organic matter in general, which agrees with the findings of Reeves et al. (2000) and Soriano-Disla et al. (2014). The inability to predict the potential nitrification rate was not unexpected, as it is an indicator of the soil nitrifying bacterial community which are autotrophic and use inorganic carbon sources. Our relatively poor prediction for acid phosphate activity agrees with the findings of (Reeves et al., 2000). This enzyme was assayed at a significantly lower pH than the pH of the soils assayed. Finally, one possible explanation of the low predictability of dehydrogenase activity is that it strongly depends on the physiological status of the microbial community, a characteristic unlikely to influence the spectra enough to register a measurable effect.

Prediction of β-glucosidase was reasonably good, probably because of the close relationship between this soil enzyme and SOM (0.49 correlation with statistical significance $P < 0.001$) and the influence of SOM on the NIR spectral profile, as found by Zornoza et al. (2008a). It is worth mentioning that despite the high number of latent variables employed, the models did not seem to be over-fitted, since RMSECV are not significantly higher than RMSEC except in potential nitrification.

The best prediction results were found for the GMea index (Figure 5) with an RPD close to 2 and a regression coefficient of 0.73 ($P < 0.001$). This suggests that models based on NIR spectroscopy have a better predictive capacity for estimating overall soil quality with combined indicators such as the GMea index. Although near infrared spectroscopy seems to present certain limitations for predicting biological variables, especially enzymatic activities, the results presented indicated that NIR reflectance spectroscopy might be useful for rapid determination of general soil biological activity when extreme accuracy is not determinant, as Gmae was highly predictable. This is of particular interest, because combined biological quality indices are more useful in assessing overall soil quality , as they summarize information from pools of all enzymatic activities studied (García-Ruiz et al., 2008; Bastida et al., 2008), and they are also a good tool for monitoring agroagro-system soil quality.

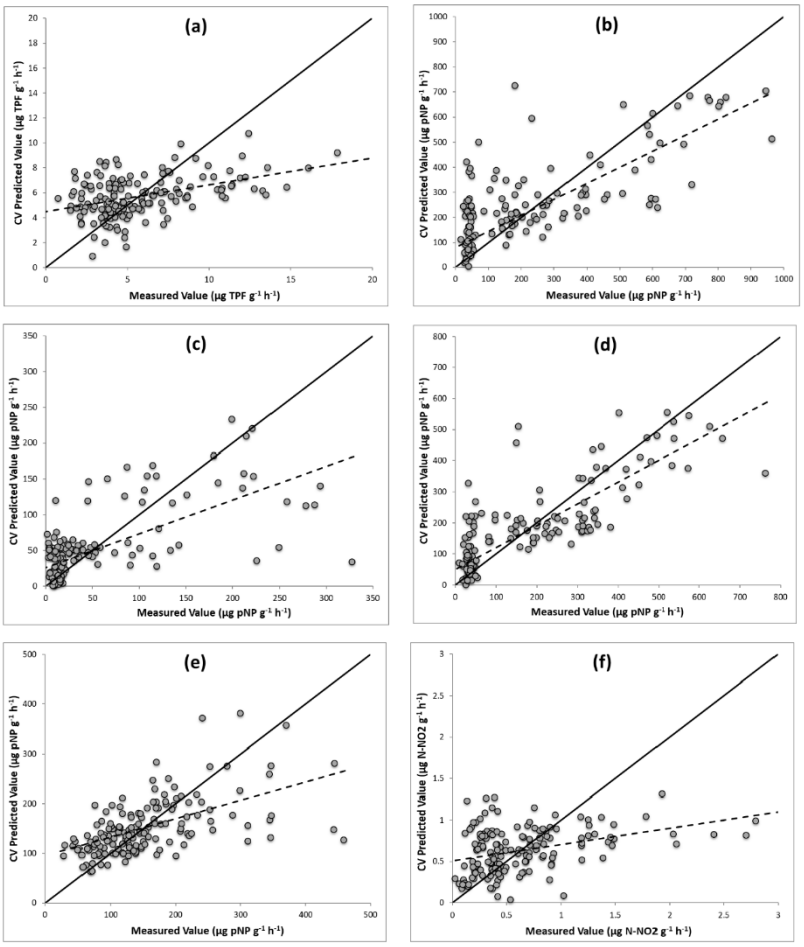

Figure 4. PLS predicted vs measured for (a) dehydrogenase, (b) B-glucosidase, (c) arylsulphatase, (d) acid phosphatase, (e) alkaline phosphatase, (f) potential nitrification. Solid line represents perfect fit and dashed line represents model fit.

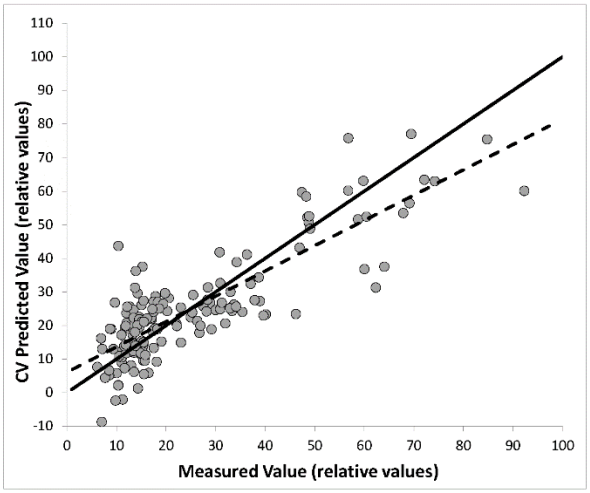

Figure 5. PLS predicted vs measured values for GMea index. Solid line represents perfect fit and dashed line represents model fit.

4. Conclusions

Combined soil indexes, such as the GMea, which is based on biological properties like individual soil enzymes, can be used as indicators of agro-environmental soil state as revealed in this study comparing organic and conventional olive groves. In addition, infrared spectroscopy proved to be quite sensitive not only to physicochemical but also to biological properties. NIR spectral features enabled relatively good discrimination among soils differing in biological quality, mainly due to soil type. Despite some limitations for quantitative prediction, especially individual enzymatic activities, this technique can be used to estimate the GMea index of different soils. Thus, NIR proved to be a technique sensitive to biological properties of the soil, useful for fast nondestructive assessment of soil biological quality. NIR spectroscopy can be especially useful for the expeditious identification of soils suffering severe degradation of biological activity. Hence, it would respond to the need for quickly estimated indicators of biological soil quality globally applicable to agro-environmental sustainability. However, further work is needed to develop more reliable models including a larger number of samples from different soil types and zones with a wide range of soil characteristics.

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Capítulo 8
Effect of irrigation water quality on soil properties and infrared spectroscopic signatures

Este capítulo se corresponde con el artículo "Effect of irrigation water quality on soil properties and infrared spectroscopic signatures", pendiente de envío a la revista Spanish Journal of Agricultural Research. En este artículo se estudian los efectos sobre el suelo de una irrigación con agua de baja calidad, y como esto se refleja en el espectro infrarrojo.

Esta publicación está pendiente de envío.
F. Comino, V. Aranda, M.J. Ayora-Cañada, A. Díaz, A. Domínguez-Vidal

Abstract

Irrigation is a common technique in agriculture that usually generates a production increase. This is particularly true for crops growing in semi-arid environments as it is the case of olive trees. However, the use of poor quality irrigation water can generate salinity and sodicity problems in soils, principally in the mid-long term. In this work, we study the case of an olive farm that has been irrigated for fifteen years with medium-low quality water where after some years of increase in olives production and olive oil yield, a decrease in both quantity and quality of the production have occurred in the last years leading to productivity values even lower than before irrigation. We studied soil samples of this farm, developed over siliceous and carbonated material. Half of the samples of each type were collected from areas directly affected by the irrigation bulb, and the other half from intercanopy. We found that soil physical properties have been clearly affected by irrigation, with low infiltration rates and structure destruction, being this effects more marked in the samples directly affected by the irrigation bulb. Chemical properties were also affected, showing alkaline pH, low amounts of OC and N, and what is especially concerning, high proportions of sodium. Again, this effect was more marked in the bulb samples. In addition, we also find that siliceous soils were more affected that carbonated ones, which can be explained by the positive effect in chemical properties of the higher calcium proportions in the latter soil typology. Finally, we investigated the use of infrared spectroscopy to asses sodicity and salinity problems in soils. We found that it is possible to discriminate theses samples affected by sodicity problems from similar soil samples from Jaen province, proving that this technique could be useful for a quick assessment of soil quality and soil degradation by salinity and sodicity problems.

1. Introduction

Soil salinity and sodicity is a growing problem that affect soils worldwide, being considered one of the major soil degradation threats occurring in Europe (Daliakopoulos et al., 2016). Saline and sodic soils cover 932.2 Mha globally, and about 31 Mha only in Europe (Rengasamy, 2006). Soil salinity generate unbalance in nutrients amount, normally with deficit for most of them (Grattan and Grieve, 1998), as well as a decrease of biological activity also unbalancing biogeochemical cycles (Rietz and Haynes, 2003; Zeng et al., 2013). Soil salinity is a problem of great importance for agriculture, since it causes water stress in the plant by reducing the osmotic potential of the soil, which ends up affecting vegetative growth (Setia et al., 2013; Shannon and Grieve, 1998) and even causing the death of some plants (Aragüés et al., 2005). Furthermore, high concentration of some ions such as sodium, chlorine or boron can cause toxicity and a reduced uptake of nutrients (Hardie and Doyle, 2012). By other side, high levels of sodium generate other problems, such as the dispersion of clays, when the sodium ion is introduced into the interlayer of phyllosilicates (Sumner, 1993) and the rapid decomposition of organic matter, decreasing its levels in the soil (Nelson and Oades, 1998). Soil sodicity, in addition, promotes destruction of the soil structure leading to a decrease in the infiltration rate, which in turn can generate soil crusting and sealing (Amezketa et al., 2003) limiting the posiblity of water infiltration.

The Mediterranean basin is encompassed within the semi-arid zones, and one of its most important crops is the olive grove. This climatic zone is characterized by rainfall scarcity and a highly irregular spatial and temporal distribution of rainfall events. Water availability is thus the main limiting factor of production in olive groves (García-Tejero et al., 2017). For this reason, olive trees respond very favorably to small water contributions complementary to rainfall, such as deficit irrigation (Ruiz-Sanchez et al., 2010). However, it is important to characterize the waters used for irrigation, in order to know their effects on the crop (Melgar et al., 2009), since the use of poor quality irrigation water with a high concentration of salts, particularly sodium, could cause salinization and/or sodification of soils (Jalali et al., 2008; Qadir and Oster, 2004). This problem is accentuated in regions of arid or semi-arid climate (Gorji et al., 2015) where the leaching and transport of soluble salts is not as efficient as in humid regions. It also has high economic importance when it occurs in previously non-saline soils, which become saline as a result of irrigation, affecting their fertility (Singh, 2015).

Salinity is not an unfamiliar problem for the olive tree since its productivity is affected by the quality of the soil despite its great adaptability to the environment. The influence of soil salinity in olives has been studied, demonstrating that can cause a decrease in the quality of the olive fruit (Zeleke et al., 2012), as well as problems in its vegetative growth (Rahemi et al., 2017), leading in extreme cases to the death of young plants (Aragüés et al., 2005; Benlloch et al., 1991). Luckily, the presence of sodic soils in the Andalusian olive groves (the main olive growing area in the world) is scarce, being almost non-existent in the rain-fed olive grove, but starting to show up in areas where high-water irrigation is carried out. Thus, it must be taken into account that the Mediterranean semi-arid zones are quite susceptible to salinization and sodification due to inadequate irrigation programs (Calero et al., 2013).

Considering the enormous agro-environmental and economic importance of the salinization and sodification problems in agricultural soils, it would be interesting to develop tools for an early diagnostic, in order to maintain a sustainable agriculture and environment (Herrero and Snyder, 1997). A possibility could be the use of infrared spectroscopy, a fast and robust analytical technology, tipically used for the assessment of different soil properties like carbon and nitrogen content, as well as other physical and physicochemical variables (Soriano-Disla et al., 2014; Vohland et al., 2014) including some biochemical properties (Comino et al., 2018; Reeves et al., 2000). Infrared spectroscopy has demostrated to be effective in discriminating between types of soils and management (Aranda et al., 2014). Some studies exist about the prediction of salinity and sodicity by using mapping techniques and satellite data (Castrignanò et al., 2008; El Harti et al., 2016), but few studies exist about the use of laboratory IR spectroscopy for the determination of salinity and sodicity related parameters in soils.

Thus the objective of this work was double: first, to study the effects in the medium term of the use of irrigation water with relative risk of sodicity and salinity on the quality of the soil. This study was performed in an olive grove in the province of Jaén (South Spain) where two different types of soil, siliceous and carbonated, can be found enabling a comparison of the effects. Second, to study the feasibility of infrared spectroscopy to detect salinity and sodicity in different types of soil.

2. Materials and methods

2.1. Study site and soil sampling

The study was conducted on an olive grove located in the municipality of Guarromán (Jaén), which is included in the central zone of the Sierra Morena region of Jaén. At the geological level, Guarromán is located in the border area of two major morphological domains: Sierra Morena and the Guadalquivir Depression, which in these latitudes manifests as a narrow depression known as the tectonic pit of Bailén-La Carolina. The predominant landscape consists of meadows of pastures with holm oaks, Mediterranean scrubland and forest species of repopulation. Climatologically the region is characterized, by its mild winters, with minimums of few degrees below zero, and hot, dry summers, with maximums above 40ºC. The average annual rainfall is about 560 mm.

The farm consists of 14.5 hectares of olive groves of the picual cultivar, with slopes ranging from 8% on average in the upper part, to 5% average in the lower part. The management consists of non-tillage with bare soil, with application of residual herbicide in autumn (first of October usually) and post-emergence herbicide in spring (last days of March usually), with the objective of avoiding nutrients and water competition by eliminating spontaneous vegetation. Fertilization is carried out through the application of mineral fertilizers, on the one hand nitrogenous compounds added to the soil through the irrigation water (fertirrigation system), in several applications made at spring, and on the other hand two foliar fertilizations, mainly phosphorus and potassium, along with micronutrients, which take place in autumn (October) and spring (April-May) respectively, coinciding with phytosanitary treatments. Productivity after irrigating increased considerably to 105 Mg. olives with an oil yield of 19.5-20.75%. After 15 years of irrigation, there was an important decrease in the quantity of the production to an average of 30 Mg of olives with an oil yield of 16.5-19%.

Two soil types were found in the study site, according to the Andalusian soil map (Junta de Andalucía, 2005) and the WRB for soil resources (FAO, 2006). Thus, in the farm we found a change of lithology, with calcareous (carbonated) and siliceous soils derived from Miocene calcarenitic and Triassic quartzitic parent material, respectively. The dominant soils in the calcareous area were Eutric Regosols and in the siliceous area Dystric Leptosols. Sampling consisted of a random selection of 10 locations, 5 corresponding to calcareous soils and 5 to siliceous soils. In each sampling area, two different samples were taken, one corresponding to the soil directly affected by the irrigation bulb (B) and the other one corresponding to the intercanopy soil, without direct irrigation influence (C). Samples were taken from 30 cm depth, being always composed of 4 different subsamples taken randomly. For further comparison, we will include in the study 71 olive grove soil samples from the Jaen province, with similar lithological material but different management systems, showing no salinization or sodicity problems.

2.2. Irrigation conditions and water analysis

The farm was put under irrigation in 1995 through the drip irrigation system, using water from its own well. Each tree is irrigated by two emitters of self-compensating type of 8 L/h, located on both sides of the trunk. The irrigation is extended for 8 hours every 3 days, each olive receiving 128 L. The irrigations begin between March and May, depending on the rainfall, and extend until the middle or end of September. The water coming from the proper sounding of the plot, which is used for irrigation and fertirrigation was analyzed to know its chemical characteristics. pH was measured using a CRISON micropH 2001 pHmeter. Electrical conductivity using a CRISON-522 conductivity meter. The cations (Ca, Mg, K and Na), by atomic absorption spectrometry (AAS), using a Perkin-Elmer AANLIYST800. Chlorides by titration with silver nitrate using as indicator potassium chromate. Sulphates by precipitation with acetone, centrifugation and resolving in the water, evaluating the calcium of the calcium sulphate formed with versanate (solution 0.001 N of EDTA). Carbonate and bicarbonate by titration with sulfuric acid to the equivalence points of carbonate anion (pH 8.3) and bicarbonate anion (pH 4.0). Nitrates by ionic chromatography. Boron by molecular absorption spectroscopy in UV-Vis. Likewise, different ratios were calculated, such as the sodium adsorption ratio (SAR), the residual sodium carbonate (RSC), the exchangeable sodium percentage (ESP), the total hardness, the Langelier saturation index (LSI), the Ca / Na ratio and the Ca / Mg ratio.

2.3. Soil analysis

Soil samples were air dried for 24h and sieved at 2 mm. Soil analysis were carried out following American Society of Agronomy and Soil Science Society of America official methods (Klute, 1986; Page, 1982). The pH was determined by potentiometry in distilled water (1:2.5). Electrical conductivity was measured with a CRISON-522 conductivity meter. Cations and anions were determined either in water or in the saturation extract. Cations, exchangeable bases and cation exchange capacity (CEC) were determined by the ammonium acetate method (pH 7) and the sodium chloride method, and then the concentrations were measured. The cations (Ca, Mg, K and Na) were determined by atomic absorption spectrometry (AAS), using a Perkin-Elmer AANLIYST800. Chlorides by titration with silver nitrate using as indicator potassium chromate. Sulphates were determined by precipitation as calcium sulphate with acetone, centrifugation and redisolution in water to finally titrate the calcium of the released with EDTA. Carbonate and bicarbonate

were determined by titration with sulfuric acid to the equivalence points of carbonate anion (pH 8.3) and bicarbonate anion (pH 4.0). Nitrates by ionic chromatography. Boron by molecular absorption spectroscopy in UV-Vis. Likewise, different ratios were calculated, such as the sodium adsorption ratio (SAR), the residual sodium carbonate (RSC), the exchangeable sodium percentage (ESP), degree of base saturation (S), the total hardness, the Langelier saturation index (LSI), the Ca / Na ratio and the Ca / Mg ratio. Furthermore, the organic carbon (OC) content was determined by the Tyurin method with dichromate oxidation and organic matter calculated through Waskman coefficient. Total N was measured with the Kjeldhal method. Equivalent CaCO3 was found by volumetry with a Bernard calcimeter.

Clay, lime and sand contents were determined by the Robinson´s pipette method after elimination of organic matter with H2O2 and dispersion with sodium polyphosphate. Saturated hydraulic conductivity (Ks, cm h−1) was measured by a constant-head permeameter (Eijkelkamp Agrisearch Equipment, Giesbeek, NL) in laboratory, using unaltered cores taken in the field. Soil bulk density was measured using the cylindrical core of known volume method. The soil aggregate stability index (ASI) was determined with the method described by Kemper and Rosenau (1986), using a wet sieving apparatus (Eijkelkamp Agrisearch Equipment, Giesbeek, NL).
Soil erodibility (USLE K factor) was calculated following (Wischmeier and Smith, 1978) equation:
K= 2.8 M1.14 (10-7) (12-OM) + 4.3 (10-3) (S-2) + 3.3 (10-3) (P-3)
where M is the particle size parameter calculated as (silt, %+very fine sand, %)
(100-clay, %), OM organic matter (%); S soil structure code (1=very fine granular,
2=fine granular, 3=medium or coarse granular, 4=block, platy, or massive); and P
the profile-permeability class (1=rapid, 2=moderate to rapid, 3=moderate, 4=low
to moderate, 5=slow, 6=very slow).
Spontaneous dispersion test (SDC), that analyzes the dispersion of clays induced by wetting of the soil samples, and mechanical dispersion test (MDC), that analyzes the dispersion of clays provoked by wetting and mechanical stirring of the soil samples (Rengasamy et al., 1984).

2.4. Infrared spectroscopy

Diffuse reflectance NIR spectra between 4000 and 12000 cm^{-1} were registered using an Antaris FT-NIR analyzer (Thermo Nicolet Corporation) with gold integrating sphere with internal reference, 4 cm^{-1} resolution, and 128 accumulations. The sample cup, with the sample finely grounded in an agate mortar, was placed on top of the integrating sphere optics and rotated during measurement. Samples were measured in triplicate, increasing the amount of sample scanned. The mean spectrum was used for each sample.

Mid-IR spectra were registered between 650 and 4000 cm^{-1} with a Varian 660 FTIR spectrometer with an attenuated total reflection (ATR, Pike Technologies) accessory with a three-reflection diamond crystal and a torque-limited pressure applicator to keep the sample in contact with the crystal. Samples finely ground in an agate mortar were placed directly on the ATR crystal and then pressure was applied to ensure good contact between the sample and the ATR element. Resolution was set to 2 cm^{-1} and 128 accumulations were recorded for each spectrum. The spectra of air on the clean dry ATR crystal was used as background. Bands interpretation was made according to (Ben-Dor et al., 1997; Viscarra Rossel et al., 2011) for near infrared region and (Changwen et al., 2007; Madari et al., 2006) for mid infrared region.

2.5. Statistical analysis

Data analysis included correlations between variables (Pearson's correlation coefficient) and ANOVA. Assumptions of normality were tested using Saphiro-Wilk test. For variables fitting a normal distribution, F-test Anova was applied, while an alternative non-parametric test (Kruskal-Wallis ANOVA) was applied for the rest of variables. Bonferroni test was used to investigate differences between means. These statistic procedures were carried out using Statgraphics Centurion XVI software (StatPoint Technologies, Inc.). Principal component analysis (PCA) technique was used to investigate the grouping between the samples. Prior to applying PCA, different spectra preprocessing were tested, including derivation, smoothing, detrend, baseline correction and mean centering in order to increase the signal/noise ratio. All chemometric treatments were performed using MATLAB 2008R (MathWorks, Natik, USA) employing the PLS-toolbox from Eigenvector Research Inc. (Wenatchee, USA).

3. Results

3.1 Irrigation water analysis

The results of the chemical analysis of the aquifer water used for irrigation can be seen in Table 1. We are facing a water of medium-low salinity as indicated by its low EC, as well as its content in total salts. It has a high sodium concentration compared to the rest of cations, although without showing high SAR. The most negative parameter for its use for irrigation is its high pH, due to the high concentration of bicarbonates, which causes high CSR values. According to water quality recommendations (Ayers and Westcot, 1985), the use of this water should not be restricted, since there is no risk of salinity, chloride or sodium toxicity. Nevertheless, there is a slight to moderate risk of decrease in the infiltration rate due to the combination of high concentration of bicarbonate and high SAR and EC that can affect the crop. With all these data, we can say this water is suitable for irrigation, however, when using it, it is necessary to control the possible effects that high bicarbonate concentrations, moderate sodium concentrations and high amount of total salts could have on the soil and the plant in the medium to long term. This condition must be particularly taken into account, in the case of being used for fertirrigation, where the quantity of salts is even higher.+.

Table 1. Results of chemical analysis of irrigation water

Parameter	Result	Parameter	Result
pH	8.3	Chloride (ppm)	29.6
C.E (dS/m)	0.6	Chloride (%)	13
Nitrates (ppm)	2.2	Sulfates (ppm)	23.2
Boron (ppm)	0.6	Sulfates (%)	8
Total Salts (g/L)	0.4	Carbonates (ppm)	0
Ca (ppm)	29.6	Carbonates (%)	0
Ca (%)	25	Bicarbonates (ppm)	297.5
Mg (ppm)	14.6	Bicarbonates (%)	79
Mg (%)	21	SAR	2.3
K (ppm)	9.5	RSC (%)	2.2
K (%)	4	ESP (%)	2
Na (ppm)	66.7	Ratio Ca/Na	0.5
Na (%)	50	Ratio Ca/Mg	1.2
Durity (dGH)	13.4	Langlier Index	0.24

3.2 Soil physical properties

The results of the physical properties of the soils studied are shown in Table 2. Different textures are found, ranging from sandy loam to clay. However, the dominant texture was sandy clay loam. No significant differences were observed in texture regarding neither the parent material nor the influence of irrigation (bulb-intercanopy difference). The bulk density values ranged from adequate to values that indicate compaction problems (Arshad et al., 1996). The values of bulk density are significantly higher for siliceous soil samples what could be related to the lower content of carbonates. There is also a trend towards higher values of bulk density in intercanopy soils, subject to the pressure of machinery transit. The soil aggregate stability index is far from the recommended value for all the samples, which is 0.7 according to Amezketa, 2009, which indicates poor structural stability throughout the entire farm studied. Values ranging from medium structural stability (0.4-0.6) to low (0.3-0.4) and very low (<0.3) can be found, being the latter range most common in the areas affected by the irrigation bulb. None of the studied soils were susceptible to spontaneous clay dispersion when wetted in absence of mechanical stress, which could be indicative of a high content of illite-like clays. On the contray, mechanical dispersion of clays at very low levels was observed, for siliceous soils located at the irrigation bulb. The infiltration behaviour, analyzed through the saturated hydraulic conductivity (Ks), is very low for all the samples. It is worth noting that water infiltration is generally lower for siliceous samples located at the bulb than for siliceous samples from intercanopy areas. Meanwhile, in carbonated samples we found the opposite results. According to Landon (1984), the levels of infiltration of some siliceous samples at the bulb could be classified as waterproof whereas those of carbonated samples can be regarded as very slow, indicating problems of infiltration along the whole farm. Finally, considering the low values of soil erodibility (K factor) found for all the samples there is no high risk of erosion regarding this parameter of the USLE equation, although a significant tendency to greater erodibility appears in the case of siliceous samples at the bulb.

Table 2. Mean, SD, t-test and ANOVA of physical properties of samples divided according to parent material, irrigation influence, and material + irrigation influence. Different letters stand for significance differences (p<0.005).

	Material		Irrigation influence		Material + Irrigation influence			
	Siliceous	Carbonated	Bulb	Intercanopy	Siliceous bulb	Siliceous intercanopy	Carbonated Bulb	Carbonated intercanopy
Sand (%)	61 ± 12 a	65 ± 7 a	65 ± 10 a	60 ± 9 a	64 ± 12 a	57 ± 11 a	67 ± 9 a	64 ± 4 a
Silt (%)	12 ± 6 a	13 ± 4 a	13 ± 4 a	12 ± 6 a	13 ± 4 a	11 ± 8 a	13 ± 5 a	13 ± 4 a
Clay (%)	27 ± 10 a	22 ± 6 a	22 ± 10 a	27 ± 6 a	23 ± 12 a	31 ± 6 a	21 ± 9 a	23 ± 2 a
Bulk density (gr cm^{-3})	1.62 ± 0.19 a	1.35 ± 0.15 b	1.44 ± 0.26 a	1.53 ± 0.16 a	1.6 ± 0.27 b	1.63 ± 0.08 b	1.28 ± 0.12 a	1.43 ± 0.15 ab
ASI	0.49 ± 0.18 a	0.42 ± 0.13 a	0.42 ± 0.19 a	0.49 ± 0.12 a	0.44 ± 0.25 a	0.55 ± 0.08 a	0.4 ± 0.14 a	0.43 ± 0.13 a
SDC (%)	0.5 ± 1.6 a	0.2 ± 0.4 a	0.7 ± 1.6 a	0 ± 0 a	1 ± 2.2 a	0 ± 0 a	0.4 ± 0.5 a	0 ± 0 a
MDC (%)	11.5 ± 9.4 a	3.1 ± 2.3 b	9 ± 11 a	5.6 ± 2.5 a	16 ± 12.1 b	7 ± 1.6 a	2 ± 1.6 a	4.2 ± 2.5 a
Ks (cm h^{-1})	1.35 ± 1.04 a	1.43 ± 1.71 a	1.59 ± 1.66 a	1.19 ± 1.08 a	0.82 ± 0.66 ab	1.89 ± 1.12 ab	2.37 ± 2.06 b	0.49 ± 0.34 a
Erodibility K factor	0.184 ± 0.092 a	0.168 ± 0.023 a	0.192 ± 0.087 a	0.16 ± 0.032 a	0.232 ± 0.112 b	0.136 ± 0.023 a	0.152 ± 0.016 a	0.184 ± 0.018 ab

Bulb: wetted bulb from drip irrigation system; ASI: aggregate stability index; SDC: spontaneously dispersed clay; MDC: mechanically dispersed clay; Ks: saturated hydraulic conductivity

3.3. Soil chemical properties

The analysis of different chemical properties of the studied soils are shown in Table 3. All soil samples have alkaline pH, many of them with values higher than 8.5, one of the main indicators of salinity and sodicity (Daliakopoulos et al., 2016). In addition, the samples affected by the irrigation bulb, and especially in the case of siliceous soils, have higher pH values than their equivalent intercanopy samples. The values of electrical conductivity are generally low, being higher for the samples affected by the irrigation bulb, but never close to worrying values that may indicate soil salinization (> 4dS/m). The studied soils are not very calcareous, with almost total absence of carbonates in the case of siliceous samples, and a relatively low amount in carbonated soils. Organic carbon values are very low, indicating a clear loss of organic material in these soils. The difference in the amount of organic matter regarding the area of sampling is probably related to the presence of olive roots in the bulb samples. Closely related to the OC is the total nitrogen, and in the same way higher values are found for bulb samples, although the content is low, in general, for all the samples. This entails a high C/N ratio for most samples (>12), characteristic of slightly humified organic matter, and soils with little nitrogen release.

The total level of cations in the soil solution is low. Sodium stands out for its greater proportion in siliceous soils, being the second cation in concentration in carbonated soils. It is also remarkable that the samples affected by the irrigation bulb have the highest sodium concentrations, always significantly higher than those in the intercanopy samples. Calcium appears as the predominant cation in carbonated soils, as expected, and second in importance in siliceous soils, while magnesium and potassium have lower concentrations. On the other hand, high bicarbonate contents are found in all soils, especially in those affected by the irrigation bulb. This also occurs with other anions, with relatively high concentrations of chlorides and to a lesser extent sulfates. These values begin to approach those of saline soils. All samples have an adequate CEC, although this is significantly lower in siliceous samples. The sodium adsorption ratio (SAR) has low values in most cases, but the mean is significantly higher for the samples located under the irrigation bulb. ESP, on the other hand, does have very high values in all samples, being especially high for siliceous samples, and particularly for those under the influence of the irrigation bulb. High levels of residual sodium carbonate (CSR) are also observed in the areas of the irrigation bulb, especially in siliceous samples, indicating an excessive concentration of bicarbonates. The degree of saturation with bases presents similar values for the different soils studied, being higher than 50% (eutric character), although a very marked imbalance is observed in the proportion of each of the cations within the exchange complex. According to Navarro Blaya and Navarro García (2003) values ranging 70-75% Ca, 6-15% Mg, 2-2.5% K and 7.5-20% Na should be found. In our soils a very low proportion of calcium is observed in the exchange complex, whereas higher amounts of sodium are found. This high proportion of sodium occurs more markedly in the samples under the irrigation bulb, as well as in the samples on siliceous material, being the samples that fulfill both characteristics those that show more worrying values reaching over 45% of sodium.

Table 3. Mean, SD, t-test and ANOVA of chemical properties of samples divided according to parent material, irrigation influence, and material + irrigation influence. Different letters stand for significance differences (p<0.005).

	Material		Irrigation influence		Material + Irrigation influence			
	Siliceous	Carbonated	Bulb	Intercanopy	Siliceous bulb	Siliceous intercanopy	Carbonated Bulb	Carbonated intercanopy
pH	8.5 ± 0.6 a	8.5 ± 0.1 a	8.8 ± 0.4 a	8.3 ± 0.3 b	9 ± 0.4 c	8.1 ± 0.4 a	8.6 ± 0.1 b	8.5 ± 0.1 b
EC (dS m^{-1})	0.68 ± 0.33 a	0.69 ± 0.2 a	0.88 ± 0.24 a	0.49 ± 0.09 b	0.91 ± 0.32 b	0.44 ± 0.1 a	0.84 ± 0.17 b	0.53 ± 0.04 a
OC (%)	0.7 ± 0.24 a	0.88 ± 0.24 a	0.9 ± 0.26 a	0.68 ± 0.2 b	0.76 ± 0.24 ab	0.65 ± 0.25 a	1.05 ± 0.21 b	0.72 ± 0.15 a
N (%)	0.05 ± 0.01 a	0.07 ± 0.02 b	0.07 ± 0.02 a	0.05 ± 0.01 b	0.05 ± 0.01 a	0.05 ± 0.01 a	0.08 ± 0.01 b	0.05 ± 0.01 a
C/N	13.7 ± 3.2 a	13.6 ± 2.6 a	13.6 ± 2.7 a	13.7 ± 3.1 a	14.2 ± 3.7 a	13.2 ± 2.9 a	13.1 ± 1.5 a	14.2 ± 3.6 a
Carbonates (%)	0.11 ± 0.22 a	6.99 ± 4.53 b	3.03 ± 3.95 a	4.07 ± 5.54 a	0.17 ± 0.3 a	0.04 ± 0.08 a	5.89 ± 3.83 b	8.1 ± 5.34 b
Ca^{2+} (ppm)*	27 ± 12 a	47 ± 13 b	35 ± 17 a	39 ± 15 a	29 ± 17 a	26 ± 6 a	41 ± 16 ab	52 ± 6 a
Mg^{2+} (ppm)*	11 ± 5 a	12 ± 7 a	15 ± 6 a	7 ± 1 b	13 ± 6 bc	8 ± 2 ab	17 ± 6 c	7 ± 1 a
K^{+} (ppm)*	17 ± 27 a	8 ± 2 a	9 ± 2 a	16 ± 27 a	9 ± 2 a	25 ± 38 a	9 ± 3 a	7 ± 1 a
Na^{+} (ppm)*	87 ± 56 a	70 ± 40 a	114 ± 44 a	43 ± 13 b	129 ± 49 b	46 ± 18 a	100 ± 37 b	40 ± 5 a
HCO_3^{-} (ppm)*	190 ± 100 a	205 ± 50 a	255 ± 63 a	140 ± 37 b	267 ± 83 b	113 ± 30 a	243 ± 41 b	167 ± 16 a
Chlorides (ppm)*	100 ± 59 a	91 ± 32 a	122 ± 53 a	68 ± 11 b	131 ± 72 b	68 ± 13 a	113 ± 30 ab	68 ± 10 a
Sulfates (ppm)*	29 ± 18 a	40 ± 44 a	42 ± 46 a	27 ± 8 a	32 ± 23 a	26 ± 11 a	53 ± 63 a	28 ± 5 a
SAR*	3.5 ± 1.87 a	2.46 ± 1.67 a	4.28 ± 1.69 a	1.69 ± 0.59 b	5.01 ± 1.31 c	2 ± 0.72 ab	3.54 ± 1.83 bc	1.38 ± 0.18 a
RSC*	0.97 ± 0.97 a	0.29 ± 0.51 a	1.2 ± 0.83 a	0.06 ± 0.13 b	1.82 ± 0.49 c	0.11 ± 0.17 ab	0.58 ± 0.61 b	0 ± 0 a
ESP (%)	21.9 ± 3.9 a	14.6 ± 1.4 b	19.8 ± 5.1 a	16.7 ± 3.8 a	24.1 ± 3.4 c	19.6 ± 3.2 b	15.5 ± 0.9 a	13.8 ± 1.3 a
CEC	18 ± 4.5 a	24.4 ± 1.2 b	21 ± 5 a	21.4 ± 4.4 a	17.6 ± 5 a	18.4 ± 4.5 a	24.4 ± 1.5 b	24.4 ± 0.9 b
Base Saturation (%)	51.6 ± 4 a	50.8 ± 2.3 a	52.4 ± 3.1 a	50 ± 3 a	52.6 ± 4.3 a	50.6 ± 4 a	52.2 ± 1.8 a	49.4 ± 1.8 a
Ca^{2+} ex.	3.74 ± 1.68 a	7.39 ± 0.97 b	4.97 ± 2.27 a	6.16 ± 2.29 a	3.05 ± 1.23 a	4.44 ± 1.91 a	6.89 ± 0.91 b	7.88 ± 0.83 b
Ca^{2+} ex. (%)	38.9 ± 9.5 a	59.6 ± 6.9 b	42.9 ± 11.9 a	55.6 ± 11.9 b	32.1 ± 4.2 a	45.8 ± 8.1 b	53.7 ± 2.7 c	65.5 ± 3.6 d

3.4. Infrared spectra characterization

The mean near-infrared spectra calculated after baseline correction for each of the four types of samples included in the study, are shown in Figure 1. The NIR spectra are dominated by absorptions due to the vibration modes (overtones and combination bands) of water molecules and lattice hydroxyl groups. Three main bands are observed. The first at 7062 cm^{-1} can be assigned to the with the 2nd overtone of OH stretching in clays like kaolinite. The second one at 5230 cm^{-1} is a combination band of the stretching vibration of OH in phyllosilicates and the bending vibration of interlayer water molecules. Additionally, the 2nd overtone of the carbonyl stretching (C=O) associated to different organic compounds of recalcitrant nature such as lignins or humic acids can also contribute to absorptions in this spectral region. Finally, the band at 4524 cm^{-1} band can be associated with the combination of OH stretching and deformation of Al-OH bonds in clays like kaolinite, illite and motmorillonite. Vibration bands of NH and OH bonds of organic matter can also contribute in this region. We can find slight differences related to the geological material, thus siliceous soils present higher intensity for the bands of 4524 and 5230 cm^{-1}, while the band at 7062 cm^{-1} associated with kaolinite is similar between the two types. In addition, a greater signal can be observed in siliceous samples in the range between 4250-4000 cm^{-1}, which indicates a higher concentration of illite. The presence of this mineral explains the low values of spontaneous dispersion of clays, as previously mentioned, differences in spectra according to irrigation influence are difficult to detect by simple visual inspection of the spectra.

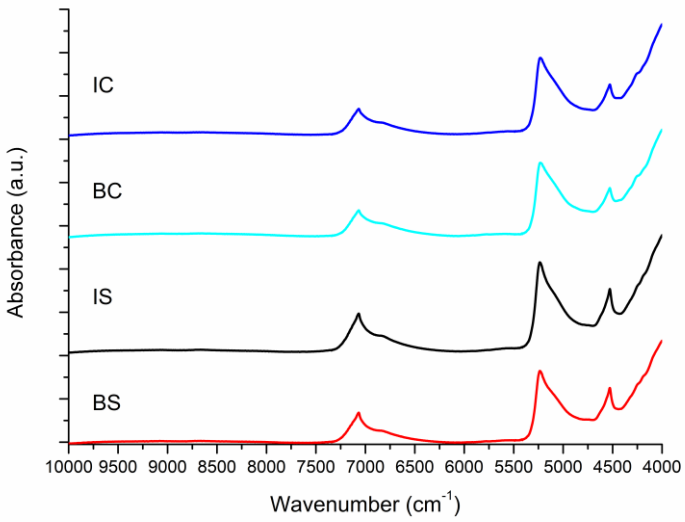

Figure 1. Mean FT-NIR spectra of four soil typologies studied. IC: intercanopy carbonated; BC: bulb carbonated; IS: intercanopy siliceous; BC: bulb siliceous.

On the other side, comparing the mean of the mid-infrared spectra of the four sample typologies (Figure 2) the most remarkable difference is due to the carbonate bands located at 1795, 1425, 872 and 710 cm^{-1}, clearly present only in the carbonated soils. In addition, we can observe how siliceous samples have a greater signal intensity in bands that can be associated to clays: 3695 cm^{-1}, attributed to OH-stretching of interlayer hydroxyl groups in kaolinite, 3620 cm^{-1} assigned to OH stretching of lattice hydroxyl groups in kaolinite, illite and montmorillonite as well as the band at 910 cm^{-1}, associated with kaolinite. It can also be

seen that there are slight frequency and intensity differences in the Si-O stretching mode, which is located at approximately 1030 cm^{-1} for carbonated samples and about 1000 cm^{-1} for siliceous soils. Polysaccharides also contribute to differences in the absorption bands in this region. In addition, the band located at 1640 cm^{-1} in all samples can be attributed to the bending vibration of interlayer water molecules. As in the case of NIR spectra differences between the intercanopy and bulb samples are not clearly visible to naked eye.

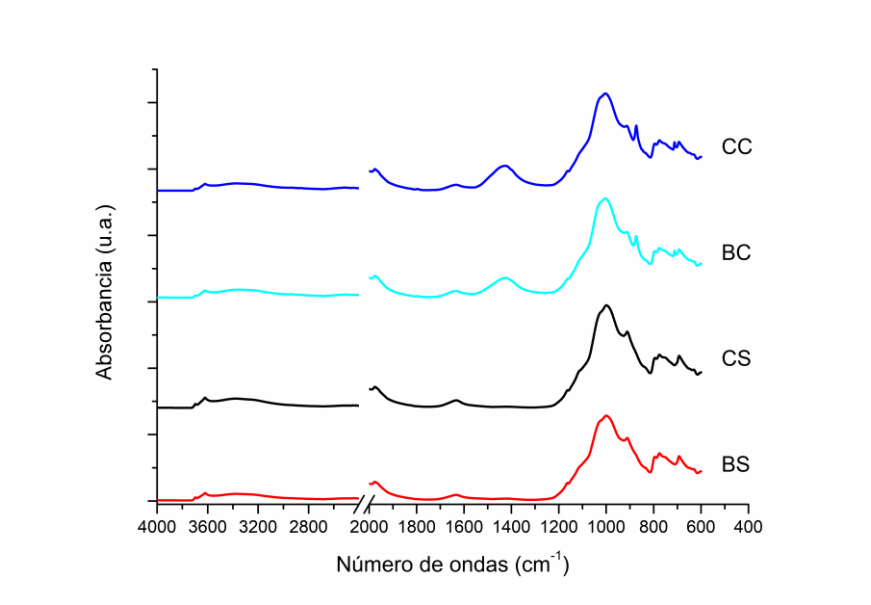

Figure 2. Mean FTIR spectra of four soil typologies studied. IC: intercanopy carbonated; BC: bulb carbonated; IS: intercanopy siliceous; BC: bulb siliceous.

In order to highlight the possible differences in the infrared spectra of the different sample typologies, a principal component analysis (PCA) was performed. To do this, the FTIR and FT-NIR spectra were concatenated after proper scaling. The score plot in the space defined by the first (PC1) and third principal (PC3) components is shown in Figure 3. We can see how samples are separated according to the type of soil mainly along PC3 (4.96% Variance). Positive values for PC3 are mainly associated to carbonated soils. The inspection of the loading vector of this component reveal the bands with more weight in this PC are the bands of carbonates (711, 871 and 1423 cm^{-1}), and a band at 1029 cm^{-1} that can be attributed to silicates and organic compounds little evolved like polysaccharides. On the contrary, siliceous soils with negative values for this PC, are characterized by the importance of bands of kaolinite and other clays (4528 and 7073 cm^{-1}) and illite (4204 cm^{-1}). The grouping of samples according to a combination of lithology and the influence of the wet bulb, is not so clear.

To verify the ability of infrared spectroscopy to discriminate between the soils studied, affected by salinity problems in different degrees, with typical soils of the province of Jaén not suffering from such problems, a PCA was performed on FTIR and FT-NIR spectra of the samples studied together with spectra of a wide database of soil samples from Jaén. An analytical summary of the samples included in this database is shown in

Table 4, where it can be seen that the basic pH predominates among the samples included, although there is a wide variety for most parameters, with levels of organic matter ranging from very low to acceptable levels and ions and cations contents varying in a wide range. In general, in these soils there are no problems of salinity or sodicity, although some of them have high values for some of the indicator parameters, such as SAR, ESP and EC.

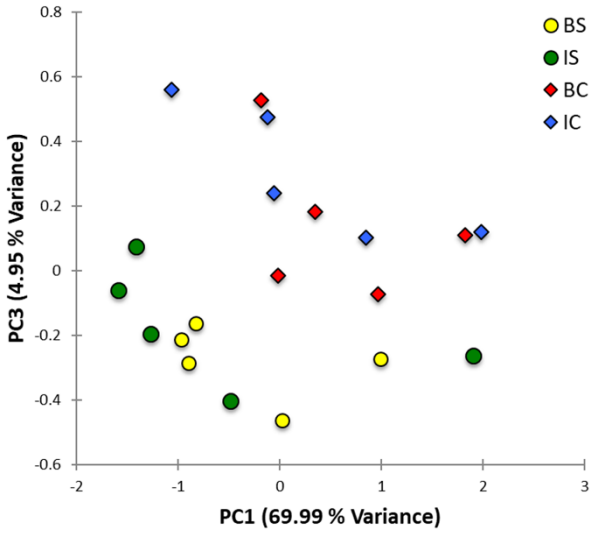

Figure 3. Scores for PCA of FTIR and FT-NIR spectra of study samples. IC: intercanopy carbonated; BC: bulb carbonated; IS: intercanopy siliceous; BC: bulb siliceous.

Table 4. Range and Mean ± SD of physical and chemical properties of soil samples in Jaén database (n = 70).

Parameter	Result	Parameter	Result
Sand (%)	30 - 78 50.1 ± 10.7	Chlorides (ppm)*	36 - 379 102 ± 69
Silt (%)	3 - 25 12.2 ± 3.8	Sulfates (ppm)*	12 - 1428 288 ± 380
Clay (%)	12 - 60 37.6 ± 9.6	Ca2+ (ppm)*	22.8 - 1114.2 146.9 ± 197
pH	6.8 - 9.5 8.3 ± 0.4	Mg2+ (ppm)*	4.7 - 203.8 24.5 ± 30.5
EC (dS m-1)	0.4 - 3.9 1.1 ± 0.8	K+ (ppm)*	38.2 - 598.7 125.3 ± 113.5
OM (%)	0.2 - 2.6 0.9 ± 0.7	Na+ (ppm)*	0.9 - 141.9 10.2 ± 22.1
Carbonates (%)	0.2 - 83.8 29.2 ± 18.8	SAR	0.3 - 8.3 1.9 ± 1.5
HCO3- (meq L-1)*	0 - 376 153 ± 62	ESP (%)	0 - 57 4.3 ± 9.3
		CEC (cmol+ Kg-1)	4.1 - 34.5 17.4 ± 6.8

EC: electrical conductivity at 25°C; OM: total organic matter

**: soil solution parameters*

In the score plot of the PCA analysis (Figure 4) we can see now a clear grouping of the studied samples along the PC2 (28.08% Variance), indicating a significant difference in the spectral signatures between the samples studied and the rest of soil samples from our database. These grouping also match reasonably with the values of ESP parameter, which is high in all the samples of the farm in Guarroman, and also high for the two samples from the database that show similar values for PC2 to these samples. According to the loadings of this PC, this discrimination is based on the greater weight for Jaén database samples of bands due to polysaccharides and silicates (1000 cm^{-1}) as well as those of carbonates (711, 871 and 1423 cm^{-1}). In a less extent certain influence of bands associated with more evolved organic materials (5227 cm^{-1}), illite (3620, 4000 and 4265 cm^{-1}) and kaolinite (4440 and 7000 cm^{-1}).

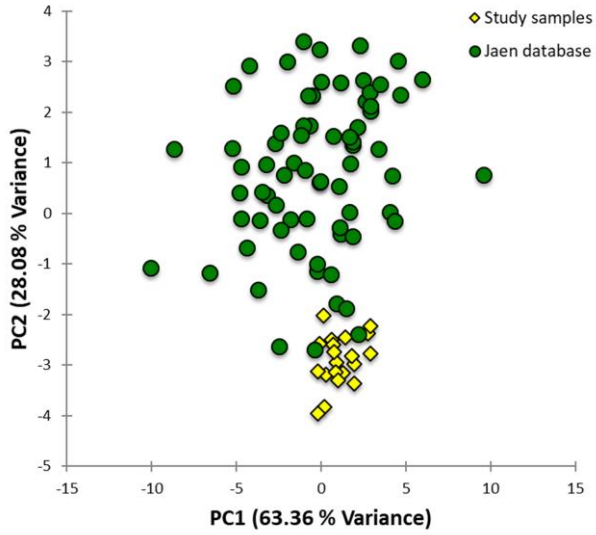

Figure 4. Scores for PCA of FTIR and FT-NIR spectra of study samples and Jaen database samples. IC: intercanopy carbonated; BC: bulb carbonated; IS: intercanopy siliceous; BC: bulb siliceous.

4. Discussion

4.1. Effect of irrigation on soil type

The results obtained show how the irrigation water used, although a priori should not pose any risk to the soil, with its continued addition has caused problems due to the large amount of sodium that is added to the soil through it, as well as because of its high bicarbonate content, which has caused sodification of the soil. To this we must add that, although this water alone was suitable for irrigation with slight restrictions, when used for fertirrigation, the amount of dissolved salts increases, which has probably aggravated and accelerated the problem of salinity and sodicity.

If we compare the effect according to the type of soil, we observe the great importance of this when it comes to responding to the application of irrigation water. We see how carbonated soils, as expected, have a higher concentration of calcium, both in the extract and in the exchange complex, as well as carbonate, due to the influence of the parent material. This causes, on the one hand, a tendency towards better maintenance of

the physical properties of the soil, with a lower bulk density, indicating the important role of calcium in improving and maintaining the structure, as opposed to sodium, in greater concentration in siliceous samples, which causes a destruction of the structure, making the soil more susceptible to compaction (M. Sumner, 1993). On the other, this greater amount of sodium in the siliceous samples, causes in these a greater dispersion of the clays, generating a greater destruction of the structure in these samples. This sodification has an even clearer effect on the chemical properties, producing a clear tendency to lower values of organic matter and nitrogen. This seems to indicate that sodium causes a greater decomposition of organic matter (Nelson and Oades, 1998), which is lost over time, and causes a clear loss of fertility in these soils. In addition, high sodium concentrations give rise to very high values of exchangeable sodium, in both soil typologies, but especially in siliceous soils, where it reaches values higher than 20%, which cause problems not only for soil, but also affect the health of the plant (Benlloch et al., 1991). Therefore, irrigation with this water causes degradation in both types of soil, being much more evident in siliceous soils than in carbonated soils, due to the attenuating effect of sodicity that produces in the latter its higher concentration of calcium (Li et al., 2013).

4.2. Effect of irrigation influence zone

No significant differences in physical parameters are found when looking for the effect of the irrigation bulb, although there are clear trends in the average values for bulk density, higher in intercanopy samples, and hydraulic conductivity, higher in the bulb areas. These differences indicate that the structure is equally affected throughout the entire farm, although the problems of infiltration are concentrated to a greater extent in the intercanopy areas, because it is through these where the agricultural machinery passes, compacting the soil, which causes both the increase in its bulk density, and a sealing process, which decreases infiltration. On the other hand, the practice of irrigation with sodium bicarbonate waters in this farm, has had very negative consequences on the chemical status of the soil, as can be seen in the samples affected by the irrigation bulb that have, on the one hand, higher pH values, SAR and especially the greater amount of RSC. This indicates that these areas are more strongly sodified as a consequence of irrigation, although the entire farm presents this symptomatology, as can be seen by its high ESP, somewhat higher again in the irrigation bulb samples. But in addition they can also be observed in the samples directly affected by the irrigation, higher values of EC, chlorides and bicarbonates, which indicates that a process of soil salinization is being generated, although it does not yet present such worrying values. It also stands out that in the bulb samples there is a higher concentration of sodium due to the more direct influence of irrigation, but also a higher percentage of magnesium in the exchange complex, which has replaced calcium to a greater extent in these samples, thus increasing all degradation processes previously described, by having the soil less calcium that helps generate and maintain the structure. Finally, it is curious the higher levels of organic matter and nitrogen in the bulb area, values that may be due to traces of the roots present in the soil profile.

4.3. Effect of irrigation influence zone on soil type

The pernicious effect of the use of this water on different types of soil has been proven, as well as the variation of this effect between the soils located right in the area of influence of the irrigation bulb with respect to the farm's more distant soils. The sodification and salinization is more accentuated in those samples located in siliceous soils and under the influence of the wet bulb. These samples show the highest values of bulk density, clay dispersion and erodibility, having theses samples the highest physical degradation, but they also have the highest values of pH, EC, bicarbonates, chlorides, sodium both in the saturation extract and in the exchange complex, and especially SAR and RSC. All these parameters seem to indicate these samples are very alkalinized, severely sodified, and also in a process of salinization, which is clearly subjecting the soil to degradation and loss of fertility. In addition, it is affecting the plants, which, as previously mentioned, have drastically reduced their production. Therefore, irrigation seems to be the main cause of this loss of production, both due to the physical affectation of the soil, and due to the probable blocking of nutrients due to the high pH and the high concentration of sodium. It is also noteworthy that bulb samples of carbonated soils, although presenting equally worrisome values in the previously mentioned parameters, have better resisted the process of sodification thanks to the effect of calcium, which on the one hand acts as a flocculating agent, helping the formation of structure, improving physical properties, and on the other hand when it is in higher concentration decreases the effect of sodium cations. Finally, it should be noted that the soils of both material, although not directly affected by the irrigation bulb, also show clear signs of sodification, indicating that the high concentration of sodium, over time, is affecting the entire farm, being able to turn it into sodic soils. However, in these samples there are no signs of salinization, since they present lower values of chlorides, EC and bicarbonates, indicating that the salinization process, at least for the moment, is only concentrated in the area affected by the irrigation bulb.

4.4. Infrared spectra for assesing salinity and sodicity state

Infrared spectra of siliceous samples are dominated by inorganic bands associated with illite and kaolinite, as well as bands associated the more evolved organic matter, while in carbonated samples carbonate bands and little evolved organic matter bands predominate. This highlight the great influence of the inorganic bands associated to parent material in the spectra. By another side, the lower degree of evolution of organic matter in carbonated soils could be due to a greater amount of total organic matter in these soils, due to a lower degree of decomposition of fresh OM, which may be associated with the protective effect exerted by calcium on the freshest organic matter. This fact, as described by (Duchaufour, 1976), could be caused by the physical retention of particulate organic matter, expected from a substrate particularly rich in carbonates. On the other hand, in siliceous soils the greater amount of sodium may have caused a more intense destruction of fresh organic matter, causing a relative accumulation of the most recalcitrant fraction. Irrigation influence also can be seen in the spectra, as intercanopy samples generally show a greater signal in most of the bands, especially those associated with organic compounds, which seems to indicate a better conservation of organic matter in these soils. Although this amount of organic matter does not coincide with the analytical data previously shown, this difference may be due to the fact that in the infrared spectra only some organic compounds are reflected, and when the ground soil is measured, the IR spectra does not take into account small roots and other contributions of organic matter. Finally, infrared spectra also show some

differences between the 4 types of soil studied. Intercanopy siliceous soils presents the largest amount of kaolinite and illite minerals, as well as some organic compounds that are little evolved, such as phenols and polysaccharides, and also more evolved and recalcitrant, indicating the best state of this soils respect to the soils of bulb siliceous, which presents the lowest signal for all this type of compounds. This seems to indicate the high degradation produced in the siliceous soil with irrigation bulb influence, where soil salinization and sodification due to irrigation have caused a loss of organic matter, generating a decrease in fertility. On the other hand, both carbonated soils typologies have a better state than siliceous bulb soil, but worse than intercanopy siliceous soils. This indicates that the sample of irrigation bulb located carbonated soils, responds much better to the effects of salinity and sodicity, largely due to the protective effect of calcium, which has mitigated the harmful effect of the addition of sodium. This is also seen in the comparison between the different carbonated soils, which shows a decrease in the amount of carbonates in bulb carbonated soils, probably because this carbonate has been depleted over time to alleviate the effect on the soil of the addition of sodium. Finally, it highlights the ability of infrared spectroscopy to detect these soils with low fertility due to salinity and salinity, when compared with soils with no problems but similar parent material like those included in Jaén database. This discrimination is made based on the low intensity of the bands of organic compounds, as well as minerals and carbonates. Although the IR spectra is not directly sensitive to the processes of salinity and sodicity, if it is able to detect these by its correlation with other compounds, and is a useful technique to discriminate soils with a decreased fertility, indicating that infrared spectroscopy could be used as a diagnostic tool for this kind of problem.

5. Conclusions

Irrigation is a practice that generates important increases in agricultural production, especially in semi-arid areas where water is usually a limiting factor. However, the use of low quality water can cause damage to soil degradation and productivity. In our study it was proved how the use of medium-low quality irrigation water affected the physical properties, generatin infiltration problems and structure destruction, and especially the chemical properties of the soil, generating salinization and sodification processes that decreased fertility. When comparing two contrasting soil types, it was found that soils developed on siliceous materials are much more vulnerable to this effect, while carbonated soils attenuate this effect due to the greater presence of calcium. In addition, as this effect was much greater in the area directly affected by the irrigation bulb, although the pernicious effect over time extends throughout the farm. We also demonstrated that infrared spectroscopy is a very useful technique to characterize soils and detect degradation, that is reflected specially in the organic phase of the soil samples. In addition, it proves to be a quick and economical technique, capable of detecting soil with lower fertility due to sodification and salinization processes.

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Capítulo 9
Near-infrared spectroscopy and X-ray fluorescence data fusion for olive leaf analysis and crop nutritional status determination

Este capítulo se corresponde con el artículo "Near-infrared spectroscopy and X-ray fluorescence data fusion for olive leaf analysis and crop nutritional status determination", publicado en la revista Talanta. En este artículo se estudia la capacidad de la espectroscopía FT-NIR y EDXRF para determinar el estado nutricional del olivo, aplicando diferentes estrategias de fusión de datos.

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Abstract

Leaf analysis is a useful way of diagnosing the nutritional status of the plants and therefore fast methods of analysis are demanded to aid in fertilization management decisions. In this work, a strategy based on the combined use of near-infrared spectroscopy (NIR) and portable energy dispersive X-Ray Fluorescence (EDXRF) is proposed as a suitable cheap and rapid alternative to traditional wet analytical methodologies. The approach has the major benefit of minimal sample preparation since leaves need to be only dried and ground. The ability of both techniques individually and applying two strategies of data fusion for the prediction of the most important plant nutrients, namely N, P, K, Ca, Mg, Mn, Zn, and B was tested. Predictive models were constructed using Partial Least Squares (PLS) to correlate the spectra with the nutrient contents. Models of unequal prediction performance in terms of the ratio of predictive deviation (RPD) were obtained for the different parameters when considering both techniques separately. Low-level data fusion, which consists of a concatenation of the raw data from both techniques, showed little improvement and even decreased the predictive ability for some elements. Better results were obtained with mid-level data fusion, that is, merging data after a feature extraction step performed by means of Principal components analysis (PCA). The results show that a fair quantitative prediction is possible for Ca, K and Mn with RPDs ≥2 for external validation, whereas models for N and P allowed a semiquantitative estimation. Mg and B models were less satisfactory and can be used only for distinguish between low and high levels, while Zn content cannot be predicted. Finally, the potential of the fusion of FT-NIR and EDXRF spectroscopic data for the fast screening of olive crop nutritional status has been tested. Deficiencies in important elements like N and K has been successfully detected.

1. Introduction

Efficient nutrient management is a major concern for the future global crop production, particularly in areas of intensive production practices. This is the case of olive cultivation in many areas of the Mediterranean countries, especially south European Union countries. Suitable fertilization decisions require characterization of plant nutritional status. The leaf-nutrient analysis provides an indication of tree nutritional status that can reveal deficiencies and represents an important tool for determining fertilization requirements. The determination of macro and micronutrients normally include wet chemical methods including titrations, colorimetry and atomic techniques like atomic absorption spectroscopy (AA) or inductively coupled plasma (ICP) with optical o mass spectrometric detection (1,2). All these methods have in common long and tedious sample preparation steps resulting in an expensive labor-intensive and time-consuming analysis. In the context of sustainable precision agriculture, there is a demand for the introduction of more efficient analytical methods that can replace the traditional laboratory routine analysis allowing for effective crop nutrition monitoring (3).

The use of spectroscopic techniques is increasingly extended as an alternative for traditional laboratory wet methods because they could provide similar results with the benefits of being much faster and simpler. In addition, these techniques are more environmentally friendly, since no chemicals or disposables are needed. In the case of vegetable tissues study, one of the most promising techniques is near infrared spectroscopy (NIR) with several examples of application in different plant leaves. Most of the studies have focused on the determination of N, with good results for apple, cotton and cucumber leaves (4–6). Other macronutrients, like P and K, have also been successfully predicted in sugarcane (7), and with more difficulties in other plants like tea, rice and fingered citron (8,9). The determination of micronutrients using FT-NIR spectroscopy is less common and in general, calibrations are poorer than for macronutrients (10–13). These previous studies indicated that the efficiency of FT-NIR spectroscopy in nutrition diagnosis differs among plant species (10). In the case of olive leaves, few studies are available for characterization of the nutritional status using NIR spectroscopy, only one for determination of N content (14) and another for detection of N and K deficiencies without quantifying (15). By another side, X-ray Fluorescence (XRF) is a technique that has been widely used for elemental analysis in very different fields (16), but its use in organic samples is much less extended. However, XRF, and particularly energy dispersive X-ray fluorescence (EDXRF) with portable instruments can be advantageous as a lower-cost alternative to ICP-OES and ICP–MS for direct analysis of leaf samples. In recent years, several studies of the application of this technique for element determination have been published for plants leaves (17–20). Generally, for EDXRF, heavy trace metals such as Mn, Fe, Cu, Ni, and Zn can be detected even with limits of quantification down to a few ppm. Higher concentrations are needed to quantify S, P, K, Mg, and Ca, whereas light elements like B and N are generally not detectable. In most of these studies, the sample was prepared by grinding to a very fine particle size, mixing it with a binder and pressing to produce a homogeneous sample pellet. The use of EDXRF with olive leaves has been reduced to the determination of elevated iron levels in plants infected by "sooty mold" (21) and determination of some elements in olive residual biomass including leaves (22). Up to our knowledge, no studies have been attempted to the determination of the nutrients in olive leaves using EDXRF.

In the context of leaf analysis for nutrient evaluation, it is clear that both spectroscopic techniques, FT-NIR and EDXRF spectroscopies, can provide complementary information, sharing the benefit of little sample preparation. Furthermore, since they are non-destructive techniques, the same sample can be easily analyzed by both instruments. However, it seems also clear that none of these techniques alone has the potential to provide complete nutrient status characterization, whereas their application in parallel is an interesting focus of research. In this work, we address the question of how to merge the information obtained from both techniques in the most efficient way. There are different strategies of data fusion (23), namely low-level data fusion, which consists in a fusion or concatenation of both raw data, mid-level data fusion, which consists in a previous variable selection or transformation using chemometrics, and to fuse these new variables to construct a new matrix and high level data fusion, a strategy better suited to gain qualitative information (24). These fusion strategies have provided good results when working with spectroscopic data from different sources, including visible, NIR, and Raman spectra, as well as compositional data obtained by XRF (24–28). However, no attempts have been made for the use of multiple spectra to characterize the chemical composition of plant leaves. In this work, we will evaluate for the first time the feasibility of combining the spectral information related to both molecular and elemental composition of olive leaves to determine the nutritional status by using different data fusion strategies.

2. Materials and methods

2.1. Leaf sampling and preparation

Leaves samples were obtained from foliar analysis laboratory Agronutrientes Jaén S.C.A. and correspond to more than 30 different municipalities of the provinces of Jaén, Granada, Córdoba, and Girona (Spain). The sampling procedure followed the Specific regulations of integrated olive crop production, BOJA, 2008, Junta de Andalucía (29). Each sample was composed by at least eighty healthy leaves caught from no less than ten olive trees. These leaves were always picked from branches at eye level, and taking the third and fourth pair of leaves of every branch. From each tree, leaves were harvested from the five orientations of the tree (Center, North, South, East, and West). Samples were collected from 2013 to 2016 years and include different olive cultivars (Picual, Arbequina, and Koroneiki). Leaf samples were washed with phosphate-free soap and distilled water to remove dust and any other contaminants. Samples were oven dried at 60 °C for 24 h and ground using an electric grinder. Samples were stored at 4 °C in the dark until use.

2.2. Spectroscopic techniques

Fourier transform NIR spectra were registered using an Antaris FTNIR analyzer (Thermo Nicolet Corp.) equipped with an integrating sphere module for diffuse reflectance measurements. Spectra were collected between 4000 and 10,000 cm−1, at 8cm−1 resolution with 128 accumulations. Sampling cups of 30mm were filled with ground leaf samples and placed on top of the integrating sphere window and rotated for measurement. The background was automatically recorded using the internal gold reference of the sphere. X-ray fluorescence spectra collection was carried out with a handheld Niton XL3t GOLDD+ (Thermo Fisher Scientific Inc.) equipped with a Rh tube anode of 45 kV and 40 uA maximum and a Geometrically Optimized Large Drift Detector (GOLDD) and placed in the shielded SmartStand (Thermo Fisher Scientific Inc.) to avoid

any scattered radiation. The instrument is provided with multiple programmable excitation filters that are optimized for specific ranges: main (40 kV), low (20 kV) and light ranges (10 kV). Approximately 3 g of sample were placed into XRF polyethylene sample cups of 32mm (24mm height and 26mm inner diameter) and covered with a polypropylene film. Infinitely thick geometry is assumed and "soils and minerals" measuring mode was used with 45 s for main range and 30 s for low and light ranges.

2.3. Nutrients determination

The most important plant macro and micronutrients were determined in dry leaf samples (2). N content was assessed by the Kjeldahl method which involves wet acid digestion. For the rest of the parameters, leaf samples calcination in a muffle furnace at 450 °C during at least 8 h was carried out. Phosphorous and B contents were then measured by colorimetry using the molybdovanadate and azomethine H methods, respectively. K was determined by flame atomic emission spectroscopy and Ca, Mg, Mn and Zn contents were determined by flame atomic absorption spectroscopy.

2.4. Chemometrics

All chemometric treatments were performed using MATLAB 2010a (MathWorks, Natick, USA) employing the PLS-toolbox from Eigenvector Research Inc. (Wenatchee, USA).

Hierarchical cluster analysis (HCA) was used to select representative samples to build the calibration dataset. To do this, four matrixes containing the analytical parameters of nutrients corresponding to each year were considered. The Ward's minimum variance method was used to create the dendrogram joining the existing clusters such that the resulting pooled within-cluster variance (with respect to each cluster's centroid) is minimized.

Different preprocessing methods were tested for FT-NIR and EDXRF spectra namely mean center, baseline correction, second derivative and detrend.

Partial least squares (PLS) regression method was used to build predictive models for nutrients from the spectra by calculating a small number of orthogonal factors (latent variables) that provide the maximum correlation with the dependent variable (concentration of the nutrients). Cross-validation by random subsets (5 groups and 5 iterations) was used to estimate the performance of the models when applied to new data. This involves that 27 samples are removed in each iteration and used as test set.

Two different data fusion strategies were applied and subsequent PLS regression for prediction of nutrients was tested. In low-level data fusion, FT-NIR and EDXRF spectra were fused by concatenation of the signals after scaling. In mid-level data fusion, a previous variable selection step is performed over the spectra obtained from each technique. Here, principal components analysis (PCA) was used to extract the most relevant features of EDXRF and FT-NIR spectra. Then, the scores of the principal components were autoscaled and joined to construct the fused data matrix.

The accuracy of all these predictive models was estimated by means of root mean square error of cross-validation (RMSECV) and prediction (RMSEP) and the ratio of predictive deviation (RPD) which is calculated as:

Eq 1. $RPD = \frac{SDv}{RMSEv\ x\ \sqrt{n/(n-1)}}$

where SDv is the standard deviation of the variable entered, RMSEv is the deviation in the model cross-validation/prediction and n is the number of samples.

3. Results and discussion

3.1. Chemical characterization of leaf samples and selection of the calibration dataset

In this study, a large population of olive leaf samples was available from crop years 2013–2016 and from different geographic origin. They were analyzed in Agronutrientes Jaén S.C.A. laboratory (Jaen, Spain) and a summary of the results is presented in Table 1S (Supplementary information). This large database provides valuable information about the concentration variations of the different macro and micronutrients in the area under study and constitutes a good estimate of the expected variation in the samples to be predicted. A relatively wide range of concentrations was observed for all nutrients, including low levels that can be considered as a deficiency in all cases except Ca, probably due to the presence of important quantities of calcium carbonate in the soils of these provinces. By another side, K, Ca, Mg, Mn, and Zn present very high values for some samples but never reaching the toxicity range. N is the only element showing a mean value near to deficiency (< 1.4%). This is typical of the Andalusian region (Jaén, Granada, and Córdoba) where this nutrient is, together with water, the main limitation (30).

However, when exploring the data, we found many samples with very similar ranges for some nutrients, which probably would not be beneficial for the construction of good predictive models. Furthermore, such a large dataset is not computationally effective and the model complexity may increase rapidly when more 'non-relevant' spectral variability is included in the exhaustive calibration, needing additional latent variables or introducing non-linearities that may require nonlinear calibration techniques (31). In order to make a selection of an adequate calibration dataset for the feasibility study, we applied a selection method based on Hierarchical Cluster Analysis to get a representative number of samples from each year. Dendrograms of each year were obtained (see Figure S, Supplementary information) and a distance that established between 12 and 18 groups was selected. The size of the groups differs from 15 to 20 samples to about 100 samples, depending on the total number of samples available for each year. For groups larger than 50, three samples were randomly selected for calibration, while for groups up to 50 samples, two of them were selected.

This resulted in a calibration set of 133 samples where excessive influence of the years with a large sampling in the models was prevented. Analytical data of these samples are also shown in Table 1S. If we compare these samples with the entire dataset, we can observe very similar ranges as well as mean values and standard deviation. On the other hand, an external set of validation was built with 60 samples randomly chosen from the entire dataset. In this way, we have a calibration set of approximately 2/3 and a validation set of 1/3 of the considered samples

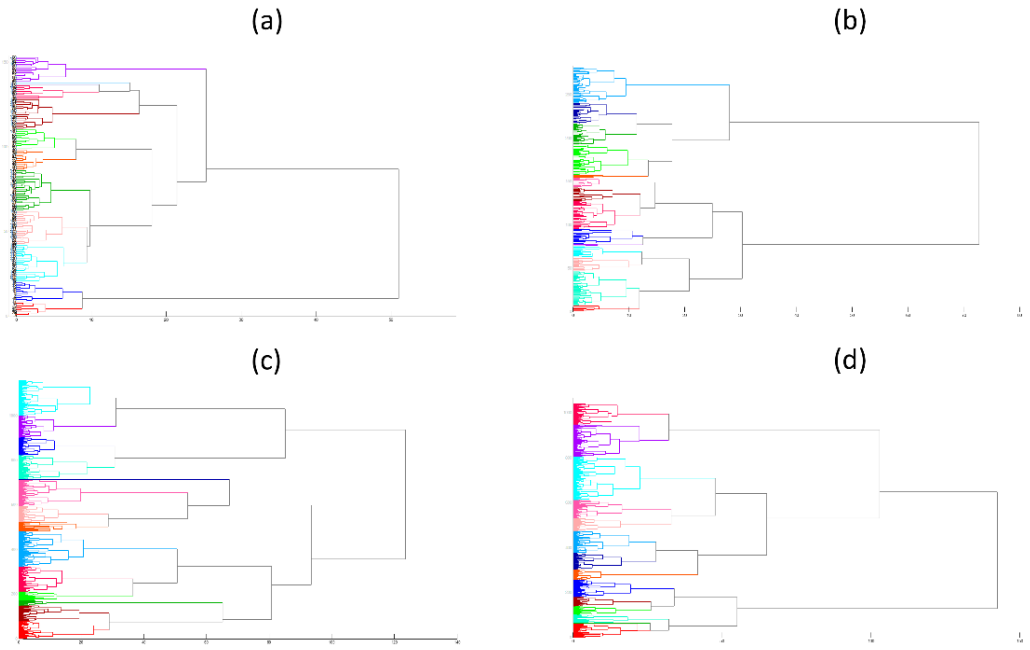

Figure 1S. Dendrograms obtained from the hierarchical cluster analysis applied to the chemical parameters of the years a) 2013, b) 2014, c) 2015 and d) 2016.

3.2. Nutrients prediction by regression models based on independent FT-NIR and EDXRF spectra

In a first approach, we built predictive models to test individual FTNIR and EDXRF capacity to estimate the contents of the different elements responsible for plant nutritional status. Different preprocessing treatments were applied to the spectra in order to improve the subsequent multivariate regression. The ratio of predictive deviation (RPD) is useful to compare the performance of the different models as it standardizes the corresponding RMSECV and RMSEP with respect to population spread. However, there is no statistical basis to determine the threshold in order to classify models according to their reliability and therefore there is no agreement in literature about the significance of a certain RPD value. Some authors estimate very high thresholds considering good model performances with RPD values from 5 (32,33). However, others consider that RPD values around to 2 already correspond to models with good predictive ability (34–36). Thus, in this work, we have considered this parameter in terms of comparison in between the performance of the different models tested.

Table 1S. Chemical characterization of nutritional state of leaf samples. Range, average and standard deviation are presented for each parameter.

Year	n		N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)	Zn (ppm)	B (ppm)
2013	152	Min - Max	0.94 - 1.75	0.047 - 0.198	0.42 - 1.42	0.61 - 2.52	0.067 - 0.21	11 - 71	7 - 57	13 - 55
		Mean ± SD	1.4 ± 0.1	0.11 ± 0.03	0.8 ± 0.2	1.2 ± 0.4	0.12 ± 0.03	27 ± 14	13 ± 4	34 ± 7
2014	281	Min - Max	0.86 - 1.78	0.046 - 0.174	0.33 - 1.31	0.63 - 2.62	0.048 - 0.224	11 - 69	6 - 36	13 - 39
		Mean ± SD	1.4 ± 0.1	0.08 ± 0.01	0.6 ± 0.2	1.6 ± 0.5	0.13 ± 0.04	26 ± 12	13 ± 3	23 ± 5
2015	1156	Min - Max	0.71 - 1.99	0.036 - 0.171	0.27 - 1.22	0.59 - 2.73	0.051 - 0.232	10 - 70	6 - 53	11 - 52
		Mean ± SD	1.4 ± 0.2	0.07 ± 0.02	0.6 ± 0.1	1.3 ± 0.3	0.12 ± 0.04	26 ± 11	13 ± 4	26 ± 7
2016	1042	Min - Max	0.67 - 1.99	0.037 - 0.175	0.28 - 1.44	0.63 - 2.73	0.056 - 0.234	9 - 75	5 - 56	11 - 49
		Mean ± SD	1.5 ± 0.2	0.09 ± 0.02	0.7 ± 0.2	1.4 ± 0.4	0.14 ± 0.05	29 ± 13	15 ± 6	28 ± 7
Overall	2530	Min - Max	0.67 - 1.99	0.036 - 0.198	0.27 - 1.44	0.59 - 2.73	0.048 - 0.234	9 - 75	5 - 57	11 - 55
		Mean ± SD	1.5 ± 0.2	0.08 ± 0.02	0.7 ± 0.2	1.4 ± 0.4	0.13 ± 0.04	27 ± 12	13 ± 5	27 ± 7
Calibration Subset	133	Min - Max	0.71 - 1.98	0.047 - 0.198	0.3 - 1.42	0.63 - 2.71	0.048 - 0.211	11 - 69	6 - 57	13 - 47
		Mean ± SD	1.4 ± 0.2	0.09 ± 0.02	0.7 ± 0.2	1.4 ± 0.5	0.13 ± 0.04	28 ± 13	13 ± 4	27 ± 7
Validation Subset	60	Min - Max	1.15 - 1.99	0.044 - 0.163	0.3 - 1.26	0.66 - 2.72	0.05 - 0.228	6 - 61	6 - 22	11 - 50
		Mean ± SD	1.5 ± 0.2	0.09 ± 0.02	0.7 ± 0.2	1.3 ± 0.4	0.12 ± 0.04	27 ± 11	12 ± 3	28 ± 8

In the case of FT-NIR, best results were obtained using spectral detrending and mean centering to eliminate baseline offset effects. In Figure 1. Typical FT-NIR spectra and b) Preprocessed FT-NIR spectra after detrend and mean center of leaf samples.raw and preprocessed FT-NIR spectra for the calibration samples are shown. The best results obtained for prediction of each element are shown in Table 1. Looking at the results, it can be deduced that Ca is the best-predicted element with RPD values of 2.5 and 2.1 for cross-validation and prediction performances, respectively. N and K models showed RPD values close to 2 that can be considered as good results. In the case of K, however, this result must be taken with caution because the model shows signs of overfitting as can be seen when comparing RMSEC with RMSECV and RMSEP values. Elements that are found in minor concentration showed inferior prediction results using the FTNIR spectra especially in the case of Zn. It is worth to mention that B was the only micronutrient with remarkable prediction results. This can be related to the influence of B status in the plant phenolic metabolism. Thus, B deficiency has been reported to be responsible for the accumulation of a distinct phenolic metabolite in olive leaves (37). Since FT-NIR spectroscopy is rather sensitive to phenolic compounds, this effect could be the reason for the better prediction results obtained for B in comparison with the other micronutrients at the ppm level.

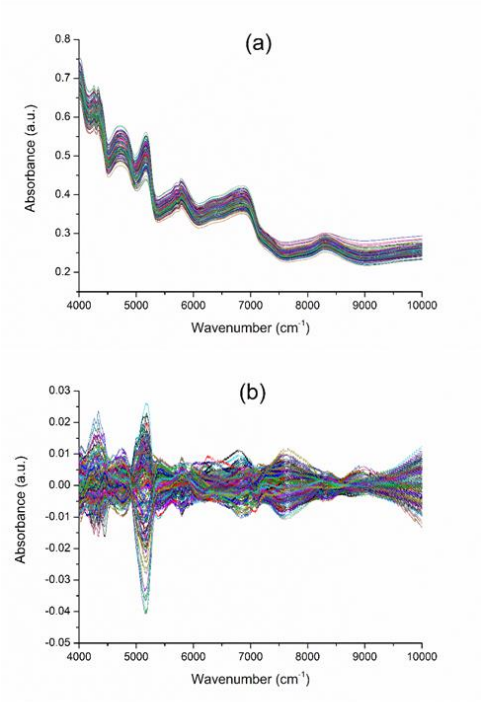

Figure 1. Typical FT-NIR spectra and b) Preprocessed FT-NIR spectra after detrend and mean center of leaf samples.

In the case of EDXRF, the X-ray fluorescence intensities are usually employed to evaluate the concentration of each element using either the fundamental parameters or empirical coefficients methods (38) based on the assumptions of sample homogeneity, plain surface, negligible particle size effects and a priori knowledge of the sample matrix composition. Such assumptions are not valid for the complex biological matrix of leaves composed mostly of low-Z elements. Recently, the use of PLS regression for EDXRF data has been proposed as a good alternative to deal with samples showing severe matrix effects, enhanced Compton scatter, elevated background as well as poor signal to noise ratio and overlapping bands (39). PLS

regression models for EDXRF data in the different measurements ranges of the Niton analyzer (Figure 2) were tested. As can be seen in Table 1, excellent predictive models were obtained with cross-validation for Ca and K, with RPD close to 3. However, the performance in external validation was notably lower particularly for K. P prediction is also reasonably accurate with RPD higher than 2 for CV and 1.6 for the external validation. This is particularly interesting because the measurement of this light element in EDXRF is difficult and no helium purge was used. However, in the case of Mg, the prediction ability was rather poor. The low contents of Mn and Zn in the samples (ppm level) lead also to limited prediction ability. Results obtained for most of micronutrients, as well as P and K were better than using FT-NIR. In general, single spectral ranges with optimized measurement conditions performed better than using a combination of the spectral ranges except in the case of N. This element cannot be determined directly by the use of EDXRF due to its low-Z, as also occurs with the B. However, maybe they could be estimated either by correlation with other elements or on the basis of different X-ray scattering peaks present in the spectra, as reported previously for several parameters of sugarcane (40). In any case, predictive models of limited ability were obtained for both elements using EDXRF.

3.3. FT-NIR and EDXRF data fusion for nutrients prediction

The main goal of data fusion is to exploit the synergies of individual information provided by different techniques by merging them to optimize the prediction of the parameters of interest (41). Here, to test if better results can be obtained with the fusion of FT-NIR and EDXRF spectra, two different data fusion strategies were used, namely low level and mid-level data fusion. Measurements level or low-level data fusion consists in a concatenation of raw data, with previous application of data processing to scale them. Thus, to fuse FT-NIR and EDXRF spectra, normalization was required as a first step, thereby reducing magnitude effects from larger signal intensities. Two different normalization procedures were investigated, namely scaling to unit length and scaling to unit area. FT-NIR data were fused with the three different measurement ranges of EDXRF (light, low and main) as well as with the combination of the three ranges. The number of variables in the x block was 1557 in the case of the FT-NIR spectra and 1999 in the case of EDXRF spectra.

Table 1. Summary of calibration and cross validation results of best PLS models constructed using FT-NIR and XRF individually.

				NIR				
Element	**N (%)**	**P (%)**	**K (%)**	**Ca (%)**	**Mg (%)**	**Mn (ppm)**	**Zn (ppm)**	**B (ppm)**
Number of LVs	9	8	13	8	12	12	3	14
RMSEC	0.086	0.011	0.016	0.151	0.011	4.145	1.957	0.807
RMSECV	0.111	0.016	0.103	0.191	0.029	11.372	6.241	4.511
RMSEP	0.091	0.025	0.120	0.198	0.030	9.075	5.660	7.358
R2 Cal	0.82	0.8	0.99	0.9	0.91	0.89	0.88	0.99
R2 CV	0.71	0.62	0.73	0.85	0.39	0.26	0	0.63
R2 Pred	0.73	0.22	0.65	0.78	0.3	0.41	0	0.33
RPD CV	1.8	1.6	1.9	2.5	1.3	1.1	0.9	1.6
RPD Pred	1.8	0.9	1.5	2.1	1.2	1.3	0.6	1.1
				XRF				
Range	**Light Range**	**Light Range**	**Low Range**	**Low Range**	**Light + Low + Main Range**	**Light + Low + Main Range**	**Light + Low + Main Range**	**Light + Low + Main Range**
Element	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)	Zn (ppm)	B (ppm)
Number of LVs	6	8	3	3	5	8	12	5
RMSEC	0.119	0.007	0.060	0.158	0.030	5.492	0.747	4.767
RMSECV	0.135	0.012	0.064	0.161	0.032	8.817	5.575	5.199
RMSEP	0.166	0.015	0.111	0.182	0.033	7.041	3.333	6.747
R2 Cal	0.65	0.93	0.9	0.89	0.35	0.81	0.98	0.56
R2 CV	0.56	0.78	0.89	0.89	0.28	0.54	0.09	0.5
R2 Pred	0.28	0.59	0.62	0.8	0.11	0.65	0.21	0.28
RPD CV	1.5	2.1	3.1	3.0	1.2	1.4	1.0	1.4
RPD Pred	1.0	1.6	1.6	2.2	1.1	1.6	0.9	1.2

Cross validation was performed by random subsets with 5 groups and 5 iterations. FT-NIR spectra were preprocessed using detrend and mean centering and EDXRF spectra were preprocessed using mean centering.

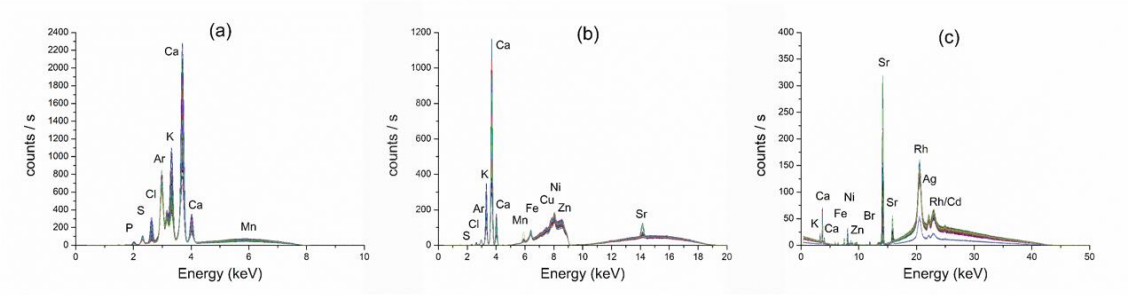

Figure 2. Typical EDXRF spectra of leaf samples showing the different energy filters available on the soils mode of the instrument, a) light, b) low and c) main ranges.

Different data treatments were also tested for fused spectra to improve model performance. In general, detrend and mean center were the most useful ones. The performance of the models showing the best results for each nutrient is shown in Table 2. Comparing these results with those obtained with each spectral technique individually, a certain decrease in model performance is observed for N and in lesser extent to B in comparison with FT-NIR results. This is probably because of the low sensitivity of EDXRF to these elements that cannot be directly determined by using this technique. The prediction for Mn is in between the provided by both techniques separately. The improvement observed for Zn, with 10% increase on RPD (CV) must be taken with caution because the difference in the values of RMSEC, RMSECV, and RMSEP indicates model overfitting. This can be also detected with the coefficients of determination (R2). Therefore, the robustness of the predictive model is questionable. The prediction performance for the rest of the elements is similar to the best obtained for either FT-NIR or EDXRF individually. These results seem to indicate that this data fusion level is not the best option to improve the predictive potential of FT-NIR and EDXRF spectra.

Table 2. Summary of calibration and cross validation results of best PLS model constructed using low-level data fusion of FT-NIR and XRF data.

Element	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)	Zn (ppm)	B (ppm)
Number of LVs	5	8	3	3	5	4	11	8
RMSEC	0.115	0.006	0.062	0.160	0.027	7.626	1.613	2.305
RMSECV	0.130	0.012	0.064	0.166	0.031	10.670	5.073	4.915
RMSEP	0.159	0.014	0.113	0.177	0.039	7.797	3.998	7.092
R2 Cal	0.67	0.94	0.9	0.89	0.46	0.63	0.92	0.9
R2 CV	0.59	0.79	0.89	0.88	0.32	0.41	0.2	0.56
R2 Pred	0.32	0.58	0.61	0.81	0.05	0.54	0.04	0.25
RPD CV	1.6	2.1	3.0	2.9	1.2	1.2	1.1	1.5
RPD Pred	1.0	1.6	1.6	2.3	0.9	1.5	0.8	1.1

Cross validation performed by random subsets with 5 groups and 5 iterations. Matrix preprocessed using normalize.

Another strategy for data fusion is the feature level or mid-level. This involves selecting or reducing variables before fusion and building the regression model. The most common approach for variable reduction is to fuse the information from a reduced number of latent variables obtained independently from the signals of each instrument. Here, the scores from the principal component analysis (PCA) were fused. PCA was applied to FT-NIR and EDXRF data. For EDXRF spectra, models including each energy range individually and the combination of the three ranges were considered. Autoscaling was applied to PCA scores prior to their fusion to generate new data matrices that combine the most relevant EDXRF and FT-NIR features. Considering the different possibilities of PCA scores for each technique (combining spectral pre-processing and the different measurement ranges available for EDXRF), we finally generated 33 fused data matrices (see Table 2S. *Summary of the different pretreatments used to perform PCA for feature selection in mid-level data fusion.* supplementary information). The number of PCs retained for each spectroscopic technique was determined by the best prediction performance during the cross-validation process. The capability of the model for the prediction of unknown samples the mid-level strategy was tested projecting the spectra of the 60 independent samples on the PC loadings of FT-NIR and EDXRF to calculate the scores of each sample that were then fused. Best results of PLS models in the mid-level data fusion are shown in Table 3.

With this data fusion strategy, no decrease in prediction performance was observed for any element in comparison with the best results obtained with the individual models. Thus, it was possible to obtain reliable predictions for Ca, K and N fusing the scores of 12 FT-NIR PCs (spectra mean centered and detrend) with the scores of 8 PCs of EDXRF low energy range (normalized). This approach was also used for Mg and B with more discrete results (RPD~1-1.5). A slight increase in Mg prediction with respect to the individual models was observed with an RPD increase of 9.7 % in the cross-validation, but with no differences in the external validation. In the case of Zn, neither the individual nor fused data matrices provided acceptable results.

For the prediction of P, it was necessary to use the light energy range of EDXRF (2nd derivative and mean center retaining 10 PCs). The RPD values for this element are very similar to the results obtained with EDXRF spectra alone. Finally, Mn prediction using mid-level data fusion provides an RPD value of 2.8 in cross-validation and 1.7 in prediction. This is a significant improvement in comparison with previous results using spectra separately or low-level fusion. In this case, the second derivative of the low energy range of EDXRF spectra (retaining 4 PCs) was used to construct the fused matrix. This was necessary due to the shape of the baseline observed in the Mn region (see Figure 3).

Table 2S. Summary of the different pretreatments used to perform PCA for feature selection in mid-level data fusion.

Matrix	NIR	XRF	
	Preprocessing	Range	Preprocessing
mid1	None	Light + Low + Main Range	None
mid2	None	Light + Low + Main Range	2nd derivative + Mean center
mid3	None	Light + Low + Main Range	Normalize (area=1)
mid4	Mean center	Light + Low + Main Range	None
mid5	Mean center	Light + Low + Main Range	2nd derivative+Mean center
mid6	Mean center	Light + Low + Main Range	Normalize (area=1)
mid7	Detrend+Mean center	Light + Low + Main Range	None
mid8	Detrend+Mean center	Light + Low + Main Range	2nd derivative + Mean center
mid9	Detrend+Mean center	Light + Low + Main Range	Normalize (area =1)
mid11	None	Low Range	None
mid12	None	Low Range	2nd derivative + Mean center
mid13	None	Low Range	Normalize (area=1)
mid14	Mean center	Low Range	None
mid15	Mean center	Low Range	2nd derivative + Mean center
mid16	Mean center	Low Range	Normalize (area=1)
mid17	Detrend+Mean center	Low Range	None
mid18	Detrend+Mean center	Low Range	2nd derivative + Mean center
mid19	Detrend+Mean center	Low Range	Normalize (area=1)
mid21	None	Light Range	None
mid22	None	Light Range	2nd derivative + Mean center
mid23	None	Light Range	Normalize (area=1)
mid24	Mean center	Light Range	None
mid25	Mean center	Light Range	2nd derivative+Mean center
mid26	Mean center	Light Range	Normalize (area=1)
mid27	Detrend+Mean center	Light Range	None
mid28	Detrend+Mean center	Light Range	2nd derivative + Mean center
mid29	Detrend+Mean center	Light Range	Normalize (area =1)
mid31	none	Light + Low + Main Range*	None
mid32	mean center	Light + Low + Main Range*	None
mid33	detrend+mean center	Light + Low + Main Range*	None
mid34	none	Light + Low + Main Range*	2nd derivative + Mean center
mid35	mean center	Light + Low + Main Range*	2nd derivative + Mean center
mid36	detrend+mean center	Light + Low + Main Range*	2nd derivative + Mean center

** Scores of individual PCA for each range joined.*

Table 3. Summary of calibration and cross validation results of best PLS models constructed using FT-NIR and XRF mid-level data fusion level.

Element	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)	Zn (ppm)	B (ppm)
Number of LVs	3	6	7	2	11	7	6	2
RMSEC	0.087	0.010	0.051	0.135	0.021	2.834	4.876	4.035
RMSECV	0.110	0.013	0.067	0.162	0.027	4.588	6.376	4.769
RMSEP	0.094	0.014	0.110	0.183	0.036	6.867	5.201	6.473
R2 Cal	0.81	0.84	0.93	0.92	0.67	0.94	0.23	0.69
R2 CV	0.71	0.74	0.89	0.89	0.5	0.85	0	0.58
R2 Pred	0.68	0.6	0.63	0.8	0.25	0.71	0	0.34
RPD CV	1.8	1.9	2.9	3.0	1.4	2.8	0.9	1.5
RPD Pred	1.7	1.6	1.6	2.2	1.0	1.7	0.6	1.2

Cross validation performed by random subsets with 5 groups and 5 iterations. Matrix preprocessed using autoscale.

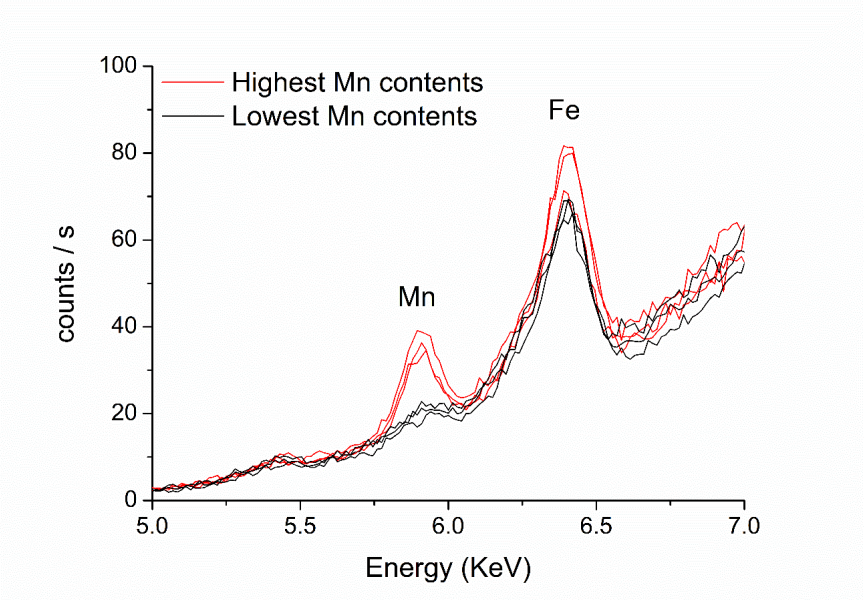

Figure 3. EDXRF spectra (lowest and highest Mn contents) focused in the region of Mn emission lines.

Summarizing, the strategy of fusing EDXRF and FT-NIR spectra at feature level provides the best results in terms of prediction even when the improvement with respect to individual models is slight. Taking into account the RPD values and according to the criteria proposed by Viscarra-Rosell et al. (36), models for Ca, K and Mn can be considered good for quantitative predictions, while the models for N and P, can be considered fair models, useful only for assessment and correlation, with room for improvement. In the cases of Mg and B models, they present capacity only for distinguish between low and high levels. The model for Zn has scarce predictive capacity and it cannot be considered useful. Although RPD is a useful parameter to evaluate the prediction errors that enables comparison between the qualities of models for different constituents with different variation ranges, a deeper analysis can be done by plotting the predicted values in front the actual values in test samples (see Figure 4). For Ca and Mn, the line of adjust of cross-validation (black line) and external validation (red line) appear near of each other, and close to the line of the perfect adjust (green line). The slopes ranged from 0.8 to 0.9 with confidence intervals (95%) of 0.05-0.1. In the case, K, the slope of the line of cross-validation is also 0.91, close to perfect adjust, although for external validation the results are more spread. For N and P, the slopes are 0.7-0.8, which shows the low ability of these models to quantify with enough reliability but still provides a semiquantitative estimation of the content of each nutrient. The results for the rest of models are more limited and particularly for Zn, there is no correlation.

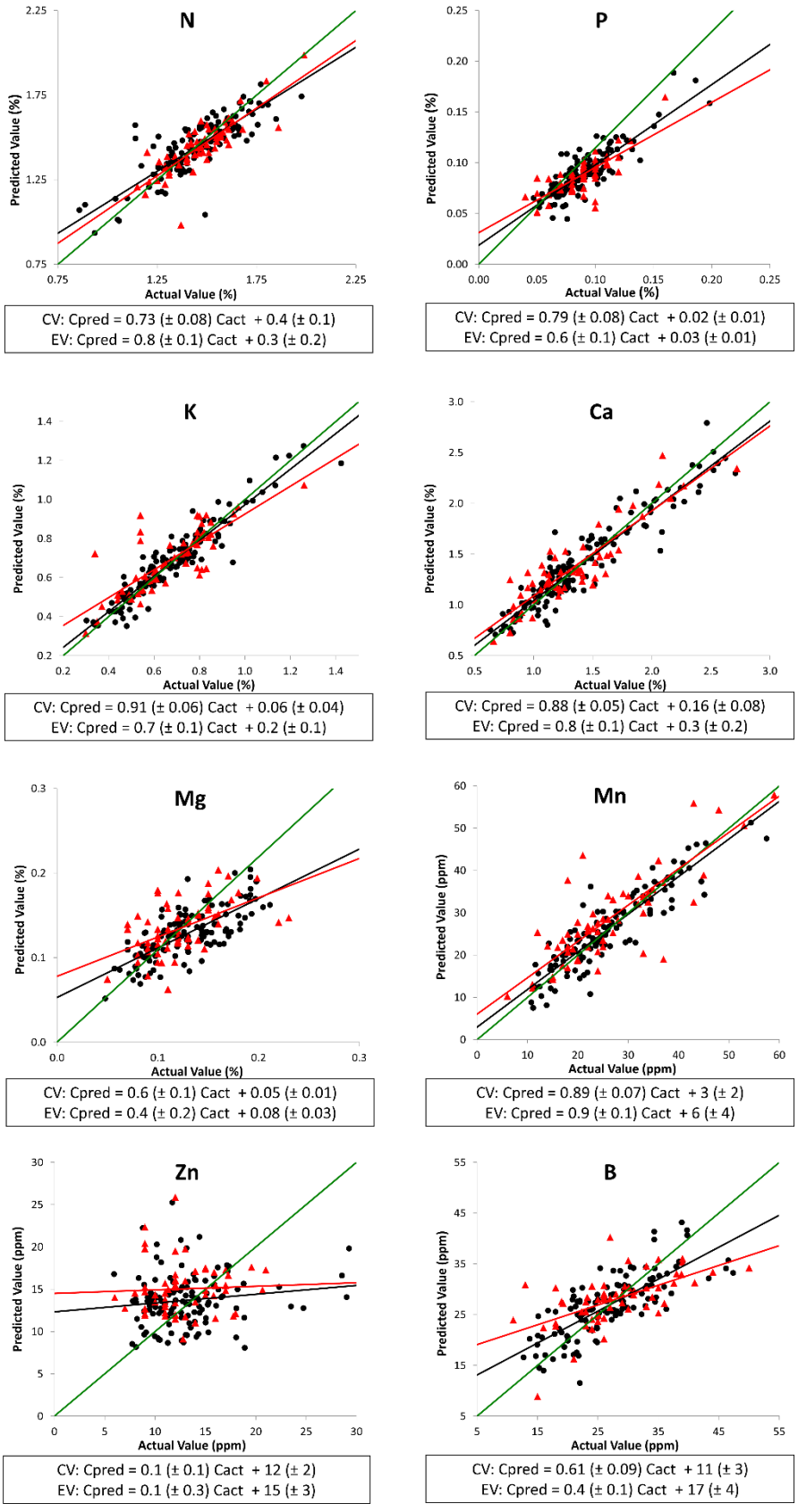

Figure 4. Cross-validation and external validation results using the mid-level data fusion for all the studied nutrients. Equations of the regression lines between predicted (Cpred) and actual (Cact) contents are given with confidence intervals for the intercept and slope at 95% probability. Cpred =intercept (± confidence interval) + slope (± confidence interval) Cact.

Despite these limitations in the quantitative assessment of many nutrients, it is interesting to evaluate the possibility of using the contents predicted by FT-NIR and EDXRF fused data to perform a fast screening to detect deficiencies of a certain element in order to decide fertilization needs. To do this, we selected those nutrients with deficiency problems in our sample set that are N, K, B, and Mg. The predicted nutrient content, provided by fused models described above, together with the threshold level for nutrient deficiency have been used to classify the samples in two levels: deficient, thus need fertilization and no deficient. In Figure 5, these samples have been labelled in color accordingly, and the actual nutrient content values are plotted in Y-axis for all the samples for comparison. Furthermore, samples with predicted contents in the superior error margin of the approach (deficiency threshold + RMECV or RMSEP, doted red line in Figure 5) have been labelled in grey and considered as suspicious of needing fertilization.

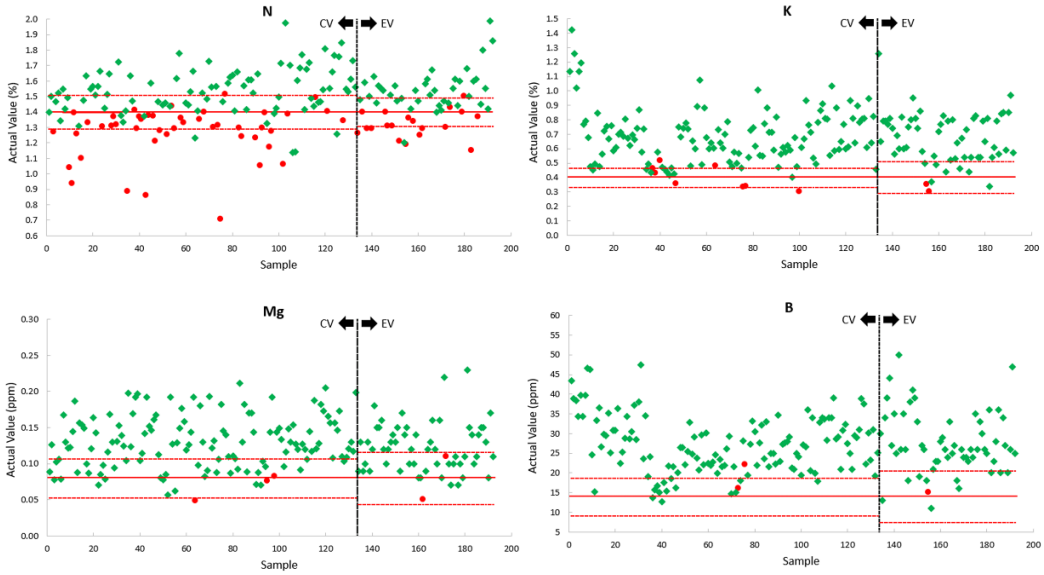

Figure 5. Screening for deficiencies in N, K, Mg and B. Actual contents vs sample number. Red line marks the deficiency level and dotted red line marks the decision level (deficiency level + RMSECV or RMSEP). Color labelling of samples is according to prediction results being red circle: deficient (< deficiency level), grey triangle: below decision level; and green diamond above decision level.

N is the nutrient with more deficiency problems being almost a third of the samples below the deficiency threshold (1.4%). With the proposed approach, most of the samples are correctly identified and only 2 samples (in cross validation) have not been identified as deficient (false negatives). It is worth mentioning that using the RMSECV or RMSEP as decision level reduces considerably the amount of false negatives, which is considered the most critical issue in fertilization programs. Of course, this leads to the classification of some samples with an actual content above the deficiency level as suspicious of needing fertilization. However, as can be seen in Figure 5 they have an actual content close to deficiency and, in all cases, far below the level considered the most appropriate for optimum yield (2.0 % in the case of N). Only few samples (4 in CV and 2 in EV) clearly no deficient were classified as deficient (false positives).

For the rest of the cases, the results are less conclusive because there are far fewer samples under the critical level. However, the results can be promising since only few false negative and false positives are found and again very close to the deficiency level.

4. Conclusions

The presented approach based on the combined use of FT-NIR and EDXRF spectroscopies can provide fast information about the nutrient content in plant leaves. The ability of both techniques for the prediction of nutrients separately has been evaluated. In general, EDXRF spectroscopy was more powerful with the exception of light elements like N and B that were better assessed using FT-NIR. This is consistent with the fact that FT-NIR predictions are generally based on secondary correlations between elemental contents and spectroscopically active compounds. However, none of the techniques is capable to predict successfully all the elements, therefore both have to be used. The strategy of feature fusion (mid-level data fusion) based on principal component scores showed to be superior to measurement fusion (low-level data fusion). Even when the results of the mid-level fusion were comparable to the best obtained with the most suitable individual technique, it was especially useful for Mn with a clear improvement in RPD for the cross-validation. The results show that a fast screening is feasible since the fusion of FT-NIR and EDXRF spectra is able to detect samples with deficiency in N and the results are promising for K. This would be very useful from the agricultural point of view since these two nutrients are considered one of the main limiting nutrients in olive production (15,42). In comparison with conventional wet chemical analysis, the proposed approach has the important advantage of minimal sample preparation. Furthermore, since both spectroscopic techniques can be implemented in portable devices further improvements involving field measurements can be envisioned. This would be of particular interest in the context of precision agriculture to support fertilization decision-making.

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Capítulo 10
Thermal destruction of organic waste hydrophobicity for agricultural soils application

Este capítulo se corresponde con el artículo "Thermal destruction of organic waste hydrophobicity for agricultural soils application", publicado en la revista Journal of Environmental Management. En este artículo se aplica un tratamiento térmico a diferentes enmiendas orgánica con el objetivo de eliminar su repelencia al agua.

La referencia de esta publicación, que ha sido citada 1 vez, es:
Comino, F., Aranda, V., Domínguez-Vidal, A., Ayora-Cañada, M.J.
Thermal destruction of organic waste hydrophobicity for agricultural soils application.
J. Environ. Manage. (2017) 202, 94-105. doi:10.1016/j.jenvman.2017.07.024

Abstract

Use of organic amendments is a good strategy for combating the growing problem of soil degradation due to deterioration of organic matter content, particularly severe in semi-arid European Mediterranean regions, while at the same time providing an opportunity for recycling organic wastes. Olive mill pomace (OMP), the main by-product of the olive oil industry, is being used increasingly in olive grove soils for this purpose. Although the positive effects of OMP amendments have been widely studied, they also have some negative effects on soil. One of the most critical is that they increase water repellency (WR) due to the presence of poorly evolved, strongly aliphatic compounds. This detrimental effect has received very little attention, although it may impair plant water availability and infiltration rates, increase erosion and lower long-term soil quality. This study proposed, for the first time, thermal treatment as an effective way of reducing WR in organic amendments (i.e. mixtures of OMP, olive tree pruning, chicken manure and spent coffee grounds) prior to their application to soil. Thermal treatment at 275ºC proved effective in removing WR, while lower temperatures (175 or 225ºC) can even increase it. Changes by thermal treatment in the characteristics of the organic amendments studied with FTIR and UV-Vis spectroscopy and thermogravimetric analysis showed that it strongly reduced the aliphatic compounds mainly responsible for their hydrophobicity, concentrated aromatic compounds and increased thermostability. Heating also reduced phytotoxicity, making all of the organic amendments usable in the field (germination index over 100%). Therefore, heating at 275ºC could be an acceptable option for removing WR from organic amendments, enhancing their quality with more stable evolved characteristics.

1. Introduction

Olive groves are of enormous importance in the Mediterranean economy. They produce around 2.5 million Mg of olive oil per year, which is over 90% of the world production (COI). Unfortunately, poor agricultural practices have reduced soil organic matter levels, leading to severe soil degradation. One possible solution is to restore organic matter in the soil with the addition of appropriate organic amendments (Pérez-Lomas et al., 2010). As underlined by Almendros et al., (2005) the assessment of the quality of soil organic matter, including the amendment incorporated to the soil, rather than its total concentration, must be considered.
On the other hand, the extensive olive cultivation leads to a huge annual production of olive mill pomace (OMP), the main by-product of the two-phase olive oil extraction system. Due to its high polyphenol, lipid and organic acid concentrations, it is phytotoxic and antimicrobial (Serramiá et al., 2010), and therefore, highly pollutant. This makes OMP a considerable management problem in the olive agro-industry (López-Piñeiro et al., 2007). However, it has interesting possibilities for use as organic amendment after composting, which eliminates the harmful substances (Fernández-Hernández et al., 2014). The positive effects of the use of composted olive mill pomace (COMP) on soil fertility have been widely studied (García-Ruiz et al., 2012; Lozano-García and Parras-Alcántara, 2013), but there are still some disadvantages like high pH (often above 8). However, the most critical factor is increased soil water repellency (WR) due to hydrophobicity generated by accumulation of hydrophobic compounds from the decomposition of fresh organic matter with little alteration or humification. This affects the soil water regime, reducing the infiltration capacity (Mohawesh et al., 2014), hydraulic conductivity and plant water availability (Doerr and Thomas, 2000). It could therefore generate or increase soil surface flow, and consequently, significantly increase risk of erosion (Diamantopoulos et al., 2013), especially at the beginning of the rainy season. This would be amplified on sloping surfaces, for example, by ridges in ploughed agricultural soils (Ahn et al., 2013). WR could induce a preferential flow in soil, causing irregular distribution of water and nutrients, and accelerating pollutant transport to groundwater (Arye et al., 2011). This can affect large areas and thus cause serious problems in agricultural production and plant growth (Vogelmann et al., 2013). Increased WR is also observed in other types of organic amendments (Liyanage and Leelamanie, 2016). In Mediterranean soils, this is a particularly severe problem, because the climate is characterized by very irregular rains and strong seasonal contrasts, which could exacerbate these adverse processes. High WR in Mediterranean agricultural soils has been found to be induced not only by soil type, but also by organic amendments (Aranda et al., 2016). Study of fire-induced changes in soil properties has revealed that water repellency can be diminished at temperatures above 250ºC, and similar findings have been reported in laboratory simulations with raw organic matter (Mataix-Solera and Doerr, 2004). High temperature diminishes WR due to thermal degradation or vaporisation of the organic substances responsible for hydrophobicity (Simkovic et al., 2008). These findings suggest that thermal treatment of OMP amendments before application could reduce the aforementioned problems associated with the increase in WR. Composted OMP for use as an organic amendment can also be improved by adding other organic matter sources, like spent coffee grounds, another waste by-product, which arouses much scientific interest and an industry that produces millions of tonnes worldwide. Part is recycled by processes such as composting, although its use as a soil amendment has not been well studied (Cruz and Marques dos Santos Cordovil, 2015).

This study evaluated the feasibility of using different thermal treatments to increase the quality of several organic amendments based on olive mill pomace by eliminating their hydrophobicity. The functional characterisation and stability of these materials was studied using ATR-FTIR, UV-Vis spectroscopy and thermogravimetric analysis (TG-DTA).

2. Material and methods

2.1. Sample description

Ten samples of compost containing olive mill pomace and other wastes were collected as described below and summarized in

Table *1*. Samples COMP1 to COMP4 were composted (mixed and heaped in 3-m-high x 6-m-diameter piles) and turned regularly every 15 days to avoid anaerobic processes for a maturation period of seven months. For better composting, olive tree prunings were added to all samples as a bulking agent, and chicken manure was also added to COMP1 to COMP4 to accelerate and improve composting. Sample COMP5 was prepared by mobile stacking, turning every fortnight for the first four months of bio-oxidation and then allowed to stand without turning for a two more months. Samples COMP5-CF1 to COMP5-CF4 were mixtures of sample COMP5 with different proportions of spent coffee grounds. An additional sample of uncomposted dried olive mill pomace (OMP) and another of spent coffee grounds (CF) were also included. Each compost sample consisted of five subsamples taken from five different positions within the heap in order to ensure representative results of the material.

Table 1. Description of the organic waste samples.

Sample	Composition	Origin
COMP1	50% OMP 25% Olive tree pruning 25% Chicken Manure	OMP produced and composted in Hermejor de la Reina organic oil factory[1]
COMP2	50% OMP 25% Olive tree pruning 25% Chicken Manure	OMP produced and composted in Hermejor de la Reina organic oil factory[1]
COMP3	80% OMP 10% Olive tree pruning 10% Chicken Manure	OMP produced and composted in olive-growing cooperative N. S. Remedios[2]
COMP4	80% OMP 10% Olive tree pruning 10% Chicken Manure	OMP produced and composted in Alcanova S.L. oil factory[3]
COMP5	30% OMP 70% Olive tree pruning	OMP produced and composted in IFAPA[4]
COMP5-CF1	24% OMP 56% Olive tree pruning 20% Coffee residues	Mix of COMP5 with coffee residues
COMP5-CF2	18% OMP 42% Olive tree pruning 40% Coffee residues	Mix of COMP5 with coffee residues
COMP5-CF3	12% OMP 28% Olive tree pruning 60% Coffee residues	Mix of COMP5 with coffee residues
COMP5-CF4	6% OMP 14% Olive tree pruning 80% Coffee residues	Mix of COMP5 with coffee residues
OMP	Dried OMP	OMP produced in Hermejor de la Reina organic oil factory1
CF	Coffee residues	Coffee residues obtained from University cafe

1 (Villanueva de la Reina, Jaén); 2 (Noguerones, Jaén); 3 (Alcaudete, Jaén); 4 Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA) (Mengibar, Jaén). COMP: composted olive mill pomace; OMP: olive mill pomace

2.2. Thermal treatment

After desiccation at 65ºC, samples were ground with a ball mill and sieved to 200 μm. Then they were subjected to thermal treatment by placing about 60 g of each in porcelain containers and heating to 175, 225 or 275ºC in a pre-heated oven (Binder FED GmbH) by ramping temperature up on a 5ºC/min gradient and maintaining the temperature for five hours (Simkovic et al., 2008). A total of 42 thermally treated samples were obtained in this way. It should be noted that OMP samples burned at temperatures above 200ºC, probably due to their high residual olive oil content.

2.3. Chemical characterisation and elemental analysis

Electrical conductivity and pH were measured in a 1:10 water soluble extract (w/v) with a CRISON conductimeter and a CRISON pH-meter respectively. A LECO TruSpec CHN 620-100-400 analyser was used to determine the total organic carbon, total nitrogen and ash content. P, K, Ca and Mg were determined after digesting samples with H2O2, HNO3 and HF in an ETHOS microwave digester, then adding H3BO3 for elemental determination in an ICP-OES VARIAN 715-ES.

2.4. Spectroscopy

Mid-IR spectra of the solid samples were recorded using a Varian 660 FTIR spectrometer (Varian, Inc.) equipped with an attenuated total reflection accessory (ATR, Pike Technologies) with a three-reflection diamond crystal. Samples were finely ground in an agate mortar and placed directly on the ATR crystal. Controlled pressure was applied to ensure good contact between the sample and the ATR element. The spectra ranged from 700 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and 128 accumulations. The spectra of air on the clean dry ATR crystal was used as background.

The hydrophobicity index, as the hydrophobic/hydrophilic ratio (Matějková and Šimon, 2012) was calculated using the area under the 3000-2800 cm^{-1} band corresponding to aliphatic CH bonds as a measure of hydrophobic functional groups, and the total area of the 1740-1600 cm^{-1} band, corresponding to the C=O of aromatic functional groups, as a measure of hydrophilicity. Areas were calculated using OPUS 7.0 spectroscopy software (Bruker Optics). The ratio between absorbance values at 1606/1740 cm^{-1} was also calculated as an indicator of the relative concentration of aromatic compounds.

UV-Vis spectra of sample NaOH extracts were recorded from 200 to 800 nm. Extracts were obtained by mixing 0.1 g of each sample with 25 mL of 0.5 M NaOH in polyethylene flasks. These suspensions were stirred for two hours and then let stand for 12 h. Finally, the samples were centrifuged for 25 minutes at 3000 rpm. Dilutions from 1:5 to 1:20 were made to record spectra in the appropriate measurement range and avoid detector saturation. Measurements were later corrected with dilution factor in order to make them comparable as 1:5 dilutions.

2.5. Thermogravimetric analysis

Thermogravimetric (TG) and simultaneous differential thermal analysis (SDTA) were carried out using a TGA/SDTA 851e Mettler Toledo instrument. 20 mg of each sample were dried and weighed into a 70-µL aluminium oxide capsule and subjected to temperatures of 25 to 850ºC, increasing 5ºC/min with a 100 mL/min synthetic air flow. The thermostability index (TS index) of samples was calculated following Lopez-Capel et al., (2005), by dividing the recalcitrant fraction mass loss, by the mass loss of the labile and recalcitrant fractions. The labile fraction was calculated as the mass loss band area between 200 and 400ºC in SDTA, while the recalcitrant fraction was calculated as the band area corresponding to mass loss between 400 and 600ºC in SDTA.

2.6. Water repellency estimation

Persistence of soil WR in organic waste samples was assessed by the water drop penetration time (WDPT) test (Wessel, 1988). The WDPT test, which measures how long repellency persists on a porous surface, consists of placing a drop of distilled water on the soil surface and recording the time it takes for the drop to penetrate completely in the material (Jordán et al., 2011). WDPT was determined by pouring 50 µl of distilled water at 25ºC in five drops from a height of 5 mm to prevent excess kinetic energy. This method was used to distribute samples in five classes according to the classification by Bisdom et al., (1993): wettable (≤ 5 s); slightly water repellent (6-60 s); strongly water repellent (61-600 s); severely water repellent (601-3600 s) and extremely water repellent (> 3600 s).

2.7. Phytotoxicity measurement

Phytotoxicity of organic wastes was determined according to the germination index using the Zucconi et al. (1981) test. 4 g of dry samples were moistened with deionised water to 60% field capacity. The mixture was shaken and let stand for 30 minutes. After that, more deionised water was added to a 1:10 w/v concentration and stirred mechanically for 30 minutes, centrifuged at 3000 rpm for 15 minutes and filtered (0.45 µm). 1 ml of the extract was added to a Petri dish with filter paper and 10 Raphanus sativus L. seeds were incubated at 28ºC for 72 hours in the dark. Then the number of seeds germinated was counted and the root length of those germinated measured. Results are expressed as the germination index resulting from the percentage of germination and root elongation over the control sample.

2.8. Statistics

Data analysis included correlations of variables (Pearson's correlation coefficient) and ANOVA. Normality assumptions were tested using the Saphiro-Wilk test. The F-test (ANOVA) was applied to variables fitting a normal distribution, while an alternative nonparametric test (Kruskal-Wallis ANOVA) was applied to the rest. The Bonferroni test was used to find differences between means. All statistical procedures were carried out using Statgraphics Centurion XVI software (StatPoint Technologies, Inc.).

Principal component analysis (PCA) was used for clustering samples and their relationships to specific bands in the mid-IR region. PCA was performed on the mid-IR spectra data matrix, preprocessed with

application of a second derivative and mean centring. This statistical treatment was performed in MATLAB 2008R (MathWorks, Natik, USA) using the Eigenvector Research Inc. (Wenatchee, USA) PLS-toolbox.

3. Results and discussion

3.1. Organic amendment characterisation

Chemical characterisation of the samples is shown in

Table 2, where it may be observed that composting raises pH, as expected for this type of materials (Cayuela et al., 2007). The chicken manure may also be in part responsible for the higher pH observed in COMP1, COMP2, COMP3 and COMP4 samples due to its high concentration in calcium carbonate. This can also be related to their higher ash content. Electrical conductivity was relatively high, in agreement with other compost studies (Gómez-Muñoz et al., 2013). Composting also decreases organic carbon concentration, with the consequent decrease in C/N ratio and increase in P, K, Ca and Mg concentrations. Thus, raw organic matter appears to be easily decomposed by soil microorganisms, improving organic amendment quality.

Spent coffee grounds (CF) and OMP samples show lower ash contents and higher organic carbon concentrations. Thus, these organic wastes need to be composted or mixed to reduce the C/N ratio and make them easier for soil microorganisms to decompose.

Table 2. Chemical characterisation of organic amendments. Mean ± Standard Deviation (n=3).

Sample	pH	EC (dS/m)	Ash (%)	O. C (%)	N (%)	C/N	P (%)	K (%)	Ca (%)	Mg (%)
COMP1	8,8 ± 0,2	1,2 ± 0,2	11,8 ± 1	27,9 ± 0,3	1,7 ± 0,1	16,5 ± 0,9	0,5 ± 0,07	1,52 ± 0,15	5,58 ± 0,25	0,71 ± 0,08
COMP2	8,1 ± 0,2	1,5 ± 0,4	11,8 ± 0,6	31 ± 1,2	1,8 ± 0,1	17,2 ± 0,5	0,57 ± 0,03	1,64 ± 0,11	6,23 ± 0,32	0,67 ± 0,09
COMP3	9 ± 0,2	1,1 ± 0,2	16,6 ± 1	22,6 ± 0,5	1,8 ± 0,1	12,9 ± 0,9	0,51 ± 0,09	1,3 ± 0,19	6,45 ± 0,19	0,26 ± 0,05
COMP4	8,9 ± 0,2	0,9 ± 0,1	9,8 ± 0,8	28,8 ± 1	1,7 ± 0,1	16,9 ± 1,3	0,8 ± 0,12	1,95 ± 0,09	8,41 ± 0,19	0,65 ± 0,04
COMP5	6,1 ± 0,4	3,5 ± 0,5	7 ± 0,4	51,9 ± 0,5	1,5 ± 0	34,6 ± 1,3	0,94 ± 0,07	1,33 ± 0,21	3,23 ± 0,15	0,89 ± 0,04
OMP	5,8 ± 0,3	0,9 ± 0,2	0,8 ± 0,2	54,6 ± 0,8	1,5 ± 0,1	36,4 ± 1,8	0,28 ± 0,07	1,03 ± 0,12	0,66 ± 0,14	0,1 ± 0,02
CF	5 ± 0,2	2,1 ± 0,2	1,6 ± 0,2	52,9 ± 0,5	2,3 ± 0,1	23 ± 1,1	1,06 ± 0,15	0,3 ± 0,1	0,51 ± 0,03	0 ± 0,01
COMP5-CF1	7 ± 0,3	0,8 ± 0,1	2,2 ± 0,2	54,2 ± 0,6	1,7 ± 0,1	32,5 ± 1,2	0,27 ± 0,05	1,15 ± 0,18	2,04 ± 0,13	0,27 ± 0,03
COMP5-CF2	6,9 ± 0,2	0,7 ± 0,1	1,6 ± 0,2	54,2 ± 1	1,7 ± 0,1	32,5 ± 1,2	0,27 ± 0,06	1,05 ± 0,08	1,73 ± 0,15	0,2 ± 0,03
COMP5-CF3	6,7 ± 0,2	0,6 ± 0,1	0,8 ± 0,1	53,8 ± 0,9	1,7 ± 0,1	31,1 ± 1	0,26 ± 0,04	0,73 ± 0,1	1,23 ± 0,11	0,2 ± 0,06
COMP5-CF4	6,3 ± 0,2	0,5 ± 0,1	2,8 ± 0,5	52,6 ± 1,4	1,8 ± 0,1	28,9 ± 0,8	0,27 ± 0,04	0,51 ± 0,1	0,78 ± 0,14	0,22 ± 0,05

EC: electrical conductivity; OC: total organic carbon; N: total nitrogen; P: total phosphorus; K: total potassium; Ca: total calcium; Mg: total magnesium.

Representative parameters of interest for the study of compost (organic amendments) are summarized in Table 3. The relationship between variables is shown by the Pearson´s coefficient matrix (Table 4). A4 was studied taking into account that range of 460–480 nm reflects the organic material at the beginning of humification while absorbance at 600–670 nm is associated with strongly humified material with a highly condensed aromatic groups (Zbytniewski and Buszewski, 2005). Results for A6 are very similar for all samples (Table 3), which indicates similar aromatic condensation, size and weight of organic molecules in the humic-like fraction extracted. Samples COMP1 to COMP4 show higher A4, suggesting more partly humified material, and coinciding with a dominant aromatic domain in the humic-like macromolecules extracted, probably related to intensified humification.

Table 3. Specific parameters for the characterisation of organic amendments. Mean ± Standard Deviation (n=3).

Sample	E4 (472nm)	E6 (680nm)	E4/E6 Ratio	Hydrophobic Index	1606/1740 Ratio	Thermostability Index	Germination Index (%)	WDPT (s)
COMP1	0,38 ± 0,03	0,11 ± 0,01	3,53 ± 0,12	0,068 ± 0,005	1,22 ± 0,06	0,754 ± 0,017	165,6 ± 6,3	345 ± 14
COMP2	0,39 ± 0,06	0,11 ± 0,01	3,4 ± 0,55	0,068 ± 0,004	1,24 ± 0,03	0,755 ± 0,011	151,5 ± 4,5	550 ± 13
COMP3	0,45 ± 0,05	0,12 ± 0,01	3,7 ± 0,36	0,172 ± 0,013	1,21 ± 0,03	0,809 ± 0,011	168,9 ± 4,4	65 ± 6
COMP4	0,34 ± 0,04	0,11 ± 0,01	3,14 ± 0,18	0,179 ± 0,008	1,17 ± 0,02	0,754 ± 0,012	110,4 ± 3,3	150 ± 16
COMP5	0,31 ± 0,04	0,12 ± 0,01	2,49 ± 0,16	0,424 ± 0,017	1,12 ± 0,02	0,687 ± 0,019	93 ± 4,5	930 ± 29
OMP	0,2 ± 0,02	0,08 ± 0,01	2,37 ± 0,15	0,576 ± 0,023	0,91 ± 0,07	0,58 ± 0,012	43,7 ± 2,2	2580 ± 52
CF	0,22 ± 0,04	0,09 ± 0	2,62 ± 0,4	0,53 ± 0,012	0,95 ± 0,04	0,358 ± 0,015	77,4 ± 2,6	3180 ± 62
COMP5-CF1	0,28 ± 0,03	0,11 ± 0,01	2,47 ± 0,21	0,48 ± 0,016	1,07 ± 0,03	0,628 ± 0,018	113,7 ± 4,4	2010 ± 33
COMP5-CF2	0,26 ± 0,02	0,1 ± 0,01	2,59 ± 0,1	0,552 ± 0,018	1 ± 0,03	0,635 ± 0,015	111,1 ± 3,5	2280 ± 48
COMP5-CF3	0,27 ± 0,03	0,1 ± 0,01	2,66 ± 0,07	0,579 ± 0,013	0,99 ± 0,04	0,607 ± 0,014	116,7 ± 5,7	2580 ± 55
COMP5-CF4	0,23 ± 0,02	0,09 ± 0,01	2,63 ± 0,13	0,583 ± 0,017	0,95 ± 0,04	0,523 ± 0,011	118,5 ± 4	3120 ± 53

ATR-FTIR spectra of selected samples are shown in Figure . Assignments of the main IR spectra bands are based mainly on, Madari et al., (2006) and White et al., (2011). The uncomposted materials (OMP and CF samples) have a broad absorption band with a maximum close to 3300 cm−1 corresponding to hydrogen-bonded hydroxyl (–OH) groups in polysaccharides and phenolic compounds. The strong bands at 2920 and 2850 cm^{-1} are due to symmetric and anti-symmetric aliphatic CH stretching, where the methylene group (CH2) is the predominant contributor. In the 1800-700 cm^{-1} region, the spectra of uncomposted materials are rich in spectral features, reflecting a variety of organic compounds. Although detailed interpretation is not possible, the different bands in the 1750-1600 cm^{-1} region can be attributed to overlapping of several carbonyl compounds. Thus, CF is characterised by a strong sharp carboxylic acid band at 1710 cm^{-1}, whereas the broad band centred at about 1630 cm^{-1} is probably due to overlapping of an Amide I protein band, antisymmetric carboxylate stretching and aromatic and alkene C=C stretching. The strong wide band in the 1000-1100 cm^{-1} region is due to C-O and C-C stretching vibrations in polysaccharides. Comparing these spectra with those of compost samples, clear differences can be observed in COMP1, COMP2, COMP3 and

COMP4. As shown in Figure (COMP1 and COMP3 spectra), they are dominated by bands of inorganic materials like carbonates (1795 cm^{-1}, 1410 cm^{-1} and 870 cm^{-1}). Furthermore, the band at 1020 cm^{-1}, typically associated to polysaccharides, could also be influenced by the strong absorption of phosphates, usually present in chicken manure (Changwen et al., 2010). Overlapping of the strong inorganic component absorption bands impedes detailed interpretation of the spectra. However, it is clear that they present a smoother profile poorer in differentiated functional groups. Particularly interesting is the 2850-2920 cm^{-1} region where low intensity bands due to aliphatic CH reveal the progress of composting. COMP5 is more similar to uncomposted OMP, although bands of aliphatic CH present lower relative intensity. This fact is indicative of less mature composting of this sample.

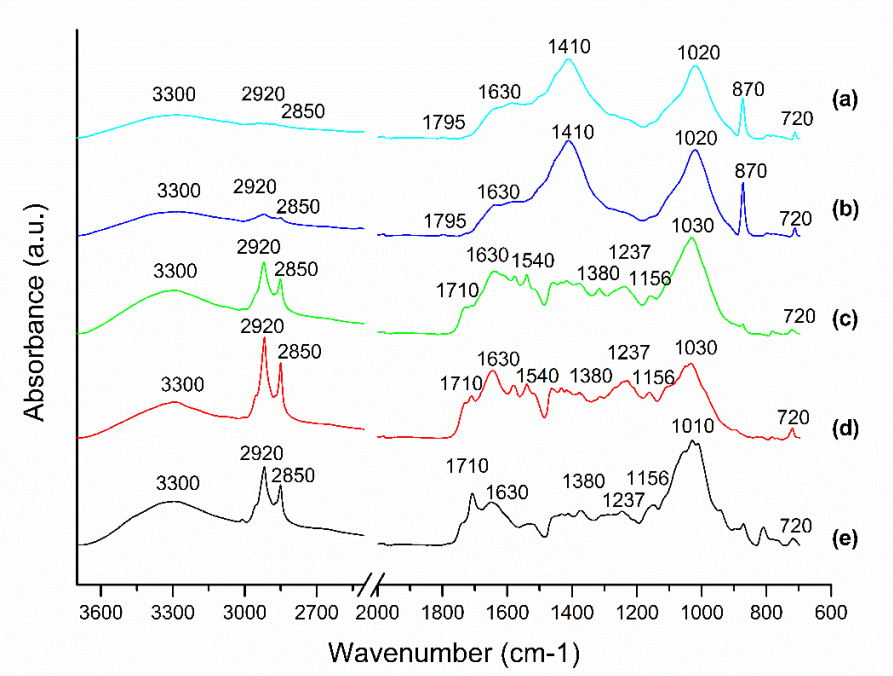

Figure 1. FTIR spectra of (a) COMP1; (b) COMP3; (c) COMP5; (d) OMP; (e) CF. Spectral traces have been stacked for clarity.

As the bands at 2920 and 2850 cm^{-1} are associated with the aliphatic compounds responsible for the hydrophobicity of organic matter (Matějková and Šimon, 2012), the area of these bands was used to estimate the hydrophobicity index (

Table *3*). This index was higher in COMP5 and lower in COMP1 to COMP4. It was highest in uncomposted organic wastes, showing the importance of composting in reducing aliphatic compounds, because this will probably strongly influence water repellency. Similar trends in aliphatic C/aromatic C ratio reduction, and the decomposition of readily organic matter, such as aliphatic components, polysaccharides and alcohols, have been reported during composting (Wu et al., 2011).

Therefore, according to the IR spectra, we are dealing with two groups of samples. COMP1 to COMP4 is a group of more evolved (mature compost), less aliphatic (reflected in low bands at 2920 and 2850 cm^{-1}) organic amendments with fewer carbohydrate and polysaccharide functional groups (1170-1050 cm^{-1}). The other group, comprised of less evolved samples, is made up of COMP5 and the mixtures (COMP5-CF1-4). These materials are poorly composted and therefore much more similar to the uncomposted organic waste

(CF and OMP). Hence, the concept of characterising the quality of SOM by its hydrophobicity as established by Capriel (1997) could be applied to these organic materials.

Finally, thermogravimetric (TG) and simultaneous differential thermal analysis (SDTA) were applied to study the response of these organic amendments to temperature as well as compost maturity (Baffi et al., 2007; Dell'Abate et al., 2000). TG and SDTA provide information on sample mass loss as a function of temperature, which is empirically interpreted as the progressive destruction of plant biopolymers with differing structural stability of interest in evaluating resistance to biodegradation (Blanco and Almendros, 1994). With these data, the TG curves may be divided into two regions, which are represented as two bands in SDTA. The first region/band, at around 300-400ºC, corresponds to more labile materials, such as aliphatic components like the cellulosic constituents of organic matter, carbohydrates and other less-condensed structures (Carballo et al., 2008; Gascó et al., 2012). The second region/band, at around 400-500ºC, is associated with more complex and recalcitrant OM, such as lignin and polyphenols, and resistant aromatic structures and higher-molecular-weight polynuclear systems (Carballo et al., 2008; Gascó et al., 2012). At about 700ºC there was another loss of mass, but very small and only in samples containing chicken manure (COMP1, 2, 3, 4) due to carbonate decomposition.

Figure 2 presents thermograms of a selection of representative samples. COMP5, CF and OMP showed higher weight loss in the first region, as reflected in SDTA, with a more intense first band for COMP5, and even stronger for OMP. However, Sample CF, even with a higher mass loss in TG, had a low SDTA first band signal. Samples COMP3 and COMP4 had the lowest signal in this band, and moderate in COMP1 and COMP2. This indicates a higher concentration of labile material in uncomposted samples (OMP and CF) and in COMP5. In the second region, the percentages of mass loss were similar for all samples. However, differences are found in SDTA, with a more intense band for all COMP samples, while OMP was intermediate, and CF the weakest. This suggests that more evolved and recalcitrant organic components are present in OMP in increasing amounts as composting progresses.

The thermostability index can be calculated using the first two SDTA bands (Table 3). This index reflects the proportion of labile and recalcitrant chemical structures. Samples COMP1 to COMP4 showed higher thermostability index, which is related to the presence of more complex and evolved organic matter. The index for COMP5 sample was still higher than the uncomposted samples (OMP and CF), showing that composting increases thermostability. In samples made with mixtures of COMP5 and CF this index depends on the percentage of each.

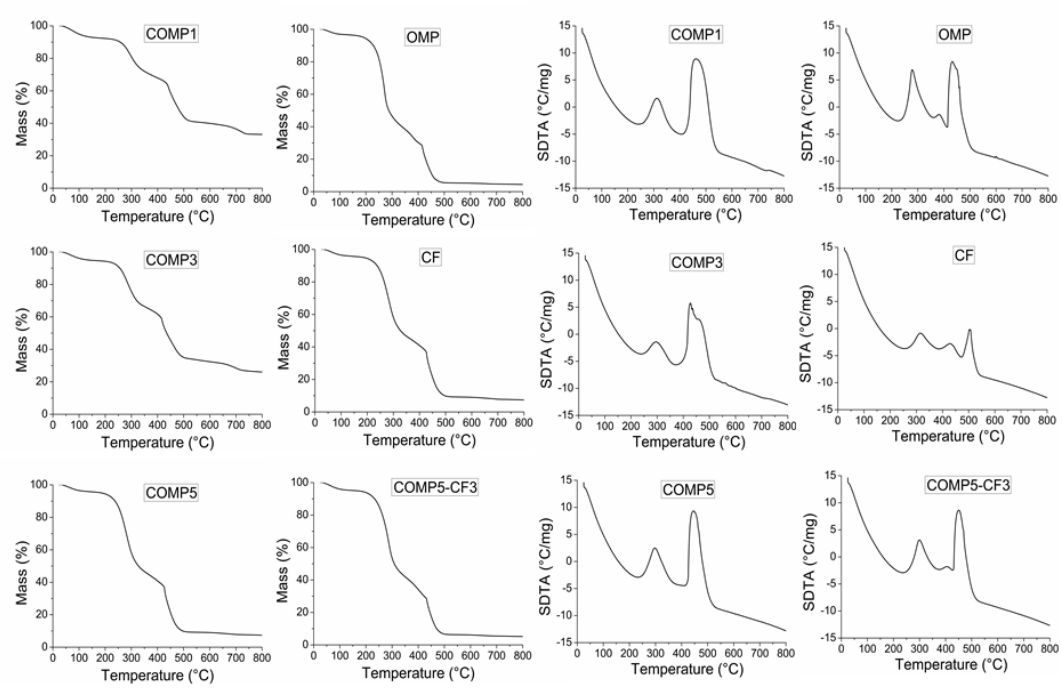

Figure 2. Thermogravimetric (TG) and differential thermal analysis (SDTA) curves of representative samples.

The results of the water-drop penetration time test (WDPT) employed to estimate the hydrophobic behaviour of the samples are also shown in

Table 3. All samples are strong or severely water repellent according to the Bisdom classification (Bisdom et al., 1993). It is worth mentioning that COMP3 and COMP4 are the organic amendments with the least WR (strong WR) whereas COMP5 had the highest WR of the composted samples. This could suggest that longer composting contributes to eliminating hydrophobicity. Sample CF had the strongest WR probably because of its high content in organic matter, as shown in Table 4, where a clear correlation between OC and WR is observed. This is in agreement with the trend observed in agricultural (Aranda et al., 2016) and forest soils (Ellerbrock et al., 2005), where an increase in organic carbon (and the C/N ratio) is accompanied by an increase in WR. The high OMP WR should also be stressed, because when directly applied as an organic amendment without composting, it increases soil hydrophobicity, reducing its ability to retain water, as shown by Lozano-García et al., (2011). According to de Blas et al., (2010), this WR could be produced by hydrophobic surfaces, such as those in undecomposed organic matter particles, including polyalkyl biomacromolecules (e.g., cutins and suberins), or by the accumulation of long-chain organic compounds (Doerr et al., 2000), more frequent in uncomposted organic material and less humified compost. In general, fresh and partly decomposed organic matter had a higher WR than more completely humified fragments. During composting of the biomass, organic macromolecules are broken down and WR is reduced (Bisdom et al., 1993).

As seen in Table 4, WDPT is clearly directly correlated with the hydrophobicity index (0.8505) and inversely with the thermostability index (-0.8198), which shows that these analyses are useful for predicting hydrophobicity in compost. According to Wu et al., (2011), the hydrophobicity index, i.e. the aliphatic C/aromatic C ratio, can provide information on change in organic matter and track the biological stability during composting. WR results are in agreement with this, as samples with a higher hydrophobicity index and lower thermostability show the highest WR.
Visible spectroscopy A4 and A6 optical densities were highly correlated negatively with the hydrophobic index and WDPT (Table 4), and therefore, show a clear trend towards a lighter colour (less aromaticity and humification) in humic-like substances extracted from samples with a strong tendency towards hydrophobicity.

The presence of carbonates (associated with higher pH) could also have an influence on the lower hydrophobicity in samples enriched with chicken manure, especially rich in this compound. This inverse relationship between WR and carbonates in soils has been described by several authors (Cerdá and Doerr, 2005; Aranda et al., 2016), but here it is described for the first time in organic amendments. Cerdá and Doerr (2005) explained these differences in soils in terms of soil acidity, and suggested that the alkalinity of calcareous soils is responsible for low hydrophobicity.
Finally, another important parameter that should be studied in organic amendments is their phytotoxicity, especially because the presence of caffeine and polyphenols could affect plant growth. We used the germination index (GI) to do this. Over 100% means not only the absence of toxicity, but also that it is a good substrate for plant growth.

Table *3* shows that the most evolved compost samples had the highest germination indexes, especially COMP1, COMP2 and COMP3. Sample COMP5 was not an especially good substrate for plant growth, but with a GI over 80 it may be regarded as a mature compost, practically free of phytotoxic substances (Selim et al., 2012). OMP with a GI of 44 was the only sample under 60 (Zucconi et al., 1981) or 50 (Eshetu, 2013), and therefore phytotoxic, showing its capacity to affect plant growth. However, the phytotoxicity of this raw material disappeared during composting. Hence, direct application of solid olive-mill waste to soil may have negative agronomical and environmental implications due to its acidic pH and high content in potentially phytotoxic and antimicrobial compounds, such as phenols, tannins, fatty acids, and low molecular weight organic acids (Nasini et al., 2013). The correlation between the hydrophobicity index and the germination index (-0.7234), and between the germination index and the thermostability index (0.6628), suggest that the evolution of the organic material reduced the potential phytotoxicity of these organic amendments.
It is also significant that even though COMP5 and CF seem not to be as good as the other composted samples as substrates, they showed better characteristics for plant growth with higher GI when mixed. This suggests that mixing OMP materials with spent coffee grounds is a good option for improving compost.

Table 4. Pearson´s correlation coefficients for organic waste variables (n=11).

	O.C. (%)	C/N	E4	E6	E4/E6	Hydrophobic Index	1606/1740 Ratio	TermostabilityIndex	Germination Index	Log WDPT (s)
O.C.(%)										
C/N	0.9243***									
E4	-0.9042***	-0.7867***								
E6	-0.5877	-0.4485	0.8239*							
E4/E6	-0.9664***	-0.9357***	0.9105***	0.5605						
Hydrophobic Index	0.9438***	0.8499***	-0.8961***	-0.6547*	-0.9068***					
1606/1740 Ratio	-0.8699	-0.7372**	0.9482***	0.8509***	0.8376**	-0.9496***				
Termostability Index	-0.7564**	-0.4805	0.8560***	0.7584**	0.6974*	-0.7690**	0.8509***			
Germination Index	-0.7406**	-0.7225*	0.8488***	0.6681*	0.8458***	-0.7234*	0.7771**	0.6628*		
Log WDPT (s)	0.9631***	0.7995***	-0.9057***	-0.7180*	-0.8591***	0.8505***	-0.8495***	-0.8198**	-0.6432*	

*Statistical significance: * P< 0.05; ** P < 0.01; *** P < 0.001*

3.2. Effects of thermal treatment on compost quality

Several different thermal treatments were applied to the organic amendment samples to reduce their hydrophobic compounds and hence water infiltration. According to some authors, long heating at around 200-250ºC can reduce or even eliminate WR in soil organic matter (Mataix-Solera and Doerr, 2004; Simkovic et al., 2008). Therefore, samples were subjected to temperatures of 175, 225 and 275ºC. The 200 to 300ºC temperature range in this thermochemical treatment was similar to torrefaction (also called mild pyrolysis) (Ren et al., 2013), but was carried out in the presence of oxygen.

In general, progressive smoothing of the biogenic signature in FTIR spectra was observed with increasing temperature. FTIR spectra of samples COMP3 and COMP5 at different temperatures are shown in Figure 3. Wide spectral changes may be observed in COMP5. The bands at 3300 cm^{-1} and 1030 cm^{-1} decreased with heating at 175ºC as a consequence of the thermal destruction of labile organic compounds like the polysaccharides of cellulose from vegetable matter. At 225ºC these bands seemed to stabilize. CH stretching bands were also reduced at 175ºC, but the greatest decrease was observed above 225ºC and still continued decreasing considerably at 275ºC. In the 1750-1200 cm^{-1} region, there were also several, more difficult to interpret, spectral changes. It seems that heating increased the relative concentration of certain carboxylic compounds and lignin, but decreased the variety of spectral features, resulting in a smoothed profile with few broad bands.

In sample COMP3, the strong mineral fraction bands complicated observation of band evolution, especially in the 1500-700 cm^{-1} region. For example, the decrease in polysaccharides in these samples could not be assessed due to strong overlapping with the phosphate/silicate band at 1020 cm^{-1}. Carbonates (at 1795 cm^{-1}, 1410 cm^{-1} and 870 cm^{-1}) and phosphate/silicate bands, in general, intensified with thermal treatment due to their relative accumulation with mass loss.

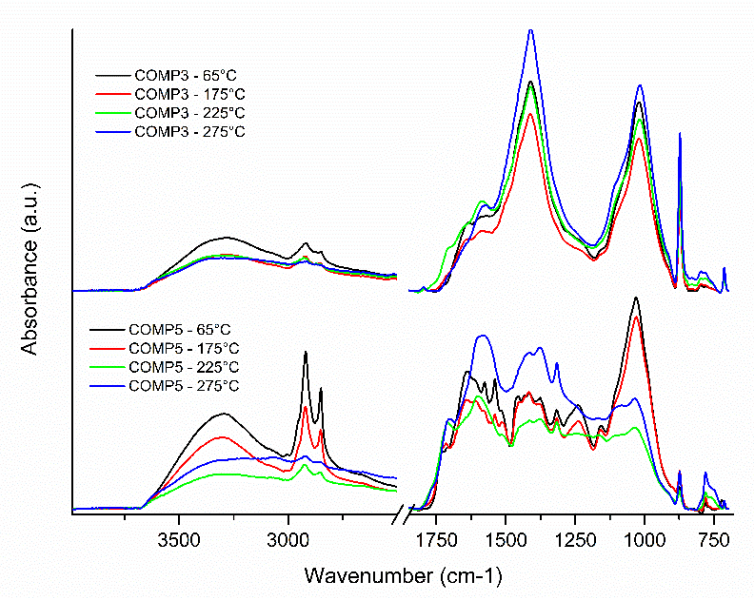

Figure 3. FTIR-ATR spectra of COMP3 and COMP5 at different temperatures.

To analyse the effect of heating in more detail, PCA was performed with the FTIR spectra of the 42 samples (Figure 4). As seen in Figure 4a, PC1 (70% of variance) clearly separated two groups of samples that match the observations of the unheated samples (65ºC). The composted samples (COMP1-4) had negative scores for this component, whereas samples based on COMP5 and uncomposted materials (OMP and CF) were positive. The loading vector mainly reflected the presence of mineral compounds in COMP1-4 and the larger amount of aliphatic compounds (with bands at 2920 and 2850 cm^{-1}) in uncomposted materials, COMP5 and mixtures of COMP5 with CF. In fact, this component separated samples by raw material composition regardless of the thermal treatment temperature. However, within each group of samples the influence of temperature is evident. The effect of the temperature is even more clearly reflected in the PC2 scores (17.25% of variance). This component behaved differently in the two clusters defined by PC1. In the cluster of uncomposted materials (left side of the plane defined by PC1 and PC2), the samples appear clearly clustered by heating temperature along the axis defined by PC2. At 65ºC, the samples in this group show negative PC2 scores and positive PC1. As temperature increases, the scores on PC2 increase and become positive, whereas PC1 scores decrease. The behaviour of the cluster formed by the mature compost samples is not so clear. This PC loading is mainly dominated by bands related to aliphatic (2920 and 2850 cm^{-1}), carboxylic and aromatic compounds (1710, 1606, 1598, 800 and 720 cm 1), suggesting that this PC separated samples by change in organic matter. The presence of polysaccharide bands (1010 and 1030 cm^{-1}) and compounds derived from vegetal materials such as lignin (1580, 1540, 1510, 1465 and 1410 cm 1) in this component loading is evident. This is also the reason why this PC had some problems differentiating between thermal treatments in COMP1 to COMP4, since they had lower organic matter content. Finally, PC3 (Figure 4b) only accounted for the main differences between the two uncomposted organic wastes, OMP with positive scores and CF with negative, and scores for mature compost were close to zero. Increasing temperatures of immature compost and organic wastes also reduced the scores of this component to values close to zero. This shows that samples of less evolved materials become more like mature composted materials with heating. In this PC, organic compounds derived from vegetal materials from 1560 to 1010 cm^{-1}, present in a higher proportion in sample COMP5, were positive and tended to be reduced and almost eliminated at higher temperatures. In addition, bands with negative weights (like 1480, 890 and 820 cm^{-1}) are only present in samples with spent coffee grounds (also tended to disappear when thermal treatment was applied.

Finally, it should be noted that although PCA showed clear association of samples by temperature, it revealed a very different response to thermal heating depending on the original sample composition.
Furthermore, taking into account the PCA results, the ratio between absorbance at 1606 cm^{-1} (characteristic of aromatic C=C vibrations, Painter et al., 1981) and 1740 cm^{-1} (typical of acetyl groups in hemicelluloses, Bui et al., 2015) can be proposed as an estimation of the relative amount of aromatic compounds.

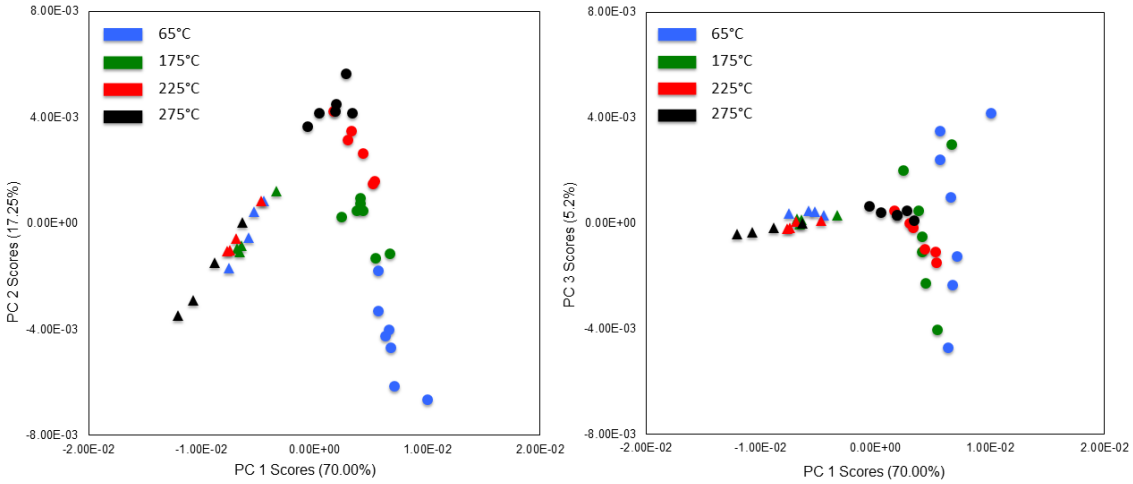

Figure 4. PCA Scores for sample FTIR spectra. Samples are represented by temperature.

A selection of representative TG and SDT sample analyses are shown in Figure 5. Both TG and SDTA showed few changes in the amounts of labile and recalcitrant compounds with thermal treatment at 175ºC, and practically no differences in percentage of mass loss. However, wider differences were found at this temperature in more evolved samples (COMP1 to COMP4), with a small decrease in labile compound mass, showing the stronger effect of the 175ºC thermal treatment in these samples. Thermal treatment at 225ºC, as expected, generated stronger change in organic fractions, reducing the labile fraction by 15% and the recalcitrant fraction by 10% with respect to untreated samples. This shows a reduction in the total organic fraction, but with a relative increase in the recalcitrant fraction, demonstrating that treatment at this temperature positively affected organic matter maturation. This effect was stronger in less evolved samples (COMP5 and mixtures), which originally had more organic matter, and suggests that, while a less aggressive treatment induces changes in organic matter in evolved samples, higher temperatures are required in less evolved samples, but these changes are then more pronounced. Finally, the effect of the 275ºC thermal treatment was similar to 225ºC, but reduced both fractions even more and generated a larger proportion of evolved organic matter. In CF sample a clear decrease of labile compounds in the TG can be observed, while more slight changes are observed for the recalcitrant fraction. However, the reduction in labile compounds does not produce an increase of the recalcitrant fraction, particularly when high temperatures are applied. This indicates that the thermal treatment is less useful for spent coffee grounds.

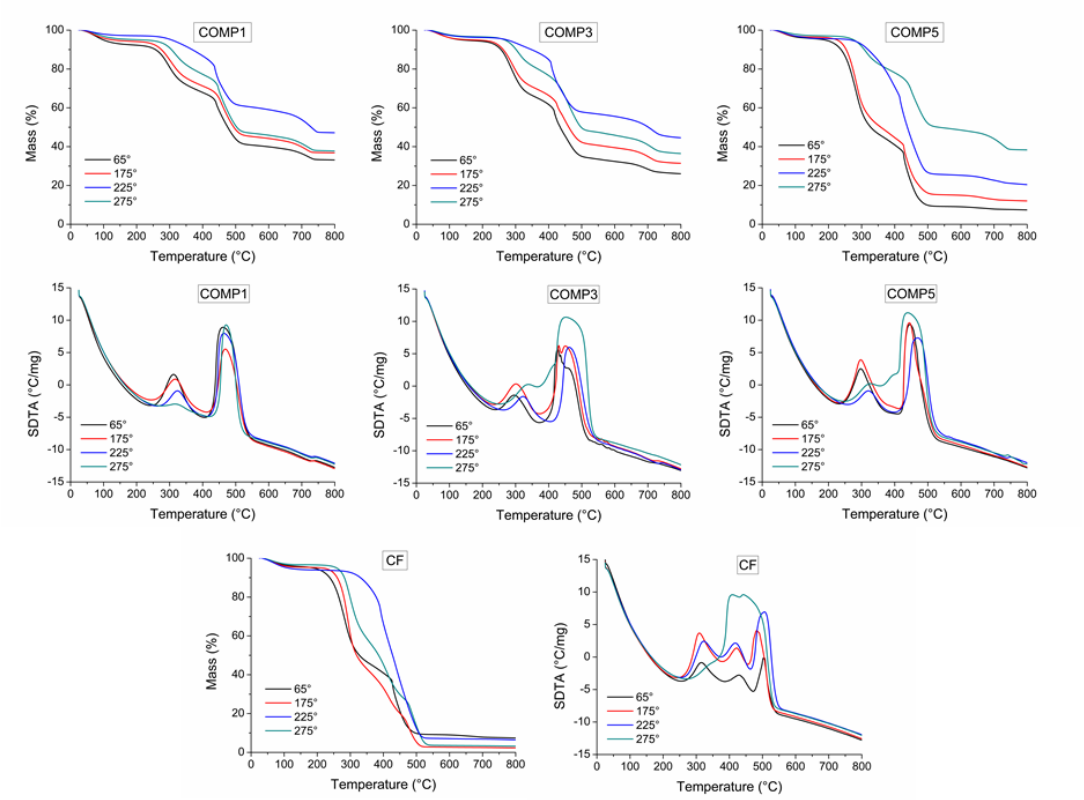

Figure 5. Thermogravimetric (TG) and differential thermal analysis (SDTA) curves of representative samples at different temperatures.

Table 5 presents a summary of the parameters characterising the organic amendments by thermal treatment temperature. Clearer trends in calculating the means at each temperature were observed when samples had been divided into the two groups according to the PCA results (evolved and little evolved). Heating reduced the hydrophobicity index, reflecting a relative increase in aromatic compounds due to degradation of hydrophobic compounds. However, this effect was observed at 175ºC only in less evolved samples, which suggests that at this temperature only fresh and less evolved material is transformed. For other parameters, such as the thermostability index, a clear effect was observed only at higher temperatures. In non-heated samples, the higher ratio 1606/1740 observed in evolved composted samples revealed an increase in the proportion of aromatic compounds when composting is adequate. When thermal treatment is applied, this ratio has different behaviour for the two groups of samples showing only a clear increase when higher temperatures are applied. A clear trend in A4 results showed an increasing with thermal treatment in proportion with the material during humification. This increase was greatest in the less evolved samples.

Table 5. Results for all samples grouped by temperature and distributed by degree of evolution. Mean ± Standard Deviation.

Samples	E4 (472nm)	Hydrophobic Index	1606/1740 Ratio	Thermostability Index	Germination Index (%)	Log WDPT (s)
65ºC	0.31 ± 0.08	0.383 ± 0.215	1.08 ± 0.12	0.644 ± 0.130	115.5 ± 37.2	3 ± 0.6
65ºC Evolved	0.39 ± 0.04	0.122 ± 0.062	1.21 ± 0.03	0.768 ± 0.027	149.1 ± 26.9	2.3 ± 0.4
65ºC Little Evolved	0.25 ± 0.04	0.532 ± 0.06	1.00 ± 0.07	0.574 ± 0.108	96.3 ± 27.6	3.4 ± 0.2
175ºC	0.35 ± 0.08	0.255 ± 0.134	1.03 ± 0.15	0.626 ± 0.135	119 ± 27.9	3.4 ± 0.2
175ºC Evolved	0.44 ± 0.04	0.128 ± 0.043	1.18 ± 0.04	0.745 ± 0.028	148.9 ± 22.6	3.4 ± 0.2
175ºC Little Evolved	0.30 ± 0.02	0.328 ± 0.110	0.95 ± 0.12	0.558 ± 0.124	101.9 ± 10.1	3.4 ± 0.2
225ºC	1.42 ± 0.45	0.129 ± 0.08	1.08 ± 0.18	0.775 ± 0.112	122.9 ± 20.1	3.3 ± 0.5
225ºC Evolved	1.21 ± 0.10	0.055 ± 0.012	1.23 ± 0.03	0.839 ± 0.005	138.1 ± 14	2.9 ± 0.5
225ºC Little Evolved	1.56 ± 0.56	0.178 ± 0.065	0.98 ± 0.16	0.733 ± 0.131	112.7 ± 17.5	3.5 ± 0.0
275ºC	1.64 ± 0.64	0.032 ± 0.016	1.3 ± 0.09	0.915 ± 0.059	132.3 ± 17.2	< 1
275ºC Evolved	1.04 ± 0.21	0.026 ± 0.012	1.27 ± 0.03	0.944 ± 0.008	140.3 ± 19.8	< 1
275ºC Little Evolved	2.04 ± 0.49	0.036 ± 0.018	1.32 ± 0.11	0.896 ± 0.071	126.9 ± 14.5	< 1

Evolved: COMP1. COMP2. COMP3 and COMP4. Little evolved: COMP5. OMP1. CF. COMP5-CF1. COMP5-CF2. COMP5-CF3. COMP5-CF4.

The statistical significance of the different thermal treatment parameters at different temperatures (all samples) are shown in Figure 6. Results of treatment of untreated samples (65ºC) at 175ºC were statistically significant only for the hydrophobicity index and WR, which interestingly, increased at this temperature. Thermal treatment at 225ºC led to a clear reduction of the hydrophobicity index and an increase in the thermostability index. These changes are most evident in less evolved samples, where changes were stronger, while still more hydrophobic and less thermostable than evolved samples. This shows that this temperature has a stronger effect on fresher and less evolved material. However, this treatment again reflected an increase in WR when all the samples were taken together in the statistical tests. Finally, treatment at 275ºC yielded the lowest hydrophobicity index and highest 1606/1740 ratio and thermostability index. These results suggest that this temperature is the most suitable for transforming organic matter into more aromatic and evolved forms, and therefore, the best quality for application to soil to avoid hydrophobicity, as reflected in the abrupt decrease in WR observed. At this temperature, both groups had similar hydrophobicity and thermostability, suggesting that this temperature is the best option for improving organic amendment quality. Although the GI was not statistically significant, the variability of the data was strongly reduced, and the mean index tended to be higher, which reinforces the conclusion that heating at 275º C is the best option for favouring crop development and eliminating toxicity. It should be underlined that heating OMP at 175ºC already eliminated phytotoxicity, reaching a GI near 100, which

indicates that this treatment could be a good option for eliminating the toxicity of olive mill pomace. The GI over 100% observed after heating, although not significant, shows that germination is promoted (Table 5;Figure 5), and therefore, this treatment seems suitable for agriculture. An explanation for the variable effects on GI could be that the nutrient content (with different release rates) of "torrefacted" organic wastes is controlled by both biomass type and combustion conditions, similar to biochar (Mukherjee and Zimmerman, 2013).

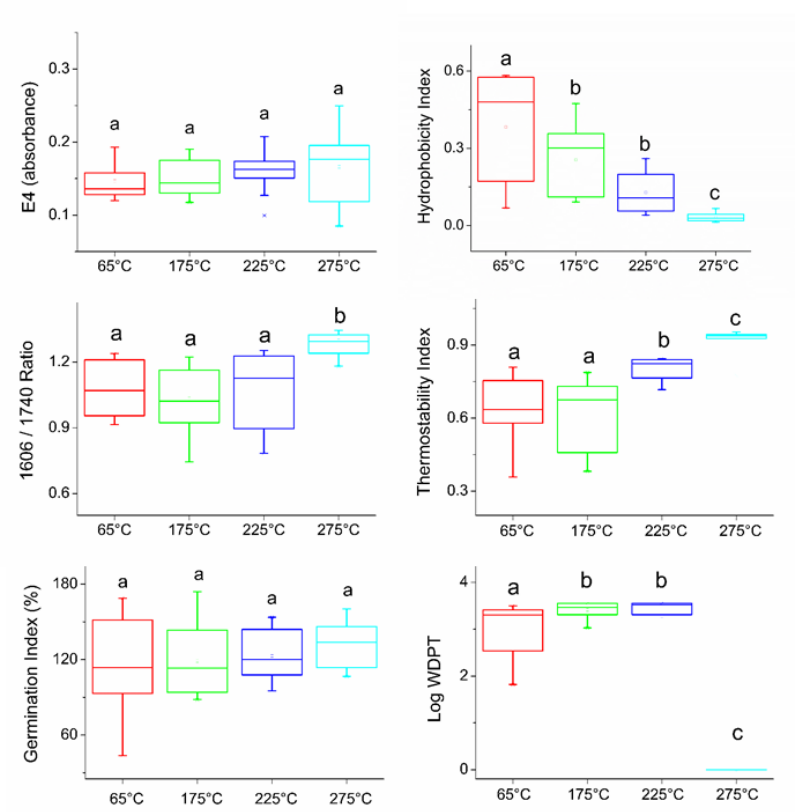

Figure 6. Means and Standard deviations of results with statistical differences of temperatures. All values with different letters are significant at P < 0.001 for ANOVA (1606/1740 ratio) or ANOVA Kruskal-Wallis test (A4, Hydrophobicity index, thermostability index, germination index and log WDPT).

3.3. Thermal effects on compost water repellency

Thermal treatment demonstrated an improvement in organic matter quality, making it more aromatic and recalcitrant by reducing the aliphatic fraction. It is important to interpret these findings in terms of WR behaviour. Table 5 and Figure 6 showed a clear increase in WR (WDPT) at 175ºC. This could be explained by the decrease in cellulose hydroxyl groups (3300-3400 cm^{-1} band in FTIR) and carboxylic acids (1710 cm^{-1} band in FTIR), which are hydrophilic. However, it could also be due to a relative concentration in aliphatic compounds by volatilisation and later solidification on surface organic particles, as described by Zavala et al., (2010). This would increase hydrophobicity even when the aliphatic fraction was reduced and the aromatic fraction increased. 225ºC treatment reduced WR but without reaching original levels, showing evident destruction of hydrophobic compounds at this temperature, but insufficient to cause higher water repellency than the original samples as the 175ºC treatment does due to the disappearance of carboxylic acid and hydroxyl groups, as well as possible migration of hydrophobic compounds to the sample surface.

Finally, the 275ºC treatment reduced WR to practically 0, so it destroyed most of the hydrophobic compounds present in the samples. This coincides with the disappearance of aliphatic groups in the FTIR spectra at 2920 and 2850 cm^{-1}, or at least many of them, leaving the rest far from the surface, and resulting in no hydrophobicity in the sample. Therefore, sample behaviour can be attributed to the mineralisation, volatilisation, or transformation of readily degradable molecules and metabolites of the organic matter studied. Similar results were found by Jordán et al., (2011) in soil samples. That study concluded that WR changed little at soil temperatures below 175°C, but increased considerably between 175 and 200°C. At temperatures above 280°C, it was eliminated. Highly evolved organic materials (decomposed), before and after heating, were clearly more stable (from thermostability data), although in less evolved materials, the differences were narrower as temperature increased. Hence, once applied to the soil, it could be more efficient for organic C sequestration.

Pearson's correlation coefficients (Table 6) showed that tools used in this study are strongly correlated with WR estimation (WDPT), positively with the hydrophobicity index (0.5805) and inversely with the 1606/1740 ratio (0.7078) and thermostability index (0.6965), showing they could be used to assess WR of organic samples. The thermostability index, hydrophobicity index and 1606/1740 ratio are all highly correlated (Table 6), suggesting that they provide very similar information and are good techniques for studying the organic composition of compost. The germination index is also correlated with the other indexes calculated, showing that the evolution of organic matter samples, in which thermostability increased and hydrophobicity decreased, also contributed to making the organic amendments less phytotoxic.

Table 6. Pearson's correlation coefficients of variables for organic amendment samples with their different treatments (n=42).

	E4 (472nm)	**Hydrophobic Index**	**1606/1740 Ratio**	**Thermostability Index**	**Germination Index**	**Log WDPT (s)**
E4 (472nm)						
Hydrophobic Index	-0.5083***					
1606/1740 Ratio	0.2931	-0.7527***				
Thermostability Index	0.4607*	-0.7784***	0.8156***			
Germination Index	0.3852*	-0.6320***	0.6252***	0.5929***		
Log WDPT (s)	-0.2863	0.5805***	-0.7078***	-0.6965***	-0.3177*	

*Statistical significance: * P< 0.05; ** P < 0.01; *** P < 0.001*

Summarizing, thermal treatment eliminates aliphatic and labile compounds, while aromatic and recalcitrant compounds are reduced in much smaller proportion, leading to a decrease of water repellency. This effect is more marked when higher temperatures are applied and depends of organic amendment initial characteristics. For less evolved olive mill pomace compost samples and spent coffee grounds, the thermal treatment is not as efficient as for more evolved organic amendments based on olive mill pomace.

4. Conclusions

Organic amendments, especially OMP, are extremely hydrophobic and can have a very negative effect on different soil properties, increasing WR and favouring erosion. This hydrophobicity can be successfully monitored by FTIR and TG-SDTA analysis, which clearly reflect sample content in fractions responsible for their hydrophilic, and above all, hydrophobic character. Adequate composting helps reduce WR. But even when properly done, it appears to be insufficient. Several different thermal treatments for eliminating WR from organic amendments were tested, but only treatment at 275ºC proved to be effective for this purpose, reducing the material's WR to near 0. Treatment at 225ºC, and to a lesser extent 175ºC, clearly improved organic matter quality, but appeared not to be a good option for eliminating sample hydrophobicity, even generating higher WR. Spent coffee grounds and COMP mixtures increase WR due to the high WR of spent coffee grounds, but could make organic matter less phytotoxic. This type of organic amendment based on OMP and/or spent coffee grounds, which have been shown to be relatively resistant to degradation, can enrich the soil with very different compounds not easily degradable, and could also be an appropriate strategy for organic carbon sequestration in the soil. Nevertheless, these results and findings should be supported by field research, because many factors, such as microtopography, management, interaction with natural organic matter, carbonates and soil texture could affect the soil hydrological response.

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Capítulo 11
Impact of spent coffee grounds over soil organic matter functionality and stability in two contrasted Mediterranean agricultural soils

Este capítulo se corresponde con el artículo "Impact of spent coffee grounds over soil organic matter functionality and stability in two contrasted Mediterranean agricultural soils", enviada a la revista European Journal of Soil Science. En este artículo se estudian los efectos sobre la materia orgánica del suelo y sus diferentes fracciones de la adición de posos de café.

Esta publicación está enviada.
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Abstract

The effects of spent coffee grounds (SCG) application on the quantity and quality of soil organic matter (SOM) in two Mediterranean agricultural soils (Vega soil, SV and Red soil, SR) were studied in soil microcosm conditions. The impact of this biowaste on SOM has been barely studied and it arouses great interest due to the huge amount of SCG produced around the world. SOM fractionation, spectroscopic UV-Vis and Mid-IR, thermogravimetric and simultaneous differential thermal analysis as well as scanning electron microscopy (SEM), were applied in this study. The addition of SCG on both type of soils had a similar effect; SCG increased all SOM fractions, especially the levels of SOM with more labile character (as hot water soluble carbon, increased to around 600-700%) and total extractable carbon (increased to around 200%), although with a lower increment of the more recalcitrant, aromatic and oxidized functional groups (the amounts of humic acids and fulvic acids increased to around 200%). Even when the addition of SCG clearly increased the levels of humic acids, its functionality were affected since a reduction of the proportion of functional groups with more recalcitrant and stable character was observed. The soils tested differed from each other in terms of physical, physicochemical and mineralogical properties (SV has a more clayey texture and higher content of smectite in its clay fraction than SR), which made the behavior of the soils to be different when SCG was added to them. The different degree of incorporation of SCG into the soils' interstices and the interaction between soil and SCG particles (observed by SEM) affected carbon (C) retention under stable forms, increasing C stabilization in SV with respect to SR. This was confirmed by the increase in thermostability index and carbonyl functional groups in humic acids. Consequently, soil type is a key factor in increasing long-term C stability when an amendment is incorporated. On the other hand, (30 and 60 days) seems to have had no effect on the SOM of the soils studied. The application of SCG to soils could lead to important short and mid-to-long-term benefits in soil quality, and can be a valuable alternative use of a widespread polluting waste worldwide.

1. Introduction

More than 6 million tons of spent coffee grounds (SCG, grounds that remain after brewing the beverage) are generated each year worldwide (Hardgrove and Livesley, 2015). SCG is mainly composed of polysaccharides (38% hemicelluloses and 9% cellulose) and protein (14%) (Murthy et al., 2012). With reference to sugar content, SCG is composed of mannose, galactose, glucose and arabinose, which when summed up, is made up of a great variety of functional groups including phenolic hydroxyl, aliphatic hydroxyl, methoxyl, carboxyl and sulfonate functional group (Ballesteros et al., 2014). SCG also contain significant amounts of lignin (which is composed of a great variety of functional groups) (Ballesteros et al., 2014; Zarrinbaksh et al., 2016). Its ash content is a minority component (1.3%), and potassium is the most predominant element in SCG. SCG also contains melanoidins (which is considered as synthetic humic susbtances) macromolecules formed by Maillard reaction during the roasting process (Cosovic et al., 2010). Many studies have corroborated that SCG also contains toxic substances such as caffeine, polyphenols, and tannins (Mussatto et al., 2011).

The composition of SCG makes it possible to use it as soil organic amendment. On the one hand, from soil fertility point of view, some studies have revealed, that SCG has positive effects since it increased nitrogen, phosphorous, potassium and organic carbon contents in soils (Yamane et al., 2015; Cervera-Mata el al., 2018). On the other hand, some studies have shown that SCG limits plant growth (Yamane et al., 2015; Hardgrove and Livesley, 2015; Cervera-Mata et al., 2018).

There are many studies that have described the influence of organic amendments on the composition and quality of organic matter (OM). For instance, the review of Senesi et al. (2007) described the humic-like fractions in different organic amendments, such as animal manures, sewage sludges and municipal solid waste compost, and their effects on soil humic substances. However, very little is known about the influence of SCG on OM. Hachicha et al. (2012) studied the co-composting of SCG with olive mil wastewater sludge and poultry manure and found out that SCG provided a highly compostable feedstock. Zhang et al. (2017) also reported an increase in humic substances when cow dung and SCG were mixed; this organic amendment stimulated the enzymes involved in lignocelluloses degradation, resulting in an increase in the concentrations of low molecular weight compounds, enhancing the maturity and the stability of the compost.

When the fate of an organic amendment in the soil is studied, it must be taken into account that the nature of the soils (components and structure) decisively intervene in the decomposition, evolution and stabilization of the organic compounds present in the soil (Baldock and Skjemstad, 2000; Feng et al., 2013; Pérez-Lomas et al., 2010; Rakhsh et al., 2017; Six et al., 2002). These authors reported the importance of fine particles (silt and clay fractions) and their mineral nature in the soil, as well as the presence of 2:1 phyllosilicates (such as smectite), CaCO3 and Fe- and Al- oxides. Agricultural practices that include the systematic incorporation of OM of different origins and natures are essential in the arid and semiarid Mediterranean regions, because of their irregular climatic conditions and the fact that they are subjected to high human pressure, which results in very negative consequences on soil degradation. In these regions, it

would be necessary to minimize the impact of an environmentally unsustainable agriculture. An example is found in Andalusia (South Spain), where 20% of its surface area is classified as "high erosion risk" with a mean annual soil loss of 23.2 Mg ha-1 (Rodríguez Martín et al., 2016). Water deficit conditions and land use, mainly extensive, are predominant factors that limit soil organic carbon built up in Southern Spain and result in low OM contents in soils which reduces the global carbon stock. In this context, organic amendments have been widely requested for in other to restore soil biological, physical and chemical fertility (Reichstein et al., 2013).

As is stated above, the SCG has been used as soil organic amendment; however, no systematic research has been carried out on the changes in the different organic fractions of SCG in the course of their transformation in the soil. Moreover, the problem explained above with regard to Mediterranean soils, requires organic amendments that have improving effects on the soil quality in the short and medium terms. The composition of SCG, being rich in proteins, carbohydrates and substances similar to humic acids, such as melanoidins, makes it possible to believe that SCG can modify the composition of soil organic matter (SOM) in a short period of time. Thus, this research was aimed at studying, in soil microcosm (controlled laboratory conditions), the SOM evolution in two mineralogically constrasted agricultural Mediterranean soils amended with SCG. The experimental design involved the analysis of three factors: the dose of SCG, the soil types and the duration of incubation. This paper focuses on the evolution of SOM fractions, as well as their thermal stability and functionality to evaluate their evolution and maturity after the application of SCG into the soil.

2. Materials and methods

2.1. Soil sampling

Two Mediterranean agricultural soils from Granada province (Andalusia, Southern Spain) were selected. Vega soil (SV) is typically used for growing corn, alfalfa and horticultural crops under irrigation and was classified as Cambic Calcisol (Aric, Ochric) in WRB (IUSS Working Group WRB, 2014). Red soil (SR) is used for cereal crops under rain-fed conditions and was classified as Chromic Luvisol (Cutanic, Differentic, Hypereutric, Ochric) in WRB (IUSS Working Group WRB, 2014). The sampling of soils consisted of a random selection of three locations comparable in terms of climate, slope and, orientation. In each spot, a soil sample that is composed of four sub-samples (from the surface 0-20 cm layer) was randomly taken within a 5 m radius. The soil samples (by triplicate) were air-dried and sieved (<2 mm) for further analysis.

2.2. Spent coffee grounds

The coffee beans (100% Arabica) were supplied by a Spanish producer (Café Cumbal, S.A.). The SCG were obtained by grinding 50 g of coffee beans and mixing them with 1 L of boiled distilled water, followed by filtration and air drying (Rufián-Henares and de la Cueva, 2009; Cervera-Mata et al., 2018). The particle size distribution (by sieving) of SCG was heterogeneous: 21% between 1000 and 500 μm, 60% between 500 and 250 μm, 12% between 250 and 200 μm and 7% less than 200 μm.

2.3. Experimental design: soil microcosms

Microcosms were prepared using sieved air-dried soil samples (<2 mm), and mixing them with 0, 2.5 and 10% w:w of SCG (corresponding to 0, 60 and 240 Mg ha-1, respectively) with a rotating shaker in order to obtain 400 g of the soil-SCG mixtures. The soil-SCG mixtures were transferred to PVC pots of 300 mL capacity (height 8.5 cm, superior diameter 11 cm, base diameter 7.5 cm) and each PVC pot was closed with a mesh of fiber glass at the base and covered with a dish to avoid the entrance of light but not of air. The assay was performed in a growth chamber under controlled conditions with a relative humidity of 60-80% and temperature of 22/18°C (day/night). To prevent leaching and water stress the moisture of the pots was maintained between field capacity and permanent wilting point. The microcosms were irrigated once a week with distilled water (mean value of added water: 35 mL). The irrigation requirements were calculated by weighting (Dumroese et al., 2015). A total of 90 samples were obtained, which included sampling at 30 and 60 days, with six replicates for each condition, and three replicates for each case at time 0, as initial control samples. The microcosm samples were air-dried and stored in a sterile bag and kept in a refrigerator at 5 °C.

2.4. Characterization of soil and spent coffee grounds

The methods of soil analyses of the American Society of Agronomy and Soil Science Society of America (Soil Survey Staff, 2014) were adopted to determine granulometry, pH, electrical conductivity at 25°C (EC25), carbonates as CaCO3 (CO32-), available phosphorus and potassium, cation exchange capacity (CEC), bulk density, water retention at -33 kPa (field capacity) and -1500 kPa (permanent wilting point) and available water content (AW). Organic carbon (OC) was determined by wet digestion with K2Cr2O7 and H2SO4 at 155°C for 30 min (Mingorance et al., 2007), and further determination by measuring absorbance at 600 nm in a Spectrophotometer BOECO-S200. Total nitrogen was determined with a Truspec CN analyzer (LECO Corporation, Saint Joseph, MI, USA). The mineralogical analysis of the fine earth (<2 mm) and clay (<2 μm) was carried out by X-ray diffraction (XRD; crystalline powder method). XRD spectra of the fine earth and clay fractions (disoriented powder) were obtained using a Bruker D8 Advance diffractometer (radiation Cu Kα; λ = 0.15406 nm; range 3° to 70° 2θ; 40 kV; 40 mA).

2.5. Soil humus fractions and organic carbon content determination

For OM fractionation and characterization from the 90 samples, including replicates, new samples were composed by mixing an equal weight of three of each case replicates, for a total of 30 composed samples which included 5 of each combination of the type of soil and SCG amount.

Fractionation of soil organic matter was done by following the IHSS procedure (Swift, 1996). Total extractable carbon (TEC) for humic and non-humic substances was extracted from soil by mechanically shaking the samples with a solution of 0.1 M NaOH and Na4P2O7 (pH 14) for 24 h at 60°C (1:10, w/v). The extracts were centrifuged and filtered (Millipore 0.45 mm) separating into TEC and humins. The TEC fraction was separated into humic acids (HA) and fulvic acids (FA) by precipitation with H2SO4 (for HA determination) and purification with polyvinylpolypyrrolidone to eliminate non-humic carbon, respectively. Hot water soluble carbon (HWSC) was extracted in distilled water (1:10, w/v) for 18 h at 80°C (Ghani et al., 2003). The C content in humins, TEC, FA, HA and HWSC was determined by wet digestion with

$K_2Cr_2O_7$ and H_2SO_4 at 155°C for 30 min (Mingorance et al., 2007), and further determination by measuring absorbance at 600 nm in a Spectrophotometer BOECO-S200. HA/FA ratio and Degree of humification = ((HA+FA)/TEC) ×100 were also calculated.

2.6. Spectroscopic and thermogravimetric analysis

UV-Vis spectra of 0.2 mg C mL−1 humic acids solutions in 0.1 M $NaHCO_3$ (Senesi et al., 2003) were recorded from 200 to 800 nm in a computer controlled Varian Cary 50 spectrophotometer with the Varian spectroscopy software, WIN-UV. Mid-IR spectra of the soil samples and the humic acids extracted were recorded using a Varian 660 FTIR spectrometer (Varian, Inc.), equipped with an attenuated total reflection accessory (ATR, Pike Technologies) with a three-reflection diamond crystal. The soil samples were finely ground in an agate mortar. The samples were placed directly on the ATR crystal. Controlled pressure was applied to ensure good contact between the sample and the ATR element. The spectra ranged from 700 to 4000 cm-1 with a resolution of 4 cm-1 and 128 accumulations. The spectra of air on the clean dry ATR crystal was used as background. For better comparison, the relative peak absorbance was calculated by dividing the absorbance of each individual peak by the sum of the absorbances of all peaks in each spectral region of interest (3700-2500 cm−1 and 1850-800 cm−1) and were expressed as percentages. Areas were calculated using OPUS 7.0 spectroscopy software (Bruker Optics).

Thermogravimetric (TG) and simultaneous differential thermal analysis (SDTA) were carried out using a TGA/DSC1 Mettler-Toledo instrument. 20 mg of each sample was weighed and subjected to temperatures of 25 to 1000°C, increasing 5°C/min with a 50 mL/min synthetic air flow. The thermostability index (TS index) of samples was calculated by dividing the recalcitrant fraction mass loss, by the mass loss of the labile and recalcitrant fractions (Lopez-Capel et al., 2005). The labile fraction was calculated as the mass loss band area between 200 and 400°C in SDTA, while the recalcitrant fraction was calculated as the band area corresponding to mass loss between 400 and 600°C in SDTA.

2.7. Scanning electron microscopy analysis

SEM images were captured from the soil aggregates (1-2 mm) fixed with carbon double-sided adhesive tape, metalized with carbon and analyzed with a variable pressure and high resolution scanning electron microscope (VPESEM-FESEM) SUPRA40VP (ZEISS, Oberkochen, Germany), that was equipped with an energy dispersive X-ray (EDX) elemental analyzer. SEM analyses were performed during the first week after the end of the assay.

2.8. Statistical analysis

Data analysis included correlations of variables (Pearson's correlation coefficient) and ANOVA. Normality assumptions were tested using the Saphiro-Wilk test. The F-test (ANOVA) was applied to the variables that

fit into a normal distribution, while an alternative non-parametric test (Kruskal-Wallis ANOVA) was applied to the rest. Student T-test, Fisher Least Significant Difference and Turkey´s Honestly Significant tests were used in the appropriate case for the search for differences between means. All statistical procedures were carried out using Statgraphics Centurion XVI software (StatPoint Technologies, Inc.). Principal component analysis (PCA) was used for clustering samples and their relationships with specific bands in the mid-IR region. PCA was performed on the mid-IR spectra data matrix, pre-processed with the application of a second derivative and mean centering. This statistical treatment was performed in MATLAB 2008R (MathWorks, Natik, USA) using the Eigenvector Research Inc. (Wenatchee, USA) PLS-toolbox.

3. Results and Discussion

3.1. Characterization of soil samples and spent coffee grounds

3.1.1. Properties of soils and spent coffee grounds before incubation

The soils tested and the SCG presented very different characteristics in the parameters studied (Table 1). The soils tested also differed from each other, mainly in their content of CO32- (higher in SV than in SR), which is related to their mineralogical properties, i.e., higher content of calcite and dolomite in SV inherited from its soil parent material. According to granulometry data, both soils had a clayey textural class, although the fine particles (silt + clay) of SV represented 87.9% of the fine soil (<2mm) while in the case of SR the percentage was 61%. As a consequence of this granulometric difference, together with the slightly higher amount of OC in SV, the CEC, the capacity of water retention at both potentials and plant available water content (AW) were greater in SV than in SR, while the reverse was observed in terms of BD. Regarding mineralogy (Table 1), the fine-earth of the SV was rich in phyllosilicates and carbonates (calcite and dolomite), while the SR was composed of a higher content of phyllosilicates, quartz and iron oxides, and the carbonates present was in amounts that were less than 1% (Table 1). SV contained a higher quantity of smectites (swelling clays) in clay fraction. SR contained more phyllosilicates with d00l > 1.4 nm (such as chlorite) and kaolinite in the clay fraction, which is indicative of a greater age and more mineralogical evolution (Martín-García et al., 1999). The clay content and mineralogy may be important in the interactions between the organic matter and mineral fraction (Baldock and Sjemstad, 2000; Six et al., 2002; Rakhsh et al., 2017). SCG was moderately acidic, with high organic carbon and nitrogen contents, low bulk density, and high water retention capacities at -33 kPa and -1500 kPa. SCG also had a high C/N ratio, which indicates that it is a little humified waste.

Table 1. Soils and Spent Coffee Ground properties before incubation. Values are the mean ± standard deviation.

Chemical and physicochemical properties															
sample	Dry color (Munsell)	CF (%)	Clay (%)	Silt (%)	pH	EC_{25} (dS m^{-1})	OC (%)	N (%)	C/N	CO_3^{2-} (%)	CEC (cmol$^+$ kg^{-1})	BD (g cm^{-3})	W_{33} (%)	W_{1500} (%)	AW (mm cm^{-1})
Spent Coffee Ground					5.76 ± 0.26	4.5 ± 0.5	59.38 ± 0.62	1.87 ± 0.10	32 ± 1	nd	58.28 ± 1.6	0.49 ± 0.01	118.3 ± 3.7	110.4 ± 6.1	0.39 ± 0.02
Vega Soil	10YR 5.5/2	2.0 ± 0.3	58.0 ± 1.7	29.9 ± 0.9	8.2 ± 0.17	1.3 ± 0.2	1.36 ± 0.08	0.11 ± 0.05	13 ± 1	39.0 ± 3.2	26.61 ± 0.5	1.20 ± 0.04	26.4 ± 0.4	15.6 ± 0.7	1.30 ± 0.07
Red Soil	2.5YR 4/6	6.0 ± 0.2	43.2 ± 0.9	18.0 ± 1.2	7.2 ± 0.15	0.6 ± 0.1	1.16 ± 0.11	0.11 ± 0.03	10 ± 1	1.6 ± 0.5	23.73 ± 0.7	1.27 ± 0.04	20.0 ± 0.6	11.7 ± 0.6	1.05 ± 0.03

Mineralogical properties																
	Fine earth (< 2mm)						Clay (< 2 µm)									
	Phy	Qz	Fd	Iron ox	Cal	Dol	Ill	Pa	Sm	Int 1-1.4 nm	Phy >1.4 nm	Ka	Qz	Fd	Iron ox	Cal	Dol
Vega Soil	47 ± 2	11 ± 1	<1	<1	34 ± 2	8 ± 0	29 ± 2	3 ± 0	17 ± 1	12 ± 1	3 ± 0	6 ± 0	12 ± 1	2 ± 0		14 ± 1	2 ± 0
Red Soil	63 ± 3	33 ± 2	2 ± 0	2 ± 0	<1	<1	33 ± 2	2 ± 0	3 ± 0	5 ± 0	11 ± 1	27 ± 1	13 ± 1	1 ± 0	5 ± 0	<1	<1

EC25: electrical conductivity measured at 25ºC; OC: organic carbon; N: total nitrogen; CO32-: carbonates as CaCO3; BD: bulk density; W33 and W1500: water retention at -33 and -1500 kPa, respectively; AW: available water; nd: not detected; Phy: phyllosilicates; Qz: quartz; Fd: Feldspar; Iron ox: iron oxides (goethite and hematite); Cal: calcite; dol: dolomite; Ill: illite; Pa: paragonite; Sm: smectites; Int 1-1.4 nm: interstratified phyllosilicates with d00l between 1 and 1.4 nm; Phy >1.4 nm: phyllosilicates with d00l >1.4 nm; Ka: kaolinite; CF: coarse fragments (whole soil).

3.1.2. Effects of spent coffee grounds on soil physicochemical properties

The main chemical and physicochemical properties of the different mixtures of both soils with SCG before and after incubation are shown in Table 2. Soil amended with SCG significantly improved the chemical fertility of SV and SR samples, since increased additions of SCG significantly raised OC, N, K and Cu contents. These results are in agreement with Yamane et al. (2011) and Cervera-Mata et al. (2018). Moreover, the addition of SCG increased other micronutrients such as Fe and Zn, but not significantly. Soil material significantly influenced the properties that are related to the presence of carbonates, such as CO_3^{2-} and pH, and other properties such as EC, P, K and Fe, which were higher in SV than in SR. In contrast to this, more Zn was detected in SR. The time of incubation significantly affected pH, EC, P and Cu, which decreased during the cultivation time. In general, SCG improved soil chemical fertility in both soils, corroborated by CEC data, although its effect was only significant when 10% SCG was added.

3.1.3. FTIR analysis of soil samples and spent coffee grounds

The Mid-IR spectra of the initial samples are shown in Fig. 1. The assignments of the main IR spectra bands are mainly based on Madari et al. (2006) and White et al. (2011) reports. The main differences between the soils and the SCG were the mineral bands, only present in soil samples, as expected, mainly due to the presence of kaolinite (3695 and 910 cm^{-1}) and illite (3620 cm^{-1}) in both soils, but more marked in SR; and carbonates (1795, 1415 and 870 cm^{-1}) which are only present in SV and which coincided with the XRD results, where they constituted about 40% of the total composition (Table 2). These mineral bands reduced their intensities with the addition of SCG, due to the relative decrease of the mineral contents. In SCG samples, as observed previously in raw materials like olive mill pomace and SCG (Comino et al., 2017), it is remarkable the high intensity bands for symmetric and asymmetric stretching of C–H (2920, 2850 cm^{-1}), typically associated to aliphatic acids, and the broad band at 1740 cm^{-1} of stretching absorption of carbonyl (C=O) bond, associated with the ester group of triglyceride in lipid (Craig et al., 2012). However, the intensities of these three bands can also be associated with the presence of caffeine and lipids (Li et al., 2014). Also, a series of bands appeared between 1500 and 500 cm^{-1}, and most of them were associated with the hemicellulose and lignin functional groups (Chen et al., 2012), but were also associated with some proteins and lipids, whose results are difficult to assign due to the overlap between them (Zarrinbakhsh et al., 2016). These results indicate the main composition of the soils, the SCG and the mixtures, which are very important to better understanding of the OM composition.

Table 2. Chemical and physicochemical characteristics of soil samples before and after incubation. Values are the mean of 0, 30 and 60 days ± standard deviation.

Soil	SCG %	n	pH	EC (dS m^{-1})	OC (%)	N (%)	C/N	CO_3^{2-} (%)	CEC $cmol^+ kg^{-1}$	P	K	Cu	Zn	Fe
										(mg kg^{-1})				
Red	0	15	7.5 ± 0.1 b	0.5 ± 0.3 a	1.23 ± 0.17 a	0.109 ± 0.023 b	11 ± 2 a	1.0 ± 0.4 a	21.07 ± 0.63 a	47 ± 6 b	233 ± 24 a	4.24 ± 0.34 a	2.17 ± 0.16 c	5.20 ± 0.69 a
Red	2.5	15	7.4 ± 0.1 b	0.3 ± 0.2 a	2.28 ± 0.17 b	0.161 ± 0.023 b	15 ± 2 bc	1.2 ± 1.1 a	21.75 ± 0.63 a	49 ± 5 b	295 ± 17 b	4.65 ± 0.53 b	2.36 ± 0.25 d	5.66 ± 1.14 ab
Red	10	15	6.7 ± 0.2 a	0.4 ± 0.2 a	5.09 ± 0.98 d	0.293 ± 0.022 c	17 ± 3 de	1.0 ± 0.2 a	24.81 ± 0.75 b	54 ± 7 b	405 ± 64 c	5.51 ± 0.89 c	2.61 ± 0.52 e	6.88 ± 1.63 bc
Vega	0	15	8.3 ± 0.1 d	0.9 ± 0.3 bc	1.47 ± 0.16 a	0.113 ± 0.022 a	13 ± 2 ab	40.2 ± 1.2 d	26.95 ± 1.89 c	58 ± 10 a	406 ± 65 c	4.20 ± 0.39 a	0.82 ± 0.08 a	8.77 ± 2.08 d
Vega	2.5	15	8.3 ± 0.2 d	0.8 ± 0.4 b	2.66 ± 0.30 c	0.180 ± 0.062 b	16 ± 4 cd	39.3 ± 1.6 c	26.52 ± 0.67 c	61 ± 12 a	486 ± 25 d	4.47 ± 0.30 ab	0.96 ± 0.13 a	8.80 ± 1.51 d
Vega	10	15	8.0 ± 0.1 c	1.0 ± 0.2 c	5.62 ± 1.74 e	0.304 ± 0.091 c	19 ± 3 e	36.8 ± 1.3 b	29.28 ± 1.96 d	69 ± 12 ab	639 ± 73 e	4.71 ± 0.29 c	1.17 ± 0.17 b	7.87 ± 3.00 cd
Soil type			SV>SR	SV>SR	SR=SV	SR=SV	SV>SR	SV>SR	SV>SR	SV>SR	SV>SR	SR=SV	SR>SV	SV>SR
SCG %			0%=2.5	0%=2.5%=1	10%>2.5%>	10%>2.5%>0	10%>2.5%>	0%=2.5%=10	0%=2.5%<	0%=2.5%<1	10%>2.5%>	10%>2.5%>	0%≤2.5%=1	0%=2.5%=1
Maduration			0d>30d	0d>30d=60d	0d=30d=60d	0d=30d=60d	0d≥30d≤60d	0d=30d=60d	0d=30d=60	0d>30d=60d	0d=30d=60d	0d>30d=60d	0d=30d=60d	0d>30d<60d

EC25: electrical conductivity measured at 25ºC; OC: organic carbon; N: total nitrogen; CO32-: carbonates as CaCO3; CEC: cation exchange capacity; SV: Vega soil; SR: Red soil. Different letters denote significant differences (P < 0.05). Statistical differences (>, < or =) of samples grouped according to soil type, %SCG addition and maduration time are also shown.

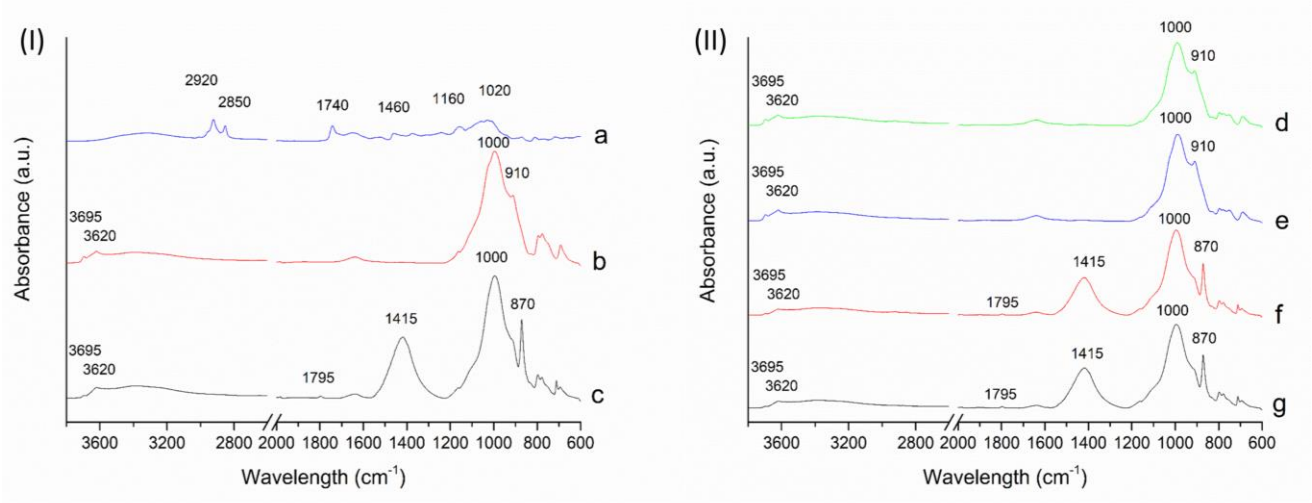

Figure 1. Typical FT mid-IR spectra of samples at time 0. I: initial samples: a) spent coffee grounds (SCG), b) Red soil and c) Vega soil. II: mixed samples: d) Red soil with 2.5% SCG, e) Red soil with 10% SCG, f) Vega soil with 2.5% SCG, and g) Vega soil with 10% SCG.

3.2. Effect of spent coffee grounds and soil type on SOM

The SOM quality is of vital importance from the perspective of its agro-ecological and environmental functions. The addition of an organic material, such as the raw SCG, could affect the functionality and stability of SOM in soils. For this reason, and mainly for the case of this type of organic amendments of little evolved nature, and without composting, the evaluation of SOM quality through its fractionation and physicochemical nature is more important than the quantification of the material provided to the soil. This evaluation has been carried out mainly through a thermogravimetric analysis, to know its stability and decomposability, as well as the maturity and functionality of its extracted HA. Coinciding with Almendros et al. (2005), the interest of assessing SOM quality rather than its total concentration must be underlined.

3.2.1. SOM fractionation

Different fractions of SOM and its quantification before and after incubation are shown in Table 3. These fractions include native soil humic substances co-extracted with SCG derived humic-like substances. The results obtained show a clear increase in all SOM fractions with the addition of SCG. The increment is especially higher in TEC and HWSC fractions, while the increase in HA and FA fractions is lower, but also significant. The humins increment is only significant in the soils with 10% of SCG. These increments indicate an increase in total organic matter in the soil with the addition of SCG, but especially in the more labile pool of SOM, which includes microbial biomass as well as soluble soil carbohydrates and amines (Ghani et al., 2003), with a less substantial gain of the more evolved and recalcitrant fractions of soil organic matter, especially in the case of humins, which represents a more stabilized SOM. The days of maduration appear to have had no effect on organic fractions, therefore, up to 60 days appears to be no time enough to detect changes due to decomposition or evolution in any of the organic matter fractions.

The indexes calculated (Table 3) prompted more discussion about the degree of evolution of organic matter in the different samples. The HA/FA ratio (degree of polymerization) was similar among all samples, although it shows a trend of higher values in the SV samples with the addition of SCG. On the other hand, lower values for the degree of humification were obtained for SR samples when compared with SV samples, and also a trend of lower values for this ratio when SCG is incorporated in both kind of soils. This shows that the addition of SCG tend to increase the proportion of the non-humified fraction, i.e., substances with few humic characteristics, such as peptides, sugars, nucleic acids, fats, etc. (Vergnoux et al., 2011) in this soil with respect to the more evolved forms of organic matter. But it also showed some differences between the soil typologies. Soil capacity for storage resistant plant components, or accumulating and protecting humic substances are influenced by soil type, mainly granulometry and mineralogy (Six et al., 2002; Feng et al., 2013; Rakhsh et al., 2017). This appears to explain what happened in the SV samples, with a more clayey texture (higher content in clay and silt) and a higher content in smectite in clay fraction, that favored the presence of this kind of organic compound. This may be due to overlapping of the effect of OM incorporation in the form of SCG and its biogenic background, where pedogenic development is more intense, corroborated by a higher content of HA, FA and humin in SV native soil. These results showed that the OM stabilization intensified in the samples from the SV in comparison with those from SR.

The SEM images (Fig. 2) show an interesting result with respect to the interaction between SCG particles and the soil. In SR samples (Fig. 2a), the SCG particles adhered to the surface of the soil aggregates and interacted with some silt/clay particles on the surface. In SV samples (Fig. 2b), the SCG particles were introduced into the soil interstices and were more coated with mineral particles. In summary, it appears that SCG interacts with soil particles more intensively in SV than in SR. Hence, SV samples showed an improvement of soil structure, as a consequence of microaggregate-associated soil C and silt and clay-associated soil C, i.e., and according to Six et al. (2002), physically protected soil C has the main stabilization mechanism.

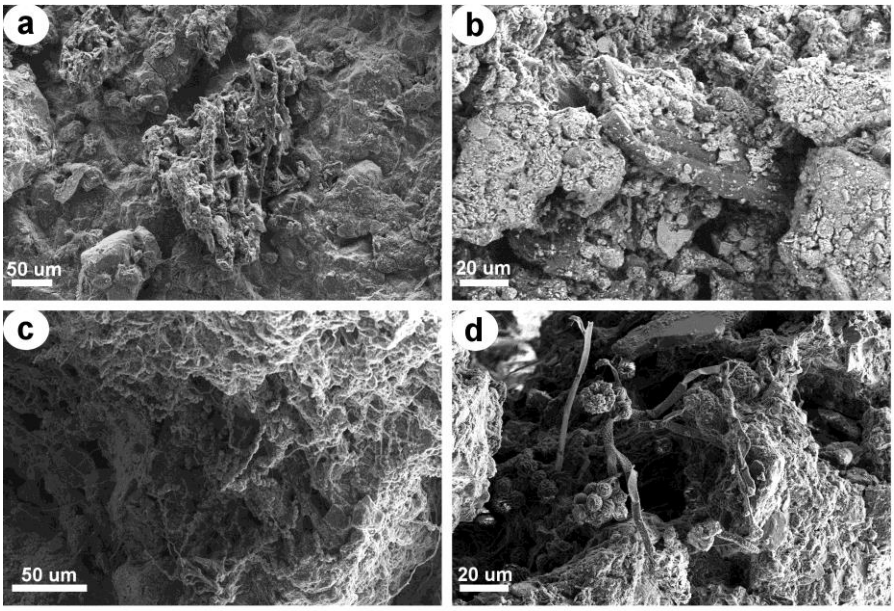

Figure 2. SEM images from the surface of aggregates of Vega soil and Red soil added with 10% Spent coffee ground (SCG) incubated during 60 days (SV10 and SR10, respectively). a) Sample SR10: SCG particle little incorporated in the soil matrix in the center of the photograph. b) Sample SV10: SCG particle (dark gray color) embedded in the soil matrix and surrounded to a large extent by mineral particles of silt and clay size (light gray color). c) Sample SR10: fungal hyphae with conidiophores can be observed. d) Sample SV10: fungal hyphae with conidiophores can be observed. Images a, c and d were captured with secondary electrons. Image b was captured with backscattered electrons.

Table 3. Fractionation of the SOM before and after incubation. Values are the mean of 0, 30 and 60 days ± standard deviation.

Soil	SCG %	n	TEC (mg C kg^{-1})	HWSC (mg C kg^{-1})	Humins (mg C kg^{-1})	HA (mg C kg^{-1})	FA (mg C kg^{-1})	Ratio HA/FA	Degree of humification
Red	0	5	5198 ± 1154 a	228 ± 132 ab	819 ± 348 a	2640 ± 658 a	229 ± 33 a	11.6 ± 3.1 a	55.4 ± 8.8 ab
Red	2.5	5	8571 ± 1492 b	702 ± 124 c	1722 ± 800 a	4326 ± 1176 b	374 ± 108 b	11.7 ± 1.9 a	54.2 ± 5.5 a
Red	10	5	18382 ± 2831 c	1574 ± 499 d	14247 ± 3244 b	8515 ± 2176 c	754 ± 107 c	11.5 ± 3.3 a	50.2 ± 7.2 a
Vega	0	5	5342 ± 510 a	173 ± 88 a	1943 ± 1110 a	3222 ± 851 ab	274 ± 43 ab	11.8 ± 2.8 a	65.2 ± 12.5 b
Vega	2.5	5	8224 ± 1093 b	511 ± 68 bc	2918 ± 787 a	4334 ± 599 b	370 ± 81 b	12.1 ± 2.4 a	57.3 ± 3.8 ab
Vega	10	5	16964 ± 1756 c	1394 ± 249 d	15084 ± 3488 b	8846 ± 1071 c	680 ± 159 c	13.5 ± 3.1 a	56.2 ± 4.5 ab
Soil type			SR=SV	SR=SV	SR=SV	SR=SV	SR=SV	SR=SV	SV>SR
SCG %			10%>2.5%>0	10%>2.5%>0	10%>2.5%=0	10%>2.5%>0	10%>2.5%>0	0%=2.5%=10	0%=2.5%=10
Maduration			0d=30d=60d	0d=30d=60d	0d=30d=60d	0d=30d=60d	0d=30d=60d	0d=30d=60d	0d=30d=60d

TEC: total extractable carbon; HWSC: hot water soluble carbon; HA: humic acids; FA: fulvic acids; SV, Vega soil; SR: Red soil. Different letters denote significant differences (P < 0.05). Statistical differences (>, < or =) of samples grouped according to soil type, %SCG addition and maduration time are also shown.

3.2.2. Thermogravimetric analysis

TG and SDTA were applied in order to obtain more information about OM composition and evolution, and especially about its stability and decomposability. The area of SDT was integrated and the thermostability index calculated (Comino et al., 2017; Lopez-Capel et al., 2005). The areas of SDT and thermostability index are shown in Fig. 3. The results obtained show that the addition of SCG increased the recalcitrant fraction when 10% of SCG was added, but that labile fraction was greatly increased with both 2.5 and 10% addition. This led to a lower thermostability index in the samples with more SCG amount and indicated less stability of the organic matter in them. This indicates that the OM provided by SCG is very labile and easily decomposable by soil microorganisms (Plante et al., 2009), mainly by those of a fungal nature, as shown in Fig. 2c and 2d. By comparing the different soils, a slightly higher amount of labile fraction was found in SV samples, especially in the samples with no SCG addition. This indicates a higher concentration of more labile compounds only in these samples, but when SCG were added, the values obtained were similar to those of SR samples, which indicates an overestimation in this case. This is also reflected in the thermostability index (Fig. 3b), where the addition of SCG increases the thermostability of SV samples with respect to SR samples, which could be justified according to the higher contents of smectite and carbonates in SV, which have the role of stabilizing the SOM (Six et al., 2010; Pérez-Lomas et al., 2010).

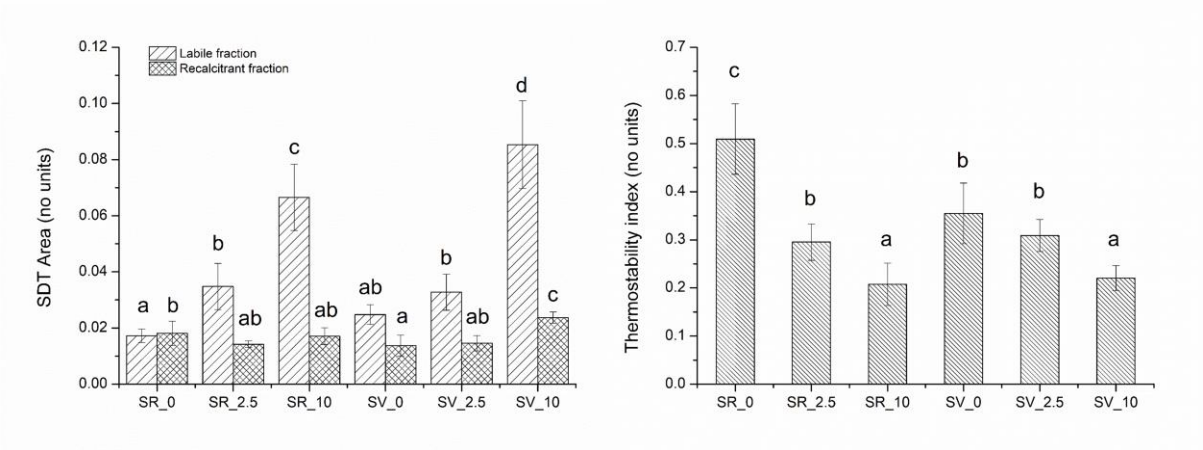

Figure 3. a) Simultaneous differential thermal (SDT) areas of labile and recalcitrant fractions. b) Thermostability index. SR0: Red soil without spent coffee grounds (SCG); SR2.5: Red soil with 2.5% SCG; SR10; Red soil with 10% SCG; SV0: Vega soil without SCG; SV2.5: Vega soil with 2.5% SCG; SV10: Vega soil with 10% SCG. Different letters denote significant differences (P < 0.05)

In summary, the addition of SCG generated an increase in all the fractions of SOM. This increase was especially higher in the less evolved and labile organic matter, hence, SCG could be an amendment that provides the soil with organic matter that is poorly developed and is easily decomposable. However, the addition of SCG to the soil have also shown to increase the levels of the more evolved and recalcitrant organic matter in the soil. In this sense, recalcitrance connotes the preservation of OM caused by structures inherently resistant to biochemical decay such as condensed and lignin-derived aromatic carbons, melanoidins, some tannins or aliphatic compounds (Mikutta et al., 2006); and would represent the biochemically protected soil C. These recalcitrant compounds might contribute to a low rate of carbon

decomposition of the amendment when applied to soils under field conditions, improving the physical and chemical fertility (García-Ruiz et al., 2012), and coinciding with Aranda et al. (2015), could also be an appropriate strategy for sequestering organic carbon into the soil.

3.3. Study of humic acids after amendments with spent coffee grounds

Humic substances, in particular HA, which clearly represents SOM decomposition pathways and stage of humification, and as a proxy for SOM, are assumed to be chemically more homogeneous and stable than the total SOM (Buurman et al., 2009). To determine the main composition of HA, UV-Vis spectra is of interest, when contributing to the information about the degree of maturity and aromaticity of these substances, being comparatively low in those coming from compost and raw materials (Traina et al., 1990). On the other hand, the high sensitivity of IR spectroscopy to the changes induced by soil management and the incorporation of organic amendments in crop soils is of special interest, providing structural details with the identification of useful functional groups to evaluate the transformation of the incorporated OM.

3.3.1. Characterization of humic acids

The results obtained, as shown in Table 4, indicate that the main differences in the HA absorbance is due to the type of soil. SV samples show higher A4 and A6, which indicate both a greater presence of partly humified material (A4) and a higher degree of aromatic condensation and bigger size and weights of organic molecules in the humic fraction extracted (A6) (Zbytniewski and Buszewski, 2005); which could be due to the fact that smectite-associated SOM is rich in aromatic compounds (Wattel-Koekkoek et al., 2001). The SCG addition shows a tendency to reduce these values in SR and SV, but in the latter only when the incorporation of SCG is 10%. This could mean that the application of SCG made this fraction to present less aromatic condensation, with the dominance of smaller organic molecules and more aliphatic structures, more typical of the initial state of humification. The A4/A6 ratio is considered to be inversely related to the degree of condensation and aromaticity of the humic substances and to their degree of humification (Stevenson, 1994). This ratio indicates differences between soil typologies, with lower values in SV, showing a better distribution in partial and total humified humic acids, and also a higher evolution of HA in this soil. According to Ding et al. (2002), an increase of aromatic-C in HA implied that the humification processes were more advanced, clearly observed in SV without SCG amendment. The addition of SCG makes the values of this ratio to increase, which indicates again that its addition to soil makes HA fraction to have a less evolved character, increasing the partly humified material with a lower molecular size and weight. This also reflects a lower aromaticity with SCG addition and less resistance of SOM.

Table 4. Spectroscopic data of humic acids of soil samples before and after incubation. Values are the mean of 0, 30 and 60 days ± standard deviation.

			Absorbance in UV-Vis			Relative band area (%) of main band of infrared spectra (wavenumber cm^{-1})										
Soil	SCG %	n	A4 (472nm)	A6 (680nm)	A4/A6	3300	2920	2850	1710	1651	1543	1454	1412	1260	1105	595
Red	0	5	0.57 ± 0.04 a	0.13 ± 0.01 a	4.5 ± 0.2 b	70.48 ± 1.75 b	3.07 ± 0.2 bc	0.34 ± 0.03 bc	0.85 ± 0.46 a	11.45 ± 2.17 a	2.62 ± 0.88 ab	1.21 ± 0.1 cd	0.33 ± 0.02 c	0.21 ± 0.05 d	2.12 ± 0.6 bc	1.12 ± 0.96 b
Red	2.5	5	0.53 ± 0.03 a	0.1 ± 0.01 a	5.2 ± 0.2 c	65.99 ± 1.49 a	3.81 ± 0.64 c	0.44 ± 0.14 c	1.4 ± 0.3 ab	13.27 ± 1.28 b	3.23 ± 0.25 b	1.4 ± 0.16 d	0.22 ± 0.03 b	0.18 ± 0.04 cd	1.13 ± 0.6 ab	0.5 ± 0.11 ab
Red	10	5	0.51 ± 0.04 a	0.1 ± 0.01 a	5.1 ± 0.3 c	67.41 ± 4.73 ab	3.58 ± 1.09 c	0.32 ± 0.21 bc	1.2 ± 0.66 ab	12.12 ± 3.37 b	3.63 ± 1.42 b	1.23 ± 0.29 cd	0.09 ± 0.06 a	0.08 ± 0.04 a	1.37 ± 0.79 ab	0.58 ± 0.22 ab
Vega	0	5	0.88 ± 0.29 b	0.22 ± 0.08 b	4 ± 0.2 a	74.63 ± 2.75 c	1.97 ± 0.21 a	0.11 ± 0.05 a	1.57 ± 0.53 abc	6.26 ± 1.37 b	1.87 ± 1.31 a	0.76 ± 0.14 a	0.23 ± 0.05 b	0.15 ± 0.04 bc	2.48 ± 0.53 c	0.69 ± 0.65 ab
Vega	2.5	5	0.92 ± 0.16 b	0.23 ± 0.04 b	4.1 ± 0.1 a	70.08 ± 3.6 b	2.34 ± 0.31 ab	0.16 ± 0.05 a	2.13 ± 0.46 c	10.19 ± 2.15 b	2.48 ± 0.59 ab	0.82 ± 0.23 ab	0.25 ± 0.04 b	0.14 ± 0.03 bc	1.24 ± 1.09 ab	0.38 ± 0.22 a
Vega	10	5	0.82 ± 0.08 b	0.18 ± 0.01 b	4.4 ± 0.2 b	66.72 ± 1.64 ab	3.13 ± 0.57 c	0.24 ± 0.08 ab	1.73 ± 0.81 bc	12.56 ± 3.17 b	2.94 ± 0.67 ab	1.01 ± 0.12 bc	0.11 ± 0.04 a	0.1 ± 0.04 ab	1.04 ± 0.83 ab	0.49 ± 0.28 ab
	Soil type		SV>SR	SV>SR	SR>SV	SR=SV	SR>SV	SR>SV	SV>SR	SR>SV	SR=SV	SR=SV	SR=SV	SR=SV	SR=SV	SR=SV
	SCG %		0%=2.5%=10%	0%=2.5%=10%	10%≥2.5%≥0%	0%>2.5%=10%	10%≥2.5%≥0%	0%=2.5%=10%	0%=2.5%=10%	10%=2.5%>0%	10%≥2.5%≥0%	0%=2.5%=10%	0%>2.5%=10%	0%>2.5%=10%	0%=2.5%=10%	0%≥10%≥2.5%
	Maduration		0d=30d=60d	0d=30d=60d	0d=30d=60d	0d=30d=60d	0d≥30d≥60d	0d≥30d≥60d	0d=30d=60d	0d=30d=60d	0d=30d=60d	0d=30d=60d	0d=30d=60d	0d=30d=60d	0d=30d=60d	0d=30d=60d

SV, Vega soil; SR: Red soil. Different letters denote significant differences (P < 0.05). Statistical differences (>, < or =) of samples grouped according to soil type, %SCG addition and maduration time are also shown.

3.3.2. Functionality of humic acids

To determine functional groups dominance, FT mid-IR spectra of HA was measured and the relative band area of the main bands was calculated (Table 4). Association of bands was made following the method of Madari et al. (2006) and Tatzber et al. (2011). The band with the major absorbance was that associated with the -OH bond of phenols at 3300 cm-1, which appeared to have a higher absorbance in soils without SCG amendment, and also tend to be higher in SV. However, the most significant differences were found in the antisymmetric C–H stretching vibrations in methylene and terminal methyl groups (2920 and 2850 cm-1), associated with the aliphatic compounds, and amide I (1651 cm-1) and amide II bands (1543 cm-1), associated with the little evolved forms of nitrogen. These bands presented higher signal in the SR samples and increased its values in the samples with higher content of SCG, and therefore, denotes the presence of the less evolved HA in these samples. The samples with no SCG amendment presented more signal in the band related with amide III and the more recalcitrant forms of aliphatic acids derived from lignin (1412 cm-1), and also for a band characteristic of phenol and native lignin (1260 cm-1). On the other hand, the band at 1454 cm-1, normally associated to lignin, showed a tendency to increase with the addition of SCG, mainly in SV samples. These results clearly show that the presence of functional groups are associated with less evolved forms of organic matter mainly in the SR samples. No differences were obtained for the band at 1105 cm-1, which was made up of a combination of polysaccharides, phenols and hemicellulose derivatives compounds. Also, a higher signal was obtained at 595 cm-1 for no amendment samples, which indicates a higher presence of aromatic groups, but this must be carefully interpreted because this band could also reflect some clay particles as consequence of impurities in the organic matter extraction.

Therefore, functional groups differences were found when comparing the soil typologies. SR samples showed a trend similar to SCG addition, with higher presence of more labile aliphatic groups and amide I band, while SV presented more absorbance in band associated to C=O bond of the carboxylic acids and the ester carbonyl oxidized functional groups (1710 cm-1), and a tendency of a higher concentration of more recalcitrant groups and aromatics, but these were not statistically significant. This tendency of oxidation confirmed an increase in the resistance of C-fractions in SV, which indicates more efficiency in the stabilization of C. A similar trend was reported in soils on colluvial limestones with organic management (Aranda et al., 2011). These results are probably due to the fact that SR samples have poorer porosity and structural development, as seen in Fig. 2a and 2b. Therefore, the presence of carbonyl functional groups decreased in HA during organic matter stabilization in these soils and the explanation for these results could be oxygen limitation due to reduced diffusion through soil pores. The results of SV, with a better structural development, may show the degradation process of the readily degradable molecules, such as aliphatic components, polysaccharides and alcohols, and can explain the decrease in the concentration of the labile C fraction HWSC. At the same time, it appears that melanoidins and other aromatic constituents from SCG were concentrated, as seen in SV samples.

3.4. Relations between physicochemical and chemical-structural properties of OM

Pearson's correlation coefficients were calculated for all parameters (Table 5). Also, PCA was applied to a matrix containing all the data obtained, and the scores for each sample were represented superimposed with loadings of each parameter (Fig. 4). The different fractions of SOM appeared together, with higher scores in PC2, near the samples with 10% of SCG; which shows a strong correlation between the addition of SCG and the amount of all fractions of SOM. Hence, high correlations (>0.9) between the amount of SCG and SOM fractions individually (TEC, HWSC, Humins, HA, and FA) with $p<0.001$ were found. Samples with SCG addition also appeared near the SDTA labile and recalcitrant area, which shows a positive correlation with the labile compounds total area (0.918), and in a lesser extent with the recalcitrant area (0.5171). However, thermostability index appeared to be closer to samples without amendment and was highly correlated with them (-0.6556), which indicates a higher thermostability in the samples with no amendment treatment. SOM fractions also show correlation with the SDTA areas, which indicate that the main factor in the analysis is the increase of total SOM and all its fractions when SCG were incorporated, and that organic matter functionality changes cannot be clearly appreciated by only a fraction quantification.

However, spectroscopic techniques and especially mid-IR spectra appear to be more sensitive to SOM functionality changes. 1412 and 1260 cm-1 bands appeared close to the samples with no SCG addition, and SCG % showed an inverse correlation with them (-0.8401 and -0.6886, respectively), which indicate a decrease of these functional groups in HA when SCG were incorporated into the soils. These bands also showed a strong correlation with thermostability index, which indicates that these functional groups are the best to reflect SOM stability and recalcitrance. On the other hand, nearer to the samples with 10% of SCG, A4/A6 ratio showed a high correlation with more labile functional groups in mid-IR spectra (2920, 2850, 1651, 1543 and 1454 cm-1); which shows that these functional groups are associated and could be a good indicator of more aliphatic and less stable humic fraction, which is in greater amount in the amended samples. Also, differences between soils can be appreciated, especially a higher concentration of all forms of SOM in SV, as well as a higher macromolecular complexity in their HA (A4 and A6 results). The negative correlation between A4/A6 supports this argument. The results obtained demonstrate that mid-IR spectra of HA contain valuable information and that the characteristics of SOM functionality may be represented by compressing mid-IR into a few vectors that are sufficient to describe its characteristics. Finally, it should be noted that the days of maturation are related only to a certain reduction in aliphaticity in HA (negative correlation with 2920 and 2850 cm-1).

Table 5. Pearson's correlation coefficients of selected variables (n=30).

	% SCG	Days of maduration	HWSC	Humins	TEC	HA	FA	A4/A6	A6	A4	SDTA Labile area	SDTA Recalcitrant area	Thermostability index	2920cm^{-1} (FTIR)	2850cm^{-1} (FTIR)	1412cm^{-1} (FTIR)
% SCG																
Days of maduration	0															
HWSC	0.9158***	-0.0645														
Humins	0.9383***	0.0184	0.7897***													
TEC	0.9605***	-0.1598	0.9013***	0.8952***												
HA	0.9122***	-0.2092	0.8771***	0.864***	0.9604***											
FA	0.9074***	-0.1171	0.8144***	0.8623***	0.9061***	0.863***										
A4/A6	0.3869*	0.0261	0.4749**	0.2688	0.4086*	0.3389	0.3991*									
A6	-0.211	-0.1091	-0.2701	-0.1325	-0.2367	-0.1636	-0.2354	-0.8274***								
A4	-0.1232	-0.135	-0.1799	-0.0589	-0.1546	-0.0808	-0.1698	-0.7421***	0.987***							
SDTA Labile area	0.918***	-0.0517	0.7861***	0.8806***	0.9056***	0.862***	0.8499***	0.2058	-0.0778	-0.0046						
SDTA Recalcitrant area	0.5171**	-0.1115	0.4932**	0.5186**	0.5097**	0.5537**	0.4892**	0.0768	-0.1418	-0.0993	0.5763***					
Thermostability index	-0.6556***	-0.0169	-0.5813***	-0.5801***	-0.6248***	-0.5962***	-0.6062***	-0.1877	-0.1207	-0.1955	-0.6807***	0.0993				
2920 cm^{-1} (FTIR)	0.372*	-0.3911*	0.524**	0.2486	0.4706**	0.4735**	0.299	0.7217***	-0.535**	-0.4395*	0.2994	0.2514	-0.1482			
2850 cm^{-1}(FTIR)	0.1091	-0.3943*	0.3158	-0.0224	0.2211	0.236	0.0556	0.6695***	-0.5419**	-0.4672**	0.0535	0.1322	0.0334	0.9512***		
1412 cm^{-1}(FTIR)	-0.8401***	0.0386	-0.7695***	-0.8187***	-0.8772***	-0.8757***	-0.7705***	-0.328	0.1472	0.0797	-0.8376***	-0.3727*	0.7239***	-0.3596	-0.1262	
1260 cm^{-1}(FTIR)	-0.6886***	-0.1494	-0.5244**	-0.6573***	-0.6228***	-0.6169***	-0.6498***	-0.141	-0.0463	-0.0979	-0.5986***	-0.1136	0.6685***	0.0563	0.2848	0.684***

*TEC: total extractable carbon; HWSC: hot water soluble carbon; HA: humic acids; FA: fulvic acids; SDTA: simultaneous differential thermal analysis. Statistical significance: * P< 0.05; ** P < 0.01; *** P < 0.001*

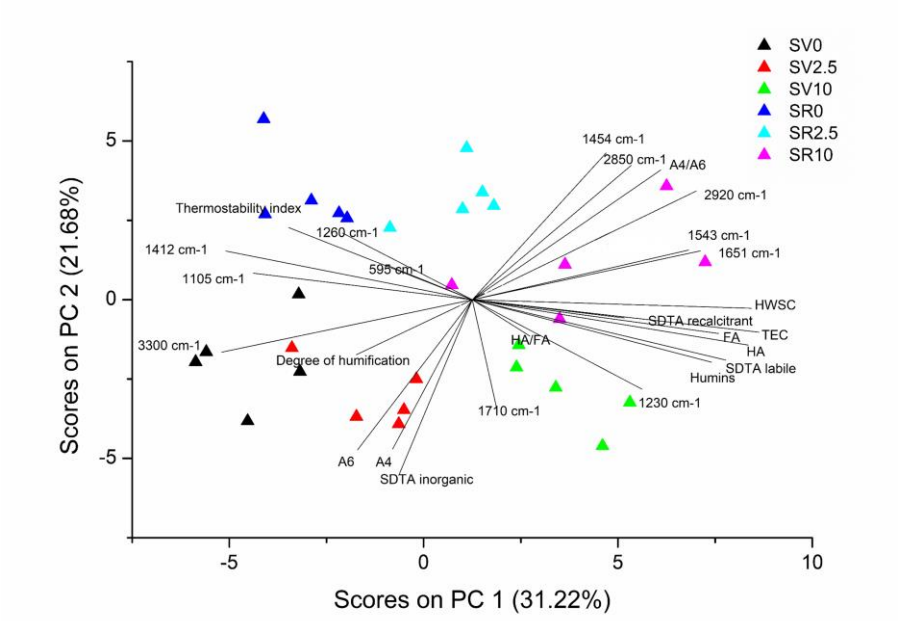

Figure 4. Superimposed graph of principal component analysis (PCA) scores obtained for samples and loadings of each parameter analyzed. SR0, Red soil without Spent Coffee Ground (SCG); SR2.5: Red soil with 2.5% SCG; SR10; Red soil with 10% SCG; SV0: Vega soil without SCG; SV2.5: Vega soil with 2.5% SCG; SV10: Vega soil with 10% SCG; SDTA: simultaneous differential thermal analysis; HA: humic acids; FA: fulvic acids; TEC: total extractable carbon; HWSC: hot water soluble carbon.

4. Conclusions

The addition of 2.5 and 10% SCG as an amendment clearly generated an increase in organic matter levels in two contrasted Mediterranean soils, which led to an improvement of soils physicochemical properties, increasing soil fertility and carbon stock. The type of soil plays a key role: the smectitic and more clayey soil (SV) maintained, in general, a higher quality of organic matter with regard to the more illitic and less carbonated soil (SR), probably because of their higher SOM evolution, and/or because of their background as originally higher quality soil. SCG addition greatly improved the levels of all SOM fractions, but it does so especially in more labile fractions, while the increase in the more complex and recalcitrant material is lower. This affects the SOM functionality because the proportion of more recalcitrant and aromatic compounds is reduced, which makes organic matter to reduce its stability, which in turn can affect its nutrient affordability in the long term. This effect is attenuated in SV, where recalcitrant fraction increased in a higher level due to its mineralogical composition and aggregation status. Incubation days assayed (30 and 60 days) seems to have had no effect on the SOM studied, which leads to consideration of the necessity of a longer microcosm assays, and the need for field studies.

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Capítulo 12
Improvement of quality and agronomic properties of raw organic material mixtures by thermal treatment

Este capítulo se corresponde con el artículo "Improvement of quality and agronomic properties of raw organic material mixtures by thermal treatment", pendiente de envío a la revista Waste and Biomass Valorization. En este artículo se estudian los cambios en la calidad de diferentes enminedas orgánicas, y mezclas de ellas, tras aplicar un tratamiento térmico.

Esta publicación está pendiente de envío.
F. Comino, V. Aranda, A. Domínguez-Vidal, M.J. Ayora-Cañada

Abstract

Soil quality degradation processes constitute an important and growing problem in the Mediterranean area due to agricultural practices. Organic amendments are a suitable option to recover soil fertility by increasing soil organic matter. Composted olive mill pomace and spent coffee grounds are two examples of organic amendments which can be used for this purpose, but both individual amendments have some disadvantages like pH and the organic matter quality. For improving these amendments, we propose to mix them in different proportions, which show to generate an amendment with some improved properties, like neutral pH, reduced EC and more complex and evolved organic matter, due to COMP and SCG different functional groups predominance. However, the final quality of this amendments could be upgraded. Thermal treatment shows to be a good option for improving the quality of organic amendments, especially treatments at 275ºC, and in lesser extent 225ºC. Thermal treatment transforms organic matter into one more recalcitrant, with more presence of aromatic and stable functional groups, and a decreased presence of more labile and easily degradable forms. This treatment shows to be more effective for mixes of COMP and SCG, generating an even richer organic amendment.

1. Introduction

The improvement of soil quality has become an interesting task to study since the soil degradation processes constitute an important and growing problem in the Mediterranean area, mainly due to agricultural practices [1]. The use of organic amendments has been revealed as an efficient way to help recover adequate levels of organic matter, which is a key factor in this task [2]. In this sense, the use of by-products is always a good option because it achieves the reduction of residues together with the increase of quality of soils. Extensive olive cultivation in the Mediterranean area leads to a huge annual production of by-products, like the olive tree pruning (OTP), a yearly generated lignocellulosic agricultural residue, with

a production estimated at 3000 kg/ha worldwide [3]. It is mainly composed by a woody fraction and a remaining portion containing leaves and fine branches. The transformation of olive tree pruning biomass is necessary to keep fields clean and to avoid propagation of vegetal diseases [4]. Biomass from pruning of olive trees is one of the most widely available lignocellulosic materials particularly in the Mediterranean countries. By another side, olive mill pomace (OMP) is the main by-product of the extraction process of olive oil. This waste is highly pollutant and difficult to handle due to its high moisture, which makes it an important problem for olive agro-industry [5]. The possible recycling opportunity for these by-products could be its use as an organic amendment after an adequate composting process [6].

Spent coffee grounds (SCG) can be also examined from the same point of view. More than 6 million tons of these grounds that remain after brewing the beverage are generated each year worldwide [7]. Thus, the use of this by-product as a source of organic matter and nutrients for agricultural soils has to be considered [8]. The use of SCG directly can arise problems of acidity and toxicity [9] due to the presence of some compounds as polyphenols and tannins. That is why an adequate composting process [6] is necessary. Furthermore, the mixing with OMP and OTP compost seems like a good option that could correct typical high pH and C/N ratio of olive mill pomace compost amendments [10]. By the other side, thermal treatments have been found to change organic matter fractions, stabilizing the organic matter to chemical and biological degradation [11]. This can yield in organic amendments more suitable to improve soil fertility [12]. The use of this kind of organic amendments can not only increase soil organic matter levels [13], but also encourage soil physical, chemical and biological properties [14], without the need of use any industrial fertilizer. But it is necessary to further study effects and changes in soils, especially in organic matter functionality [15], since the application of organic matter that is not sufficiently mature and stable may adversely affect soil properties, and especially the content and quality of native soil organic matter pools [16]. In this way we could knowhow soil carbon stock may change, as well as the nutrient availability in the short, mid and long-term, and the resistance to degradation of this organic matter [17].

The objective of this work is to study organic matter quality of amendments of OMP, OTP, SCG and mixes of them by means of ATR-FTIR spectroscopy and UV-Vis spectroscopy. Thermogravimetric analysis (TG-DTA) and phytotoxicity tests have been also approached. In order to improve the quality of the proposed amendments a thermal treatment was applied.

2. Material and methods

2.1. Sample description

Six samples containing olive mill pomace compost and spent coffee grounds were used in this study. One sample of COMP, composed by a 30% of OMP and 70% of OTP, prepared by mobile stacking, turning every fortnight for the first four months of bio-oxidation and then allowed to stand without turning for two more months; one sample of SCG and four samples (M1 to M4) that are mixtures of COMP and SCG with different proportions (20, 40, 60 and 80% of SCG respectively). SCG (100% Arabica) were obtained from a local coffee shop.

2.2. Thermal treatment

Samples were desiccated at 65ºC and then ground and sieved to 200 µm. Thermal treatment was applied to about 60 g of each sample, in a pre-heated oven (Binder FED GmbH) with a 5ºC/min gradient reaching temperatures of 175, 225 or 275ºC respectively, and maintaining the temperature for five hours [18].

2.3. Chemical characterisation and elemental analysis

Electrical conductivity and pH were measured in 1:10 (w/v) water soluble extracts with a CRISON conductimeter and a CRISON pH-meter respectively. Total organic carbon, total nitrogen and ash contents were determined using a LECO TruSpec CHN 620-100-400 analyser. P, K, Ca and Mg were determined in an ICP-OES VARIAN 715-ES after digesting samples with H2O2, HNO3 and HF in an ETHOS microwave digester.

2.4. Spectroscopy

UV-Vis spectra of sample NaOH extracts were recorded in a Varian Cary 50 (Varian, Inc.) spectrometer. Extracts were obtained by mixing 0.1 g of each sample with 25 mL of 0.5 M NaOH in polyethylene flasks. These suspensions were stirred for two hours and then let stand for 12 h. Finally, the samples were centrifuged for 25 minutes at 3000 rpm. Dilutions from 1:5 to 1:20 were prepared and then spectra were recorded from 200 to 800 nm The A4/A6 ratio was calculated using absorbance at 472 nm (A4) and 664 nm (A6) [19]. Mid-IR spectra of the solid samples were registered using a Varian 660 FTIR spectrometer (Varian, inc.) equipped with an attenuated total reflection accessory (ATR, Pike Technologies) with a three-reflection diamond crystal. Samples were finely ground in an agate mortar and placed directly on the ATR crystal. Spectra were collected from 700 to 4000 cm-1 with a resolution of 4 cm-1 and 128 accumulations. The spectrum of air was used as background. The hydrophobicity index, as the hydrophobic/hydrophilic ratio [20] was calculated using the area under the 3000-2800 cm-1 band corresponding to aliphatic CH bonds as a measure of hydrophobic functional groups, and the total area of the 1740-1600 cm-1 band, corresponding to the C=O of aromatic functional groups, as a measure of hydrophilicity. Areas were calculated using OPUS 7.0 spectroscopy software (Bruker Optics). The ratios between absorbance values at 1606/1740 cm-1 was also calculated as an indicator of the relative concentration of aromatic compounds [12], and between 1606 and maximum absorbance between 1150-1000 cm-1 as an indicator of the concentration of polysaccharides compounds.

2.5. Thermogravimetric analysis

Thermogravimetric (TG) and simultaneous differential thermal analysis (SDTA) were carried out using a TGA/SDTA 851e Mettler Toledo instrument. 20 mg of each sample were dried and weighed into a 70-µL aluminium oxide capsule and subjected to temperatures of 25 to 850ºC, increasing 5ºC/min with a 100 mL/min synthetic air flow. The thermostability index of samples was calculated following [21], by dividing the recalcitrant fraction mass loss, by the mass loss of the labile and recalcitrant fractions. The labile fraction was calculated as the band area of the mass loss between 200 and 400ºC in SDTA, while the recalcitrant fraction was calculated as the band area corresponding to mass loss between 400 and 600ºC in SDTA.600ºC in SDTA.

2.6. Phytotoxicity measurement

Phytotoxicity of organic wastes was determined according to the germination index using the Zucconi test [22]. 4 g of dry samples were moistened with deionised water to 60% water holding capacity. The mixture was shaken and let stand for 30 minutes. After that, more deionised water was added to a 1:10 w/v concentration and stirred mechanically for 30 minutes, centrifuged at 3000 rpm for 15 minutes and filtered (0.45 μm). 1 ml of the extract was added to a Petri dish with filter paper and 10 Raphanus sativus L. seeds were incubated at 28ºC for 72 hours in the dark. Root length was measured and results were expressed as the germination index resulting from the percentage of germination and root elongation over the control sample.

2.7. Statistics

Normality assumptions were tested using the Saphiro-Wilk test. The F-test (ANOVA) was applied to variables fitting a normal distribution, while an alternative non-parametric test (Kruskal-Wallis ANOVA) was applied to variables with no normal distribution. The Bonferroni test was used to find differences between means. All statistical procedures were carried out using Statgraphics Centurion XVI software (StatPoint Technologies, Inc.).

Principal component analysis (PCA) was used for clustering samples and their relationships to specific bands in the mid-IR region. This statistical treatment was performed in MATLAB 2008R (MathWorks, Natik, USA) using the Eigenvector Research Inc. (Wenatchee, USA) PLS-toolbox.

3. Results and discussion

3.1. Effect of spent coffee grounds in amendment quality

COMP sample stand out cause of its high electrical conductivity (3.5 ± 0.5 dS/m), in part associated to a higher concentration of some cations (Ca 3.23 ± 0.15 %, Mg 0.89 ± 0.04 % and K 1.33 ± 0.21 % respectively), but especially because of its low pH (6.1 ± 0.4), due that typical olive mill pomace compost pH values range between 7.5 and 8.5 [23], which could indicate an inadequate composting process, and therefore an inmature compost By another side, SCG show lower ash content (only 1.6 ± 0.2 % for the 7 ± 0.4 % of COMP sample), and higher N and P concentrations (2.3 ± 0.15 % and 1.1 ± 0.07 % respectively). This differences in the concentration of nutrients could make the mix of COMP and SCG to generate an improved amendment, with balanced amount of nutrients. Mid-IR spectra plot of the COMP, SCG and M1 and M3 (20 and 60% SCG)

samples are represented in

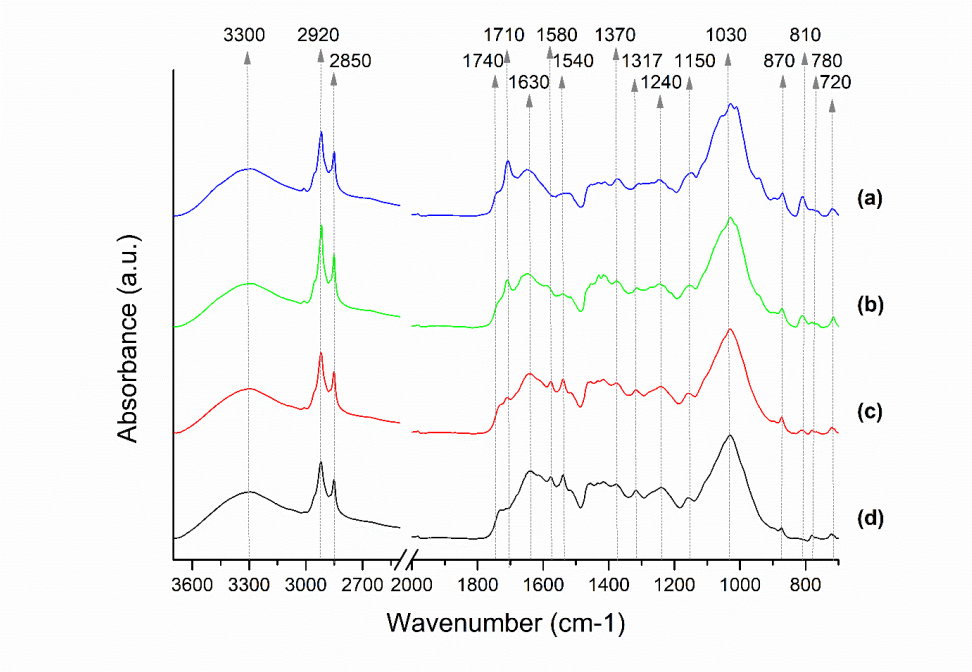

Figure 1. Assignments of the main IR spectra bands are based mainly on [24–26]. OH stretching of H-bonded hydroxyl groups associated to phenols of OMP, and also celluloses derived compounds (3300 cm^{-1}), and symmetric and asymmetric aliphatic C-H stretching (2920 and 2850 cm^{-1}), appear as two of the most dominant bands. The signal in them is higher for SCG M4 and M3 samples, indicating a high concentration of this more labile organic matter in the SCG. The absorbance of bands at 2920 and 2850 cm^{-1} could also be associated with caffeine and lipids, but the presence of these compounds is too low to think they could be highly responsible for that signal. Same occurs with 1710 cm^{-1} -C=O stretching of –COOH band, typically associated with carboxylic acids, but also to caffeine [25]. By another side, it is clear the higher aromatic character of samples with SCG, which show higher absorbance in bands between 900-700 cm^{-1}, related to aromatic C-H out of plane bend. COMP sample show higher signal in the bands between 1600 and 1200 cm-1, most of them associated to functional groups indicating proteins, hemicellulose and lignin presence [27]. This make samples with COMP to show higher presence of amide I (1651 cm^{-1}) and amide II bands (1543 cm^{-1}), associated to little evolved forms of nitrogen, amide III and more recalcitrant forms of aliphatic acids derived from lignin (1412 cm^{-1}), and also for a band characteristic of phenol and native lignin (1260 cm^{-1}). This show a high variety of SOM in samples with COMP, with both labile and recalcitrant character. Finally, bands at 1150 and 1030 cm^{-1}, associated to carbohydrates and polysaccharides show similar absorbance in

all samples, but 1150 cm^{-1} band seems to be broader in samples with SCG, and 1030 cm^{-1} band show various small bands, which indicate more variety of these type of compounds in SCG samples.

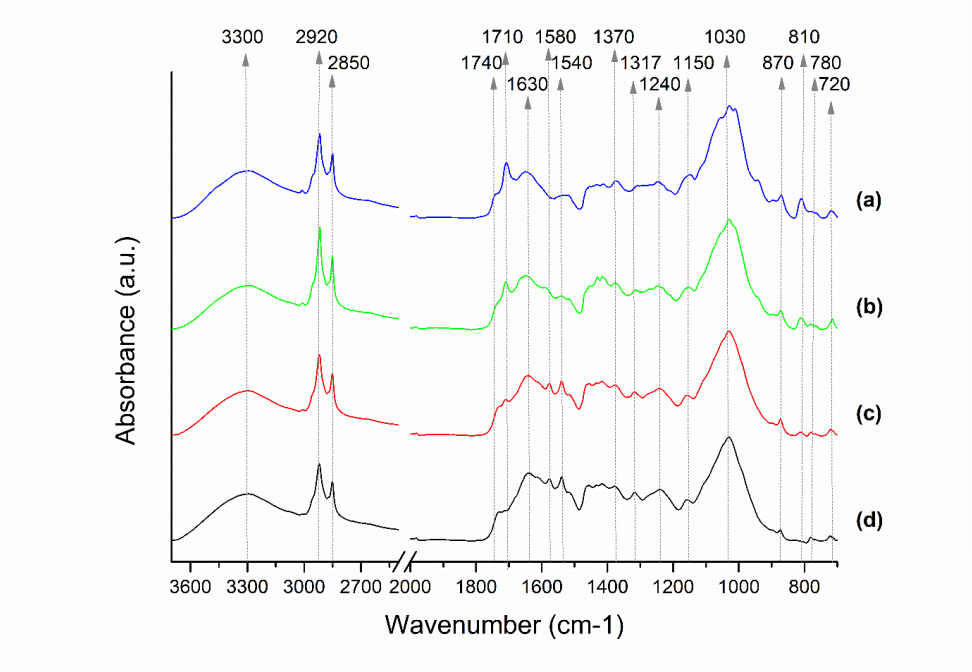

Figure 1. Mid-IR spectra representation of initial amendment samples. a) SCG; b) M3; c) M1; d) COMP.

Mid-IR spectra interpretation afford relevant information about the organic matter composition of amendments, but for a deeper characterization, IR ratios were calculated, and UV-Vis, TG-SDTA and phytotoxicity index were measured. Results are shown in Table 1. We can observe higher values for A4 and A6 in COMP sample, indicating both more presence of material more complex and evolved, and material with less complex character and more decomposable. Also, COMP sample, show a higher thermostability index and 1606/1740 ratio and lower 1606/Polysaccharides ratio and hydrophobic index. This indicates, that COMP sample have more recalcitrant and evolved organic matter in comparison with SCG sample, and make this amendment to be more stable and useful as soil nutrient storage, but also show a higher presence of OM that could be easily degradable by microorganisms, and serve as a font of nutrients in a short term. By another side, SCG sample shows a higher hydrophobic index, indicating more presence of aliphatic compounds in relation to more hydrophilic compounds, and to be an amendment with rawer OM, as show with its lower 1606/Polysaccharides ratio, which is inversely related to the amount of polysaccharides in the sample. M1-M4 samples show values that clearly relates to the amount of each amendment they have, except for thermostability that shows values nearer to COMP that SCG, indicating the mix is a good option to improve OM stability whatever the quantity of each amendment we use. Aside, germination index, that is indicative of amendment phytotoxicity, show that both amendments have a little harmful effect, due that germination index under 100%. However according to [28], none of them should be considered to have a phytotoxic effect, and also that COMP sample should be considered as a mature compost [29]. Lower germination index of SCG is probably due to its acidic character, and the presence of phytotoxic substances contained in this waste. When mixing both organic amendments, germination index elevate over 100%, indicating not only absence of toxicity, but also that it is a good

substrate for plant growth, probably due to presence of more nutrients in the mixes, but maybe also due to some toxic compound block when mixing the samples.

3.2. Thermal effects on amendment quality

After thermal treatment of samples, mid-IR spectra of all samples were measured. For better studying samples similarities and differences PCA was applied (Figure 2). We can observe PC1 (90.71% variance) group samples according to temperature treatment. Main loadings in this PC are the bands associated to amide II (1650 cm^{-1}) and derives from lignin (1400-1200 cm^{-1}), and in a lesser extent, aromatics (900-700 cm^{-1}) and carboxylic acids (1720 cm^{-1}). This indicates thermal treatment increase concentration of more recalcitrant and aromatic functional groups, being the treatment at 275ºC who show the greater effect, while treatment at 225 and 175ºC produce very light changes. Negative loadings in this PC are associated with polysaccharides concentration (1020 cm^{-1}), but also with phenol and aliphatic acids concentration (3300, 2920 and 2850 cm^{-1}). Therefore, thermal treatment also decreases the concentration of this more labile fraction of organic matter. By another side, while PC2 (5.18% variance) do not show any clear grouping, PC3 (2.07% variance) was able to group sample by its composition. Samples with more COMP have the higher values in this PC, which are associated to amide II and lignin derivatives, while samples with more SCG have the negative values, that according to PC loadings, are due to some carbohydrates (1150 cm^{-1}), carbonyl stretching of esters (1740 cm^{-1}), and in lesser extent aliphatic compounds (2920 and 2850 cm^{-1}). These indicate that even when thermal treatment decrease this more labile groups, amendments with SCG still have a higher remainder of aliphatic acids and carbohydrates.

Table 1. Results of different analysis used for the characterisation of organic amendments.

Sample	A4 (472nm)	A6 (680nm)	A4/A6 Ratio	Hydrophobic Index	1606/1740 Ratio	1606/polysaccharides Ratio	Thermostability Index	Germination Index (%)
COMP	0,31 ± 0,04c	0,12 ± 0,01c	2,49 ± 0,16a	0,424 ± 0,017a	1,12 ± 0,02b	0.60 ± 0,03 d	0,687 ± 0,019d	93 ± 4,5b
SCG	0,22 ± 0,04a	0,09 ± 0a	2,62 ± 0,4a	0,53 ± 0,012c	0,95 ± 0,04a	0.29 ± 0,02 a	0,358 ± 0,015a	77,4 ± 2,6a
M1	0,28 ± 0,03bc	0,11 ± 0,01bc	2,47 ± 0,21a	0,48 ± 0,016b	1,07 ± 0,03b	0.50 ± 0,04 cd	0,628 ± 0,018c	113,7 ± 4,4c
M2	0,26 ± 0,02abc	0,1 ± 0,01ab	2,59 ± 0,1a	0,552 ± 0,018cd	1 ± 0,03a	0.44 ± 0,04 bc	0,635 ± 0,015c	111,1 ± 3,5c
M3	0,27 ± 0,03abc	0,1 ± 0,01ab	2,66 ± 0,07a	0,579 ± 0,013de	0,99 ± 0,04a	0.38 ± 0,04 b	0,607 ± 0,014c	116,7 ± 5,7c
M4	0,23 ± 0,02ab	0,09 ± 0,01a	2,63 ± 0,13a	0,583 ± 0,017e	0,95 ± 0,04a	0.34 ± 0,03 b	0,523 ± 0,011b	118,5 ± 4c

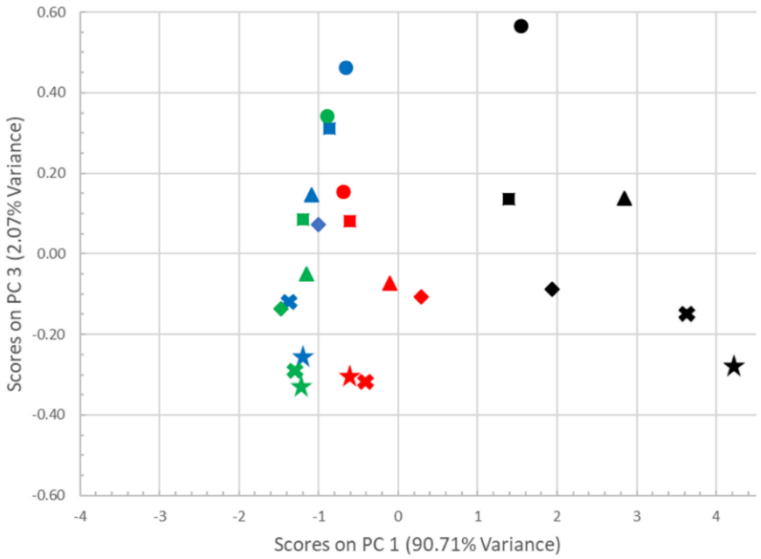

Figure 2. PCA representation according to type of sample and thermal treatment. Colors represent temperatures of thermal treatment: blue: 65ºC; green: 175ºC; red: 225ºC; black: 275ºC. Forms represent samples: circle: COMP; square: COMP-CF1; triangle: COMP-CF2; diamond: COMP-CF3; cross: COMP-CF4; star: CF.

The evolution of the different parameters studied to characterizing the organic amendments are shown in Figure 3. A clear increase is appreciated in both A4 and A6 absorbance with thermal treatment. This increase is especially relevant with 225ºC, while with 275ºC the maximum values are reached for all samples, except for A4 in COMP sample. This seems to indicate an increase in the most complex fractions of OM of all samples [30], being 225 the temperature most suitable to reach this improvement. Melanoidins, macromolecules formed by Maillard reaction and considered as synthetic humic substances [31], derived from SCG, as well as other aromatic constituents, such as condensed and lignin-derived aromatic carbons, could be concentrated during the thermal treatment, being structures inherently stable against degradation. The decrease of A4 in COMP sample at 275 appears to indicate that this temperature begins to also eliminate some of these more complex compounds. However, this temperature seems to be the best option to improve these two fractions in the SCG sample that have a way greater increase. Mixes of COMP and SCG show the highest values, especially for A6 at 275ºC, indicating this thermal treatment is even more effective when the two amendments are combined. The increase of A4/A6 ratio with thermal treatment indicates, that even when the two fractions increase, more decomposable material (but still form by complex compounds) increase in a higher way. This is less accentuated in samples with more COMP, whereas previously said, with 275ºC treatment A4 begin to descend and results in a lower A4/A6 ratio.

The hydrophobic index shows a clear tendency to decrease with temperature, while 1606/1740 and 1606/Polysaccharides ratios increase, especially with 225 and 275ºC. These ratios show a clear transformation of functional groups, reducing those associated to a more aliphatic character, and especially the carbohydrates and polysaccharides, with an increasing of those with more aromatic character. This transformation is way higher in samples with more COMP for the rise of aromatics, and reduction of polysaccharides, while for the samples with SCG the decrease of aliphatic acids is more marked. These results show in general, a better amendment quality for COMP samples, and especially for M1-M3 due to a higher aromaticity and complex functional groups presence [32]. This reduction in hydrophobic character of organic amendments with the thermal treatment could have a favorable effect on plant water availability and infiltration, once applied to the soil, as stated by Aranda et al. [33].

The changes in organic matter composition are also reflected in the thermostability index. M1-3 and COMP samples show a great increase with 225ºC treatment, while SCG sample, and in lesser extent M4 sample, which need of 275ºC to reach stability levels similar to the other samples. Temperature show to improve organic matter stability and recalcitrance of organic amendments, but samples with more amount of SCG need treatment with higher temperatures. Finally, regarding the phytotoxicity values obtained via germination index, we can observe how 175ºC treatment raise germination index in COMP sample, making it higher than 100%, and eliminating any toxicity of the sample. However, for completely eliminate the toxicity of SCG sample, 275ºC treatment is needed. Mixes show improvement with all thermal treatment and reach the highest index with 275ºC, indicating this temperature get changes in organic matter that clearly favours plant growth and eliminate possible toxicity problems.

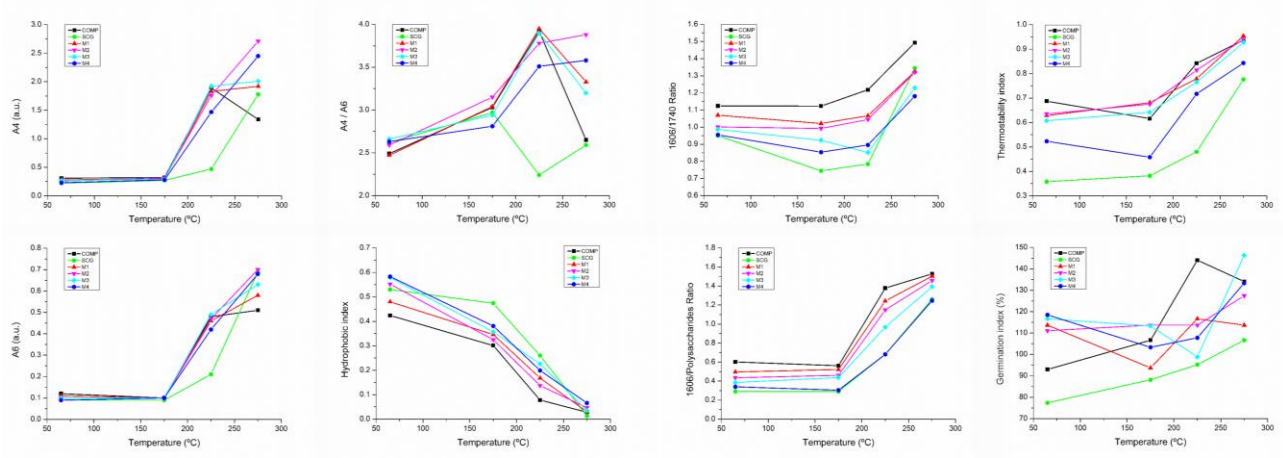

Figure 3. Diagram of parameters evolution for every sample with the different temperature of thermal treatment.

4. Conclusions

Organic amendments are a suitable option to increase soil organic matter while affording important nutrients. COMP and SCG amendments show to be good for this purpose, and the mixing of them generate an amendment with better properties than both individually. Mixes of COMP and SCG show to be better for plant growth than both individually, and also to afford more complex and evolved organic matter, due to COMP and SCG different functional groups predominance. However, the final quality of this amendments could be upgraded. Thermal treatment shows to be a good option for improving the quality of organic amendments, especially treatments at 275ºC, and in lesser extent 225ºC. Thermal treatment transforms organic matter into one more recalcitrant, with more presence of aromatic and stable functional groups, and a decreased presence of more labile and easily degradable forms. This treatment shows to be more effective for mixes of COMP and SCG, generating an even richer organic amendment. In this way, we can conclude than mixing different organic amendments seem to be a good strategy to generate a better substrate for soil, and that this kind of amendment can get an important quality improvement by thermal treatment.

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Capítulo 13
Conclusions / Conclusiones

Conclusions

I. Soil management and soil typology clearly affect soil quality, being organic management the best option to maintain and increase soil quality, while conventional management, and irrigation with poor quality water cause soil degradation and loss of fertility.

II. Infrared spectroscopy shows to be a technique really useful to characterize soils, being especially sensitive to soil minerals, but also to the organic fraction in soils, showing the ability to cluster samples according to soil quality.

III. Infrared spectroscopy demonstrates to have the capacity to almost perfectly discriminate different soil typologies, but also a good capacity to discriminate between different soil managements.

IV. Predictive models built using infrared spectroscopy could be used to predict most of more usual soil physical-chemical properties. Those results indicate some capacity of this technique to substitute or complement typical laboratory analysis. By other side, infrared spectroscopy shows less capacity to predict biological variables, but a good performance in predicting soil biological quality index, which could be really good tool for a rapid quality assessment of soils.

V. FT-NIR and EDXRF techniques demonstrate to be able to assess olive nutritional status in a more rapid and economic way than traditional laboratory analysis, through prediction of most of main nutrients in leave analysis. Those results are even better when a mid level data fusion strategy is applied, demonstrating the viability of utilize this kind of strategy when using different techniques.

VI. The use of spent coffee grounds as an organic amendment in soils show to clearly increase all soil organic matter fractions, but the higher increase in more labile fraction could affect organic matter functionality. By other side, the mix of this amendment with olive mil pomace compost shows as a great way to generate an improved amendment that palliate the previous disadvantage, among others like pH and C/N.

VII. Thermal treatment of 275ºC completely eliminate water repellency of different organic amendments, also improving the quality of their organic fraction. This effect is even better for the mixes of spent coffee grounds and olive mill pomace compost.

Conclusiones

I. El manejo y la tipología de suelo son dos factores que afectan claramente a la calidad del suelo. De los diferentes manejos, el manejo ecológico se demuestra como la mejor opción para mantener y mejorar la calidad del suelo, mientras manejos convencionales y sistemas de irrigación con aguas de mala calidad causan degradación y pérdida de fertilidad.

II. La espectroscopía infrarroja es una técnica muy útil para caracterizar suelos, ya que es muy sensible al contenido mineralógico del suelo, pero también sirve para estudiar su fracción orgánica, siendo en definitiva capaz de diagnosticar la calidad del suelo.

III. La espectroscopía infrarroja demuestra tener la capacidad para discriminar perfectamente entre diferentes tipologías de suelo, y con una fiabilidad muy alta entre diferentes manejos del suelo.

IV. Los modelos predictivos construidos utilizando espectros infrarrojos con capaces de predecir gran cantidad de parámetros físico-químicos en suelos. Estos resultados indican que esta técnica puede en parte ustituir o complementar a los análisis tradicionales de laboratorio. La capacidad de predecir variables biológicas es inferior, aunque si se revelan como más robustos los modelos para predecir el índice de calidad biológica global (GMea), lo que sería una herramienta muy útil para la rápida estimación de la calidad del suelo.

V. El uso combinado de FT-NIR y EDXRF permite determinar el estado nutricional del olivo, de manera más rápida y barata que el análisis tradicional de laboratorio, a través de la predicción de la mayoría de los nutrientes en la hoja. Los modelos predictivos son más robustos cuando se utiliza una estrategia de fusión de datos *mid level*, lo que demuestra la viabilidad de este tipo de estrategia cuando se usan diferentes técnicas.

VI. La adición de posos de café al suelo como enmienda orgánica incrementa en gran medida las diferentes fracciones de materia orgánica del suelo, pero el mayor aumento de compuestos más lábiles, afecta a la funcionalidad de la materia orgánica. Por otro lado, mezclar esta enmienda con compost de alperujo consigue paliar este perjuicio, mejorando tambien otros parámetros como el pH y el C/N.

VII. El tratamiento térmico a 275ºC consigue eliminar por complete la repelencia al agua de las enmiendas orgánicas, mejorando además la calidad de su fracción orgánica. Este efecto es aún mayor cuando se aplica el tratamiento térmico sobre mezclas de posos de café y compost de alperujo

Anexo I

Contribuciones científicas generadas durante el desarrollo de la tesis doctoral

En este capítulo se exponen las producciones de índole científico generadas durante el desarrollo de la tesis doctoral, incluyendo las diferentes publicaciones científicas realizadas, incluidas alguna no presente en esta tesis doctoral, así como los diferentes congresos a los que se han aportado comunicaciones y las estancias de investigación realizadas en centros diferentes a la Universidad de Jaén.

Publicaciones

1. Autores: Víctor Aranda Sanjuán; Ana Domínguez Vidal; Francisco Comino Romero; Julio Calero González; María José Ayora Cañada.
Título: Agro-environmental characterization of semi-arid Mediterranean soils using NIR reflection and mid-IR-attenuated total reflection spectroscopies. Revista: Vibrational Spectroscopy. 74, pp. 88 - 97. ELSEVIER, 07/08/2014.
Tipo de producción: Artículo científico. Autor de correspondencia: No

2. Autores: Francisco Comino Romero; Víctor Aranda Sanjuán; Ana Domínguez Vidal; María José Ayora Cañada.
Título: Thermal destruction of organic waste hydrophobicity for agricultural soils application.
Revista: Journal of Environmental Management. 202, pp. 94 - 105. ELSEVIER, 01/11/2017.
Tipo de producción: Artículo científico. Autor de correspondencia: Si.

3. Autores: Francisco Comino Romero; Víctor Aranda Sanjuán; Roberto García Ruiz; María José Ayora Cañada; Ana Domínguez Vidal.
Título: Infrared spectroscopy as a tool for the assessment of soil biological quality in agricultural soils under contrasting management practices.
Revista: Ecological Indicators. 87, pp. 117 - 126. ELSEVIER, 04/01/2018.
Tipo de producción: Artículo científico. Autor de correspondencia: Si

4. Autores: Francisco Comino Romero; María José Ayora Cañada; Víctor Aranda Sanjuán; Antonio Díaz; Ana Domínguez Vidal.
Título: Near-infrared spectroscopy and X-ray fluorescence data fusion for olive leaf analysis and crop nutritional status determination.
Revista: Talanta. 188, pp. 676 - 684. ELSEVIER, 01/10/2018.
Tipo de producción: Artículo científico. Autor de correspondencia: No
European Journal of Soil Science

5. Autores: Francisco Comino Romero; Ana Cervera Mata; Víctor Aranda Sanjuán; Gabriel Delgado Calvo-Flores.
Título: Impact of spent coffee grounds over soil organic matter functionality and stability in two contrasted Mediterranean agricultural soils.
Revista: European Journal of Soil Science. Enviado.
Tipo de producción: Artículo científico. Autor de correspondencia: No

6. Autores: Francisco Comino Romero; Víctor Aranda Sanjuán María José Ayora Cañada; Antonio Díaz; Ana Domínguez Vidal.
Título: Effect of irrigation water quality on soil properties and infrared spectroscopic signatures
Revista: Spanish Journal of Agricultural Research. Pendiente de envío.
Tipo de producción: Artículo científico. Autor de correspondencia: Si

7. Autores: Francisco Comino Romero; Víctor Aranda Sanjuán; Ana Domínguez Vidal; María José Ayora Cañada.
Título: Improvement of quality and agronomic properties of raw organic material mixtures by thermal treatment.
Revista: Waste and Biomass Valorization. Pendiente de envío.
Tipo de producción: Artículo científico. Autor de correspondencia: Si

Publicaciones no incluidas en esta tesis

1. Autores: Víctor Aranda Sanjuán; Francisco Comino Romero.
Título: Soil organic matter quality in three Mediterranean environments (a first barrier against desertification in Europe).
Revista: Journal of Soil Science and Plant Nutrition. 14 - 3, SCIELO,
Tipo de producción: Artículo científico. Autor de correspondencia: No.

Comunicaciones presentadas en congresos nacionales o internacionales

1. Soil biological quality determination in agricultural soils using near infrared spectroscopy. EGU General Assembly 2018. Viena, Austria. 08/04/2018. Comunicación en formato póster.
2. Spectroscopic study of effects of spent coffee grounds addition on soil organic matter in two Mediterranean agricultural soils. EGU General Assembly 2018. Viena, Austria. 08/04/2018. Comunicación en formato póster.
3. Near infrared spectroscopy and X-ray fluorescence data fusion for olive nutritional status determination. Colloquium Spectroscopicum Internationale - XL PIsa 2017. Pisa, Italia. 11/06/2017. Comunicación oral.
4. Destrucción térmica de la hidrofobicidad del alperujo compostado para su aplicación como enmienda orgánica. evaluación mediante espectroscopía FTIR. V Jornadas de la Red Española de Compostaje REC 2016. Sevilla, Andalucía, España. 16/11/2016. Comunicación oral.
5. Nutrient assessment in olive leaves through NIR spectroscopy. XXV Reunión Nacional de Espectroscopía (XXV RNE) - X Congreso Ibérico de Espectroscopía (IX CIE). Alicante, Comunidad Valenciana, España. 20/07/2016. Comunicación en formato póster.
6. Estudio Preliminar de la Aplicación de la Espectroscopía de Infrarrojo Cercano a la Predicción del Estado Nutricional del Olivo. VII Jornadas de Jóvenes Investigadores en Física Atómica y Molecular. Jaén, Andalucía, España. 18/03/2015. Comunicación en formato póster.
7. Estudio Preliminar Sobre la Capacidad de la Espectroscopía de Infrarrojo Cercano Para la Predicción de Nutrientes en Hoja de Olivo. XV Reunión del Grupo Regional Andaluz de la Sociedad Española de Química Analítica (GRASEQA 2016). Almería, Andalucía, España. 30/06/2016. Comunicación en formato póster.
8. Efectos a largo plazo del co-compost de alperujo sobre la repelencia al agua y otras propiedades físicas y fisicoquímicas en suelos de olivar. IV Jornadas de la Red Española de Compostaje (REC 2014). Murcia, Región de Murcia, España. 12/11/2014. Comunicación en formato póster.
9. Knowledge fusion in the Agro-environmental Field: A Global Index for Soil Quality in Olive groves from Quantitative and Qualitative Variables. 17th International Conference on Information Fusion (Fusion 2014). Salamanca, Castilla y León, España. 07/07/2014. Comunicación en forma de resumen.
10. Predicción de parámetros edáficos de fertilidad en olivar mediante espectroscopía infrarroja. VI Congreso Ibérico de la Ciencia del Suelo. Santiago de Compostela, Galicia, España. 22/06/2014. Comunicación oral.
11. Una nueva propuesta de evaluación de la calidad del suelo y su aplicación en el olivar de Jaén. VI Congreso Ibérico de la Ciencia del Suelo. Santiago de Compostela, Galicia, España. 22/06/2014. Comunicación en formato póster.
12. Predicción de parámetros edáficos de fertilidad en olivar mediante espectroscopía infrarroja. I Reunión de Investigadores en Formación de la Universidad de Jaén. Jaén, Andalucía, España. 29/11/2013. Comunicación oral.

13. Caracterización agro-ambiental de suelos semiáridos mediterráneos mediante espectroscopía infrarroja. XXIII Reunión Nacional de Espectroscopía – VII Congresso Ibérico de Espectroscopía. Córdoba, Andalucía, España. 17/09/2012. Comunicación en formato póster.

Estancias en centros de I+D+i públicos o privados

1. Entidad de realización: CSIRO Land and Water. Tipo de entidad: Agencia Estatal. Ciudad entidad realización: Canberra, Australia. Fecha de inicio-fin: 24/09/2018 - 28/12/2018 Duración: 3 meses. Objetivos de la estancia: Doctorado/a. Tareas contrastables: Desarrollo de modelos predictivos para la predicción de variables en suelos mediante técnicas de data fusion aplicacadas a espectroscopía infrarroja.

2. Entidad de realización: AGRONUTRIENTES JAEN, S.COOP AND. Ciudad entidad realización: Jaén, Andalucía, España. Fecha de inicio-fin: 14/07/2014 - 10/10/2014 Duración: 3 meses. Objetivos de la estancia: Doctorado/a. Tareas contrastables: Desarrollo de modelos predictivos de nutrientes en hoja mediante espectroscopía. Infrarroja.