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# Environmental forensics evaluation of residual soybean sludge using trees of Brazilian savannah

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ABSTRACT

Truck and train are the primary transport means for soybean shipments to national or international markets from Brazil. Substantial amounts of grain residues are dispersed throughout the transport and may accumulate in various places by the runoff. The objective of this study was to evaluate the contamination and mortality of Brazilian savannah trees due to the accumulation of soybean runoff that comes from a grain storage yard. Xylem samples were collected from 28 dead trees in a forest where residues severely polluted the natural environment. Increment cores at breast height were also taken from 25 living trees for control procedure. All samples were analyzed by X-ray densitometry and microfluorescence (XRF) techniques. No differences were observed in the xylem densitometry profile from bark to pith between dead and living trees, indicating that the stagnation in cell production and tree growth occurred drastically after the accumulation of soybean residual sludge, which was confirmed by temporal analysis of satellite images. Significantly higher S, P, Ca and Fe concentrations were observed close to the bark in dead trees. The roots of dead trees accumulated higher amounts of Al, Si, S, Ca and Mn. Higher K, Ca and Si concentrations and lower Mn concentrations were observed in trees closer to the polluted area, and it indicates the need for soil remediation. However, As, Pb and Cd, markers of fertilizers and pesticides in soybean production, were not detected by the analyses performed. It is likely that the residual soybean sludge and its fermentation process during the rainy season (tree - growing season) have generated toxic concentrations of inorganic constituents, that together with warmer soil conditions caused the death of many trees. This study provides tools for the assessment of the environmental impact of soybean production through a novel protocol for monitoring the physical and chemical patterns of tree growth using rapid microscopic scale X-ray techniques.

## 1. Introduction

The soybean production area in Brazil has almost doubled from 2000 to 2019, from approximately 13 Mha to 34 Mha (Song et al., 2021). In less than 20 years, soybeans have become the most produced grain in the country, and the market has been in continued to expand given the global demand for food and animal feed (Da Silva et al., 2021; Cunha et al., 2022). The territorial expansion of soy crops has improved the welfare of some rural areas with the increase in purchasing power and access to education and health. However, environmental impacts such as deforestation for the expansion of the agricultural frontier, the change in soil properties and the use of pesticides are affecting many ecological processes of natural resources that go beyond the crop areas (Garrett and Rausch, 2016; Da Silva et al., 2021).

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In Brazil, the transport of soybean products is mainly by road or in a multimodal way by trucks and railroad (Lopes et al., 2017). During the transport, some grains are lost on the way due to over-loading and sealing failures of the trailers, as well as during the loading and unloading of the trucks at the storage sites and silos (Wilhelmi et al., 2014). Garrett and Rausch (2016) reported that there was little knowledge about the total environmental impacts of the management of soybean production worldwide, especially those associated with pollution caused by the runoff of production areas to adjacent environments.

Environmental ecotoxicity of fertilizers and pesticides used in soybean farming is one of the main negative impacts of this crop, mainly because these products contain heavy metals in their origin (e.g., Ni, Hg, Cd, Pb, Cr, Cu, Zn) (Da Silva et al., 2010). However, the soybean grains lost during transportation have the capacity to be a pollutant in the environment, although the risk is less than in the storage and silo areas and their vicinity because of the lower quantity of soybean residues along roads. These residues under high temperature and humidity conditions decompose, and aerobic degradation releases carbon dioxide, phosphate, nitrate and ammonia into the environment (Wong et al., 2001; Li et al., 2012). Also, soybeans contain carbohydrates, lipids and minerals, and their decomposition can release various nutrient elements (e.g., N, P, K, Na, Ca, Mg, Fe, Cu, Mn, Zn and Cr), whose concentrations depend on the origin of products (i.e., genotype and environment) (Li et al., 2012; Wijewardana et al., 2019). These substances in high concentrations could be toxic in the environment and need to be evaluated and remediated under certain circumstances of high production or transport rates that cause an undesirable change in the physical, chemical and biological characteristics of the environment, which affect living beings and human activities (Ashraf et al., 2014).

The most common methods widely used in agricultural areas for determination of contaminants analyze the physical, chemical and biological characteristics of soils, sediments and waters (Hela et al., 2005; Filizola et al., 2006). The analysis of these samples requires good planning since the materials must be well packaged and collected at the right time, and the laboratory analysis must be carried out in a few hours, depending on the contaminant to be analyzed (Filizola et al., 2006). In addition, because pollutants are carried by and leach from the runoff to the water table and streamflow, the analysis of these materials may not detect the harmful substances after a few months or days of the event, as well as being difficult to associate them with the location and source (Hela et al., 2005; Jergentz et al., 2005). Thus, alternative methods are necessary to allow assessment of contamination after long periods that the impact occurred, mainly those relative to the agriculture and industries in rural areas, which is not detected by government control agencies when it happens. In this context, trees and shrubs can be used for evaluation, considering that during secondary growth, record information from the past are associated with changes in the environmental conditions during the formation of the xylem, which can be analyzed by novel techniques to extract chemical changes in the tree rings with high spatial and temporal resolution (Hevia et al., 2018; De Micco et al., 2019; Binda et al., 2021; Ortega-Rodríguez et al., 2022).

The temporal analysis of fluctuations of the physical characteristics of the xylem, such as growth or density, can indicate plant physiological changes and thus provide important information with an annual resolution about the impacts of environmental changes on the plant over its lifespan or until the date of sample collection (Fritts, 2001; Cailleret et al., 2016; De Micco et al., 2019; Santini-Junior et al., 2019). Density is related to the anatomical characteristics of the wood and varies according to the dimensions, proportions, shapes and thickness of the fiber cell walls, vessels and parenchyma, and may vary depending on the species, functional group, life stage and tree height. In general, inverse relationships are expected between tree density and tree mortality, i.e., a reduction in tree density before death occurs. However, some species could show great variability from one individual to another, as well as variations could be expected between fast-growing (pioneer) and slow-growing species, low- and high-density wood species, and could depend on the life stage and the height of the wood in the trunk (Woodcock and Shier, 2002; Beeckman, 2016).

In addition, the temporal analysis of chemical fluctuations (dendrochemistry, chemical analysis of tree rings) can also indicate physiological changes, mainly due to the changes in nutrients, heavy metals, and stable isotopes in the xylem. This technique has been presented as a promising approach to investigate the spatial and temporal impacts of contaminants, as well as for monitoring pollution in urban, industrial and mining areas (Locosselli et al., 2019; Locosselli et al., 2020; Semeraro et al., 2020; Amais et al., 2021; Binda et al., 2021; Clackett et al., 2021; Dobrzanska et al., 2021). Accordingly, Hietz et al. (2015) and Santini-Junior et al. (2019) observed that a relationship exists between density and chemical fluctuations in the xylem, and it can be used to identify the occurrence of deterioration in the growing conditions, the mobilization of substances used for defense, and/or the alteration in the nutritional supply for plants.

Under the current global change context, monitoring and evaluating the environmental impacts of production industries on different ecosystems is increasingly necessary following the 2030 Sustainable Development Goals (SDGs), especially in goals 9 (Industry, Innovation and Infrastructure), 12 (Responsible consumption and production), 13 (Climate action) and 15 (Life on land) (United Nations General Assembly, 2015). According to these goals, in this study, we investigated the responses of trees of different species growing in a Brazilian savanna (cerrado) affected by residual soybean sludge pollution. Our objectives were carried out by (1) identifying alterations in the growth and density patterns of trees growing under polluted vs. control conditions and (2) determining the chemical traces in the wood of living and dead trees. We also analyzed the possible tipping point in the growth and physiology of trees that led to the death of many individuals in this savanna. To analyze physical and chemical patterns, we used fast, low-cost and environmentally friendly X-ray densitometry and X-ray fluorescence techniques (see Hevia et al., 2018). We hypothesize that the trees died because of (1) toxicity of elements related to fertilizers and pesticides or (2) the high concentration of inorganic constituents and warming conditions in the soil due fermentation of soybean residues. These novel approaches will provide new insights into monitoring and evaluating vegetation responses to contamination in tropical environments.

## 2. Material and methods

### 2.1. Study site

The study was conducted in a forest of the municipality of Alto Araguaia, in the southeast of the state of Mato Grosso, Brazil, at the geographic coordinates 17°15′46″ S and 53°18′42″ W (Fig. 1A). The climate is classified as Aw, according to the Köppen system (Alvares et al., 2013), with two well-defined seasons, one dry between the months May and August and one rainy from September to April (Fig. 1A). The average annual rainfall is 1582 mm and mean temperature is 22 °C (Instituto Nacional de Meteorologia, 2022). The soil of the area is classified as arenosols (IUSS Working Group WRB, 2015). The vegetation is characterized by the presence of woody arboreal/shrub individuals of species of the genera *Myrcia*, *Miconia*, *Qualea*, *Emmotum*, *Diptychandra*, *Styrax*, *Byrsonima*, *Pterodon* and *Bowdichia,* among others (Schardong et al., 2020).

### 2.2. Affected area characteristics

An area of 0.3 ha was monitored between 2019 and 2021 (Fig. 1B, 1C and 1D). This area was flooded with residual soybean sludge. The runoff of this sludge occurred from the loading area (where the mode of transport is changed from road to the rail system) to the forest after the rains and the water used to clean the trucks dragged soybean residues (straw and grains) through the drainage system of the storage. The transport of soybean residues occurred through water by force of gravity

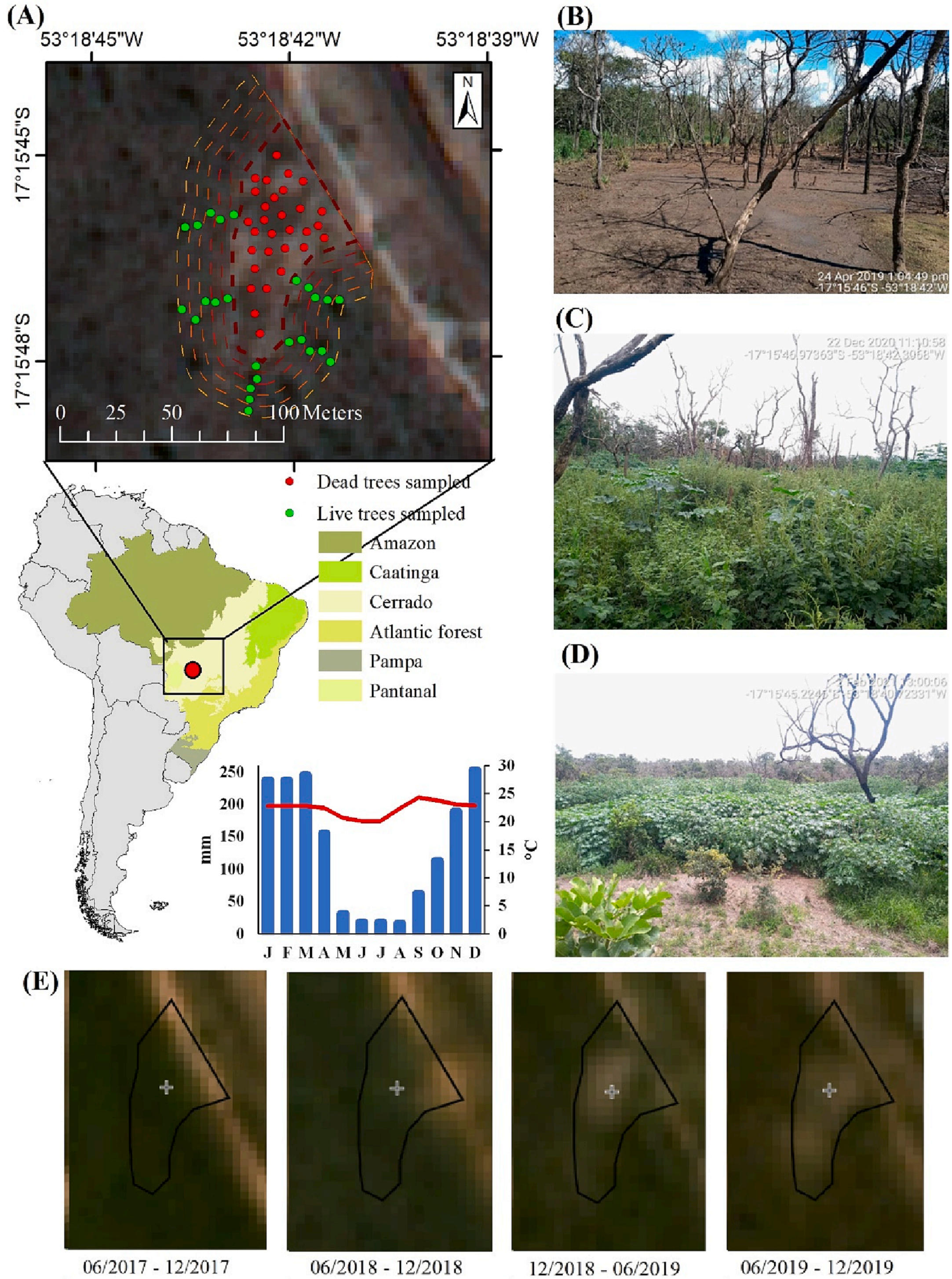


**Fig. 1.** A) Location, experimental design of sample collection and diagram of monthly precipitation and temperature, average data for the period 2011 to 2021 (Instituto Nacional de Meteorologia, 2022). Photograph of: B) the contaminated area in April 2019, C) same area colonized by *Amaranthus sp*. in December 2020, and D) same area colonized by *Ricinus communis* L. in February 2021. E) Temporal analysis between 2017 and 2019 of the contaminated area using high spatial resolution images from PlanetScope satellites. .
Source: https://www.globalforestwatch.org

along the drainage system because of the topography of surface. The waste then accumulated in the natural vegetation obstacles (trees and grasses) on the rural property adjacent to the storage area. It was observed that the residues in the yard of the storage were consisted of intact and broken soybeans; however, in the area where the death of trees was observed and along the drainage system, the residues could not be differentiated because they were in a high degree of decomposition (Fig. 2).

The change in vegetation cover was investigated between the years 2016 and 2022 through the temporal analysis of high spatial resolution images from the PlanetScope satellite, freely available on the Global Forest Watch platform (https://www.globalforestwatch.org) (Fig. 1E).

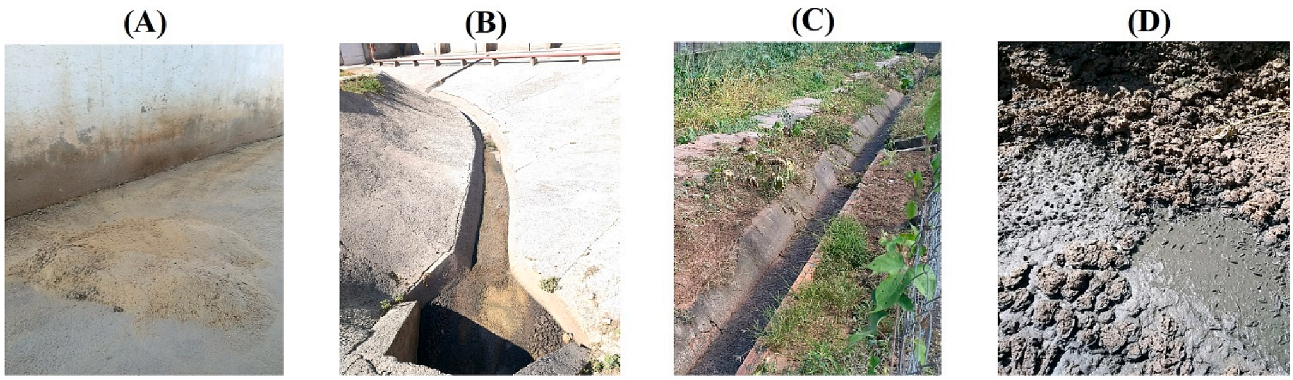


**Fig. 2.** Characteristics of the soybean residues: (A) intact and broken soybeans on the yard of the storage grains; (B) soybean residues into drainage system; (C) decomposed soybean residues in drainage system; and (D) high degree decomposition of soybean residues on area where were observed dead trees and insect larvae.

The mortality of trees occurred since 2018, when the spectral responses of the vegetation (loss of the treés leaves) in the contaminated area were observed, shortly before the first field monitoring in April 2019 (Fig. 1B). In the first field monitoring (2019), the visit was carried out to investigate the storage area with the aim of identifying eventual environmental damage, and we verified the death of native trees in the location where the residual soybean sludge had accumulated, as well as the presence of high humidity and abundant insect larvae in the soil surface, with a heavy odor of methane (Fig. 1B; Fig. 2C). On the second monitoring visit during December 2020, the scope was to demonstrate that the dead trees still were in the contaminated area and that it was possible to sample them. Furthermore, we found that the drainage system of the warehouse was suitable for directing the waste to containment basins, no longer being carried to the study site and that the contaminated area was colonized by alien species of *Amaranthus* sp. (Fig. 1C). On the third monitoring in February 2021, the growth of *Amaranthus* sp. was suppressed because of colonization by another alien species, *Ricinus communis* L (Fig. 1D), and xylem samples were collected from dead and living trees. Topsoil (0–20 cm) characteristics were evaluated in the affected area and surrounding natural forest in the first (2019) and third (2021) monitoring (see Table S1).

### *2.3. Sample collection and preparation*

Trees of different species were selected considering a minimum diameter at breast height (DBH, at 1.30 m from the base) of 5 cm (see Table 1). Twenty-eight dead trees in the contaminated area were felled with a chainsaw, and 5-cm discs were removed in three positions: at the base of the trunk, 10 cm from the ground and 1.30 m from the base. The roots were extracted with a backhoe tractor and samples with diameters between 2 and 10 cm were cut at about 50 cm from the base of the trunk. The samples were marked, organized in unsealed plastic boxes to facilitate air drying and transported to the laboratory for preparation and analysis.

Furthermore, one to two radial samples were taken with a Pressler borer (5.5 mm) from the trunk of 25 living trees in the surroundings of the area where the death of trees occurred (Table 1). The selected trees were spaced at the edges of the polluted area and approximately every 5 m from each other, up to a distance of 25 m (Fig. 1A). Radial samples were placed in plastic tubes and transported to the laboratory for air drying, preparation and analysis. The holes that resulted from the collection were treated with Bordeaux mixture (antifungal) and sealed with wooden dowels and carnauba wax to aid in the healing process of the trees and avoid infestation of pathogens.

In the laboratory, the identification (at least up to the genus level) of living trees were performed using vegetative parts and wood anatomy and for dead trees using only wood anatomy. The xylem samples obtained at 1.30 m (DBH) from both dead and living trees were polished gradually with sandpaper (from 80 to 600 grains inch$^{-2}$) to distinguish anatomical elements. Digital images at the macroscopic scale (2.5x) were then obtained under a light microscope coupled to a Zeiss Axio Cam (Carl ZeissMicroscopy, Gottingen, Germany) and distinction of growth ring boundaries was characterized (Table 1; Fig. 3) (Ortega-Rodríguez et al., 2022). One to three radial samples (2 cm wide and 2 cm thick) from the discs of dead trees (trunk and root) were cut with a band saw and, like the radial samples from living trees, were glued onto wooden supports. Thin wood slice (0.4 mm thickness) for radial samples of living and dead trees were cut transversely with a precision sectioning circular saw ISOMET 4000 (Buehler, IL, USA) to analyze wood density by X-ray densitometry and the traces of elements by X-ray fluorescence.

**Table 1**
Species and number of trees selected for analyzing. Leaf habit and growth-rings boundaries distinction were included and characterized based on Silva Júnior (2005) and Silva et al. (2019), respectively.

| Species | Family | Live trees | Dead trees | Leaf habit | Ring Visualization |
|---|---|---|---|---|---|
| *Qualea* sp. | Vochysiaceae | 14 | 14 | Deciduous | Distinct |
| *Pouteria* sp. | Sapotaceae | 1 | 2 | Deciduous/Semideciduous | Little distinct |
| *Bowdichia* sp. | Fabaceae | | 3 | Deciduous | Distinct |
| *Euplassa* sp. | Proteaceae | 3 | 3 | Semideciduous | Indistinct or absent |
| *Byrsonima* sp. | Malpighiaceae | | 1 | Deciduous/Evergreen | Indistinct or absent |
| *Machaerium* sp. | Fabaceae | | 1 | Evergreen | Little distinct |
| *Eriotheca* sp. | Malvaceae | 1 | | Semideciduous | Little distinct |
| *Kielmeyera* sp. | Calophyllaceae | 2 | | Deciduous | Little distinct |
| *Tachigali* sp. | Fabaceae | 1 | | Deciduous | Little distinct |
| *Eugenia* sp. | Myrtaceae | 1 | | Deciduous | Indistinct or absent |
| *Vochysia* sp. | Vochysiaceae | 1 | 1 | Semideciduous | Distinct |
| *Ficus* sp. | Moraceae | | 1 | Deciduous | Distinct |
| – | Myrtaceae | | 1 | … | Distinct |
| – | Euphorbiaceae | 1 | | … | Little distinct |
| – | N.I | | 1 | … | Indistinct or absent |

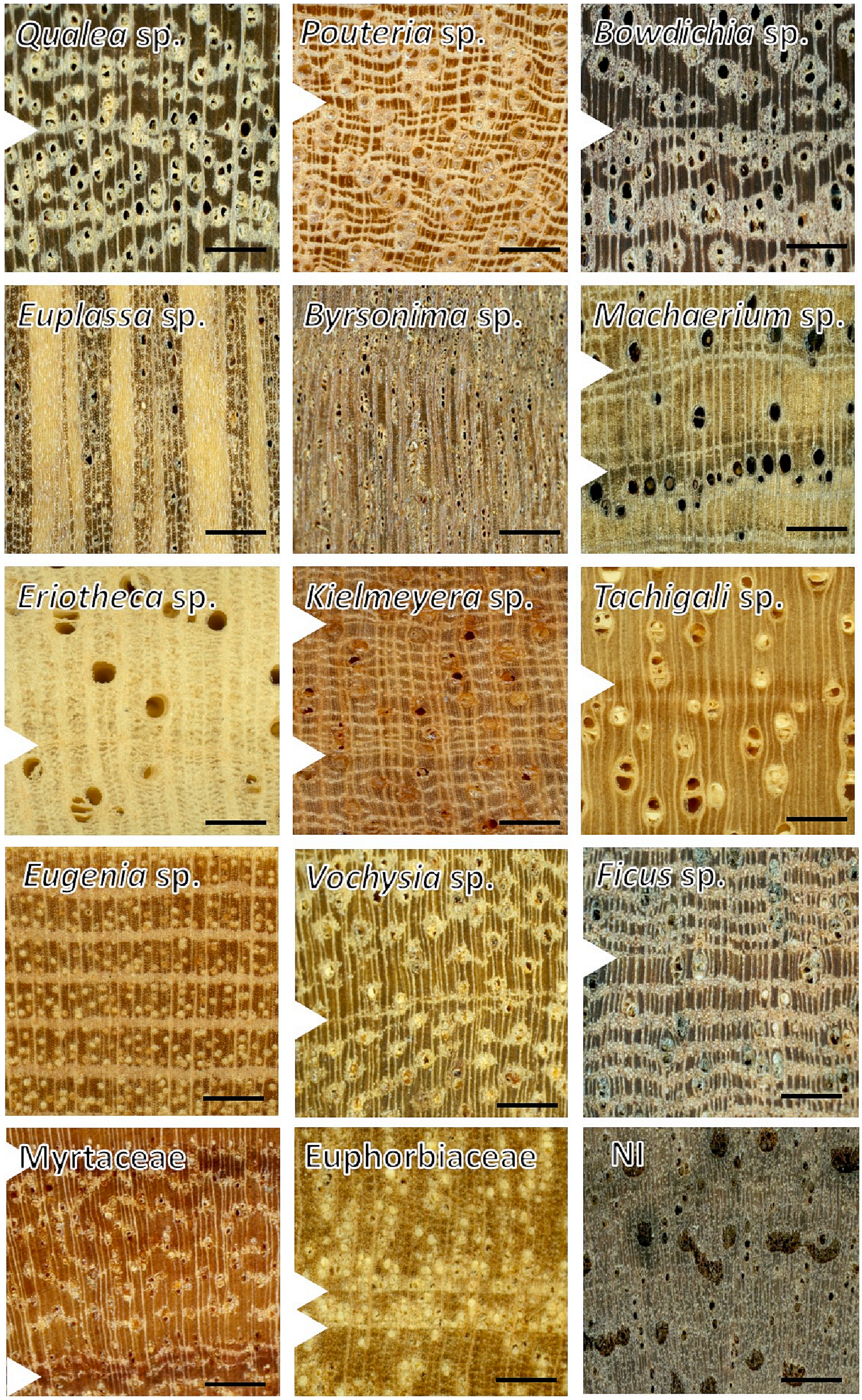


**Fig. 3.** Characterization of the growth rings of the species sampled in the study area: distinct: *Qualea* sp., *Bowdichia* sp., *Myrtaceae*, *Vochysia* sp., *Ficus* sp.; little distinct: *Pouteria* sp., *Machaerium* sp., *Eriotheca* sp., *Kielmeyera* sp., *Tachigali* sp., *Euphorbiaceae*; and indistinct or absent: *Euplassa* sp. and *Byrsonima* sp., and *Eugenia* sp. NI- non identified. Note: Bar is 500 μm.

### *2.4. X-ray densitometric analysis*

The wood slice samples were conditioned for 48 h to 12% moisture content in a room with constant temperature and relative air humidity (20 °C and 60%, respectively) (Quintilhan et al., 2021; Tomazello-Filho et al., 2008). The samples were then scanned with a calibration scale of cellulose acetate using an X-ray densitometry chamber (Faxitron MX20-DC12, Faxitron X-Ray, Illinois, USA). The digital images of cross-section samples were analyzed with WINDENDRO Density 2017a® software (Regent Instruments Inc.), obtaining the density profile (interval of 15 μm).

### 2.5. X-ray fluorescence analysis

Chemical signatures in the wood slice samples were determined from bark to pith, spaced 0.5 cm from bark to first spot, 1 cm from first to second spot, 1.5 cm from second to third spot, and every 2 cm after the third spot until the pith. The measurements were carried out using a portable spectrometer pXRF, Tracer III-SD model (Bruker AXS, Madison, WI, USA), equipped with a 4-W Rh X-ray tube and 10-mm$^2$ X-Flash® Peltier-cooled silicon drift detector. The tube was configured for a voltage of 40 kV and current of 30 μA (Costa et al. 2020). The scanning time was 60 s, which was performed under vacuum. The acquired spectra were normalized by the detector live time (38 s) and evaluated in counts of photons per second (CPS) (Tavares et al. 2021). The K-lines of Al, S, P, K, Ca, Fe, Mn and Si were normalized by the Compton peak. XRF emission lines were obtained using Artax® software (Bruker AXS) and processed in Excel (Microsoft Corporation, Redmond, WA, USA).

### 2.6. Data organization and analysis

To analyze the effects of the contamination on wood density, radial profiles of trees were constructed (Fig. S1). For dead trees, trends in density profiles at different positions (root, stem base and DBH) were compared. Furthermore, the trends in density profiles between the DBH samples of dead and living trees at different distances from the contaminated area were visually compared. In addition to visual comparison, a quantitative analysis of the variation in density between dead and living trees was made for the species *Qualea* sp. This species was more abundant in the area, and its growth rings were distinct, allowing the measurement of their width and wood density. Annual growth and wood density for the period 2014–2020 were determined and the common five years (2014–2018) prior to contamination for both variables were analyzed. The means of ring width and wood density in each year were compared by a non-parametric bootstrap method using 1000 repetitions with replacements. Precipitation, temperature and vapor pressure data for the period 2014–2020 were plotted against the means of width and wood density to analyze potential effects of climate variability. Climate data were obtained using version 4 of the CRU TS monthly high-resolution gridded multivariate climate dataset (Harris et al., 2020). We selected the grid encompassing the CRU TS 4.06 grid-box data for the 17.25°S, 53.25°W region.

To analyze potential chemical contamination of trees, means comparison was performed for the identified elements by a non-parametric bootstrap method using 1000 repetitions with replacements. We compared means of elements measured (1) at points distributed from bark to pith for each xylem location of the dead trees (root, stem base and DBH) (2) at different positions of the dead trees (root, stem base and DBH), (3) at points distributed from bark to pith in dead and live trees growing at different distances from the contaminated area and (4) in dead and living trees growing at different distances from the contaminated area. This analysis was prioritized for the first four points measured in the bark-pith direction (Fig. S2-S9).

## 3. Results

The analysis of the wood density showed that almost all living and dead trees showed stable density values along the radial profile with a slight decrease in the pith-bark direction related to age and size effects (Fig. S1). The average wood density values showed mainly a decrease in the last 10 mm close to the bark of living trees and in different locations in dead trees (root, stem base and DBH). The most abrupt decrease was observed in the density profile of roots of dead trees. No differences were observed in the density profile between heartwood and sapwood of trees, except for one dead *Bowdichia* sp. tree with the largest diameter. Regarding the analysis of growth and wood density variation of *Qualea* sp. trees for the period between 2014 and 2020, we observed that the species displayed a positive (weak) relationship between the width and density of the ring. No significant differences were observed between the average annual growth and wood density of *Qualea* sp. trees between 2014 and 2018 (Fig. 4), except in 2016, where the annual growth of dead trees was significantly higher than the growth of live trees (Fig. 4A). The non-significant difference in the annual density between living and dead trees indicates that the physiological stress did not occur for an extended period (over a year) before the death of the trees, which was confirmed by the dynamics of pollution and defoliation of the vegetation observed in the satellite images in Fig. 1E.

Significantly higher S, P, Ca and Fe concentrations were observed at positions close to the bark in the xylem of dead trees (root, stem base and/or DBH) in at least one location (Fig. 5A). Non-significantly higher Al and Mn concentrations at positions close to the bark were observed in the stem base and root. Non-significantly lower Si concentrations at positions close to the bark were observed in the three xylem positions of dead trees. No trends were observed for K (Fig. 5A). Non-significantly higher Al and Si concentrations and significantly higher S, Ca and Mn concentrations were observed in the roots compared to other xylem locations of dead trees (Fig. 5B). Non-significantly higher P and K

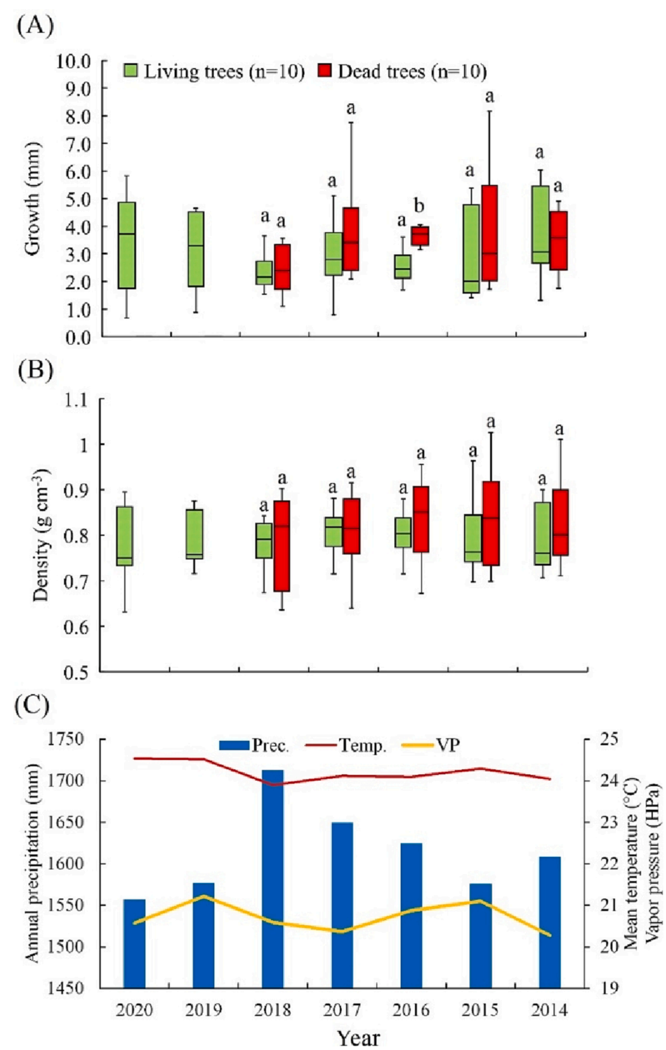


**Fig. 4.** Boxplot of mean annual (A) growth and (B) wood density of dead and living *Qualea* sp. trees. (C) Meteorological annual average data for precipitation, air temperature, and vapour pressure for the period between 2014 and 2020 (Instituto Nacional de Meteorologia, 2022). The letters "a" and "b" indicate mean differences at 5% of significance between treatments (dead and living trees).

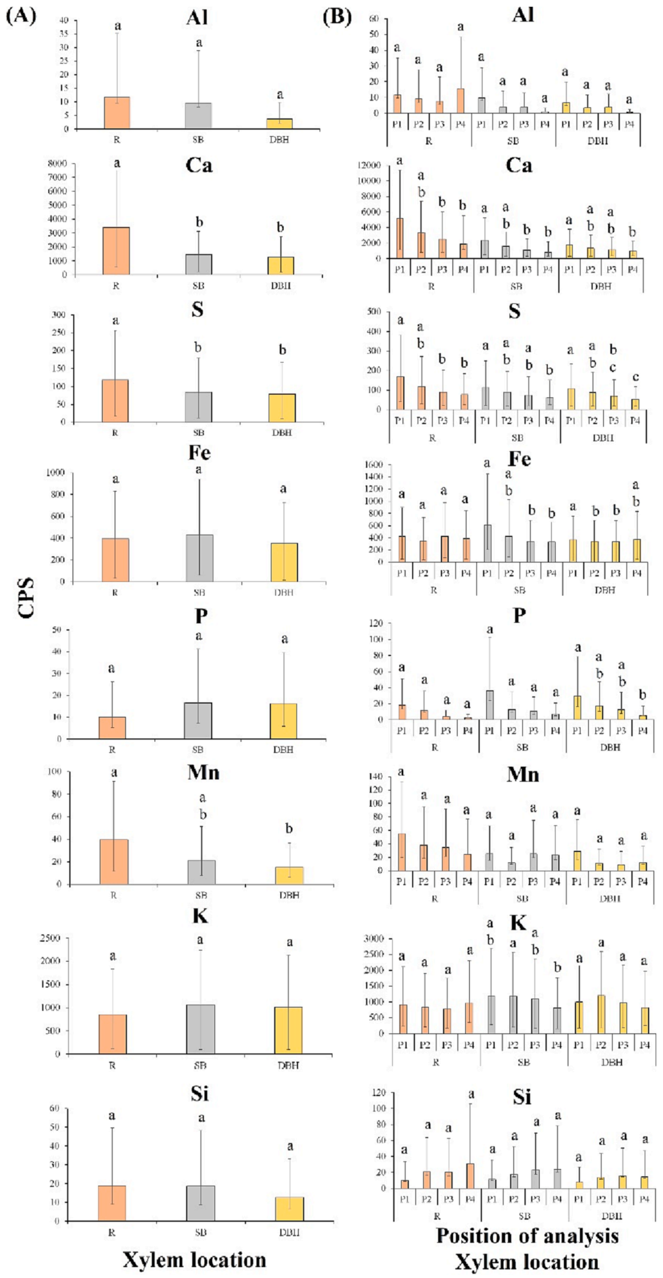


**Fig. 5.** Mean comparisons of elements measured (A) at different positions of the dead trees (R = roots, SB = stem base; and DBH = diameter at the breast heigh); (B) at points distributed from bark to pith (P1 = 0.5 cm, P2 = 1.5 cm, P3 = 2.5 cm, P4 = 4.5 cm) for each xylem location of the dead trees (R, SB, and DBH). The letters “a”, “b”, and “c” indicate mean differences at 5% of significance between treatments. CPS = counts per second.

concentrations were observed in the stem base and the DBH compared to the root of dead trees (Fig. 5B).

Non-significantly higher concentrations of Al and significantly higher concentrations of S and P at positions close to the bark were observed in DBH of live trees located at different distances from the contaminated area (Fig. 6A). Higher concentrations of Ca at positions close to the bark were observed in the DBH of live trees at distances up to 10 m from the contaminated area. After 10 m, non-significantly lower Ca concentrations at positions close to the bark were observed in live trees. Non-significantly lower Mn concentrations at positions close to the bark were observed in live trees located more than 5 m from the contaminated area (Fig. 6A). Significantly higher K, Ca and Si concentrations and significantly lower Mn concentrations were observed in trees closer to the contamination area (Fig. 6B).

## 4. Discussion

This study is the first case reported about the environmental ecotoxicity caused by soybean residue accumulation after the runoff carrying it out from grain storage, resulting in a death event in the native vegetation of the Brazilian savanna. Cases of environmental ecotoxicity in soybeans and other crops has already been reported worldwide (Ashraf et al., 2014; Da Silva et al., 2010; Garrett and Rausch, 2016). However, its impact was related to heavy metals and mineral salts from pesticides and fertilizers through wrong application, and because of their transport by the runoff to streamflow and adjacent areas (see Da Silva et al., 2010). This study showed how soybean residues can result in pollution if there is no control and adequate disposal of these residues. However, studies must be carried out to answer questions such as: (1) at what amount of soybean residues does the problem of deforestation start to appear? (2) does the percentage of broken beans affect the rapidity of the phenomena? (3) are only soybean residues capable of causing environmental pollution or are other factors involved? (4) is the heat generated from fermentation of the soybean residues responsible for death by hydraulic failure?

In the monitoring carried out between 2019 and 2021 in the contaminated area, we found that the accumulation of soybean runoff that came from the grain storage yard had been tidied up. This allowed the colonization of the contaminated area by plants of the genera *Amaranthus* and *Ricinus* (Fig. 1), exotic species with phytoremediation potential (Pires, 2003; Romeiro et al., 2006; Borin, 2010). Furthermore, the chemical evaluation of soil allowed us to observe a decrease in pH, nutrients and organic matter between 2019 and 2021 (Table S1). Complementary to these evaluations, the study of environmental ecotoxicity, the causes of death of trees and the extent of environmental damage was carried out through the evaluation of the xylem of trees given their potential for environmental forensics (Balouet and Chalot, 2015; Balouet et al., 2009).

For the experimental design, we prioritized the distance of the trees from the contaminated area, which led to the collection of samples of different species, sizes and probably ages; without considering a minimum number of trees per species (Fig. 1A, Table 1). This was reflected in the variability of density values (Fig. S1) and chemical elements (Fig. S2-S10). This variability responds to ontogenetic effects that influence physical properties (De Mil et al., 2018) and the distribution and concentration of elements in the xylem (Ortega-Rodriguez et al., 2022), facilitating their differentiation and identification with high precision (Boeschoten et al. 2022; Shugar et al., 2021). Despite this variability, similar patterns were observed between the species, mainly in the concentration of elements along the pith-bark position, along the parts of the tree and at different distances from the contaminated area (Figs. 5 and 6) (Smith et al., 2014).

Regarding the wood density profiles in the radial bark-pith distance, no significant changes were observed over the medium to long term, which suggests that the stress and mortality of trees was not a gradual process, considering the xylem density reflects the physiological conditions of the trees (Cailleret et al., 2016; De Micco et al., 2019). Reductions in xylem density in regions less than 10 mm away from the bark were similar in both treatments (dead and living trees), and it could be related to cellular immaturity (lack of lignification) during the growth phase in the rainy season; we will evaluate this hypothesis from histological analysis in further studies. This decrease in density close to the bark observed for living and dead trees was the only pattern of variations observed, but it must be considered that the variety of anatomical characteristics of the species and the low number of individuals per species may have impaired observations of specific patterns of variation between dead and living trees. Thus, further studies are recommended with a larger number of individuals per species, and at different stages of

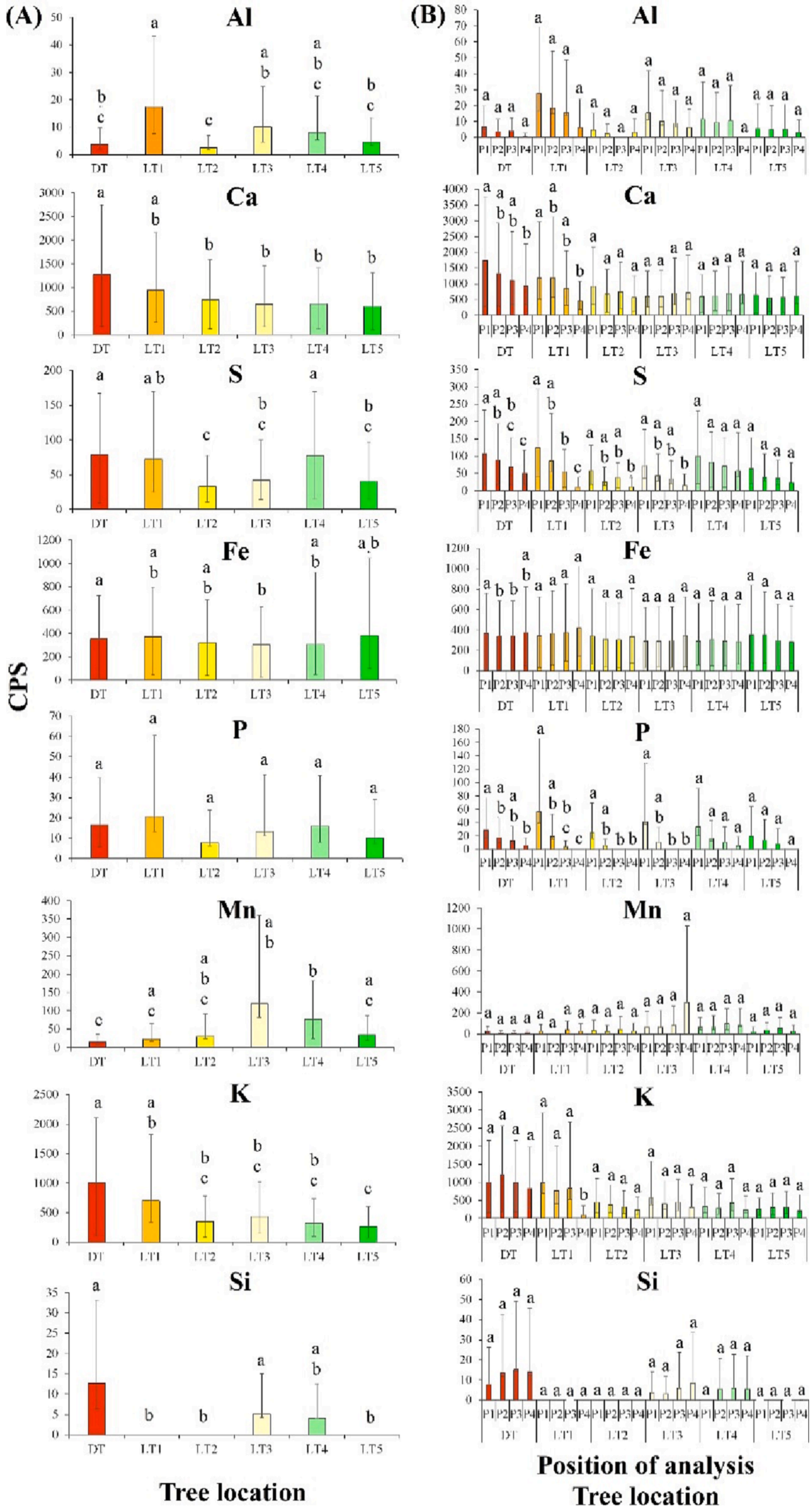


**Fig. 6.** Mean comparisons of elements measured (A) in dead (DT) and living trees growing at different distances from the contaminated area (LT1 = 5 m, LT2 = 10 m, LT3 = 15 m, LT4 = 20 m, LT5 = 25 m); and (B) at points distributed from bark to pith (P1 = 0.5 cm, P2 = 1.5 cm, P3 = 2.5 cm, P4 = 4.5 cm) in dead and living trees growing at different distances from the contaminated area. The letters "a", "b", and "c" indicate mean differences at 5% of significance between treatments. CPS = counts per second.

growth, so that clearer information can be extracted for a better understanding of the density profiles of each species studied.

Chemical analysis did not confirm our first hypothesis that the death of trees occurred due to the heavy metals from pesticides and fertilizers in soybean residues, which are greatly used in agriculture (Jiao et al., 2012). However, the higher concentrations of K, Ca, S and Si in the trees closest to the contaminated area (Fig. 6) may indicate that the trees accumulated toxic levels of these elements (Ashraf et al., 2014), previously limited in the soil. As in the case of overfertilization, the excessive concentration of nutrients (e.g., NPK) in the soil can be harmful to plants, and the amount of damage varies according to the species, type of substances and moment of alteration (Kozlowski and Pallardy, 1997). Arenosols in Mato Grosso are low in organic matter (less than20 g $dm^{-3}$) and have an acidic pH (5.8), and their high sand content causes poor water retention (Table S1, Speratti et al. 2017). A contribution of nutrients such as N (~54 kg/t), P (~4.8 kg/t), K (~18 kg/t), Ca (~2.8 kg/t), Mg (~2.5 kg/t), S (~2.8 kg/t), B (~31 g/t), Cu (~11.5 g/t), Fe (~65 g/t), Mn (~39 g/ t) and Zn (~41 g/t) are expected with the soybean residues (Oliveira Junior et al. 2020). After the incorporation of soybean sludge, the acidity of the soil decreased, being confirmed by the increase in pH (6.9) of the contaminated area in 2019 associated with the decrease in Al in the soil (Table S1). With the decrease in soil acidity, the solubility of minerals such as Al, Fe and Mn decreases, facilitating the absorption of Ca and Mg in the soil (Fageria and Nascente, 2014). It is possible that the decrease in the solubility of these elements in soil had decreased their transport in the plant, being associated with a lower contraction of Mn and Al in the trees closest to the contaminated area (Fig. 6) and a higher concentration of Mn and Al in the roots compared to other parts of the stem of the dead trees (Fig. 5). Despite the initial improvements in soil characteristics and the promotion of nutrient uptake by various tree organs, excessive concentrations of salts (mainly associated with Ca) in the soil solution may have reduced its osmotic potential, enough to decrease water uptake, leading to leaf dehydration, stomatal closure, reduced photosynthesis, leaf damage, and root cell plasmolysis (Kozlowski and Pallardy. 1997; Weng et al. 2022; Palani and Raju, 2020). Ca was the main element indicating the effect of contamination on trees, and its excess can be lethal to plants by causing apoptosis, among other physiological and biochemical disorders during cell formation (Levine et al., 1996; White and Broadley, 2003). Excess Ca in the soil also reduces the absorption of other nutrients by plants, such as P, K and Mg, leading to deficiencies of these nutrients (Fromm, 2010; Maathuis and Diatloff, 2013). Since Ca is immobile in the phloem and it is mainly deposited in the cell walls after leaving the xylem cells (Brett and Waldron, 1996), it acts as an important regulator in many processes related to xylogenesis and nutrient uptake responses to environmental stresses (Lautner and Fromm, 2010; Sánchez-Salguero et al., 2019). However, chemical analysis showed a high concentration of K in the dead trees, which suggests that the excess of these nutrients may have compensated for the antagonistic relationships with the Ca excess in the soil. Change in P concentrations was not observed when comparing dead and living trees, which suggests that the concentration of this element should be related to another factor (Hevia et al., 2019). On the other hand, the undetected Mg in the xylem may have been induced by Ca excess, considering that under normal conditions its presence in a similar concentration was expected (Meerts, 2002).

The assessment of pollution could be done over more than 100 years using an extensive growth ring time series from trees, demonstrating the potential of species with annual growth rings for pollution evaluation (Balouet et al., 2009; Hevia et al., 2018; Clackett et al., 2021). Thus, sampling species with distinct growth rings in the xylem must be preferred, where the pollution occurred over long periods. The growth ring analysis could complement the temporal investigation to identify the year of the beginning of the environmental impact, mainly when other inputs are unavailable (e.g., satellite images). Some species sampled in this study were not suitable for temporal evaluation of changes in physicochemical properties in the xylem, which suggests the need for further studies on the trees species in the region to define the potential for this type of investigation to understand the pollution processes caused by overexploitation of natural resources, like what soybean is causing in the Brazilian cerrado.

## 5. Conclusions

Our results suggested that the mortality of the trees occurred during the rainy season due to the physiological stress caused by the excess of nutrients. Mortality could also be related to a physical process caused by the fermentation of soybean residues in conditions of heat and humidity, which could negatively affect the radicular zone; however, it must be better studied. Furthermore, the use of trees for the evaluation and monitoring of pollution proved to be helpful in understanding the processes even two years after the beginning of the contamination (Balouet et al., 2009). From the xylem of the trees, it was possible to detect the change in the concentration of chemical elements in the contaminated area, indicating the need for soil remediation due to the excess of nutrients from the decomposition of soybean residues. In addition, the sampling of living trees in the surroundings allowed us to assess the extent of the contaminated area and can be useful to monitor nutrient leaching and environmental ecotoxicity over time in similar case of pollution because of intensive agricultural practices around the globe. Thus, woody vegetation analysis is an interesting option to deal with the problems of conventional analysis using soil, sediment or water, providing advantages regarding spatio-temporal analysis limitations.

## CRediT authorship contribution statement

**José Guilherme Roquette:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Daigard Ricardo Ortega-Rodriguez:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Leif Armando Portal-Cahuana:** Investigation, Visualization. **Francisco de Almeida Lobo:** Methodology, Formal analysis. **Andrea Hevia:** Writing – review & editing, Visualization. **Raúl Sánchez-Salguero:** Writing – review & editing, Visualization. **Hudson Wallace Pereira de Carvalho:** Resources, Writing – review & editing. **Mario Tomazello-Filho:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.enmm.2023.100814.

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