University of São Paulo
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Sulfur speciation in a tropical soil under long-term no-till amended with lime and phosphogypsum

Rodolfo Fagundes Costa

Dissertation presented to obtain the degree of Master in Science. Area: Soil and Plant Nutrition

Piracicaba
2020

Rodolfo Fagundes Costa
Agronomist

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **LUÍS REYNALDO FERRACCIÚ ALLEONI**

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To my parents Flávia and Adriano, for all the support during my whole life.

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I dedicate.

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"Share your knowledge. It is a way to achieve immortality."

– Dalai Lama XIV

CONTENTS

RESUMO

Especiação de enxofre em um solo tropical sob sistema plantio direto de longo prazo com aplicação de calcário e fosfogesso

O enxofre (S) é um nutriente requerido em grandes quantidades por culturas de alta produtividade. As plantas absorvem o S principalmente como sulfato inorgânico (SO_4^{2-}). Porém, esse elemento se apresenta em grande parte em formas orgânicas (> 90%) nas camadas mais superficiais de solos bem drenados. A conversão de formas orgânicas de S em SO_4^{2-} é mediada por microrganismos e é uma fonte importante de S para plantas, especialmente em sistemas naturais ou agrícolas que receberam pouca ou nenhuma adição de S. A diminuição do teor de S solo está associada à diminuição dos teores de matéria orgânica do solo (MOS) após a conversão de florestas naturais em áreas agrícolas, e a adoção do sistema plantio direto (PD) é uma alternativa para aumentá-lo. O calcário é aplicado em solos tropicais altamente intemperizados para aumentar o pH e servir como fonte de Ca e de Mg. O fosfogesso é um condicionador que atua como fonte de Ca e de S e promove a diminuição na toxidez por Al em camadas mais produndas do perfil do solo. Nesse estudo, objetivou-se verificar os efeitos da aplicação de fosfogesso, associado ou não ao calcário, na dinâmica do S, nos atributos químicos do solo e na atividade enzimática num solo sob sistema PD quando comparado ao solo de uma floresta nativa. A espectroscopia de absorção de raios-X baseada em luz síncrotron (XANES) foi usada como ferramenta não invasiva para determinar as formas de S em solos após uma análise de ajuste de combinação linear (LCF) nos espectros de XANES na borda de absorção do S. Foram determinados pH do solo, teores de MOS, SO_4^{2-}, cátions trocáveis (K, Ca e Mg) e enzimas do solo (ß-glucosidase e arilsulfatase). O teor de MOS foi afetado pela mudança no uso da terra e pela aplicação de corretivos. A aplicação de fosfogesso aumentou o conteúdo de S inorgânico nas camadas mais profundas, alterando a distribuição das frações S orgânicas e inorgânicas. O LCF foi um bom indicador das frações S, mas os resultados não corresponderam à proporção de frações orgânicas e inorgânicas de S obtidos pela extração química. O calcário aumentou o pH do solo até 40 cm de profundidade e diminuiu o teor trocável de Al^{3+} quando comparado ao fosfogesso e ao solo da floresta nativa. O fosfogesso diminuiu o teor de K trocável na superfície, quando comparado ao controle e ao solo da floresta nativa. A combinação de calcário e fosfogesso promoveu alta translocação de Ca aplicado à superfície para camadas mais profundas. O teor de Mg trocável diminuiu quando apenas fosfogesso foi aplicado. A atividade da ß-glucosidase diminuiu com a profundidade em todos os tratamentos e no solo da floresta nativa, mas aumentou após aplicação de calcário e fosfogesso. A atividade da arilsulfatase aumentou nos tratamentos que receberam calcário e diminuiu com a aplicação de fosfogesso quando comparados ao controle e foram semelhantes ao solo da floresta nativa até 20 cm de profundidade.

Palavras-chave: Enxofre, Fosfogesso, XANES: Sistema de Plantio Direto, Solos tropicais, Matéria orgânica do solo, Atividade enzimática, Arilsulfatase, ß-glucosidase

ABSTRACT

Sulfur speciation in a tropical soil under long-term no-till amended with lime and phosphogypsum

Sulfur (S) is a plant nutrient usually required in great amounts by yielding crops. Plants absorb S mainly as inorganic sulfate (SO_4^{2-}), but this element is presented greatly in organic forms (> 90 %) in the most superficial layers of well-drained soils. The conversion of organic S forms to SO_4^{2-} is mediated by microrganisms and is an important source of S for plants, especially in natural systems or agricultural systems that received little or no S input. A decrease in S content in soil is related to the depletion of soil organic matter (SOM) after converting natural forest to agriculture fields, and the adoption of no-tillage (NT) system is an alternative to increase it. Lime is applied to highly wheathered tropical soils to increase pH and serve as a source of Ca and Mg. On the other hand, phosphogypsum acts as a source of Ca and S in depth and decrease Al tocixity in depper leayers. In this study, the effects of these greater recommendation rates of phosphogypsum, in association or not with lime, were evaluated on S dynamics, soil chemical attributes and enzyme activity of a soil under NT as compared to a native forest soil. Synchrotron-based X-ray absorption near-edge structure spectroscopy (XANES) was used as noninvasive tool to direct determination of S forms in soils after a linear combination fitting (LCF) analysis on S K-edge XANES spectra of soil samples. The following attributes were evaluated: soil pH, contents of SOM, sulfate, exchangeable cations (K, Ca and Mg) and soil enzymes (ß-glucosidase and arylsulfatase) activity. SOM was affected by land use change and amendments´ application. Phosphogypsum application increased contents of inorganic sulfur in depper layers and changed the distribution of organic and inorganic S fractions. LCF analyses were a good indicator of S fractions, but did not match the proportion of organic and inorganic S fractions to the wet-chemical analysis. Lime increased soil pH until 40 cm depth and also decreased exchangeable Al^{3+} when compared to phosphogypsum and native forest soil. Phosphogypsum decreased exchangeable K contents in surface, when compared to control and native forest soil. The combination of amendments promoted a great translocation of Ca applied to the surface to depper depths. Exchangeable Mg content decreased when only phosphogypsum was applied. ß-glucosidase activity decreased with depth for all treatments and native forest soil and increased with lime and phosphogypsum application. Arylsulfatase activity increased in treatments that received lime and deacresed with application of phosphogypsum alone when compared to control and were similar to native forest soil down to 20 cm depth.

Keywords: Sulfur, Phosphogypsum, XANES: No-tillage system, Tropical soil, Soil organic matter, Soil enzyme activity, Arylsulfatase, ß-glucosidase

1. GENERAL INTRODUCTION

Sulfur (S) is found in various oxidation states in soils, either in organic or inorganic forms, with number of oxidation ranging from -2 in inorganic sulphides to +6 in sulfates. It is essential for the formation of organic compounds such as amino acids (cysteine, methionine, cystine, taurine) and enzymatic cofactors (Sfredo and Lantmann, 2007; Alvarez V. et al., 2007; Prietzel et al., 2011).

Although S deficiency in crops has not been largely reported in the past, various researchers show the increase of S deficiency in the last decades, and it may be attributed to: (i) the reduction on the emission of sulfur dioxide in the atmosphere, (ii) use of highly concentrated fertilizer formulations containing little or no S, and (iii) increased S removal of soils under intensive management systems and increased crop yields (Blum et al., 2013).

In natural or agricultural soils, more than 90% of S is found in organic forms in temperate and tropical ecosystems (Solomon, 2003). The conversion of native vegetation to agriculture changes the dynamics of soil organic matter in soil (Sá et al., 2014) and conservation systems such as no-tillage (NT) are alternatives to increase SOM contents when compared to conventional tillage alongside soil profile including lower depths (Balota et al., 2014; da Costa and Crusciol, 2016).

In highly wheathered tropical soils under NT, lime is applied to the surface to amelioriate soil chemical attributes for plant growth such as elevation of pH, increase of exchangle Ca and Mg and deacrease of Al^{3+} (Caires et al., 2004; Rampim et al., 2011). As lime has a low solubility, phosphgypsum is used as a soil ammendment in agriculture areas, serving as source of calcium (Ca) and S for plants in the most subsuperficial soil layers, and its use is related to a better root development and distribuition of plants by reducing Al^{3+} (Alvarez V. et al., 2007).

The main form of S absorbed by plants is sulfate (SO_4^{2-}) and the flux between organic and inorganic S forms is mediated in great part by the soil microbiota (Stevenson and Cole, 1999). One of the ways to investigate biological activity is to measure enzyme activity, such as arylsulfatase, an enzyme linked to the S cycle and responsible to convert organic S forms to inorganic sulfate (Gupta et al., 1993). This enzyme was reported to be afftected by lime and phosphogypsum application (Inagaki et al., 2016).

Most part of the studies focused on understanding the dynamics of S in soils are based on the wet chemical fractionation technique, in which organic compounds are reduced by hydriodic acid (Solomon et al., 2003; de Bona and Monteiro, 2010). This methodology gives

an in indirect measure of the organic S and may not represent the real content of S in soil (Solomon, 2003). Thus, X-ray absorption near-edge structure spectroscopy (XANES) can be used on the speciation and quantification of various organic and inorganic S compounds in different oxidation states in soil samples (Prietzel et al., 2011).

The aim in the study was to evaluate the influence of surface application of lime and phosphogypsum in S pools, accesed by S K-edge XANES technique, in soil chemical attributes and in enzyme activity in a humid tropical soil under long term no-til compared to a native vegetation area.

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2. SULFUR SPECIATION IN A TROPICAL SOIL UNDER LONG TERM NO-TILL ASSESSED BY S K-EDGE XANES

Abstract

Sulfur (S) is presented greatly in organic forms (> 90 %) in the most superficial layers of well-drained soils. Conversely, inorganic sulfate (SO_4^{2-}) is the main form of S absorbed by plants, and the conversion os organic S to SO_4^{2-} is mediated microrganisms. The depletion of soil organic matter (SOM) after land use change can decrease S contents in soil, and the adoption of no-tillage (NT) system is an alternative to increase them. Lime is applied to highly wheathered tropical soils to increase pH and serve as source of Ca and Mg. Because of its low solubility, it is often combined with phosphogypsum. Both amendments have been reported to increase SOM contents. The inputs SO_4^{2-} via phosphogypsum application changes S dynamics in soil, and techniques based on wet-chemical to distinguish S fractions usually creates artefacts. Synchrotron-based X-ray absorption near-edge structure spectroscopy (XANES) is an important noninvasive tool to direct determination of S forms in soils. We carried out this study to assess the effects of lime and phosphogypsum application under NT system when compared to control and native forest (NF) site. To attain the objectives, we proceeded a linear combination fitting (LCF) analysis on S K-edge XANES spectra of soil samples to separate obtain S fractions. We compared the results to total S accessed by soil acid digestion and inorganic S extracted with calcium phosphate. NT system presented a smaller SOM content than NF on the first 20 cm. Lime and phosphogypsum affected SOM contents on the uppermost soil layer when compared to the control. Phosphogypsum application increased contents of inorganic sulfur in depper layers and changed the distribution of organic and inorganic S fractions. Fairly good fits of S K edge XANES spectra with the selected reference compounds were obtained. LCF analyses were a good indicator of S fractions, but did not match the proportion of organic and inorganic S fractions to the wet-chemical analysis.

Keywords: Sulfur; Phosphogypsum; XANES; No-tillage system; Tropical soil; Soil organic matter

2.1. Introduction

Sulfur (S) is a plant nutrient that participate on the composition of aminoacids such as cysteine, cystine and methionine and on the synthesis of essential vitamins and cofactors (Kertesz and Mirleau, 2004; Blum et al., 2014). On the other hand, S deficiency affects the efficiency of other plant nutrients, such as nitrogen (De Bona e Monteiro, 2010). Although

uncommon in the past, sulfur deficiency has been reported worldwide in the last decade, and the cause was attributed to: the reduction of sulfur dioxide emissions due to anthropogenic activities; the increasing the use of highly concentrated fertilizers with low S concentration in the formula; decrease in use of S containing pesticides; high yield crop varieties; depletion of soil organic matter (SOM) due to the intensive for cultivation (Eriksen et al., 2004; Scherer, 2009; Blum et al., 2013).

Sulfur can be found in a range of various oxidation states as organic and inorganic compounds in the soil, but is presented greatly in organic forms (> 90 %) in the most superficial layers of well-drained soils (Schoenau and Malhi, 2008). Conversely, the main S form absorbed by plants is inorganic sulfate (SO_4^{2-}), present in the soil solution or adsorbed to soil particles (Solomon, 2003; Alvarez V. et al., 2007). The realease of SO_4^{2-} from organic S forms is mediated by microrganisms and is an important source of S for plants, especially in agricultural systems that received low or none S input besides atmospheric deposition (Tabatabai and Bremner, 1970; Scherer, 2009).

Sulfur concentrations are closely related to SOM content, and the inadequate management of soils can deplete it. So, conservation systems such as no-tillage (NT) are an alternative to increase SOM contents. Previous studies on long-term NT experiments reported a net increase of SOM content alongside the profile, including lower depths (da Costa and Crusciol, 2016).

In humid tropical soils, chemical attributes such as low pH, deficiencies of nutrients (Ca^{2+} and Mg^{2+}) and Al^{3+} toxicity are main factors that reduce plant productivity and hence SOM input (Soratto and Crusciol, 2008; Garbuio et al., 2011). To amelioriate soil conditions for plant growth, lime is widely applied to soil surface in NT systems. Because of the low solubility of lime in soils, a reduced effect of this amendment was reported in subsoil layers (Caires et al., 2016). Phosphogypsum, a more soluble salt than lime and a by-product of phosphate fertilizer production, is used as soil conditioner in order to reduce Al toxicity and serve as a source of Ca and S in subsoil (da Costa and Crusciol, 2016).

Phosphoypsum recommendation rates were historically based on the clay content of the 20 – 40 cm layer (van Raij et al., 1996), but a new method proposed by Caires and Guimarães (2018) is based on the elevation of Ca saturation to 60% of the effective cation exchange capacity (ECEC) in the 20 – 40 cm layer. This new formulation commonly results in much greater rates of phosphogypsum. As phosphogysum is composed by 16% of inorganic S-SO_4^{2-}, this large input might change S dynamics in soil.

Most of the past studies concerning S speciation in soils were conducted using a wet-chemical fractionation technique that separates S fractions based on the acid reduction of organic S compounds in soil (Solomon et al., 2003). As this method is operationally defined, it might create artefacts and cause change in oxidation of S species (Solomon et al., 2005; Prietzel et al., 2007). This way, Synchrotron-based X-ray absorption near-edge structure spectroscopy (XANES) emerged as an important noninvasive tool to direct determination of S forms in soils (Blum et al., 2013). XANES can help overcome the traditional methodology limitations and provide molecular-level information about S species in soil (Xu et al., 2016).

The objective aimed with this study was to evaluate the long-term effects of lime and phosphogypsum application on the distribution of S species accessed by S K-edge XANES in comparison to a native forest soil. Our hypothesis was that the content of organic S increases after sulfate application as phosphogypsum because of the conversion from inorganic form.

2.2. Material and Methods

2.2.1. Study sites location and experimental design

Soil samples from a sandy clay loam kaolinitic and thermic Typic Haplorthox (USDA, 2014) were collected in a field-scale study carried out in Southeast Brazil (Botucatu, State of São Paulo – 48º 23’ W, 22º 51’ S and 765 m) for 15 years under no-till system. The area was cultivated with a variety of plants in a long term crop rotation, mixing species with different C/N relations in order to increase SOM content. The area in which the soil samples were collected was cultivated with soybean (*Glycine max* L.) during the growing season. The complete list of all cultivated species can be found in the table S1 of the supplementary material.

The experiment was in a completely randomized block design with four replicates for each treatment. Four treatments had been evaluated: control (C) in which no amendments have been applied during the whole duration of the experiment, and lime (L) application, phosphogypsum (P) and lime + phosphogypsum (L+P) that received the amendments four times during the time that the experiment were carried out.

The rates of lime for the first three lime applications were calculate to raise the base saturation (BS) of the 0 - 20 cm layer to 70 %, as proposed by Cantarella et al. (1997), while the rate of phosphogypsum was based on the clay content of the 20 – 40 cm layer as described by van Raij et al (1996). The previous rates of the amendments applied can be found in the table S1.

In 2015, the rate of lime was also calculated based on the BS elevation of the most superficial soil layer (0 – 20 cm) to 70% (Cantarella et al., 1997), but phosphogypsum rate was based in a method previously proposed and described by Caires and Guimarães (2018) that intended to increase Ca^{2+} saturation (Ca%) to 60% of the effective cation exchange capacity (ECEC) in the 20 – 40 cm layer of soil profile. In this 2016/2017 season application, the L tretament received 6,300 kg ha^{-1} of lime, P received 10,000 kg ha^{-1} of phosphogypsum and L+P received 6,300 kg ha^{-1} of lime and 10,000 kg ha^{-1} of phosphogypsum.

A dolomite limestone - an anhydrous grounded carbonate rock - composed of 23.3% CaO and 17.5% MgO was used. The limestone had 68.8, 92.4, and 99.7% of particles passed 50, 20, and 10 mesh sieves, respectively. The phosphogypsum was a by product of the phosphate fertilizer production and was composed of 20% Ca, 16% S and residual P and F (0.1%).

For the last growing season, soil cropped with soybean (*Glycine max*) (cv. Soy 5917) was fertilized with 300 kg ha^{-1} of NPK 04–20–20 + 18 kg ha^{-1} of N, 3 kg of ha^{-1} S + 3 kg of ha^{-1} B + 1 kg of ha^{-1} Cu + 1 kg of ha^{-1} Mn + 10 kg of ha^{-1} Zn + 0.2 kg of ha^{-1} Mo.

2.2.2. Soil sampling

Twelve samples were taken randomly from each plot 24 months after amendments application, between the rows and in rows to form a composite sample for each replicate of the four treatments (C, L, P and L+P). The soil was sampled with an steel auger in eight layers in depths of 0 – 5, 5 – 10, 10 – 20, 20 – 40, 40 – 60, 60 – 90, 90 – 120 and 120 – 150 cm. We also collected soil samples of native forest (NF) area with an ombrophilous dense forest (Veloso et al., 1991), using the same criteria, to serve as reference of an undisturbed system.

2.2.3. Soil chemical and physical attributes

The soil samples used for chemical and physical characterization were oven dried at 60 ºC and sieved (2-mm). The chemical attributes (pH, exchangeable Al^{3+}, Ca^{2+}, and Mg^{2+}, and base saturation) were analyzed according to van Raij et al. (2001). The content of soil organic matter was determined by the Walkley–Black method (Walkley and Black, 1934). The results can be seen in table S2 of the supplementary material.

The soil texture was determined according to Teixeira et al. (2017) and the results for each depth can be seen in table S3 of the supplementary material. The soil texture was obtained for the most constrasting treatment L+P and NF, and the results did not differ for any of the soil depths collected. At the uppermost layer (0 – 5 cm), the soil has around 37% clay, 8% silt, and

55% sand. At the deepest layer (120 – 150 cm) the soil has around 43% clay, 10% silt, and 47% sand.

2.2.4. Mineralogical composition

To evaluate the main mineralogical phases component in soil, it was measured the sand fraction and the concentrated in iron oxides using the method described by Singh and Gilkes (1991). The difratograms were obtained usin a X-ray diffractometer (Rigaku Geigerflux) with CuKα radiation (λ = 1.5418 Å) generated at 30 kV and 15 mA. The X-ray diffractogram (XRD) patterns were recorded in the range of 3 to 65°2θ with a scanning speed of 0.015°2θ and 1s counting time per step.

2.2.5. Total, inorganic and organic sulfur

Pseudototal sulfur determination was conducted according to EPA 3051a protocol (USEPA 2007) and will be treated as total S for this study. Briefly, 0.5 g of soil was mixed with a mixture of nitric and hydrochloric acid (HNO_3+HCl, 3:1 (v/v)), digested in a microwave and the extract analyzed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (iCAP 6300 Duo, Thermofisher). An internal reference sample was used to guarantee that no volatilization of sulfur would occur in the samples.

The water-souble and adsorbed SO_4^{2-} which represent the plant available sulfur were extracted using a 0.01 mol L^{-1} calcium phosphate ($Ca(H_2PO_4)_2 \cdot H2O$) as proposed by Fox et al. (1964), which is indicated for highly weathered soils and used as the reference methodology for São Paulo State, Brazil.

As the samples were collected from a well-drained soil, the SO_4^{2-} extracted by the $Ca(H_2PO_4)_2 \cdot H_2O$ solution can be considered as the inorganic S. Organic S was obtained by the difference of total S and inorganic S.

2.2.6. X-ray absorsoption near-edge structuture spectroscopy (XANES)

An aliquote of fresh bulk soil samples was stored in a sealed plastic bag inside refrigerated box during collection and within 9 h was stored in a freezer under – 50 °C. An aliquot of each sample was freeze-dried under vacuum condition and finely grounded (<100 mesh) in a glove box with N_2 environment in order to preserve reduced S forms as proposed by Luo et al. (2014).

To optimize the available beamline time, soil samples of soil layers (0 – 5, 5 – 10, 10 – 20, 20 – 40, 40 – 60 and 90 – 120 cm depth) were used for the S K-edge XANES analysis.

The layers were selected to represent the soil profile in depth, but it was limited to 120 cm due to the low total S content present in layer 120 – 150 cm of control and lime treatments and native forest soil.

The homogenized powder samples were spread on sulfur-free Kapton tape (Lanmar Inc., Northbrook, IL) mounted on a sample holder available at SXS beamline of the Brazilian Synchrotron Light Laboratory (LNLS) located in Campinas, State of São Paulo, Brazil. The beamline was equipped with a Si (111) monochromator with an energy resolution of 0.5 eV. Energy calibration was performed using elemental sulfur white-line with E_0 adjusted to 2472 eV. The range of data collection started from 50 eV below the edge to 80 eV above the edge to improve background subtraction.The spectra were measured under vaccum condition in fluorescence mode, in the energy range between 2420 and 2600 eV energy. The steps used where different for each region of the spectrum, as follows: 2420 - 2465 eV and 2521 - 2600 eV: 1 eV; 2465.5 - 2490 eV: 0.20 eV and 2490.5 – 2520 eV: 0.5 eV. Each XANES spectrum of soil samples is an average of five to 12 scans, depending on spectrum quality.

We selected ten organic and inorganic sulfur reference compounds that could be present in soils to be latter used for linear combination fitting (LCF) analysis, as follows: L-cystine, L- cysteine, elemental sulfur, L-methionine, sodium sulfide, L- Cysteic acid, chondroitin sulfate, calcium sulfate, magnesium sulfate.

Additionally, we performed a sulfate adsorption study as described by Prietzel et al. (2008) to different aluminum and iron oxihydroxides minerals present in soil clay fraction of highly weathered tropical soils. Hematite (Fe_2O_3) and goethite (a-FeOOH) and gibbsite [$Al(OH)_3$] were purchased. For the adsorption procedure according to Prietzel et al. (2008), 2.5 g of each mineral were mixed with a 50 ml solution of 0.003 M Na_2SO_4 and 100 ml solution 0.1 M HCl. The HCl solution was mixed to ensure sufficient positive charge of the mineral surfaces for the adsorption SO_4^{2-} to occur. The mixtures were shaken for 90 min in an end-over-end shaker and the suspensions were filtered through a cellulose acetate filter (Schleicher & Schuell Comp.; pore size 0.45 mm). The residues in the filters were rinsed with 1 ml ethanol, washed two times with 5 ml deionised water to remove the excess of Na_2SO_4 solution, and dried in a desiccator.

All reference compounds and SO_4^{2-} minerals that served as standards for LCF procedure were diluted using boron nitride to avoid self absorption and to achieve a concentration similar to the soil samples of around 0.02% of S. XANES spectra for all standards were collected in the same conditions as were for the soil samples. Each XANES spectrum of a standard is an average of three to seven scans, depending on spectrum quality.

2.2.7. Linear combination fitting (LCF)

Background correction and normalization of the acquired spectra and linear combination fitting (LCF) were performed using Athena program in DEMETER software package (Ravel and Newville, 2005). The maximum value in the first-derivative spectra was set as the edge reference energy (E_0). LCF was done over the range of E_0 – 20 eV to E_0 + 50 eV, that comprised all major spectra features of the soil samples. Fitting was conducted using two approaches. The first was performed as described by Colzato et al. (2017) and consisted in fitting all possible combinations of 12 standards with a maximum of four standards per fit in all soil samples selected for XANES analysis. In this procedure no energy shift was used to identify the standards present in the fits with the lowest residual, considered the best fits.

The second procedure has been previously described in Manceau et al. (2012). Each soil sample spectrum was fit to a sum of all reference compounds and the standards with negative weighting coefficient were sequentially being eliminated, starting from the most negative coefficient. After all negative coefficient standards were eliminated, the remaining standards with weighting of less than 0.1 (10%) were also eliminated and the final result included a maximum of four standards (no energy shift allowed).

In general, both approaches converged to the same result. For the approach described by Manceau et al. (2012), S_0 was one of the four standards to remain after the procedure was completed in 0 – 5, 5 – 10, 10 – 20 cm samples of different treatments and for native forest soil. S_0 can occur naturally in soil if anaerobic conditions are set and associated with marine or marsh situations, or from fertilizer application and aerial deposition (Kowalenko and Grimmett, 2007). None of conditions for either have naturally occurring S_0 or reduction of SO_4^{2-} to S_0 are present in an agricultural well-drained soil, so the results in which S0 was present as been part of the fit was not considered, and the second best result was selected. After selecting the set of maximum four standards for each sample, an energy shift of ≤ 0.5 eV for each standard was allowed.

The Origin 2017 software (OriginLab, USA) was used for plotting the spectra.

2.2.8. Statistical analysis

Statistical analysis was conducted using the software Sisvar (Ferreira, 2011). A one-way ANOVA with a Tukey pairwise was used to compare chemical attributes in treatments and native forest soil samples. Differences between values at $p < 0.05$ were considered significant.

2.3. Results and discussion

2.3.1. Soil Organic Matter content

Soil organic matter (SOM) content presented a deacrese with increase depth for all treatments and for native forest soil (figure 1). The native forest soil had a greater SOM content than all treatments in layers 0 – 5, 5 – 10 and 10 – 20 cm and remains with similar values to the treatments throught the soil profile. This shows that the agricultural use of soil, even in a no-till system where ammendments were applied for chemical attributes amelioriation, and SOM was depleted in first 20 cm when compared to the native forest soil. SOM input happens to the surface via plant residue deposition or in depth by rhizodeposition and dead root residues (Briedis et al., 2012), therefore SOM tends to be greater in surface and in root zone rather than in subsurface.

Blum et al. (2013) reported similar results when comparing the first 5 cm of an Oxisol under no-tillage system for 19 years to natural forest area. For the same study site, Carmeis Filho et al. (2017) observed a higher proportion of easily degradable SOM in treatment lime + phosphogypsum which increased soil microbial activity and C mineralization in 0 – 20 cm layer. Therefore, in a short term, the ameloriation of soil chemical attributes by lime and phosphogypsum application tends to decrease the SOM in the upper soil layer.

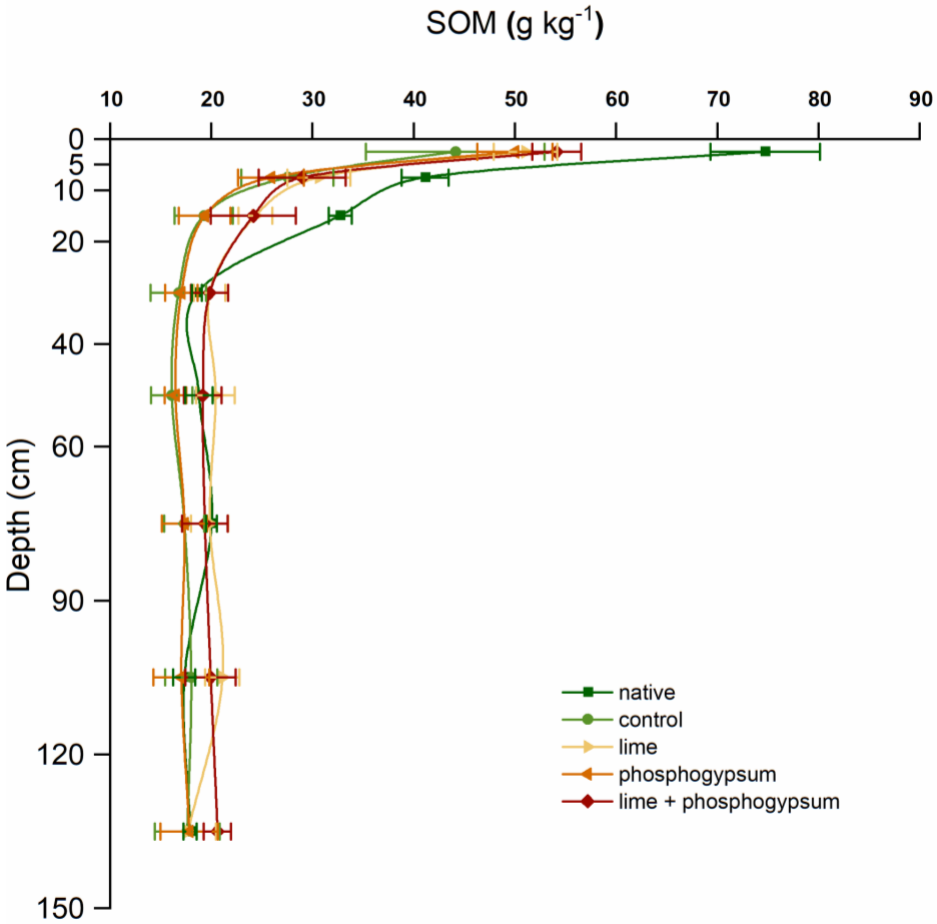

Figure 1. Variation in soil organic matter (SOM) contents as a function of the treatments. Horizontal bars represent the standard deviation of each layer.

The control treatment had the lowest SOM content in the layer 0 – 5 cm, but it did not differ from the SOM contents in the treatments lime, phosphogypsum and lime + phosphogypsum in greater depths (Figure 1). Previous study carried out in the same area by Carmeis Filho et al. (2017), with samples collected in 2014/2015 growing season, showed that treatments containing lime had a greater total organic carbon content than control and phosphogypsum alone, for depths of 0 – 5, 5 – 10, 10 – 20 and 20 to 40 cm. The values of SOM of the 0 – 5 cm layer of our study were greater than previous studies conducted in the area (Carmeis Filho et al., 2017; da Costa and Crusciol, 2016).

Depper layers, down to 60 cm, had a similar SOM contents to previous study (da Costa and Crusciol, 2016), with soil samples collected 60 months after the amendments application. Conversely, for our soil samples, SOM content did not differ between treatments after 5 cm. As the soil samples for the present study were collected 24 months after the application of lime

and/or phosphogypsum, the long-term effects of lime on promoting the accumulation of SOM was not noticed (Briedis et al., 2012; Hati et al., 2008). The C accumulation in long-term in the area was related to a greater biomass production as a result of an amelioriation of the soil chemical attributes, such as increased pH, an adequate supply of Ca and Mg, and Al^{3+} reduction (da Costa and Crusciol, 2016).

2.3.2. Total, inorganic and organic S

The amount of total S content in soil decreased with increasing depth for all treatments and for native forest soil (Figure 2). As a great part of total S in soil is represented by organic forms, (Prietzel et al. 2007, Luo et al. 2014, Xu et. al 2016) reported the same trend and attributed it to the decrease of SOM content with increase in depth. These results agree with the SOM content (Figure 1), and total S had a sharp decrease on the first centimeters for all treatments, with a smoother decrease in native forest soil.

The highest amount of total S was 365 mg kg^{-1} in 0 – 5 cm layer of both lime + phosphpogypsum treatment and native forest soil. The lowest value was 81 mg kg^{-1} in the 120 – 150 cm layer of the control treatment. The concentrations of total S in the 0 – 5 cm layer were similar to what was described by Blum et al. (2013) for an Oxisol in under no-till system for 19 years in which lime and phosphogypsum had been previously applied, but was smaller from what was found under the native forest area.

After the first 20 cm, the total S content did not differ for the treatments control, lime and the native forest soil. The amount of total S in phosphogypsum and lime + phosphogypsum after 20 cm is greater than the previous treatments and remains with similar values throughout the profile. Kar et al. (2019) reported an increase in total S content after gypsum application (20 kg ha^{-1} rate) when compared to control for different soils. Kar et al. (2019) also showed an overall decline of total S in the top 15 cm of the first eight weeks after application of fertilizers and gypsum and attributed to plant uptake and/or leaching out of the system. This observation is in agreement with our data for treatments that received phosphogypsum, with a decrease of total S in first centimeters and an increase in depth.

Organic S followed the same trendline of total S and SOM (figures 1 and 2), being greater close to the surface and decreasing with as depth increased. In the first 10 cm, organic S represented more than 90% of total S content for all treatments and native forest soil (figure 2) as previously described for tropical soils by Solomon et al. (2001, 2003) and Schmidt et al. (2012).

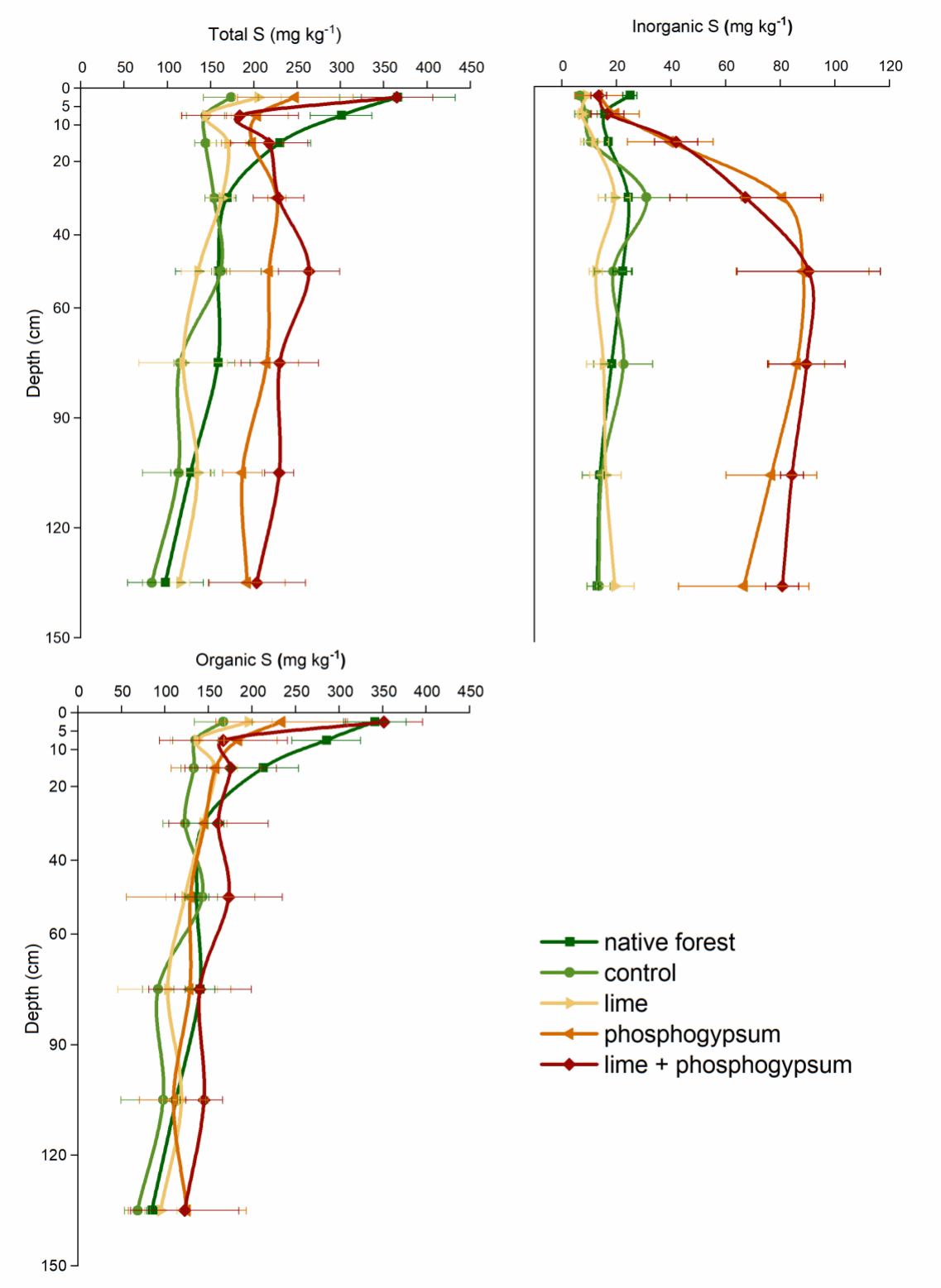

Figure 2. Variation in total S contents as a function of the treatments. Inorganic S extracted by $Ca(H_2PO_4)\ 2{\cdot}H_2O$, and Organic S calculated by the difference between total and inorganic S. Horizontal bars represent the standard deviation of each layer.

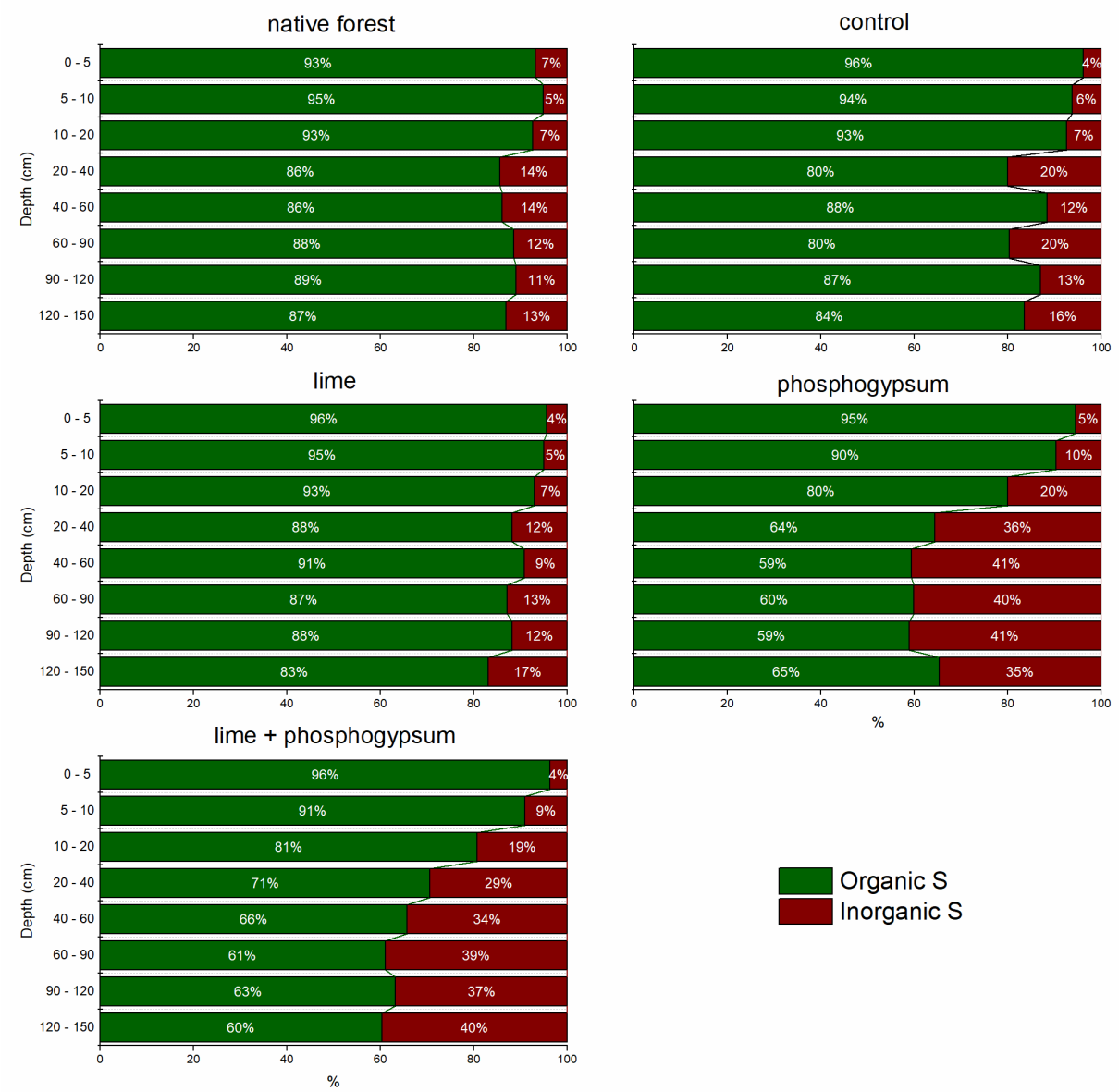

Figure 3. Porcentages of organic and inorganic S in total S contents as a function of the treatments in depth.

In deepest layers, the treatments control and lime presented a percentage of organic S close the native forest, around 80% of the total S content. For treatments phosphogypsum and lime + phosphogypsum, the organic S content had a smaller contribution to the total S content, ranging from 80% on layer 10 – 20 cm to 60% on layer 120 – 150 cm (figure 3). These results indicate that, although a great amount of inorganic S was applied as SO_4^{2-} in the treatments phosphogypsum and lime + phosphogypsum, it was either translocated to greater depths, which agrees with the inorganic S trendline, or transformed in organic S and absorbed by plants or microrganisms (McGill and Cole, 1981; Schimdt et al. 2012).

The amount of inorganic S was smaller on the first 10 cm of soil for all treatments, representing less than 10% of total S content (figures 2 and 3). This also agrees with as

presented by other authors for tropical soils (Solomon et al. 2001, Schmidt et al. 2012). The small retention of SO_4^{2-} close to the surface is due to higher pH values that promoted an increase in the negative charge of the soil and high SOM, thus increasing the translocation of SO_4^{2-} to deeper layers (Camargo and van Raij, 1989; Quaggio et al. 1993).

The layer 10 – 20 cm of all treatments and the native forest soil presented an increase in inorganic S content, reaching a maximum value on layer 20 – 40 cm and stabilizing with increasing depth (Figure 2). The intensity of the increase was different between treatments, with greater values in the ones in which phoshphogypsum was applied. This indicates that the inorganic S applied as SO_4^{2-} in phosphogypsum was leached to lower depths. Previous study on the same area showed that phosphogypsum had a residual effect on available and adsorbed SO_4^{2-} with high concentrations in subsoil after 60 months of application (da Costa and Crusciol, 2016), and similar results were found 53 months after phosphogypsum application in a clayey Rhodic Hapludox (Caires et al. 2006).

2.3.3. S K-edge XANES

Syncrotron-based XANES is a powerfull technique to show differences in oxidation states of S, based on the energy position of the white line that results from s to p photoelectron transition (Solomon et al., 2005). This relation between white line peak shift and energy position for compounds with S in different oxidation states has been extensively discussed previously (Maceau and Nagy, 2012; Blum et al. 2013; Luo et al., 2014).

Table 1 shows the oxidation state, first order peak and additional peaks energy positions obtained by S K-edge XANES spectra for 12 reference compounds in this study using the same conditions for data collection as soil samples, as follows: elemental sulfur (S_0) L-cystine ($HOOCCH(NH_2)_2CH_2S_2$), L- cysteine ($HSCH_2CH(NH2)COOH$), L-methionine ($HO_2CCH(NH_2)CH_2CH_2SCH_3$), sodium sulfide ($Na_2S$), L- Cysteic acid ($C_3H_7NO_5S$), chondroitin sulfate ($C_{14}H_{19}NO_{14}SNa_2$), calcium sulfate ($CaSO_4 \bullet 2\ H_2O$), magnesium sulfate ($MgSO_4 \bullet 7\ H_2O$), SO_4^{2-} adsorbed hematite ($Fe2O_3\ SO_4$), SO_4^{2-} adsorbed gibbsite ($Al(OH)_3\ SO_4$) and SO_4^{2-} adsorbed goethite ($FeO(OH)\ SO_4$). As conditions vary in different studies, we also compared the results with what was found by Prietzel et al. (2007, 2008) and Boye et al. (2011), and the white-line of the spectra of the reference compounds in the present study agrees with what can be found in bibliographic references.

Table 1. Oxidation state, first order peak positions and additional peaks obtained by S K-edge XANES in this study and bibliographic references.

Reference Compounds	Oxidation State	This study		Boye et al (2011)		Prietzel et al. (2007*, 2008**)	
		First order peak (eV)	Additional peaks (eV)	First order peak (eV)	Additional peaks (eV)	First order peak (eV)	Additional peaks (eV)
Elemental Sulfur*	0	2472.9				2472.5	
L-Cystine	+0.2	2473.5	2474.5	2472.6	2474.1		
L-Cysteine	+0.5	2473.9		2473.4			
L-Methionine	+2	2474.3		2473.6			
Sodium sulfide*	+3.7	2478.7	2482.7			2478.7	
L-Cysteic acid	+5	2481.3				2481.3	
Chondroitin sulfate*	+6	2482.3	2481.3	2482.7	2481.2	2482.5	
Calcium sulfate**	+6	2482.9	2486.1, 2492.9			2482.5	2486.0, 2492.0
Magnesium sulfate**	+6	2483.1	2492			2482.5	2487.0, 2492.0
SO_{42-} adsorbed gibbisite**	+6	2482.5				2482.5	
SO_{42-} adsorbed hematite**	+6	2482.7				2482.5	
SO_{42-} adsorbed goethite**	+6	2483.2				2482.4	

There is an increase in energy of the first order peak with increase in oxidation state of S in the coumpounds (Figure 4). Boye et al. (2011) grouped the reference compounds into three groups based on the energy peak position: reduced (<2475), intermediate (2475–2479) and highly oxidized S forms (>2479). So, reference compounds were chosen in order to comprise all the main regions of a typical S K-edge XANES spectra.

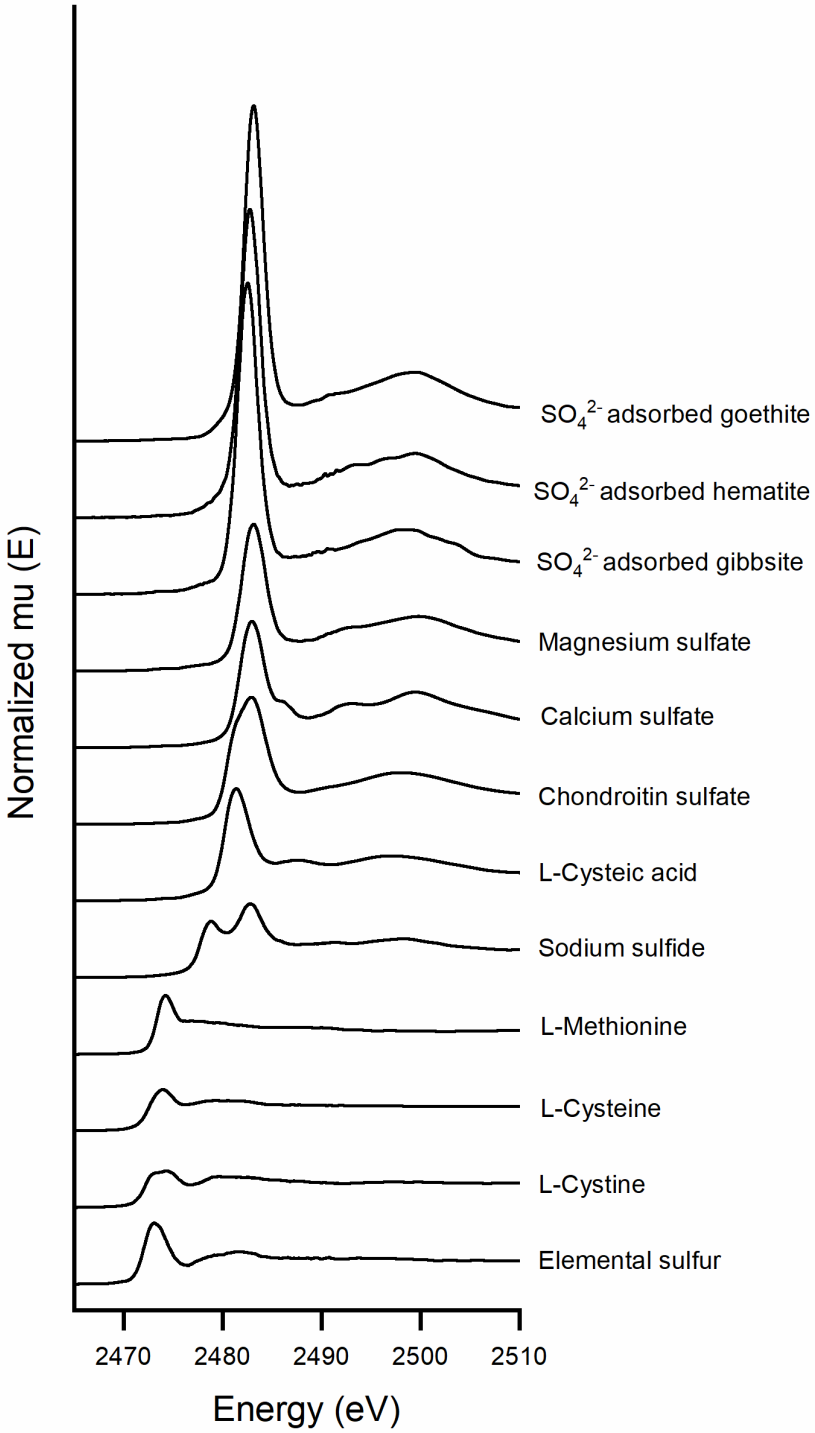

Figure 4. S K-edge XANES spectra of reference compounds

As S is present in soil in a variety of organic and inorganic compounds, it is unlikely that reference compounds will match perfectly the soil samples, but inferences can be done about the oxidation state and the chemical functions of S in soil samples. Along with the white-line energy peak position, additional peaks and other pre and post edge features can be used to distinguish reference compounds and latter converge to a proportion in soil samples using LCF.

Chondroitin sulfate, an ester sulfate, presented a clear pre-peak shoulder at (2481.3 eV), as also described by Boye et al. (2011). Features like this are not considered when using GCF approach (Prietzel et al., 2007). Chondroitin sulfate also presented a broader peak when compared to inorganic sulfate compounds and SO_4^{2-} adsorbed minerals (Figure 4).

Inorganic sulfate compounds such as calcium and magnsesium sulfate had additional post edge features (Table 1), that was not present in chondroitin sulfate and SO_4^{2-} adsorbed minerals. For the latter, the post edge oscillation around 2499 eV is broad and less pronounced, not showing distinct peaks. This was also seen by Prietzel et al. (2008), as adsorbed SO_4^{2-} is not part of a three dimensional cristal system, so the distance between SO_4^{2-} and all neighbour atoms are not well defined.

Additionally, at same concentration as the organic and inorganic sulfates, SO_4^{2-} adsorbed gibbsite, SO_4^{2-} asorbed hematite and SO_4^{2-} asorbed goethite presented a sharper and more intense peak, making possible to differentiate between organic and inorganic SO_4^{2-} compounds. This result agrees to what was observed by Boye et al. (2011) and Prietzel et al (2008). The spectra obtained for SO_4^{2-} adsorbed minerals have similar spectra features with slightly differences in peak position (Table 1). Prietzel et al. (2008) calculated the height ratio of the white-line compared to the post-edge feature (WL/PEF) around 2499 eV for different minerals adsorbed with SO_4^{2-}. The WL/PEF varied between minerals, SO_4^{2-} adsorbed goethite presented a greater ratio around 11.9, SO_4^{2-} adsorbed hematite a ratio of 10.7 and SO_4^{2-} adsorbed gibbsite the smaller ratio around 8.1. The same trend can be seen in spectra collected in this study (Figure 4).

The S K-edge XANES spectra of all treatments and the native forest soil had a relatively increase in signal in two regions of the spectra for all depths. The first peak was around 2474.0 eV and the second peak appeared around 2482.0 eV (Figure 5). No distintictive post edge features appeared in any of the samples, showing a broad peak around 2499 eV.

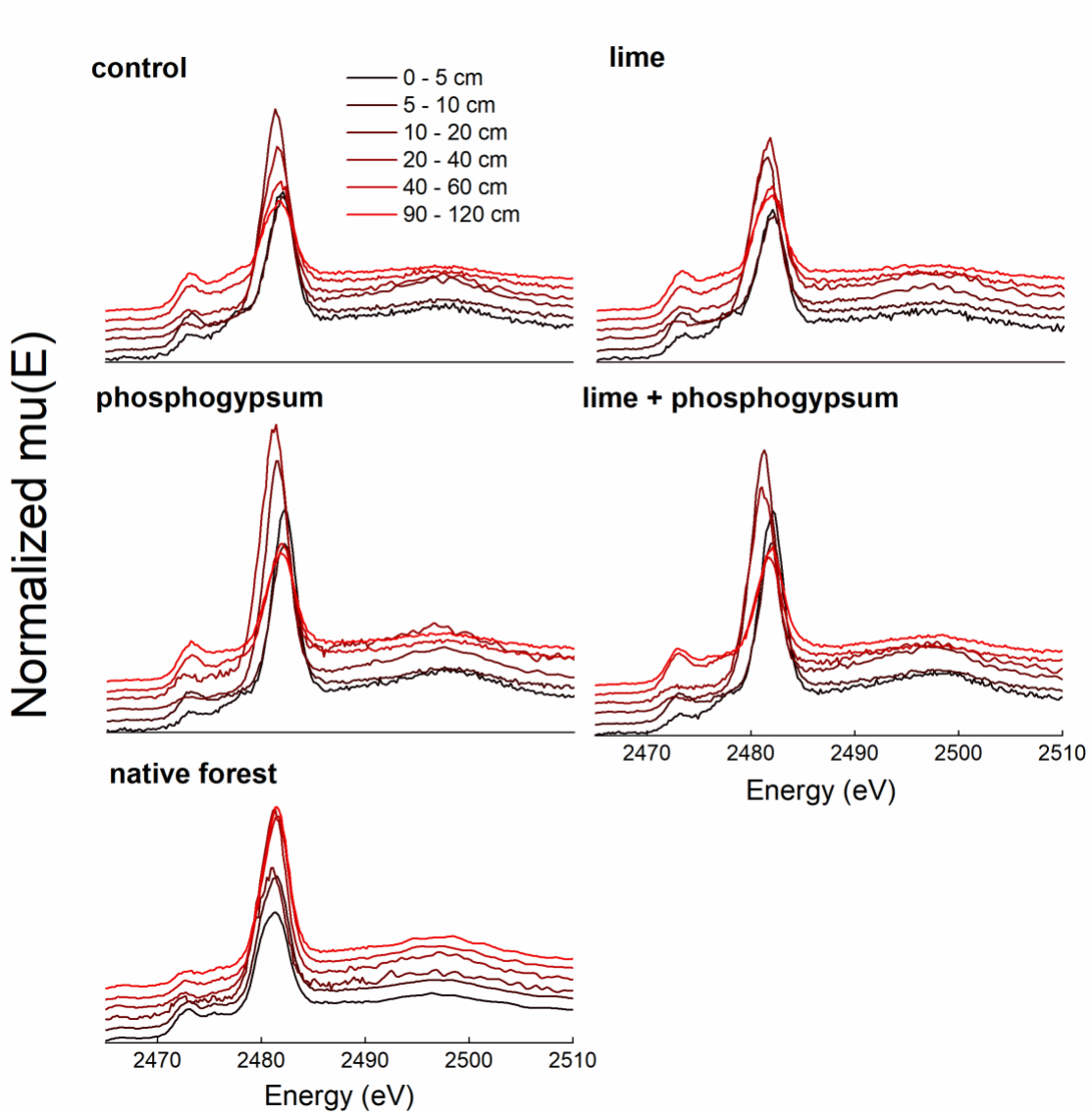

Figure 5. XANES S K-edge of soil samples as a function of the treatments.

For the native forest soil, the first peak was sharper for depths close to the surface, such as 0 – 5 and 5 – 10 cm, and this peak decreased with increase in depth. Conversely, the second peak gets sharper with increasing depth for native forest soil samples. Even though the native forest presented a spectra behavior close to what was expected, all the treatments showed a sharper peak of reduced organic S forms for the depths of 40 – 60 and 90 – 120 cm. All of the treatments also presented sharper peaks of the oxidized forms at depths of 10 – 20 and 20 – 40 cm (Figure 5).

As the environmental conditions do not favour the ocorrence of S_0, it is expected that this region of the spectra represent reduced organic S forms, in which S is directly bounded to C. The reduction of the peak with increase in depth agrees with the decrease of SOM content

in depth (figure 1). Luo et al. (2014) showed a decrease in reduced organic S forms with increasing depth for an Inceptisol in control treatment, but markedly for the treatment that receive sewage sludge (rich in C-bounded S) application in surface.

On the other hand, the second peak increase in intensity and present a sharper format with increase in depth, indicating that oxidized S forms increase with increase in depth. This result agrees with the distribution of organic and inorganic S with increasing depth (Figure 3). Luo et al. (2014) and Xu et al. (2016) also reported an increase in intensity and sharper format of the peak around 2482.0 eV with increase in depth.

A broad post-edge oscillation is also present in the spectra of soil samples in all depths (Figure 5). No post-edge features related to $CaSO_4 \bullet 2\ H_2O$ appeared in the spectra of soil samples, including the treatment that received phosphogypsum. As $CaSO_4 \bullet 2\ H_2O$ is relatively sobluble salt (Tiecher et al., 2018), a great part might have solubilized before soil sample was collected. Kar et al. (2019) showed an increase in SO_4^{2-} - S after one week of $CaSO_4 \bullet 2\ H_2O$ application, indicating that the salt solubilized rapdly in soil. The broad post-edge oscillation (~2499 eV) is similar to the spectra of SO_4^{2-}adsorbed Al and Fe oxyhydroxides (figure 4). XRD results of oxide concentrated sample of native forest soil and all treatments in a composite sample of the first 10 cm shows a predominance of hematite in soil (Figure 6). So, SO_4^{2-}that is not in organic form might be bound to hematite.

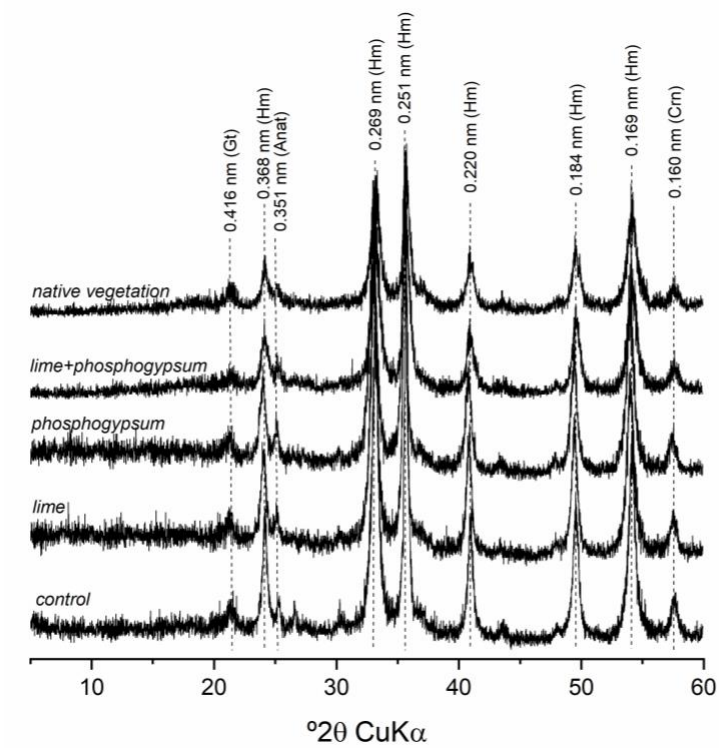

Figure 6. XRD spectrum of oxide concentrated samples dor each treatment and native forest soil. Hm = hematite; Gt = Goethite; Anat = anatase.

Although qualitative information can be retrieved from fingerprint analysis of spectra behavior of the soil samples, a quantitative approach to determine sulfur fractions can be challenging. Many authors have conducted different approaches to reach an quantitative understandment of the S fractions in soil, either by using Gaussian Curve Fitting – GCF (Luo et al. 2014,) or Linear Combination Fitting – LCF (Boye et al., 2011) analysis.

Using GCF approach, the spectrum is divided into six regions representing S oxidation states by using Gaussian curves and the scale of each peak is given by two arctangent functions (Solomon et al., 2011; Xu, 2016). No spectra of reference compounds are needed to retrieve the collaboration to calculate the relative proportions of the different S moieties, and the results are based in the distribution of oxidation states.

LCF approach is based on the use of least squares linear combination fits of normalized reference compounds spectra, allowing a precise separation of functional groups with similar oxidation state by tanking into account different spectral features such as pre-edge peaks, peak shoulders and post-edge features (Vairavamurthy et al., 1994). Prietzel et al. (2011) tested both methods of spectrum decovolution, GCF and LCF, by applying both techniques in define misturex of S reference compounds and to soil samples. Prietzel et al. (2011) stated that if dilute reference compounds of species of interest are available, LCF must be preferred, as it showed to be more helpful on the identification and quantification of C-bonded S species and S_0.

One of the main concerns on using LCF approach is the selection of the reference compounds to perform LCF. Previous authors used different methods to model S XANES spectra to eliminate the necessity of previous assumptions about the composition of the sample, such as principal component analysis – PCA (Beauchemin et al., 2002) and use of aqueous diluted samples (Almkvist et al., 2010)

We used two approaches as described by Colzato et al. (2017) and Manceau et al. (2012) to select the reference compounds to perform the fit. All fits were set to be performed by a combination of a maximum of four reference compounds, and only factions greater than 0.1 (10%) were considered. Both approaches converged to similar results, fits with the smaller R-factor were considered, and the results can be seen in table S4. All soil samples were a combination in different proportions of six main S reference compounds in order of oxidation state of the S atom present in the molecule: L- cysteine (+0.5), L-methionine (+2), , L- Cysteic acid (+5), Chondroitin sulfate (+6), SO_4^{2-} adsorbed to gibbsite (+6) and SO_4^{2-} adsorbed to hematite (+6).

Reasonable fits were attained for all soil samples spectra, both for the peaks and for the post-edge feature intensity, being a good indication that the reference compounds spectra

were representative of the S forms in soil. Figure 7 displays examples of the fits for the native forest soil and lime + phosphogypsum treatment for the layers 0 – 5 and 20 – 40 cm depth.

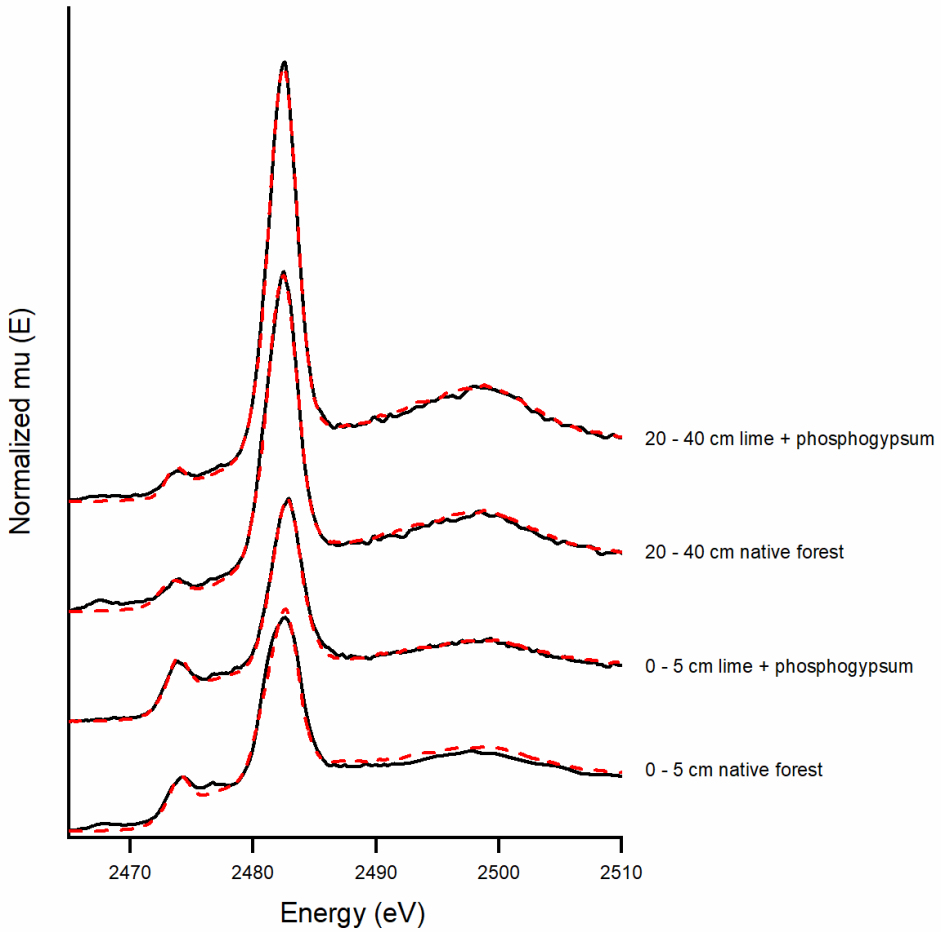

Figure 7. XANES S K-edge spectra (black) and the best fits (red) fits for the native forest soil and lime + phosphogypsum treatment for the layers 0 – 5 and 20 – 40 cm depth.

The result of the LCF analysis can be seen in figure 8 and more detailed information can be found in table S4 of the supplementary material. Residuals or the R-factors calculated by Athena software (Ravel and Newville, 2005) were between 0.0012 and 0.0224. The sums of the values attributed to reference compounds, in percentage, were between 91.6 and 106.0%. The only sum that exceed the maximum value were the one of 10 – 20 cm layer of phosphogypsum treatment, which represented 122%. This error might be attributed to the fact that a poor normalization procedure was conducted with the spectra available of the sample.

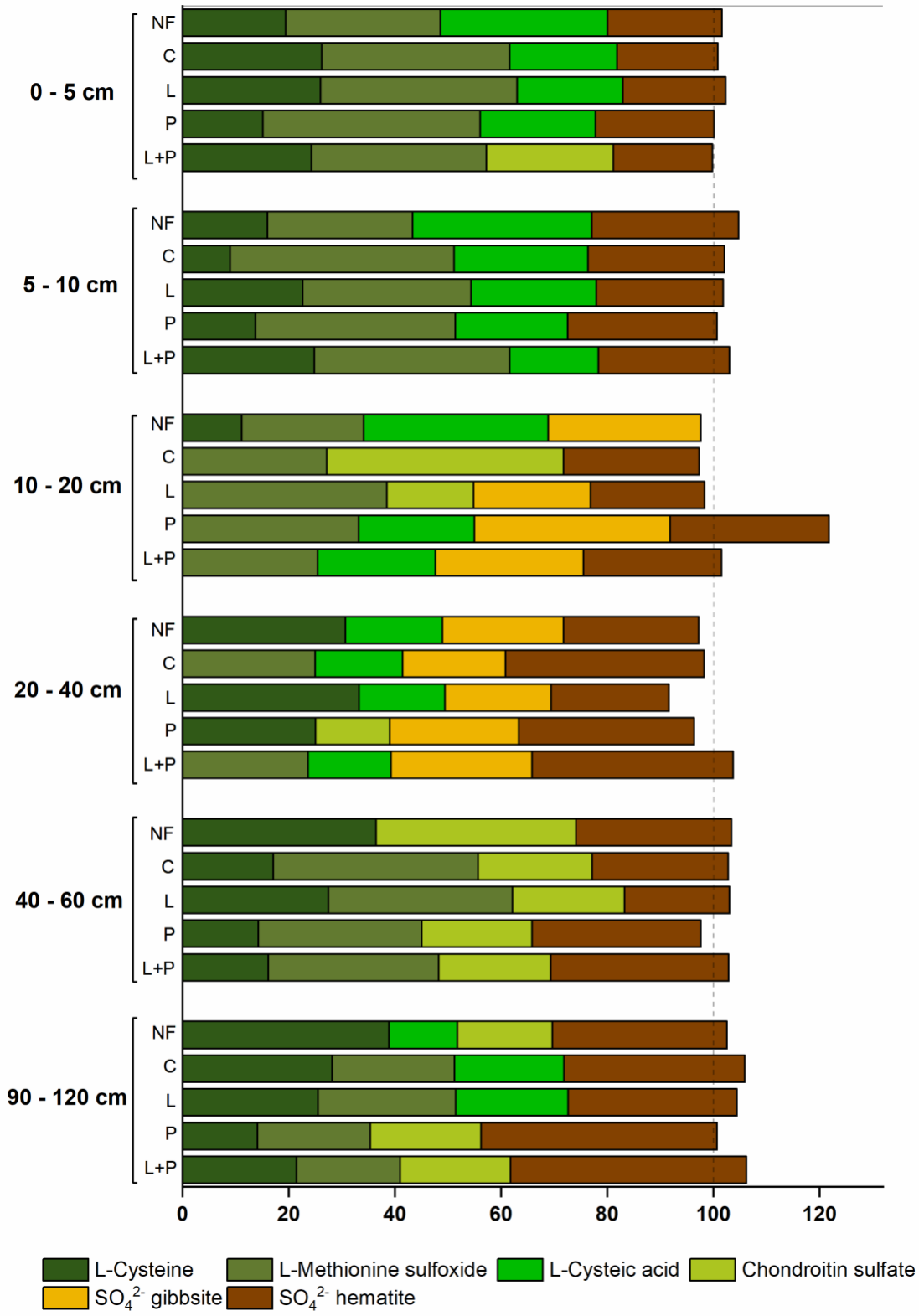

Figure 8. Linear combination fitting (LCF) of spectra of soil samples for the treatments: control (C), lime (L), phosphogypsum (P), lime +phophogypsum (L+P) and native forest (NF).

The uppermost layer (0 – 5 cm) had a greater proportion of reduced organic S forms, represented by L-Cysteine and L-Methionine (> 50% of the total S) (figure 8). The proportion these reduced organic S forms decreases with increasing depth until the 20 – 40 cm (~25%) has a subtle increase in 40 – 60 cm depth and stabilizes around 40% in 90 – 120 cm. Luo et al. (2014) and Xu et al. (2016) reported a decrease in reduced organic forms with increase in depth accessed by S K-edge XANES, which was related to SOM depletion in the subsoil.

L-Cysteic acid, an intermediate S form, was greater in the first 10 cm for all treatments besides for lime + phosphogypsum in layer 0 – 5 cm that presented chondroitin sulfate, a more oxidized organic form (figure 8). The proportion of this intermediate form of S decrease with increase in depth, especially for treatments in which phosphogypsum was applied. Gypsum application showed a deacrese in intermediate S (sulfonate) forms in first centimeters of soil after one week of application, but the proportions of intermediate S increased after eight weeks of application (Kar et al., 2019). Xu at al. (2016) reported a decrease in intermediate forms in depth 20 – 40 cm when compared to 0 – 20 cm of a subtropical agriculture soil in China.

Figure 8 displays an increase in oxidated (+6) S forms with increase in depth for all treatments and native forest soil (figure 8). The proportions are greater in treatments that received phosphogypsum. Kar et al. (2019) reported an increase in oxidized forms after gypsum application in soil surface. Luo et al. (2014) and Xu et al. (2016) observed an increase in oxidized S forms with increase in depth). On the first 10 cm, oxidized forms represent ranges from 18 to 30% in proportion and are mainly related to SO_4^{2-} adsorbed hematite. This result agrees with the XRD of oxyhydroxides concentraded samples (Figure 7) for all treatments, in which hematite is the main Fe oxyhydroxide present in soil.

Both layers of 10 – 20 and 20 – 40 cm depth had a greater proportion of inorganic SO_4^{2-} adorsorbed to Fe and Al oxyhydroxides minerals. These results agree with what can be seen in figures 6 and 7, in that the intensity of the peak around 2482.0 increase in these layers. Our results (Figures 3 and 4) and previous study conducted in the area (da Costa and Crusciol, 2016), showed an increase in absolute values of SO_4^{2-}in comparison to organic S fractions going depper in soil profile in treatments that received phosphogypsum. These conditions indicate that applied SO_4^{2-} was leached to depper layers and is bounded to oxyhydroxides minerals. The SO_4^{2-} adsorbed to gibbsite appeared only in these two soil layers, and our hypothesis is that the pH conditions and SOM content on first centimeters favoured Al^{3+} leaching to subsurface where it might bound to SO_4^{2-}, creating a chemical environment close to SO_4^{2-} adsorbed to gibbsite.

The 40 – 60 and 90 – 120 cm depth had a greater proportion of oxidized forms of S, represented by chondroitin sulfate and SO_4^{2-} adsorbed hematite. For these layers, the proportion

of inorganic and organic S followed the same trendline, with a greater participation of inorganic S, but did not agree in absolut values with what was presented in figure 2.

Although the results of LCF analysis followed the same trendline as presented by wet chemical fractionation of S (Figures 2 and 3), the proportion of organic and inorganic materials did not match exactly. As showed before, organic S represented more than 90% of the total S present in soil for superficial layers for all treatments, so the results from LCF were slightly different. The layers 10 – 20 and 20 – 40 cm depth seems to have an overestimated result for inorganic S forms. The depper layers show a similar proportion of organic and inorganic S forms to what was found in wet chemical extraction.

2.4. Conclusions

The agricultural use of soil under no-tillage system decreased SOM content when compared to native forest soil on the first 20 cm. Lime and phosphogypsum affected SOM contents on the uppermost soil layer when compared to the control. Phosphogypsum application increased contents of inorganic sulfur in depper layers and changed the distribution of organic and inorganic S fractions. Sulfur K-edge XANES was a powerfull technique to evaluate changes in S oxidation when comparing soil depths. LCF analyses provided fair fits for the selected S reference compounds and seems to be a good indicator of S fractions, but did not match the proportion of organic and inorganic S fractions to the wet-chemical analysis.

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Supplementary information

Table S1. Crop species, fertilization and amendments´ application during the 15 years of the field experiment conduction under no till.

Season		Crops	Amendment Application
2002/2003	**Summer**	***Oriza Sativa* (cv. Caiapó)** BF: 300 kg ha_{-1} of NPK 08–28–16 + 4.5% S + 0.5% Zn. TF: 50 N ha of N	Lime: 2,700 kg ha_{-1} (71%ECCE) + Phosphogypsum 2,100 kg ha_{-1}
	Autumn-winter-Spring	***Avena Strigosa* (cv. Comum)** BF: 200 kg ha_{-1} of NPK 10–20–10 + 4.5% S	
2003/2004	**Summer**	***Phaseolus vulgaris* (cv. Pérola)** BF: 300 kg ha_{-1} of NPK 08– 28–16 + 4.5%S + 0.5% Zn. TF: 110 kg ha_{-1} of N	
	Autumn-winter-Spring	***Avena Strigosa* (cv. Comum)** BF: 200 kg ha_{-1} of NPK 04–20–20 + 7% S	
2004/2005	**Summer**	***Arachis Hypogaea* (cv. Runner IAC 886)** BF: 24 kg kg ha_{-1} N + 84 kg ha_{-1} P_2O_5 + 48 kg ha_{-1} of K_2O + 10% S + 0.5% Zn	Lime: 2,700 kg ha_{-1} (71%ECCE) + Phosphogypsum 2,100 kg ha_{-1}
	Autumn-winter-Spring	***Avena sativa* (cv. IAC 7)** BF: 300 kg ha_{-1} of NPK 08–28–16 + 4.5% S + 0.5% Zn. TF: 110 kg ha_{-1} N	
2005/2006	**Summer**	***Arachis Hypogaea* (cv. Runner IAC 886)** BF: 24 kg kg ha_{-1} N + 84 kg ha_{-1} P_2O_5 + 48 kg ha_{-1} of K_2O + 10% S + 0.5% Zn	
	Autumn-winter-Spring	***Avena sativa* (cv. IAC 7)** BF: 8 kg ha_{-1} of N + 40 kg ha_{-1} P_2O_5 + 20 kg ha_{-1} of K_2O + 7% S	
2006/2007	**Summer**	***Zea mays* (cv. 2B570)** BF: 24 kg ha_{-1} of N + 84 kg ha_{-1} P_2O_5 + 48 kg ha_{-1} of K_2O + 10% S + 0.5% Zn. TF: 90 kg kg ha_{-1} N	
	Autumn-winter-Spring	***Uruchloa brizantha* (cv. Marandu)** The forage seeds were simultaneously sown with corn.	
2007/2008	**Summer**	***Zea mays* (cv. 2B570)** BF: 24 kg ha_{-1} of N + 84 kg ha_{-1} P_2O_5 + 48 kg ha_{-1} of K_2O + 10% S + 0.5% Zn. TF: 90 kg kg ha_{-1} N	
	Autumn-winter-Spring	***Uruchloa brizantha* (cv. Marandu)** The forage seeds were simultaneously sown with corn.	
2008/2009	**Summer crop**	***Glycine max* (cv MGBR-46)** BF: 250 kg ha_{-1} of NPK 04–20–20 + 4.5%S + 0.5% Zn.	
	Autumn-winter-Spring crop	***Avena Strigosa* (cv. Comum)** No fertilizer was applied	

2009/2010	**Summer**	***Glycine max* (cv MGBR-46)** BF: 250 kg ha$_{-1}$ of NPK 04–20–20 + 4.5%S + 0.5% Zn.	
	Autumn-winter-Spring	***Sorghum Vulgare* (cv. AG1020)** No fertilizer was applied	
2010/2011	**Summer**	***Zea mays* (cv. 2B433)** BF: 350 kg ha$_{-1}$ of NPK 08–28–16. TF: 150 kg kg ha$_{-1}$ N	Lime: 2,000 kg ha$_{-1}$ (71%ECCE) + Phosphogypsum 2,100 kg ha$_{-1}$
	Autumn-winter-Spring	***Crambe abyssinica* (cv. FMS Brilhante)** BF: 150 kg ha$_{-1}$ of NPK 08–28–16	
2011/2012	**Summer**	***Zea mays* (cv. 2B433)** BF: 350 kg ha$_{-1}$ of NPK 08–28–16. TF: 150 kg kg ha$_{-1}$ N	
	Autumn-winter-Spring	***Crambe abyssinica* (cv. FMS Brilhante)** BF: 150 kg ha$_{-1}$ of NPK 08–28–16	
2012/2013	**Summer**	***Pennisetum glaucum* (cv. ADR300)** No fertilizer was applied	
	Autumn-winter-Spring	***Triticum aestivum* (cv. CD116)** BF: 35 kg ha$_{-1}$ N + 70 kg ha$_{-1}$ P2O5 + 40 kg ha$_{-1}$ of K_2O + kg ha$_{-1}$ S + 12 kg kg ha$_{-1}$ Zn	
2013/2014	**Summer**	***Phaseolus vulgaris* (cv. Pérola)** BF: 10 kg ha$_{-1}$ of N + 50 kg ha$_{-1}$ P_2O_5 + 50 kg kg ha$_{-1}$ K_2O + 11 kg ha$_{-1}$ S + 12 kg ha$_{-1}$ Zn. TF: 100 kg ha$_{-1}$ N	
	Autumn-winter-Spring	***Triticum aestivum* (cv. CD116)** BF: 35 kg ha$_{-1}$ N + 70 kg ha$_{-1}$ P2O5 + 40 kg ha$_{-1}$ of K_2O + kg ha$_{-1}$ S + 12 kg kg ha$_{-1}$ Zn	
2014/2015	**Summer crop**	***Phaseolus vulgaris* (cv. Pérola)** BF: 10 kg ha$_{-1}$ of N + 50 kg ha$_{-1}$ P_2O_5 + 50 kg kg ha$_{-1}$ K_2O + 11 kg ha$_{-1}$ S + 12 kg ha$_{-1}$ Zn. TF: 100 kg ha$_{-1}$ N	
	Autumn-winter-Spring crop	***Uruchloa brizantha* (cv. Marandu)**No fertilizer was applied	
2015/2016	**Summer crop**	***Uruchloa brizantha* (cv. Marandu)**No fertilizer was applied	
	Autumn-winter-Spring crop	***Uruchloa brizantha (cv. Marandu)*** *The forage seeds were simultaneously sown with corn.*	
2016/2017	**Summer**	***Glycine max* (cv. Soy5917)** BF: 300 kg ha$_{-1}$ of NPK 04–20–20 + 18 kg ha$_{-1}$ N+ 3 kg ha$_{-1}$ S + 3 1 kg of ha–1 B + 1 1 kg of ha–1 Cu + , Mn + 1 kg of ha$_{-1}$ Zn + 0,2 kg of ha$_{-1}$ Mo	Lime: 6,300 kg ha-1 (71%ECCE) + Phosphogypsum 10,000 kg ha$_{-1}$

	Autumn-winter-Spring	***Zea mays* (cv. 2A401PW)** BF: 220 kg of ha_{-1} of NPK 08–28–16 + 14 kg kg of ha_{-1} N + 3 kg of ha_{-1} S + 3 kg of ha_{-1} B + 1 kg of ha_{-1} Cu + 1 kg of ha_{-1} + 1 Mn 10 kg of ha_{-1} Zn + 0,2 kg of ha_{-1} Mo

Table S2. Soil chemical attributes of all treatments.

Depth	Treat.	pH 0.01 M $CaCl_2$	SOM	S	K	Ca	Mg	Al	H+Al	ECEC	CEC
			g kg^{-1}	mg kg^{-1}	mmol$_c$ kg^{-1}						
0 - 5	NF	3.9 c	75 a	25 ab	2.3 a	10 d	4 b	18 d	149 c	35 c	165 a
	C	4.0 c	44 c	7 b	2.3 a	7 d	5 b	9 b	67 b	23 b	81 c
	L	5.7 a	51 b	9 b	1.9 ab	64 b	56 a	1 ab	20 a	123 a	142 b
	P	4.5 b	50 b	14 a	1.8 ab	27 c	2 b	6 bc	51 b	36 b	82 c
	L+P	5.8 a	54 b	14 ab	1.6 b	80 a	34 a	1 a	18 a	117 a	134 b
5 - 10	NF	3.9 d	41 a	16 a	1.3 c	4 c	2 b	21 c	100 c	28 ab	107 a
	C	4.0 cd	28 b	9 a	1.9 ab	4 c	2 b	14 b	66 b	22 b	74 b
	L	4.8 b	31 b	7 a	2.2 a	18 b	10 a	2 a	39 a	32 ab	69 b
	P	4.1 c	26 b	20 a	1.5 bc	10 c	1 b	10 b	59 b	22 b	71 b
	L+P	5.0 a	29 b	17 a	1.4 bc	27 a	7 a	2 a	33 a	36 a	68 b
10 - 20	NF	3.8 c	33 a	17 b	0.9 b	2 c	1 ab	20 c	79 c	24 a	84 a
	C	4.0 b	19 b	11 b	1.6 a	10 bc	2 ab	16 bc	58 b	30 a	71 ab
	L	4.5 a	24 b	12 b	1.4 a	16 ab	6 a	6 a	42 ab	28 a	65 b
	P	4.1 b	19 b	40 ab	1.2 ab	4 c	1 b	13 b	53 b	19 a	59 b
	L+P	4.7 a	24 b	42 a	1.3 ab	19 a	5 ab	3 a	35 a	28 a	60 b

	NF	4.0	c	19	a	24	b	0.5	b	2	b	1	a	17	b	62	a	20	a	65	a
	C	4.1	c	17	a	31	b	1.5	a	1	b	1	a	17	b	57	a	20	a	61	a
20 - 40	L	4.3	b	20	a	19	b	1.5	a	7	ab	4	a	10	a	54	a	23	a	67	a
	P	4.1	c	17	a	81	a	1.5	a	4	b	0	a	12	a	61	a	18	a	67	a
	L+P	4.6	a	20	a	67	a	1.1	a	14	a	4	a	8	a	48	a	27	a	67	a
	NF	4.0	b	19	a	22	b	0.3	b	2	b	2	a	16	ab	53	b	20	a	57	b
	C	4.1	ab	16	a	19	b	1.3	a	1	b	1	a	16	ab	71	a	19	a	74	ab
40 - 60	L	4.3	a	20	a	12	b	0.9	a	7	ab	3	a	13	a	75	a	25	a	87	a
	P	4.0	b	16	a	88	a	1.1	a	5	ab	0	a	19	b	71	a	26	a	78	a
	L+P	4.3	a	19	a	91	a	1.0	a	11	a	4	a	12	a	70	a	28	a	86	a
	NF	4.0	b	20	a	18	b	0.1	c	2	a	1	a	20	a	72	a	24	a	76	b
	C	4.1	ab	17	a	22	b	1.1	ab	2	a	1	a	20	a	92	b	24	a	95	a
60 - 90	L	4.2	ab	20	a	15	b	0.6	bc	5	a	3	a	21	a	92	b	29	a	100	a
	P	4.1	ab	17	a	86	a	1.2	a	4	a	0	a	20	a	92	b	26	a	98	a
	L+P	4.2	a	19	a	90	a	0.9	ab	6	a	3	a	17	a	92	b	27	a	102	a
	NF	4.0	a	17	a	14	b	0.1	b	2	a	0	a	22	b	88	a	25	a	91	b
	C	4.0	a	18	a	15	b	0.8	ab	2	a	1	a	20	ab	108	b	23	a	112	ab
90 - 120	L	4.1	a	21	a	16	b	0.4	b	4	a	2	a	20	ab	106	ab	27	a	113	a
	P	4.1	a	17	a	77	a	1.0	a	5	a	1	a	18	ab	98	ab	25	a	105	ab
	L+P	4.2	a	20	a	84	a	0.6	ab	6	a	2	a	18	a	95	ab	27	a	104	ab

	NF	4.0	b	18	a	13	b	0.2	a	2	a	0	a	22	a	82	b	25	a	85	b
	C	4.0	b	18	a	13	b	0.3	a	2	a	1	a	21	a	100	a	23	a	102	ab
120 - 150	L	4.0	ab	18	a	19	b	0.3	a	3	a	2	a	20	a	101	a	25	a	105	a
	P	4.1	ab	18	a	67	a	0.6	a	4	a	1	a	19	a	81	b	25	a	87	b
	L+P	4.2	a	21	a	81	a	0.3	a	6	a	3	a	18	a	91	ab	27	a	100	ab

* control (C), lime (L), phosphogypsum (P), lime +phophogypsum (L+P) and native forest (NF).

**Means followed by the same letters in the line indicate difference at Tukeys's test $p \leq 0.05$

Table S3. Soil granulometry of samples from the treatments: lime + phosphogypsum and native forest.

Treatment	Depth	Clay	Silt	Sand
			%	
lime + phosphogypsum	0 - 5	37	5	58
	5 - 10	36	8	56
	10 - 20	38	8	54
	20 - 40	45	6	49
	40 - 60	47	6	47
	60 - 90	47	7	46
	90 - 120	47	5	48
	120 - 150	44	8	48
native forest	0 - 5	37	8	55
	5 - 10	38	5	57
	10 - 20	40	4	56
	20 - 40	46	7	47
	40 - 60	45	10	45
	60 - 90	46	8	46
	90 - 120	43	9	48
	120 - 150	43	10	47

Table S4. LCF results for all treatments and native forest soil

Depth (cm)	Treatments	L-methionine	L-cysteine	L-cysteic acid	SO_4^{2-} adsorbed hematite	Chondroitin sulfate	SO_4^{2-} adsorbed gibbsite	Sum	R-factor
		----------% ----------							
0 – 5	control	35.4 ± 2.8	26.3 ± 3.3	20.2 ± 0.9	19 ± 0.3	-	-	100.9	0.0159
	lime	37.1 ± 2.6	26.0 ± 3.1	19.9 ± 1	19.4 ± 0.4	-	-	102.5	0.0140
	phosphogypsum	40.9 ± 2.8	15.2 ± 3.2	21.7 ± 0.9	22.4 ± 0.4	-	-	100.2	0.0090
	lime + phosphogypsum	33.0 ± 1.9	24.3 ± 2.2	-	18.7 ± 0.4	23.9 ± 0.8	-	99.9	0.0045
	native forest	29.1 ± 3.5	19.5 ± 4.3	31.5 ± 1.6	21.6 ± 0.7	-	-	101.7	0.0114
5 – 10	control	42.2 ± 4.1	9.0 ± 4.8	25.2 ± 1.3	25.7 ± 0.5	-	-	102.2	0.0144
	lime	31.7 ± 3.1	22.7 ± 3.6	23.6 ± 1.1	23.9 ± 0.5	-	-	101.8	0.0138
	phosphogypsum	37.6 ± 2.9	13.8 ± 3.4	21.2 ± 1	28.1 ± 0.4	-	-	100.8	0.0075
	lime + phosphogypsum	36.8 ± 2.8	24.9 ± 3.2	16.7 ± 0.9	24.7 ± 0.4	-	-	103.1	0.0084
	native forest	27.4 ± 3.9	16.0 ± 4.7	33.7 ± 1.7	27.7 ± 0.7	-	-	104.8	0.0096
10 – 20	control	27.2 ± 1.1	-	-	25.6 ± 0.8	44.6 ± 1.5	-	97.5	0.0069
	lime	38.5 ± 1.0	-	-	21.5 ± 0.7	16.4 ± 1.6	22 ± 0.4	98.4	0.0058
	phosphogypsum	33.2 ± 1.4	-	21.8 ± 1.7	29.9 ± 1.4	-	36.9 ± 1.9	121.8	0.0053
	lime + phosphogypsum	25.5 ± 0.9	-	22.2 ± 1.1	26.0 ± 0.8	-	27.9 ± 1.2	101.6	0.0034
	native forest	23.0 ± 5.6	11.2 ± 6.5	34.7 ± 1.6	-	-	28.8 ± 0.5	97.6	0.0150

	control	25.0 ± 0.8	-	16.5 ± 1.4	37.4 ± 0.7	-	19.4 ± 1.2	98.2	0.0022
	lime	-	33.3 ± 1	16.2 ± 1.4	22.2 ± 0.7	-	20 ± 1.8	91.6	0.0040
20 – 40	phosphogypsum	-	25.1 ± 0.8	-	33.0 ± 1.4	14.0 ± 1.4	24.3 ± 1.3	96.4	0.0012
	lime + phosphogypsum	23.7 ± 0.8	-	15.6 ± 0.9	37.9 ± 0.9	-	26.6 ± 1.2	103.8	0.0018
	native forest	-	30.7 ± 1.1	18.3 ± 1.2	25.5 ± 0.9	-	22.8 ± 1.2	97.3	0.0040
	control	38.6 ± 2.9	17.1 ± 3.4	-	25.6 ± 0.5	21.5 ± 1.1	-	102.9	0.0087
	lime	34.7 ± 2.4	27.5 ± 2.8	-	19.8 ± 0.5	21.1 ± 1	-	103.0	0.0102
40 – 60	phosphogypsum	30.8 ± 2.5	14.3 ± 2.9	-	31.8 ± 0.5	20.8 ± 1	-	97.6	0.0047
	lime + phosphogypsum	32.1 ± 2.8	16.2 ± 3.2	-	33.5 ± 0.6	21.1 ± 1.2	-	102.8	0.0056
	native forest	-	36.5 ± 1.4	-	29.3 ± 2.2	37.7 ± 8.4	-	103.6	0.0058
	control	23.1 ± 3.0	28.2 ± 3.2	20.6 ± 1.3	34.1 ± 0.5	-	-	106.0	0.0224
	lime	25.9 ± 2.9	25.6 ± 3.2	21.2 ± 1.3	31.8 ± 0.5	-	-	104.4	0.0234
90 – 120	phosphogypsum	21.2 ± 5.6	14.2 ± 6.7	-	44.4 ± 0.6	20.9 ± 1.5	-	100.8	0.0074
	lime + phosphogypsum	19.5 ± 2.4	21.5 ± 2.6	-	44.5 ± 0.7	20.8 ± 1.4	-	106.3	0.0088
	native forest	-	38.9 ± 1.5	12.9 ± 7.1	32.9 ± 2.3	17.9 ± 8.9	-	102.6	0.0073

3. CHEMICAL ATTRIBUTES AND ENZYME ACTIVITY OF A TROPICAL SOIL AMENDED WITH LIME AND PHOSPHOGYPSUM

Abstract

The conversion of native forest area to agricultural systems is usually linked to a depletion of soil organic matter (SOM) contents and changes in nutrient distribution and in microbial activity. The adoption of conservation systems such as no-tillage (NT) is an alternative to increase SOM contents. Lime is applied to highly wheathered tropical soils to increase pH and serve as source of Ca and Mg. Because of its low solubility, it is often combined with phosphogypsum that acts as source of Ca and S in depth and decrease Al tocixity in depper leayers. In the present study, we verified the effects of rates of phosphogypsum, in association or not with lime, in soil chemical attributes and enzyme activity when compared to soil samples from a native forest. The following chemical soil attributes were evaluated: soil pH, and SOM, sulfate, K, Ca and Mg contents. Soil enzymes (ß-glucosidase and arylsulfate) activity were measured as indicators of ammelioriation of soil chemical attributes after amendments´s application. The change in land use from native forest to NT system decreased SOM content on the first 20 cm. Soil samples that received lime had an increase in pH until 40 cm depth and also decreased exchangeable Al^{3+} when compared to phosphogypsum and native forest soil. Phosphogypsum decreased exchangeable K contents in the most superficial layer when compared to control and native forest soil. The combination of amendments promoted a great translocation of Ca to depper depths. Exchangeable Mg content decreased when only phosphogypsum was applied. Enzyme activity was greatly affected by depth and amendments application. ß-glucosidase activity decreased with depth for all treatments and native forest soil and increased with lime and phosphogypsum application. Arylsulfatase activity increased in treatments that received lime, deacresed with application of phosphogypsum alone when compared to control and was similar to native forest soil down to 20 cm depth.

Keywords: Lime; Phosphgypsum; Soil chemical attributes; Soil enzyme activity; Arylsulfatase; ß-glucosidase

3.1. Introduction

The conversion of native vegetation to agricultural systems leads to changes soil organic matter (SOM) dynamics, nutrient distribution and microbial activity (Sá et al., 2014, Balota et al., 2014; Inagaki et al., 2016). Soils under no tillage (NT) system are widespread in Brazil and are an alternative to increase SOM contents, as shown in soil from long-term field

experiments (Costa and Crusciol, 2016). As NT areas are not plowed for growing crops, a chemical gradient is created by the deposition of nutrients and organic matter to the soil surface (Tiecher et al., 2018).

Lime is often applied to the surface in NT to amelioriate soil chemical attributes for plant growth such as elevation of pH, increase of exchangle Ca and Mg contents and decrease of toxic Al^{3+} contents (Caires et al., 2004; Rampim et al., 2011). Although long-term effects of lime can be seen in subsurface layers, it is less marked than in the surface layer, so high subsurface acidity can restrict root growth and development and, therefore, lead to lower crop yields (Sousa et al., 2007; Dalla Nora and Amado, 2013).

Phosphogypsum is a highly pure and fine-textured material of non-geological origin, originated as an industrial waste from the production of phosphoric acid from apatite (Shainberg et al., 1989). Because of its greater solubility when compared to lime, phosphogypsum is used as a soil conditioner that carries cations to greater depths where the increase in Ca content along with the deacrease in Al toxicity leads to an improvement of the soil chemical properties (Alvarez V. et al., 2007). These factors contribute to a better root development and distribution, increasing the amount of plant residue in greater depths (Carmeis Filho et al., 2017).

Phosphoypsum recommendation rates were historically based on the clay content of the 20 – 40 cm layer (van Raij et al., 1996), but a new method proposed by Caires and Guimarães (2018) is based on the elevation of Ca saturation to 60% of the effective cation exchange capacity (ECEC) in the 20 – 40 cm layer. This new formulation commonly results in much greater rates of phosphogypsum.

Soil enzyme activity assays are a good and sensitive indicator of land use change and soil sustentability (Deng and Tabatabai, 1997; Jin et al., 2009; Wang et al., 2012; Balota et al., 2011). Enzyme acitvity is affected by soil temperature and humidity (Steinweg et al., 2012; Zhou et al., 2013), fertilizer application (Prietzel, 2001); SOM content (Dou et al., 2016), lime and phosphogypsum application (Ekenler and Tabatabai, 2003, Inagaki et al., 2016). NT system was linked to an increase in enzyme activity when compared to conventional tillage (Sá et al., 2018).

The main questions raised by this study are: (i) How do lime and phosphogypsum application affects soil chemical attributes? (ii) Are enzyme activity impacted by amendments application? Our major hypothesis is that the great amount of phosphogypsum applied will deacrease arylsulfatase activity in soil samples from a long-term field experiment under no-till when compared to samples from a native forest soil.

3.2. Material and methods

3.2.1. Study sites location and experimental design

Soil samples came from a sandy clay loam kaolinitic and thermic Typic Haplorthox soil (USDA, 1999) collected from field scale study that has been carried out in southeast Brazil (Botucatu, State of São Paulo – 48° 23’ W, 22° 51’ S and 765 m) for 15 years under NT system (Figure 1). According to Köeppen’s classification, the climate is Cwa (tropical climate with dry winter and a hot and rainy summer).

Figure 1. Geografic location of the Botucatu (green) located in southeast of Brazil.

The area was cultivated with a variety of plants in a long-term crop rotation, mixing grasses with legumes such as soybeans (*Glycine max*), this last been the cultivated crop during soil sampling (all cultivated species can be found in the table S1 of the supplementary material of the first chapter).

The experimental design was composed of completely randomized blocks with four replicates. Four treatments had been evaluated: control (no amendments), lime (L) application, phosphogypsum (P) and lime + phosphogypsum (L+P) that received the amendments four times during the whole period of cultivation.

The rates of the first three lime applications were calculate to raise the base saturation of 0 -20 cm to 70%, as proposed by Cantarella et al. (1998) (previous rates can be found in the supplementary material). On the other hand, rates of phophogypsum were calculated based on the clay content of the 20 – 40 cm layer (van Raij et al., 1996).

In 2015, the rate of phosphogypsum were based in a method intended to increase Ca^{2+} saturation to 60% of the effective cation exchange capacity at the layer of 20 – 40 cm of soil profile (Caires and Guimarães, 2018). In this last application, the treatment LM received 6,300 kg ha^{-1} of lime, PG received 10,000 kg ha^{-1} of phosphogypsum, and LM+PG received 6,300 kg ha^{-1} of lime and 10,000 kg ha^{-1} of phosphogypsum.

A dolomite limestone - an anhydrous grounded carbonate rock - composed of 23.3% CaO and 17.5% MgO was used. The limestone had 69, 92, and 100% of particles passed 50, 20, and 10 mesh sieves, respectively. The phosphogypsum was a by product of the phosphate fertilizer production and was composed of 20% Ca, 16% S and residual P and F (0.1%).

For the last growing season, soil cropped with soybean (cv. Soy 5917) was fertilized with 300 kg ha^{-1} of NPK 04–20–20 + 18 kg ha^{-1} of N, 3 kg of ha^{-1} S + 3 kg of ha^{-1} B + 1 kg of ha^{-1} Cu^{+} + 1 kg of ha^{-1} Mn + 10 kg of ha^{-1} Zn + 0.2 kg of ha^{-1} Mo.

3.2.2. Soil sampling

Twelve samples were taken randomly from each plot, between the rows and in rows to form a composite sample for each replicate of the four treatments (C, L, P and L+P). The soil was sampled with an steel auger in eight layers in depths of 0 – 5, 5 – 10, 10 – 20, 20 – 40, 40 – 60, 60 – 90, 90 – 120 and 120 – 150 cm. We also collected soil samples of native forest (NF) area with an ombrophilous dense forest (Veloso et al., 1991), using the same criteria, in order to have a sample to serve as reference of an undisturbed system.

3.2.3. Soil chemical attributes

The soil pH was determined using a 0.01 mol L^{-1} $CaCl_2$ suspension (1:2.5 soil/solution). Soil organic matter (SOM) content was determined according to the method proposed by Walkley and Black (1934). The water-souble and adsorbed SO_4^{2-} were extracted using a 0.01 mol L^{-1} calcium phosphate [$Ca(H_2PO_4)_2 \cdot H_2O$], as proposed by Fox et al. (1964), and determined by the turbidimetric method, in which it precipitates as $BaSO_4$. Exchangeable contents of K, Ca and Mg were extracted with an ion-exchange resin, which is the standard method for São Paulo State, and the determination of their contents was performed in an atomic absorption spectrometer (van Raij et al., 2001). The exchangeable content of Al was extracted

using a 1 mol L^{-1} KCl (1:10 soil/solution) and determined by titration with a 0.025 mol L^{-1} NaOH solution (van Raij et al., 2001). The total acidity at pH 7.0 (H+Al) was estimated by the SMP-buffer solution method (van Raij et al., 2001).

The effective cation exchange capacity (ECEC) was calculated by the sum of the basic cations (K, Ca and Mg) and exchangeable Al^{3+}. The cation exchange capacity (CEC) was calculated by the sum of basic cations and H+Al. The base saturation (BS) was calculated as follows: BS = 100 (Ca^{2+} + Mg^{2+} + K^{+}/CEC). Aluminum saturation (m) was acquired by: m= 100(Al^{3+}/ECEC). Ca saturation was calculated by dividing the Ca content by ECEC and multiplied by 100.

3.2.4. Enzyme activity

We evaluated the activity of two enzymes: β-glucosidase and arylsufatase. The first is responsible for degrading the SOM by converting cellobiose to glucose monomers and was classified as a good indicatior of C mineralization. The second is related to S cycle in soil, responsible for mineralization of organic S into SO_4^{2-}.

β-glucosidase activity was determined using the methodology described by Eivazi and Tabatabai (1988), while and arylsulfatase activity was determined using the methodologies described by Tabatabai and Bremner (1970). Both methods use the base substrate p-nitrophenol, that bounds to either glucose or SO_4^{2-}. Soil samples were kept in the refrigerator at 5 ºC and it was passed through a 4 mm sieve. The analyses were carried out in triplicates with one blank for each sample. One gram of soil was weighed, and 4 mL of a buffered solution of calcium acetate with pH of 5.8 and 1 mL of p-nitrofenol sulfate solution were added (not added to the blanks). The flasked was shaken in a rotate shaker and incubated in a water bath at 37 ºC for 1 h. After that, the pH was adjusted using 1 mL of $CaCl_2$ (0,5 mol L^{-1}), and 4 mL of NaOH 0.5 mol L^{-1} were added to the arylsulfatase flasks and/or 4 mL of 0.5 mol L^{-1} trishydroxymethylaminomethane for β-d-glucosidase flasks. One mL of p-nitrofenol was also added to the blank flasks. The solutions were filtered in a filter paper Whatman no. 2 and measured in a spectrophotometer at 410 nm.

3.3. Results and discussion

3.3.1. Soil chemical attributes

The soil chemical attributes were greatly affected by surface lime and phosphogypsum application (Table 1). Soil samples from the control treatment had similar pH to native forest soil in all evaluated layers (Figure 2). Soil pH was greater in treatments that received lime application until 40 cm. Previous results from the same area showed an increase in pH in the first 20 cm of treatments that received lime (2,000 kg ha^{-1} rate) 48 months after the application (da Costa and Crusciol, 2016). As the rate of lime applied in the last application (6,300 kg ha^{-1}) was much greater, the increase in pH can be seen in depper layers.

The potencial acidity at pH 7.0 (H+Al) was greatly affected by lime application on the first 20 cm (figure 2). The acidity was corrected by the increase in hydroxyl in soil solution via limestone dissolution. This hydroxyl group reacts with the excess of H^+, increasing soil pH and precipitates exchangeable Al^{3+} as $Al(OH)_3$ (de Oliveira and Pavan, 1996). Caires et al. (2003) attributed to the pH correction in surface the increased movement in HCO_3^- ions along with Ca and Mg. Phosphogypsum alone had a slightly influenced the pH when compared to control on the first 5 cm of soil (Figure 2). Phosphogypsum application affected the Al^{3+} availability (Figure 2), as Al^{3+} bounds to SO_4^{2-} to form $AlSO_4^+$ oxocomplexes (Pavan et al, 1984). The values of Al^{3+} was affected by lime and phosphogypsum application in the first 40 cm, remaining close to values of control and native forest soils in depper layers.

Table 1. Soil chemical attributes as a function of the treatments.

Depth	Treat.	pH 0.01 M $CaCl_2$		SOM		S		K		Ca		Mg		Al		H+Al		ECEC		CEC		BS		m		Ca	
				g kg^{-1}		mg kg^{-1}		mmol$_c$ kg^{-1}														%					
0 - 5	NF	3.9	c	75	a	25	ab	2.3	a	10	d	4	b	18	d	149	c	35	c	165	a	10	c	52	c	30	b
	C	4.0	c	44	c	7	b	2.3	a	7	d	5	b	9	b	67	b	23	b	81	c	17	c	40	c	30	b
	L	5.7	a	51	b	9	b	1.9	ab	64	b	56	a	1	ab	20	a	123	a	142	b	86	a	1	a	52	ab
	P	4.5	b	50	b	14	a	1.8	ab	27	c	2	b	6	bc	51	b	36	b	82	c	38	b	15	b	74	a
	L+P	5.8	a	54	b	14	ab	1.6	b	80	a	34	a	1	a	18	a	117	a	134	b	87	a	1	a	69	a
5 - 10	NF	3.9	d	41	a	16	a	1.3	c	4	c	2	b	21	c	100	c	28	ab	107	a	7	c	75	c	14	c
	C	4.0	cd	28	b	9	a	1.9	ab	4	c	2	b	14	b	66	b	22	b	74	b	11	bc	64	c	18	c
	L	4.8	b	31	b	7	a	2.2	a	18	b	10	a	2	a	39	a	32	ab	69	b	44	a	7	a	54	ab
	P	4.1	c	26	b	20	a	1.5	bc	10	c	1	b	10	b	59	b	22	b	71	b	17	b	45	b	43	b
	L+P	5.0	a	29	b	17	a	1.4	bc	27	a	7	a	2	a	33	a	36	a	68	b	51	a	5	a	73	a
10 - 20	NF	3.8	c	33	a	17	b	0.9	b	2	c	1	ab	20	c	79	c	24	a	84	a	5	c	82	c	10	c
	C	4.0	b	19	b	11	b	1.6	a	10	bc	2	ab	16	bc	58	b	30	a	71	ab	19	b	54	b	33	b
	L	4.5	a	24	b	12	b	1.4	a	16	ab	6	a	6	a	42	ab	28	a	65	b	35	a	20	a	55	ab
	P	4.1	b	19	b	40	ab	1.2	ab	4	c	1	b	13	b	53	b	19	a	59	b	10	c	68	b	22	bc

	L+P	4.7	a	24	b	42	a	1.3	ab	19	a	5	ab	3	a	35	a	28	a	60	b	41	a	12	a	66	a
20 - 40	NF	4.0	c	19	a	24	b	0.5	b	2	b	1	a	17	b	62	a	20	a	65	a	5	c	84	c	8	c
	C	4.1	c	17	a	31	b	1.5	a	1	b	1	a	17	b	57	a	20	a	61	a	6	c	83	c	7	c
	L	4.3	b	20	a	19	b	1.5	a	7	ab	4	a	10	a	54	a	23	a	67	a	18	b	46	b	31	b
	P	4.1	c	17	a	81	a	1.5	a	4	b	0	a	12	a	61	a	18	a	67	a	9	c	65	bc	24	b
	L+P	4.6	a	20	a	67	a	1.1	a	14	a	4	a	8	a	48	a	27	a	67	a	28	a	30	a	51	a
40 - 60	NF	4.0	b	19	a	22	b	0.3	b	2	b	2	a	16	ab	53	b	20	a	57	b	7	bc	79	c	11	c
	C	4.1	ab	16	a	19	b	1.3	a	1	b	1	a	16	ab	71	a	19	a	74	ab	4	c	85	c	6	c
	L	4.3	a	20	a	12	b	0.9	a	7	ab	3	a	13	a	75	a	25	a	87	a	13	ab	54	b	29	b
	P	4.0	b	16	a	88	a	1.1	a	5	ab	0	a	19	b	71	a	26	a	78	a	9	bc	74	c	21	b
	L+P	4.3	a	19	a	91	a	1.0	a	11	a	4	a	12	a	70	a	28	a	86	a	18	a	44	a	39	a
60 - 90	NF	4.0	b	20	a	18	b	0.1	c	2	a	1	a	20	a	72	a	24	a	76	b	5	a	84	b	9	c
	C	4.1	ab	17	a	22	b	1.1	ab	2	a	1	a	20	a	92	b	24	a	95	a	4	a	84	b	9	c
	L	4.2	ab	20	a	15	b	0.6	bc	5	a	3	a	21	a	92	b	29	a	100	a	8	a	71	b	18	b
	P	4.1	ab	17	a	86	a	1.2	a	4	a	0	a	20	a	92	b	26	a	98	a	6	a	77	b	17	b
	L+P	4.2	a	19	a	90	a	0.9	ab	6	a	3	a	17	a	92	b	27	a	102	a	10	a	63	a	24	a
90 - 120	NF	4.0	a	17	a	14	b	0.1	b	2	a	0	a	22	b	88	a	25	a	91	b	3	a	90	a	8	b
	C	4.0	a	18	a	15	b	0.8	ab	2	a	1	a	20	ab	108	b	23	a	112	ab	3	a	84	a	8	b

	L	4.1	a	21	a	16	b	0.4	b	4	a	2	a	20	ab	106	ab	27	a	113	a	6	a	75	a	16	a
	P	4.1	a	17	a	77	a	1.0	a	5	a	1	a	18	ab	98	ab	25	a	105	ab	7	a	72	a	20	a
	L+P	4.2	a	20	a	84	a	0.6	ab	6	a	2	a	18	a	95	ab	27	a	104	ab	9	a	66	a	23	a
120 - 150	NF	4.0	b	18	a	13	b	0.2	a	2	a	0	a	22	a	82	b	25	a	85	b	3	a	88	a	9	b
	C	4.0	b	18	a	13	b	0.3	a	2	a	1	a	21	a	100	a	23	a	102	ab	3	a	89	a	7	b
	L	4.0	ab	18	a	19	b	0.3	a	3	a	2	a	20	a	101	a	25	a	105	a	5	a	80	a	11	b
	P	4.1	ab	18	a	67	a	0.6	a	4	a	1	a	19	a	81	b	25	a	87	b	7	a	76	a	18	ab
	L+P	4.2	a	21	a	81	a	0.3	a	6	a	3	a	18	a	91	ab	27	a	100	ab	9	a	66	a	23	a

* control (C), lime (L), phosphogypsum (P), lime +phophogypsum (L+P) and native forest (NF).

**Means followed by the same letters in the line indicate difference at Tukeys's test $p \leq 0.05$

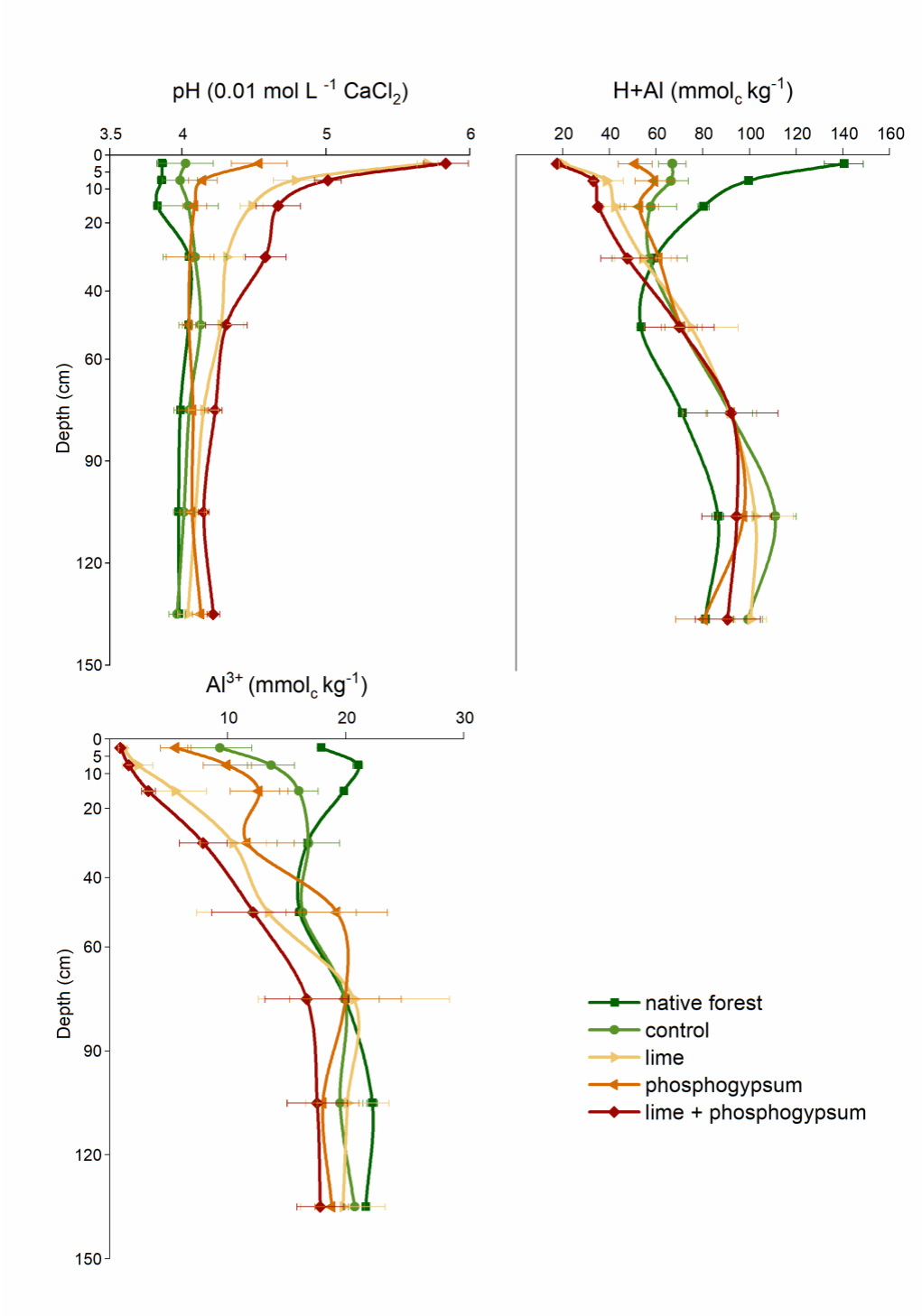

Figure 2. Variation in pH, exchangeable Al3+ and total acidity (H+Al) contents of the soil in each soil layer as a function of the treatments. Horizontal bars represent the standard deviation of each layer.

SOM content decreased with increasing depth for all treatments and for native forest soil (Table 1). Soil samples from the native forest had a greater SOM content than all treatments until 20 cm and remained with similar values to the treatments throught the soil profile. Both

lime and lime + phosphogypsum treatments had different SOM contents when compared to control only on first 5 cm of soil. High rates of lime (4,000 kg ha^{-1}) in tropical soils was linked to a greater root density of cultivated crops and higher grain yield even four years after application due to soil chemical amelioriation (Carmeis Filho et al., 2017).

Even though the apport of SOM in treatments that received lime was greater, the change in soil chemical attributes may favor the soil microbiota activity that, in the first moment will accelerate SOM decomposition (Chan and Heenan, 1999; Ekenler and Tabatabai, 2003). As this SOM depletion was attributed to be an important effect only in the short term, lime application was linked to the promotion of SOM in the long-term (da Costa and Crusciol, 2016).

In the first 10 cm of soil, the water soluble + adsorbed SO_4^{2-}, extracted with calcium phosphate and considered bioavailable, did not differ for treatments that received phosphogypsum application and native forest soil and had smaller content on control and lime alone (Table 1). In deeper layers, SO_4^{2-} content was greater in treatments that received phosphogypsum application when compared to other treatments and native forest soil. These results indicate that after dissolution of phosphogypsum, SO_4^{2-} has been leached to greater depths. Camargo and van Raij (1989) and Quaggio et al. (1993) related a small retention of SO_4^{2-}in surface layers due to higher pH and high SOM that promoted an increase in the negative charge of the soil

All treatments and the native forest soil had a greater amount of exchangeable potassium (Kex) in the uppermost layer that decrease with increasing depth (Figure 3). The decrease was sharper for the native forest soil on the first 40 cm and then stabilized on the 40 – 60 cm layer. Potassium is an element with biogenic profile, i.e. it shows depletion at depth in natural systems (with concentrations in equilibrium with parent material) and enrichment at the surface due to biological fluxes (Brantley et al., 2007).

All treatments received K via fertilization all over the duration of the experiment. Control had similar amounts of Kex in the native forest soil on the first layer but had grater Kex content in depper layers, showing that K is mobile in soil and has been leached to depper layers. Limed soil had similar Kex values to control in until 40 cm and then deacreased. Carmeis Filho et al (2017) presented similar results in area where lime was applied and attributed to the displacement of K to the input of polyvalent cations (i.e. Ca and Mg). Kex content contents did not differ for all treatments in the 120 – 150 cm layer, and it indicates that the K applied in fertilization might not been leached out of the system but it is adsorbed to soil minerals in a region that already have a small root density.

Exchangable Ca (Ca_{ex}) contents were greater close to the surface and decreased rapidly with increase in depth (Figure 3). The amount of Ca_{ex} in the uppermost layer was greater for the limed soil than the soil samples that received phosphogypsum alone which might be due to greater solubility of phosphogypsum when compared to lime. The combination of lime + phosphogypsum increased Ca_{ex} until 60 cm depth. In depper layers all treatments and native forest soil present similar Ca_{ex} values. Caires et al. (2000) and Soratto and Crusciol (2008) also reported an increase in Ca availability after surface lime application, but the contents decreased in surface with time and increase in depper layers. As lime has a low solubility, the combined surface application with phosphogypsum can accelerate the rate of transport of Ca to lower depths.

Exchangable Mg (Mg_{ex}) had a similar trend to other basic cations, and its content was greater close to the surface and decreased with increasing depth (figure 3). As dolomitic lime is a source of Mg, limed soil had a greater Mg_{ex} in surface. The same amount was applied in both treatments that received lime, but Mg_{ex} content was greater in lime alone than lime + phosphogypsum in the first 10 cm, and both presented similar values until 40 – 60 cm layer. Even though Mg_{ex} contents after application of phosphogypsum was not statistically different from control and native forest soil, it presented a tendency of decrease close to the surface. The same trend was observed by Caires et al. (2006), Soratto and Crusciol (2008) and da Costa and Crusciol (2016), and it was attributed to the formation of soluble ionic pairs with SO_4^{2-}, thus increasing the mobility of cations in soil (Silva et al., 1998). Caires and Guimarães (2018) suggested that phosphogypsum application should be recommended only to soils with high Mg_{ex} content in surface layers.

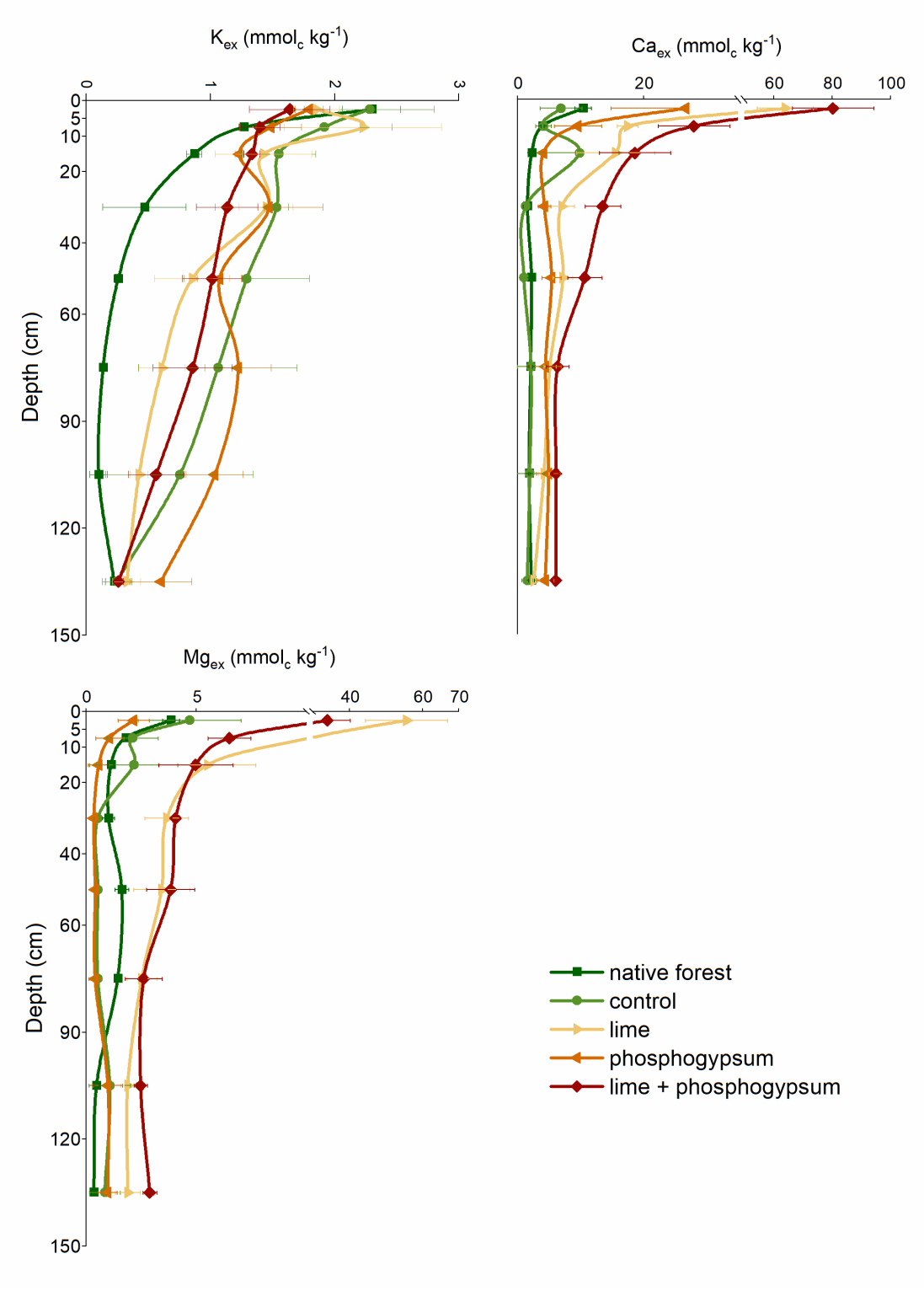

Figure 3. Variation in K, Ca and Mg contents in soil layers as a function of the treatments. Horizontal bars represent the standard deviation of each layer.

The effective cation exchange capacity (ECEC) was greater for treatments that received lime application in the first 10 cm (table 1). As Al^{3+} decreased in these treatments, the

increase in ECEC can be attributed to the input of base cations by fertilization and amendments applications. Conversely, the cation exchange capacity (CEC) was greater in native forest soil than all treatments in the first 20 cm (Table 1). This followed the same trendline of SOM contents, which was greater for native forest soil with a smoother decrease until 20 cm depth. The CEC in acid tropical soils is greatly correlated to SOM contents (Kraus et al., 2004).

Base saturation (BS) was greater for tretaments that received lime application until 60 cm (table 1). Phosphogypsum increased BS only in the uppermost layer when compared to control treatment. Control and native forest soil had similar BS values throughout the whole soil profile. Inversely, the amount of Al^{3+} saturation of ECEC was close to zero in the uppermost layer for limed soil. Phosphogypsum had smaller Al^{3+} saturation on the first 10 cm when compared to control treatment. Liming seems to have an influence in Al^{3+} saturation until 60 cm depth. As Al^{3+} is a toxic Al species to plants and was linked to a reduction on root growth (Parker et al. 1988; da Costa and Crusciol, 2016), the reduction on Al^{3+} saturation in subsurface promoted by lime is important for root development (Carmeis Filho et al., 2017).

The Ca saturation (%Ca) represents the amount of Ca in the ECEC of each soil layer, and its value was greatest close to surface and decreased with increase in depth (Table 1). The %Ca is greater in all soil depths for the treatment lime + phosphogypsum. The rate of phosphogypsum application was based on the formula proposed by Caires and Guimarães (2018) to increase the %Ca of the 20 – 40 cm to 60% of ECEC if lower than 54%, which the authors attributed to be the limiting factor for increasing productivity in soils of subtropical area in Brazil. Caires and Guimarães (2018) used samples collected up to 12 months after phosphogypsum application. For the present study, lime + phosphogypsum application increased %Ca in the 20 – 40 cm to 51% after 24 months. So even treatment lime + phosphogypsum increased %Ca in the 20 – 40 cm layer, the recommended dose was not sufficient to increase the %Ca in this layer to 60% within the analysed period.

3.3.2. Enzyme activity

ß-glucosidase activity decreased with increase in depth for all treatments and native forest soil (Figure 4). Extracellular enzimes, such as B-glucosidase, arylsulfatase and phosphatases, are responsible for cycling nutrients in soil by decomposing SOM and and supplying inorganic nutrients to soil to be latter absorbed by plants. These enzymes are an indicative of heterotrophic microbial activity (Dou et al., 2016).

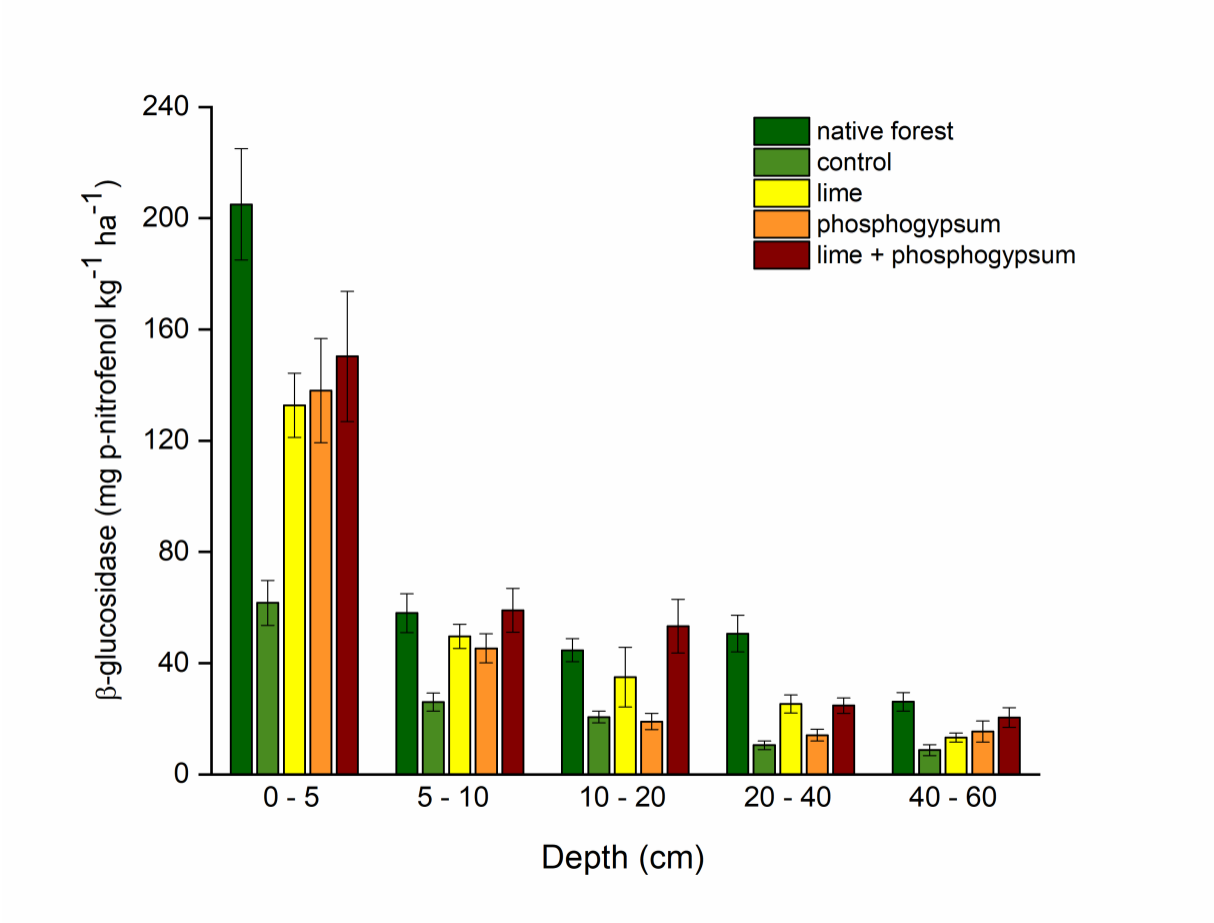

Figure 4. ß-glucosidase activity as a function of the treatments. Vertical bars represent the standard deviation of each layer.

Dou et al. (2016) reported a decrease of 52% in ß-glucosidase activity between 0 – 7.5 and 7.5 to 15 cm layer, and attributted this difference to a natural stratification of nutrients and SOM deposition by crop rotation. In our study, ß-glucosidase activity decreased by 74% in 0 – 5 to 5 – 10 cm layers of native forest soil and agrees with the sharp decrease in SOM between the same layers, as C mineralization is the main source of nutrients in natural ecosystems.

The control treatment had the smallest ß-glucosidase activity in the first 10 cm and presented similar values to phosphogypsum application alone. Both lime and phosphogypsum application affected ß-glucosidase activity, and the combination of both ammendments promoted a slightly increase in the first 20 cm. Inagaki et al. (2016) reported an increase in ß-glucosidase activity in the first 20 cm of an Oxisol when either lime alone or the combination of amendments were applied, with different rates of phosphogypsum. Conversely, the treatment that received phosphogypsym only affected the first 10 cm. Fuentes et al. (2016) attributed the increase in soil respiration and microbial biomass after lime application to the amelioriation of soil chemical attributes, such as raising pH and decrease in Al^{3+} availability.

Arylsulfatase activity had a similar trend to ß-glucosidase and decreased with increasing depth for all treatments and native forest soil (Figure 5). Balota et al. (2014) and Inagaki et al. (2016, 2017) reported a positive relationship between arylsulfatase activity and depth and C dynamics, in special for the labile fractions. Unlike the ß-glucosidase, arysulfatase activity decrease was smoother, stabilizing from 5 to 40 cm depth before drops for all treatments at layer 40 – 60 cm depth.

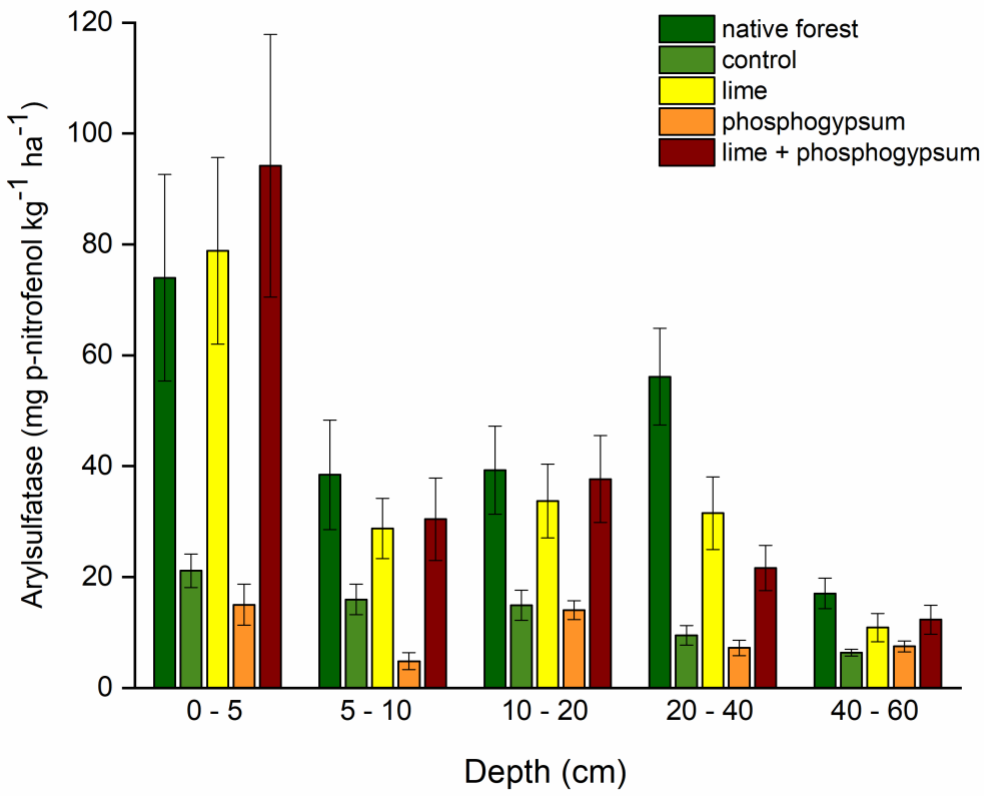

Figure 5. Arylsulfatase activity as a function of the treatments in soil layers. Vertical bars represent the standard deviation of each layer.

Native forest soils had a greater arylsulfatase activity than control in all depths, meaning that this enzyme activity was highly influenced by land use change. Balota et al. (2014) reported a deacrease in arylsulfatase activity when comparing NT system to natural forest area in an Oxisol. As no major soil disturbance event occurred in native forest sites, and has been minimized in NT, these areas usually present greater SOM content, capable of supporting microbial community (Balota et al., 2014). The greater arylsulfatase activity was linked to the vegetation complexity of an area when comparing different land uses in a Cambisol (Sá et al.,

2018). Balota et al. (2014) also reported a greater enzyme activity for NT rather than conventional tillage system and attributed it to mulching and the lack of plowing.

Treatments lime and lime + phosphogypsum had a similar arylsulfatase activity in soil samples from the native forest soil until 20 cm and were greater than control treatment in all depths. Inagaki et al. (2016) reported an increase in arylsulfatase activity in the first 10 cm in an Oxisol after the application of lime in surface and incorporated with different rates of phosphogypsum. The increase in arylsulfatase activity was previously linked to pH incrasse after lime application (Inagaki et al., 2016).

In the present study, phosphogypsum application decreased arylsulfatase activity in the first 10 cm and did not differ from the control in depper layers. A long-term effect of phosphogypsum application increasing arylsulfatase activity was reported by Inagaki et al. (2016), but the authors also found that arylsulfatase activity was highly influenced by soil organic carbon pools, wich also increased with phosphogypsum application when compared to control. Nogueira and Melo (2003) reported that phophogypsum rates ranging from 67 to 1067 kg ha^{-1} did not affect arylsulfatase activity. As phosphogypsum application did not increased pH, as in combination with lime, and increased the amount of available SO_4^{2-} in soil, it probably caused the deacrease arylsulfatase activity in first centimeters of soil. The link between arylsulfatase activity and the amount of available SO_4^{2-} vary a lot in the literature, some authors found a negative correlation (Galstyan and Bozoyan, 1974; Prietzel, 2001) and others found no correlation (Tabatabai and Bremner, 1970; Kowalenko and Low, 1975).

3.4. Conclusions

The land use change from a native forest to NT system had decreased SOM content on the first 20 cm. Lime and phosphogypsum application affected soil chemical attributes. Limed soil samples had an increase in pH until 40 cm depth and also decreased exchangeable Al^{3+} when compared to native forest soil, control and phosphogypsum alone. Exchangeable K contents were affected by amendments application, with lower content in surface, especially in treatments that received phosphogypsum application. Exchangeable Ca was greater in treatments that received either lime or phosphogypsum in the first centimeters of soil, but the combination of amendments was more effective in translocate Ca in the soil profile. Exchangeable Mg decreased with phosphogypsum application, and was smaller than the native forest soil on uppermost layer. ß-glucosidase activity decreased with depth for all treatments and native forest soil and increased with lime and phosphogypsum application. Arylsulfatase activity increased in treatments that received lime and deacresed with application of

phosphogypsum alone when compared to control and were similar to native forest soil down to 20 cm depth.

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4. FINAL REMARKS

Sulfur dynamics in soil was closely related to SOM content in soil. The content of organic S was greater close to the most superficial soil layer, following the same trend of SOM in treatments that did not receive ammendements. The application of phosphogypsum increased the amount of inorganic S in lower depths, but did not change S dynamics in the first 10 cm, showing that lime is either leached or absorbed by plants and immobilized by microrganisms. Enzyme activity also followed the same trend as SOM, being is greater in the most superficial soil layers and decreasing with depth. Arylsulfatase activity was particularly sensitive to ammendments application, especially on the first 5 cm. Lime had a greater impact in arylsulfatase activity than phosphogypsum because pH raised with lime application.