University of Sao Paulo
College of Agriculture "Luiz de Queiroz"

Forms of sulfur in Brazilian Oxisols under no-tillage system

Susana Churka Blum

Thesis presented to obtain the degree of Doctor in Science.
Area: Soils and Plant Nutrition

Piracicaba
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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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Aos meus pais Paulo e Alice,

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The most beautiful experience we can have is the mysterious. It is the fundamental emotion which stands at the cradle of true art and true science.

Albert Einstein (1879 –1955), physicist.

The scientist does not study nature because it is useful; he studies it because he delights in it, and he delights in it because it is beautiful. If nature were not beautiful, it would not be worth knowing, and if nature were not worth knowing, life would not be worth living.

Jules Henri Poincaré (1854-1912), mathematician.

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ABSTRACT

Forms of sulfur in Brazilian Oxisols under no-tillage system

Soil is a critical component of the global sulfur (S) cycle acting as source and a sink for various S species and mediating changes of S oxidation states. However, very little is known about the identities of these specific organic and inorganic S containing molecules, especially in Brazilian soils under no-tillage (NT) system. Thus, the objectives of this study were: (i) to report long and medium term effects of the application of lime and up to 12 t ha^{-1} of phosphogypsum (PG) in samples from two Oxisols collected in field experiments carried out under no-tillage (NT) systems; (ii) to assess the effect of PG application on the amount, form and distribution of organic and inorganic S pools in soils under NT comparing to a natural forest ; (iii) to investigate C, N and S mineralization/immobilization in soil samples affected by plant residues and biochar, thus identifying the major transformations in the S fractions. After 13 years of lime application in a loamy Oxisol, changes in soil acidity and exchangeable Ca^{2+} ad Mg^{2+} supply were visible up to 80 cm depth. The effect of PG providing Ca^{2+} and S-SO_4^{2-} were more pronounced at lower depths. S-SO_4^{2-} distribution in the soil profile was mostly correlated with contents of "free" dithionite extractable Fe (Fe_d) and "amorphous" dithionite extractable Fe minus oxalate extractable Fe (Fe_d-Fe_o). There was no effect of lime and PG in maize grain yield, showing that the plants were unable to benefit of the soil ameliorations. An appreciable movement of sulfate to the subsoil was observed in soil samples from a clayey Oxisol, even in a medium-term (after 3.5 years), despite the fact that the soil had presented high SO_4^{2-} adsorption capacity. In this area, PG had positive benefits in cereal crop yield due to the supply of Ca^{2+} and S-SO_4^{2-}. PG application and changes in land-use practice alters both quantitative (evaluated by the wet-chemical technique) and qualitative (evaluated by means of X-Ray Near Edge Structure - XANES spectroscopy) composition of S functional groups in this wet subtropical Brazilian soil under NT. Compared to the forest site, soils under NT system had lower amounts of organic C, total N and total S Sulfur losses due to cultivation were higher than losses of C and N, and the use of fertilizers containing S seems to be vital for the recovery of S moieties. The dynamics of the plant materials decomposition was mostly governed by the C/S ratio. Sulfur mineralization was driven by the form of S in the starting materials rather than S concentration in the tissue, as revealed by XANES. The incorporation of plant residues and biochar led to an increase in the amount of ester sulfate in the early stage of incubation, which was further mineralized and had a steady decline at the end of evaluation. These findings encourage further investigations of biochar as S source, involving particularly chars produced from different feedstocks and pyrolysis temperatures.

Keywords: Sulphur forms; Sulfate retention; Hydriodic acid digestion/reduction; XANES; Open incubation; Plant residues; Mineralization; Biochar

RESUMO

Formas de enxofre em Latossolos sob sistema plantio direto

O solo é um componente crítico do ciclo global de enxofre (S), agindo como fonte e dreno das várias espécies de S e mediando mudanças nos seus estados de oxidação. No entanto, pouco se sabe sobre as identidades dos compostos orgânicos e inorgânicos de S, especialmente em solos brasileiros sob plantio direto (PD). Assim, os objetivos deste estudo foram: (i) relatar os efeitos a longo e médio prazo da aplicação de calcário e de até 12 t ha^{-1} de fosfogesso (FG) em amostras de dois Latossolos coletadas em experimentos de campo realizados sob PD; (ii) avaliar o efeito da aplicação de PG na quantidade, forma e distribuição das frações orgânicas e inorgânicas de S em solos sob PD em comparação com uma floresta natural; (iii) investigar a mineralização/imobilização de C, N e S em amostras de solo afetadas por fontes de resíduos vegetais e biocarvão, identificando as transformações nas frações de S. Após 13 anos de aplicação de calcário em um Latossolo Vermelho distrófico de textura média, foi verificado efeito na correção da acidez do solo e no fornecimento de Mg^{2+} até 80 cm de profundidade enquanto os efeitos do FG fornecendo Ca^{2+} e $S\text{-}SO_4^{2-}$ foram mais pronunciados em profundidade. A distribuição de $S\text{-}SO_4^{2-}$ no perfil deste solo foi correlacionada com o conteúdo de Fe-ditionito (Fe_d) e Fe-ditionito menos Fe-oxalato (Fe_d, Fe_o). Não houve efeito do calcário e do FG na produção de grãos de milho, mostrando que as plantas não puderam se beneficiar das melhorias no solo. Foi observado apreciável movimento de S para o subsolo em amostras de um Latossolo Vermelho distrófico muito argiloso, mesmo em médio prazo (após 3,5 anos), apesar de o solo apresentar alta capacidade de adsorção de sulfato. Nesta área, o FG teve efeitos positivos no rendimento das culturas de cereais, devido ao fornecimento de Ca^{2+} e $S\text{-}SO_4^{2-}$. A aplicação de FG e as mudanças no uso da terra alteraram tanto a composição quantitativa (avaliada pela técnica de fracionamento químico) quanto qualitativa (avaliada por meio da espectroscopia de raios-X próxima à borda de absorção - XANES) dos grupos funcionais de S em um solo subtropical da região úmida sob PD. Em comparação com o PD, os solos sob floresta apresentaram maior conteúdo de C orgânico, N e S total. As perdas de S devido ao cultivo foram superiores às perdas de C e N, e o uso de fertilizantes sulfatados parece ser vital para a recuperação do S do solo. A dinâmica de decomposição dos materiais vegetais foi principalmente regida pela razão C/S. A mineralização de enxofre foi impulsionada pela forma de S nos materiais iniciais, e não pela concentração S no tecido, conforme revelado por XANES. A incorporação de resíduos vegetais e biocarvão levou ao aumento nos ésteres de sulfato na fase inicial de incubação, fração posteriormente mineralizada, apresentando declínio no final da avaliação. Estas descobertas incentivam mais investigações do biocarvão como fonte de S, envolvendo carvões produzidos a partir de diferentes matérias-primas e temperaturas de pirólise.

Palavras-chave: Formas de enxofre; Retenção de sulfato; Digestão/redução por ácido Iodídrico; XANES; Incubação aberta; Resíduos vegetais; Mineralização; Biocarvão

1 INTRODUCTION

Sulfur (S) is ranked as the 13th most abundant element in the Earth's crust (TABATABAI, 2005). It is essential for all forms of life since it plays a critical role in the synthesis of several compounds like amino acids, chlorophyll and enzyme cofactors and its cycling provides a key to understanding changes in the biosphere (KERTESZ; MIRLEAU, 2004; FLEET 2005). Sulfur is ubiquitous and it presents a large variety of species and valence states from -2 (in sulfides) to +6 (in sulfates) (JALILEHVAND, 2006). This provides S the ability to form multiple S-S linkages and to exist in a variety of organic and inorganic forms in terrestrial ecosystems, making its pathways highly complex and intricately intertwined with other elements (MARTÍNEZ et al., 2002; SOLOMON et al., 2011).

The soil environment is a critical component of the global S cycle, acting as a source and a sink for various S species and mediating changes of oxidation states (WANG et al., 2006). In the tropical and temperate soils, S is present in organic and inorganic forms. The organic form is predominant, in general accounting for more than 90% of the total S (NEPTUNE; TABATABAI; HANWAY, 1975; SOLOMON et al., 2001; WANG et al., 2006; ERIKSEN et al., 2009) in surface horizons. In humid tropical soils, however, the amount of total sulfur is frequently lower than in temperate systems (NEPTUNE; TABATABAI; HANWAY, 1975) due to higher rates of decomposition caused by weathering. Inorganic sulfate generally makes up less than 10% of the total S present in surface horizons (KERTESZ; MIRLEAU, 2004). It is, however, the most important form of sulfur in the plants point of view.

Crop plants have become increasingly dependent on the soil to supply the sulfur that they need since recently many actions have been taken concerning the reduction in atmospheric deposition of sulfur (KERTESZ; MIRLEAU, 2004; LEHMANN et al., 2008). However, decreases in sulfur inputs in the soil system (by the use of concentrate fertilizers, without S in its constitution) and increases in sulfur outputs (losses by erosion or leaching and S removal by intensive cropping systems and crops with high exigency in nutrient supplies) have depleted the level of S in soils, increasing S deficiencies all around the world (SCHERER, 2001; 2009). Large areas of the globe present S deficient soils, specially the most weathered ones, as Oxisols and Ultisols (ALVAREZ V. et al., 2007). These are the predominant soil orders in Brazil, representing almost 60% of the Brazilian territory (COELHO et al., 2002). Based on this, we can infer that most agricultural areas in Brazil are in need of S fertilization. However, little information is available regarding the speciation and dynamics of the various organic S functionalities in Brazilian soils (NEPTUNE;

TABATABAI; HANWAI, 1975; LILIENFEN et al., 2001; LEHMANN et al., 2001; DE BONA; MONTEIRO, 2010).

Sulfur fertilization can increase crop yields and quality and result in significant economic returns to growers. Positive responses to S application have been recently shown (CHEN; KOST; DICK, 2008; CAIRES et al., 2011; ERCOLI et al., 2011; YIN et al., 2011), further emphasizing the role of sulfur fertilization in crop production. Many fertilizers can be used as source of sulfur to the crops, as elemental S, ammonium sulfate, single superphosphate (SSP), potassium sulfate and calcium sulfate (in the form of gypsum and phosphogypsum). The use of gypsum as a soil conditioner, improving the root environment in the subsurface, reinforces the importance of better understanding the knowledge of S; this knowledge is not only related to the plant nutrition, but it is related to the sulfate functioning (ALVAREZ V. et al., 2007), because it carries cations to the lower depths, where the enrichment in Ca and the decrease in Al toxicity leads to amelioration of the subsurface soil layers. As a consequence, roots have a greater exploitation of soil and absorb greater amounts of water and nutrients (TOMA et al., 1999; FARINA; CHANNON; THIBAUD, 2000a; b).

Sulfate rarely remains in surface soil layer. So, it is important to evaluate deeper soil layers for S diagnosis. Liming, accumulation of organic matter (OM) and the use of P fertilizers contribute for S leaching to the lower depths by the increase in net negative charge of the soil, causing SO_4^{2-} repulsion and reducing SO_4^{2-} adsorption in iron and aluminum oxides (COUTO; LATHWELL; BOULDIN, 1979). However, lower amounts of OM combined with higher amounts of iron/aluminum oxides can result in net positive charge and S retention in the subsurface layer. Despite almost 90% of the plant roots are concentrated in the topsoil, absorbing most part of water and nutrients, soil subsurface layers can represent a volume of soil insufficiently explored, especially in periods of low rainfall.

Changes in management practices imposed on natural systems (as extensive deforestation) and on agricultural lands (as fertilizer application and forms of soil tillage) play a big role in the soil environment, influencing the microbial community structure and functioning and consequently affecting S dynamics in short- and long-term. Deforestation of natural forests and subsequent conversion into low-input agriculture has caused extensive soil degradation and thereby a decline in agricultural land productivity (SOLOMON et al., 2001). Conservation agriculture as no-till (NT) system has emerged from the necessity of meeting future food demands, while also contributing to sustainable agriculture and rural development (HUGGINS; REGANOLD, 2008). No-till is a very effective measurement of erosion control

and improves water and fertilizer use efficiency so that many crops present higher yields under NT than under tilled systems (TRIPLETT; DICK, 2008).

The adoption of NT in Brazil has been remarkable in the last few years, representing more than 60% of the Brazilian grain production and bringing the country as one of the leading in the world in terms of NT adoption (DERPSCH; FRIEDRICH, 2009). NT system, which preserves plant residues on the soil surface, can supply sulfur to the subsequent crop due to straw mineralization. The knowledge of S behavior in soils, especially in areas under NT, is very helpful to evaluate the availability of the element to plants as well as its efficient management in agricultural systems.

Organic matter and plant residues are major sources of plant-available sulfur in the soil (DICK; KOST; CHEN, 2008). Despite plant sulfur nutrition is primarily dependent on the uptake of inorganic sulfate, researches have demonstrated that the sulfate-ester and sulfonate pools of soil sulfur are also plant bioavailable, probably due to interconversion of carbon bounded sulfur and sulfate ester sulfur by soil microbes (FRENEY; MELVILLE; WILLIAMS, 1975; KERTESZ; MIRLEAU, 2004; DE BONA; MONTEIRO, 2010).

Although it is well known that S in soils is present in organic and inorganic forms, very little is known about the identities of these specific organic S containing molecules (KERTESZ; MIRLEAU, 2004; TABATABAI, 2005). Knowledge of the speciation of S in soils can provide a clear understanding of the amount, form and biochemical transformations of S in the ecosystem (SOLOMON; LEHMANN; MARTINEZ, 2003). Our knowledge of the biochemical S cycling is, however, severely limited by crude analytical techniques used in S measurement and speciation (SOLOMON; LEHMANN; MARTINEZ, 2003; SCHROTH et al., 2007). Most of the studies on S fractionation were based in destructive methods of extraction through preferential reduction of S phases using the principles of the classical method suggested initially by Johnson and Nishita (1952), which are indirect in their characterization and thus limited in accuracy and detail of S species measurements (PRIETZEL et al., 2003; SOLOMON; LEHMANN; MARTINEZ, 2003; SCHROTH et al., 2007).

Synchrotron techniques provide an excellent option to overcome the impasse created by this lack of accurate and meaningful methods in the speciation of several elements in soils (LOMBI; SUSINI, 2009). Recently, studies using noninvasive synchrotron-based sulfur K-edge X-ray Near-edge Structure (XANES) spectroscopy has been successfully used to distinguish and measure multiple S fractions in coals (SPIRO et al., 1984), marine sediments (VAIRAVAMURTHY et al., 1997), humic substances (XIA et al., 1998; SOLOMON et al.,

2003; 2005; 2009), organic matter (SCHROTH et al., 2007) and bulk soils (PRIETZEL et al., 2003; 2007). XANES provides specific information on the functional groups containing sulfur because of the sensitivity to the electronic structure, oxidation state, and the geometry of the neighboring atoms (VAIRAVAMURTHY et al., 1997). The advantage XANES affords over traditional wet-chemical methods is that intermediate oxidation states can be identified. The two combined techniques (wet-chemical fractionation and XANES) can provide valuable information regarding the S dynamics in Brazilian soils under NT. This understanding is vital to effectively describe S fluxes from various pools of Brazilian soils, to design and promote sustainable agricultural systems in the country and also to comprehend the transfers of S between the soil environment and other reservoirs during the biogeochemical S cycle in the tropical agro-ecosystems. Thus, the objectives of this study were:

- to report long and medium term effects of phosphogypsum (PG) application in two field trials conducted in NT system that received the application of up to 12 t ha^{-1} of PG and the factors related to S permanence in these soils;
- to assess the impacts of land use changes comparing undisturbed native forest and NT systems and to assess the role of PG application in NT systems on the amount, form and distribution of organic and inorganic S pools in soils in NT-based agricultural systems of Brazil using both degradative wet-chemical and solid-state X-Ray Near-Edge Structure spectroscopy (XANES) techniques;
- to investigate C, N and S mineralization/immobilization in NT affected by sources of plant residue and to evaluate S species dynamics during decomposition identifying the major transformations in the S fractions.

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2 LONG AND MEDIUM TERM EFFECTS OF LIME AND PHOSPHOGYPSUM APPLICATION AND SULFATE RETENTION IN SUBTROPICAL SOILS UNDER NO-TILL

Abstract

In highly weathered tropical soils, lime and phosphogypsum (PG) are applied as soil amendments to alleviate aluminum toxicity and improve root environment, increasing the efficiency of applied nutrients and benefiting crop growth and yield. We still face a lack of information concerning long term PG effects, acidity conditions and ion leaching in subsurface layers. This study reports long and medium term effects of the application of up to 12 t ha^{-1} of PG in two field trials conducted under no-till systems. One was located in Ponta Grossa, state of Paraná (PR), Brazil, in a loamy Oxisol, which received lime and PG applications in 1993. Another area, located in Guarapuava (PR), in a clayey Oxisol, received PG in 2005. Some soil chemical attributes were evaluated: pH (0.01 M $CaCl_2$), Δ pH (pH_{KCl} - pH_{H2O}), Al^{3+}, Ca^{2+} and Mg^{+} exchangeable contents extracted by 1 M KCl, and SO_4^{2-}-S content extracted by 0.01 M calcium phosphate up to 200 cm depth, the factors related to S permanence in these soils [organic C, dithionite extractable Fe (Fe_d), oxalate extractable Fe (Fe_o), oxalate extractable Al (Al_o), pH_{NaF}, remaining P (Prem) and remaining S (Srem)] and the effect of PG on grain yield of cereal crops [maize (*Zea mays*), wheat (*Triticum aestivum*) and triticale (x *Triticosecale*)]. After 13 years of lime and PG applications in the loamy Oxisol we still observed effects along the soil profile. Lime effects, as reducing the soil acidity and providing exchangeable Ca^{2+} ad Mg^{2+}, were visible up to 80 cm depth. The effect of PG providing Ca^{2+} and S-SO_4^{2-}, were more pronounced at lower depths. S-SO_4^{2-} distribution in the soil profile was mostly correlated with Fe_d and Fe_d-Fe_o. Despite the lack of rain occurred in December of 2008, we did not observe differences in the maize grain yield, showing that the plants were unable to benefit of the subsoil ameliorations provided by PG application. An appreciable movement of S to the subsoil was observed in soil samples from the clayey Oxisol in Guarapuava, even in a medium-term (after 3.5 years), despite the fact that the soil had presented higher SO_4^{2-} adsorption capacity. Surface-applied PG had positive benefits in cereal crop yield (maize and triticale) due to the supply of Ca^{2+} and S-SO_4^{2-}.

Keywords: No-tillage system; Oxisol; Sulfur; Soil acidity; Sulfate adsorption; Weathered soil; Subsoil acidity; Cereal crops

2.1 Introduction

Acidity amelioration is one of the most important factors in the management of highly weathered soils for adequate crop growth. Approximately 70% of the Brazilian cultivated soils present high levels of acidity, often presenting toxic levels of aluminum (Al^{3+}) and manganese (Mn^{2+}) and low availability of exchangeable bases (RITCHEY; SILVA; COSTA, 1982). Hence, the application of soil amendments is a primary strategy to achieve satisfactory yields in Brazilian systems.

In such weathered tropical soils, lime and phosphogypsum (PG) are applied as soil amendments to alleviate Al toxicity and improve the root environment, increasing the efficiency of applied nutrients and benefiting crop growth and yield (TOMA et al., 1999;

FARINA; CHANNON; THIBAUD, 2000a; CAIRES; JORIS; CHURKA, 2011). Under no-till (NT) systems, soil acidity is alleviated by surface applied lime, since crop growers decide do not disturb the first layers of the soil, keeping soil aggregation beyond another benefits brought throughout NT. Nevertheless, in many cases, the amelioration of soil acidity by lime application may be restricted to the first soil layers (0 to 10 cm) due to soil conditions and the low mobility of the products of lime reaction. Subsoil acidity, however, is also one of the major yield-limiting factors in acid soils (TOMA et al., 1999), since it restricts root growth, decreasing the exploitation of water and nutrients. Improving soil layers below the topsoil can be benefic for grain yield (RITCHEY; SOUZA; COSTA et al., 1980), mainly under water deficient situations.

When surface-applied, phosphogypsum ($CaSO_4.2H_2O$) is an alternative to provide Ca to crops and to decrease Al toxicity in soil subsurface layers (OATES; CALDWELL, 1985) due to its relative higher solubility (0.24g/100g H_2O) when compared to lime. It is also a source of sulfur and provides long-term residual effect when applied in large amounts, mainly in clayey soils (TOMA et al., 1999; CAIRES; JORIS; CHURKA, 2011; BLUM et al., 2011).

The current recommendation of PG requirements in most of Brazilian agricultural soils is based on the clay content. Because of the high operational costs of gypsum application (TOMA et al., 1999), high rates are applied in the first year in order to promote long term effects, especially in clayey soils. However, sulfate and cation losses should be accounted in the gypsum recommendation (ALVES; LAVORENTI, 2004b), since there are many other factors governing sulfate adsorption and leaching as (i) soil pH (HUE; ADAMS; EVANS, 1985; ZHANG; YU, 1997), (ii) presence of other anions as phosphate (BOLAN et al., 1988), (iii) presence of another cations in the soil solution (CHAO; HARWARD; FANG, 1963; CICHOTA et al., 2007) and (iv) organic matter content (ENSMINGER, 1954; COUTO; LATHWELL; BOULDIN, 1979). As the soil surface layer presents unfavorable conditions for sulfate adsorption due to additions of phosphate fertilizers, lime application and the adoption of practices that increase the soil organic matter content as NT system, it is important to evaluate the subsoil conditions to measure SO_4^{2-} and cation leaching mainly when high amounts of gypsum are applied aiming long term effects.

Researches involving PG in agriculture were intensified in the 70’s and 80’s, coupled with the concern about phosphoric acid industry residues. In those decades many benefits of gypsum application were brought up, as the increase in exchangeable Ca, as the reduction of exchangeable Al, together with deep rooting in the subsoil (REEVE; SUMNER, 1972; RITCHEY; SOUZA; COSTA, 1980; SUMNER et al., 1986). Currently, many field studies

report the effects of gypsum application (ZAMBROSI; ALLEONI; CAIRES, 2007; SORATTO; CRUSCIOL, 2008; CAIRES; JORIS; CHURKA, 2011; CAIRES et al., 2011a; b), but the depth of sampling is commonly restricted to 60-80 cm, since 90% of the roots of the main crops are concentrated up to this layer. However, we still face a lack of information concerning long term PG effects in soil acidity conditions and ion leaching in subsurface layers. This study reports long and medium term effects of PG application in two field trials conducted under NT system that received up to 12 t ha^{-1} of phosphogypsum. Soil chemical attributes were evaluated up to 200 cm depth, the factors related to S permanence in these soils [organic C, dithionite extractable Fe (Fe_d), oxalate extractable Fe (Fe_o), oxalate extractable Al (Al_o), pH_{NaF}, remaining P (P-rem) and remaining S (Srem)] and the effect of PG on the grain yield of cereal crops (maize - *Zea mays*, wheat - *Triticum aestivum* or triticale - *x Triticosecale*).

2.2 Material and Methods

2.2.1 Site characterization

The experiments were performed in two Brazilian locations, in the State of Parana: (i) Ponta Grossa (25º10'S, 50º18W, 794 m) on a loamy Typic Hapludox, and Guarapuava (25°17'S e 51°48'W, 997 m) on a clayey Typic Hapludox. The state of Parana is the leader in the production of cereals, legumes and oilseeds in Brazil harvesting 64.1 millions of tons in 2010, what represented 21.6% of the national production (BRAZIL, 2011). These Oxisols are representative of the region since they are widely distributed in this area. Table 1 presents the results on chemical and particle size distribution analyses (0-20 cm) and some mineralogical attributes for the diagnostic soil horizon (around 120 cm depth). Iron (Fe) and Al were extracted by a 9 M H_2SO_4 solution, and Si was extracted with NaOH 30% (v/v) solution. Contents of Fe and Al were determined by atomic absorption spectrophotometry (AAS), and Si was quantified by gravimetry (CAMARGO et al., 1986). It was assumed that Fe, Al, Mn and Ti contents associated with secondary minerals are solubilized by a 9 M H_2SO_4 solution, while Si is solubilized by a NaOH 30% (v/v) solution. Taking these values into account, the weathering indexes Ki $(SiO_2/60)/(Al_2O_3/102)$ and Kr $(SiO_2/60)/[(Al_2O_3/102)+(Fe_2O_3/160)]$ were calculated. Mineralogical analysis of the clay fraction, after treatment with Na-CBD (citrate-bicarbonate-dithionite), was conducted using X-ray analysis of oriented mounts on glass slides. Samples were saturated with either Mg or K, solvated with glycerol, heated to 350 and 550 ºC and then X-rayed. X-ray diffractograms were obtained in a diffractometer (Philips X'pert System) operating at 40 kV and 40mA using Ni-filtered Cu-Kα radiation, at

1.2 ° 2 θ min-1 in the 3 to 45° 2 θ range. We also estimate the amounts of the minerals kaolinite, gibbsite, goethite and hematite according to Kämpf; Schwertmann (1983).

Table 1 - Soil attributes for different depths, before the establishment of the experiments

Attribute	Typic Hapludox (Ponta Grossa, Parana)	Typic Hapludox (Guarapuava, Parana)
	---------- **Surface layer (0-20 cm)**----------	
pH (0.01 M $CaCl_2$)	4.5	5.9
[1]Organic Carbon (g dm^{-3})	19	28
[2]Exchangeable cations ($mmol_{(+)}$ dm^3)		
Ca^{2+}	16	48
Mg^{2+}	10	22
K^+	1.4	4.3
Al^{3+}	6	0
Cation Exchange Capacity pH 7.0 (CEC) ($mmol_{(+)}$ dm^3)	85.4	119
P, Melich-1 (mg dm^{-3})	8.9	4.7
S-SO_4^{2-}, 0.01 M $Ca(H_2PO_4)_2$ (mg dm^{-3})	19.1	5.9
Soil Texture	Loamy	Clayey
	----------**Subsurface layer (≅120 cm)**----------	
Organic Carbon (g dm^{-3})	8.1	10.2
SiO_2 (g kg^{-1})	38	101
Al_2O_3	144.4	294.3
Fe_2O_3	53.7	175
MnO	0.05	15.6
TiO_2	2.1	482.4
Ki (SiO_2/60)/(Al_2O_3/102)	0.45	0.58
Kr (SiO_2/60)/[(Al_2O_3/102)+(Fe_2O_3/160)]	0.36	0.43
Mineralogy (relative distribution)	Gb>Kt>Hm=Gt	Gb>Kt>Gt>Hm
Kaolinite (%)	12.9	25.2
Gibbsite (%)	26.8	37.1
Hematite (%)	2.2	7.4
Goethite (%)	2.9	9.9

[1] Walkley-Black
[2] Ca, Mg e Al extracted by 1 M KCl and K extracted by Mehlich-1.
[3] Kt (kaolinite), Gb (gibbsite), Hm (hematite), Gt (goethite)

Both soils are highly weathered, but the Typic Hapludox from Guarapuava presented higher amounts of organic carbon, higher degree of fertility and had no exchangeable Al. However, the soil had narrow Ca/Mg ratio (CAIRES et al., 2004) and low contents of sulfate. The Typic Hapludox from Ponta Grossa was highly acidic, with toxic amounts of Al^{3+} and low amounts of exchangeable bases. Both soils had the predominance of gibbsite in the clay fraction.

2.2.2 Experimental design, treatments and cropping studies

Before the establishment of the experiment, both field sites had been cultivated with grain crops under NT during 15 yr. The experiment in Ponta Grossa was established in 1993 on a complete block design with three replications. Plots of 50.4 m^2 (6.3 x 8 m), received lime

and PG on 0 and 0 t ha^{-1}; 0 plus 12 t ha^{-1}, 6 plus 0 t ha^{-1} and 6 plus 12 t ha^{-1}, respectively, at the beginning of the experiment in 1993. Lime rate was calculated to increase the base saturation in topsoil (0-20 cm) to 70%, as shown by CAIRES et al. (2005) and the PG rate was calculated to achieve the same equivalent in calcium content of the lime treatments. The experiment in Guarapuava was established in 2005 with gypsum application from 0, 4, 8, and 12 t ha^{-1} with four replications. Plot size was 49.0 m^2 (7.0 x 7.0 m) and PG rates were calculated to raise the exchangeable calcium/magnesium (Ca/Mg) ratio of the topsoil (0-20cm), at values between 4 and 8 according to data from previous studies (CAIRES et al., 1999, 2004). More details concerning the experiment site in Guarapuava can be found in Caires et al. (2011b).

In Ponta Grossa, maize (*Zea mays*) was sown on September 25th 2008 (15 years after lime and PG application) using the hybrid Pioneer 30F53. Grain was harvested from a 10.8 m^2 plot. In July 2009 wheat (*Triticum aestivum*) (cv. Supera) was sown in a density of 175 kg ha^{-1} and row spacing of 22 cm. The harvest of the crop was in November 2009, from a 4 m^2 plot. Fertilizer rates varied with crops and years (Table 2), according to soil test recommendations for the State of Parana. Nitrogen, P, and K were applied, most times, as urea, triple superphosphate, and potassium chloride, respectively.

In Guarapuava, maize was sown in September 2008 (Pioneer 30R50 hybrid), using 74,480 plants per hectare. The harvest was performed in April 2009, from a 8.4 m^2 plot. In the winter of 2009, triticale (x *Triticosecale*) was grown (cv. IPR 111), seeded at a density of 240 kg ha^{-1}. The harvest also happened in November, from a 4 m^2 plot. Crop grain yields were corrected to 130 g kg^{-1} moisture content and the plant residues were left on the soil surface following grain harvest. In both areas we decided to evaluate the yield of cereal plants (maize, wheat or triticale), which have presented higher response to gypsum application than legume plants, as soybean or bean (RAIJ et al., 1998; TOMA et al., 1999; CAIRES et al., 1999; 2004; 2011a;b; FARINA; CHANNON; THIBAUD., 2000a; RHEINHEIMER et al., 2005; SORATTO; CRUSCIOL, 2008; CAIRES; JORIS; CHURKA, 2011).

Seasonal rainfall data for the two areas, during the development of the crops are presented in Figure 1. A lower rainfall rate was registered in December of 2008, only in Ponta Grossa, during the maize flowering. Comparing the both areas, Guarapuava presented higher rainfall per year (average of 1,922 mm) than Ponta Grossa (average of 1,554 mm).

Table 2 - Cropping history and amounts of N, P, and K applied since the establishment of the experiments

Agricultural year	Crop	N	P	K
			kg ha^{-1}	
		----------Ponta Grossa----------		
1993-1994	soybean	*0*	*22*	*42*
1994-1995	maize	*65*	*30*	*33*
1995-1996	soybean	*0*	*22*	*42*
1996	wheat	*58*	*27*	*52*
1996-1997	soybean	*0*	*0*	*40*
1997	triticale	*60*	*32*	*62*
1997-1998	soybean	*0*	*0*	*40*
1998-1999	soybean	*0*	*28*	*54*
1999-2000	soybean	*0*	*13*	*25*
2000-2001	maize	*132*	*28*	*82*
2001-2002	soybean	*5*	*20*	*36*
2002-2003	soybean	*0*	*23*	*46*
2003	wheat	*80*	*0*	*33*
2003-2004	soybean	*0*	*26*	*50*
2004-2005	maize	*120*	*40*	*55*
2005-2006	soybean	*6*	*36*	*50*
2006-2007	soybean	*0*	*22*	*42*
2007-2008	soybean	*0*	*26*	*42*
2008-2009	maize	*134*	*36*	*89*
2009	wheat	*80*	*0*	*33*
		----------Guarapuava----------		
2005-2006	maize	*195*	*39*	*62*
2006-2007	soybean	*0*	*0*	*0*
2007-2008	soybean	*5*	*22*	*42*
2008-2009	maize	*204*	*45*	*94*
2009	triticale	*66*	*24*	*60*

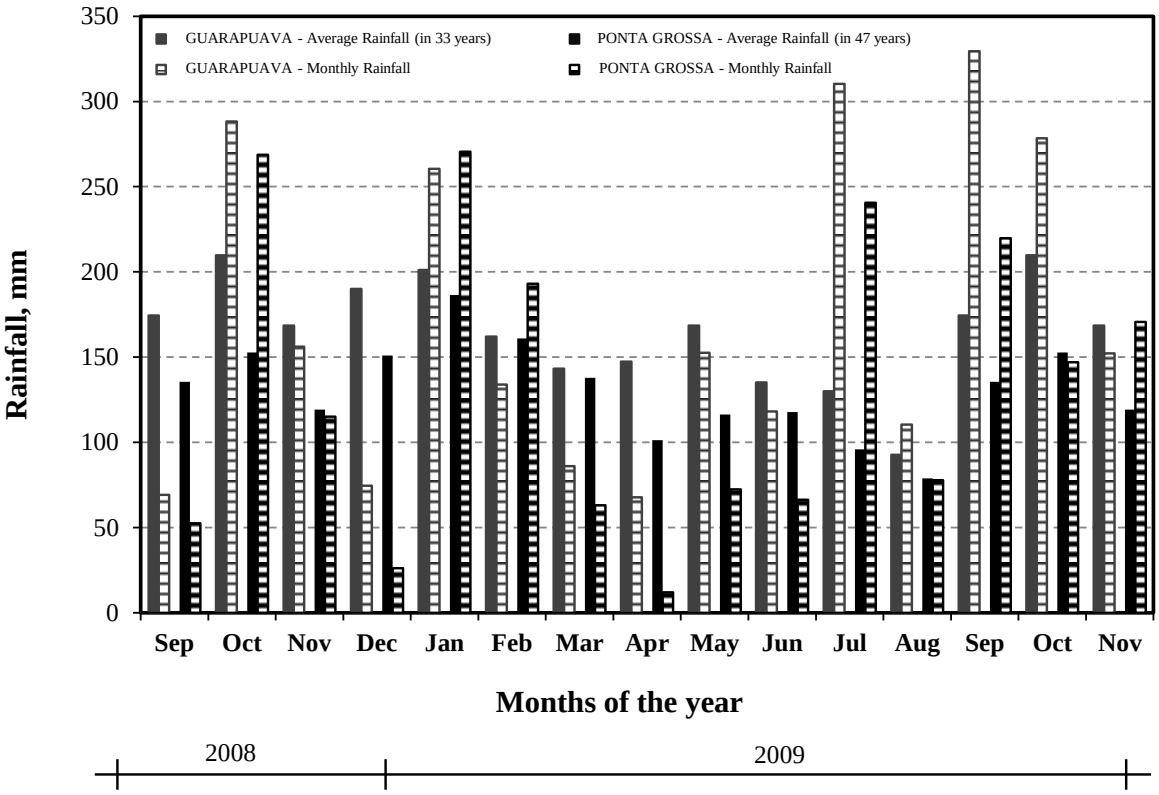

Figure 1 - Monthly rainfall registered during the experiments and average monthly rainfall recorded for Ponta Grossa (47 years) and Guarapuava (33 years)

2.2.3 Soil sampling and chemical analyses

Soil samples were collected in Ponta Grossa in 2007, 13 yr after gypsum application. In Guarapuava samples were collected in 2009, 3.5 yr after gypsum application. Twelve soil core samples per plot were taken with a soil probe sampler to obtain a composite sample of the 0-5, 5-10, and 10-20 cm depths, and five cores of the 20-40 up to 180-200 cm depths. Samples were dried (40ºC), ground and sieved to pass a 2 mm sieve and stored in permeable plastic bags.

To evaluate the chemical alterations after long and medium term of the treatments in the both areas were determined the soil pH (using a 0.01 M $CaCl_2$ suspension and a 1:2.5 v/v, soil/solution); Δ pH (calculated by subtracting the pH measured in water from the one measured in 1 M KCl) according to Mekaru and Uehara (1972); exchangeable Al^{3+} (extracted by 1 M KCl solution and determined by titration with 0.025 M NaOH solution); Ca^{2+} and Mg^{2+} (also extracted by 1 M KCl solution and determined by atomic absorption spectroscopy). Soil S-SO_4^{2-} contents were extracted with 0.01 M calcium phosphate (FOX; OLSON; RHOADES, 1964) in a 1:2.5 (v/v) soil/solution ratio. The suspension (soil plus extracting solution) was shaken by 30 min, filtered and determined by turbidimetry. The calcium phosphate solution was first suggested by Fox et al. (1964) and it contains sufficient P to displace most of the adsorbed sulfate in weathered soils. In selected layers of the control plots (that did not receive lime or PG application) we built adsorption isotherms by shaking 2.5 g of soil for 24 h with 25 ml of $CaCl_2$ solutions containing SO_4^{2-} added as K_2SO_4. After shaking, the suspensions were filtered through a Whatman nº 42 filter paper, and the solutions were analyzed for sulfate by $BaSO_4$ turbidimetry. The amount of sulfate adsorbed was calculated from the difference between the initial and final sulfate concentrations. The results were fitted to the Langmuir and Freundlich isotherms, widely used for this purpose.

The Langmuir equation (1) is given by:

$$q = \frac{k\,c\,b}{1 + kc} \qquad (1)$$

Where q = adsorbed sulfate (mg kg^{-1}); k = parameter related to affinity of the soil by sulfate (L g^{-1}); c = equilibrium concentration of sulfate (mg L^{-1}); b = maximum capacity of sulfate adsorption (mg kg^{-1}).

The Freundlich equation (2) is given by:

$$q = kc^{1/n} \qquad (2)$$

Where q = adsorbed sulfate (mg kg^{-1}); k = constant ($mg^{(1-1/n)}$ kg^{-1} $L^{(1/n)}$; L g^{-1}); c = equilibrium concentration of sulfate (mg L^{-1}) and n = constant, dimensionless.

Other chemical attributes were obtained: organic carbon (Walkley-Black) and clay content using the densimeter method (GEE; OR, 2002). “Free” iron oxides (Fe_d) were extracted using Na-CBD solution according to Mehra and Jackson (1960). Oxalate-extractable Fe and Al (representing the poorly crystalline oxides) were determined according to Loeppert and Inskeep (1996). Soil was equilibrated with 0.3 M ammonium oxalate at pH 3.25 on an end-over-end shaker. After centrifugation, the concentration of Fe and A1 in the equilibrium solution was measured by atomic absorption spectrophotometry. These forms of Fe and A1 have been described as reactive or amorphous and are considered to exert a major influence on the P sorption capacity of many soils (SINGH; GILKES, 1991). Additionally the pH_{NaF} (shaking 0.5 g of soil for 1 h with 20 ml of 1 M NaF) was determined according to BOLLAND et al. (1996). This index was proposed by Bolland et al. (1996) as a quick and economical procedure to access the P sorption capacity of soils in acid soils from Australia. Thus, the increase in pH of the extracts is an indirect measure of the P sorption capacity of soils (SINGH; GILKES, 1991). The same index was also used by Alves and Lavorenti (2004 a, b) and provided a suitable estimation of sulfate adsorption capacity for weathered subsoils. Remaining P (P-rem) was obtained according to Alvarez et al. (2000), and remaining S (S-rem) according to Alvarez et al. (1983). The indexes Prem and Srem also can be used to predict S adsorption capacity, comparing to the pH_{NaF}.. The determination of S-rem consisted in shaking soil samples with 0.01 M $CaCl_2$ containing 20 mg L^{-1} of S in the proportion 1:10 (soil/solution). Samples were centrifuged, filtered and the suspensions were quantified by turbidimetry.

2.2.4 Statistical analyses

The effects of soil amendments (in both experiments) on soil chemical attributes and on crop grain yields were analyzed by analysis of variance using a randomized complete block design. Means were compared using the Tukey test ($p \leq 0.05$) for the experiment conducted in Ponta Grossa and using polynomial regression for the experiment conducted in Guarapuava. We used Pearson product moment to correlate soil properties and sulfate retained in the profile. Also correlations coefficients were used to explain increases in yield for the Guarapuava experiment.

2.3 Results and Discussion

2.3.1 Soil chemical attributes - 13 years after lime and phosphogypsum application on a loamy Oxisol under no-till

When the soil sampling was performed, after surface application of lime and phosphogypsum, the soil had high acidity and low pH values in all depths evaluated (Figure 2). Higher pH values were observed from 0-80 cm on the treatments that received lime application, either with or without PG.

The effects of surface-applied lime in alleviating soil acidity bellow the point of placement are related in many field studies under NT (CAIRES et al., 2005; 2008; CAIRES; JORIS; CHURKA, 2011; SORATTO; CRUSCIOL, 2008) and its occurrence has been attributed to the benefits brought with the adoption of NT system, as the use of plant residues left on the surface (MIYAZAWA; PAVAN; FRANCHINI, 2002; FRANCHINI et al., 2003) and the improvement in soil aggregation and aggregate stability (SIX et al., 1999) letting fine particles of applied lime to move into and down the profile along with water infiltration to alleviate subsoil acidity (AMARAL et al., 2004). Regardless the mechanism involved, surface lime application is, in many cases, as effective as incorporated lime in reducing subsoil acidity in a variable charge soil (CAIRES; JORIS; CHURKA, 2011).

The main effects of the treatments on the acidity alleviation were observed up to 80 cm depth, and the soil pH of the plots that did not receive any amendment (control) was higher on the subsoil, compared with the surface. It can be explained by the acidification caused by the use of high rates of N fertilizers (Table 2). Soils of the humid tropics used for grain cropping under NT have become more acidic because of the use of increased rates of ammonium-based N fertilizers to obtain more intensive production of cereal crops, like wheat and maize (JUO; DABIRI; FRANZLUEBBERS, 1995; CAIRES et al., 2005; GARBUIO et al., 2011). On subsurface soil samples (120-140 cm), slightly increases on pH were observed in the plots that received PG. This increase can be attributed to the adsorption of sulfate on the surface of Fe and Al oxides, displacing OH^- and thus promoting partial neutralization of soil acidity (REEVE; SUMNER, 1972), what is called “self-liming effect”, also reported by Toma et al. (1999) and Farina; Channon; Thibaud (2000b).

ΔpH (Figure 2) can be used to predict net soil charge (MEKARU; UEHARA, 1972), and was affected by lime and PG application. Up to 80 cm depth the soil profile had a negative ΔpH, since management strategies employed in the area - like liming, fertilizer application, and conservation agriculture (preserving crop residues on the soil surface and

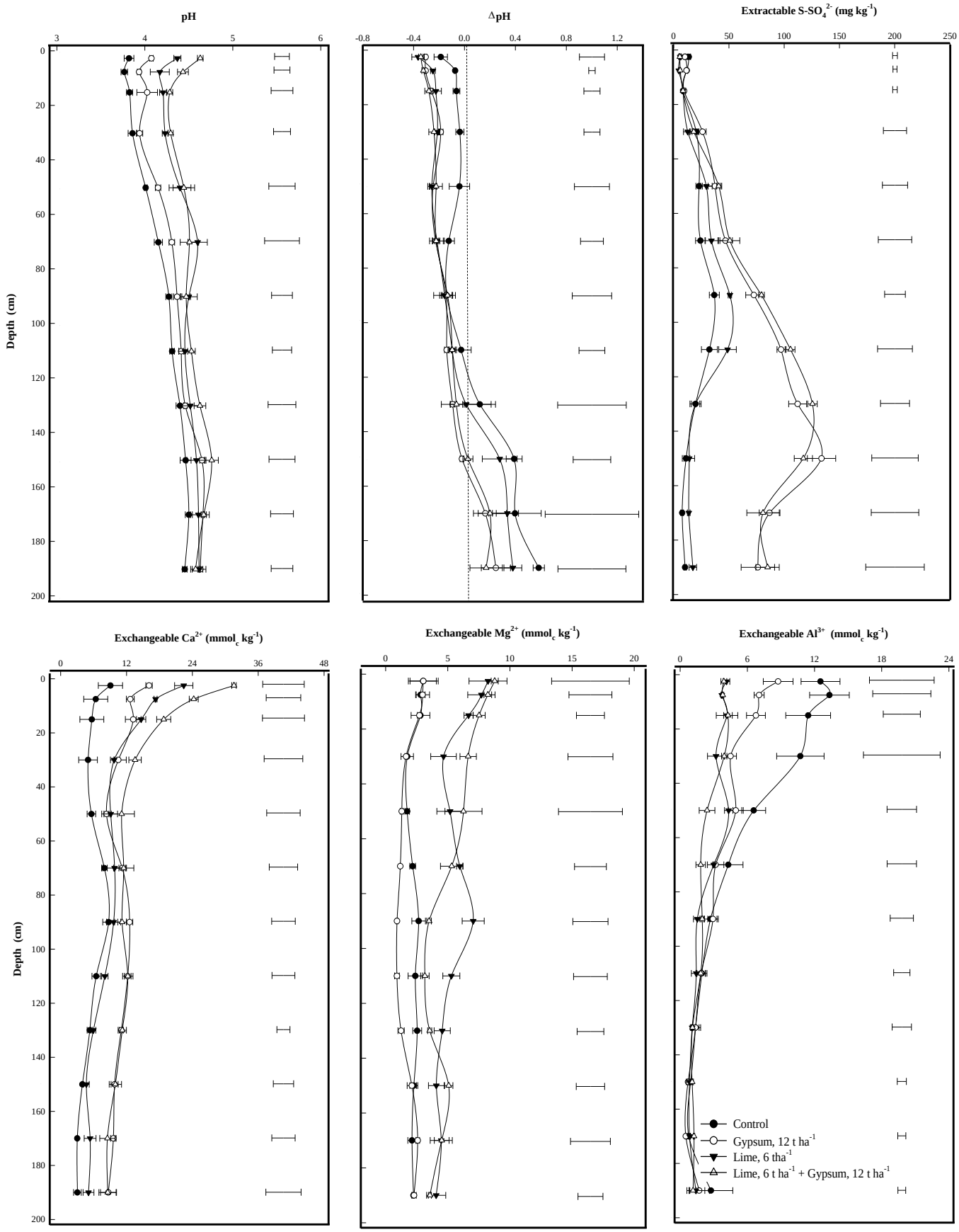

Figure 2 – Effects of surface applied lime and phosphogypsum on soil attributes at different depths, 13 years after application. The bars at each depth indicate the LSD at $p = 0.05$

increasing contents of soil organic matter) interfere on variable charge soil colloids, increasing the net negative charge. At depths greater than 120 cm we observed the dominance of positive charges, due to high amounts of iron/aluminum oxides and lower content of organic matter (Table 1). In such layers, a ΔpH displacement (to the left region of the figure) was observed with to the application of PG, due to the sulfate adsorption discussed above. Lime effects on ΔpH were observed up to 80 cm depth.

After 13 yr of phosphogypsum application, sulfur (SO_4^{2-}-S) extracted by calcium phosphate was more concentrated on 100-160 cm depth (Figure 2). Lime application in 1993 increased SO_4^{2-}-S leaching, due to increases in pH thus promoting more negative charge and subsequent sulfate repulsion. A previous study evaluating the same experiment after 8 months of PG application showed that around 60% of the SO_4^{2-} - S extracted by 0.5 M ammonium acetate in 0.25 M acetic acid that came from PG had leached to depths lower than 60 cm (CAIRES et al., 1998). We opted by the extractant 0.01 M $Ca(H_2PO_4)_2$ introduced by Fox et al. (1964), that has enough phosphate to displace most of the adsorbed sulfate in many soils, since we were working with highly weathered soils. Under this condition, Ca can keep the ionic strength of the solution and also depress the extraction of soil organic matter, thus eliminating contamination from extractable organic S (BEATON; BURNS; PLATOU, 1968). The higher sulfate adsorption in lower depths (between 120-160 cm), remaining for many years after application can be attributed to the positive charges (as showed by ΔpH - Figure 2) attracting the negatively charged SO_4^{2-} ions. This result agrees with the observed by Farina; Channon; Thibaud (2000b) that have attributed the retardation of gypsum movement to the reversion of electric charge in subsurface layers of an Ultisol in South Africa, as reflected by the differences in ΔpH. In our case, part of this fashion can be also attributed to the increase in clay content and at lower depths associated with the mineralogy (Table 1). Because of the solubility of PG and the soil texture, we would have expected that after 13 yr of application, it would have been completely leached from the soil profile.

We observed effects of the treatments on Ca^{2+} e Mg^{2+} distribution in all soil profile (Figure 2). The higher increase in Ca^{2+} content in both surface and subsurface layers was observed when lime + PG were applied. The surface application of lime in 1993 still had residual effect up to 100 cm depth, while Ca^{2+} movement up to 200 cm depth was verified in plots that had received PG. Increases in Ca^{2+} content due to PG application are frequently reported, since each ton of PG provides around 5 $mmol_c dm^{-3}$ of this nutrient. However, the duration of the effect varies with the amount of product applied to the soil and the soil texture. In this experimental area, a previous work have reported fast leaching of Ca^{2+} 24 months after

PG application, where 40% of the applied Ca^{2+} had leached bellow 80 cm depth (CAIRES et al., 2001).

There was an increase in the Mg^{2+} content by the surface application of lime up to 100 cm depth (Figure 2) and Mg^{2+} leaching on 80-120 cm depth was observed when lime + PG were applied. After 13 yr of PG application, we were not able to see suitable effects of this amendment on the contents of exchangeable Mg^{2+}. In the same experiment higher losses of Mg^{2+} were reported even only 12 months after liming and 8 months after PG application (CAIRES et al., 1998), since liming had increased Mg^{2+} levels on the soil solution, making it very susceptible to leaching. At 8 months after application of PG at 12 t ha^{-1} Mg^{2+} contents were higher than the observed for this experiment (around 10 $mmol_c dm^{-3}$ on the control plots and 30 $mmol_c dm^{-3}$ in the ones that had received lime application). In a long term, losses of exchangeable Mg^{2+} from the surface layers caused by PG application were relatively small when compared to the application of lime (alone). These results are in agreement with those obtained by Caires, Joris and Churka (2011).

We observed toxic levels of Al^{3+} up to 100 cm, except for the treatments that had received lime application (Figure 2). Exchangeable Al is responsible for the harmful effects of soil acidity, since it is released in low pH conditions. The increase in the pH due to lime application (Figure 2) causes precipitation of the Al ions, producing $Al(OH)_3$, thus reducing its toxicity. Even after long term of application, we can clearly see the effects of soil amendments on exchangeable Al^{3+} reduction up to 80 cm depth. Below this layer, Al contents were smaller than the ones observed in the samples from the surface layer. The combined surface application of lime and PG under NT system was effective to maintain the levels of exchangeable Al^{3+} as non-toxic to the crops in a long term.

2.3.2 Soil chemical attributes - 3.5 years after phosphogypsum application on a clayey Oxisol under no-till

Surface-applied PG in 2005 did not affect the values of soil pH (measured in 0.01 M $CaCl_2$) up to 60 cm depth (Figure 3) of the samples collected in 2009. The lack of effect of PG on the soil acidity was expected, considering that gypsum is a neutral salt, without the ability of consuming H^+ protons. Conversely, a linear increase in pH was observed in the lower depths (from 60 to 200 cm) as a response of PG rates, and it can also be attributed to the self-liming mechanism as discussed previously. It is noteworthy that this increase in pH due to surface gypsum application was of small magnitude, and the soil utilized in this study had low acidity. Consequently, very small amounts of exchangeable Al^{3+} were found throughout

the investigated soil profile (Figure 3). The soil presented net negative charge (represented by ΔpH) up to 80 cm depth. Bellow this depth, the remaining charge was found to be predominantly positive. The effect of PG in the soil net charge took place from 80 to 200 cm, reducing the ΔpH (Figure 3). Differences between gypsum treated and control plots in terms of ΔpH lend further support to the important role played by SO_4 sorption-precipitation reactions and the ΔpH differentials are clearly related to the quantities of SO_4 held in the soil, as found by Farina, Channon, Thibaud (2000b).

Contents of sulfate extracted with monocalcium phosphate increased for most depths evaluated, after the application of PG rates ($p \leq 0.05$) (Figure 3). The soil had high clay content (700 g kg^{-1}) in most part of the profile where the samples were taken. Unlike the soil of the first experiment, also discussed here, the levels of Fe_d were constant along the profile (Table 3), and the prevalence of sulfate in the lower depths can be linked primarily to the effect of organic matter, generating negative charges and promoting repulsion of the anion that moved to lower depths. Since the values of ΔpH were zero or positive in the subsurface layers (Figure 3), combined with higher amounts of Al_2O_3 and Fe_2O_3 (175.0 and 294.3 g kg^{-1}, respectively) and lower Ki and Kr (Table 1), we can assert that the soil had reached an advanced degree of weathering. Under these circumstances, SO_4^{2-}-S was rapidly leached to the subsoil layers. Sulfate movement is dependent on particle size distribution of the soil; intensity of rainfall after application, amount of S supplied, and the presence of organic matter and another ions. In this case, the movement of sulfate in the soil profile can be attributed to low soil acidity (Figure 3) and high levels of organic C (Table 1), beyond fertilization with P, especially in the first layers of the soil.

We observed a linear increase in exchangeable Ca^{2+} up to 80 cm depth and leaching of Mg^{2+} up to 120 cm depth after application of PG rates (Figure 3). When high rates of PG are applied to clayey soils, the effects of increasing contents of Ca^{2+} in the profile are very durable. In Georgia (USA), Toma et al. (1999) found an increase in levels of exchangeable Ca^{2+} up to 1.20 m, after 16 yr of the application of 35 t ha^{-1} of gypsum in a kaolinitic soil. The levels of exchangeable Mg^{2+} were reduced linearly with the PG rates applied from 0 to 40 cm depth (Figure 3). Leaching of exchangeable Mg^{2+} from the soil surface resulted in an increase in the contents of the nutrient at 60-120 cm depth after 3.5 yr of PG application. Leaching of exchangeable Mg contents in soil with the addition of PG is facilitated by the formation of the ionic-pair $MgSO_4^0$ (ZAMBROSI et al., 2007) and has been related in several studies. In the study performed by Toma et al. (1999) Mg leaching was observed from the 20-60 cm layers to the 100-120 cm after 16 yr of PG application.

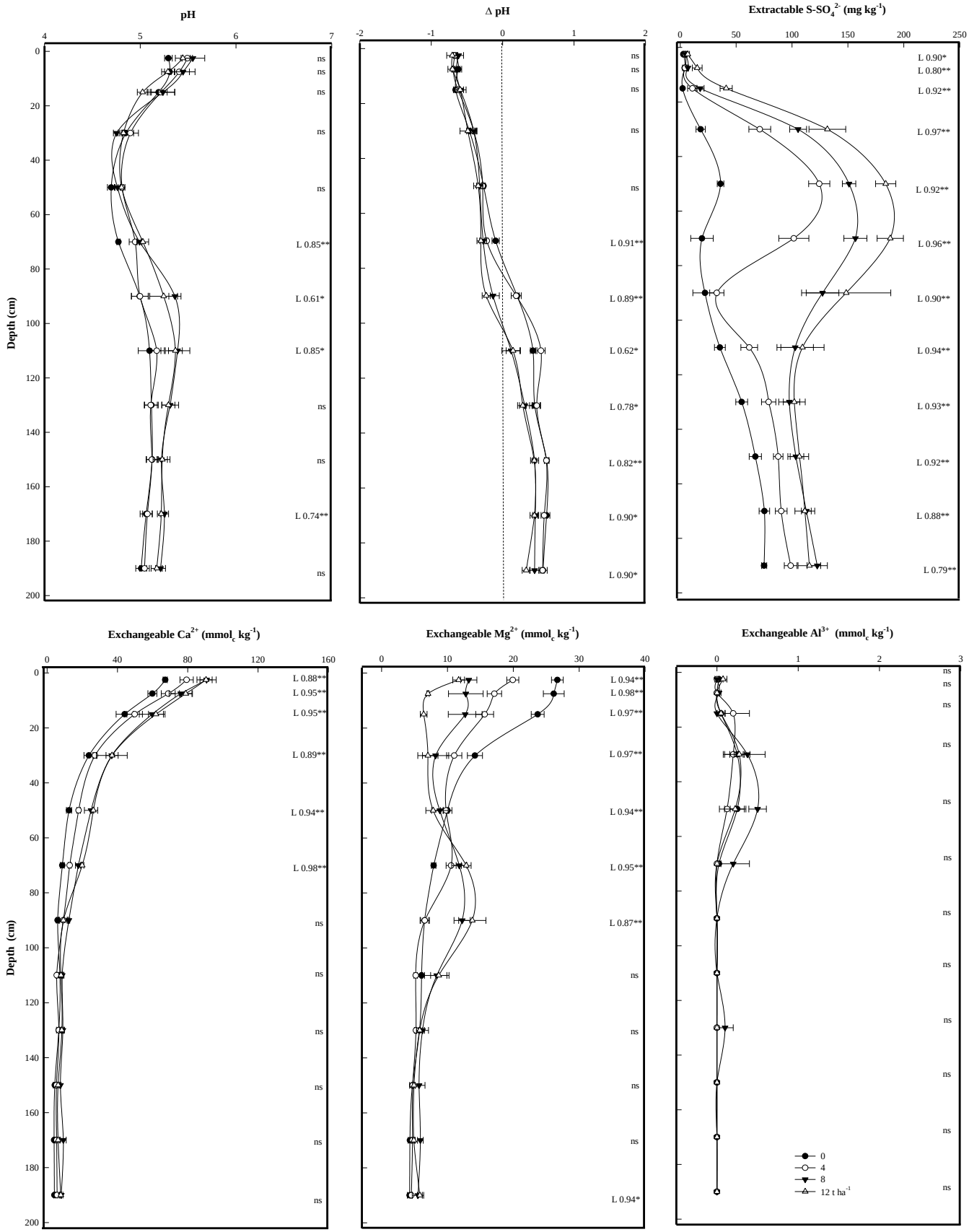

Figure 3 - Effects of phosphogypsum rates on soil attributes at different depths, 3.5 years after application .
L = significant by linear regression; * $p \leq 0.05$; ** $p \leq 0.01$; ns – non significant

The alleviation of soil acidity with dolomitic lime may be a viable alternative to minimize the losses of exchangeable Mg caused by PG application (CAIRES; JORIS; CHURKA, 2011).

Table 3 – Soil attributes related to sulfate adsorption

Depth	Srem	Prem	Organic Carbon	Fe_d	Fe_o	Al_o	pH NaF
cm	-------mg L^{-1}-------		----------g kg^{-1}----------				
			Loamy Hapludox – Ponta Grossa/PR				
0-5	14.9	21.9	23.6	36.2	1.7	3.9	9.5
5-10	14.7	18.0	19.3	38.0	1.7	3.5	9.8
10-20	14.8	15.7	17.4	37.3	1.9	4.4	9.9
20-40	12.2	13.2	14.6	42.0	1.6	4.6	10.0
40-60	10.6	8.7	11.6	45.9	1.5	4.7	10.3
60-80	10.0	7.0	10.9	46.2	1.4	6.1	10.4
80-100	9.4	5.1	10.4	47.5	1.4	5.8	10.5
100-120	7.7	3.9	9.2	50.8	1.6	6.0	10.5
120-140	6.5	2.8	8.1	50.1	1.6	4.7	10.6
140-160	4.8	1.6	7.4	49.8	1.6	4.8	10.5
160-180	4.2	1.3	8.2	48.3	1.5	3.6	10.4
180-200	4.1	1.4	7.4	49.4	1.6	4.7	10.5
			Clayey Hapludox – Guarapuava/PR				
0-5	15.0	2.5	44.2	168.0	5.2	10.7	10.3
5-10	13.6	1.7	33.4	172.7	4.9	9.6	10.5
10-20	12.3	1.4	29.4	167.6	5.1	9.6	10.6
20-40	9.2	1.4	25.1	173.1	5.2	11.7	10.8
40-60	6.3	0.7	20.2	164.9	5.3	12.0	10.9
60-80	4.7	0.5	17.7	165.3	5.8	13.7	11.0
80-100	3.6	0.3	14.3	166.1	5.8	10.3	11.0
100-120	3.7	0.1	11.4	173.1	6.1	10.4	10.9
120-140	3.8	0.2	10.2	168.8	6.2	10.1	10.9
140-160	3.5	0.1	8.7	172.7	6.0	9.5	10.9
160-180	3.7	0.1	7.3	179.8	6.0	9.2	10.9
180-200	3.7	0.1	7.3	192.7	6.0	8.5	10.8

As the soil in this study presented low acidity, the levels of exchangeable Al^{3+} found in the soil were lower than 1 $mmol_c$ kg^{-1} and were not influenced by the gypsum rates (Figure 3). After 3.5 yr of the PG application, we did not observe effect of the treatments on soil exchangeable K, although it has been observed pronounced leaching of potassium in the first years of evaluation (CAIRES et al., 2011).

2.3.3 Factors related to sulfate retention

After 13 yr of PG application, 52% (503.6 mg kg^{-1}) of the sulfate was recovered in the soil profile evaluated in the experiment carried out in Ponta Grossa, (0-200 cm) (Figure 2), whereas the remaining sulfate had leached to lower depths. In the experiment carried out in Gurapuava, we recovered 100% of sulfate provided by the PG application in the soil profile evaluated (0-200 cm), and a higher sulfate concentration was found in the 20-60 cm depth

(Figure 3). The inorganic sulfate can be leached from soils or adsorbed by soil colloids, and it may occur as precipitates or insoluble forms (TABATABAI, 2005). Sulfate leaching losses may reduce the long-term possibility of maintaining the S supply of crops in low input farming (ERIKSEN; ASKEGAARD, 2000) and also may remove cationic nutrients from the root zone. Sulfate losses are a concern in agricultural areas, although it is not considered as a serious environmental problem. The presence of excessive amounts of sulfate can be a concern in water supplies because of its cathartic effect upon humans (JANG; TOWNSEND, 2001). Also, it may convert to hydrogen sulfide during some reuse scenarios that causes noticeable odors and tastes.

Table 3 presents some soil attributes related to sulfate adsorption. Srem varied from 4.1 to 14.9 mg L^{-1} in Ponta Grossa and from 3.5 to 15 mg L^{-1} in Guarapuava soils, while Prem showed a wide variation between soil depths and the two experimental areas varying from 1.3 to 21.9 mg L^{-1} in Ponta Grossa and from 0.1 to 2.5 mg L^{-1} in Guarapuava (Table 3). Srem as well as Prem are soil attributes that normally present a narrow correlation with the maximum sulfate adsorption capacity in soil, however Prem had higher discriminatory capacity for the adsorption in the soil profile. Based on these results, we selected depths with similar adsorption capacities and fitted adsorption isotherms for both experiments (Figures 4 and 5).

The data presented a better fit to the Freundlich equation, agreeing with the results presented by Bolan et al. (1988) and Alves and Lavorenti (2004b). The adsorption capacity was increased with the depths for both soils evaluated, and higher adsorption capacity was registered for the soil from Guarapuava, agreeing with the results previously showed by Prem. Despite the fact that Prem have shown higher discriminatory capacity for adsorption, the results must be carefully evaluated, especially when practical implications are envisaged. As phosphate and sulfate do not present the same mechanism of adsorption (GOLDBERG, 2010) they can present different behaviors in agricultural and environmental systems.

Higher levels of organic C were observed for the soil surface layer, and the clayey Oxisol (Guarapuava) presented 55% more organic C than the loamy Oxisol (Ponta Grossa) in the whole profile. Higher amounts of organic C favor sulfate leaching due to the increase in negative charges and repulsion of SO_4^{2-} (COUTO; LATHWELL; BOULDIN, 1979). The amounts of Fe_d, Fe_o and Al_o were higher in the clayey Oxisol (Guarapuava). In both sites, the ratio Fe_o/Fe_d was lower than 0.07, what indicates the dominance of crystalline iron hydroxides (SCWERTMANN et al., 1982). Oxalate-soluble Fe and Al play an important role in sulfate adsorption in highly weathered subsoils (ALVES; LAVORENTI, 2004b).

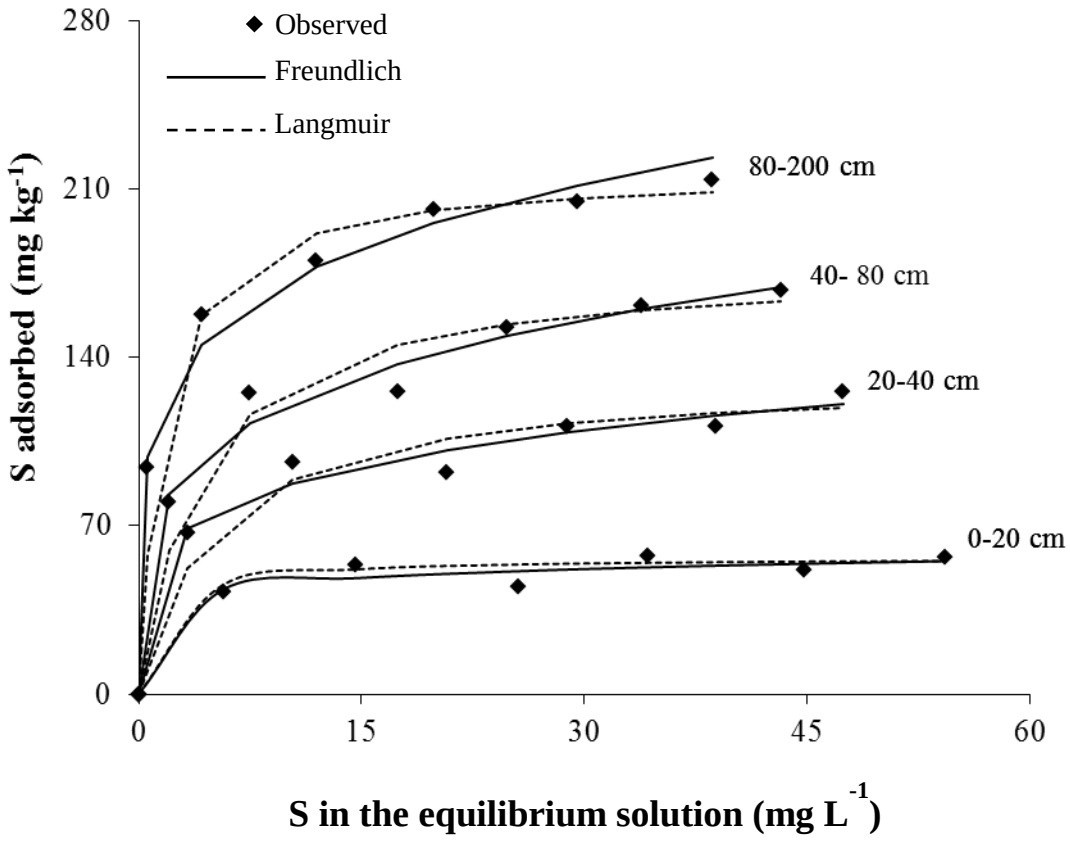

Figure 4 - Experimental data of sulfate adsorption for the loamy Oxisol (Ponta Grossa/PR) at different depths and adsorption curves drawn according to the Freundlich and Langmuir equations

The retention of sulfate in soils is dependent on the nature of the colloidal system, soil pH, concentration of sulfate and the concentration of other ions in solution. As a consequence, sulfate leaching may be subjected to considerable variations caused by differences in soil attributes or climatic conditions and by the differences in agricultural management (ERIKSEN; ASKEGAARD, 2000).

The pH_{NaF} values ranged from 9.5 to 10.6 for Ponta Grossa and from 10.3 to 11.0 for Guarapuava, suggesting that the F^- replaces the OH^- from soil minerals, thus increasing the pH value. However, pH_{NaF} also had a small discriminatory capacity for both soils, when compared to Prem agreeing with Alves and Lavorenti (2004a).

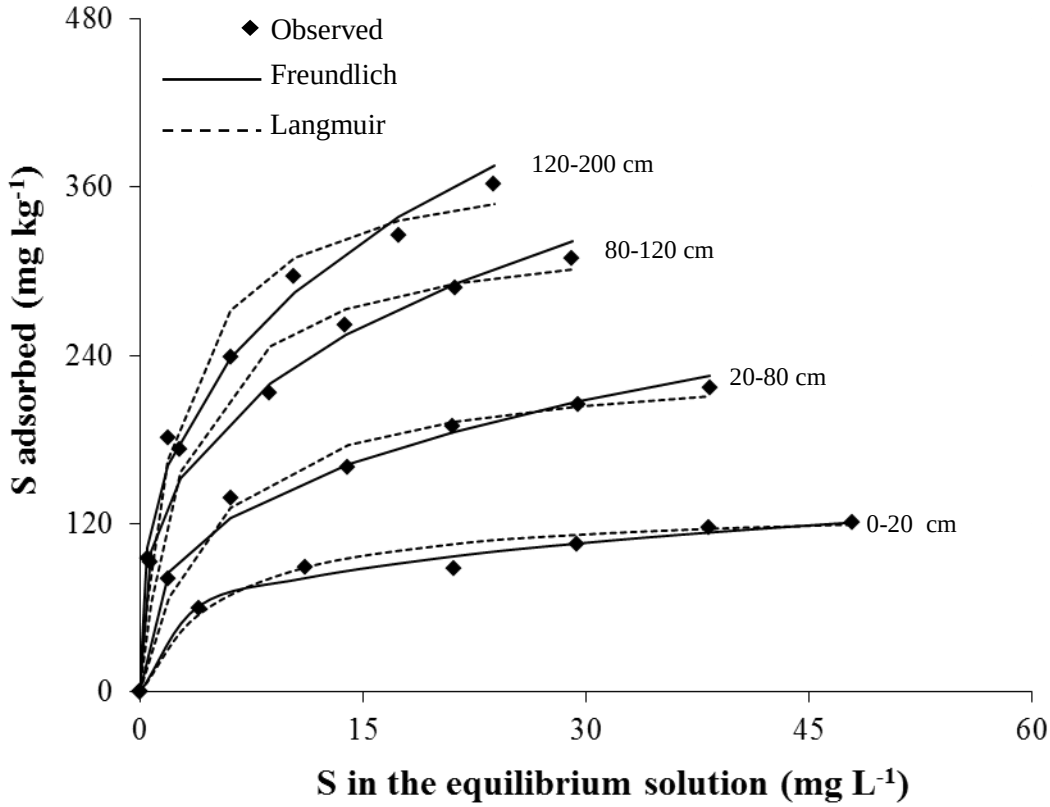

Figure 5 - Experimental data of sulfate adsorption for the clayey Oxisol (Guarapuava/PR) at different depths and adsorption curves drawn according to the Freundlich and Langmuir equations

Even for soils with a marked capacity to retain sulfate, the strength of the retention seems weak. Chao; Harward; Fang et al. (1962) found that adsorbed sulfate could be removed by repeated extraction with water. Since almost half of the applied sulfate was already leached to depths lower than 200 cm in the experiment conducted in Ponta Grossa, the sulfate extracted by calcium phosphate represents the soluble plus the retained S remaining in the profile, and the fashion of its distribution is due to the soil characteristics. By correlating the amounts of sulfate extracted by calcium sulfate and some soil attributes (Table 4), we verified that Fe_d, Fe_d-Fe_o and pH_{NaF} had positive correlations with SO_4^{2-}-S, while Srem, organic C, and Prem were negatively correlated. We obtained lower coefficients of correlation for the Oxisol of Guarapuava (Table 5), since the PG was applied 3.5 yr ago and it is still coming down in the profile. However, we still observed higher correlation coefficients between SO_4^{2-} and the pH measured in NaF, organic C and Srem and Prem.

Table 4 – Pearson Product Moment Correlation between the SO_4^{2-} in the soil profile and soil attributes related to S retention (n=12) for Ponta Grossa

	SO_4^{2-}	Organic C	Fe_d	Fe_o	Al_o	Fe_d-Fe_o	pH_{NaF}	P-rem	S-rem
SO_4^{2-}	-	-0.86**	0.90**	-0.39ns	0.34ns	0.90**	0.84**	-0.84**	-0.89**
Organic C		-	-0.96**	-0.56ns	-0.46ns	-0.96**	-0.98**	0.99**	0.92**
Fe_d			-	-0.62*	0.53ns	0.99**	0.97**	-0.97**	-0.92**
Fe_o				-	-0.51ns	-0.63*	-0.61*	0.57ns	0.48ns
Al_o					-	0.53ns	-0.22ns	-0.43ns	0.58*
Fe_d-Fe_o						-	0.97**	-0.97**	-0.91**
pH_{NaF}							-	-0.97**	-0.86**
P-rem								-	0.96**
S-rem									-

Table 5 – Pearson Product Moment Correlation between the SO_4^{2-} in the soil profile and soil attributes related to S retention (n=12) for Guarapuava

	SO_4^{2-}	Organic C	Fe_d	Fe_o	Al_o	Fe_d-Fe_o	pH_{NaF}	P-rem	S-rem
SO_4^{2-}	-	-0.62*	-0.09ns	0.45ns	0.54ns	-0.11ns	0.89**	0.66*	-0.75*
Organic C		-	-0.42ns	-0.88**	0.22ns	-0.37ns	-0.86**	0.99**	0.96**
Fe_d			-	0.33ns	-0.64*	0.99**	0.00ns	-0.32ns	-0.26ns
Fe_o				-	-0.18ns	0.27ns	0.70**	-0.87**	-0.90**
Al_o					-	-0.64*	0.24ns	0.16ns	0.03ns
Fe_d-Fe_o						-	-0.03ns	-0.27ns	-0.21ns
pH_{NaF}							-	-0.89**	-0.92**
P-rem								-	0.97**
S-rem									-

2.3.4 Effects on the yield of annual crops

Despite the decrease in exchangeable Al^{3+} and the increase in exchangeable Ca^{2+} contents after long-term applications of lime and PG (Figure 2), we did not see increase (p = 0.76) in cereal yield in Ponta Grossa (Figure 6). Many field trials conducted under NT system have shown increases in cereal grain yield with applications of lime (ERNANI; BAYER; MAESTRI, 2002; CAIRES et al., 2005; 2008) and gypsum (TOMA et al., 1999; FARINA, CHANNON, THIBAUD, 2000a; CAIRES et al., 2011a; b).

In our study, both crops achieved good grain yields; the average was 9,016 kg ha^{-1} for maize and 2,553 kg ha^{-1} for wheat. Based on the limitation in rainfall occurred in December 2008 (Figure 1), we would expect to see positive effects of the lime and gypsum on maize grain yields, but it did not happen. Water deficiency usually potentiates the positive effects of lime and gypsum application, especially when cereal crops are cultivated (CAIRES et al., 2008). Some authors have reported high crop grain yield in acid soils under NT system even in the absence of lime (BROWN et al., 2008; CAIRES et al., 2005; 2011a), such authors attribute the large grain yield in acidic conditions to the lower concentration of toxic species of Al^{3+} and higher concentration of Al complexed with organic matter (BROWN et al., 2008;

ALLEONI et al. 2010). Another possible explanation for the absence on the response in our experiment is the short range of pH values (between 3.9 and 4.7) since the amendments were applied to the soil in 1993 and no longer had such significant effects. In a trial conducted under similar soil and climate conditions to our study, Joris (2010) has obtained the higher maize grain yield at pH (0.01 M $CaCl_2$) of 6.1. Ernani, Bayer and Maestry (2002) observed that pH values above the required to the Al^{3+} neutralization also have contributed to the increase in maize yield.

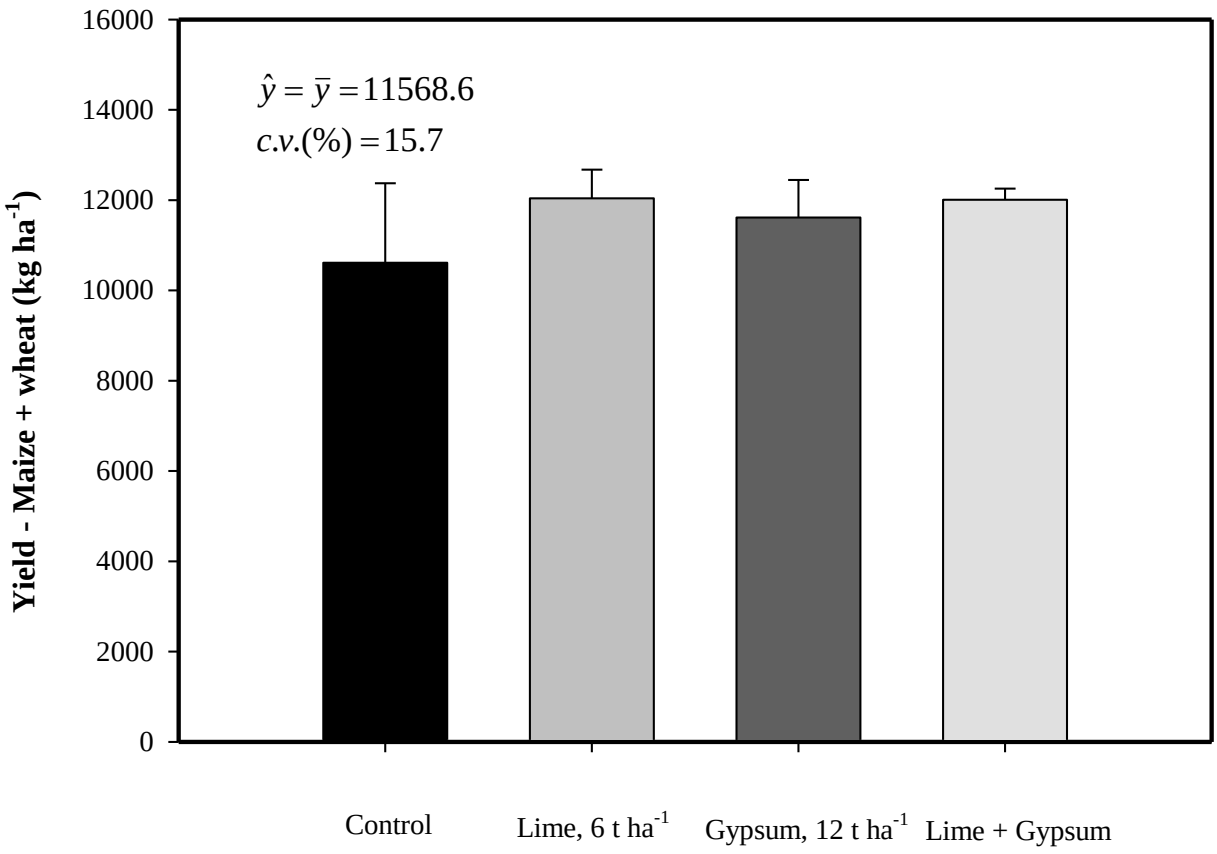

Figure 6 – Acumulated yield (maize+wheat), grown in 2008-2009 in response to lime and phosphogypsum application in 1993 (Ponta Grossa)

Phosphogypsum rates applied on the soil surface increased linearly the cereal grain yield in Guarapuava (Figure 7) providing an increase of approximately 12.9 % in grain yield. Positive responses of grasses, especially maize has been obtained in many other studies conducted in a wide variety of soil and climate (RAIJ et al., 1998; TOMA et al., 1999; FARINA et al., 2000a; CAIRES et al., 1999; 2004; 2011a;b). In the case of the soil under study, which had high exchangeable Ca^{2+} levels and and low exchangeable Al^{3+} levels in the

whole profile, the response of PG due to the improvement of the subsurface environment was difficult to be expected. Unlike the region of Ponta Grossa, no rainfall limitation was verified during the crop growth (Figure 1). In our experiment, the increase in grain yield can be explained by the changes in the soil chemical attributes due to PG application. The attributes more correlated to crop grain yield were (i) increases in SO_4^{2-} content; (ii) increases in Ca^{2+} content and in the Ca/Mg ratio (iii) decreases in Mg^{2+} and K^+ content (Table 6). K data were not discussed here, since we did not observe effects of the soil conditioner on this ion even after 3.5 years after PG application. However, intense leaching of K was registered in the first years after application of PG (CAIRES et al., 2011b).

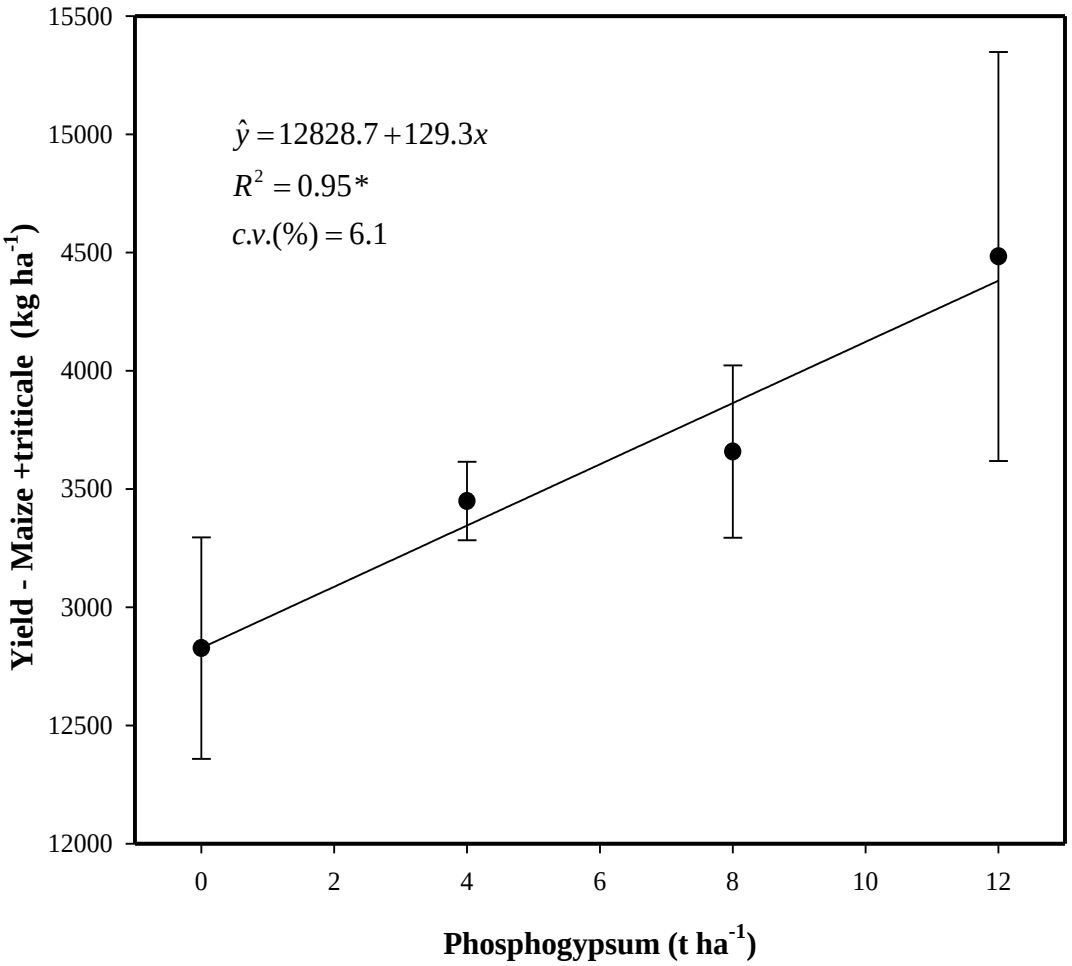

Figure 7 – Accumulated yield (maize+triticale), grown in 2008-2009 in response to phosphogypsum rates applied on the soil surface in 2005 (Guarapuava)

The attributes that most contributed to explain the increases in cereal yield occurred at 0-20 cm depth, although effects of PG were observed at lower depths. Higher correlation coefficients were verified for the Ca and Mg contents extracted by KCl and for the S-SO_4^{2-} extracted by calcium phosphate. The data presented here reinforce what was previously

discussed by Caires et al. (2011b) and Caires; Joris and Churka (2011), i.e. increases in grain yield observed for the grass plants grown is soils without limitations imposed by acidic conditions are mostly related to the supply of calcium and sulfur.

Table 6 – Pearson Product Moment Correlation between the accumulated cereal yield and soil chemical attributes at different depths (n=16)

Depth (cm)	**S-SO_4^{2-} $Ca(H_2PO_4)_2$**	**Ca^{2+} KCl**	**Mg^{2+} KCl**	**K^+ Mehlich- 1**	**Ca/Mg KCl**
0-5	0.69**	0.60*	-0.42ns	-0.50*	0.52*
5-10	0.69**	0.66**	-0.43ns	-0.42ns	0.59*
10-20	0.63**	0.65**	-0.46ns	-0.62**	0.57*
20-40	0.29ns	0.39ns	-0.14ns	-0.46ns	0.09ns
40-60	0.39ns	0.48ns	0.14ns	0.27ns	0.20ns
60-80	0.46ns	0.40ns	0.70**	-0.13ns	0.01ns
80-100	0.14ns	-0.06ns	0.21ns	-0.27ns	-0.30ns
100-120	0.21ns	-0.31ns	0.15ns	-0.22ns	-0.38ns
120-140	0.35ns	0.47ns	0.49ns	-0.18ns	0.12ns
140-160	0.26ns	0.51*	0.46ns	-0.15ns	0.16ns
160-180	0.24ns	0.05ns	0.26ns	-0.30ns	-0.03ns
180-200	0.21ns	0.50ns	0.54*	-0.39ns	0.15ns
0-20	**0.67****	**0.67****	**-0.45ns**	**-0.59***	**0.58***

2.4 Conclusions

Lime and phosphogypsum provided amelioration of soil acidity and root environment in the whole soil profile of the loamy Typic Hapludox even after 13 years. Lime effects reducing the soil acidity and providing exchangeable Ca^{2+} and Mg^{2+} were visible up to 80 cm depth, while phosphogypsum effects (providing Ca^{2+} and S-SO_4^{2-}) were more pronounced at lower depths. S-SO_4^{2-} distribution in the soil profile was mostly correlated with Fe_d, Fe_d-Fe_o and pH_{NaF}. Despite the lack of rain, no differences were found in maize + wheat yields in Ponta Grossa, showing that the plants were unable to benefit of the subsoil ameliorations provided by lime and PG applications in a long-term. Medium term PG effects (after 3.5 years) in Guarapuava showed rapidly SO_4^{2-}-S movement to the subsoil, despite the fact that soil had presented higher SO_4^{2-} adsorption capacity. Surface-applied PG had positive benefits in maize + triticale yields due to the supply of Ca^{2+} and SO_4^{2-}-S. Although the soils had presented different capacities for sulfate adsorption and had been evaluated at different times, the responses of crops to PG application under NT are associated not only to the improvement of the subsoil conditions and may be related to the improvement of the chemical conditions of the soil surface layers.

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3 SOIL SULFUR FRACTIONS INFLUENCED BY PHOSPHOGYPSUM APPLICATION IN A BRAZILIAN OXISOL ACCESSED BY WET-DEGRADATIVE AND XANES TECHNIQUE

Abstract

Little information is available regarding the speciation and dynamics of organic S functionalities in highly weathered Brazilian soils. One of the main sources of sulfur in agricultural systems is the phosphogypsum (PG), but there are not many results in the literature showing the effects PG application on soil sulfur fractions. We carried out this study in order to assess the effect of PG application on the amount, form and distribution of organic and inorganic S pools in soils under no-tillage (NT) and under a native forest site. To attain the objectives, both degradative wet-chemical (using hydriodic acid digestion/distillation) and solid-state XANES (X-Ray Near Edge Structure) spectroscopy techniques in the humic substances extracted by 0.1 M NaOH/0.4 M NaF were employed. Using wet-chemical technique it showed that contents of total S were linearly increased in all depths because of PG application, and the fraction bonded to C was increased at 0.20-0.40 m. After four years of PG application there was an increase in the content of inorganic S extracted either by $CaCl_2$ or by $Ca(H_2PO_4)_2$ in all depths, and mostly part of the inorganic S was present as adsorbed S. Contents of sulfur bonded to ester increased in the soil surface layer (0-0.1 m) showing the sulfate-S being incorporated into organic matter. Compared to the forest site, soils under NT system had lower amounts of organic C, total N and total S. Sulfur losses due to cultivation were higher than losses from C and N, suggesting that NT system was able to preserve a large amount of C by the addition of crop residues, although there was an intense removal of S, since no S mineral fertilizer had been applied in the area. Most part of the organic sulfur in both areas was present mainly in the form of ester sulfate, and relatively larger proportion of soil organic sulfur was lost from ester-SO_4^{2-} than C bonded S. This fraction may represent the more labile form of organic S. By means of XANES, we observed that the white-line of sulfur increased with the formal oxidation number of the sulfur ion, and the peak positions varied from 2473.4 eV to 2483.4 eV. There were more oxidized S species (sulfate ester - S^{6+}) in the forest area and in the PG area (rate of PG = 12 t ha^{-1}) than in the control treatment (agricultural area without PG application), in accordance with the observed data obtained by the wet-chemical technique. However, in all areas evaluated in this experiment, reduced and intermediate S species (S^0 to S^{5+}) were dominant when compared to the strongly oxidized ones (S^{6+}). Using XANES technique for the speciation of organic sulfur, we were able to see the presence of more S bounded to C than sulfate esters in all depths evaluated, contradicting the results obtained by wet-chemical fractionation. Changes in land-use practice alter both quantitative and qualitative composition of S functional groups in this humid subtropical Brazilian soil under NTS. The use of fertilizers containing S seems to be vital for the recovery of S moieties.

Key words: Gypsum; HI reduced S; Wet-chemical sulfur fractionation; XANES; No-tillage system; Southern Brazil agriculture; Triticale; Sulfur extractants

3.1 Introduction

Sulfur (S) is a component of amino acids, enzymes, vitamins and other biomolecules and is involved in a large number of biochemical processes; it is an essential element for the growth and development of microorganisms, plants and animals. Soil is the primary

component of the global biogeochemical cycling of this nutrient, acting as a source and a sink for various S species and mediating changes of oxidation states (WANG et al., 2006). Therefore, knowledge of S cycling is a key component to understanding changes in the biosphere (ZHAO, 1996; SOLOMON et al., 2005). However, despite the fact that the plant requirements for S are sometimes similar to those for phosphorus in agriculture, sulfur is still considered a secondary nutrient in plant nutrition. Although considerable efforts have been made to promote greater S research and S utilization in agriculture, sufficient knowledge concerning sulfur and its role in environmental and agricultural systems is scarce.

Soils of the wet tropical and subtropical regions, like Brazil, generally have low total S compared to temperate soils (NEPTUNE; TABATABAI; HANWAI, 1975) because of low S contents in parent materials or because of the extreme degree of weathering, beyond leaching losses (ACQUAYE; KAN, 1987). Plants absorb this element mainly as sulfate (SO_4^{2-}), but the organic pool accounts in general for more than 90% of the total S (NEPTUNE; TABATABAI; HANWAY, 1975; SOLOMON et al., 2001; WANG et al., 2006; ERIKSEN, 2009) on surface horizons, and organic S fractions govern the release of plant-available S. The availability of S in cropping systems has been dramatically reduced due to increases in S removal from soils by intensive cropping system coupled with increased crop yield, soil organic matter (SOM) depletion, leaching and erosion losses, the use of highly concentrated non-S fertilizers, and reduced anthropogenic S deposition through enhanced emission control measures and improved environmental regulations that lead to the reduction of sulfur dioxide emission to the atmosphere (e.g. the burning of coal). As a result, S deficiencies and crop responses to S fertilizers have been reported worldwide in the last ten years (SCHERER, 2001; 2009; CHEN; KOST; DICK, 2008; CAIRES et al., 2011; ERCOLI et al., 2011). These changes have made plants and soil biota increasingly dependent on soil organic S for the supply of this critical nutrient (KERTESZ; MIRLEAU, 2004; SOLOMON et al., 2009). The changes in management practices imposed on agricultural lands (e.g. tillage and fertilizer application) can also influence the soil microbial community structure and functioning and, consequently, S dynamics.

Conservation agriculture in the form of no-till (NT) or zero-tillage systems have been developed in many tropical agro-ecosystems as an alternative to improve the sustainability of farming systems (HUGGINS; REGANOLD, 2008). This sustainable agricultural approach relies on growing crops throughout the growing season without disturbing the soil through tillage operations and by keeping crop residues in the surface to reduce soil and nutrient losses by erosion. It also preserves and/or attempts to increase the level of organic matter in soils

(LAL, 1995). In Brazil, these systems have rapidly increased from almost nothing in the 1990's to the current level where they occupy up to 41% of the cultivated area, making Brazil one of the leading countries in the world in terms of adoption of the NT (DERPSCH; FRIEDRICH, 2009). However, most Brazilian soils contain a large proportion of variable-charge minerals and usually have high acidity and low nutrient reserves, requiring remediation before establishing NT systems for grain crop production (CAIRES; JORIS; CHURKA, 2011). Gypsum, a mined and industrial by-product material also called phosphogypsum (PG), is used for ameliorating these acid soils (SUMNER, 1993).

Besides acting as a soil conditioner, PG is also a valuable source of S and provides this nutrient to plants as SO_4^{2-}. However, there are not detailed investigations on the role of PG in the biogeochemical cycling of S in the wet tropics. Also, there is not enough information about this soil amelioration practice in Brazilian NT systems. Moreover, the few available studies report S transformations in soil due to application of S containing fertilizers (CASTELLANO; DICK, 1991; CURTIN; BEARE; McCALLUM, 2007; DE BONA; MONTEIRO, 2010) were evaluated only by the classical wet-chemical method using digestion/distillation with hydriodic acid. Thus, little information is available regarding the speciation and dynamics of organic S functionalities in Brazilian soils (NEPTUNE; TABATABAI; HANWAI, 1975; LILIENFEN et al., 2000; LEHMANN et al., 2001; DE BONA; MONTEIRO, 2010). This knowledge is vital to effectively describe S fluxes from various pools of Brazilian soils to design and promote sustainable agricultural systems in the country, and also to better evaluate the transfer of S between the soil environment and other reservoirs during the biogeochemical S cycle in the tropical agro-ecosystems following PG-based soil amelioration (JANZEN; ELLERT, 1998; SCHROTH et al., 2007; SOLOMON et al., 2009). Therefore, there is a need to carry out a detailed investigation to understand speciation, long- and short-term biogeochemical dynamics, oxidative state, and accompanying functional group chemistry changes of the various organic S moieties following PG application in NT systems in Brazil.

Although S is present mainly in organic forms in soils (>95%), very little is known about the identities of these specific S containing molecules, especially in tropical soils (SOLOMON et al., 2001; KERTESZ; MIRLEAU, 2004). Until recently, advances in our understanding of S speciation were associated with destructive methods of extracting S compounds using the principles of the classical method suggested initially by Johnson and Nishita (1952). Most recently, studies using noninvasive synchrotron-based sulfur K-edge X-ray Near-edge Structure spectroscopy (XANES) has been successfully used to identify

multiple S fractions in a variety of environmental and geochemical samples originating in humic substances from marine sediments (VAIRAVAMURTHY et al., 1997), humic substances (XIA et al., 1998; SOLOMON et al., 2003; 2005; 2009), organic matter (SCHROTH et al., 2007) and bulk soils (PRIETZEL et al., 2003; 2007). XANES provides specific information on the functional groups containing sulfur because of the sensitivity to the electronic structure, oxidation state, and the geometry of the neighboring atoms (VAIRAVAMURTHY et al., 1997). The advantage XANES affords over traditional wet-chemical methods is that intermediate oxidation states can be identified. The two combined techniques (wet-chemical fractionation and XANES) can provide valuable information regarding the S dynamics in NT systems.

The present study has the following objectives: (i) to assess the impacts of land use changes comparing undisturbed native forest and no-tillage systems, and (ii) to assess the role of PG application in NT systems on the amount, form and distribution of organic and inorganic S pools in soils in NT-based agricultural systems of Brazil using both degradative wet-chemical and solid-state XANES techniques. The study reported here formed part of a field study designed to examine the effects of phosphogypsum (PG) application to improve soil fertility and design more sustainable agronomic systems for crop production under NT. More details regarding the effects of PG application on soil chemical attributes crop yield and nutrition can be found in CAIRES et al. (2011).

3.2 Material and Methods

3.2.1 Site description and soil samples

The present study was carried out using soil samples collected from a PG field experiment in an agricultural area in the southern Brazil (Guarapuava, state of Parana - 25°17’ S e 51°48’ W, 997m) and also from an adjacent soil site collected in a native forest near the cultivated area, considered a reference of undisturbed system. The experiment was performed to evaluate the effects of PG application on soil and plant attributes (CAIRES et al., 2011). It was established in 2005, and the rates were: 4, 8, and 12 t ha^{-1} plus the control treatment (without PG application) with four replications. The forest site was in a fragment of the ombrophilous (high-rainfall tolerant) mixed forest where the canopy was dominated by Parana pine (*Araucaria angustifolia*) also referred to as *Araucaria* forest (VELOSO; RANGEL FILHO; LIMA, 1991). According to Köppen-Geiger System (PEEL; FINLAYSON; McMAHON, 2007) the climate of the region is Cfb, with mild summer and

frequent frosts during the winter. The average of precipitation and temperature registered for 33 years were 1,923 mm and 17.1 º C, respectively.

Soil is a clayey Typic Hapludox that has been kept under NTS for 19 years. The soil analysis of the 0-0.20 m layer performed in 2005, before the establishment of the PG experiment revealed low acidity (pH measured in 0.01 M $CaCl_2$ of 5.9; no exchangeable Al^{3+}) and high fertility (base saturation of 62%, Ca^{2+} e Mg^{2+} extracted by 1 M KCl of 48 and 22 $mmol_c\ dm^{-3}$, respectively, and K^+ extracted by Mehlich-1 of 4.3 $mmol_c\ dm^{-3}$). The mineralogy was mainly composed by kaolinite and gibbsite, with presence of hematite, goethite, and some clay minerals with hydroxy interlayers. The soil had 168.0 g kg^{-1} of ditionite-citrate-bicarbonate extractable Fe; 5.2 g kg^{-1} of oxalate extractable Fe; and 10.7 g kg^{-1} of oxalate extractable Al. More characteristics of the both soils (0-0.2 m), measured for this experiment are expressed in Table 1. Organic Carbon and total Nitrogen were measured by dry combustion in a Thermo-Finnigan Flash 1112 elemental analyzer. Total Sulfur was obtained by alkaline digestion with NaOBr (TABATABAI; BREMNER, 1970) and quantified using the colorimetric method as bismuth sulfide (KOWALENKO, 1993a).

Table 1 - Selected properties of the Oxisol collected in an agricultural area under NTS and under a native forest in the southern Brazil

Soils	pH $CaCl_2$	Total S	S $Ca(H_2PO_4)_2$	Organic C	Total N	C/N	C/S	N/S
		……....mg kg^{-1}	…..…..	...….g kg^{-1}	……...			
Agricultural area	5.9	329.3	5.9	35.6	1.9	18.8	108.1	5.8
Natural Forest area	4.5	459.5	13.1	41.9	2.8	14.8	91.2	6.1

Soil samples were collected in October 2009, 4 yr. since the gypsum experiment had been started. To obtain a composite sample, 12 soil cores were taken at 0-0.05, 0.05-0.10 and 0.10-0.20 m depth, and 5 soil core samples at 0.20-0.40 m depth in each plot using a hand soil probe. The same sample scheme was adopted for the forest site with four replications. Prior to analysis, soils were oven dried at 40º C, ground to pass a 2 mm sieve (10 Mesh) and further ground using a ball grinder to pass a 0.149 mm sieve (100 Mesh) as recommended by Tabatabai (1982) for S fractionation. For the XANES analysis, however, because of limitations in the availability of instrument and allocation of beam-time, we chose the samples from the treatment with the higher PG rate (12 t ha^{-1}) and the control treatment (without PG) as the most contrasting ones, beyond the native forest soil. We also mixed soil samples for the four field replicates preparing one representative composite sample in the depths 0-0.10, 0.10-0.20 and 0.20-0.40 m.

3.2.2 Chemical analyses

Total soil organic carbon (SOC) and N concentrations were determined by dry combustion in a Thermo-Finnigan Flash 1112 elemental analyzer. Sulfur fractionation in the bulk soils were performed according to Kowalenko (1993 a; b). Total S was oxidized to sulfate by NaOBr method (Tabatabai and Bremner, 1970), quantified by hydriodic acid (HI) reduction of SO_4^{2-} to sulfide, and determination of S as bismuth sulfide by spectrophotometry at 400 nm. Soluble S was extracted with 0.01 M $CaCl_2$. As the soil presents favorable characteristics for sulfate adsorption, we opted for using the extractant 0.01 M calcium phosphate (initially proposed by Fox; Olson and Rhoades, 1964), indicated for very weathered soils (Tabatabai, 1982) instead of 0.016 M potassium phosphate. Thus, the soluble + adsorbed sulfate fraction was extracted. The extracts were also reduced by HI after evaporation in the oven at 105 ºC. Organic S was estimated as the difference between the total S and inorganic S. HI-reducible S, consisting primarily of ester sulfate and inorganic sulfate was analyzed by direct reduction with 4 ml of the hydriodic acid reducing mixture (hydriodic acid, formic acid and hypophosphorus acid in a 4:2:1 proportion) and measured on a spectrophotometer according to Kowalenko (1993b). The difference between HI-reducible S and inorganic SO_4^{2-} was considered as ester SO_4^{2-}. Because HI will not reduce the C–S (amino acid) or C–SO (sulfonate linkage), C-bonded S was considered to be that fraction of the total organic S that was not reduced by HI.

3.2.3 X-ray Absorption Near-Edge Structure (XANES) spectroscopy analysis

Solid-state characterization by XANES spectroscopy of S oxidation states in the humic substances extracted from the soils was carried out using S K-edge XANES spectroscopy at beam-line X-19A of the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory. The extraction procedure for the humic fraction followed the outline of Schnitzer (1982), as modified by Sumann et al. (1998). The finely sieved soil samples (passed through a 100 mesh sieve) were extracted three times with a mixture of 0.1 M NaOH and 0.4 M NaF solutions in a 1:5 soil to extraction solution ratio (w/v) under N_2 environment, to avoid the oxidation of the substances during the extraction. Replacement of 0.1 M $Na_4P_2O_7$ by 0.1 M NaOH - 0.4 M NaF mixture improves extraction yield compared with extraction using only 0.1 M $Na_4P_2O_7$ (SUMANN et al. 1998). The F^- ion was introduced to dissolve silicate impurities and to reduce the influence of paramagnetic metals on XANES spectra (SOLOMON et al. 2003, 2007). Then the extracts were centrifuged at 13,000 r.p.m. for 20 min, and the supernatant were stored in a refrigerator at 4°C. The extracts were

filtered twice through a 0.2-μm pore-size membrane filter to remove fine clay that may interfere with XANES measurements (SOLOMON et al. 2003), transferred into dialysis tubes (MWCO 12,000 to 14,000 Da, Spectrum Laboratories, Rancho Dominguez, California, USA), dialyzed against deionized water to eliminate soluble salts and lyophilized using a freeze dryer.

Experimental data at X-19A were collected under standard operating conditions. The X-ray energy were calibrated to the K-edge of elemental S at 2473 eV and scans ranging from 80 eV below to 150 eV above the absorption edge of S were collected with step size of 0.2 eV. We used a monochromator consisting of double-crystal Si (111) with an entrance slit of 0.5 mm and a minimum energy resolution of 2×10^{4}(≈ 0.5 eV) at the S K-edge. The monochromator was detuned to 70% at the S K-edge in order to reduce fluorescence induced by high-order harmonics (Xia et al. 1998). The spectra were recorded in fluorescence mode using a passivated implanted planar silicon (PIPS) detector (Canberra Industries, Meriden, Connecticut, USA). The beam path from incident ion chamber to the sample chamber was purged with He gas. The samples were accommodated into a 0.5 mm thick acrylic holder and covered with 3.6 μm thick Mylar film (Chemplex Industries, Palm City, Florida, USA). Background correction and normalization of the spectrum were performed using the software WinXAS following Xia et al. (1998), Martínez et al. (2002) and Solomon et al. (2003; 2005; 2009; 2011). The interpretation of the forms of S obtained by S K-edge XANES were made based on the qualitative data observed in each spectrum.

3.2.4 Statistical analyses

To verify the effects of PG in the parameters evaluated, results were submitted to polynomial regression analysis. The criterion for choosing the regression model was the magnitude of the determination coefficients significant at $p \leq 0.05$. For examining the statistical differences between the parameters related to land use, data were analyzed by one-way analysis of variance (ANOVA) along with post hoc separation of means by the Tukey's range test ($p \leq 0.05$) using the software package Sisvar version 5.3.

3.3 Results and Discussion

3.3.1 Effects of phosphogypsum on C and N contents and the sulfur pools accessed by the wet-chemical fractionation

The Oxisol had a high content of organic C, although it is a highly weathered soil of the subtropical region of Brazil (Figure 1). The content of C found in this study is comparable

with the one found by Sá et al. (2001), that had studied the same soil order cultivated under NT for more than 20 years in the same Brazilian region. N contents however, were a little lower than the ones observed by Sá et al. (2001) in samples of an Oxisol under NT for 22 years (3.3 g kg^{-1}).

After four years of PG application, soil organic C and total N contents were affected by the treatments in the first soil depths evaluated in this study (Figure 1). There was a linear increase in the contents of OC in the depths 0 - 0.05 and 0.05 - 0.10 m. Conversely, an increase for total N content was verified only in the most superficial layer. Management strategies adopted in agricultural systems like the addition of crop residues and the application of lime and fertilizers have a complex set of effects on C and N storage and cycling in NT cropping systems (GARBUIO et al., 2011).

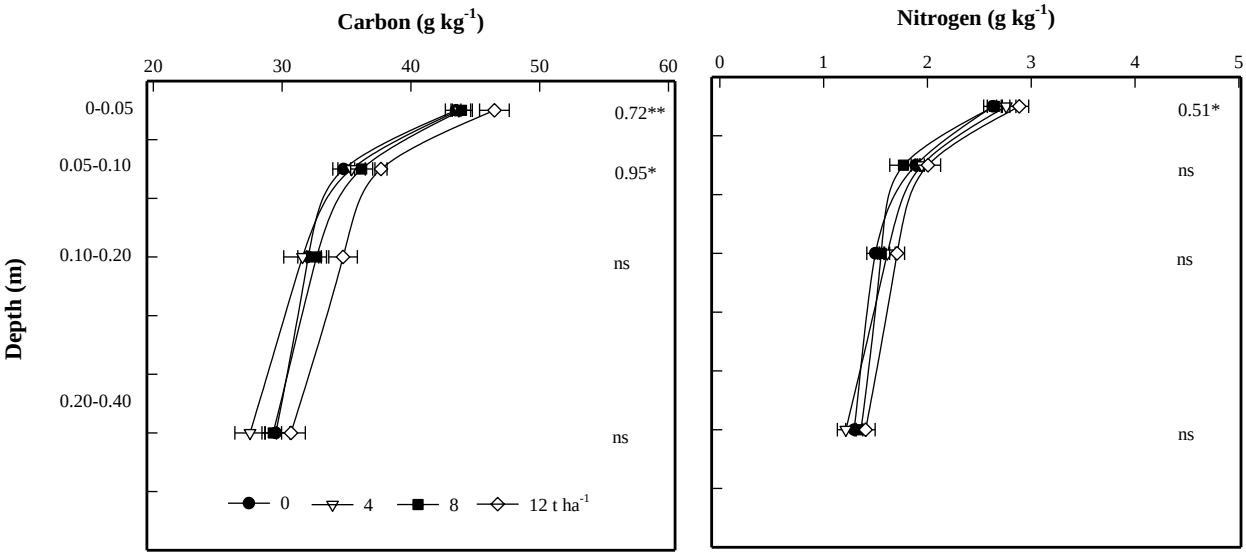

Figure 1 - C and N contents after phosphogypsum application rates from 0 to 12 t ha^{-1}. ns: non-significant; ** significant ($p \leq 0.01$); * significant ($p \leq 0.05$)

There is no detailed information in the literature about the effect of PG on C and N in soils. There are two possible explanations regarding the changes in C and N contents as a response of PG application: the increase in the biomass content of the crops, thus promoting high input of crop residues (roots and shoots) and resulting in better degree of aggregation of the soil. As a consequence, a high protection of OM inside of the aggregates is observed (TISDALL; OADES, 1982). Another possible reason is the increase in soil exchangeable Ca^{2+} contents by surface application of PG, which improved macroaggregate stability through Ca-organic matter bridges. In a previous study conducted in the experimental area, the total biomass production was not evaluated, but an increase in yield response of corn in a corn-soybean system was reported (CAIRES et al., 2011). However, increases in biomass

production with the application of gypsum or PG are reported in the literature (TOMA et al., 1999). Microaggregates are formed from organic molecules (OM) connected by clay and polyvalent cations (PC) such as calcium, resulting the formation of composite particulates (Clay-PC-OM) that combine with other particulates (Clay-PC-OM) to form macroaggregates $[(\text{Clay-PC-OM})_x]_y$ (TISDALL; OADES, 1982).

Many methods of wet-chemical fractionation are available for sulfur in soils. The most widely employed is the one suggested by Johnson and Nishita (1952) adapted by Tabatabai and Bremener, described in Tabatabai (1982). Five main fractions are obtained in this version of sulfur fractionation (Total S, Inorganic S, S bonded to ester, S bonded to Carbon, and residual S). Total S is oxidized by HNO_3, $HClO_4$ and H_2PO_4 and then reduced by hydriodic acid (HI) in the apparatus of digestion/ distillation. Inorganic S is extracted by a salt solution and reduced by HI. S bonded to ester is the S reduced by HI minus the inorganic fraction and S bonded to carbon is the fraction reduced by the Raney-Ni, which covers mainly the amino acids. Another widely employed methodology for the sulfur fractionation is suggested by Kowalenko (1993b). This method has some advantages compared to the first one described above, as less time required to conduct a determination and lower cost for the glassware fabrication. In this version of sulfur fractionation four main fractions are generated (Total S, Inorganic S, S bonded to ester and S bounded to Carbon). The main differences with the methodology proposed by Tabatabai (1982) are that the fraction bounded to carbon is obtained by difference, subtracting the fraction reduced by HI from the total S. Another methods of fractionation are also employed for the study of sulfur in soils, and they come mainly from adaptations of the P fractionation (LILIENFEIN, 2000; SINGH et al., 2004), since some authors assert that the “classical” fractionation, employing HI, does not separate the S in fractions with distinct mineralization potentials.

In this study we used the wet-chemical analysis of S fractions according to Kowalenko (1993a; b). Contents of total S increased by PG application in the four depths evaluated (Figure 2), as observed by Castellano and Dick (1991) after the application of gypsum and elemental S on a fine-silty Mollisol. However, total soil S content does not have a direct contribution as an S source for plants, since the constitutive fractions have different liabilities. The amount of total sulfur found in this study ranged from 316 to 425 mg kg^{-1} (Figure 2), similar to the ones reported by Neptune, Tabatabai and Hanway (1975) for soils from Parana/Brazil (209 and 398 mg kg^{-1}). They are higher than the ones reported by Lilienfein et al. (2000) for the savannah region in central Brazil (247 to 263 mg kg^{-1}) and the ones reported

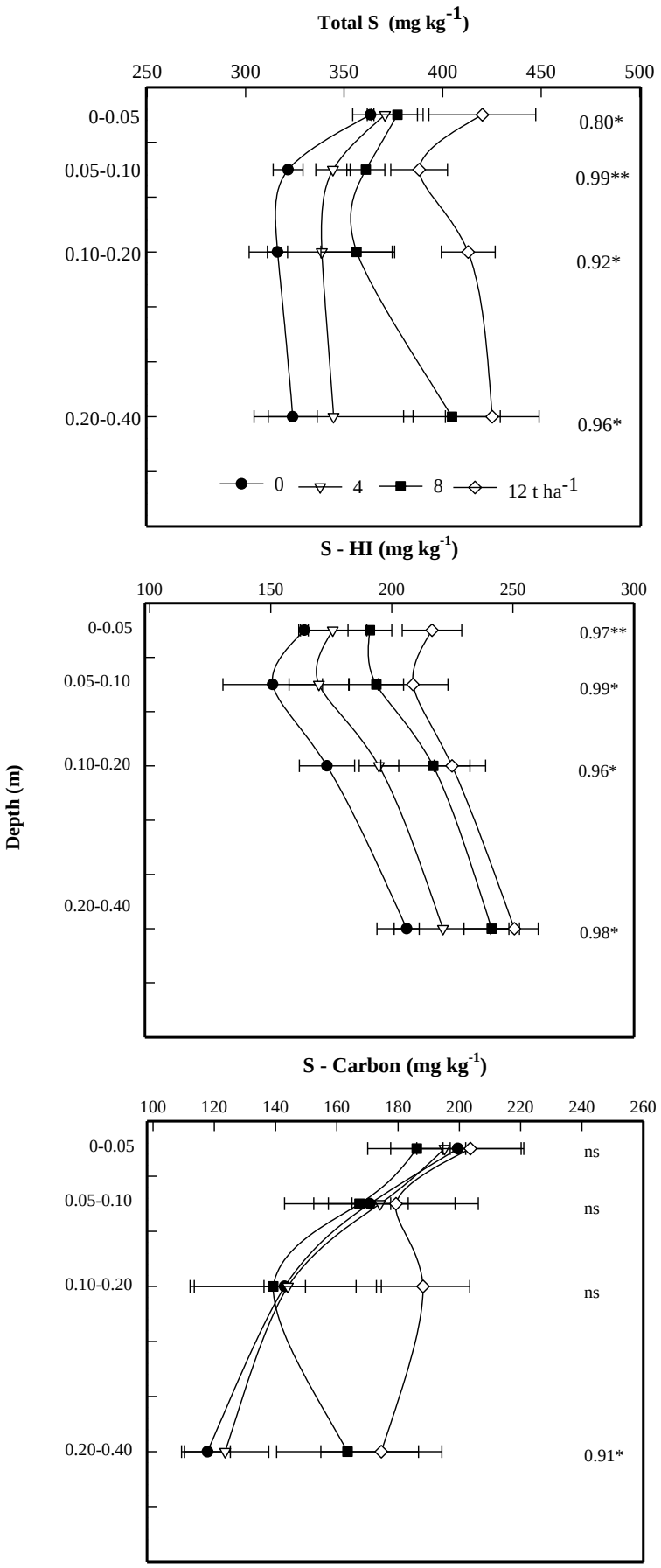

Figure 2 - Total S and S fractions according to phosphogypsum rates. *: $p \leq 0.05$, ** $p \leq 0.01$ and ns: non-significant

by De Bona and Monteiro (2010) for a Brazilian Entisol under pasture (115 to 230 mg kg^{-1}). However, higher amounts of total S (501 to 798 mg kg^{-1}) were reported by Lehmann et al. (2001) for a Xanthic Ferralsol in the Amazon. Stanko-Golden and Fitzgerald (1991) found also higher values in humid tropical soils from Puerto Rico (353 to 1231 mg kg^{-1}), and Solomon et al. (2001) observed the same trend in Ethiopian soils (520 to 1082 mg kg^{-1}). Differences between the total sulfur content among these soils are probably related to differences in soil organic matter levels because of different types of vegetation, climate, depth of sampling, soil class and management practices (ex.: fertilizers application in case of agricultural/agroforestry areas). Contents of S reduced by HI were also increased by PG application in the four depths evaluated in this study (Figure 2). Hydriodic-acid reduced method extracts the fraction of sulfur reduced to H_2S by digestion with a mixture of hydriodic, formic and hypophosphorous acid, and ester sulfate S is calculated from HI-reducible S by subtracting inorganic S, which usually occurs entirely as sulfate (FRENEY, 1961).

HI will reduce to H_2S only that organic S not directly bounded to C as in ester sulfates (-C-O-S-), sulfamic acid (-C-N-S-) and the second S* in S-sulfocysteine (-C-S-S*-) where the S and C atoms are separated by O, N or S atoms, respectively (FRENEY, 1986). In this study, the content of S reduced by HI also increased on the deeper soil layers (accounting for 45 to 64% of the total S in the soil), so as the findings of Tabatabai and Bremner (1972) and Eriksen (1996).

In the control treatment (without PG application) the percentage of S as carbon-bonded decreased with sample depth. However, an increase in this S fraction was observed at the depth of 0.20-0.40 m with the application of PG. This fraction represents the difference between the total organic S and that reduced to H_2S by HI. Carbon-bonded S is not converted to inorganic SO_4^{2-} by hot acids or alkalis and would include the S containing amino-acids, such as methionine and cysteine, mercaptans, disulfides, sulfones and sulfonic acids (FRENEY, 1986). Tabatabai; Bremner (1972); Neptune; Tabatabai; Hanway (1975) and De Bona; Monteiro (2010) consider the fraction of sulfur bonded to carbon as the one reduced by the reaction of the soil with Raney-Ni (LOWE; DELONG, 1963). This reagent reacts with the reduced S bonded to C (like in mercaptans R-C-SO-CH_3), sulfinic acids R-C-SO-OH, or sulfonic acids attached to an aromatic nucleus (sulfanilic acid, H_2N-C_6H_4-SO_2OH). However, Raney-Ni does not react with S in the form of aliphatic sulfones or aliphatic sulfonic acids (FRENEY, 1986). For those who chose to call C bonded S the fraction reduced by Raney Ni, they also present another fraction of sulfur, called residual S, unidentified organic S or

sulfonate S (NEPTUNE; TABATABAI; HANWAY, 1975; DE BONA; MONTEIRO, 2010; STANKO-GOLDEN; FITZGERALD, 1991). However, Kowalenko (1993b) asserts that the chemical reduction of S by Raney-Ni is not quantitative due to interference problems and a better estimative of the carbon bonded S is the difference between total sulfur and HI reducible sulfur instead the direct determination by Raney-Ni digestion.

For this study, we chose two sulfur extractants: $CaCl_2$, that extracts the soluble sulfate fraction, and $Ca(H_2PO_4)_2$, that extracts the soluble plus the adsorbed sulfate. Since this method of chemical fractionation is also based on obtaining some fractions by difference, the choice of an extractant play a big role in this process and modify the interpretation of the results. The S-SO_4^{2-} contents extracted with monocalcium phosphate increased along the soil profile even after four years of the PG application (Figure 3). The trends are similar to the ones reported by Caires et al. (2011), but there was a decrease in the absolute values (the maximum content of soluble + adsorbed sulfate found in this study was around 80 mg kg^{-1}, while concentrations greater than 200 mg kg^{-1} were reported by Caires et al. (2011), since great leaching of S-SO_4^{2-} was observed along time due to the low soil acidity and high soil organic carbon content.

Many solutions can be used as sulfur extractants in Brazilian soils to obtain the available contents, but the most frequently used are the monocalcium phosphate [$Ca(H_2PO_4)_2$] and the ammonium acetate in acetic acid (PROCHNOW; BOARETTO; VITTI, 1997; RIBEIRO et al., 2001; CAIRES et al., 2011). The total organic S (calculated from total S minus inorganic S extracted by $Ca(H_2PO_4)_2$ solution) ranged from 99% (in the surface layer, without PG application) to 75% (in the subsurface, with the application of 12 t ha^{-1} of PG. The range of organic S found in this study was similar to the one reported by Neptune, Tabatabai and Hanway (1975) for Brazilian soils (77 to 95%). However, the effect of PG application observed on the organic S fraction was not consistent with another results observed, as for the fraction bonded to carbon as for the one bonded to ester.

The sulfur bonded to ester (calculated from the HI reducible S minus the S extracted by $Ca(H_2PO_4)_2$) ranged from 38 to 59% in this soil and it was influenced by the PG application. In this study, different patterns in the S ester were observed. While there was an increase of this fraction of S in the first 0-0.10m, a decrease on S-ester content was observed in the 0.20-0.40 m layer. Increases in S ester content due to an increase of inorganic S in solution, as provided by PG application, are reported in the literature (FRENEY; MELVILLE; WILLIAMS, 1975; MAYNARD; STEWART; BETTANY, 1985; GHANI; McLAREN; SWIFT, 1992; CURTIN; BEARE, McCALLUM, 2007). The ester-sulfate-S pool is related to

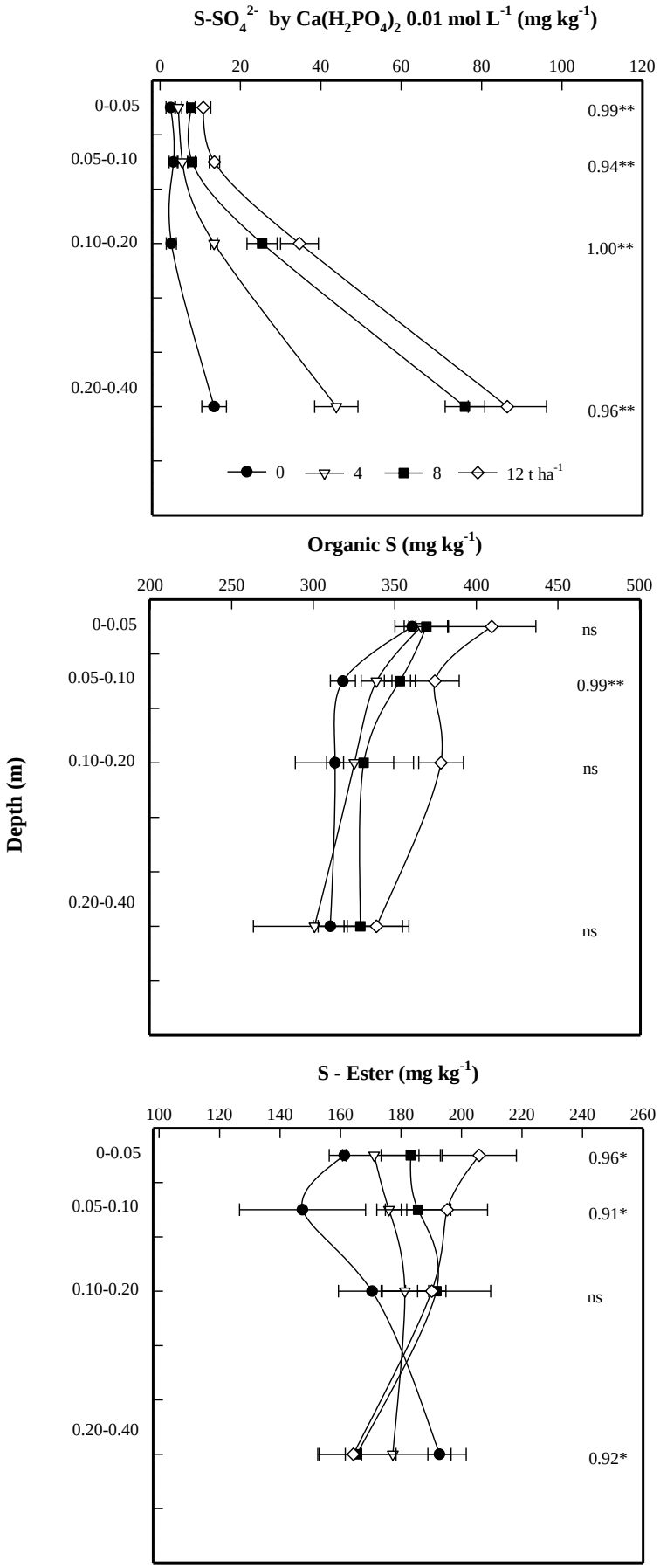

Figure 3 - Inorganic S extracted by 0.01 M $Ca(H_2PO_4)_2$ and the fractions of organic S and S bounded to ester calculated based in this content of inorganic sulfate (soluble + adsorbed S). *: $p \leq 0.05$, ** $p \leq 0.01$ and ns: non-significant

be more reactive than the C-bonded S and it forms more rapidly after S additions, as shown by Ghani; McLaren and Swift (1993), using ^{35}S labeling. The incorporation of S into ester sulfates is directly related to microbial activity, C and N contents (SCHINDLER et al., 1986; GHANI; McLAREN; SWIFT, 1992). In presence of available sulfate, some soil microorganisms have the capacity of storing intracellular S as choline sulfate (an ester sulfate) as demonstrated by Saggar, Bettany and Stewart (1981). These authors observed a gradual and significant increase in the HI-reducible S content of fungi with an increase in the amount of S in the growth medium. However, the result was not seen in bacteria, where 90% of the total S was found to be non-HI-reducible.

The decrease in the content of ester sulfate at 0.20-0.40 cm in this study is contradictory to these previous results presented in the literature and it is against the model of S cycling in soils proposed my McGill and Cole (1981). However some authors also report a decrease in the ester sulfur fraction with the application of inorganic S. Ghani; McLaren; Swift (1992) and De Bona; Monteiro (2010) determined the ester sulfate correcting for phosphate-extractable S (soluble + adsorbed), but they utilized soil from the most surface layer (0-0.10 and 0-0.20m), different from our study in which this result was found only at the 0.20-0.40 m-layer. Ghani, McLaren and Swift (1992) justified their results based on N limitations in the soil, since they found a relationship between available N and HI-reducible forms of S. In our case, the lower contents of C and N in this layer (Figure 1), associated with the high S adsorption, could have been the cause of this contradictory result. Also, it is sometimes very challenging to understand if the results revealed by the wet-chemical fractionation of S truly represent changes in the chemical S composition or if they are just errors inherent to the method of determination, since the process is quite subject to interferences, and some fractions are taken by difference (FRENEY, 1986).

Different patterns were found when the extractant calcium phosphate is replaced by calcium chloride (Figures 3 and 4). The results of the Figure 4 are much more similar with the ones presented in the literature, so agreeing with the model proposed for sulfur cycling (McGILL; COLE, 1981). Comparing the absolute values of inorganic sulfate extracted by 0.01 M $CaCl_2$ (Figure 4) and by 0.01 M $Ca(H_2PO_4)_2$ (Figure 3), it is observed that much more sulfate was extracted by the calcium phosphate, especially in the 0.20-0.40 m depth. Neptune, Tabatabai and Hanway (1975) also extracted the S-sulfate by the ions Cl^- and PO_4^{2-} in Brazilian and Iowa soils and they found more sulfate extracted by the $Ca(H_2PO_4)_2$ in the Brazilian soils, indicating that most part of the sulfate in those soils were in adsorbed forms.

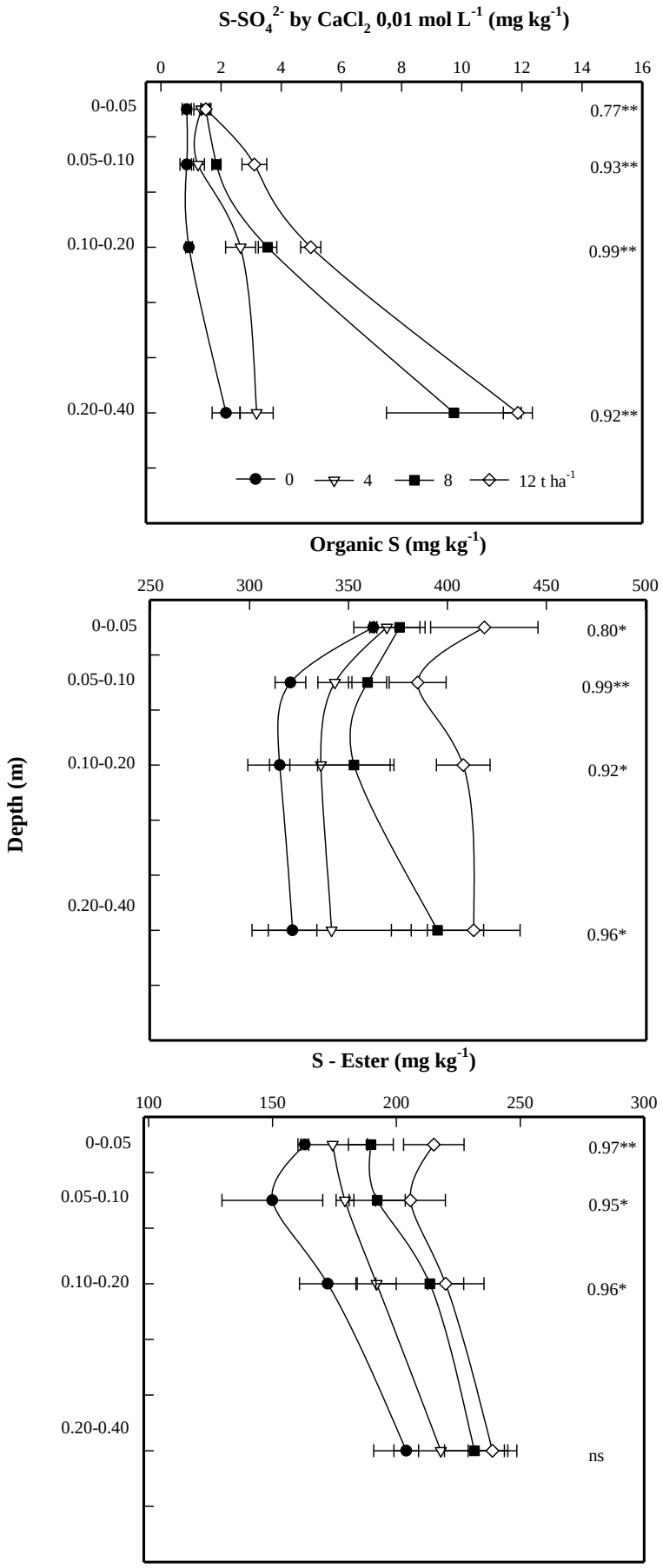

Figure 4 - Inorganic S extracted by 0.01 M $CaCl_2$ and the fractions of organic S and S bounded to ester calculated based in this content of inorganic sulfate (soluble S). *: $p \leq 0.05$, ** $p \leq 0.01$ and ns: non-significant

As the soil used in our experiment is a highly weathered soil with high amounts of Fe and Al oxides/hydroxides, the adsorbed fraction of sulfate should be seriously considered.

By means of the Langmuir isotherms we have estimated the maximum S adsorption capacity for the soil used in this study for the 0-0.20 and 0-0.40 m. We have found 133 mg kg^{-1} as the maximum adsorption for the 0-0.20 layer, and 238 mg kg^{-1} as the maximum adsorption for the deeper layer. As many results of sulfur fractionation are presented for superficial layers of temperate soils, we hypothesized that maybe the HI reducing mixture did not capture the entire adsorbed S and underestimated the results for humid tropical soils. Based on that, after the extraction with 0.01 M $Ca(H_2PO_4)_2$, the soil was placed in the oven to dry at 105 ºC and then reduced using the HI mixture. These results were compared with the ones only digested by HI, without the previous extraction (figure 5), but the results were very similar, showing that there was no underestimation of S by the HI mixture. Another point is that small amounts of organic soluble S are also extracted during the process of extraction of S by water or salt solution (BEATON; BURNS; PLATOU, 1968; STANKO-GOLDEN; FIZGERALD, 1991).

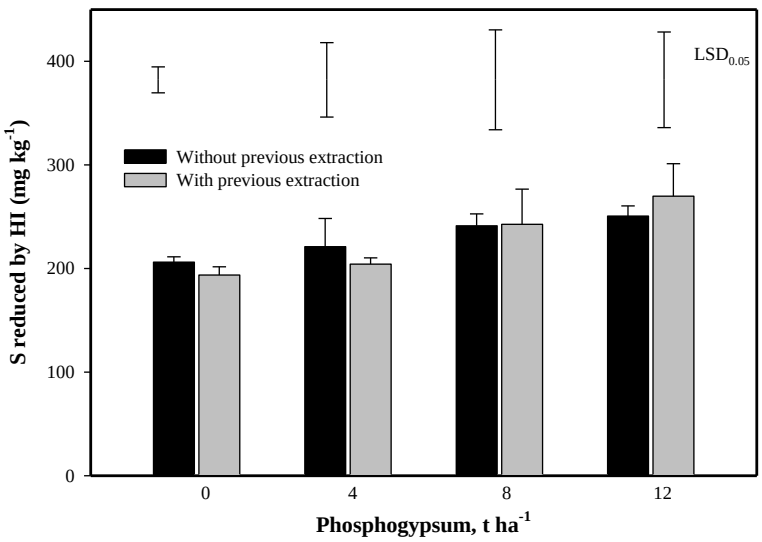

Figure 5 - S reduced by HI with and without previous extraction by calcium phosphate (0.01 M $Ca(H_2PO_4)_2$) solution. These results are presented only for the depth of 0.20-0.40m

When using $CaCl_2$ or $Ca(H_2PO_4)_2$ solutions, anions will displace the adsorbed sulfate, and calcium ions depress the extraction of soil organic matter, thus eliminating contamination from extractable organic S (BEATON; BURNS; PLATOU, 1968). Nevertheless, if the calcium phosphate had extracted significant amounts of organic S in this experiment, the controversial results would appear in the soil surface where larger amounts of organic matter are present and not at 0.20-0.40 m as observed in this work. When the fractions S-ester and S-

organic were calculated based on the soluble S extracted by $CaCl_2$, they were more consistent, but the numbers were increased by up to 20% (Figure 4) compared to the ones calculated based on the soluble + adsorbed S (Figure 3).

It is important to mention that the best method is not the one that extracts the largest content, but the one that provides the more reliable value and that correlates to plant absorption. During the soil sampling, the area had been cultivated with triticale (x *Triticosecale*) (cv. IPR 111) in the flowering stage. Leaves were collected for analysis, and the results of S leaf concentration and soil extracted S were correlated using Pearson Product Moment. After four years of the PG application the concentration of S in the triticale leaves were linearly increased (R^2=0.97**) by the treatments. Both extractants revealed good correlation between the S present in the leaves and the S extracted by the soil, especially in the first depths (Table 2).

Table 2 - Correlation between the concentration of S in the soil extracted by two extractants and the concentration of S in triticale leaves, cv. IPR 111

Depth	**Extractant**	
(m)	**0.01 M $Ca(H_2PO_4)_2$**	**0.01 M $CaCl_2$**
0-0.05	0,73**	0,69**
0.05-0.10	0,67**	0,69**
0.10-0.20	0,68**	0,82**
0.20-0.40	0,65**	0,49*
0-0.20	0,71**	0,85**

*: $p < 0.05$, ** $p < 0.01$

At 0-0.20 m-layer, however, calcium chloride presented higher coefficient of correlation compared to calcium monophosphate. Comparing 0.01 M $CaCl_2$ with another extractants for soils of Northest US, Ketterings et al. (2011) also verified that the best correlation between soil sulfur test and S fertilizer application was found for this extractant. On the other hand, when considering the S fractionation, some attempts have to be made to express the correct result of the fractions taken by difference, like the organic S and the ester S (PRIETZEL; HIRTCH, 2000). In the present study, the organic S (calculated based on the S extracted by $CaCl_2$) was overestimated, corresponding to more than 97% of the total S, even in the deepest layers (Figure 4). This is not expected for a soil presenting a large amount of Fe and Al oxides/hidroxydes and a high S adsorption capacity.

Despite the controversial results regarding the extractants, we could clearly verify in this experiment both mechanisms of sulfate retention in soils. It may occur through adsorption, in which sulfate becomes incorporated as sulfate-S into organic matter or it is associated with Fe and/or Al oxides or hydrous oxides (STANKO-GOLDEN; FITZGERALD,

1991). A high incorporation of added inorganic S in organic S fractions would increase the long-term availability of the nutrient by preventing losses by leaching and benefiting crops in medium/long term rotation.

3.3.2 Effects of land use on C and N contents and the sulfur pools accessed by the wet-chemical fractionation

Compared to the native forest site, there was depletion in the organic C and in the total N contents with the cultivation (Table 3). C and N depletion are associated with soil disturbance and reduced inputs. Decrease in SOM stocks due to conventional soil cultivation is a well-known phenomenon that is more pronounced in humid tropical and subtropical than in temperate regions (DALAL and MAYER, 1986). Is therefore not surprising that NT system had a positive impact in soil organic carbon (SOC) contents because of high crop-residue input and the lack of soil disturbance (SÁ et al., 2001).

Losses of sulfur due to cultivation were bigger (35.0%) than losses from C (13.7 %) and N (34.5 %) in the 0-0.40 m layer evaluated (Table 3). These results contradict the ones found by Solomon et al. (2001; 2005), that observed lower depletion of S comparing to C and N, suggesting that S is more resistant to mineralization. Because of its high retention, it presents lower susceptibility of losses from the ecosystem. The results obtained in the present study, however, suggest that the adoption of NT system for 19 years was able to preserve a large amount of C by the high addition of crop residues, but there was an intense removal of N and S by the crops and by leaching, indicating the necessity of a better crop rotation, including more legume plants and the constant supply of S by fertilizers.

Some considerations have to be made concerning the forest site area, since the natural vegetation that dominated the area before the conversion to agriculture was a subtropical prairie dominated by C4 species (MAACK, 1981). Since there was no natural prairie near the experiment, we choose the subtropical forest site area as a reference of undisturbed site to compare the land use effects on the S pools. However, results must be carefully interpreted, since the agricultural area did not come directly from a forest conversion.

We were able to observe differences in the total and organic sulfur contents in all depths evaluated (Table 3). Contents of S bonded to ester were affected by the treatments but the fraction bonded to C did not differ between the forest site and the cultivated area ($p < 0.05$) with or without PG application. Inorganic S (extracted by 0.01 M $Ca(H_2PO_4)_2$), that is the fraction of S most absorbed by plants, was also affected by the treatments. The inorganic S depletion in the soil surface layer agrees with the results presented previously (Table 3) and

Table 3 - Comparison between C and N contents, different amounts of S forms and C/N and C/S ratios in the native forest, no-till cultivation area and no-till after 4 yr. application of 12 t ha^{-1} of phosphogypsum

Depth (m)	Forest	No-tillage without PG	No-tillage with PG
		Organic C (g kg^{-1})	
0-0.050	60.1 b	43.5 a	45.6 a*
0.05-0.10	39.0 b	34.8 a	37.8 ab
0.10-0.20	34.3 a	32.0 a	34.7 a
0.20-0.40	32.8 a	29.5 a	30.7 a
		Total N (g kg^{-1})	
0-0.05	4.2 b	2.6 a	2.9 a
0.05-0.10	2.6 b	1.9 a	2.0 a
0.10-0.20	2.3 b	1.5 a	1.7 a
0.20-0.40	2.2 b	1.3 a	1.4 a
		Total S (mg kg^{-1})	
0-0.05	532.7 b	363.3 a	420.1 a
0.05-0.10	499.1 b	321.5 a	388.1 a
0.10-0.20	403.1 a	316.2 a	412.0 b
0.20-0.40	428.0 b	323.9 a	425.2 b
		Inorganic S (mg kg^{-1})	
0-0.05	14.6 b	2.7 a	10.8 ab
0.05-0.10	18.8 b	3.4 a	13.5 ab
0.10-0.20	18.6 b	2.8 a	34.7 c
0.20-0.40	17.9 a	13.4 a	86.4 b
		Organic S (mg kg^{-1})	
0-0.05	518.1 b	360.7 a	409.4 a
0.05-0.10	480.2 b	318.2 a	374.5 a
0.10-0.20	384.5 a	313.4 a	378.3 a
0.20-0.40	410.1 b	310.5 a	338.7 a
		HI-reducible S (mg kg^{-1})	
0-0.05	267.0 b	163.9 a	216.2 ab
0.05-0.10	278.1 b	150.8 a	208.8 ab
0.10-0.20	299.7 b	173.2 a	224.8 a
0.20-0.40	268.2 b	206.1 a	250.6 b
		Ester S (mg kg^{-1})	
0-0.05	252.4 b	161.2 a	205.8 ab
0.05-0.10	259.3 b	147.4 a	195.2 ab
0.10-0.20	281.1 b	170.4 a	190.2 a
0.20-0.40	250.3 b	192.7 a	164.2 a
		C-bonded S (mg kg^{-1})	
0-0.05	265.7 a	199.5 a	203.5 a
0.05-0.10	220.9 a	170.7 a	179.3 a
0.10-0.20	103.5 a	143.0 ab	188.1 b
0.20-0.40	159.8 a	117.8 a	174.5 a
		C/N	
0-0.05	14.3 a	16.6 b	16.3 b
0.05-0.10	15.0 a	18.3 b	19.0 b
0.10-0.20	15.2 a	21.2 b	20.4 b
0.20-0.40	15.2 a	22.5 b	22.0 b
		C/S	
0-0.05	113.9 a	119.7 a	119.7 a
0.05-0.10	79.3 a	108.2 b	97.4 b
0.10-0.20	86.3 a	101.3 a	84.4 a
0.20-0.40	76.6 a	91.3 a	72.4 a

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

has been probably caused by the high absorption of S by crops, even though it may be leached, due to high contents of organic C, low acidity and presence of another anions, like

phosphate. With the application of PG, there was an increase in the inorganic S fraction comparing with the area without application and more S-SO_4^{2-} was found in the lower depths, even after 4 years. The fact of the area under cultivation presents lower amounts of S than the forest soil can be explained by leaching losses due higher pH (Table 1) and S repulsion by negative charges of organic matter functional groups and the application of P fertilizers (CAIRES et al., 2011).

C/N and C/S ratios were not affected by PG application (Table 3), but there were differences between the cultivated area and the forest site. The wider ratios observed in the cultivated area (with and without PG application) in comparison to the forest are contrary to the results presented for studies carried out in both temperate (WANG et al., 2006) and tropical soils (SOLOMON et al., 2001). They reported decrease of C/S and N/S ratios with the cultivation, indicating that soil organic sulfur is more resistant to mineralization than soil organic carbon in those ecosystems. We can attribute these differences between our study and the literature by the fact that the cultivated site did not come directly from the forest and the litter composition in the NT area and the forest site vary considerably. Soils under NT present higher contents of C due to qualitative and quantitative changes in soil organic matter (ARSHAD et al., 1990), presenting higher aliphatic C and containing less aromatic C.

In the present study, most of the organic S present in the soil was bonded to ester (Figure 6). The possible origin of these compounds in soils are arylsulfates, sulfate esters of steroids, amino acid O-sulfates, ascorbic acid 2-O-sulfate in animal excreta, chondrointin sulfate in many invertebrates that inhabit soils, alkylsulfate esters in a variety of microorganisms, and choline sulfate in lichens, algae, fungi and plants (FRENEY, 1986). In studies conducted using the wet-chemical fractionation, it was also found that esther-SO_4 sulfur was the dominant form of organic S in most mineral soils (TABATABAI; BREMNER, 1972; NEPTUNE; TABATABAI; HANWAY, 1975; DE BONA; MONTEIRO, 2010). Nevertheless, studies conducted in temperate (WANG et al., 2006), tropical (SOLOMON et al., 2001) and subtropical climates (SOLOMON et al., 2005) found that C-bonded S was the dominant organic S fraction and constitute the biggest source of S depletion in these ecosystems.

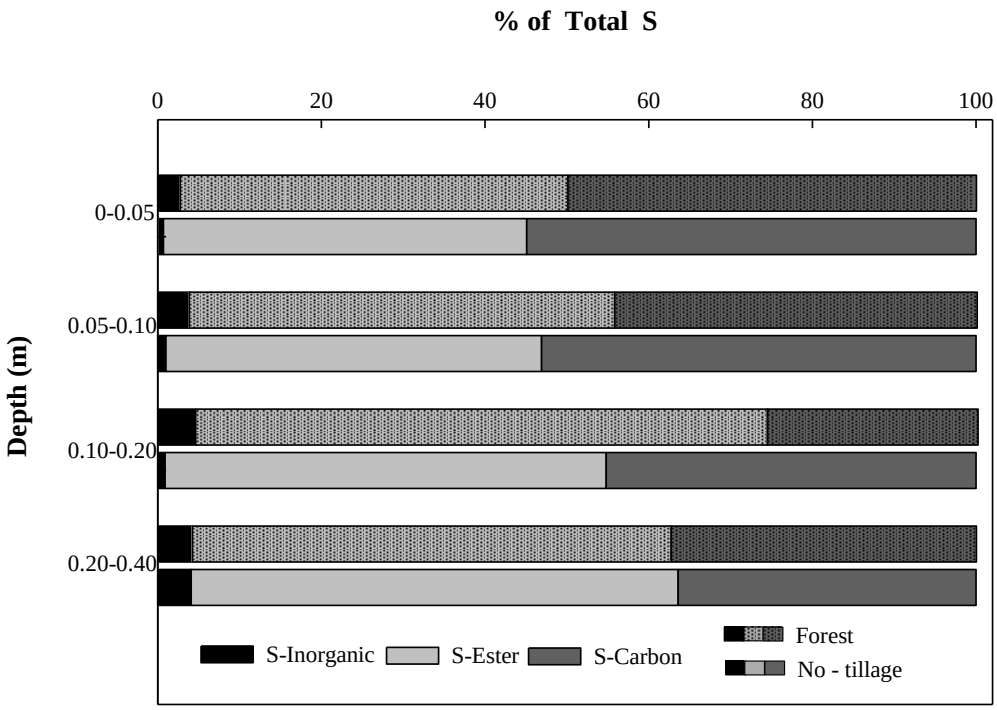

Figure 6 - Percentual distribution of the S forms in the native forest and in the no-till cultivation area

3.3.3 S K-edge XANES characterization of organic-S forms in the soil extracts

Synchrotron-based XANES spectroscopy at the S K-edge is a powerful tool that helps to determine the various oxidation states of S based in the energy position of the called "white-line" resulting from the s→p photoelectron transition. This occurs because of a loss of screening of the inner-shell electrons from the nuclear charge, which produces a larger decrease in the energy of the "s" level than the "p" level (HUFFMAN et al., 1991). The white-line of sulfur increases with the formal oxidation number of the sulfur ion (Figures 7, 8). In our experiment the peak positions varied from 2473.4 eV to 2483.4 eV, and Table 4 summarizes the peak position of the S compounds utilized in this experiment. XANES technique reflects the distribution of electrons in the valence shell of S atoms in their actual bonding environment, thus can be substantial differences between electronic and formal oxidation states (Table 4) of the S atom, especially for reduced S species in complex organic materials (XIA et al., 1998; MARTÍNEZ et al., 2002). Therefore, we reported the electronic oxidation states rather than the formal oxidation states as they reflect the actual electron density in the valence shell of S (SOLOMON et al., 2003; 2005; 2009; 2011). Likewise, XANES spectra did not allow us to distinguish between inorganic SO_4^{2-} and ester sulfate (Figure 7, Table 4), because the S electronic oxidation states and the peak energies of organic and inorganic S are identical.

We compared the s→p transition peaks obtained in this experiment with some data presented in the literature for S compounds (Table 5). The absolute values for each measurement of the same S compound differ slightly from one to another, since the measurements were taken at different beamlines, and samples in different states were used. In our experiment we utilized pure standards, as did Prietzel et al. (2007). Almkvist, Boye and Persson (2010) measured the S compounds in aqueous solution, and Prietzel et al. (2011) prepared S compounds diluted with quartz to achieve a final S concentration of 2 mg g^{-1}. The physical state of a model S compound also affects its S K-edge XANES spectrum, since S species in aqueous solutions display sharper features than for solids because of self-absorption in the particles (JALILEHVAND, 2006). On the other hand, is important that the standard XANES spectra should be obtained from sulfur groups in a similar state and surrounding as in the sample (JALILEHVAND, 2006).

Our baseline-corrected and normalized XANES spectral features recorded from soil humic fractions consistently had several white-lines in the energy range of 0 to 10 eV above the S K-edge (Figure 9). The qualitative interpretation of the data revealed several oxidation states (from -2 to +6) of the S atom in the humic substances extracted from these soils under investigation. The spectra recorded from the humic substance extracts in the present experiment generally showed four major absorption bands in the energy ranges of 2472-2476 eV, 2477-2478 eV, 2480-2483 eV and 2483-2486 eV (Figure 9). These peaks were assigned to transitions of the S 1s core electrons to the lowest unoccupied antibonding states of the S atom and possibly to the various post-edge features generally attributed to multiple scattering resonances (FLEET, 2005).

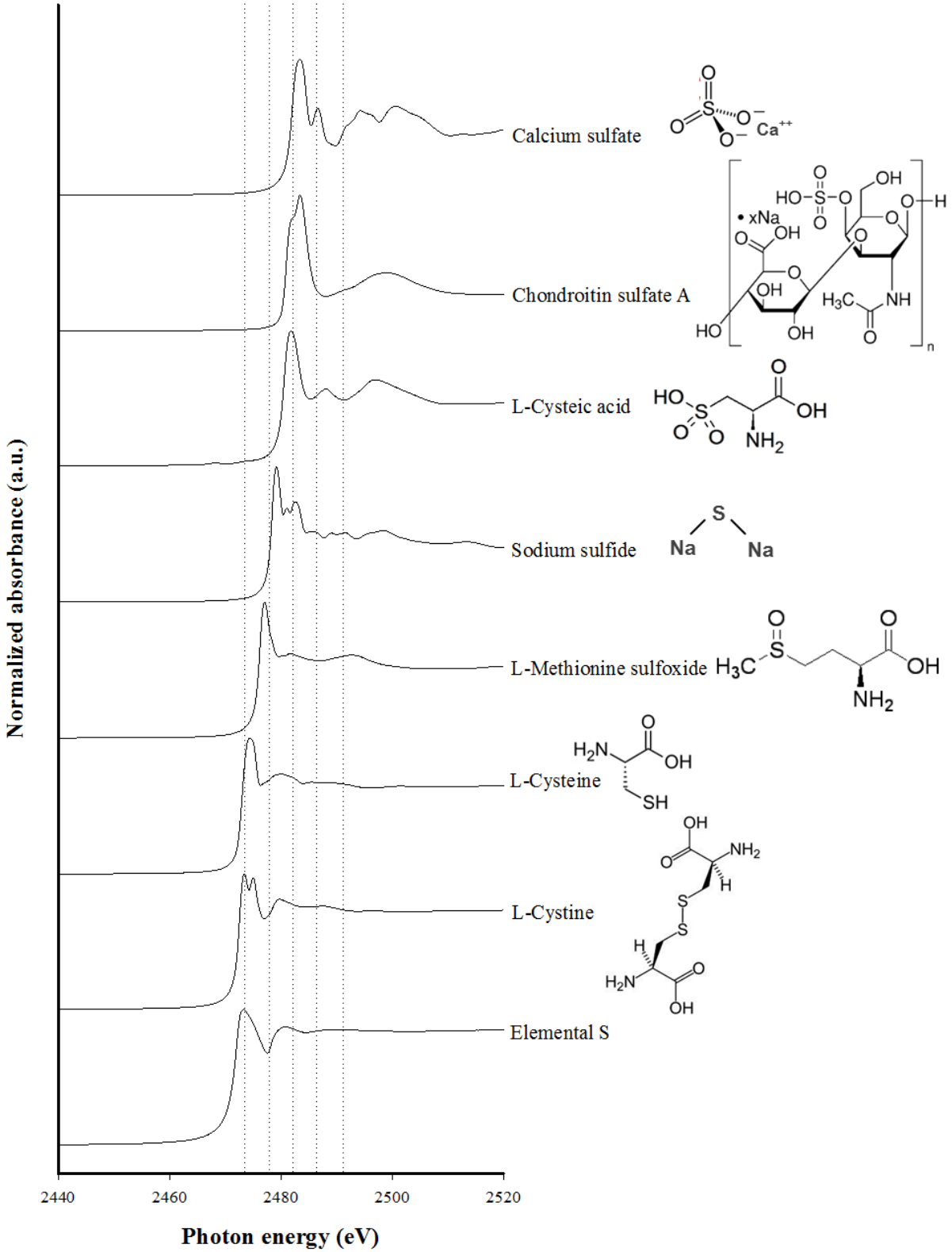

Figure 7 - Sulfur K-edge XANES spectra and structural formula of sulfur compounds in different oxidation states

Table 4 - Names, oxidation state, molecular formula, and experimental peak positions (eV) observed from Sulfur K-edge XANES Spectroscopy

Reference Compounds	Oxidation State	Molecular Formula	Energy positions	
			Peak 1	**Peak 2**
L-Cystine (-1)	+0.2	$C_6H_{12}N_2O_4S_2$	2473.40	2475.00
L-Cysteine (-2)	+0.5	$C_3H_7NO_2S$	2474.40	
Elemental sulfur	0	S	2473.40	
L-Methionine sulfoxide	+2	$C_5H_{11}NO_3S$	2477.00	
Sodium sulfide	+3.68	Na_2S	2479.20	2482.60
L-Cysteic acid	+5	$C_3H_7NO_5S.H_2O$	2481.78	
Chondroitin sulfate A	+6	$C_{14}H_{19}NO_{14}SNa_2$	2483.40	
Calcium sulfate	+6	$CaSO_4$	2483.40	2486.60

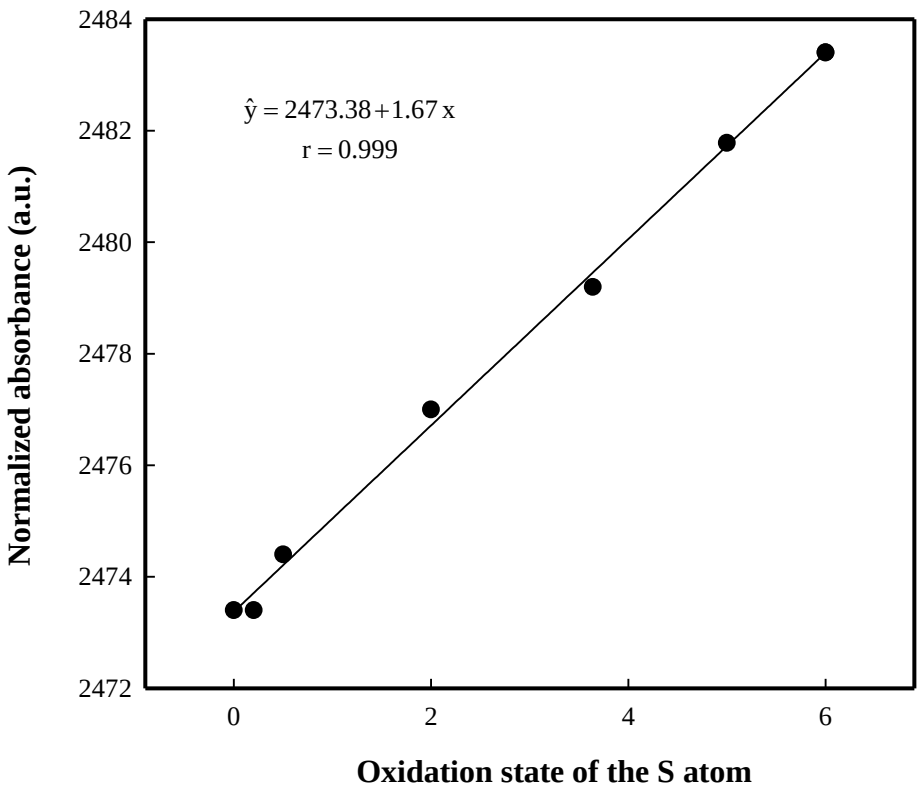

Figure 8 - Correlation between the oxidation state of the S atom in several S compounds and the peak energy of their white lines in XANES spectrograms

Table 5 - First order peak positions obtained by S K-edge XANES Spectroscopy in this study and some literature data

Reference compounds Common name	Vairavamurthy et al. (1997)	Xia et al., (1998)	Prietzel et al. (2003)	Prietzel et al. (2007)	Almkvist, Boye and Persson (2010)	Prietzel et al. (2011)	This study
L-Cystine	2473.0	2472.7	2472.6	2473.0	2472.6		2473.40
L-Cysteine	2473.5	2473.5	2473.4	2472.6	2473.4	2473.5	2474.40
Elemental sulfur	2472.5			2472.5	2472.7	2472.6	2473.40
L-Methionine sulfoxide	2476.0	2476.0	2475.8	2475.8		2476.2	2477.00
Sodium sulfide			2478.7	2478.7		2478.5	2479.20
L-Cysteic acid	2481.5	2481.2	2481.3	2481.3			2481.78
Ester sulfate	2483.5		2482.5	2482.5	2482.7		2483.40
Inorganic sulfate	2483.5	2482.6	2482.5	2482.5	2482.4	2482.7	2483.40

Based on the results of the relative energy position and predicted electronic oxidation states, we differentiated various oxidation states identified by our experimental S XANES spectra, fingerprinted the discrete organic S functionalities found in this soil extracts extracted from natural and cultivated areas and classified them into groups based on their oxidation states: (i) organic S functionalities present in strongly reduced (S^0 to S^{1+}) oxidation states, which include thiols, monosulfides, disulfides, polysulfides and thiophenes; (ii) organic S in intermediate (S^{2+} to S^{5+}) states that include sulfones, sulfoxides and sulfonates; and (iii) organic S present in strongly (S^{6+}) oxidized state, which represent ester-SO_4 sulfur (VAIRAVAMURTHY, 1997; SOLOMON et al., 2011). The inorganic S (also presenting oxidation state of S^{6+}) was not identified in these samples, even in those that received PG application, since the inorganic salts were eliminated during the purification (dialysis) of the humic substances.

For the humic substances extracted from the Control plots (without PG application) we observed that the S atom was present in reduced, intermediate and oxidized states and in the 0-0.10 cm depth the oxidized S atoms (S^{5+} and S^{6+}) are found in almost the same proportion. In samples from the 0.10-0.20 depth we observed more S in the oxidation state of S^{5+} compared to S^{6+}. On the other hand, the oxidized S atoms were found in almost equal proportion in the lower depth.

For the humic substances extracted in soil samples collected from the plots that received the application of phosphogypsum (12 t ha^{-1}) we observed less species of the most reduced and intermediate states (S^0 to S^{4+}) in the 0-0.10 m compared to the most oxidized ones (S^{5+} and S^{6+}), and we found that there are more sulfates (oxidation state of 6+) than sulfonates (S^{5+}). In 0.10-0.20 m depth the oxidized S species were prevalent but the proportion of sulfonates and sulfates were the same. In samples from the 0.20-0.40 m depth, less reduced forms of S were detected, and there were more sulfonates compared to sulfates. In the forest site we verified the dominance of the most oxidized S species (S^{5+} and S^{6+}) in comparison to the reduced ones, as also verified for the 0.10-0.20 m depth. Conversely, the proportion of oxidized S species was almost equal in soil samples from the 0.20-0.40 m. Comparing the area under native forest with the cultivated area with (PG 12 t ha^{-1}) and without (Control) PG application we verified that there were more oxidized S species (sulfate ester - S^{6+}) in the forest area and in the PG area than in the control, agreeing with the observed using the wet-chemical technique (Figures 2, 3 and 4 and Table 2).

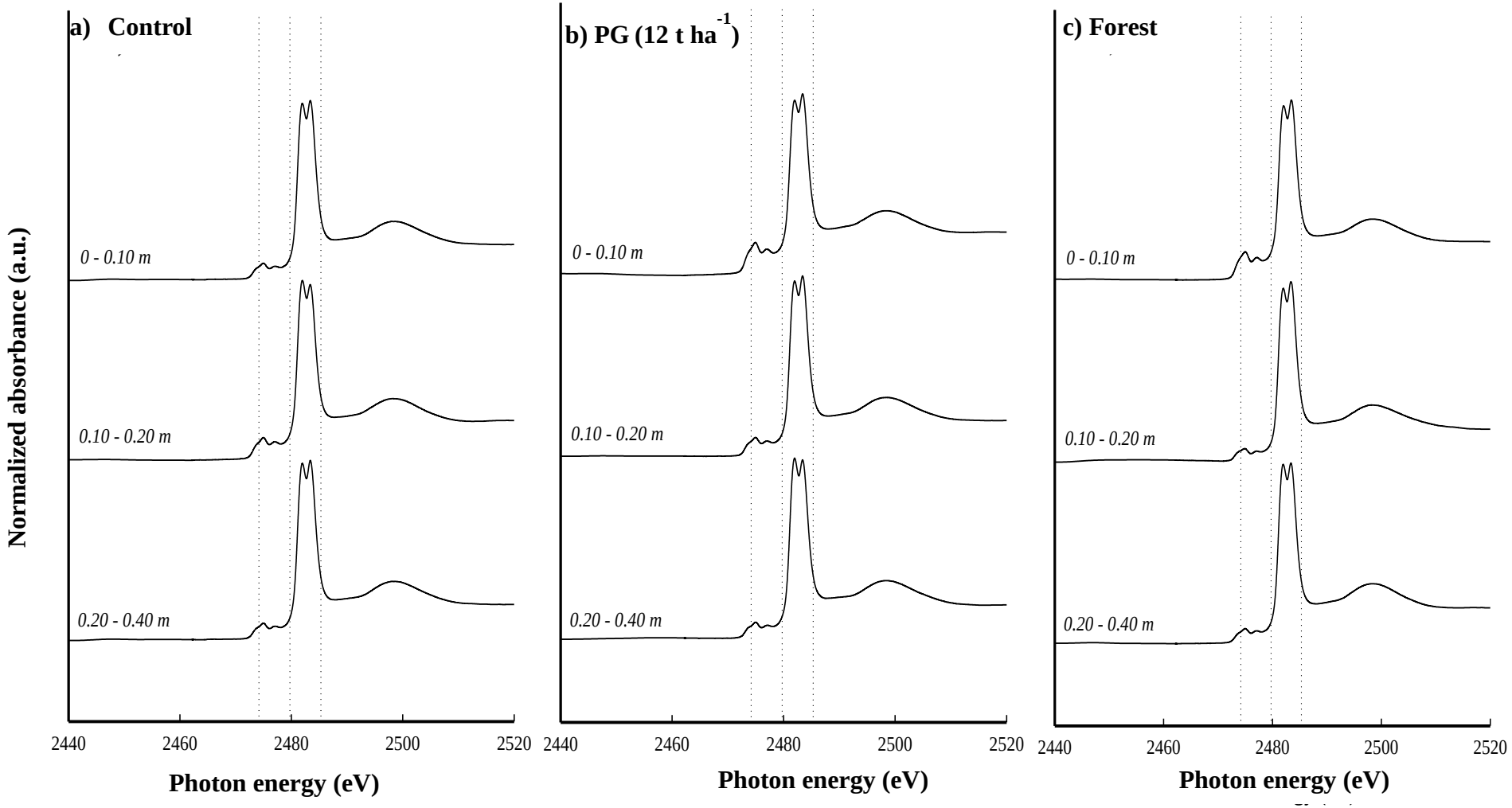

Figure 9 - S K-edge X-ray absorption near-edge structure spectra of the humic extracts of the soils collected under no-tillage system with and without phosphogypsum application and under native forest

In all areas evaluated in this experiment we verified the dominance of reduced and intermediate S species (S^0 to S^{5+}) over the strongly oxidized ones (S^{6+}). The first groups of organic S compounds - reduced and intermediate S species represent S directly linked to C in C-S or C-S-O linkages, as in the case of S-containing amino acids and sulfonates, respectively, and are commonly referred to in the conventional classification systems as C-bonded S, whereas S in highly oxidized state denotes organic S compounds where S is linked to C mostly through O atoms in the form of C-O-S linkage, as in the case of true ester sulfates ($C\text{-}OSO_3\text{-}C$).

By using XANES technique we were able to see the presence of more S bounded to C than sulfate esters in all depths evaluated, what contradicts the results obtained by the wet-chemical fractionation. One of the biggest critics concerning XANES spectroscopy is that it is generally applied to humic substances in order to improve the signal, since large background are verified when S XANES spectra is measured directly from the bulk soils, what makes very difficult the quantification of S species by standard programs (SOLOMON et al., 2003). Some authors say that the use of soil extracts may not reflect the in situ speciation of S in soil (ERIKSEN, 2009) and some artificial changes, like hydrolysis reactions or autoxidation can occur during alkali extractions (SHOENAU; BETTANY, 1987), demanding more caution when interpreting the results (ANDERSON; SHOENAU, 1993). However, studies using S K-edge XANES (HUTCHINSON et al. 2002) failed to detect any change in the oxidation states, as well as accompanying structural composition of organic S in humic substances extracts aerated at various pH levels for up to 44 h. The dominance of the C-S in the soil extracts observed in our study is in agreement with another results found using XANES spectroscopy applied to humic substances (XIA et al., 1998; MARTINEZ et al., 2002; PRIETZEL et al., 2003; SOLOMON et al., 2003; 2005; 2009; 2011), and revealed that S directly linked to C is the predominant form of organic S functional groups.

3.4 Conclusions

When compared to the forest site, the soils under no-tillage (NT) system, presented lower amount of organic C, total N and total S and the sulfur losses due to cultivation were bigger than losses from C and N suggesting that the NT was able to preserve a large amount of C by the addition of crop residues, but there was an intense removal of S by the crops, since no S fertilizer had been applied in the area. The decrease of S concentration due tillage occurred mainly by the losses from ester SO_4^{2-}-S forms supporting the conventional view that ester sulfates, which can be stabilized regardless of the main moiety of organic matter,

represents slightly more labile organic S fractions that can serves as readily available S pool through biochemical mineralization processes.

By means of XANES, we observed that there were more oxidized S species (sulfate ester - S^{6+}) in the forest area and in the PG area (rate of PG = 12 t ha^{-1}) than in the control treatment (agricultural area without PG application), in accordance with the observed data obtained by the wet-chemical technique. However, using XANES technique for the speciation of organic sulfur, we were able to see the presence of more S bounded to C than sulfate esters in all depths evaluated, contradicting the results obtained by wet-chemical fractionation.

It is concluded that changes in land-use practice and PG application alters both quantitative and qualitative composition of S functional groups in this wet subtropical Brazilian soil under NT. The use of fertilizers containing S seems to be vital for the recovery of S moieties.

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4 SUBSTRATE COMPOSITION AND SHORT-TERM DYNAMIC OF S FRACTIONS IN SOILS UNDER NO-TILL SYSTEM

Abstract

Plant residues are the major source of plant-available sulfur in soils under no-till (NT) system, but the transformations that lead to the net availability of S in this system are not well understood. In this study, the effect of sources of plant residue (black oat - *Avena strigosa* , pea - *Pisum sativum*, rape - *Raphanus sativus*, wheat - *Triticum aestivum*, corn stalks - *Zea mays* and corn stalks biochar, applied on sulfur basis) on C, N and S mineralization/immobilization was monitored in soil samples under NT. The sources of S mineralized in the soils during the incubation were determined after the peak of microbial activity and at the end of the 90-days incubation. Rape residues provided the highest cumulative normalized CO_2 evolution, as well as the highest cumulative N mineralization. Contents of inorganic S gradually increased during the incubation, and most part of the mineral S appeared in the initial extraction. The highest leaching of sulfate occurred after the application of biochar, and the main mechanism involved in this process was the abiotic release of sulfur. C, N and S mineralization were related to the C/S ratio. High correlation coefficients between C and N, C and S and N and S mineralization were observed when only the plant residues were considered. However, when the biochar was included, no significant correlations were achieved. The immediate increase in phosphate-extractable S upon the incorporation of the plant residues and biochar to the soil was related to the S forms in the starting materials as revealed by X-ray Near Edge Structure (XANES) spectroscopy. It reinforces the hypothesis that S forms rather than S concentration in the tissue plays a major role in S mineralization. The incorporation of plant residues and biochar led to an increase in the contents of ester sulfate in the early stage of incubation, which was further mineralized and had a steady decline at the 90th day of evaluation.

Keywords: Oxisol; Hydriodic Acid reduction; Wet-chemical S fractionation; S-Ester; S-Carbon; Biochar

4.1 Introduction

Along nitrogen and phosphorus, sulfur (S) is a plant nutrient and it is responsible for the synthesis of proteins and a number of essential vitamins and cofactors (KERTESZ; MIRLEAU, 2004). It contributes to increase crop yields improving direct and indirect nutritive value and ameliorating the use efficiency of other plant nutrients, like nitrogen (DE BONA; MONTEIRO, 2010). From the plants' point of view, the most important form of sulfur is the inorganic S that generally accounts for less than 10% in temperate (ERIKSEN, 2009) and humid tropical systems (NEPTUNE; TABATABAI; HANWAY, 1975; SOLOMON et al., 2001). Sulfur deficiency to plants has been reported in several parts of the world (KOST et al., 2008; SCHERER, 2001; 2009) and has been happening mainly due to the reduction of sulfur dioxide emission in the past years (LEHMANN et al., 2008), the use of highly concentrated fertilizers that contain little or no S, beyond the increase of S removal

from soils under intensive cropping systems and increased crop yields. Most part of the S in soil environments is bound to organic molecules, making up more than 90% of the sulfur present in soils. Despite plants absorb S mainly as sulfate, organic sulfur (S bonded to carbon and S bonded to ester) pools may be important sources of S to plants during their growing season (FRENEY et al., 1975; McGILL; COLE, 1981; GOH; PAMIDI, 2003; DE BONA; MONTEIRO, 2010). The process of transformation of organic sulfur to inorganic sulfate (mineralization) and the reverse process (incorporation of sulfate into soil organic compounds) plays important roles in the cycling of S within the soil and are microbiologically mediated (KERTESZ; MIRLEAU, 2004).

In agricultural systems, plant residues are the major source of organic inputs to soil (WU; O'DONNELL; SYERS, 1993), and represent the primary substrate for the replenishment of the organic matter (OM) and nutrients (JANZEN; KUCEY, 1988). Conservation agriculture in the form of no-till (NT) has enabled many areas earlier affected by erosion and nutrient depleted been sustainable and productive again (TRIPLETT; DICK, 2008). Crop rotation is a very common practice in NT systems, adding residues from different species to soil, reducing water erosion, providing OM and nutrients that can be used by the subsequent crops after the mineralization. Thus, this practice can affect sulfur cycling in soils since different amounts of C, N and S are added in the system. No-till has rapidly growth since about 1990 (DERPSCH; FRIEDRICH, 2009) and nowadays it is been adopted worldwide. In Brazil, the adoption of NT system started in the 70's due to accelerated erosion process in the southern states (TRIPLETT; DICK, 2008). Nowadays, Brazil is one of the leading countries in its adoption, having more than 60% of the production of grains obtained from this system (DERPSCH; FRIEDRICH, 2009).

The S mineralization in the soil is related with different initial S concentration (JANSEY; KUCEY, 1988), and S forms (SINGH; RENGEL; BOWDEN, 2006) in plant residues. Singh, Rengel and Bowden (2006) attributed rapid flow of sulfate immediately after application of canola (*Brassica napus*) residues to the form of S present in its tissues (soluble sulfate and readily degradable organic S forms). Since C, N and S play an important role in the sulfur cycling in agricultural systems; we can expect differences between plant residues and their chemical composition in the decomposition and in the transformations of sulfur pools in the soil. So, it is important to evaluate the changes in soil S pools after adding sources of C, N and S. Therefore, the objectives of this study are (i) to investigate C, N and S mineralization/immobilization in NT affected by sources of plant residue; (ii) to evaluate S species dynamics during decomposition identifying the major transformations in the fractions

S bounded to carbon and sulfate ester. Such studies will provide valuable information for modeling of S transformations in soil and for the management of crop residues under NT systems.

4.2 Material and Methods

4.2.1 Soil samples

The soil samples chosen for this experiment came from a clayey Typic Hapludox in the southern Brazil (Guarapuava, state of Parana - 25°17’ S e 51°48’ W, 997m) that has been cultivated under NT system for 19 years. The same soil was collected in a forest site near the cultivated area, to serve as a reference of undisturbed system. The cultivated area had recorded high crop grain yields in the last years due to the adoption of adequate varieties, agronomic practices and nutrient inputs, but the soil sulfur content were low since no source of S had been applied in the past years. Samples (0-5 cm) were collected in October 2010 from the forest area and from the control plots of an experiment that evaluated the benefits of phosphogypsum application (CAIRES et al., 2011) on soils and crops in rotation. The forest site was in a fragment of the ombrophilous (high-rainfall tolerant) mixed forest dominated by Parana pine (*Araucaria angustifolia*) also referred to as *Araucaria* forest (VELOSO; RANGEL FILHO; LIMA, 1991).

We opted by collecting the 0-0.05m depth since it is the layer most affected by the deposition of plant residues in NT, consequently presenting higher biological activity. The soils were sieved (through a 2 mm sieve), dried at 40 º C and brought to the laboratory, where a small portion was separated for the basic characterization. As a highly weathered soil, the mineralogy was mainly constituted of gibbsite and kaolinite, with presence of hematite, goethite, and some clay minerals with hydroxy interlayers. The soil had 592, 311 and 98 g kg^{-1} of clay, silt and sand, respectively; 168.0 g kg^{-1} of ditionite-citrate-bicarbonate (“free”) extractable Fe; 5.2 g kg^{-1} of oxalate (“amorphous”) extractable Fe and 10.7 g kg^{-1} of oxalate extractable Al. Other properties of both soil sites are shown in Table 1. The pH was measured in a 0.01 M $CaCl_2$ solution (soil solution ratio of 1:2.5). Organic Carbon and total Nitrogen were measured by dry combustion in a Thermo-Finnigan Flash 1112 elemental analyzer. Total Sulfur was obtained by alkaline digestion with NaOBr (TABATABAI; BREMNER, 1970) and colorimetrically quantified as bismuth sulfide (KOWALENKO, 1993a). Soluble plus adsorbed S was extracted by 0.01 M $Ca(H_2PO_4)_2 .H_2O$, which contains

sufficient phosphate to displace most of the adsorbed sulfur (FOX; OLSON; RHOADES, 1964). The inorganic sulfate was also determined by colorimetry as bismuth sulfide.

Table 1 - Selected properties of the Oxisols collected under a cultivated area under NTS and an adjacent forest in southern Brazil

Soils	pH 0.01 M $CaCl_2$	Total S	S $Ca(H_2PO_4)_2$	Organic C	Total N	C/N	C/S	N/S
		…….mg kg^{-1}……..		…….g kg^{-1}……				
Agricultural area	5.3	380.9	2.6	43.5	2.6	16.7	114.2	6.8
Natural Forest area	4.8	532.7	14.6	60.1	4.2	14.3	112.8	7.9

4.2.2 Experimental procedure and analytical techniques - Incubation and CO_2 evolution

The experiment was conducted using an open incubation system (STANFORD; SMITH, 1972; TABATABAI; AL-KHAFAJI, 1980; MAYNARD; STEWART; BETTANY, 1983; JANCEN; KUCEY, 1988; GHANI; McLAREN; SWIFT, 1992) that better represents the removal of the nutrients by plants and the leaching processes of these elements under moist field conditions (TABATABAI; AL-KHAFAJI, 1980) compared to the closed incubation system. We used 106-mL modified PVC filter units that were built attaching a nylon mesh sheet (250 μm opening) between two pieces of PVC tube. A glass microfiber filter and a piece of glass wool were added in the bottom of the PVC filter unit (VWR Scientific Products, West Chester, PA) to help in the leaching processes. Fifty grams of soil (passed through a 2 mm sieve) were homogeneously mixed with pre-acid washed sand (250-350 μm) in a 1:1 proportion (oven-dry basis). Fresh and pyrolysed crop residues (black oat - *Avena strigosa*, pea - *Pisum sativum*, rape - *Raphanus sativus*, wheat - *Triticum aestivum*, corn stalks - *Zea mays* and corn stalks biochar) were stirred into the soil and the water content was adjusted to 75% of the moisture content at field capacity (GHANI; McLAREN; SWIFT, 1992). The mixture was placed into PVC cylinders with a bulk density of 1.3 g cm^{-3} (Figure 1).

Plant residues were dried at 60 º C, grounded (< 2 mm) with a Wiley grinder and sealed in plastic bags before use. The biochar was produced using a slow pyrolysis (Daisy Reactor, Best Energies, Inc., Cashton, WI, USA). Approximately 3 kg of feedstock were manually placed into the reactor, which was thoroughly purged with N_2 (with the mixer running). The material was charred for 80 – 90 min, including rising temperature to the target with a few degrees per minute and holding at final temperature (450 ºC) for 15 – 20 min. Subsequently, the furnace was turned off, and the main chamber was allowed to cool before

collecting the biochar under N_2 purge to reduce rapid oxidation (leading to a more homogeneous product) and auto-ignition.

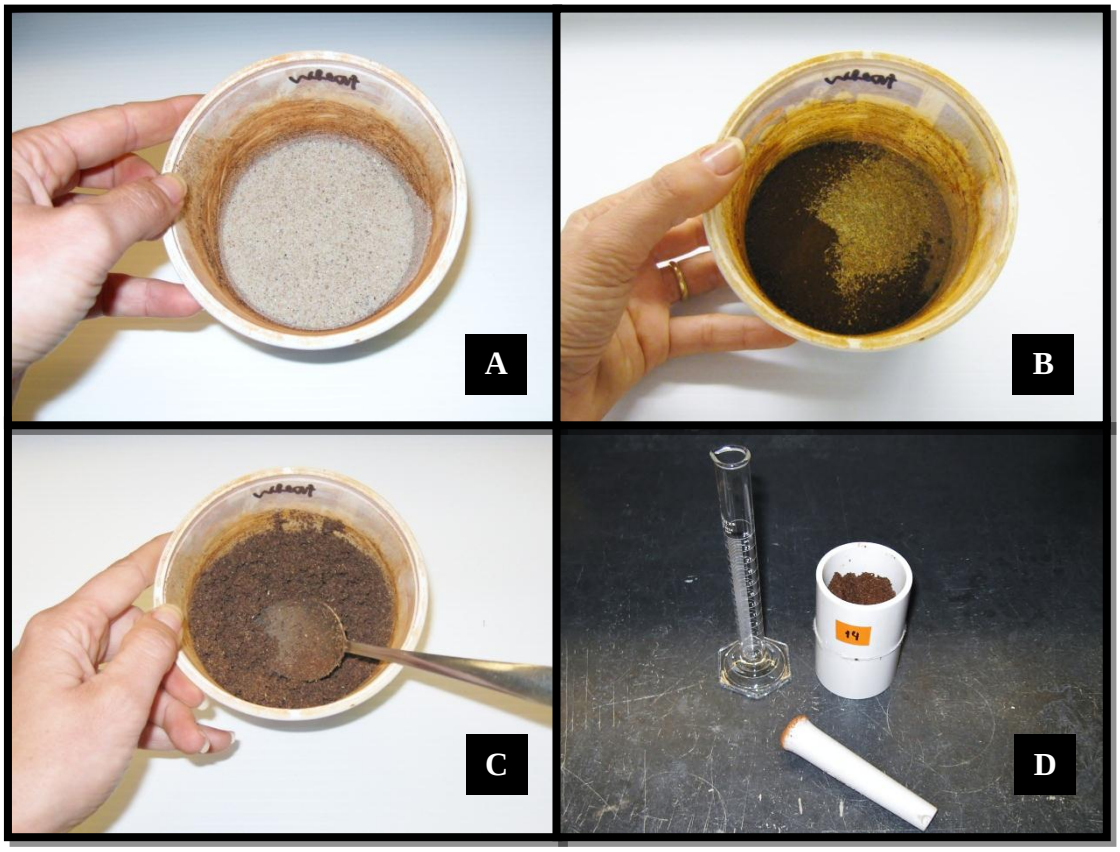

Figure 1 - Details of the open incubation experiment: Adding acid washed sand (A); Mixing with soil in equal weight and adding the plant residues (B); Bringing to 75% of the field capacity (C); Accommodating the material in PVC cylinders with a bulk density of 1.3 g cm^{-3} (D)

A portion of each residue was analyzed for some basic properties (Table 2). Contents of total C and N were measured by dry combustion using a CN-2000 (Leco Corporation, St. Joseph, MI, USA), and total S content was determined by nitric-perchloric digestion and measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES). 38 mg kg^{-1} of sulfur was applied (that corresponds to 10% of the total sulfur present in the soil) as plant residues or biochar. As the concentrations of sulfur vary in the plants and in the biochar, the amounts of residue applied to the soil followed a wide range of quantity varying from 0.3 g/100 g of soil (rape) to 8.8 g/100g of soil (corn stalks).

Table 2 - Selected properties of the materials used as sources of S

Materials	Total C	Total N	Total S	C/N	C/S	N/S
	.....g kg^{-1}……..					
Black oat	438.7	11.5	1.1	38.1	409.9	10.7
Wheat	437.3	11.1	1.4	39.5	308.4	7.8
Pea	435.6	31.9	1.8	13.7	239.3	17.5
Rape	414.8	30.0	11.1	13.8	37.3	2.7
Corn Stalks	426.2	5.3	0.4	80.4	991.2	12.3
Corn Stalks Biochar 450 ºC	673.0	11.2	0.8	60.3	851.8	14.2

The experiment was in a complete randomized design with three replicates. A total of 24 experimental units (8 treatments – black oat, pea, rape, wheat, corn stalks and corn stalks biochar; 1 control – soil collected under NT system; 1 forest soil used as a reference for comparison, and 3 replicates) plus 4 blanks (all the apparatus used for the experiment, but without soil or plant material) were placed into 500 mL sealed Mason Jars filled with 50 mL of deionized water in the bottom to maintain a water-saturated atmosphere (since the jars were periodically opened to collect the NaOH used to trap microbially respired CO_2) and the sealed jars were incubated at 30 º C in the dark. The HCO_3^- and CO_3^{2-} ions present in the 2 mL NaOH were precipitated with $BaCl_2$ solution, and total respired CO_2-C was determined by titrating the residual NaOH with 0.1 M HCl using phenolphthalein as indicator. Since the material was applied in sulfur basis, for the CO_2 evolution we normalized the data according to the equation 1:

$$\frac{C_{treat} - C_{control}}{C_{added}} \cdot \text{Average } C_{added} + \text{Average } C_{control} \qquad (1)$$

Where: C_{treat} : amount of carbon evolved by each treatment; $C_{control}$: amount of carbon evolved by the control (soil without addition of any other material); and C_{added} : amount of carbon added by plant material or biochar.

4.2.3 Experimental procedure and analytical techniques - N and S mineralization

The PVC cylinders were mounted on the top of filter units and mineral N and S were obtained by leaching the soils with 0.01 M $CaCl_2$ in 10 mL increments and the water excess was removed by vacuum filtration (60 cm Hg). A thin pad of glass wool was placed on the top of the soil to avoid disturb during the leaching events. Leachates were collected after 15, 30, 60 and 90 days of incubation. The extracts were passed through a Whatman number 42 filter paper (Whatman International, England) during the vacuum filtration and then filtered at 0.45 μm pore membrane (Pall Gelman Laboratory, Ann Arbor, Michigan, USA). The anions

SO_4^{2-}, NO_3^- and NO_2^- were determined by ion chromatography (IC) and the NH_4 was determined by the Indophenol Blue colorimetric method (KEENEY; NELSON, 1982).

4.2.4 Experimental procedure and analytical techniques - S fractionation

At 0, 3 (peak of microbial activity), and 90 days of incubation we collected soil samples to perform the sulfur fractionation according to Kowalenko (1993a; b) and obtained the fractions: S-sulfate, S-ester and S bonded to C. Content of total S was obtained by alkaline digestion (TABATABAI; BREMNER, 1970) and quantified according to Kowalenko (1993b). Soluble plus adsorbed S was extracted by 0.01 M $Ca(H_2PO_4)_2 .H_2O$ and measured by the colorimetric method as bismuth sulfide. Total organic S was calculated as the difference between Total S and phosphate extractable S. Sulfur bonded to ester was determined using HI digestion/distillation (KOWALENKO 1993b), corrected for phosphate extractable S. C-bonded S was calculated as the difference between total organic S and ester S.

4.2.5 X-ray Absorption Near-Edge Structure (XANES) spectroscopy analysis of the starting materials - plants and biochar

Solid-state characterization by XANES spectroscopy of S oxidation states in the plant samples and biochar was carried out using S K-edge XANES spectroscopy at beam-lines X-19A and X-15 B of the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory. Both materials were homogeneously mixed and finely ground using a ball grinder before the measurements. Experimental data at X-19A were collected under standard operating conditions. The X-ray energy were calibrated to the K-edge of elemental S at 2473 eV and scans ranging from 80 eV below to 150 eV above the absorption edge of S were collected with step size of 0.2 eV. We used a monochromator consisting of double-crystal Si (111) with an entrance slit of 0.5 mm and a minimum energy resolution of 2×10^{4}($\approx$ 0.5 eV) at the S K-edge. The monochromator was detuned to 70% at the S K edge in order to reduce fluorescence induced by high-order harmonics (XIA et al. 1998). The spectra were recorded in fluorescence mode using a passivated implanted planar silicon (PIPS) detector (Canberra Industries, Meriden, Connecticut, USA). The beam path from incident ion chamber to the sample chamber was purged with He gas. The samples were accommodated into a 0.5 mm thick acrylic holder and covered with 3.6 μm thick Mylar film (Chemplex Industries, Palm City, Florida, USA).

At X-15B, data from biochar sample was directly recorded under standard operating conditions. The X-ray energy was calibrated to the K-edge of elemental S at 2473 eV and

scans ranging from 80 eV below to 150 eV above the absorption edge of S were collected with step size of 0.2 eV. We used a monochromator consisting of two parallel Si (TRUST; FRY, 1992) crystals with an entrance slit of 0.5 mm and with a minimum energy resolution of 2×10^{4}(≈ 0.5 eV) at the S K-edge. High-order harmonics were eliminated by using a collimating/harmonic-rejection mirror upstream of the monochromator, rather than by detuning the monochromator. Beam was then focused to a 1 mm spot size in the sample. Beam path was in ultra-high vacuum (UHV) up to an ultra-thin Be window; the atmosphere in the sample chamber was He. Incident beam intensity was measured using a windowless He-filled ionization chamber. All spectra were recorded in fluorescence mode using a Canberra Ge detector (Canberra Industries, Meriden, Connecticut, USA) positioned 90° to the incident beam. The samples were accommodated in a thin polypropylene film (5μm thick) for XRF (Spex CertiPrep, Metuchen, NJ, USA) and attached to a thick paper sample holder. Background correction and normalization of the spectrum was done using the software WinXAS according to Xia et al. (1998), Martínez et al. (2002) and Solomon et al. (2003; 2005; 2009; 2011). The interpretation of the forms of S obtained by S K-edge XANES were made based on the qualitative data observed in each spectra.

4.2.6 Statistical analyses

All data was evaluated for normality using SigmaPlot v11 (Systat Software Inc., Chicago, IL). The appropriated analysis of variance (ANOVA) was carried out to determine the effects of plant residues on cumulative C, N and S mineralization. Where ANOVA indicated significant main effects, they were further analyzed using Tukey test ($p \leq 0.05$). Pearson Product Moment correlations and multiple regressions were used to explain the relationship between mineralization patterns and plant/biochar composition.

4.3 Results and Discussion

4.3.1 CO_2 evolution

Soil microbial activity, monitored as CO_2 evolution, varied widely among the treatments (Figure 2). During the 90 days of incubation, the cumulative amounts of C mineralized ranged from 2.65 (control) to 10.21 mg kg^{-1}(corn stalks). An apparent two-phase pattern of decomposition was observed (Figure 2). The fast and linear phase of CO_2 evolution occurred from the start of the experiment up to the 15th day, followed by a slow and more stabilized phase. CO_2 evolution increased in the untreated soils during the 90 days of the

incubation, presumably because of the decomposition of native OM and it was higher on the forest soil compared to the cultivated area ($p < 0.05$), since higher C contents and lower C/N ratios were found for this site (Table 1). Ghani, McLaren and Swift (1992) also associated the microbial activity to the native C content in the soils.

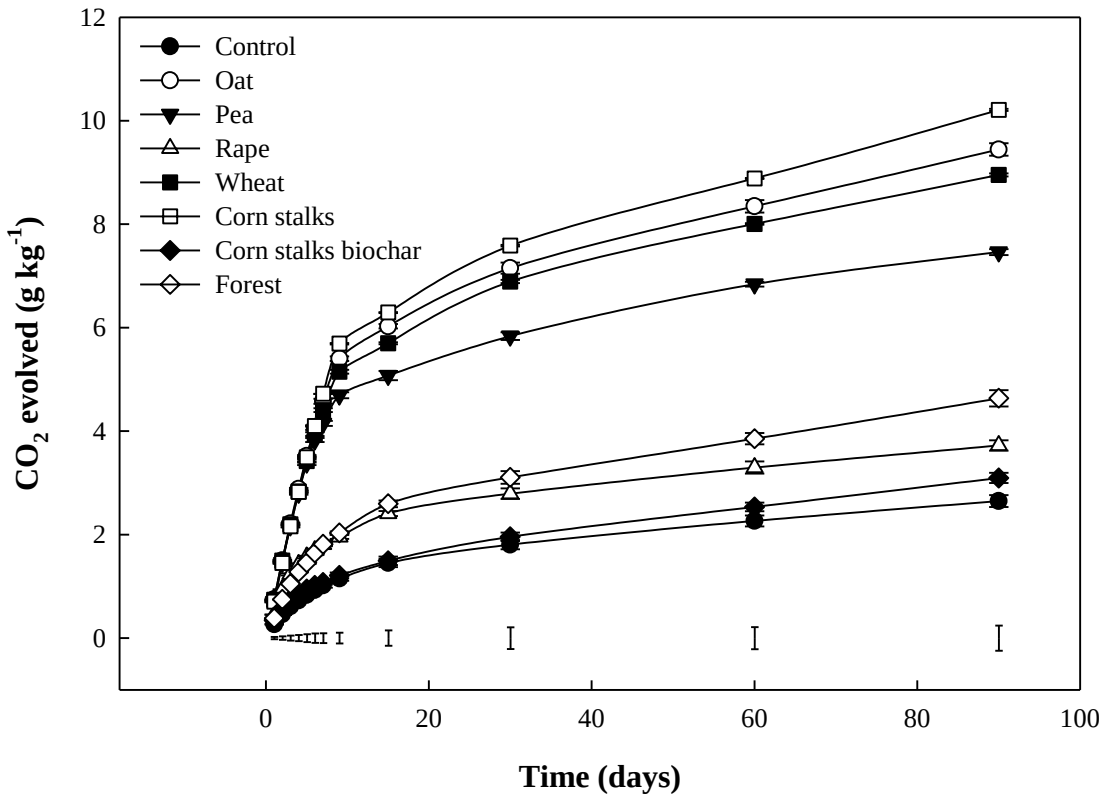

Figure 2 - Effect of the treatments on cumulative C mineralization during 90 days of incubation. Thick bars represent the $LSD_{0.05}$

Differences between corn stalks biochar and the untreated soil ($p < 0.05$) were verified only during the first 4 days of the incubation, where the biochar had increased the CO_2 evolution. Many researchers have shown positive effects of biochar application to soils, as improving soil quality (GLASER; LEHMANN; ZECH, 2002) as sequestering carbon in soils (LEHMANN; GAUNT; RONDON, 2006). Despite its high recalcitrance, as shown by the occurrence of black soils (called Terra Preta) in the Amazon basin (an environment that favors extremely rapid microbial decay), biochar application to soils have increased the CO_2 release (ZIMMERMAN, 2010) even in short-term incubations (JONES et al., 2011; LUO et al., 2011) . The increase in CO_2 release at the early stages of the incubation can be either completely derived from C contained in the biochar (SMITH et al., 2010; ZIMMERMAN, 2010; JONES et al., 2011) or attributed to positive priming effects - where biochar leads to a

stimulatory effect on soil carbon mineralization (LUO et al., 2011; ZIMMERMAN; GAO; AHN, 2011). In both cases, changes in CO_2 evolution are related to the differences in the nature of the biochar and to the conditions of the incubation.

Priming effect in the early incubation stage was observed in low carbon soils treated with biochar produced from grasses and at low temperatures (ZIMMERMAN; GAO; AHN, 2011) and for these biochars, the measurement of stable isotopic signature indicates that most part of respired CO_2 was predominantly pyrogenic. Jones et al. (2011) found that abiotic and biotic mechanisms are involved in the increase of CO_2 release derived from pyrogenic C: the release of C from mineral carbonate contained in the biochar and the breakdown of biochar soluble organic C. However, regardless of the proposed mechanism, this CO_2 release pathway only represents a small fraction of the C present in the biochar and does not compromise its potential to sequester C in soil (JONES et al., 2011). In the case of our study, we cannot know if there was a priming effect or not, since no tracers were used. However, our results are in agreement with Zimmerman; Gao; Ahn (2011), who observed that biochar made of corn stalks at relatively low temperature (450 ºC) had caused increases in CO_2 evolution at the early stages of the incubation.

For better comparison of the treatments, since they were applied in sulfur basis, we decide to normalize the data, expressing the CO_2 evolution by unit of C added (Figure 3). CO_2 production varied widely among plant species and the biochar. Highest rates of CO_2 production were observed in rape residues, followed by pea and wheat, and then oat, corn stalks and corn stalks biochar. The amounts of CO_2 evolved in our experiment were higher than the observed by Janzen and Kucey (1988) for the same crop species evaluated. In their case, there was a positive correlation between the amount of C mineralized and the N concentration of the crop residue, while a negative correlation was observed between respired C and C/N ratio and S concentration.

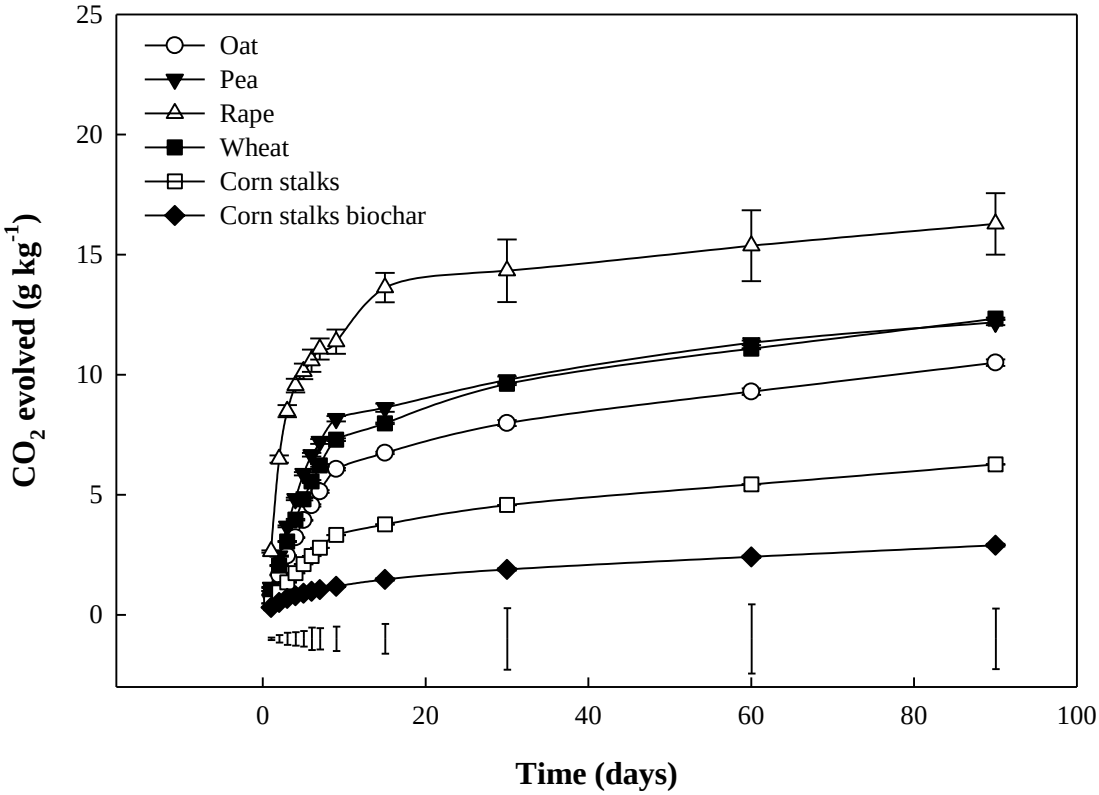

Figure 3 - Effect of the added plant material and biochar on normalized cumulative C mineralization during 90 days of incubation. Thick bars represent the LSD $_{0.05}$

The C/N ratio has long been used as an index of plant litter quality. Nitrogen is required for microbial growth and proliferation, and theoretically the optimum C/N ratio of the decomposable substrate is 25 (HEAL; ANDERSON; SWIFT 1997 cited by WANG et al., 2004). In our experiment the C/N ratio varied from 13.7 (pea) to 80.4 (corn stalks) (Table 2), but we could not establish significant linear correlations (Pearson Product Moment) between N added and evolved CO_2, when the data was not normalized. We tried to establish multiple correlations between CO_2 respired and plant and biochar properties (Table 2) variables. However, we only obtained significant relationships when the biochar was excluded from the starting materials. There was a relationship ($p < 0.01$ $R^2 = 0.99$) for cumulative CO_2 evolution and C added and C/S ratio. No other variable met the required ($p < 0.05$) significant level for entry into the model. These results indicate that under these experimental conditions the decomposition of C was influenced by S supply. Saggar, Bettany and Stewart (1981) showed that cellulose addition causes a great demand on available soil sulfur. To support these results, they also verified that in the treatments that received cellulose and S was found higher biomass C and S contents than the ones not amended with cellulose indicating that the

decomposition of added cellulose in soils is mediated by the supply of S available for new microbial synthesis.

During the first 15 days of the incubation (linear phase observed in the cumulative data), the maximum respiration rate was verified in day 2 (Figure 4) and the mineralization rate of the remaining plant material C decreased with time, reflecting the decline in the quality of substrate with decomposition (WANG et al., 2004). Low changes in the daily rate of respiration of the control treatment were observed (data not showed), reflecting the low effects of soil handling and preparation in the results obtained with this incubation experiment.

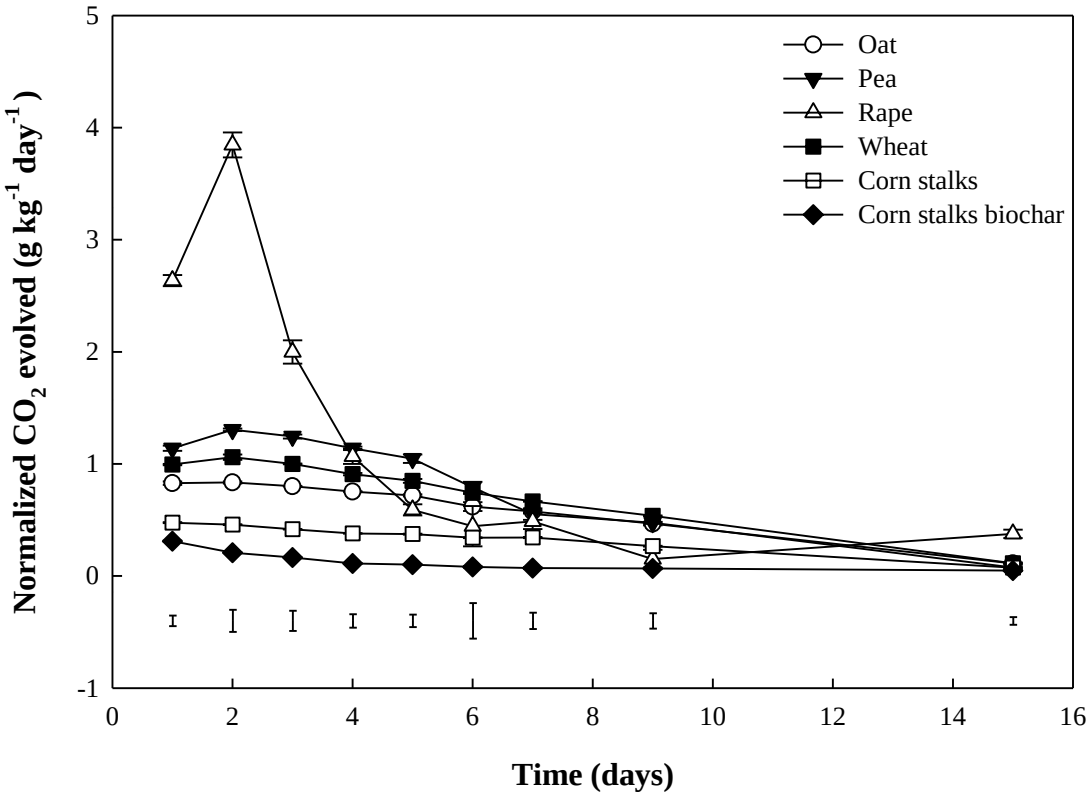

Figure 4 - Effect of the added plant material and biochar on daily C mineralization during the first 15 days of incubation. Thick bars represent the $LSD_{0.05}$

4.3.2 Nitrogen mineralization

Cumulative N mineralization (sum of mineralized NO_3^-, NO_2^- and NH_4^+) was influenced by crop residues and biochar (Figure 5). The forest soil presented high release of NH_4^+ since the first event of leaching (where was detected 126.9 mg kg^{-1} of NH_4^+ in the leachates). This higher NH_4^+ leaching was probably related to the lower pH of the soil (Table

1), favoring the leaching of ammoniacal forms. When the analysis of variance was performed including the forest soil, an increase in the variation of the data was observed, increasing the LSD (Tukey test, $p < 0.05$) and covering the differences between the other treatments. Thus, we decide to eliminate the forest soil from the statistical analysis, performing the comparisons between the control treatment, the crop species and the biochar (Figure 5).

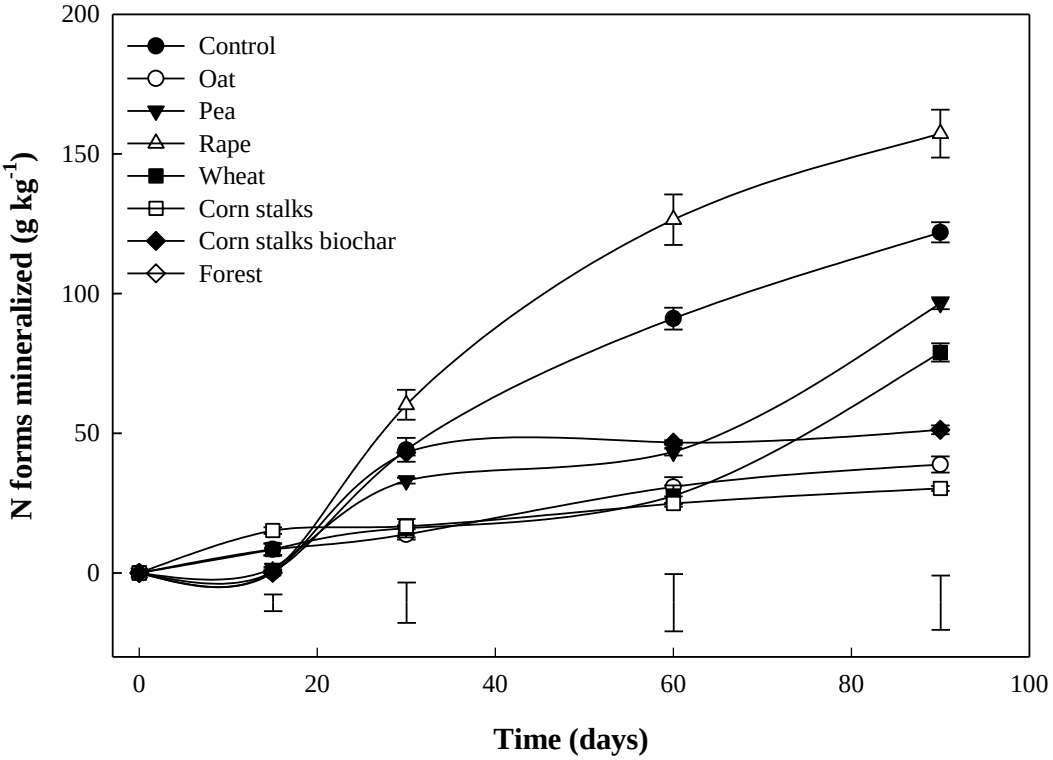

Figure 5 - Cumulative amounts of N (sum of NH_4^+, NO_3^- and NO_2^-) mineralized at 15, 30, 60 and 90 days of incubation at 30º C. Thick bars represent the LSD $_{0.05}$

A two-phase behavior was also observed for N mineralization (Figure 5). In the first event of leaching (15 days) the amount of N mineralized was influenced by the plant species added to the soil and the higher N mineralization was found for the treatment that received corn stalks addition (15.17 g kg^{-1}), followed by the one that received oat (8.46 g kg^{-1}) , wheat (8.39 g kg^{-1}), pea (1.72 g kg^{-1}), rape (0.68 g kg^{-1}), control (0.39 g kg^{-1}) and biochar (0.07 g kg^{-1}), and the dominant leached form of N was also the NH_4^+. The nitrogen mineralization presented here was not normalized by the amount of N applied, so we would expect higher N mineralization from the plant residues with higher N in their composition (like pea, for example). However, in this initial phase (15 days of incubation) of N mineralization we did not observe any correlation (linear or nonlinear) between leached inorganic N and initial added N. Linear correlations (Pearson Product Moment) were observed between the nitrogen

leached at 15 days and C/N (0.98, $P < 0.01$) and C/S ratio (0.93 $P < 0.05$). Probably in this first phase of mineralization, the inorganic N leached was the one present in the most labile fractions of organic C from the added plant materials. These findings are in agreement with the model proposed by McGill and Cole (1981) where it is primarily the need for C rather than the need for N that causes N mineralization.

In these first 15 days of incubation, N immobilization was not verified. However, after the 1st event of leaching, for all crop residues (except for the rape), there was a significant net immobilization of N (total N mineralization in the soil amended with crop residue was less than in the control soil), and higher immobilization was verified in the treatments that received corn stalks and oat due to higher C/N and C/S ratio (Table 2). In this 2nd phase, the N immobilization showed by most part of the materials revealed that there was a need of external fonts of N to decompose C rich plants (in this case consuming the native soil N). Many authors show the importance of nitrogen in the process of decomposition of plant materials (JANZEN; KUCEY, 1988; WANG et al., 2004; SINGH; RENGEL; BOWDEN, 2006), affecting the pattern and the rate of decomposition. Janzen and Kucey (1988) compared three plant species with different C/N ratio (wheat, lentil - *Lens esculenta* and rape) grown under three nutritional regimes. Janzen and Kucey (1988) observed the great importance of the nutritional regime for plant residues decomposition, where wheat plants that had received high N fertilization decomposed at a similar rate and promoted similar N mineralization as legume plants. In our experiment, we can eliminate the effects of nutritional regimes, since we obtained the plants from field areas of high soil fertility, so we can attribute the decomposition effects directly to the crop species.

Comparing the composition of the plant residues employed in this incubation experiment (Table 2) with the ones presented by Janzen and Kucey (1988) we can classify our materials as high N status crop residues. However, some attention should be taken into account when comparing the results obtained in this incubation experiment, since the amounts were applied based in the sulfur content and varied from 0.3 to 8.8 g of plant in 100 g of soil. The inorganic N produced with the application of biochar was higher than the one obtained by the application of corn stalks, despite the similar C/N and C/S ratio presented by these materials. Also, the patterns exhibited by the biochar in the processes of CO_2 evolution and N mineralization do not show a close relationship. Based on these results, we can infer that the inorganic N forms leached from the biochar treated soils were product of not only the biological mineralization, but another mechanism was involved in the process.

The addition of rape to the soil samples promoted a net mineralization of nitrogen, that was liberated mostly in the form of nitrate (data not shown) after the second event of leaching and this treatment provided the highest cumulative mineralized N forms. As verified for CO_2 evolution, was also established a multiple regression between the cumulative N mineralized at 90 days and the C/S ratio and C added ($p < 0.05$, $R^2 = 0.98$). In the case of the treatments that had received higher C/S residues, a longer incubation period (> 6 months) would be required to elucidate the timing of release of immobilized-N in the soils.

4.3.3 Sulfur mineralization and changes in S pools

As in the case with C and N, organic S in soils is mineralized to inorganic forms, mainly SO_4^{2-}, the form taken up by plant roots (TABATABAI, 2005). Some authors observed that the variations in sulfur mineralization were influenced by initial S tissue concentration (JANSEN; KUCEY, 1988; WU; O'DONNELL; SYERS, 1993; SINGH; RENGEL; BOWDEN, 2006). In our case, the treatments were added in sulfur basis (38 g kg^{-1} of S); nevertheless we also observed effects of the treatments on S mineralization and they presented similar patterns during the 90 days of the incubation (Figure 6). The inorganic S gradually increased during the incubation, but most part of the mineral S appeared in the initial extraction. Despite the fact that the forest soil presented higher C/S ratio (Table 1) and, consequently higher potential for S immobilization, similar S mineralization patterns were observed for the forest and the cultivated area, and the amounts of S mineralized were not different between these two areas in any of the periods evaluated ($p < 0.05$). Among the treatments that received plant residues addition, the higher amount of S leached by 1 M $CaCl_2$ was found with the application of rape (which mineralized ca. 40% of the S added in 90 days), followed by wheat and pea (Figure 6).

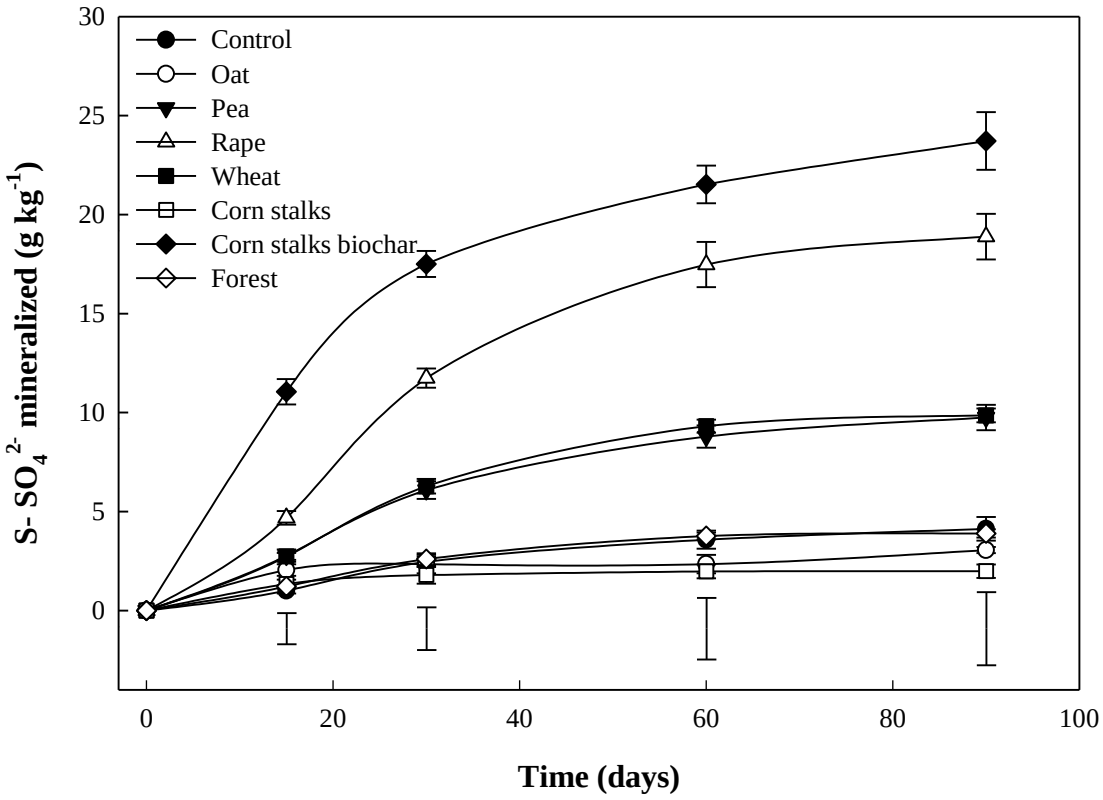

Figure 6 - Cumulative amounts of S mineralized at 15, 30, 60 and 90 days of incubation at 30º C. Thick bars represent the LSD $_{0.05}$

The samples amended with biochar presented the highest amount of S leached by $CaCl_2$, (Figure 6), however the main mechanism involved in this process was the abiotic release of S present in the biochar, since the same pattern was not observed in the CO_2 evolution (Figure 2). Besides acting as a soil conditioner, improving the structure and fertility of soils and improving biomass production, biochar can also be used as a sulfur source. It can be mixed with fertilizers and included in no-tillage methods, without the need of additional equipment (LEHMANN, 2007). Non-linear correlations were observed between cumulative S mineralization and chemical indexes of the plant materials.

When the biochar was eliminated of the analysis, we verified a relationship (expressed by multiple regression) between cumulative S mineralization and C and C/S ratio ($p < 0.05$ $R^2 = 0.96$). The biochar had a very distinct behavior from another plant residues concerning C, N and S mineralization, as verified when correlation analysis were performed with and without this treatment (Table 3). When only the plant residues were considered, we observed high positive correlation coefficients between C and N, C and S and N and S mineralization. However, when the biochar was included, no significant correlations were achieved.

Conversely, S mineralization shows less correlation with carbon decomposition as it occurs with C and N (KOWALENKO; LOWE, 1975; TABATABAI; AL-KHAFAJI, 1980). Sulfur decomposition is proposed by McGill and Cole (1981) conceptual model, in which biological and biochemical mineralization are present. Biological mineralization is controlled by microorganisms and their need for carbon to provide energy. Carbon is oxidized as CO_2, and S is liberated as a by-product in the sulfate form. Biochemical mineralization is the enzymatic hydrolysis of the ester sulfates by sulfatases (extracellular enzymes) and is controlled by the supply of S rather than the need for energy. Although the model has been criticized as "oversimplified" or "artificial" it gives us responses about S mineralization patterns, because different from C and N, sulfur is not only dependent on biological mineralization.

Table 3 - Pearson Product Moment Correlation between the cumulative C, N and S mineralization at 90 days of the incubation

	With Biochar (n=6)			**Without Biochar (n=5)**		
	Cumulative CO_2 evolution	Cumulative N mineralized	Cumulative S mineralized	Cumulative CO_2 evolution	Cumulative N mineralized	Cumulative S mineralized
Cumulative CO_2 evolution	-	0.77ns	0.14ns	-	0.92*	0.92*
Cumulative N mineralized		-	0.60ns		-	0.99**
Cumulative S mineralized			-			-

There was an immediate (after 1 h of incubation) increase in the phosphate-extractable S upon the incorporation of the plant residues and biochar to the soil (Figure 7), and higher amounts of phosphate-extractable S were found by the application of rape, wheat and corn stalks biochar. This initial liberation of S is in agreement with the findings of Singh; Rengel and Bowden (2006), indicating that much of the tissue S in these materials existed in soluble inorganic sulfate and readily degradable organic-S forms (S in amino-acids). In our experiment we employed X-ray Near Edge Spectroscopy (XANES) to examine the forms of sulfur in the plant material and in the biochar. The baseline corrected and normalized experimental S K-edge XANES spectra of the plants and the biochar were characterized by the presence of three prominent absorption edge- and post-edge bands in the energy ranges of 2471 to 2476, 2476 to 2479 and 2479 to 2486 eV (Figure 8).

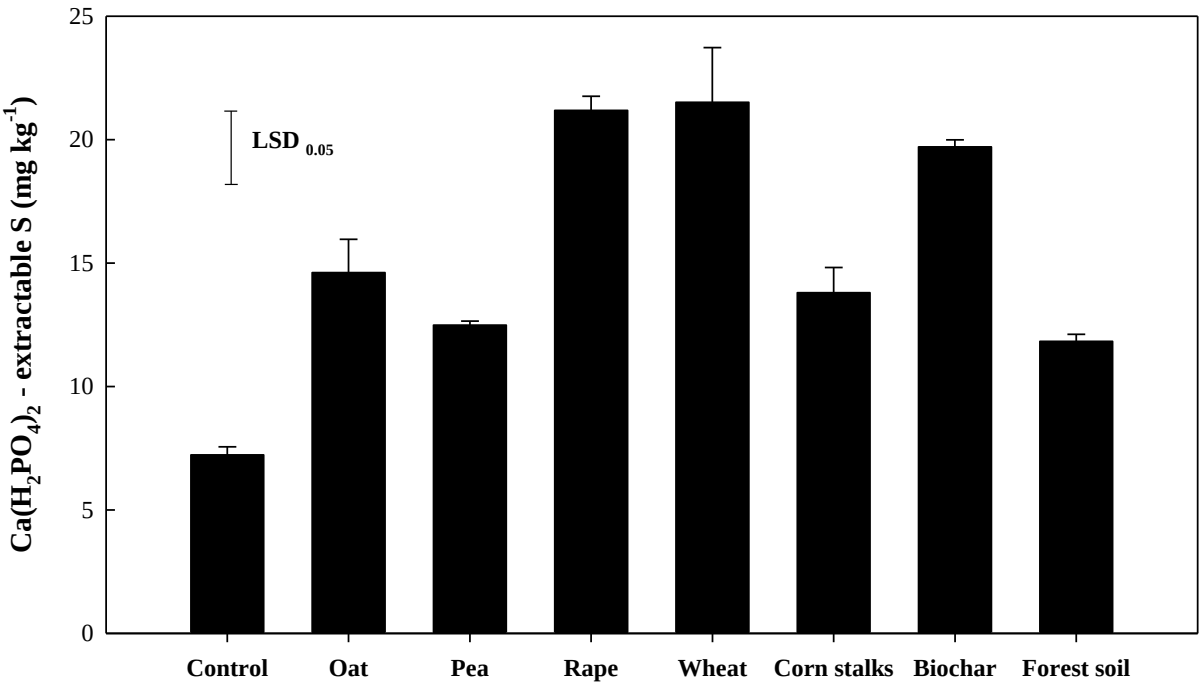

Figure 7 - Influence of plant residues and biochar addition to soil on 0.01 M $Ca(H_2PO_4)$ - extractable S during the first hour of incubation

The peak regions (Figure 8) were ascribed to transitions of the S 1*s* core electrons to the lowest unoccupied antibonding states of the S atom and possibly to the various post-edge features generally attributed to multiple scattering resonances (FLEET, 2005). These features were consistent throughout our experimental XANES spectra, indicating the presence of numerous oxidation states in the evaluated samples. The qualitative features of the XANES spectra allow us to observe that between the plant residues, rape and wheat presented sulfur in the most oxidized state (as sulfate) while pea, black oat and corn stalks presented sulfur in the most reduced and intermediate states.

The spectrum obtained from corn stalks presented a large background due to the low amount of total S present in the sample (Table 2). Comparing the spectra of corn stalks and corn stalks biochar we can observe a decrease in the sulfur from highly reduced and in intermediate states, being accumulated in the most oxidized states (Figure 8). Mostly of the information about S chemistry in pyrogenic compounds is inferred from information generated from the chemical nature of S in coal (SUGAWARA et al., 2001; QU et al., 2010) or crude oil particles.

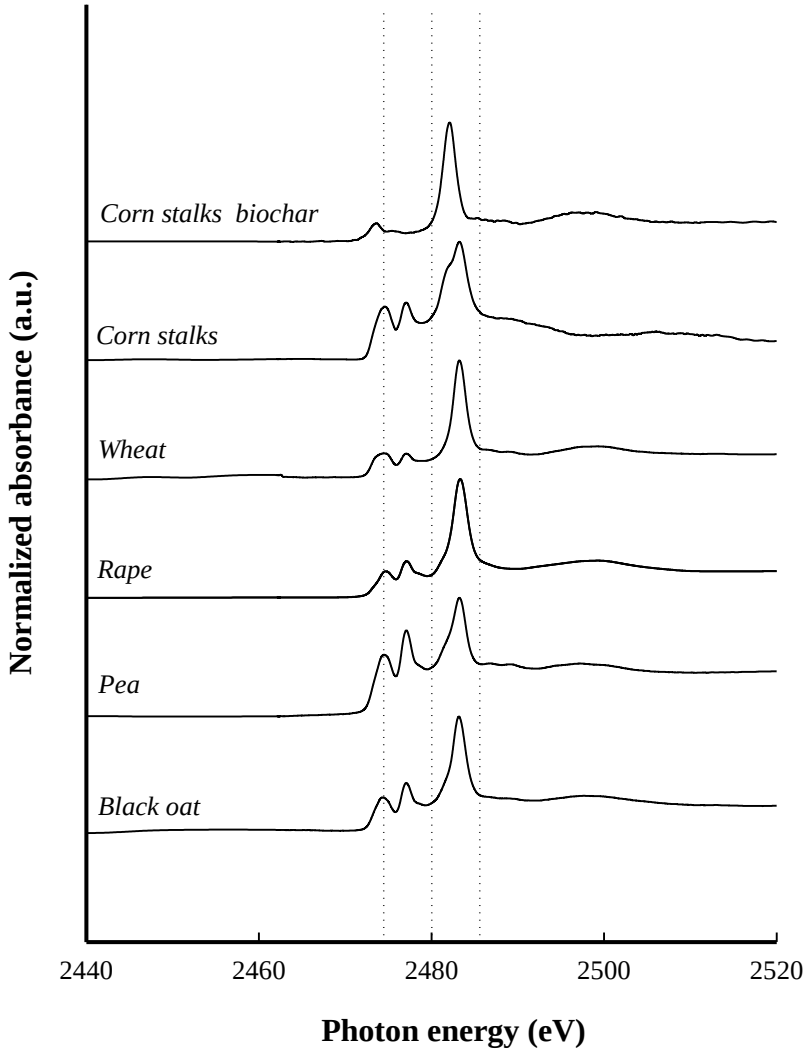

Figure 8 - Sulfur K-edge XANES spectra of the plant residues and biochar utilized in the incubation

Sugawara et al. (2001) used XANES and showed the predominance of sulfide, disulfide and thiophene with peaks ranging from 2471 to 2473 eV and sulfoxide (2474.5 eV) for raw coals. After a rapid pyrolysis at 980º C, Sugawara et al. (2001) observed a decrease in the sulfide and sulfoxide peaks because of the thermal stability of these forms and the extents of organic sulfur removal smoothly decrease with the increase in the aromatic carbon fraction. However, the form and the amount of S present in biomass varies considerably and biochars found naturally in the environment and the ones employed with agronomic purposes are produced at lower temperatures.

Some information about sulfur chemistry during biomass pyrolysis is available concerning the utilization of plant material for power generation (KHALIL; SELJESKOG; HUSTAD, 2008; WANG et al., 2010). As sulfur is present in the plant biomass as organically bound and inorganic sulfates, it is believed that the organically bound sulfur has a lower

stability, which will result in decomposition at low temperatures (< 450 °C) (KHALIL; SELJESKOG; HUSTAD, 2008), promoting the emission of sulfur species COS and H_2S. The transformation of inorganic sulfate, however, only occurs at higher temperatures, ca. 950º C (WANG et al., 2010), emitting SO_2. So, during the pyrolysis of the corn stalks at 450 ºC, most part of the S bounded to carbon from aminoacids and sulfonates was decomposed, while sulfate was preserved and concentrated in the final material. These observed features derived from XANES help to explain the differentiated initial release of inorganic sulfate by the application of the same amount of total sulfur contained in plant residues and biochar.

The net changes in the main S fractions (S-C and S-ester) were evaluated at the end of the experiment (90 days) and at three days of the incubation (Figure 9), after the peak of microbial activity (Figure 4). After the maximum microbial activity (Figure 9) we observed effects of the treatments on the organic and inorganic fractions of S. According to Ghani, McLaren and Swift (1992), any gains of S by a particular pool or pools should be balanced by losses of S from the remaining pools. For some treatments, however, the effects were not as enlightening as expected due to the errors associated in the wet-chemical fractionation. Net positive increments in inorganic S were observed from T_0 to T_3 in almost all treatments, except for the plots that had received addition of pea residues. With the increments in inorganic S, there was an increase in S bounded to ester during the first three days of the incubation.

For most part of the treatments, the increase in ester sulfate was in the same proportion as the increase in inorganic sulfate, extracted by calcium phosphate, and it was accompanied by decreases in the carbon bounded fraction. Several authors have shown increases in ester sulfates due to the addition of SO_4^{2-}-S to the soil (FRENEY; MELVILLE; WILLIAMS, 1975; SAGGAR; BETTANY; STEWART, 1981; GHANI; McLAREN; SWIFT, 1992; 1993), confirming that inorganic S is readily converted into organic forms in the soils during incubation (FRENEY; MELVILLE; WILLIAMS, 1975). The soil microbial community is the major player in this process, and the accumulation of ester sulfates is related to the mechanism used by soil microbes to store S without altering the pH of their surroundings (FITZGERALD, 1978).

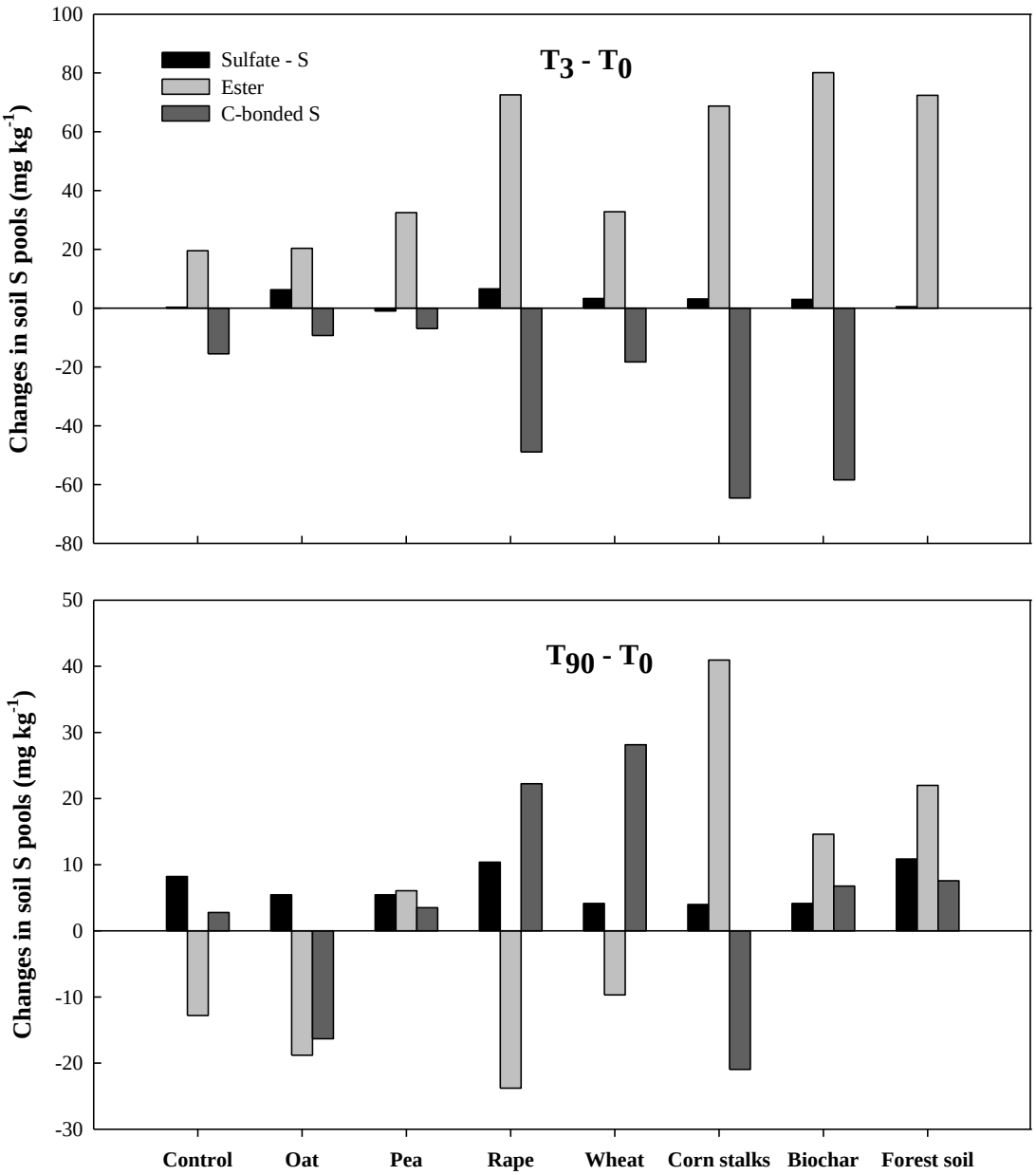

Figure 9 - Effects of plant residues and biochar addition to soil on changes in soil S pools during the incubation

The incorporation of sulfur into the organic pool observed in the treatment that received corn stalks (Figure 9) agrees with the findings of Eriksen (1997), who found higher incorporation of S in the presence of cellulose, while the addition of C and extra nitrogen stimulates S immobilization, as observed in the treatment that had received pea addition since low inorganic S appeared immediately after the incubation (Figure 7) and in the first event of leaching (Figure 6). Our results are in agreement with the ones obtained by Vong et al. (2003) in a calcareous soil, where the addition of ammonium nitrate together with glucose slightly stimulates S immobilization.

Despite the fact that we have found most part of the S immobilized in the ester sulfate fractions, some authors have found different results using both wet degradative (GHANI; McLAREN; SWIFT, 1991) or solid state XANES techniques (ZHAO et al., 2006; SOLOMON et al., 2011). Solomon et al. (2011) observed up to 40% more C-bonded S after three days of the addition of C-bonded S rich sugarcane (*Saccharum oficinarum*) residues compared with the unamended baseline (0 day), indicating that C-bonded S or organic S in reduced and intermediate states is the main source for S mineralization.

Organic S in soil environment is not static, been rapidly interconverted from one organosulfur form to another by microbial action (KERTESZ; MIRLEAU, 2004). So, under aerobic incubations it is common to observe losses of C-bonded forms in the intercourse of the process, where microorganisms act transforming C-bonded S functionalities to organosulfur bonded to O as an intermediate product before being released as inorganic sulfate (McGILL; COLE, 1981; GHANI, McLAREN; SWIFT, 1992; SOLOMON et al., 2011). However, in our experiment, differently of the data observed in the first three days, there was a high variation and few specific trends could be established at the 90 days of the incubation (Figure 9). We could verify an increase in the net inorganic sulfate, stimulated by the periodic events of leaching, but the transformations in the organic sulfur pools were complexes and difficult of explain. Part of this can be attributed to the errors involved in the wet-chemical fractionation, as commented previously, since many fractions are obtained by difference, instead of direct measurement.

Based on several studies, Kertesz and Mirleau (2004) suggested that the most rapidly mineralized pool of organic S has been most recently immobilized and that immobilization and mineralization are occurring concurrently. We could observe a decline in S bounded to ester in T_{90} compared to T_3, where the immobilization was verified, agreeing with the statement proposed, but the findings showing increase of S in the fraction bounded to C are contradictory. Castellano and Dick (1991) have proposed that immobilized S is incorporated into the S-ester pool, and then slowly converted by microbial activity into C-bonded S. This trend was observed for the treatments that have received rape and wheat additions, which have shown net S mineralization (Figure 6). Maybe if we have included one or two more evaluations of the S pools during the intercourse of the incubation (between 3 and 90 days) we could see clear trends in the S transformation driven by C, N and S additions. Regardless of the discrepancies between our study and McGill and Cole (1981) model, we could clearly see the importance of the S forms in the residue for S mineralization as well as the decomposability of the material (WU; O'DONNELL; SYERS, 1993). These forms of S when

incorporated to the soil rapidly increased the size of the soil available-S pool, showing that the incorporation of rape residues and biochar into soil can directly provide plant available-S and improve the S-supplying potential of the soil. This is in contrast to the incorporation residues of higher C/S ratio and results in a net loss of plant available-S through microbial immobilization and transformation reactions. The incorporation of such plant residues (even the same sulfur basis) may result in S-deficiency in soil, making it advisable to use S fertilizers.

4.4 Conclusions

The decomposition dynamics of the plant materials tested varied substantially, mostly governed by the C/S ratio. Sulfur mineralization was driven by the form of S in the starting materials, rather than S concentration in the tissue. Synchrotron-based spectroscopic technique proved to be an ideal nondestructive tool to identify S functionalities in plant materials as well as helped to understand the modifications occurred during the slow pyrolysis of the corn stalks, where S functionalities in highly reduced and intermediate states were thermally broken down, prevailing the S in highly oxidized states. These findings encourage further investigations of biochar as S source, involving particularly chars produced from different feedstocks and pyrolysis temperatures.

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