RAFAEL SILVA SANTOS

ORGANIC MATTER DYNAMICS IN DISTINCT SOIL ZONES FOLLOWING RIDGE PLANTING OF EUCALYPT IN SOUTHERN BRAZIL GRASSLANDS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Solos e Nutrição de Plantas, para obtenção do título de *Magister Scientiae*.

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APROVADA: 26 de junho de 2018.

Teógenes Senna de Oliveira

Maurício Dutra Costa

Edson Marcio Mattiello

Ivo Ribeiro da Silva
(Orientador)

Ao meu avô, Antenor (*in memoriam*), por

me ensinar que a felicidade está na

simplicidade em que a vida é conduzida,

dedico.

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BIOGRAFIA

Rafael Silva Santos, filho de Elizabethe Silva Santos e Danilo José da Silva Coelho, nasceu em 18 de julho de 1989, no município de Teófilo Otoni, Minas Gerais.

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Em agosto de 2016, ingressou no Programa de Pós-graduação em Solos e Nutrição de Plantas da Universidade Federal de Viçosa, em nível de mestrado, sob orientação do Professor Ivo Ribeiro da Silva, submetendo-se à defesa em junho de 2018.

SUMÁRIO

ABSTRACT

SANTOS, Rafael Silva, M.Sc., Universidade Federal de Viçosa, June, 2018. **Organic matter dynamics in distinct soil zones following ridge planting of eucalypt in Southern Brazil grasslands**. Adviser: Ivo Ribeiro da Silva. Co-advisers: Emanuelle Mercês Barros Soares, Leonardus Vergütz and Samuel Vasconcelos Valadares.

The global demand for energy, fiber, and food has intensified land use of several areas worldwide, altering soil carbon (C) dynamics. Grasslands are considered strategic to mitigate climate changes due to its ability to store C on soil, but their management (e.g. ridge tillage) and native vegetation replacement may lead to net C loss to atmosphere. The Southern Brazilian grasslands (*Pampa*) have been largely replaced by eucalypt plantations, however the impact and magnitude on native (old; derived mostly from C4 plants) soil organic matter (SOM) destabilization and new SOM (derived from C3 eucalypt plants) formation is not clear so far. In this study, the objective was to access the initial changes in soil C and nitrogen (N) stocks in SOM fractions (particulate organic matter – POM; mineral associated organic matter – MAOM) in the ridge (row) and inter-ridge (inter-row) soil zones up to 1 m depth in the first cycle of a eucalypt plantation. We evaluated the soil changes in row and inter-row zones at different soil depths over a period of 32 months in a eucalypt stand ridge-planted in a native grassland field in Southern Brazil. The replacement of native *Pampa* cover by eucalypt plants reduced C and N stocks after a period of 32 months. MAOM fraction had C loss 12 times higher than that observed in the POM (0-100 cm soil depth). No changes were observed in POM-N stocks after 32 months of eucalypt planting. However, the MAOM fraction presented an annual N mineralization that reached about 780 kg ha^{-1}. Higher C depletion was observed in the row zone, indicating the occurrence of priming effects triggered by high fine roots density. The row zone contributed for greater net C loss, whereas larger net N mineralization were observed in the inter-row soil zone. The soil layers with the largest C saturation deficit (CSD) presented the highest new (eucalypt-derived) organic matter formation efficiency (OMFE), which varied from 12.2 to 22.4% in the row position. The grassland soils have a large potential (1.5 times more than currently) for further sequestering and stabilizing C on soil. The predominance of more active 2:1 minerals and the presence of less crystalline Fe and Al oxy(hydro)xides in the clay fraction may lead to greater C protection. These results suggest that the replacement of native *Pampa* biome by eucalypt stands has reduced soil organic C in the first three years and that N has been widely scavenged from more stable native SOM to sustain forest growth and new SOM

formation. This fact highlights the importance of an adequate eucalypt fertilization program, particularly for N, in order to prevent a progressive impoverishment of the SOM, which in turn may undermine the production capacity of the sites in the long term.

RESUMO

SANTOS, Rafael Silva, M.Sc., Universidade Federal de Viçosa, junho de 2018. **Organic matter dynamics in distinct soil zones following ridge planting of eucalypt in Southern Brazil grasslands**. Orientador: Ivo Ribeiro da Silva. Coorientadores: Emanuelle Mercês Barros Soares, Leonardus Vergütz e Samuel Vasconcelos Valadares.

A demanda global por energia, fibras e alimentos tem intensificado o uso da terra em diversas áreas no mundo, alterando a dinâmica do carbono (C) no solo. As áreas de *grasslands* são consideradas estratégicas frente às mudanças climáticas devido à sua capacidade de armazenar C no solo, cujo manejo e substituição da vegetação nativa podem levar à perda de C para a atmosfera. Na região sul do Brasil, as áreas de *grasslands* ou Pampas têm sido amplamente substituídas por plantios de eucalipto cultivados em camalhões (*ridge tillage*), cujo impacto e magnitude na formação e desestabilização da matéria orgânica do solo (MOS) ainda não são claros. Neste estudo, objetivou-se avaliar as mudanças iniciais nos estoques de C e nitrogênio (N) do solo (0-100 cm) nas frações da MOS (matéria orgânica particulada - MOP; matéria orgânica associada aos minerais - MAM) em duas diferentes regiões (linha e entrelinha de plantio) originadas a partir da construção dos camalhões no primeiro ciclo de plantio de eucalipto. Foram avaliadas as alterações da MOS em diferentes profundidades (0-10, 10-20, 20-40, 40-60 e 60-100 cm) do solo ao longo de um período de 32 meses, em um povoamento de eucaliptos plantados em área do bioma Pampa no sul do Brasil. A substituição da cobertura vegetal nativa do Pampa pelo eucalipto reduziu os estoques de C e N após um período de 32 meses. A fração MAM apresentou perdas de C 12 vezes maiores do que na fração MOP (0-100 cm de profundidade do solo). Nenhuma mudança foi observada nos estoques de N-MOP após 32 meses do plantio do eucalipto. No entanto, cerca de 780 kg ha^{-1} de N foram liberados anualmente da fração MAM. Foi observado maior perda de C na linha de plantio, indicando a ocorrência de efeito *priming* desencadeado pela alta densidade de raízes finas nessa região. O solo da linha de plantio contribuiu para as maiores perdas de C, enquanto que aquele da entrelinha contribuiu para as maiores perdas de N. As camadas de solo sob grande déficit de saturação de C (DSC) apresentaram as maiores taxas de eficiência de formação de matéria orgânica (EFMO) que variou entre 12,2 e 22,4% na linha de plantio. Os solos das áreas de Pampa possuem grande potencial (1,5 vezes mais do que o atual) para sequestrar e estabilizar C no solo. A predominância de argilas 2:1 e a presença de minerais amorfos de Fe e Al podem levar a uma maior proteção do C no solo, reduzindo o *turnover* da MOS. Estes resultados sugerem que a substituição da vegetação nativa do bioma Pampa por povoamentos de eucalipto reduziu o C orgânico do solo nos primeiros

três anos; e que o N tem sido amplamente extraído da fração mais estável da MOS para sustentar o crescimento da floresta e a formação da nova MOS. Este fato destaca a importância de programas adequados de manejo do solo e de fertilização para os plantios de eucalipto, particularmente em relação ao N, a fim de se evitar o empobrecimento progressivo do MOS, que por sua vez pode comprometer a capacidade produtiva dos sítios a longo prazo.

INTRODUCTION

Soils are the major carbon (C) sink among terrestrial ecosystems. The global demand for energy, fiber, and food has intensified the land use of several areas worldwide, altering soil C dynamics (Smith et al., 2016). Among these areas, grasslands are considered strategic to climate change because of their ability to store C in soil (Scurlock and Hall, 1998; Soussana et al., 2004). It is estimated that worldwide grasslands cover 52.5 million km² and store about 20% of all C present in the soils (FAO, 2010). However, intensive use and replacement of native vegetation by other crops can promote changes in soil C and N stocks, particularly when poorly managed, contributing to increase CO_2 emissions and N losses (Oberholzer et al., 2014; Ramankutty et al., 2008).

In Brazil, grasslands or *Pampa* areas occur primarily in most part of Rio Grande do Sul, covering an area that represents approximately 63% of the state (Overbeck et al., 2007). The entire area of the *Pampas* is within a macro-region, which encompasses part of Uruguay, Argentina, and Brazil, known as *Rio de la Plata* grasslands. This macro-region occupies about 3% of South America area with a C stock estimated at 5% of all C present in this territory (Modernel et al., 2016). The favorable soil attributes, climate conditions, and landscape relief have attracted the forestry sector for the region that occupies an area of approximately 284,701 ha with *Eucalyptus* plantings (IBÁ, 2017).

The conversion of agricultural systems to forests generally tends to boost the soil C stocks (Don et al., 2011; Guo and Gifford, 2002; Kim et al., 2016), mainly in the subsoil (Hernández et al., 2016). Changes on soil C stocks by replacing the *Pampa* for *Eucalyptus* spp. have not been observed (Fialho and Zinn, 2014; Godoi et al., 2016; Wink, 2009). Nonetheless, most part of these studies have not contemplated the distinct SOM fractions into their evaluations as well their origin (e.g. “old” and “new” SOM formed), which may cause misunderstandings about the dynamics of soil C stocks, because, although C stock levels remain unmodified after land use change, SOM is continuously cycled. Usually, there is a substitution of the “native” by the "new” C without affecting C stock levels in superficial soil layer (0-15 cm) (Hernández et al., 2016). However, changes in litter quality after substitution of the grassy *Pampa* vegetation by fast growing trees may trigger the SOM destabilization, contributing to reduce C mean residence time in soil and, consequently, increased CO_2 emissions (Fontaine et al., 2007).

Species of the *Eucalyptus* genus exhibit fast-grow and a high efficiency use of resources, resulting in large accumulation of root and shoot biomass (Silva et al., 2012; Stape et al., 2010). Roots and rhizodeposits, specially, are considered the most important inputs of C to the subsoil, which accounts for about 75% of the total soil C (Jobbágy and Jackson, 2000; Pries et al., 2017; Rasse et al., 2005). The reduced microbial activity and mineral soil particles protection are the main factors for preserving C in deep soil layers (Dungait et al., 2012; Fontaine et al., 2007; Six et al., 2002). Nonetheless, the intense exudation compounds (i.e. fresh C) released by roots creates a microenvironment with high microbial activity that may alters SOM dynamics by the so-called rhizosphere priming effect (RPE) (Kuzyakov, 2002; Shahzad et al., 2015). Several factors as soil management and fertilization (e.g. nitrogen addition) have been attributed as the main factors that cause this phenomenon (Kuzyakov, 2002; Zang et al., 2016). However, there are still uncertainties about the magnitude and direction of how RPE affects SOM fractions.

In annual row crops, soil functional zone management (e.g. ridge tillage) has been used to create a soil zones more favorable to crop development, which rows or ridges are intensively managed for improving soil properties and speeding soil warming and nitrogen (N) mineralization; and inter-ridges are less disturbed contributing for soil structure and organic matter preservation (Williams et al., 2016b). A similar approach has been widely used in eucalypt plantings in southern Brazil as an alternative to reduce soil drainage constraints occasioned by a distinct textural gradient between surface and subsurface horizons (kandic horizon). However, the impact of these practices on SOM formation and destabilization is not clear so far, possibly contributing to soil C unbalance as observed in others soil management approaches (Bronick and Lal, 2005).

Here, we investigated SOM dynamics in Brazilian grasslands (*Pampa*) replaced by eucalypt plantings in Southern Brazil. Because the relatively slow water infiltration rates and the common occurrence of high rainfall events, the soils are subsoiled, fertilized, and a ridge is constructed along tree planting rows. This creates distinct physical and chemical environment zones in comparison to the inter-row area, which remains virtually under no-till until the end of the rotation. Thus, in the current study we focused on initial changes in soil C and N stocks in SOM fractions in the ridge (row) and inter-ridge (inter-row) soil zones up to 1 m in the first cycle of a eucalypt plantation.

MATERIALS AND METHODS

Site description and experimental design

The study site is located in Rio Grande do Sul state, south Brazil (30º 26’ S; 54º 31’W). The region presents a humid subtropical climate (Cfa - Köeppen’s classification) with hot summer and rainy winter (Alvares et al., 2013). The rainfall is well distributed over the year (100 to 170 mm monthly) and the mean temperature is 18 ºC (Alvares et al., 2013). The site is within the *Pampa* biome, which is characterized by co-existence of herb, shrub, and treelet species in a native grass domain (Overbeck et al., 2007).

The soil is classified as an Oxyaquic Hapludalf according to the US Soil Taxonomy (Soil Survey Staff, 2014), presenting drainage constraints during rainy periods. Before eucalypt planting, native plants were controlled by spraying glyphosate. Later, the whole area received 2 Mg ha^{-1} of lime broadcast on the soil surface. Three months later, the planting rows were subsoiled up to 60-cm soil depth and a ridge (30 x 120 cm, height x width) (Figure 1) was built during the same operation. Additionally, 200 kg ha^{-1} of single superphosphate (18% P_2O_5) were applied approximately 40 cm under the ridge by a tube attached behind the subsoiler shank. Afterwards, *Eucalyptus dunnii* Maiden clonal seedlings (~100 days old) were planted (3.3 x 2.2 m) and 150 g of 06:30:06 fertilizer (NPK + 0.6 % B + 0.4 % Zn) were side-dressed to each plant. Additional side-dressing fertilization was performed one year later by applying 12 kg ha^{-1} of N as Urea. Phosphorus (P) and potassium (K) were also applied as side-dress fertilization as needed. The experiment was set up in completely randomized blocks with four repetitions. Each block occupied an area of ~1000 m^2, in which 140 trees were distributed in seven rows. Weed plants were properly managed by herbicide applications.

Soil sampling and analyses

Soil samples (0-10, 10-20, 20-40, 40-60, and 60-100 cm depth) were collected in the rows (ridge zone) and inter-rows (inter-ridge zone) of eucalypt plants at 0, 15, 25, and 32 months after the experiment implantation. In each block (n=4), five individual samples were collected using a Dutch auger and mixed to make a composite sample for each soil layer and zone (R - row and IR - inter-row portion). Soil samples were air-dried, crushed, and sieved (2 mm) to obtain the fine earth fraction for soil chemical, mineralogical and physical analyses. Soil bulk density and total porosity were estimated using intact soil cores collected at each soil layer (EMBRAPA, 2011). Average soil attributes at the beginning of the experiment are summarized in Tables 1 and 2.

Soil organic matter analyses

From the fine earth soil samples, subsamples were taken for SOM physical fractioning into Mineral Associated Organic Matter (MAOM) and Particulate Organic Matter (POM) (Cambardella and Elliott, 1992). Shortly, 5 g of soil were weighted into polypropylene centrifuge tubes and dispersed using glass beads and 15 mL of a sodium hexametaphosphate solution (5 g L^{-1}) for 16 h at 200 rpm in a horizontal shaker. Afterwards, the solution was passed through a sieve (53 µm) and deionized water was gently added until the flush was completely clear. The material retained in the sieve

(POM) and the other (MAOM), which passed through the sieve with the deionized water flush, were properly captured and air-dried in an oven at 60 ºC until they were completely dry. Subsequently, both fractions were weighted and ground (< 149 mm) using a ball mill. Subsamples were taken for carbon (C), nitrogen (N), and ^{13}C natural abundance ($\delta^{13}C$) determination using an isotope ratio mass spectrometer (EA-IRMS ANCA-GSL 20-20, Sercon, Crewe, UK).

Soil carbon and nitrogen calculations

The ^{13}C abundance in samples was calculated according to equation 1:

$$\delta^{13}\text{C}\ (‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) x\ 1000 \qquad (1)$$

where, R is the $^{13}C/^{12}C$ ratio. The $\delta^{13}C$ values were expressed in parts per mil (‰) relative to the Pee-Dee-Belemnite (PDB) international standard.

The relative eucalypt-C contribution (%) to soil organic matter fractions (POM and MAOM) was calculated according to equation 2:

$$C_{EUC} = \left(\frac{\delta^{13}C_{fra} - \delta^{13}C_{Rf}}{\delta^{13}C_{EUC} - \delta^{13}C_{Rf}}\right) x\ 100 \qquad (2)$$

where $\delta^{13}C_{fra}$ is the $\delta^{13}C$ from the POM or MAOM, $\delta^{13}C_{Rf}$ is the $\delta^{13}C$ of a given soil fraction in the reference treatment (grassland soon after eucalypt plantation – time 0), $\delta^{13}C_{EUC}$ is the $\delta^{13}C$ of the eucalypt litter and roots sampled in the area.

The native-C (C_{Native}) contribution (%) was calculated according to equation 3:

$$C_{Native} = 100 - C_{EUC} \qquad (3)$$

For each soil layer, C and N stocks were calculated as follows (Equation 4) (Ellert and Bettany, 1995)

$$\text{C}_{\text{Stock}} = C_{Layer}\ x\ BD_{Layer}\ x\ SL \qquad (4)$$

where C_{Stock} is the soil C stock of the studied layer (Mg ha^{-1}), C_{Layer} is the soil carbon content of the studied layer (%), BD_{Layer} is the soil bulk density of the studied layer (g cm^{-3}); SL is the thickness of the studied layer (cm).

The mineralized and immobilized N were calculated based on the substitution of the grass derived-C (C_{Native}) by the eucalypt derived-C (C_{EUC}) and the C:N ratio of the SOM fractions at 32 months as follows (Equation 5):

$$N_{min} = \frac{\Delta C_{Native}}{C:N} \qquad (5)$$

where, N_{min} is the N mineralized in the POM or MAOM fraction in row or inter-row position in a given soil layer (Mg ha^{-1}); ΔC_{Native} is the variation of grass derived-C between 0 and 32 months in the POM or MAOM fraction in row or inter-row position after eucalypt planting in a given soil layer (Mg ha^{-1}); $C:N$ is the C:N ratio of SOM in the POM or MAOM fraction after 32 months of eucalypt planting in a given soil layer.

$$N_{imm} = \frac{\Delta C_{EUC}}{C:N} \qquad (6)$$

where, N_{imm} is the N immobilized in the POM or MAOM fraction in row or inter-row position in a given soil layer (Mg ha^{-1}); ΔC_{EUC} is the variation of eucalypt derived-C between 0 and 32 months in the POM or MAOM fraction in row or inter-row position after eucalypt planting in a given soil layer (Mg ha^{-1}); $C:N$ is the C:N ratio of SOM in the POM or MAOM fraction after 32 months of eucalypt planting in a given soil layer.

Net N (N_{R+IR}) was calculated by mass weighting the distinct influence zone of row (36.36%) and inter-row (63.64%) positions according to ridge spacing, as follows:

$$N_{R\ or\ IR} = N_{min} - N_{imm} \qquad (7)$$

where $N_{R\ or\ IR}$ is the net N in the row or inter-row position in the POM or MAOM fraction after 32 months of eucalypt planting in a given soil layer (Mg ha^{-1}).

$$N_{R+IR} = (N_R\ x\ 0.3636)\ x\ (N_{IR}\ x\ 0.6364) \qquad (8)$$

where, N_{R+IR} is the net N in the POM or MAOM fraction in row and inter-row position after 32 months of eucalypt planting in a given soil layer (Mg ha^{-1});

Carbon saturation deficit (CSD) (Equation 10) was calculated for each soil layer based on approach developed by Six et al., (2002), as follows (Equations 8):

$$Soil\ C_{Saturation} = 14.36\ x\ 0.21(Clay + Silt)\ x\ BD\ x\ 2 \quad (9)$$

where, $Soil\ C_{Saturation}$ is the maximum soil C storage capacity in a given layer (Mg ha^{-1}); *Clay + Silt* is the content of these two fractions in a given soil layer (%), and BD is the soil bulk density in a given soil layer (kg m^{-3}).

$$CSD = Soil\ C_{Saturation} - Soil\ C_{Layer} \quad (10)$$

where, *CSD* is the carbon deficit saturation in a given soil layer (Mg ha^{-1}) and $Soil\ C_{Layer}$ is the C content in a given soil layer (Mg ha^{-1}).

Tree growth, litter and fine root biomass evaluation

Tree diameter at breast height (DBH) and height (H) were measured at 12, 18, 25, and 62 months after eucalypt planting. All trees from each plot were accounted for DBH measuring. For H determination, 26 trees were measured in each plot, which 20 were located in the central row and six trees were randomly chosen based on the average DBH from each plot.

Litter production (litterfall) was measured over the following periods: 0-18 and 18-25 months after eucalypt planting. Four nylon litter traps (6.6 x 6.6 m; one per plot), were tied to eucalypt plants and used for litterfall measuring. Environmental problems (e.g. strong wind, native animals) made unfeasible the continuous use of the litter traps. Thus, we used a tree growth simulation model to estimate litterfall and fine root production along the experimental period (see details below). In addition, at 62 months after eucalypt planting, a wood template (0.5 x 0.5 m) was used for litter sampling on the soil surface, at four different positions – two positions in the row and two in the inter-row – distributed longitudinally across the plot. These samples were mixed to yield a composite sample for each soil zone. All material was weighted, subsamples were taken, oven-dried (60 ºC; 72 h.), and the dry matter determined.

Eucalypt fine roots biomass were measured after 32 and 62 months of eucalypt planting. An auger (Ø = 5.7 cm) was used to collect fine roots samples at 0-10, 10-20, and 20-40 cm in the row and inter-row position of a representative eucalypt plant (average DBH) in each plot. The soil was collected in six pre-defined points distributed in two different directions (row – R1, R2, and R3; inter-row – I1, I2, and I3) of the eucalypt plant (Figure 2) (Ferreira et al., 2018). The sampled soil was gently crushed and roots picked by hand. Then, they were stored in paper bags and transported to laboratory, where they were washed with deionized water, oven-dried at 60 ºC (72 h), and the dry matter quantified.

Subsamples of fine roots and litterfall were taken from the samples previously described for total C determination using an isotope ratio mass spectrometer (EA-IRMS ANCA-GSL 20-20, Sercon, Crewe, UK).

Ecophysiological model (3-PG) simulation

The 3-PG model (Landsberg and Waring, 1997) was used for simulating and estimating the production of eucalypt plant compartments (e.g. stem, roots, and litterfall) over a period of 5.5 years. The parameterization of the model was based on study developed by Borges (2012). Normal climate data (1981-2010) obtained from the Instituto Nacional de Meterologia (INMET) were used for temperature (max. and min.); and a climate data base for South America was utilized for global solar radiation data (0.5 degrees resolution for latitude and longitudes) considering the latitude -29.68. The following initial parameters were considered for starting the model: biomass of leaves (W_F), stems (W_S), and roots (W_R) for *Eucalyptus dunnii* was used according to study developed by Klippel, (2015); available soil water (ASW) was assumed as 240 mm; the soil class parameter was clay (C), and soil fertility ratio was considered one (1) due to adequate soil supply of nutrients and fertilization management performed in the area.

We compared actual forest stem yield, fine root production, and litterfall deposition to those simulated by the 3-PG model (Figure 3). The reasonable agreement between them indicates that the use of some simulated data for the experimental period is valid (Figure 3).

Soil organic matter formation efficiency in POM and MAOM fractions

The organic matter formation efficiency (OMFE) was calculated for different soil layers using the data estimated by the 3-PG model (fine roots, litterfall). We assumed fine roots turnover of three months (Jourdan et al., 2008) and the contribution from rhizodeposition as the same amount as for fine roots biomass (Rasse et al., 2005).

Statistical analysis

The assumptions for homogeneity of variances and normality were tested for each sample by Levene's test and Kolmogoroff-Smirnoff test, respectively. Changes on soil C and N stocks over time were evaluated by fitting linear regressions performed in the R software. Organic matter formation efficiency (OMFE) in the SOM fractions was compared by Tukey's test ($p < 0.1$). Root density in the row and inter-row position were subject to ANOVA and compared through F-test ($p < 0.1$). Statistical analyses were performed in the STATISTICA software (Statsoft Inc.).

RESULTS

Changes in C and N stocks in SOM fractions (POM and MAOM)

Most soil layers presented C stocks loss for both fractions of the organic matter (POM and MAOM) after eucalypt planting (Figure 4). Although in some layers the POM (60-100 cm soil layer) and MAOM (10-20 cm soil layer) fractions had increases in the C stocks, the balance of the whole soil profile (0-100 cm soil depth) reveals a continuous loss of C over time (Figure 4f, l). The C loss rate in the MAOM fraction increases along the soil depth gradient. POM and MAOM presented C loss rate about 0.36 (2.96% annually) and 4.29 Mg ha year^{-1} (2.88% annually) from which, respectively, 0.96 and 11.45 Mg ha^{-1} of C were lost to atmosphere after 32 months of eucalypt planting (Figure 4f, l; p < 0.01; R^2 = 0.95). Besides the higher C loss rate (0-100 cm soil layer), MAOM fraction has also a larger C stock, representing approximately 90 % of the total carbon in soil (Figures 4f, l).

Nitrogen stocks for POM and MAOM showed a contrasting pattern over time (Figure 4). In general, POM fraction had no changes in N stocks (Figure 3f), except for the 10-20 cm soil layer that presented a slight loss (24 kg year^{-1} of N) (Figure 5b). Conversely, MAOM had high N losses rates in most soil layers, particularly at 20-40 and 40-60 cm soil layers (Figures 5i, j). After 32 months of eucalypt planting, the 20-40 cm soil layer lost approximately 200 kg ha^{-1} of N, while at 60-100 cm had a loss of 540 kg ha^{-1} of N. These high losses of N resulted in an overall reduction of N stocks on soil profile (0–100 cm) in a magnitude of 780 kg year^{-1} of N (Figure 5l; p < 0.01).

A distinct loss between C and N can be noticed in both SOM fractions at 0-100 cm layer (Figures 4f, l; 5f, l). Carbon is lost in larger quantities than N, mainly from MAOM fraction. However, in the whole soil profile (0-100 cm), the annual relative N loss (6.75 %) is much higher than that for C (2.88%) (Figures 4l and 5l).

Dynamics of C and N stocks in SOM fractions in distinct soil zones (rows and inter-rows)

Particulate organic matter in soil from eucalypt rows had higher C loss as compared to those in the inter-rows (Figure 6a-e). The C loss in the row position decreases downward soil depth. The highest C loss rate was observed at 0-10 cm layer (970 kg ha^{-1} year^{-1}; p < 0.01). The only exception was at 60-100 cm soil layer that, in general, remained unchanged over time (Figure 6e). In the inter-row position, most soil layers (0-10, 10-20, and 40-60 cm) had no changes in C stocks (Figure 6a, b, d). However, divergent results were observed between 20-40 and 60-100 cm soil layers; the former lost

168 kg $year^{-1}$ of C year, while the later gained 288 kg $year^{-1}$ of C. This distinct C dynamics on soil layers promoted an opposite pattern on whole soil profile (0-100 cm), the C stock increased in the inter-row (+804 kg ha^{-1} of C) while it decreased in the row position (-864 kg ha^{-1} of C).

The MAOM fraction presented a C stock (0-100 cm soil depth) almost 10 and 18 times greater than those for POM in the row and inter-row positions, respectively (Figure 6f, l). Despite upper MAOM soil layers (row and inter-row) increased C stocks, a negative C stock in the whole soil profile was observed (0-100 cm) (Figure 6l). Interestingly, the 20-40 cm soil layer, solely was responsible for 94% of the total C loss occurred in the row position (0-100 cm) (Figure 6i). Likewise, the effect in the inter-row position (0-100 cm) also led to C losses, but at a rate 4.7 times smaller than that occurred for the same fraction in the row position (Figure 6l; $p < 0.01$).

Nitrogen stocks of SOM fractions in the row and inter-row positions exhibited distinct dynamics (Figure 7). In general, POM had little changes in N stocks for both row and inter-row position over time. The only exceptions were observed in the row at 10-20 and 20-40 cm layers, where N stocks were reduced (Figure 7b, c). However, they were not sufficient to affect the N stock at 0-100 cm. On the other hand, there was an increase in N stock at 20-40 cm for the POM in the inter-row position that affected the N balance at 0-100 cm, contributing for an increase of 60 kg ha^{-1} $year^{-1}$ of N (Figure 7f; $p < 0.05$).

The MAOM fraction in the upper soil layers (0-10 and 10-20 cm) had no changes in N levels (Figure 71, b). For all other layers, an intense N loss was observed. The soil rows presented a linear pattern, losing N over all studied period. Inter-rows showed a similar trend until approximately 25 months when N stock had an increase, but it was not enough to compensate the previous N losses. This scenario caused a loss of N 2.3 times higher in the inter-row (0-100 cm) as compared to the row position (Figure 7f; $p < 0.01$). Only at 20-40 cm layer, N losses in the MAOM row were greater (-860 kg ha^{-1} of N) than in the inter-row (-736 kg ha^{-1} of N) after 32 months of eucalypt planting (Figure 7c). The highest N loss occurred at 60-100 cm, from which row and inter-row soils lost 860 and 1470 kg ha^{-1} of N, respectively.

C dynamics of old (native) and new (eucalypt-derived) in SOM fractions

The POM fraction in the row and inter-row position presented distinct C substitution rates. For most soil layers, rows had higher substitution rates compared to the inter-rows positions (Figure 8a-f), resulting in a substitution of the native C (0-100 cm soil layer) by the eucalypt C 1.35 times higher in the row as compared to the inter-row position (Figure 8f, l). The only exception occurred at 60-100 cm soil layer, where the inter-row had almost 10 times more native C replaced by eucalypt C as compared to the row (Figure 8e, f).

In the MAOM fraction, inter-rows from the upper soil layers (0-10 and 10-20 cm) had a C substitution slightly higher than rows positions (Figure 9a-d). For deeper soil layers, C substitution was greater in the row position at 20-40 and 40-60 cm than inter-row. Interestingly, at 60-100 cm the C substitution rate of the inter-row position was almost 2 times higher than the row (Figure 9e, k). Considering the whole soil profile (0-100 cm), rows exhibited a C substitution twice as high as that of inter-rows. This indicates an annual eucalypt-derived new SOM formation rate of 2.26% in the row and 1.12% for inter-row. Thus, assuming the influence zone of the row (36%) and inter-row (64%), new SOM has been formed at a rate of 7.76 and 1.5 % $year^{-1}$ for POM and MAOM, respectively.

C:N ratio and N mineralization at row and inter-row position

Distinct patterns between row and inter-row positions in the POM were observed for C:N ratio. Row position had no changes throughout time (Figure 10a-f). Conversely, the inter-row position had the C:N ratio reduced in a rate of 3.29 units $year^{-1}$ (0-100 cm soil layer) (Figure 10f). The highest reduction in the C:N ratio occurred in the inter-row at 20-40 cm soil layer (2.64 units year^{-1}). Although a higher C:N ratio reduction was observed in the inter-rows positions, after 32 months of eucalypt planting row and inter-row had only significant differences at 0-10 cm soil layer (Figure 10; $p < 0.1$).

The C:N ratio values of the row and inter-row position in MAOM fraction increased in all soil layers over time (Figure 10g-l). The highest rates (0.048 month) occurred in the row position at 20-40 and 60-100 cm soil layer. For the inter-row position, only at 40-60 cm the inter-row presented a higher rate than row position. Considering the dynamics in the whole soil profile (0-100 cm soil layer), the C:N ratio from the inter-row increased 1.2 times higher compared to the row position over time (Figure 10l). As well as observed in the POM, there was no statistical difference between the C:N ratio from row and inter-row position after 32 months of eucalypt planting in the MAOM fraction for most soil layers, except for 0-10 cm soil layer (Figure 10l).

Nitrogen mineralization was greater than immobilization for both SOM fractions and positions (Table 3). The MAOM fraction in the inter-row position presented a N mineralization 3.45 times higher than that for row position (126 kg ha^{-1}). On the contrary, the POM fraction in the row position had a higher N mineralization (152 kg ha^{-1}) as compared to the inter-row, which immobilized about 40 kg ha^{-1} of N (Table 3). Weighting the net N (ΔN_R and ΔN_{IR}) from SOM fractions based on the influence zone of row and inter-row position, it is possible to infer that approximately 362 kg of N was mineralized through cycling of native *Pampa* derived-SOM after of 32 months of eucalypt planting (Table 3).

Fine roots density at row and inter-row position

Consistently, inter-row position had higher fine roots density at 0-10 cm soil layer; and row position in deeper soil layers (20-60 cm) ($p < 0.1$). At 10-20 cm in the row position (after 62 months), fine roots density was at least 3.5 times higher than any other soil layer (Table 4). In addition, deeper soil layers in the row position had at least 21 and

45.6% more fine roots density compared to inter-row after 32 and 62 months, respectively.

Soil C saturation deficit (CSD) and formation efficiency rate for POM and MAOM fraction

The soil potential C storage of the present soil was about 400 Mg ha^{-1} considering the whole soil profile (0-100 cm depth) (Table 5). In general, there were no discrepancy among the soil layers since the equation proposed by Six et al., (2002) does not consider soil layers thicknesses. Taking into account the soil C stocks measured at 32 months after eucalypt planting, the soil profile filled approximately 40% of its potential storage capacity. The greatest saturation deficit was observed for 10-20 cm followed by 40-60 cm soil layer due to their lower C stocks at 32 months (Table 5).

Soil organic matter formation efficiency ($OMFE_{POM+MAOM}$) rate was consistently higher in the row position as compared to that for the inter-row. The highest OMFE values were observed at 0-10, 10-20, and 40-60 cm soil layers (Table 6). The only exception occurred at 60-100 cm soil depth, where the OMFE was 1.78 times higher in the inter-row as compared to the same row position layer. For the other soil layers, the $OMFE_{POM+MAOM}$ in the row position was at least 1.5 times higher than in the inter-row. The 10-20 cm soil layer, in special, presented the highest OMFE in the row position for both SOM fractions. Differences were also found in the OMFE rates between POM and MAOM in the row and inter-row. In both positions (row and inter-row) the $OMFE_{MAOM}$ was greater than $OMFE_{POM}$, in which the highest values were found in the upper (0-10 and 10-20 cm) and deeper (40-60 and 60-100 cm) soil layers (Table 6).

DISCUSSION

Changes in soil C and N stocks after land use change

The *Pampa* soil had approximately 2.5 dag kg^{-1} of soil organic matter (0-100 cm soil depth) (Table 1). Despite the lower OM level compared to grasslands worldwide, it is higher than most areas in Brazil (e.g. *Cerrado* - savannah grasslands), endorsing the high ability of grasslands to store C in soil (Scurlock and Hall, 1998). Land use change is generally followed by intense C loss (Guo and Gifford, 2002).The replacement of the *Pampa* by eucalypt plants reduced soil C stocks (POM and MAOM) in approximately 7.7% over a period of 32 months (Figure 4). This C loss is mainly attributed to the management adopted for eucalypt planting and substitution of the native cover (Turner and Lambert, 2000; Vassallo et al., 2013).

Subsoiling and ridge tillage, though provide better conditions for plant development (e.g. roots growth, nutrient uptake, reduce drainage constraints) (Merino et al., 2003), promote a localized, but intensive soil disturbance. These practices break up soil structure exposing the SOM protected into microaggregates and stabilized onto colloidal particles (MAOM) to microbial activity, depleting SOM (Balesdent et al., 2000). Nevertheless, ridge tillage creates different zone areas (e.g. row and inter-row) where soil processes occur under distinct intensities, affecting SOM dynamics mainly in the ridge zone (e.g. SOM turnover) (Williams et al., 2016a). These facts, associated to a low initial C input (e.g. shoot litter), inherent to the young eucalypt age, contributed to soil C reduction (Barreto et al., 2014; Gregory et al., 2016), mainly at deeper layers where the highest C loss rates were observed in the MAOM fraction (Figure 4k).

The land use change has also affected the N stocks, mainly in the MAOM fraction (Figure 5). In all soil layers the largest N losses occurred over the first 25 months (Figure 5g-l). Generally, eucalypt plants uptake a major proportion of its N requirement in the first year (50-65%) (Laclau et al., 2010). At this time, plants are highly dependent on soil supply since nutrient remobilization through biogeochemical cycle is still incipient (Laclau et al., 2010). As the dose of N applied in the area (24 kg ha^{-1}) is low compared to the content (~180 kg ha^{-1}) present in eucalypt plants at the same age (Barreto et al., 2012), the SOM becomes the main source of N for plants (Pulito et al., 2015). Laclau et al.,

(2005) observed a deficit of about 144 kg ha^{-1} of N in soil after one eucalypt rotation (7 years), which N exportation from site can be even larger depending on the harvest method. This indicates the key role developed by the SOM as N source in eucalypt plantings and the importance of an adequate fertilization management for avoiding a progressively loss of SOM.

Soil C and N dynamics at row and inter-row positions

Subsoiling and ridge tillage promoted distinct SOM dynamics between the row and inter-row position for both POM and MAOM fraction (Figure 6). As the ridge tillage creates different soil zones, the row tends to be more similar to conventional tillage and inter-rows to no-tillage. Thus, an "active turnover" is more likely to occur in the row and a "soil building" (e.g. soil structure development and C storage) in the inter-row zone (Williams et al., 2016b). This is in accordance with our results, which show consistently higher C losses in row positions for both SOM fractions; and no changes or slight increases in C stocks in inter-rows for most soil layers (Figure 6). In general, C losses for POM and MAOM in row positions were 4.7 and 2.2 times greater than those in the inter-row, respectively. Carbon losses were also observed in eucalyptus forest soil by Hopmans et al., (2005) that found a reduction of approximately 28% in the total soil C in the upper layers (0-30 cm) after physical disturbance (e.g. topsoil removing and subsoil disturbance).

The subsoiling along with phosphorus fertilizer placement in a furrow of plant row have amended the soil environment for plants development, resulting in a contrasting root density between row and inter-row zone (Table 4). The soils layers that presented higher fine roots density had also the highest C loss rates. At 20-40 cm soil layer, for example, the C-MAOM loss rate was 3.2 times higher as compared to that in the inter-row (Figure 6i). As plants grow, a large quantity and diversity of compounds (e.g. carbohydrates, lipids, proteins) are released by roots, promoting intense changes in the rhizosphere (Studer et al., 2016). These compounds can increase up to 30 times the microbial activity in the soil vicinity of roots (Grigera et al., 2007; Kuzyakov, 2002), which may lead to the occurrence of the rhizosphere priming effect and higher turnover of the SOM, mainly, in subsoil layers (Fontaine et al., 2007; Shahzad et al., 2015). The biochemical lability of the rhizodeposits make them a preferential energy source used by microorganisms, resulting in a fast-microbial biomass increase; however, as soon as this source is used up, microorganisms start to use the native organic matter as energy source, leading to OM levels decrease (Wang et al., 2015). This would explain the highest C substitution and depletion of the SOM in the row zone under higher fine roots density (Figures 8f, 9f; Table 4). Interestingly, a high C substitution in the inter-row zone was also observed in the POM and MAOM at 60-100 cm, which further supports the occurrence of RPE triggered by a fresh C source in subsoil layers (Figure 9k). Even though a higher C substitution have been observed in row positions (2.26%) in the MAOM fraction (0-100 cm soil layer), indicating a new eucalypt-derived organic matter formation, it was not enough to compensate the losses of *Pampa*-derived SOM (Figure 9).

The N stocks, unexpectedly, did not completely follow the C dynamics in the MAOM fraction (Figure 7). In general, higher N losses occurred in the inter-row zone for all soil layers. Studies have reported a differential C and N mineralization associated to a preferential synthesis of N- over C-acquiring enzymes by microorganisms in the rhizosphere promoted by roots exudates (Jilling et al., 2018 and references therein). Rousk et al., (2016) observed a reduction of 60% in C mineralization and an increase of up to 300% in N mineralization after adding glucose (simulating a labile C source released by roots) to soil, indicating a shift on microbial use of components with higher N content than C. As this differential C and N mineralization are triggered by roots compounds released in the rhizosphere, this pattern was not expected to occur in the inter row zone since C stock loss and fine roots density were higher in the row zone. However, this indicates the plasticity of root mechanisms for changing the rhizosphere environment for attending plant nutritional requirements.

Despite the uncommon pattern of N dynamics between row and inter-row position, it is clear that a large amount of N has been released from mineralized SOM in these zones (Figure 9l). As N is a limiting factor in most environments, plants and microorganisms developed different mechanisms for obtaining N from SOM, which some of them are intrinsically associated (e.g. mutualism associations, plant compounds stimulation, co-metabolism). Depending on the N availability in soil, microorganisms can promote priming effect and speed up C loss of SOM. K- and/or r-strategists microorganisms would be the drivers of SOM depletion; which K-strategists have advantages using more recalcitrant organic compounds (low N supply) and r-strategists would grow faster by using easily available C substrates (high N supply) (Chen et al., 2014; Murphy et al., 2017). In addition, hydrolytic and oxidative enzymes released by microorganisms would act upon a large variety of compounds and thus accessing the N present on them (Kieloaho et al., 2016; Zhu et al., 2014). Plants have also developed strategies for improving N acquisition from soil under limited N supply. A study performed by Hurtarte (2017) found that under N deficiency, eucalypt plant roots produced larger amount of organic acids that would release N from organic compounds bounded to the soil mineral fractions through dissolution of oxides and complexation of Fe and Al. Also, Keiluweit et al. (2015) found that releasing oxalic acid, a common root exudate, plants free organic compounds associated to minerals trough complexation and dissolutions reactions, therefore turning them more exposed to microbial access.

Considering the high root density found in our experiment and the explicit capacity of eucalypt plants to change SOM dynamics for improving N acquisition, it is expected a large occurrence of these aforementioned mechanisms. In fact, the large amount of N released from SOM, mainly in the inter-row zone (Table 3) is a result of these plant-microbial-soil processes. Based on the influence zone of row and inter-row position from the ridge spacing (Figure 1), it was estimated that roughly 360 kg ha^{-1} of N were released through native SOM decomposition after a period of 32 months of eucalypt planting (0-100 cm) (Table 3). Similar values were also observed by Teixeira (2017) in a eucalypt stand (49 months-old) in the *Cerrado* biome (0-100 cm soil depth). Also, estimates made by Smethurst et al. (2015) through a process-based model (SNAP) predicted an annual N release of 150-350 kg ha^{-1} (0-20 cm soil depth). These data indicate the high N demand by E*ucalyptus* plants and their capacity to scavenge N by inducing SOM destabilization in more active rooting zones (Valadares et al., 2017), highlighting the importance of an adequate N fertilization management, not only to ensure the potential eucalypt yields be reached, but also to warrant site production sustainability in the long term.

Soil potential C storage and OMFE

As our assessment comprises a relative short period, C losses may change as the forest grow. Studies have reported increases in soil C stocks at the final of eucalypt cycle in several biomes such as savannas (Trouve et al., 1994), Atlantic forest domain (Pegoraro et al., 2011).The increases of soil C stocks is intrinsically related to litter quality and soil carbon saturation deficit (CSD). In our study, it was estimated that at least 1.5-fold C could be stored in the studied soil (0-100 cm soil depth), which at 10-20 and 40-60 cm soil layers presented higher CSD (Table 5). The litter quality and level of CSD are important factors that affects the rate and kinetics of soil C stabilization (Castellano et al., 2015).

Since high quality litter are used more efficiently by soil microorganisms (Lavallee et al., 2018), a larger amount of microbial byproducts are produced, resulting in a greater C stabilization in the soil matrix (Cotrufo et al., 2013). Although eucalypt litter presents relatively high C:N ratio (Ferreira et al., 2016), significant OMFE rates were observed in the present study (Table 6). Similar results were found by Lavallee et al., (2018), which under moderate to high CSD, litter quality presented less influence for C stabilization in soil. This aspect was also observed in our study through the occurrence of high OMFE rates in the row zone at 0-10 cm and also in deep soil layers (10-20 and 40-60 cm), where is expected a distinct litter quality input (e.g. root compared vs shoot litter) and CSD levels compared to upper soil layers.

Another important aspect related to soil CSD levels and C stabilization is the presence of predominant 2:1 clay minerals (e.g. montmorillonite and illite) and amorphous forms of aluminum (Al_o) and iron (Fe_o) in all soil layers (Figures S1-9; Table 2) (Six et al., 2002; Souza et al., 2017). As main mineralogical characteristics, 2:1 clays present high surface area and charge density. This make possible a higher organo-mineral interaction through SOM binding to mineral surfaces, resulting in larger potential to sequester C (Table5) (Di et al., 2017). Ammonium-oxalate extractable aluminum has been also associated to SOM protection despite lesser abundance than crystalline forms (e.g. Fe and Al) in the soil (Rasmussen et al., 2006). Through a detailed scanning microscopy work with clay-organic matter particles isolated from the soil under study, we observed a strong colocalization of C mainly with Al and Fe, which along data on soil composition at distinct depths suggest that Al_o and Fe_o also have an important role on protecting soil C (Figures S1-5). Souza et al investigating SOM stabilization in contrasting Oxisols found strong correlation ($r = 0.71$) between SOM and Al_o, which 62% of the variation in the organic content explained by Al_o. Over the soil profile, Al and Fe seems to be more related to C protection in the upper soil layers (0-10, 10-20, and 20-40 cm) and Al and Si in subsoil layers (40-60 and 60-100 cm) (Figure S1-5). Such effect is expected to occur since surface soil layers are potentially more affected by weathering processes, resulting on higher mineral transformations (Figure S6). Besides the large CSD presented by soil layers, the soil has also the potentiality for C stabilization. Thus, the practices adopted before eucalypt planting and fertilization management have to be adequately planned to foster increase/preserve C in these soils.

Overall, our results suggest that the replacement of native *Pampa* biome by eucalypt stands reduced SOM after a period of 32 months, and that N has been widely mined from stable SOM fraction in order to sustain forest growth and new (eucalypt-derived) SOM formation. Most C losses occurred due to intense microbial and fine roots activity in the row zone, which accounts for one third of the total area. This fact highlights the importance of an adequate nutrient fertilization program for future eucalypt cycles in order to prevent a progressively impoverish in the SOM and sustain eucalypt yields in the long term. In addition, the large capacity of C storage of such soils makes these areas an important agent for improving C-CO_2 sequestration and mitigation of climate change.

CONCLUSIONS

The replacement of native *Pampa* cover by eucalypt plants reduced C and N stocks after a period of 32 months. The POM and MAOM fraction presented C loss rates over time (0-100 cm soil depth), which the MAOM-C loss was 12 times higher than in the POM. This pattern also occurred for N stocks, which about of 780 kg ha^{-1} of N annually were released from SOM.

The practices (subsoiling plus deep band fertilization and ridge tillage) adopted before eucalypt planting promoted a large influence on SOM dynamics, mainly in the row zone. In this region, higher C depletion was observed in comparison to inter-row zone, indicating the occurrence of priming effects triggered by high fine roots density. Nitrogen mineralization, on the other hand, was higher in the inter-row zone.

Although old native C losses have predominated upon new eucalypt-derived organic matter formation over the experimental period, soil mineralogical characteristics (e.g. surface area, charge density, Al_o) support a large potential for C storage and stabilization in these areas. Soil layers under large C saturation deficits presented the highest organic matter formation efficiency rates, mainly in the row position. However, no differences for $OMFE_{POM+MAOM}$ (0-100 cm soil depth) were found between soils from row and inter-row zones (7.42%).

Further studies investigating the soil tillage and fertilization systems on fine root growth and microbial communities and its impact on the SOM formation and destabilization in row and inter-row zones must be considered for improving the current management employed in future short-rotation eucalypt sites.

FIGURES AND TABLES

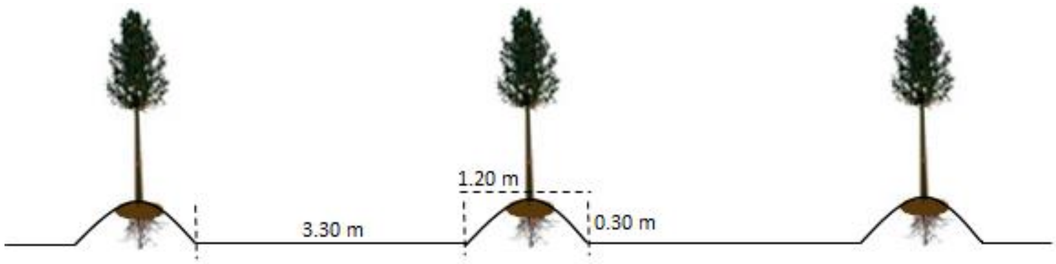

Figure 1. Ridge tillage dimensions used for eucalypt planting.

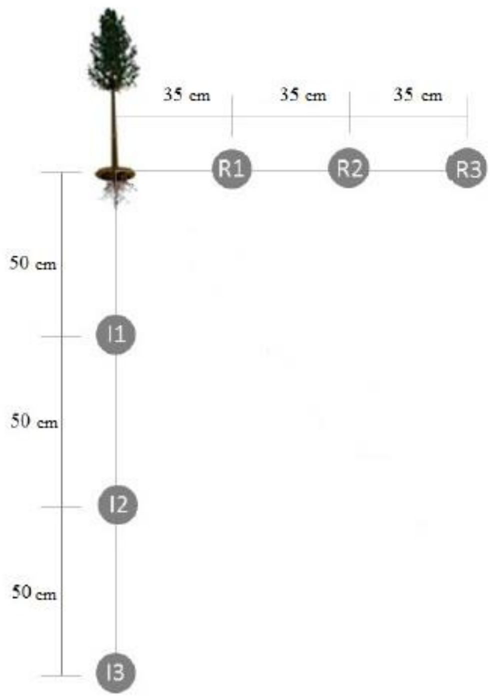

Figure 2. Distribution of fine roots sampling points in the row (R) and inter-row (I) positions of a representative eucalypt stand ridge-planted in a native grassland field in Southern Brazil. Adapted from Ferreira et al. (2018).

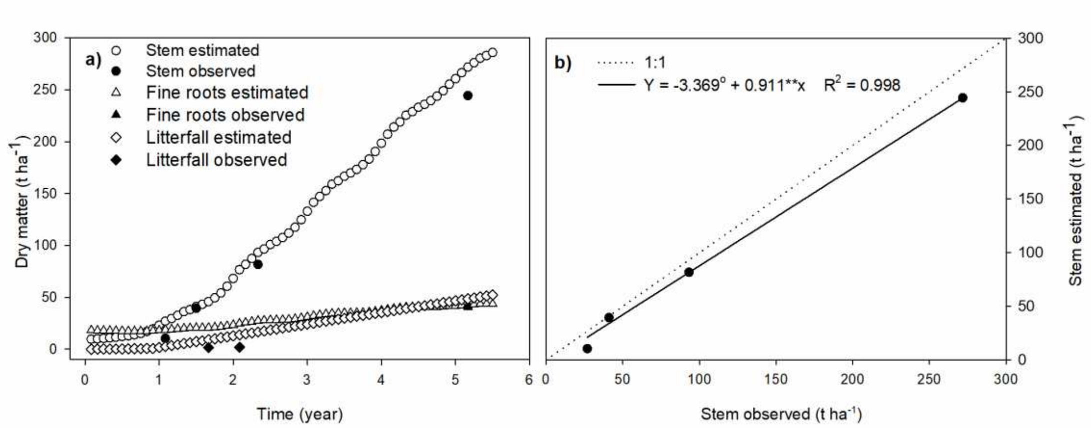

Figure 3. 3-PG model prediction of eucalypt stem, roots, and litterfall dry matter over the experimental period (5.5 years) (a); fitted regression between observed and estimated eucalypt stem (b); ^{o}, ** significant effects at $p < 0.1$ and 0.01 by-F test.

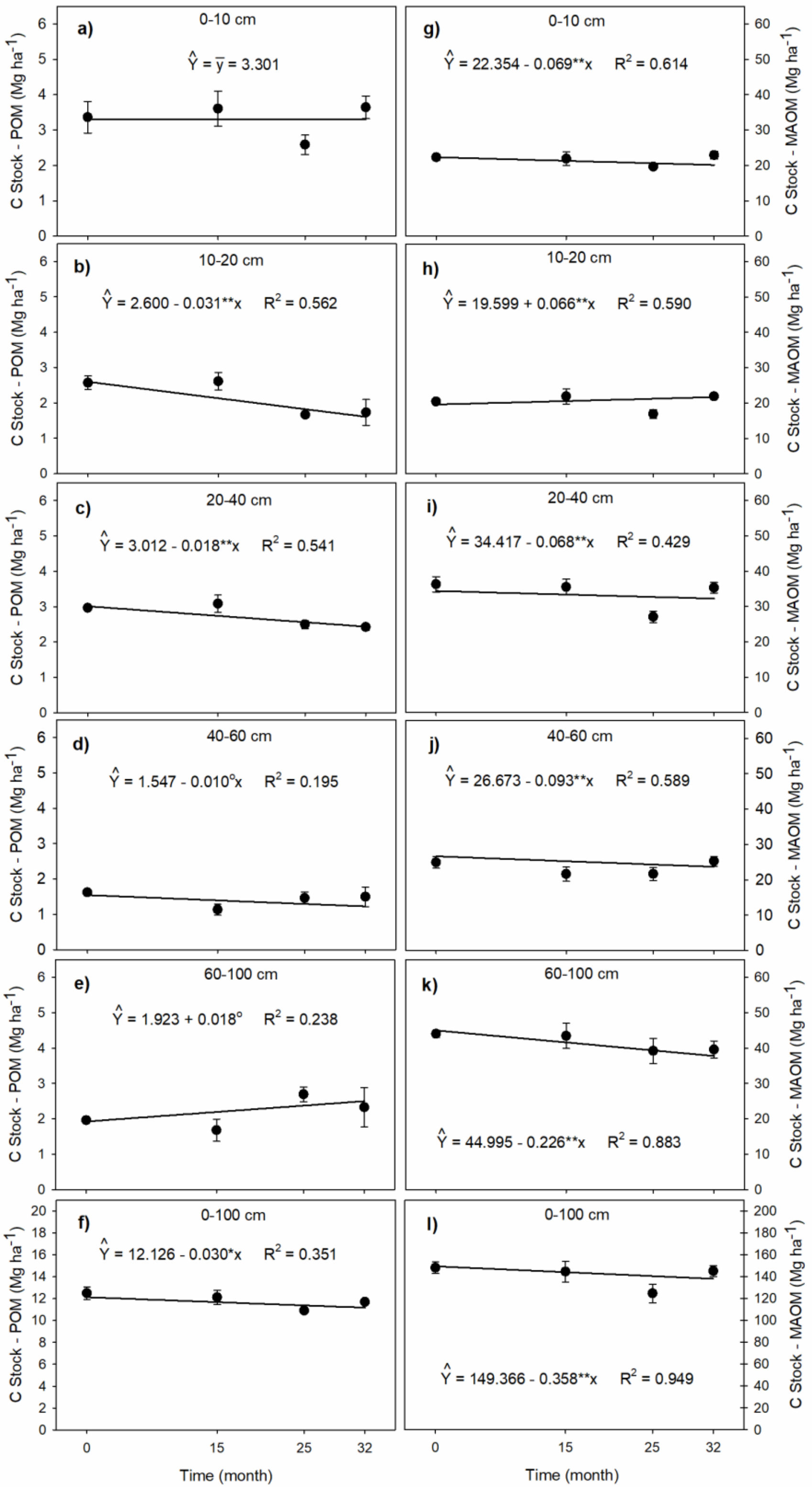

Figure 4. Soil carbon (C) stocks changes in the Particulate Organic Matter (POM) and Mineral Associated Organic Matter (MAOM) fraction at different soil depths over a period of 32 months in a eucalypt stand ridge-planted in a native grassland field in Southern Brazil. º, *, ** significant effects at $p < 0.1$, 0.05, and 0.01, respectively, by F-test. Vertical bars denote the standard error of the mean (n=4).

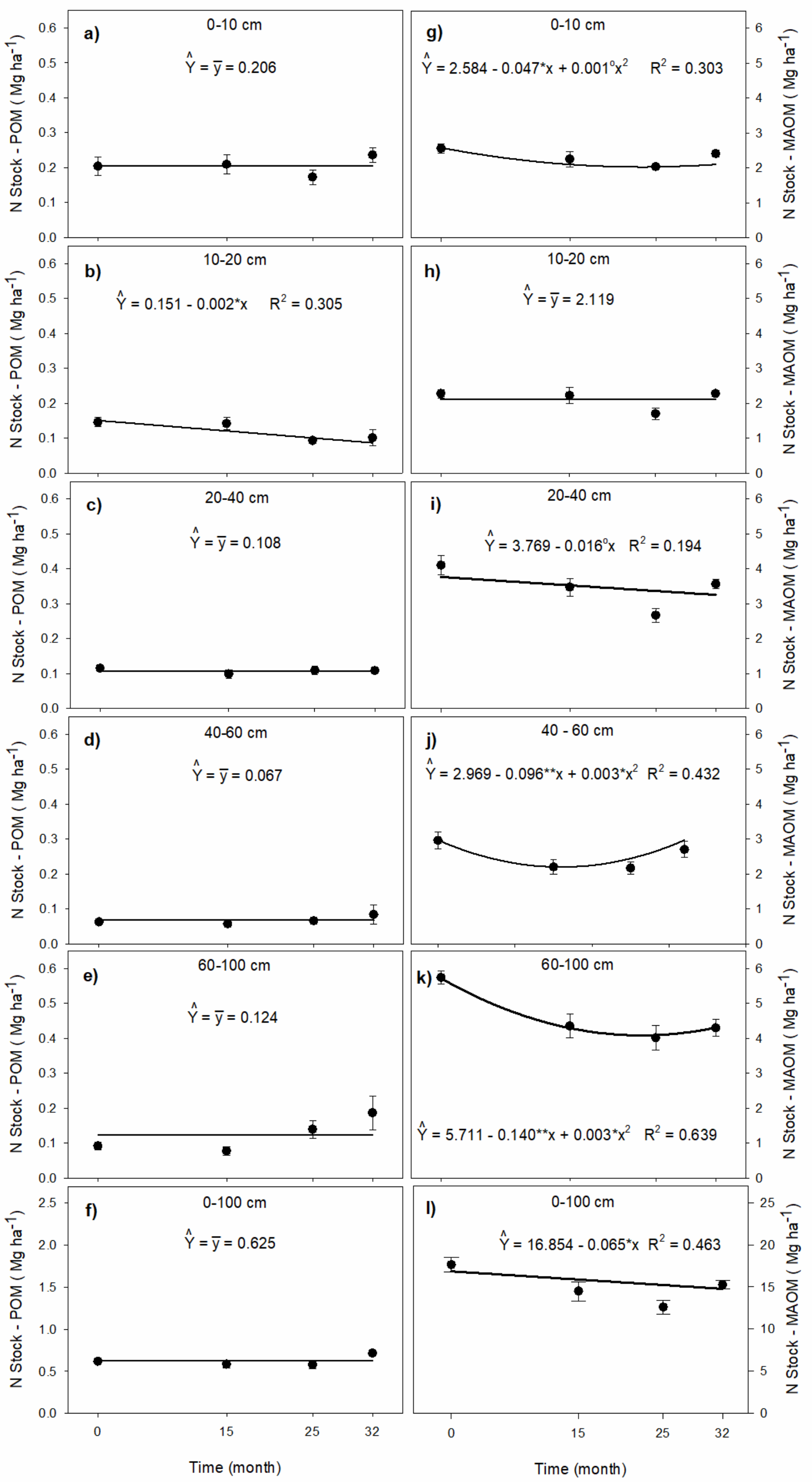

Figure 5. Soil nitrogen (N) stocks in the Particulate Organic Matter (POM) and Mineral Associated Organic Matter (MAOM) fraction at different soil depths over a period of 32 months in a eucalypt stand ridge-planted in a native grassland field in Southern Brazil. °, *, ** significant effects at $p < 0.1$, 0.05, and 0.01, respectively, by F-test. Vertical bars denote the standard error of the mean (n=4).

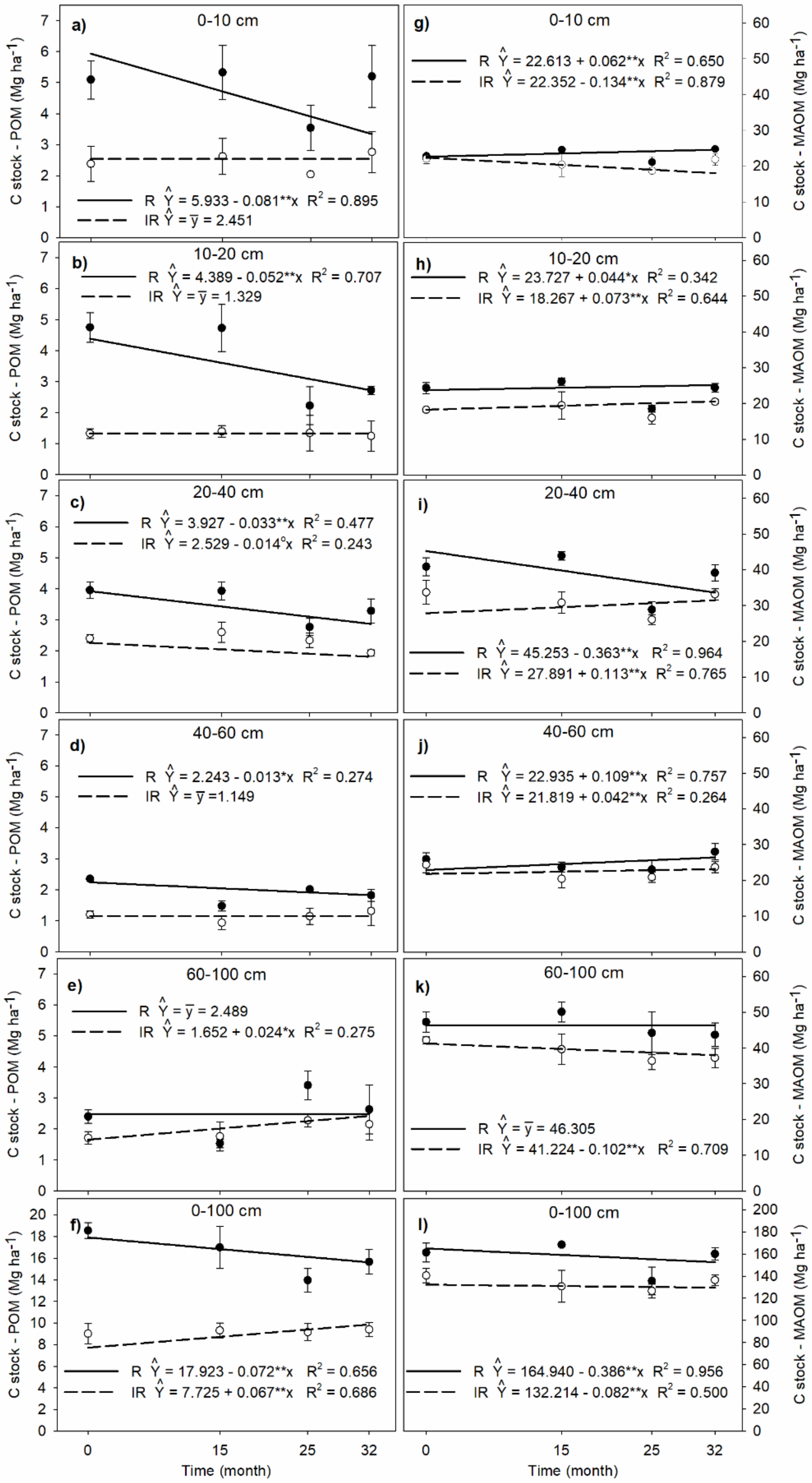

Figure 6. Soil carbon (C) stocks in the Particulate Organic Matter (POM) and Mineral Associated Organic Matter (MAOM) fraction in row (R) and inter-row (IR) position at different soil depths over a period of 32 months in a eucalypt stand ridge-planted in a native grassland field in Southern Brazil. ^{o}, *, ** significant effects at $p < 0.1$, 0.05, and 0.01, respectively, by F-test. Vertical bars denote standard error of the mean (n=4).

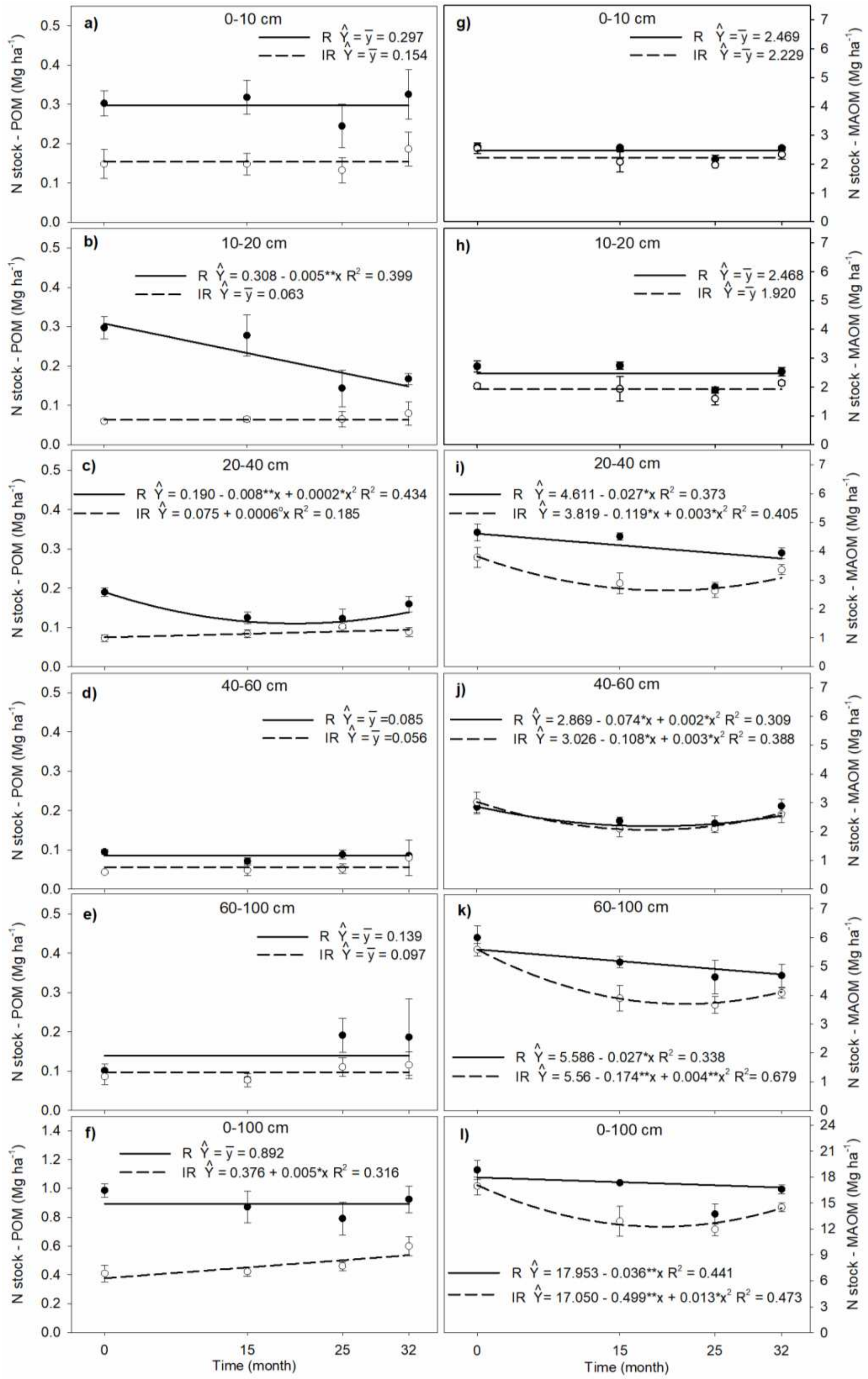

Figure 7. Soil nitrogen (N) stocks in the Particulate Organic Matter (POM) and Mineral Associated Organic Matter fraction in row (R) and inter-row (IR) position at different soil depths over a period of 32 months in a eucalypt stand ridge-planted in a native grassland field in Southern Brazil. ^{o}, *, ** significant effects at p < 0.1, 0.05, and 0.01, respectively, by F-test. Vertical bars denote the standard error of the mean (n=4).

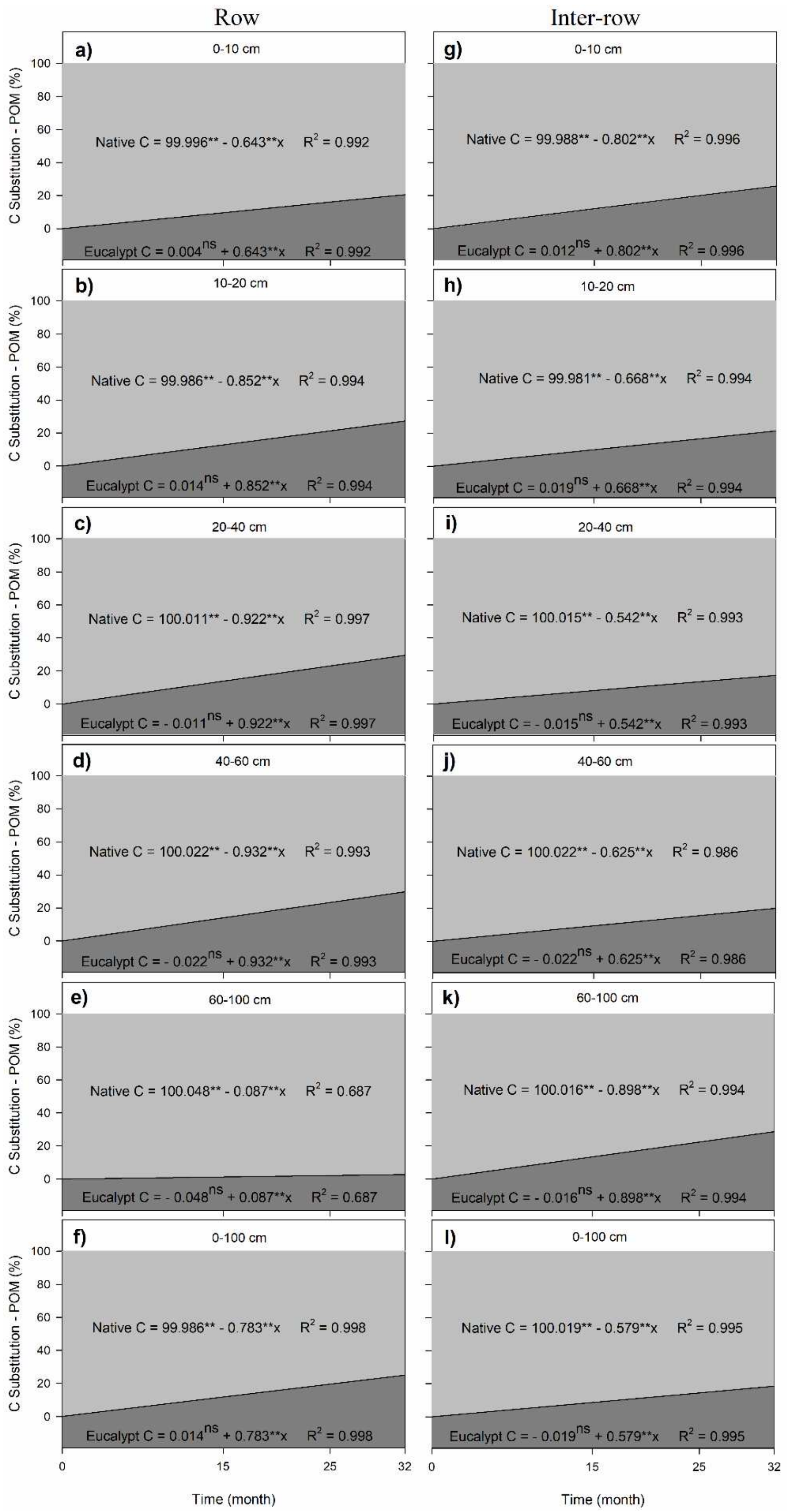

Figure 8. Native and eucalypt carbon (C) substitution in the Particulate Organic Mater (POM) in row and inter-row position at different soil layers over a period of 32 months in a eucalypt stand ridge-planted in a native grassland field in Southern Brazil. ** significant effects at $p < 0.01$and ^{ns} not significant by F-test.

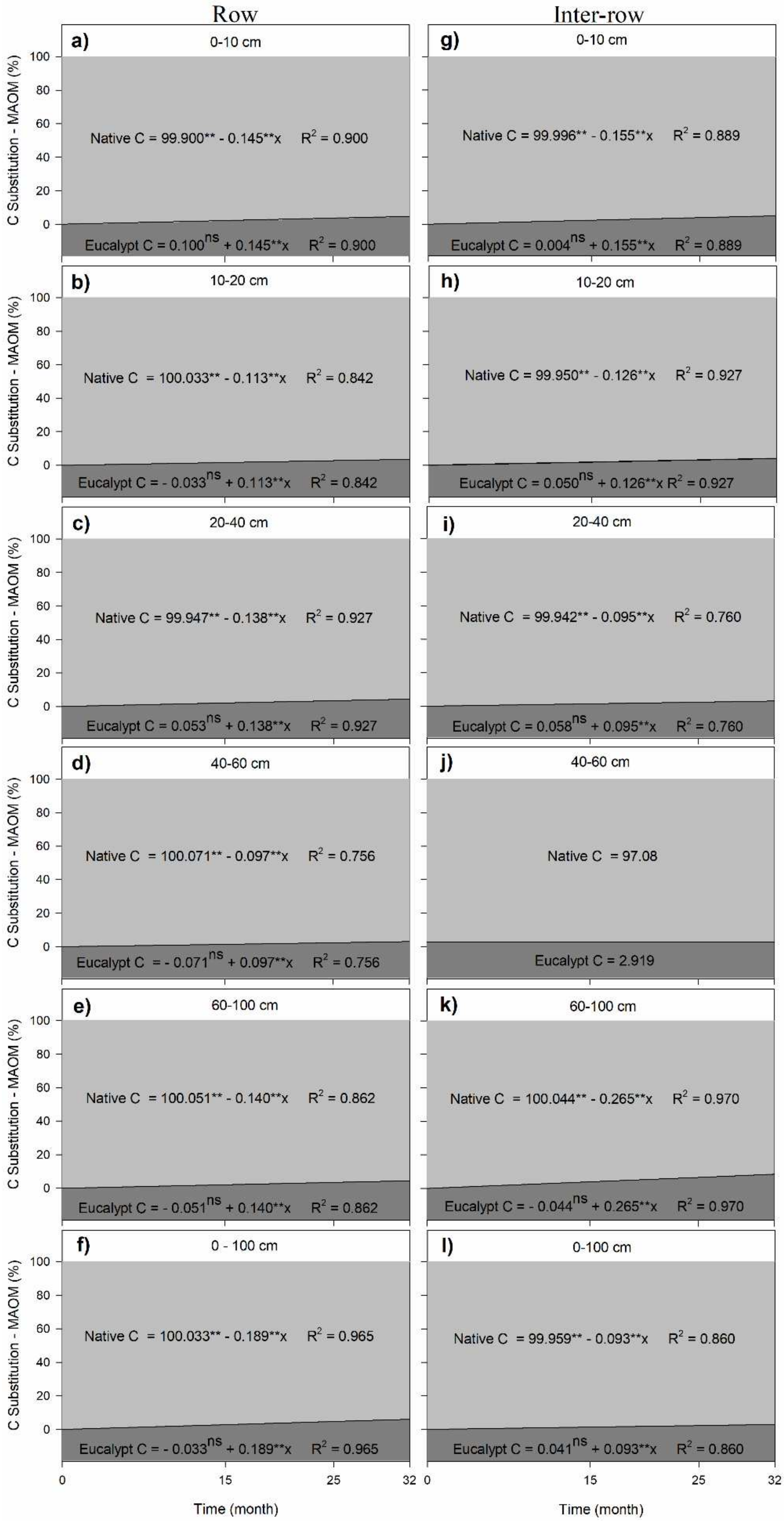

Figure 9. Native and eucalypt carbon (C) substitution in the Mineral Associated Organic Matter (MAOM) fraction in row and inter-row position at different soil layers over a period of 32 months in a eucalypt stand ridge-planted in a native grassland field in Southern Brazil. ** significant effects at p < 0.01and ^{ns} not significant by F-test.

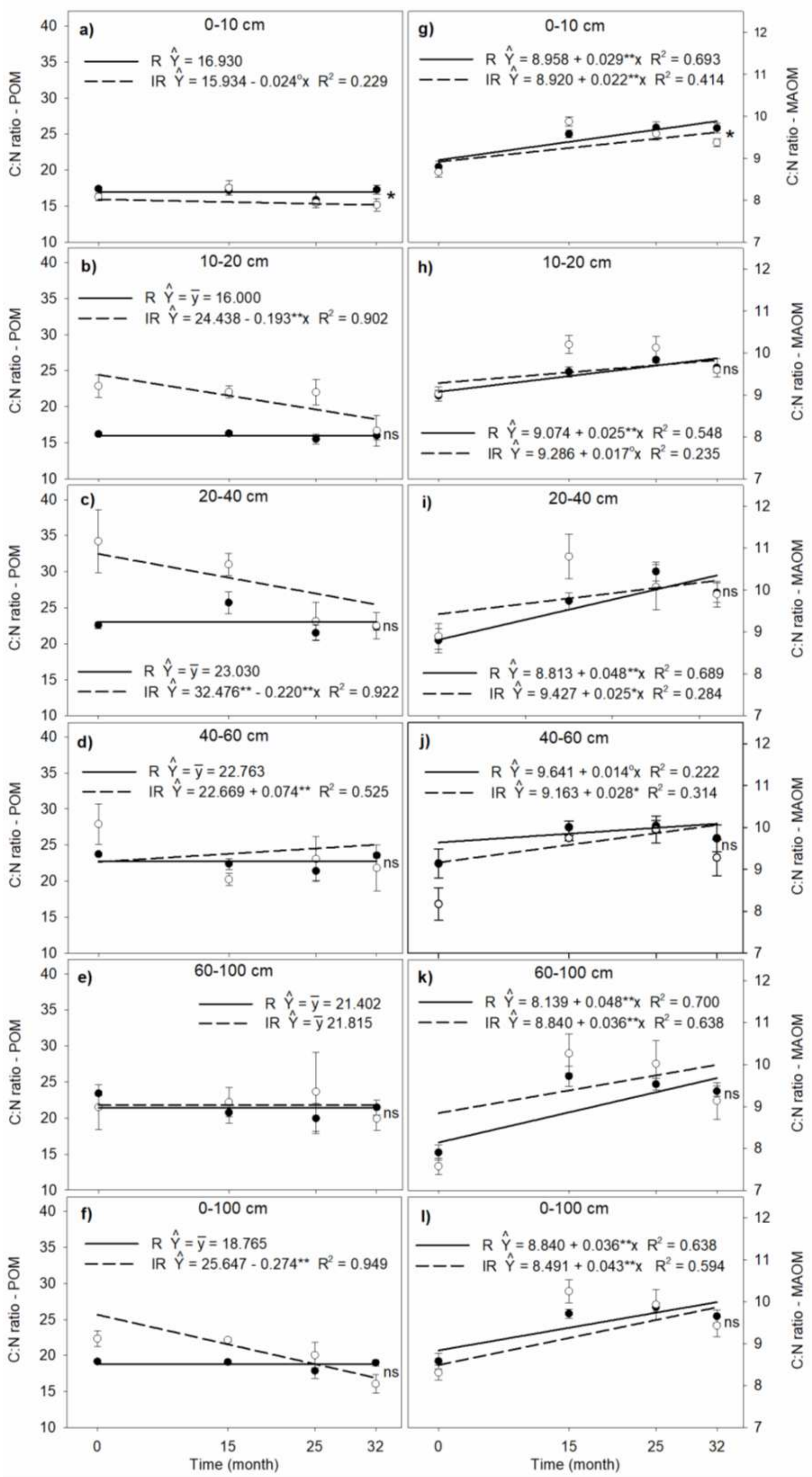

Figure 10. C:N ratio in the Particulate Organic Matter (POM) and Mineral Associated Organic Matter (MAOM) fraction in row (R) and inter-row (IR) position at different soil depths over a period of 32 months in a eucalypt stand ridge-planted in a native grassland field in Southern Brazil. * significant effects at $p < 0.01$; ^{ns} not significant by F-test ($p < 0.1$). Vertical bars denote standard error of the mean (n=4).

Table 1. Soil attributes at the beginning of the experiment.

Depth	BD	TP	Sand	Silt	Clay	pH	OM	N	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H + Al	SB	t	T	V	m	Clay activity
cm	g cm^{-3}	m m^{-3}	—— g kg^{-1}——			H_2O	— %—		— mg kg^{-1} —		———————— $cmol_c$ kg^{-1} ————————							——%——		$cmol_c$ kg^{-1}
0-10	1.15	0.53	17	55	28	4.7	4.00	0.04	3.70	73.00	5.36	2.16	0.90	8.20	7.71	8.61	15.91	48.50	10.50	56.82
10-20	1.32	0.46	23	45	32	4.8	2.7	0.11	1.80	35.00	4.97	1.77	0.90	8.40	6.83	7.73	15.23	44.80	11.60	47.59
20-40	1.36	0.45	23	40	37	5.1	1.8	0.07	0.90	29.00	5.50	2.17	1.80	8.20	7.74	9.54	15.94	48.60	18.90	43.08
40-60	1.22	0.51	17	38	45	5.1	1.7	0.14	0.50	53.00	6.11	2.49	2.90	8.90	8.74	11.64	17.64	49.50	24.90	39.20
60-100	1.28	0.48	12	26	62	5.3	1.3	0.15	5.60	164.00	9.83	4.28	2.70	9.40	14.53	12.23	23.93	60.70	15.70	38.60

BD: bulk density; TP: total porosity; pH in water (1:2.5 v v^{-1}); OM: organic matter, determined by the Walkley-Black method; TN: total nitrogen, quantified by Kjeldahl distillation; P and K extracted by $Mehlich^{-1}$ solution; Ca^{2+} and Mg^{2+} extracted by KCl (1 mol L^{-1}); Al^{3+} extracted with KCl (1 mol L^{-1}); H+Al extracted with calcium acetate (0.5 mol L^{-1}) at pH 7.0; SB: sum of bases ($SB = Ca^{2+} + Mg^{2+} + K^+$); CEC: cation-exchange capacity [$CEC = SB + Al^{3+}$]; $CEC_{pH\ 7.0}$ = cation-exchange capacity at pH 7.0 [$CEC_{pH=7.0} = SB + (H+Al)$]; V: base saturation [$V = (SB/ CEC_{pH=7.0}) \times 100$]; m: aluminum saturation [$m = (Al^{3+}/CEC)$ x 100]; Clay-activity = ($CEC_{pH=7.0}$ x 100/% clay).

Table 2. Soil chemical characterization.

Depth	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	MnO	Fe_O	Fe_D	Al_O	Al_D	Fe_o/Fe_d	Al_o/Al_d	Ki	Kr
cm	———— % ————					———— mg g^{-1} ————							
0-10	12.95	7.88	5.49	0.77	1.29	16.02	37.12	11.87	14.57	0.43	0.81	2.79	1.93
10-20	12.14	8.32	5.36	0.84	1.28	14.31	43.19	13.18	17.51	0.33	0.75	2.48	1.76
20-40	12.39	9.25	7.40	0.79	1.72	9.21	47.74	13.26	20.15	0.19	0.66	2.28	1.51
40-60	17.00	13.13	11.10	0.72	1.39	5.52	57.14	12.23	21.70	0.10	0.56	2.20	1.43
60-100	24.48	18.37	9.76	0.70	0.71	3.42	44.66	13.19	19.60	0.08	0.67	2.27	1.69

Fe_D and Al_D extracted with sodium dithionite-citrate-bicarbonate solution; Fe_O and Al_O extracted with oxalate-oxalic acid solution; $Ki = (\%SiO_2/60)/(\%Al_2O_3/102)$; $Kr = (\%SiO_2/60)/[(\%Al_2O_3/102)+(\%Fe_2O_3)]$.

Table 3. Grass derived-C variation (ΔC_{native}), C:N ratio, nitrogen mineralized (N_{min}), eucalypt derived-C ($C_{eucalypt\ derived}$), N immobilized (N_{imm}), net N mineralized ($N_{R\ or\ IR}$), and N mineralized weighted (N_{R+IR}) at different soil layers in Particulate Organic Matter (POM) and Mineral Associated Organic Matter (MAOM) fraction in row (R) and inter-row (IR) position at different soil depths after a period of 32 months in a eucalypt stand ridge-planted in a native grassland field in Southern Brazil.

	POM - Row						POM - Inter-row						
Depth	ΔC_{native}	C:N	N_{min}	$C_{eucalypt}$	N_{imm}	ΔN_R	ΔC_{native}	C:N	N_{min}	$C_{eucalypt}$	N_{imm}	ΔN_{IR}	$N_{R+IR\ POM}$
cm	Mg ha^{-1}		—— Mg ha^{-1} ——				Mg ha^{-1}		—— Mg ha-1 ——				
0-10	-0.640	17.274	0.037	0.746	-0.043	-0.006	0.075	15.187	-0.005	0.308	-0.020	-0.025	-0.018
10-20	-2.745	15.998	0.172	0.716	-0.045	0.127	-0.256	16.659	0.015	0.176	-0.011	0.005	0.049
20-40	-1.722	22.327	0.077	1.054	-0.047	0.030	-0.738	22.502	0.033	0.270	-0.012	0.021	0.024
40-60	-1.192	23.548	0.051	0.660	-0.028	0.023	-0.078	21.819	0.004	0.189	-0.009	-0.005	0.005
60-100	-0.390	21.483	0.018	0.620	-0.029	-0.011	-0.330	19.914	0.017	0.775	-0.039	-0.022	-0.018
0-100	-6.689	18.980	0.352	3.797	-0.200	0.152	-1.327	16.058	0.083	1.718	-0.107	-0.024	0.040
	MAOM - Row						MAOM - Inter-row						
Depth	ΔC_{native}	C:N	N_{min}	$C_{eucalypt}$	N_{imm}	ΔN_R	ΔC_{native}	C:N	N_{min}	$C_{eucalypt}$	N_{imm}	ΔN_{IR}	$N_{R+IR\ MAOM}$
cm	Mg ha^{-1}		—— Mg ha^{-1} ——				Mg ha^{-1}		—— Mg ha-1 ——				
0-10	0.765	9.720	-0.079	1.137	-0.117	-0.196	-0.502	9.373	0.054	1.435	-0.153	-0.100	-0.135
10-20	-1.291	9.649	0.134	1.337	-0.139	-0.005	1.663	9.599	-0.173	0.777	-0.081	-0.254	-0.163
20-40	-3.572	9.936	0.360	1.888	-0.190	0.170	-0.922	9.896	0.093	0.725	-0.073	0.020	0.074
40-60	1.443	9.740	-0.148	0.675	-0.069	-0.218	-0.829	9.278	0.089	0.606	-0.065	0.024	-0.064
60-100	-4.884	9.361	0.522	1.284	-0.137	0.385	-8.615	9.133	0.943	3.658	-0.401	0.543	0.485
0-100	-7.539	9.652	0.781	6.322	-0.655	0.126	-9.205	9.429	0.976	5.114	-0.542	0.434	0.322

$\Delta C_{native} = \Delta C_{native\ (32\ months)} - \Delta C_{native\ (0\ months)}$; $N_{min} = C_{eucalyp\ derived} / C:N$; $\Delta N = N_{min} + N_{imm}$; $N_{R+IR} = (\Delta N_R \times 0.03636) + (\Delta N_{IR} \times 0.6364)$. ΔN_{R+IR} values were calculated for POM and MAOM fraction by mass weighting based on the ridge dimensions, in which 0.3636 and 0.6364 were considered as the factors for row and inter-row position, respectively.

Table 4. Root density (g dm^{-3}) in row (R) and inter-row (IR) position at different soil layers at 32 and 62 months in a eucalypt stand ridge-planted in a native grassland field in Southern Brazil.

Depth	Root density (g dm^{-3})			
	— 32 months —		— 62 months —	
cm	R	IR	R	IR
0-10	0.22b	0.42a	0.42a	0.70a
10-20	0.26b	0.33a	3.02a	0.59b
20-40	1.17a	0.38b	1.36a	0.74b
40-60	N.D.	N.D.	1.70a	0.33b

Different lower cases letters indicate statistical differences between root density at row and inter-row positions at within a time period by F-test ($p < 0.1$). N.D. = not determined.

Table 5. Potential C stock (C $Stock_{Potential}$) and Carbon Saturation Deficit (CSD) for different soil layers at 0 and 32 months after eucalypt stand ridge-planting in a native grassland field in Southern Brazil.

Depth	C $Stock_{Potential}$	C $Stock_{0\ months}$	C $Stock_{32\ months}$	$CSD_{0\ months}$	$CSD_{32\ months}$
cm	Mg ha^{-1}				
0-10	72.77	25.67	26.56	47.10	46.21
10-20	80.20	23.03	23.68	57.17	56.52
20-40	82.63	39.26	37.75	43.37	44.88
40-60	77.20	26.56	26.78	50.64	50.42
60-100	83.69	45.97	41.87	37.72	41.82
0-100	396.50	160.49	156.64	236.01	239.86

Potential C Stock calculated according to Six et al., (2002). CSD = C $Stock_{Potential}$ – C $Stock_{0\ or\ 32\ months}$

Table 6. Organic matter formation efficiency (OMFE) in the Particulate Organic Matter (POM) and Mineral Associated Organic Matter (MAOM) fraction in row (R) and inter-row (IR) positions at different soil layers after a period of 32 months in a eucalypt stand ridge-planted in a native grassland field in Southern Brazil.

	Depth	Litterfall	Fine roots	Rhizodepozition	Total C input	POM - C_{Euc}	MAOM - C_{Euc}	$OMFE_{POM}$	$OMFE_{MAOM}$	$OMFE_{POM+MAOM}$
	cm	Mg ha^{-1} of C						%		
R	0-10	3.33	4.21	4.21	11.74	1.17a	0.73b	8.69a	5.39b	14.08a
	10-20	0.00	6.20	6.20	12.41	0.85a	1.58a	7.82a	14.57a	22.38a
	20-40	0.00	32.64	32.64	65.27	1.05a	1.37a	1.40a	1.82a	3.21a
	40-60	0.00	6.49	6.49	12.99	0.74a	1.64a	5.35a	11.91a	17.26a
	60-100	0.00	9.34	9.34	18.68	0.78b	1.66a	3.94a	8.37b	12.21b
	0-100	3.33	58.88	58.88	121.09	3.47a	7.39a	2.91a	5.56a	8.16a
IR	0-10	6.66	6.66	6.66	19.98	0.31b	1.44a	1.74b	6.75a	8.48b
	10-20	0.00	7.04	7.04	14.09	0.23b	0.78b	2.06b	6.85b	8.91b
	20-40	0.00	16.86	16.86	33.71	0.36b	0.73b	1.36b	2.75a	4.11a
	40-60	0.00	5.38	5.38	10.76	0.25b	0.61b	2.66b	6.7b	9.03b
	60-100	0.00	7.73	7.73	15.47	0.87a	2.72a	6.39a	19.91a	26.3a
	0-100	6.66	43.67	43.67	94.01	1.95b	3.55b	2.37a	4.32a	6.69a

*Root density contribution (%) at 40-60 and 60-100 cm soil layer and fine roots turnover (3 months) was determined according to Jourdan et al., (2008); Litterfall biomass was obtained from 3PG model. Carbon derived from rhizodeposition was considered as the same for fine roots as proposed by Rasse et al., (2005); $\%C_{fine\ roots} = 0.4152$; $\%C_{litterfall} = 0.4917$. Means followed by the same lower-case letters within each soil layer do not differ significantly by the Tukey's test ($p < 0.1$).

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SUPPORTING INFORMATION

Table S1. Carbon (C) stocks in the Particulate Organic Matter (POM) and Mineral Associated Organic Matter (MAOM) fraction in row and inter-row positions at different soil layers over a period of 32 months in a eucalypt stand ridge-planted in a native grassland field in Southern Brazil

	C stock POM							
	Row				Inter-row			
Depth	0 months	15 months	25 months	32 months	0 months	15 months	25 months	32 months
cm	Mg ha^{-1}							
0-10	5.09 (± 0.62)	5.33 (± 0.87)	3.54 (± 0.73)	5.20 (± 1.00)	2.38 (± 0.56)	2.62 (± 0.58)	2.04 (± 0.02)	2.76 (± 0.66)
10-20	4.75 (± 0.48)	4.73 (± 0.75)	2.23 (± 0.61)	2.72 (± 0.14)	1.33 (± 0.08)	1.40 (± 0.09)	1.34 (± 0.29)	1.25 (± 0.50)
20-40	3.96 (± 0.27)	3.94 (± 0.29)	2.77 (± 0.28)	3.29 (± 0.38)	2.40 (± 0.24)	2.60 (± 0.33)	2.34 (± 0.23)	1.93 (± 0.09)
40-60	2.35 (± 0.07)	1.48 (± 0.16)	2.01 (± 0.06)	1.82 (± 0.19)	1.20 (± 0.11)	0.94 (± 0.22)	1.15 (± 0.26)	1.31 (± 0.45)
60-100	2.40 (± 0.22)	1.53 (± 0.13)	3.41 (± 0.46)	2.63 (± 0.79)	1.71 (± 0.2)	1.76 (± 0.47)	2.28 (± 0.21)	2.15 (± 0.50)
0-100	18.55 (±0.73)	17.00 (± 1.93)	13.95 (± 1.09)	15.66 (± 1.14)	9.02 (± 0.95)	9.32 (± 0.68)	9.15 (± 0.80)	9.41 (±0.64)
	C stock MAOM							
	Row				Inter-row			
Depth	0 months	15 months	25 months	32 months	0 months	15 months	25 months	32 months
cm	Mg ha^{-1}							
0-10	22.85 (± 0.45)	24.58 (± 0.49)	21.07 (± 1.53)	24.75 (± 0.48)	22.00 (± 1.31)	20.39 (± 3.25)	18.85 (± 0.09)	21.86 (± 1.64)
10-20	24.34 (± 1.58)	26.14 (± 1.03)	18.51 (± 0.97)	24.39 (± 1.25)	18.23 (± 0.49)	19.45 (± 3.87)	15.98 (± 1.71)	20.48 (± 0.60)
20-40	40.86 (± 2.58)	43.89 (± 1.16)	28.83 (± 2.19)	39.17 (± 2.27)	33.68 (± 3.29)	30.78 (± 2.99)	26.07 (± 1.38)	33.12 (± 1.53)
40-60	25.91 (± 1.76)	23.7 (± 1.38)	23.01 (± 2.66)	28.03 (± 2.25)	24.39 (± 2.24)	20.43 (± 2.48)	20.88 (± 1.42)	23.71 (± 1.56)
60-100	47.27 (± 2.89)	50.08 (± 2.78)	44.19 (± 5.92)	43.67 (± 3.30)	42.15 (± 0.96)	39.63 (± 4.22)	36.37 (± 2.48)	37.19 (± 2.67)
0-100	161.23 (± 8.44)	168.38 (± 2.02)	135.62 (± 12.47)	160.02 (± 5.64)	140.45 (± 6.37)	130.67 (± 14.44)	118.15 (± 6.25)	136.35 (± 4.84)

Table S2. C:N ratio of the Particulate Organic Matter (POM) and Mineral Associated Organic Matter (MAOM) fraction in row (R) and inter-row (IR) positions at different soil layers over a period of 32 months in a eucalypt stand ridge-planted in a native grassland field in Southern Brazil.

	POM							
	0 months		15 months		25 months		32 months	
Depth (cm)	C:N R	C:N IR	C:N R	C:N IR	C:N R	C:N IR	C:N R	C:N IR
0-10	17.19 (± 0.19)Aa	16.30 (± 0.52)Aa	17.16 (± 0.69)Aa	17.53 (± 1.05)Aa	15.87 (± 1.10)Aa	15.48 (± 0.36)Aa	17.27 (± 0.61)Aa	15.18 (± 0.89)Ba
10-20	16.21 (± 0.32)Ba	22.88 (± 1.57)Aa	16.29 (± 0.29)Ba	22.04 (± 0.82)Aab	15.51 (± 0.70)Ba	22.01 (± 1.81)Aab	15.99 (± 0.62)Aa	16.65 (± 2.17)Ab
20-40	22.59 (± 0.47)Ba	34.22 (± 4.40)Aa	25.69 (± 1.54)Aa	30.99 (± 1.51)Aab	21.50 (± 1.04)Aa	23.12 (± 2.62)Ab	22.33 (± 0.42)Aa	22.50 (± 1.82)Ab
40-60	23.72 (± 0.03)Aa	27.90 (± 2.79)Aa	22.39 (± 0.75)Aa	20.20 (± 0.85)Aa	21.40 (± 1.32)Aa	23.04 (± 3.10)Aa	23.54 (± 0.34)Aa	21.81 (± 3.21)Aa
60-100	23.42 (± 0.32)Aa	21.50 (± 3.12)Aa	20.76 (± 1.45)Aa	22.20 (± 2.02)Aa	19.94 (± 2.08)Aa	23.64 (± 5.48)Aa	21.48 (± 1.04)Aa	19.91 (± 1.59)Aa
0-100	19.15 (± 0.04)Ba	22.33 (± 1.13)Aa	19.08 (± 0.07)Ba	22.15 (± 0.25)Aa	17.85 (± 1.03)Ba	20.04 (± 1.77)Aab	18.97 (± 0.40)Aa	16.06 (± 1.27)Bb
	MAOM							
	0 months		15 months		25 months		32 months	
Depth (cm)	C:N R	C:N IR	C:N R	C:N IR	C:N R	C:N IR	C:N R	C:N IR
0-10	8.79(± 0.14)Ab	8.67 (± 0.13)Ab	9.58 (± 0.09)Aa	9.88 (± 0.11)Aa	9.73 (± 0.14)Aa	9.59 (± 0.14)Aa	9.72 (± 0.12)Aa	9.37 (± 0.09)Ba
10-20	8.98 (± 0.13)Ab	9.02 (± 0.17)Ab	9.55 (± 0.12)Aab	10.20 (± 0.22)Aa	9.83 (± 0.07) Aa	10.13 (± 0.26)Aa	9.65 (± 0.22)Aab	9.60 (± 0.06)Aab
20-40	8.79 (± 0.29)Ab	8.90 (± 0.31)Ab	9.74 (± 0.20)Aa	10.80 (± 0.53)Aa	10.44 (± 0.22)Aa	10.07 (± 0.54)Aa	9.94 (± 0.23)Aa	9.90 (± 0.30)Aab
40-60	9.14 (± 0.34)Ab	8.17 (± 0.39)Ab	10.00 (± 0.15)Aab	9.74 (± 0.06)Aa	10.03 (± 0.13)Aa	9.95 (± 0.32)Aa	9.74 (± 0.32)Aab	9.28 (± 0.43)Aab
60-100	7.90 (± 0.18)Ab	7.57 (± 0.19)Ab	9.72 (± 0.25)Aa	10.26 (± 0.47)Aa	9.53 (± 0.14)Aa	10.02 (± 0.55)Aa	9.36 (± 0.12)Aa	9.13 (± 0.44)Aa
0-100	8.58 (± 0.19)Bb	8.31 (± 0.18)c	9.72 (± 0.11)Aa	10.25 (± 0.28)Aa	9.87 (±0.11)Aa	9.93 (± 0.36)Aab	9.65 (± 0.16)Aa	9.43 (± 0.26)Ab

Capital letters indicate statistical differences between row (R) and inter-row (IR) positions at a same time period according to F-test ($p < 0.1$); lower case letters indicate statistical differences of R and IR positions at a same soil layer among different time periods according to Tukey's test ($p < 0.1$).

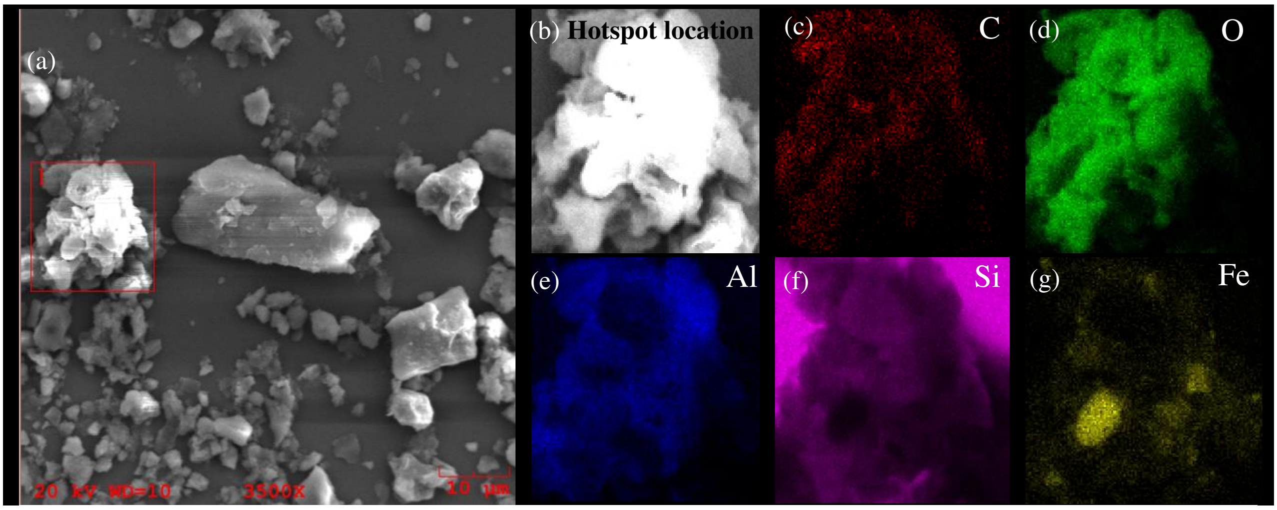

Figure S1. Carbon (C) hotspot (a and b) localization based on energy dispersive X-ray detector (EDS) mapping (c–g) at 0-10 cm soil layer.

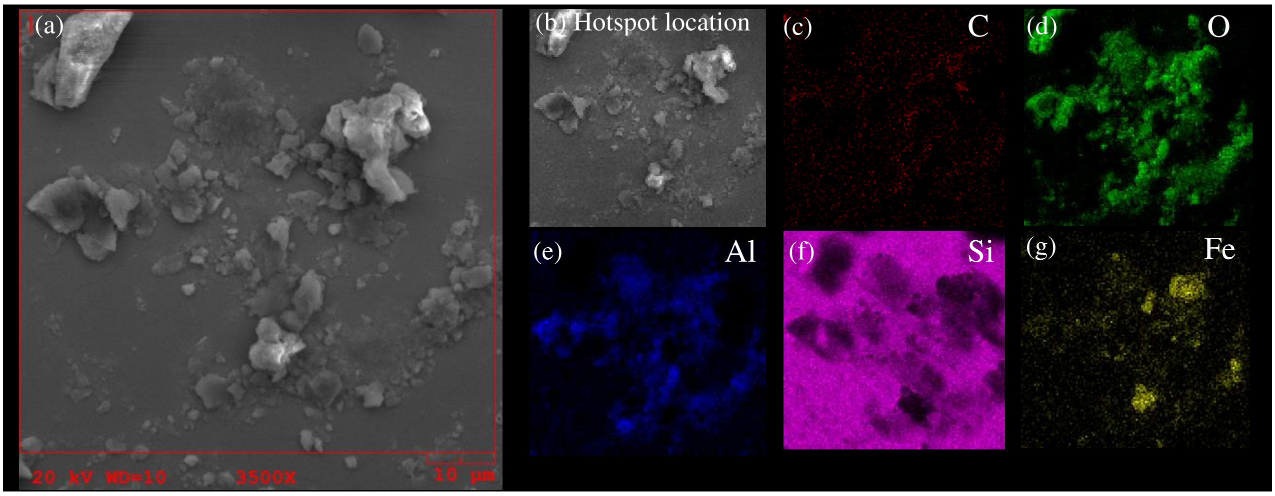

Figure S2. Carbon (C) hotspot localization (a and b) based on energy dispersive X-ray detector (EDS) mapping (c–g) at 10-20 cm soil layer. C: carbon, O: oxygen, Al: aluminum, Si: silica, and Fe: iron.

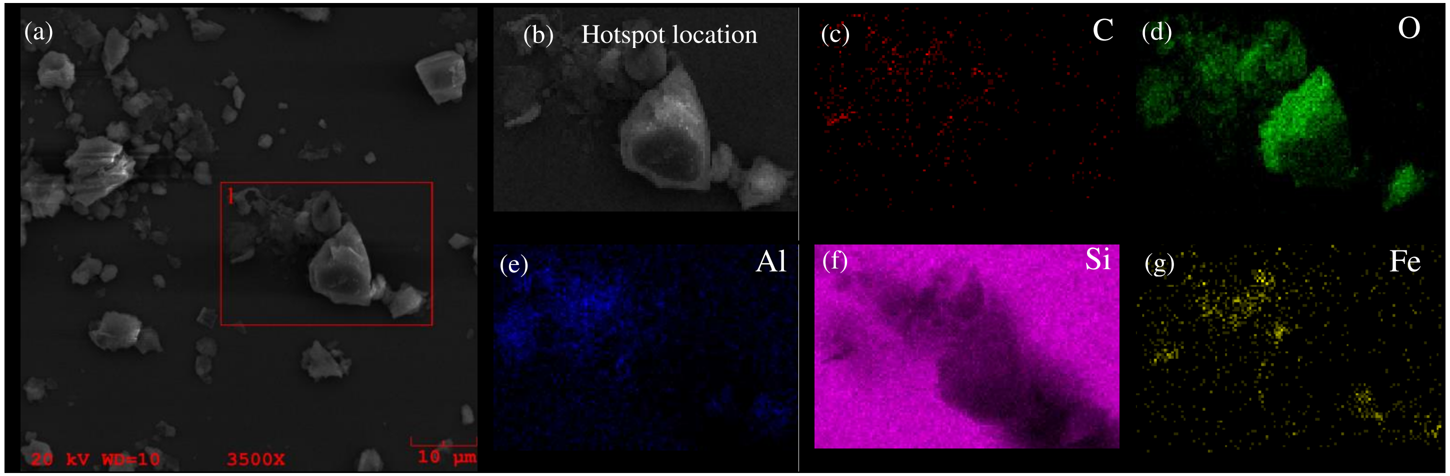

Figure S3. Carbon (C) hotspot localization (a and b) based on energy dispersive X-ray detector (EDS) mapping (c–g) at 20-40 cm soil layer. C: carbon, O: oxygen, Al: aluminum, Si: silica, and Fe: iron.

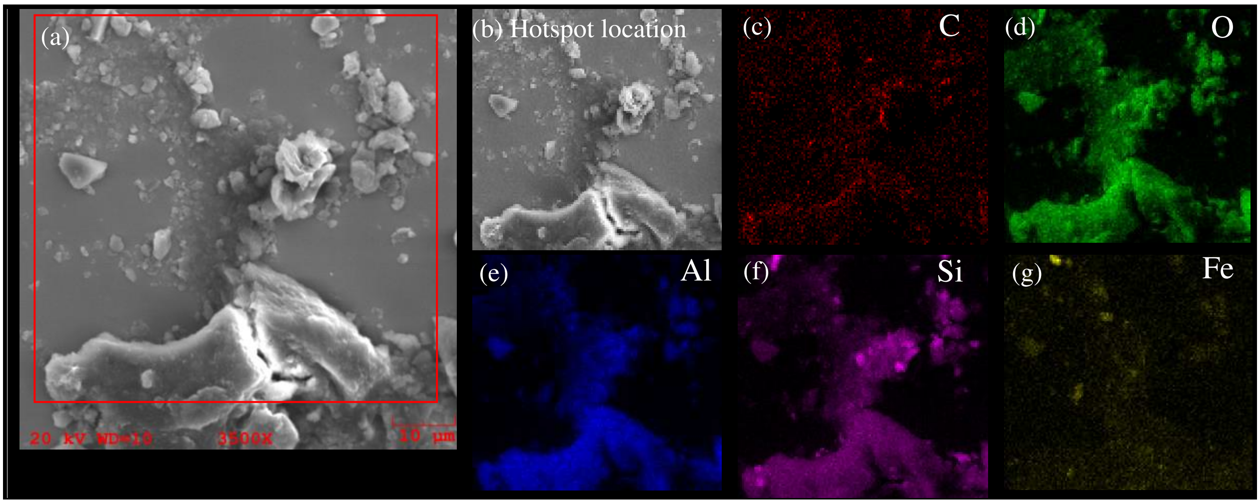

Figure S4. Carbon (C) hotspot localization (a and b) based on energy dispersive X-ray detector (EDS) mapping (c–g) at 40-60 cm soil layer. C: carbon, O: oxygen, Al: aluminum, Si: silica, and Fe: iron.

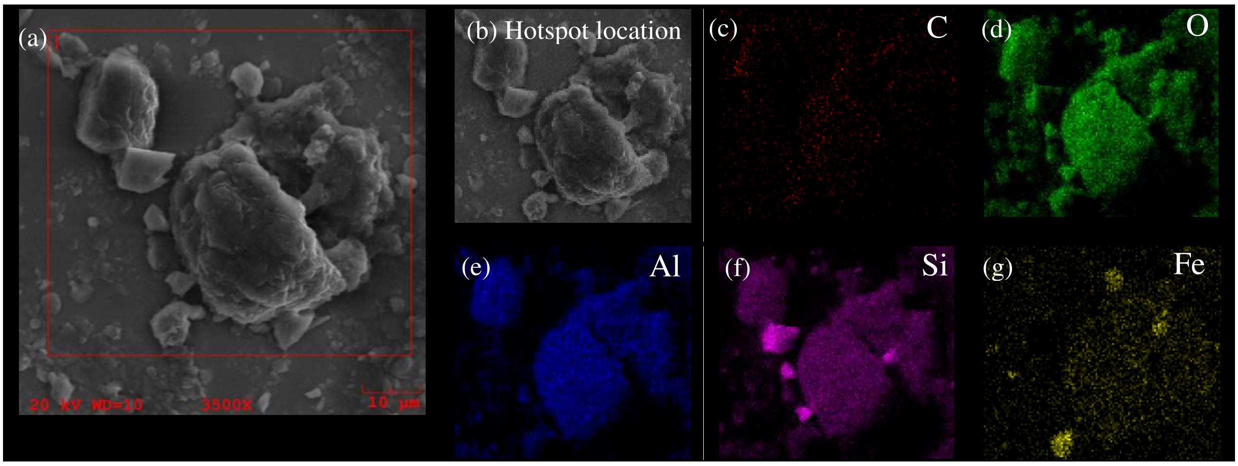

Figure S5. Carbon (C) hotspot localization (a and b) based on energy dispersive X-ray detector (EDS) mapping (c–g) at 60-100 cm soil layer. C: carbon, O: oxygen, Al: aluminum, Si: silica, and Fe: iron.

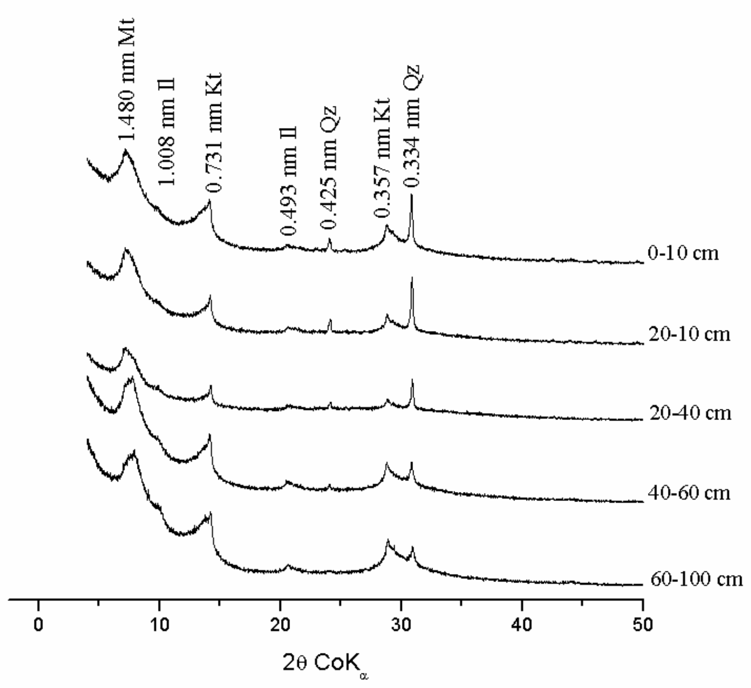

Figure S6. X-ray diffraction patterns of natural clay fraction at different soil layers in an Oxyaquic Hapludalf soil from Southern Brazil. Mt: montmorillonite; Il: illite; Qz: quartz; and Kt: kaolinite.

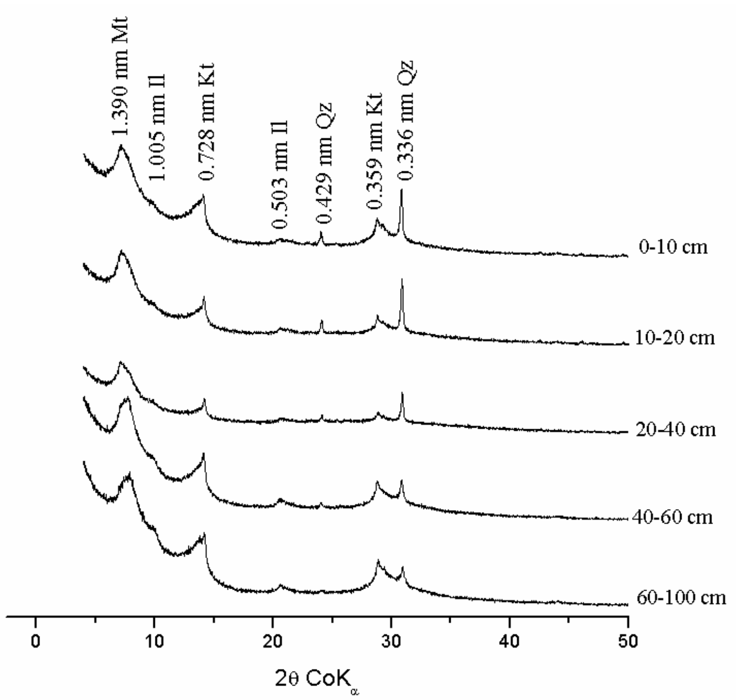

Figure S7. X-ray diffraction patterns of clay fraction after dithionite citrate bicarbonate (DCB) treatment at different soil layers in an Oxyaquic Hapludalf soil from Southern Brazil. Mt: montmorillonite; Il: illite; Qz: quartz; and Kt: kaolinite.

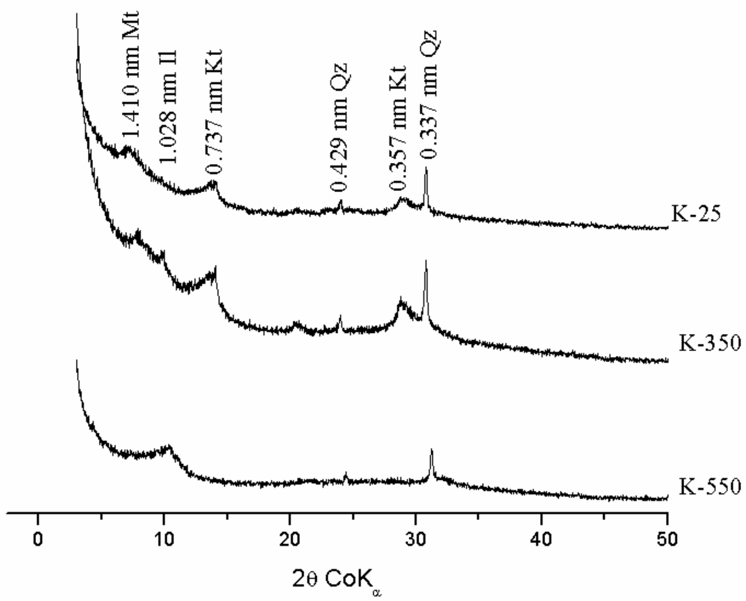

Figure S8. X-ray diffraction patterns of clay fraction after underwent saturation treatment with potassium at different heating levels: 25 °C (K-25), 350 °C (K-350), and 550 °C (K-550) in an Oxyaquic Hapludalf soil from Southern Brazil. Mt: montmorillonite; Il: illite; Qz: quartz; and Kt: kaolinite.

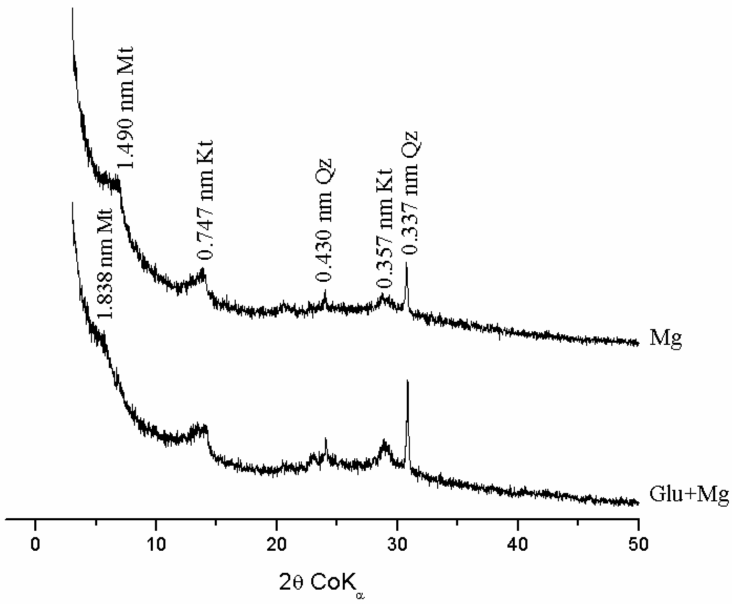

Figure S9. X-ray diffraction patterns of clay fraction after saturation treatment with magnesium and glucose in an Oxyaquic Hapludalf soil from Southern Brazil. Mt: montmorillonite; Qz: quartz; and Kt: kaolinite.