XXXV Congresso Brasileiro de Ciência do Solo
CENTRO DE CONVENÇÕES - NATAL / RN
O SOLO E SUAS MÚLTIPLAS FUNÇÕES
02 a 07 DE AGOSTO DE 2015

Eucalyptus sp. Harvest Residues: Impacts on the Dynamics of Microbial Community of Soils in Pampa Biome[(1)].

Gabriel William Dias Ferreira[(2)]; Fernanda Cristina Caparelli de Olivera[(2)]; Aline de Almeida Vasconcelos[(3)]; Jennifer A J Dungait[(4)]; Ivo Ribeiro da Silva[(5)]; Elias Frank Araújo[(6)]

[(1)]Trabalho executado com recursos da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).
[(2)]Estudante de doutorado no Programa de Pós-graduação em Solos e Nutrição de Plantas; Universidade Federal de Viçosa; Viçosa, Minas Gerais; gabrielwdf@gmail.com ;[(3)]Autônomo Doutor em Solos e Nutrição de Plantas;[(4)]Pesquisador, Rothamsted Research, UK,[(5)] Professor, Universidade Federal de Viçosa; Viçosa, Minas Gerais, [(6)] Pesquisador, CMPC, Guaíba, RS.

RESUMO: The composition and activity of soil microbial community are among the most important forces driving SOM mineralization process. The use of isotopic techniques on biomarkers of soil microbial community allows a linking between microbial community structure and your specific role on the process of SOM formation. The aim of this study was to assess the succession of soil community, through phospholipid fatty acids (PLFA) combined with ^{13}C-PLFA, mediating the decomposition of *Eucalyptus sp.* harvest residue on soil of Pampa Biome. The treatments were arranged as a randomized block design with four field replications. Treatments consisted of 3 residue management (no residue added or addition of residue without bark or with bark presence) and 2 levels of N fertilization (0 and 200 kg ha^{-1}). Soil samples were analysed in three different periods of residue decomposition: 3, 6 and 12 months. We used Principal Components Analysis (PCA) to elucidate PLFA and δ^{13}C-PLFA groups overtime. We found 24 PLFAs which account for an average concentration of 1.42 µg C-PLFA g^{-1} of dry soil. The mean of δ^{13}C-PLFA was -21.7 ‰. Differences on soil microbial community concentration caused by eucalyptus harvest residue were time dependent. Fungi was the most responsible for residue decomposition and G+ve and Actino less ones.

Index therms: harvest residue decomposition, phospholipid fatty acids (PLFA), C isotopic ratio.

INTRODUCTION

Once microbial decomposer community is close specialized to decompose a specific litter from the plants above it (Ayres et al., 2009), changes on quality of C input to soil is expected to alter soil microbial composition. According to Veen et al. (2015) the intensity of such alteration can be stronger with higher discrepancy between the new C input and former one, for example in transposition of forest to grassland (vice versa) litter, although, soil microorganisms could adapt fast to new environmental conditions.

Many studies have revealed strong correlations between microbial community structure and several environmental factors, including pH and C quantity and quality (Legg et al., 2012). Such variations can alter litter decomposition dynamics and soil C stabilization. Despite alterations on soil microbial community can be quantified, uncertainty about the identity and role of microorganism on C cycle it is difficult to experimental isolate. The use of phospholipid fatty acids (PLFA) can provide information regarding shifts in the microbial community structure resulting from management or environmental changes. PFLA analyses associated with stable isotope probing (SIP) with ^{13}C of specific biomarkers can provide a directly link among specific microbial process and the responsible organisms (Boschker and Middleburg, 2002).

The aim of this study were to asses sucession of the soil community, through phospholipid fatty acids (PLFA) combined with ^{13}C-PLFA, meadiating the decomposition of Eucalyptus sp. harvest residue in soil of Pampa Biome.

MATERIAL AND METHODS

The study was located in Southern of Brazil (30°26'S; 54°31'W), inserted in the Pampa Biome domains. Selected site is characterized by grassland (C4) to eucalyptus (C3) conversion. Experiment was established in November 2012, right after land use change. Residue management (removal of all residues, only bark removal or maintenance of all residues) and two N fertilization levels (0 and 200 kg ha^{-1}) were analyzed in a full-factorial design with four replications.

Our experimental unit consisted of polyvinyl chloride (PVC) tubes inserted 10 cm into the soil. Treatments were placed on soil surface inside the tubes. Then, tubes were closed with a plastic grid (1 cm mesh), avoiding external litter input. Three sampling were done over a year: at 3, 6 and 12 months after set up, tubes were excavated, closed and transported to laboratory for soil analyses.

Soil of 0-1 cm layer were collected and frozen at -80 ^{o}C prior to being lyophilized. Afterwards, all

visible plants debris was removed by handpicking using tweezers and the soil was ground to PLFA extraction.

A modified Bligh Dyer extraction procedure (Dungait et al., 2013) was used to extract PLFA. Briefly, Bligh Dyer solvent [monopotassium phosphate (KH_2PO_4) buffered water, chloroform ($CHCL_3$) and methanol (MeOH), 4:5:10*v/v/v*, was added to soil in a 3:1 *v/w* ratio and soil total lipids was extracted. Lipids was fractionated according with solubility differences on $CHCl_3$, acetone and MeOH, which resulted on neutral, acid and polar fractions. Nonadecane (0.1 mg mL^{-1}) was used as an internal standard before saponification withmethanolicNaOH. Methylation step were performed withdried acid MeO and methyl esters (FAMEs) formed were analyzed on GC-MS and GC-C-IRMS.

GC/MS analyses were performed using a Thermo Finnigan Trace GC/MS system (Thermo Fisher Scientific, Hemel Hempstead, UK) and GC-C-IRMS analyses were performed using a Varian 3400 GC attached to a Finnigan MAT Delta S isotope ratio MS (electron ionisation, 100 eV, 1 mA electron energy, 3 Faraday cup collectors m/z 44, 45 and 46, CuO/Pt Finnigan MAT Mark I combustion interface maintained at 940°C).

The fatty acid nomenclature used is as follow: total number carbon atoms:double bounds number followed by ω indicates double bond position; *cis/trans* geometry are indicated by suffixes c and t, respectively. The prefixes *a* and *i* refer to anteiso and iso-branching; 10 Me- indicates a methyl group on the tenth carbon atom from the carboxyl end of the molecule and *cy* refers to cyclopropane fatty acids.

Statistical analyses

Residue management and N level influences upon changes in soil microbial community structure and in microbial C source were analyzed using principal component analysis (PCA). Multivariate analyses of variance were used to test for significant differences in PLFA concentration and $\delta^{13}C$ PLFA. Time was considered as an independent factor because measurements at different dates were carried out on different samples, due to the destructive sampling arrangement.

RESULTS

Overall, FAMEs profiles contained 24 different fatty acids. When the FAME was present in insufficient quantity for a reliable measurement of their isotopic signature, data were not analyzed. The concentration of each PLFA varied overtime ($P<0.05$), but a general pattern was preserved throughout the residue decomposition: C16:0 had always the highest concentration and 18:2ω6, 16:1ω9 and 16:1ω7 the lowest ones. Likewise, treatments effects under PLFA concentration were stronger in the end of the experiment than in the begging.

At 3 months, principal component scores clustered PLFA in 2 mainly groups, 10-Me-branched groups (Actinobacteria) and another one, with 18:2w6 (Fungi) + cis/trans and mono unsaturated 16 and 18 C (G-ve bacteria) (Figure 1a). N fertilization and no eucalyptus residue addition increased Actinobacteria concentration and N fertilization also increased Fungi and 18:1ω9c concentrations ($P<0.05$). With respect to $\delta^{13}C$ PLFA, any treatments significantly altered microbial isotopic composition (Figure 1b). Slightly differences were just observed in aC16:0 (G+ve) and 10Me18:0 fatty acids (Actino) which were less ^{13}C enriched in treatments with residue and with N fertilization, respectively.

At 6 months, the first two ordination axes represented 31.2 % of the total variability in the PLFA profile (Figure 1c). Bark presence separated PLFAs 16:1w9, 16:1w7, 18:1w9c, 18:1w9t, 18:2w6 (Fungi) and i17:0 from the others. Inside this group, N fertilization resulted in increasing on mono unsaturated and double unsaturated 18 carbon fatty acids. $\delta^{13}C$ values of branched-chained *iso/anteiso* fatty acids (*i*15:0, *a*16:0 and *a*16:0) showed a tendency to be significantly more ^{13}C enriched in treatments without bark then the other groups (Figure 1d). The Fungi group was less enriched in ^{13}C in treatments with bark.

At the end of the experiment, two groups could be clustered (Figure 1e). One formed by G-ve and Fungi which residue presence enhanced PLFA concentration and the second (G+ve and Actino) which bark maintenance on residue followed for no N fertilization reduced PLFA concentrations. N additions increased $\delta^{13}C$ values of *i*15 and *a*15 PLFA, they were more 13C enriched in fertilized treatments than in unfertilized ones (Figure 1f). cy17:0 PLFA were less enriched in ^{13}C in treatments with bark than without bark and an opposite trend was observed in 10-Me18:0 PLFA.

DISCUSSION

In the current study, eucalyptus residue introduction in Pampa had two distinct features in relation to grassland vegetation. First, eucalyptus residue has different chemical composition as higher lignin and tannin and polyphenolic compounds on soluble extractives, especially in treatments with bark (Lima et al., 2013). The second one is related

to the physical structure of eucalyptus residues as greater specific surface, for example on tick roots, bark, branched. Thus, the begging of decomposition process will be more dependent of macrofauna breakdown activities, for example. This explain why we found rather effects of eucalyptus residue addition on PLFA concentration.

Basically, over the decomposition period, eucalyptus residue addition, especially bark presence, increased PLFA concentration of G-ve (*r*-strategists) and Fungi.*r*-strategists, that grow quickly and might respond faster to a new C input (Fontaine and Barot, 2005). Although it was expected that fertilized treatments had greater G-ve concentration than treatments unfertilized, no differences could be found. New theories has been postulated that stoichometric balance among substrate C:N ratios and decomposer community can be balanced by C:N ratio of bioavailable labile substrates (Kaiser et al., 2014), which in our case could ensure to G-ve substantial concentration to keep its activities independently of residue C:N ratio. However N fertilization, at 12 months, increased G+Ve concentrations. High mean temperature at 12 months (22 °C) can support greater microbial activity. In such situation, N may become the main constraint for microbial activity. Although increase on G+ve concentration was not followed by enhance on residue incorporation in this group.

Overtime, Fungal community concentrations was increased. Lupatini et al., (2013) similarly found greater Fungal community in *Eucalyptus* sp. forest than in Pampa biome. Besides being classified as *k*-strategists, Fungi had competitive advantage over G+ve and Actinobacteria, due fungal hyphae capacity to penetrate in residue tissue (Kubartová et al., 2009) and fungi enzymes capacities to decompose ligninocellulose compounds (Jiang et al., 2014). Such life style features can at the same time explain why overtime G+ve, Actinobacteria had the most ^{13}C enriched PLFA, pointing to preferential use of material originated from grasslands.

CONCLUSIONS

Eucalyptus sp. residue increased PLFA concentration of G-ve and Fungi groups in comparison to Pampa Biome.

Fungi was the main eucalyptus decomposer group followed to G-ve groups.

G+ve and Actinobacteria concentration were slightly decreased with addition of eucalyptus residue in Pampa Biome and these group had the lowest C-residue incorporation.

REFERENCES

Ayres, E., Steltzer, H., Simmons, B.L., Simpson, R.T., Steinweg, J.M., Wallenstein, M.D., Mellor, N., Parton WJ, Moore JC, Wall DH. Home-field advantage accelerates leaf litter decomposition in forests. Soil Biol Biochem 41:606–610, 2009.

Boscher HTS and Middelburg JJ. Stable isotopes and biomarkers in microbial ecology. FEMS Microb. Ecol., 45: 85-95, 2002.

Dungait JAJ, Kemmitt SJ, Michallon L, Guo S, Wen Q, Brookes PC, Evershed RP. The variable response of soil microorganisms to trace concentrations of low molecular weight organic substrates of increasing complexity. Soil Biol Biochem,64:57–64,2013.

Fontaine S, Barot S. Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. Ecol Letters 8:1075–1087, 2005.

Jiang X, Cao L, Zhang R, Yan L, Mao Y, Yang Y. Effects of nitrogen addition and litter properties on litter decomposition and enzyme activities of individual fungi. Appl Soil Ecol 80:108–115, 2014

Kaiser C, Franklin O, Dieckmann U, Richte, A. Microbial community dynamics alleviate stoichiometric constraints during litter decay. Ecol Letters 17:680–690, 2014.

Kubartová A, Ranger J, Berthelin J , BeguiristainT. Diversity and decomposing ability of saprophytic fungi from temperate forest litter. Microb Ecol 58:98–107,2009

Lima MA, Lavorente GB, Silva HK, BragattoJ, Rezende CA, Bernardinelli OD, Deazevedo ER, Gomez LD, McQueen-Mason SJ, Labate CA, PolikarpovI.Effects of pretreatment on morphology, chemical composition and enzymatic digestibility of eucalyptus bark: a potentially valuable source of fermentable sugars for biofuel production - part 1. Biotech for Biofuels 6:75,2013.

Lupatin, M, Jacques RJS, Antoniolli ZI, Suleiman AKA, Fulthorpe RR, Roesch LFW. Land-use change and soil type are drivers of fungal and archaeal communities in the Pampa biome. World J Microb Biotech 29:223–233, 2013.

Nemergut DR, Cleveland DD, Wieder WR, Washenberger CL, Townsend AR. Plot-scale manipulations of soil organic matter inputs to soil correlate with shifts in microbial community composiiton ina a lowland tropical rain forest. Soil Biol Biochem, 42: 2153-2160, 2010.

Veen GFC, Freschet GT, Ordonez A, Wardle DA. Litter quality and environmental controls of home-field advantage effects on litter decomposition. Oikos, 187–195, 2015.

ACKNOWLEDGMENTS

The authors would like to thank FAPEMIG and CNPQ for financial support to present this work at CBCS 2015 and to CAPES for financial support to develop the study.

XXXV Congresso Brasileiro de Ciência do Solo
CENTRO DE CONVENÇÕES - NATAL / RN
O SOLO E SUAS MÚLTIPLAS FUNÇÕES
02 a 07 DE AGOSTO DE 2015

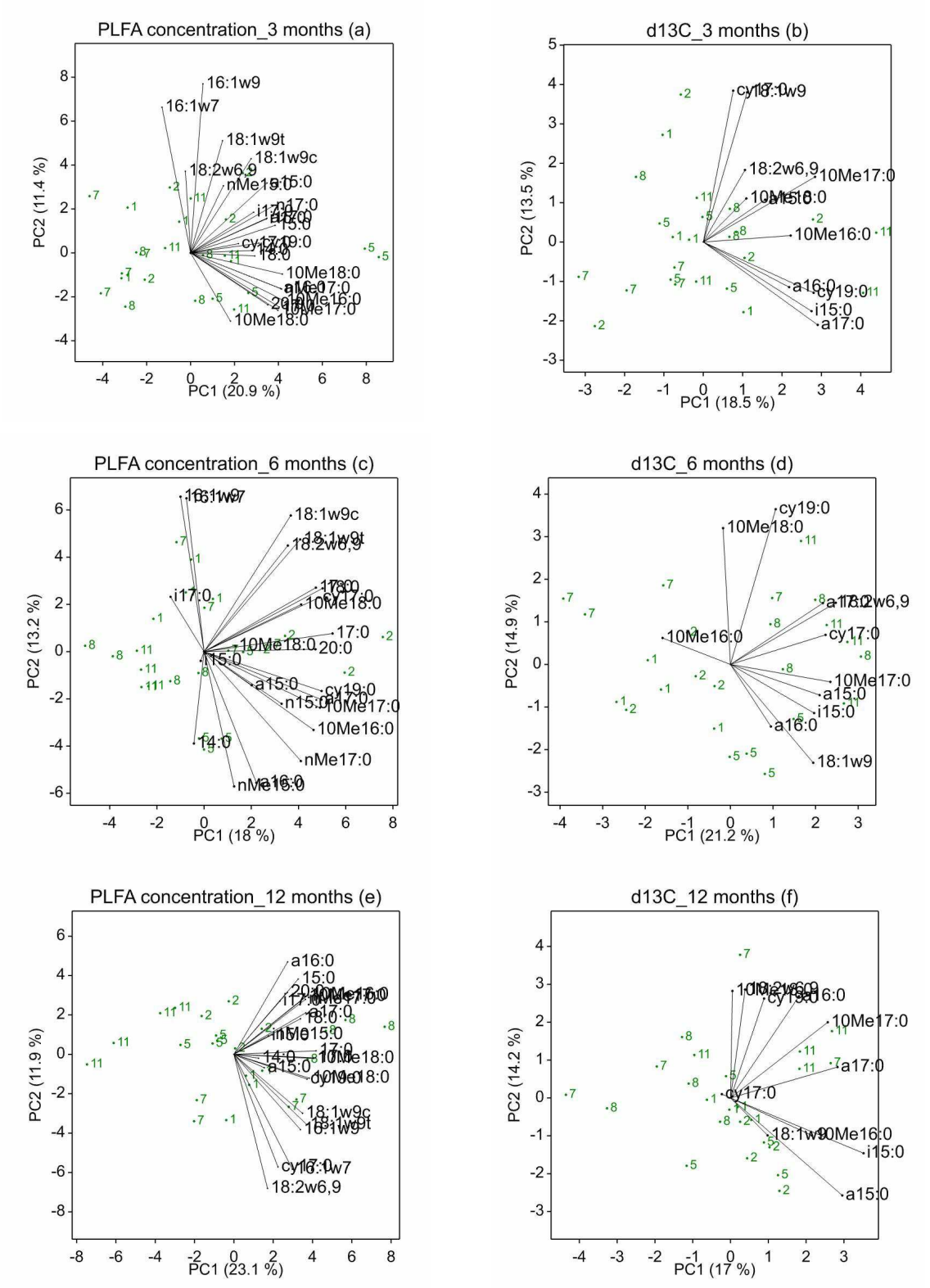

Figure 1 – Principal component analysis (PCA) of PLFA and $\delta^{13}C$ PLFA.