Geoderma 312 (2018) 121–129

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Soil organic matter formation as affected by eucalypt litter biochemistry — Evidence from an incubation study

Luís F.J. Almeida[a,*], Luis C.C. Hurtarte[b], Ivan F. Souza[a], Emanuelle M.B. Soares[a], Leonardus Vergütz[a], Ivo R. Silva[a]

[a] Departamento de Solos, Avenida P.H. Rolfs, s/n Campus Universitário, Universidade Federal de Viçosa, Viçosa, MG CEP 3657-900, Brazil
[b] Lehrstuhl für Bodenkunde, Technische Universität München (TUM), Emil-Ramann-Str. 2, Freising 85354, Germany

ARTICLE INFO

Editor: Jan Willem Van Groenigen

Keywords:
Soil organic matter
CO_2 partitioning
Biochemical fractions
Litter biochemistry

ABSTRACT

Linking plant litter biochemistry, its decomposition and soil organic matter (SOM) formation is not straightforward. In this research, we evaluated the decomposition of four biochemical fractions operationally defined as i) hot-water extractable (HWE), ii) total solvent (acetone) extractable (TSE), iii) acid-base (HNO_3-KOH) unhydrolyzable cellulosic fraction (CF), and iv) acid (H_2SO_4) unhydrolyzable (AUR) and the transfer of C from these fractions to SOM. Each biochemical fraction was Soxhlet-extracted from isotopically labeled (^{13}C) leaves, twigs, bark and roots of eucalypt plants (120 days old). The molecular composition of each fraction was inferred from thermochemolysis with tetramethylammonium hydroxide (TMAH), followed by gas chromatography coupled to mass spectrometry (GC-MS). For the incubation, we collected soil samples from the topsoil (0–20 cm) of a sandy-clay loam, kaolinitic Haplic Ferralsol. Four plant organs and four biochemical fractions were arranged into a (4 × 4) + 1 factorial scheme, including one control treatment (soil only). The samples were incubated at 80% of their water-holding capacity and kept under controlled temperature (25 °C). The decomposition of the biochemical fractions was monitored by determining the CO_2 concentration into the headspace of the vials at 1, 2, 3, 4, 7, 13, 21, 28, 38, 46, 70, 80, 92, 112, 148, 178, and 200 days after the incubation had started. After the incubation, soil samples were submitted to density followed by particle-size fractionation. HWE and CF decomposed at faster rates than TSE and AUR throughout the incubation. The soil fraction < 53 μm retained a significantly higher proportion of the initial C input of HWE (32%) and AUR (31%) than TSE (19%) or CF (15%). Light fraction of organic matter (LFOM) with density < 1.8 g cm^{-3}, retained a significant proportion of AUR (37%) and TSE (32%) while CF was mostly lost as CO_2 (79%). Selective preservation of organic materials (e.g., long-chain lipids) within the AUR and TSE fractions appears to be a significant pathway for the formation of SOM. SOM formation through a microbial-driven pathway cannot be ruled out for any biochemical fraction evaluated, but it seems more relevant for HWE and CF. In short-term, substrate biochemistry exerts a strong influence on the conversion of eucalypt litter fractions into either CO_2 or SOM. Despite inherent challenges upfront, considering such dynamics at the ecosystem level will help to improve our current understanding on C storage and CO_2 emissions from soils in long-term scales.

1. Introduction

Soil organic carbon (SOC) is mainly derived from decaying plant material such as roots and litter produced aboveground (Gleixner, 2013). Paradoxically, large variations in plant litter biochemistry constraint the connection between decomposition rates and the formation of SOM. The biochemical composition of plant litter can be summarized as a combination of different proportions of non-structural (e.g., water-soluble metabolites) and structural (water-insoluble) components including cellulose, holocellulose, lignin and lipid polymers (Cotrufo et al., 2013). Depending on the plant tissue or plant species considered, the mass proportion of structural and non-structural components can vary significantly, implying contrasting decomposition rates and potential to contribute for SOM fractions (Kögel-Knabner, 2002). For example, while carbohydrates and proteins can be easily degraded in soils, lignin, tannin, and cuticular waxes decompose slowly (Mikutta et al., 2006; Suseela et al., 2013). Nevertheless, quantitative data linking the biochemical composition of different litter components and their effect on the formation of SOM remains relatively scarce.

The relatively large number of factors that affect the decomposition

* Corresponding author.
E-mail address: luis.almeida@ufv.br (L.F.J. Almeida).

http://dx.doi.org/10.1016/j.geoderma.2017.10.004
Received 13 March 2017; Received in revised form 21 September 2017; Accepted 5 October 2017

of plant litter implies that the relationship between the decomposition of precursors' substrates and the formation of SOM is not trivial (Mueller et al., 2015). However, it is often assumed that SOM formation is a result of the progressive decomposition (PD) of plant litter (von Lützow et al., 2006) and selective preservation (SP) of specific molecules (Burdon, 2001). PD is understood as the progressive transformation of large biomolecules into small monomers and their oxidation during the decomposition of plant litter (Hedges and Keil, 1999). This also means that PD is a process tightly linked to the activity of extracellular microbial enzymes and substrate assimilation, which can be either used for catabolism or anabolism (Geyer et al., 2016; Liang et al., 2017). By contrast, SP refers to the persistence of some components of plant litter that cannot be readily assimilated by soil microbes during the decomposition process (von Lützow et al., 2006). More recently, PD and SP have been included as part of a soil continuum model, which takes into account the influence of physical and chemical processes occurring within the soil matrix that can affect SOM formation and its decomposition (Lehmann and Kleber, 2015). Thus, irrespective of PD or SP controlling litter decomposition, the retention of SOM would ultimately depend on the interactions between the organic fraction and the mineral matrix (Baldock and Skjemstad, 2000). Generally, it can be expected that the magnitude by which plant litter is converted into SOM by PD and microbial assimilation, SP or a combination of these processes can vary with soil type, biological activity and substrate biochemistry (Liang et al., 2017).

Historically, the relationship between plant litter decomposition and SOM was mainly assumed to be a function of SP, that is, materials that are difficult to decompose should accumulate as 'stable' SOM (Melillo et al., 1982). This also implied that substrates that could be easily metabolized by soil microbes would be preferentially respired as CO_2. Conversely, evidence is growing that persistent SOM can be produced from substrates that are easily metabolized by soil microbes (Bradford et al., 2013; Cotrufo et al., 2013; Miltner et al., 2012). This appears to be particularly important for the formation of mineral associated-organic matter (MAOM), which accounts for most of the persistent SOC in terrestrial ecosystems (Kleber et al., 2015). Intriguingly, SP of plant-derived materials such as lignin and lipids can account for a substantial fraction of SOM, suggesting that direct contribution of plant tissues cannot be overlooked (Angst et al., 2017). It is possible that direct contribution of plant litter for SOM may be related to its preservation by physical mechanisms e.g., aggregation and encapsulation that limit the access of microbes to substrates (Baldock and Skjemstad, 2000; Six et al., 2002). Indeed, oxygen diffusion into soil aggregates can be temporally limited and constrain SOM mineralization even in upland soils (Keiluweit et al., 2016). Importantly, it is not well understood whether the pathways linking plant litter decay to SOM formation are more affected by substrate biochemistry or by the external environment at which the decomposition occurs. Thus, evaluating the impact of litter biochemistry on the formation of SOM could help to shed light on processes underlying its persistence and dynamics in soils.

Determining the conversion of specific substrates or fractions of plant litter into SOM may enable a better understanding on soil C dynamics (Mendez-Millan et al., 2014). Our working hypothesis was that not all substrates that can be easily metabolized by soil microbes can be equally effective as precursors for the formation of mineral-associated SOM. In the present study, we evaluated the decomposition and ^{13}C transfer from chemically distinct labeled litter fractions to the particulate and MOAM fractions. The microcosm incubation experiment was carried out under controlled conditions with four biochemical fractions that were sequentially extracted (HWE, TSE, and CF or AUR) from leaves, twigs, bark and roots of eucalypt plants.

2. Materials and methods

2.1. Plant labeling and litter fractionation

Six clonal *Eucalyptus grandis* × *E. urophylla* hybrid plants (two-months old) were grown under constant aeration in a growth chamber in a 3.5 L polyethylene container supplemented with a Clark Nutrient Solution (Clark, 1975). Half of the plants were pulse-labeled with ^{13}C-CO_2 stable isotope as described by Machado et al. (2011) and the other half was submitted to the same conditions, except that no ^{13}C label was applied (unlabeled control plants). After the labeling period (126 days), all plants were harvested and fractionated into leaves, twigs, stem and roots. The stem material was only used for the extraction of bark. The plant organs were dried under a forced-draft oven at 60 °C for a week and subsequently ground in a Wiley mill and stored for the chemical fractionation.

Subsamples of each plant component (leaves, bark, twigs and roots) were ball-milled and their relative ^{13}C enrichment was accessed by analyzing the samples using an isotope ratio mass spectrometer- IRMS (ANCA-GSL, 20-20, Sercon, Crewe-UK). The isotopic composition of the components of the plant litter is given in Supplementary Table 1. The plant organs (leaves, bark, twigs and roots) were fractioned into four operationally defined biochemical fractions, namely: HWE — hot water extractable (metabolic compounds); TSE — total solvent extractable (free lipids), CF — cellulosic fraction (mostly cellulose and hemicellulose), and AUR — acid unhydrolyzable residue (mostly lignin and lipids). Briefly, the fractionation procedure was as follows: 2 g of each plant component (particle-size ≤ 0.425 mm) were Soxhlet-extracted with 150 mL of deionized water at 100 °C during 6 h to obtain the HWE fraction. Two grams of the residue generated after the HWE extraction were Soxhlet-extracted with 150 mL of acetone at 56 °C for 6 h to obtain the TSE fraction. The temperatures indicated for the extraction of HWE or TSE refer to the minimum value required to evaporate the solvent, which condensates before interacting with the sample. Both, HWE and TSE were freeze-dried and stored for later use. The residue free of HWE and TSE fractions was subsequently fractioned into the CF and the AUR in separated procedures. The CF was obtained by treating 1 g of the residue with a mixture of ethanol and aqueous HNO_3 (65%, *v/v*) at a ratio of 4:1 at 85 °C for 1 h. This extraction was repeated four times in total and the residue generated was treated with an aqueous solution of KOH 25% (*m/v*) at 100 °C for 1 h. For the isolation of the AUR fraction, 1 g of the residue free of HWE and TSE was treated with 6 mL of aqueous H_2SO_4 (72%, *v/v*) at 30 °C and kept in water bath during 1 h. Subsequently 140 mL of water was added and the temperature raised to 100 °C and kept constant during 4 h. Both CF and AUR were washed extensively with ultra-pure water and freeze-dried. The dry mass yield for each biochemical fraction from each plant component is shown in Supplementary Table 2.

Subsamples of all biochemical fractions were submitted to isotopic analysis using an IRMS in order to determine their $^{13}C/^{12}C$ ratio. The results were referred to a V-PDB international standard and are expressed as $\delta^{13}C$ in a per mill (‰) notation and are shown in Supplementary Table 3. Total C, N and H for all litter biochemical fractions were determined by dry combustion in an element analyzer (CHNS/O, Analyzer 2400 series II, Perkin Elmer; Waltham, MA, USA) and the results are given in Supplementary Table 4. Additionally, the molecular composition of the biochemical fractions from each plant organ was assessed using an off-line TMAH-mediated thermochemolysis procedure based on Hatcher et al. (1995). Afterwards, the TMAH thermochemolysis products were analyzed by gas chromatography-mass spectrometry in a Shimadzu QP 2010-SE GC-MS equipped with a Rtx-5MS column (30 m length; 0.25 mm ID; 0.25 μm film thickness). Ultrapure He was the carrier gas at a flow rate of 3 mL min^{-1}, the ion source temperature was set to 200 °C, the interface temperature to 290 °C and oven temperature was ramped from 60 °C to 300 °C at a rate of 15 °C min^{-1}, with analysis initial time at 3.50 min and final time at