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The role of lignin and cellulose in the carbon-cycling of degraded soils under semiarid climate and their relation to microbial biomass

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ABSTRACT

A high level of biological degradation is usually observed in soils under semiarid climate where the low inputs of vegetal debris constraint the development of microbiota. Among vegetal inputs, cellulose and lignin are dominant substrates but their assimilation by the microbial community of semiarid soils is yet not understood. In the present study, ^{13}C-labeled cellulose and ^{13}C-labeled lignin (75 μg ^{13}C g^{-1} soil) were added to two semiarid soils with different properties and degradation level. Abanilla soil is a bare, highly degraded soil without plant cover growing on it and a total organic C content of 5.0 g kg^{-1}; Santomera soil is covered by plants (20% coverage) based on xerophytic shrubs and has a total organic C content of 12.0 g kg^{-1}. The fate of added carbon was evaluated by analysis of the carbon isotope signature of bulk soil-derived carbon and extractable carbon fractions (water and sodium-pyrophosphate extracts). At long-term (120 days), we observed that the stability of cellulose- and lignin-derived carbon was dependent on their chemical nature. The contribution of lignin-derived carbon to the pool of humic substances was higher than that of cellulose. However, at short-term (30 days), the mineralization of the added substrates was more related to the degradation level of soils (i.e. microbial biomass). Stable isotope probing (SIP) of phospholipid fatty acids (PLFA-SIP) analysis revealed that just a minor part of the microbial community assimilated the carbon derived from cellulose and lignin. Moreover, the relative contribution of each microbial group to the assimilation of lignin-derived carbon was different in each soil.

1. Introduction

Poor vegetal cover, inadequate texture and the high salt content in arid and semiarid environments constrain the development of soil microbial communities (Albadalejo and Díaz, 1990; García et al., 1994) and cause severe biological degradation of soils, which affects the carbon cycling of these environments (Bastida et al., 2006). In this scenario, the scarce and dormant microbial biomass must efficiently deal with low inputs from the poor above-ground plant communities in order to obtain energy for its maintenance. Among such vegetal inputs, cellulose and lignin are dominant components. Lignin is a cross-linked polyphenol macromolecule with molecular masses exceeding 10.000 amu. It is relatively hydrophobic and aromatic in nature. Cellulose is a polysaccharide consisting of a linear chain of several hundred to over ten thousand β(1→4) linked D-glucose units (Updegraf, 1969). Recent evidence suggests that the stability and low degradability of lignins in soils seems to be overestimated and that their contribution to humus is exaggerated (Stevenson, 1982; Thevenot et al., 2010). Indeed, several authors have concluded that lignin is not stabilized in mineral soil horizons (Rumpel et al., 2004; Vancampenhout et al., 2012) and observed a weak contribution of lignin to the stable carbon pool (Thevenot et al., 2010). In contrast, Hoffman et al. (2009) found a relatively high persistence of lignin in soil. In any case, both soil characteristics (i.e., texture, clay content, etc.) and biotic environment can influence the stabilization of carbon in soil (Rumpel et al., 2004).

Despite the dynamics of these compounds are fundamental for terrestrial carbon cycling (Rodriguez et al., 1997; Eichorst and Kuske, 2012), the fate of lignin and cellulose are not fully understood particularly in carbon-limited soil ecosystems, i.e. semiarid climates. Previous studies have highlighted a key role for fungi in the biodegradation of cellulose (Fontaine et al., 2011). However,

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other authors have suggested an ecological succession driven firstly by bacteria and secondly by fungi (Hu & van Bruggen, 1997; Schutter and Dick, 2001). Conversely, Snajdr et al. (2010) and Schutter and Dick (2001) affirmed that fungi played a less important role than expected in lignin degradation.

In this study, the fate of cellulose and lignin was traced in degraded soils under semiarid climate conditions using stable isotope probing (SIP) methodologies. For these purposes, ^{13}C-labeled-cellulase and ^{13}C-labeled-lignin were added to two soils with different level of degradation and distinct characteristics such as texture, electrical conductivity and level of microbial biomass. The combination of SIP experiments with phospholipid fatty acids analysis (PLFA-SIP) allows to gain quantitative information on the microbial assimilation of lignin- and cellulose-derived carbon and to trace the microbial groups involved in the degradation (Boschker et al., 1998; Waldrop and Firestone, 2004; Rinnan and Baath, 2009).

We aim to shed light on the fate of cellulose and lignin in degraded soils under semiarid climate conditions and the role of the soil microbial community on these processes. The specific objectives of this study were: i) to evaluate the stability and humification of cellulose and lignin in semiarid soils with different levels of degradation at long-term; ii) to evaluate the mineralization of these substrates at short-term, and iii) to identify the microbial groups responsible for the transformation of both compounds.

We hypothesize that the fate of cellulose- and lignin-derived carbon is related to the level of degradation of both soils. Furthermore, we expect that mineralization and microbial assimilation of carbon derived from both substrates will be higher in the soil with a lower degradation level due to its initially greater microbial biomass. Moreover, the relative contribution of each particular microbial group to the assimilation of substrates is expected to be similar in both soils and dependent on the substrate type.

2. Material and methods

2.1. Study area and soil sampling

Two different soils were chosen in the Province of Murcia located in the South-East of Spain. Both soils are subjected to a semiarid climate with a mean annual rainfall of less than 300 mm and a mean annual temperature of 18 °C. Both soils were agriculturally used in the past and were subjected to natural degradation processes due to the adverse climate conditions of South-East Spain. In the basis of total organic C, nitrogen content, vegetal cover and microbial biomass and its activity, the two soils have different levels of degradation (Bastida et al., 2006), which may influence the dynamics of cellulose and lignin.

The first soil was taken in an area of 100 m^2 located in Abanilla in the Province of Murcia, SE Spain. This soil is classified as Calcaric regosol (Soil Survey Staff, 1998) and represents a highly degraded soil with no vegetation growing on it (Bastida et al., 2006). It was selected as an adequate model for studying processes of carbon transformation under pre-desertic conditions. The soil particle distribution was 34.6% clay; 7.7% silt, and 57.7% sand. Abanilla soil has a pH of 7.8, an electrical conductivity of 2.6 dS m^{-1}, a total N content of 1.3 g kg^{-1}, a total C content of 40 g kg^{-1} and total organic C was 5.0 g kg^{-1}.

The second soil was taken in an area of 100 m^2 located in Santomera in the Province of Murcia, SE Spain. The soil was classified as Haplic calcisol (Soil Survey Staff, 1998) and represents a low-degraded soil (Bastida et al., 2006). Plant cover was around 20% and was dominated by xerophytic shrubs. The soil particle distribution was 18.8% clay, 9.5% silt, and 71.7% sand. Santomera soil has a pH of 7.7, an electrical conductivity of 0.3 dS m^{-1}, a total N content of 1.0 g kg^{-1}, a total C content of 71.2 g kg^{-1} and total organic C was 12.0 g kg^{-1}. A detailed description of these soils is provided by Bastida et al. (2006).

Within these areas, three plots ($n = 3$) of 20 m^2 each were selected. Six subsamples were taken from the upper 15 cm of each plot and pooled to obtain one composite sample per plot. Soil samples were sieved by < 2 mm and stored at 4 °C until the beginning of the incubation experiments.

2.2. Experimental design and soil incubations

Two independent incubation experiments with each of the two soils were performed. The first incubation was carried out in containers with 100 g of soil. Each treatment was prepared in identical triplicates for each incubation time (1, 4, 20, 60, and 120 days). Incubation was performed in chambers at 28 °C in darkness and controlled moisture. Soil samples were used for the measurement of the content and the isotopic composition of carbon in bulk soil, water-soluble and sodium-pyrophosphate extracts at long-term, as well as for PLFA and PLFA-SIP analysis.

In the second incubation experiment, the mineralization of cellulose and lignin was studied at short-term. This experiment was carried out in 12 ml-capped glass vials (Labco Limited, Lampeter, UK) containing 1 g of soil. Each treatment was incubated in triplicates at 28 °C in darkness. The concentration and carbon isotope ratio of CO_2 were analyzed after 1, 4, 10, 20 and 30 days of incubation.

In both experiments, two solutions were prepared with uniformly ^{13}C-labeled substrates (>97 atom%) from maize, one of C^{13}-cellulose and the other of C^{13}-lignin (Isolife, Wageningen, The Netherlands). 75 μg C g^{-1} soil of cellulose or lignin was supplied. For non-labeled control experiments, the same concentrations of these substrates were prepared, but using cellulose and lignin with natural isotopic abundance. The amount of substrates is in the same range as carbon applied in field restoration experiments and in previous isotope labeling experiments (Bastida et al., 2013). Furthermore, a control experiment consisting of soil without cellulose or lignin was set up, using the same conditions as the other two treatments. The water-holding capacity of the soil was gravimetrically adjusted to 60% during incubation for all treatments. Before the substrates were added, soils were pre-incubated with distilled water during two weeks in order to avoid drastic effects of moisture on the measured parameters when adding the substrates. All treatments were performed in replicates ($n = 3$).

2.3. Analysis of carbon fractions

The total organic carbon (TOC) of soil samples was determined after acidification with 2N HCl to pH 2 in a Leco Truspec CN elemental analyzer (St. Joseph, MI) (Bastida et al., 2013). Hot water-soluble carbon was extracted with distilled water (1:5, w:v) by shaking for 2 h at 50 °C. Sodium-pyrophosphate extractable carbon was extracted with 0.1 M sodium-pyrophosphate pH 9.8 (1:5, w:v) by shaking for 4 h (Stevenson, 1982; Lucas-Borja et al., 2012). Subsequently, carbon content in both extracts was determined using a Shimadzu TOC5050A Total Organic Carbon Analyzer. Hot water and sodium pyrophosphate extractions were performed in parallel samples, not sequentially. Hot water-soluble carbon represents the easily available carbon for microorganisms, while carbon in sodium pyrophosphate extracts represents both a labile and more stable carbon presented for example in humic substances (Stevenson, 1982).