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Lignin degradation during a laboratory incubation followed by ^{13}C isotope analysis

H. Bahri [a,1], D.P. Rasse [b,*], C. Rumpel [a], M.-F. Dignac [a], G. Bardoux [a], A. Mariotti [a]

[a] UMR 7618, INRA CNRS-Université, Laboratoire de Biogéochimie et Ecologie des Milieux Continentaux, Centre INRA Versailles Grignon, Thiverval-Grignon, France
[b] Soil and Environment Division, Norwegian Institute for Agricultural and Environmental Research, Frederik A. Dahls vei 20, 1432 Ås, Norway

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ABSTRACT

Recent *in situ* ^{13}C studies suggest that lignin is not stabilised in soil in its polymerised form. However, the fate of its transformation products remains unknown. The objective of the present research was to provide the first comprehensive picture of the fate of lignin-derived C across its transformations processes: (1) C remaining as undecomposed lignin molecules, (2) C in newly formed humic substances, i.e. no longer identifiable as lignin-polymer C, (3) C in microbial biomass, (4) C mineralised as CO_2, and (5) dissolved organic C. To achieve this objective, we designed an incubation experiment with ^{13}C-labelled lignin where both elementary and molecular techniques were applied. Lignin was isolated from ^{13}C labelled maize plants (^{13}C-MMEL) and incubated in an agricultural soil for 44 weeks. Carbon mineralisation and stable isotope composition of the released CO_2 were monitored throughout the incubation. Microbial utilisation of ^{13}C-MMEL was measured seven times during the experiment. The turnover rate of the lignin polymer was assessed by ^{13}C analysis of CuO oxidation products of soil lignin molecules. After 44 incubation weeks, 6.0% of initial ^{13}C-MMEL carbon was mineralised, 0.8% was contained in the microbial biomass, and 0.1% was contained in dissolved organic C form. The compound-specific ^{13}C data suggest that the remaining 93% were overwhelmingly in the form of untransformed lignin polymer. However, limited transformation into other humic substances potentially occurred, but could not be quantified because the yield of the CuO oxidation method proved somewhat variable with incubation time. The initial bacterial growth yield efficiency for MMEL was 31% and rapidly decreased to plateau of 8%. A two-pool first-order kinetics model suggested that the vast majority (97%) of MMEL lignin had a turnover time of about 25 years, which is similar to field-estimated turnover times for soil-extractable lignin but much longer than estimated turnover times for fresh plant-residue lignin. We conclude that natural lignin structures isolated from plants are rather unreactive in soil, either due to the lack of easily available organic matter for co-metabolism or due to enhanced adsorption properties. The data also suggest that fairly undecomposed lignin structures are the main reservoir of lignin-derived C in soils.

1. Introduction

Lignin has long been suspected to be preserved in soil due to its chemical recalcitrance (Dagley, 1975; Flaig et al., 1975). However, recent studies based on direct extraction and quantification of soil lignin have shown that the chemical recalcitrance of lignin does not lead to its long-term stabilisation (van Bergen et al., 1997; Lobe et al., 2002; Kiem and Kögel-Knabner, 2003). These results have now been confirmed by advanced compound-specific ^{13}C analyses (Dignac et al., 2005; Rasse et al., 2006; Heim and Schmidt, 2007). The mean residence time of the most recalcitrant pool of soil-incorporated lignin polymer ranges from 20 to 38 years (Rasse et al., 2006; Heim and Schmidt, 2007). A fast-turnover pool of soil lignin was also identified, with a mean residence time of about 7 years (Heim and Schmidt, 2007). In addition, more than 90% of plant residue lignin would never benefit from soil protection mechanisms and display a mean residence time shorter than one year in agricultural ecosystems (Rasse et al., 2006). These studies clearly indicate that the chemical recalcitrance of the lignin polymer does not lead to its long-term stabilisation in aerobic soils. However, these studies do not elucidate the fate of the constitutive C of the decomposed lignin molecule. This C can be integrated into new molecular structures or mineralised to CO_2. The difference is particularly crucial for C storage in soils and needs to be assessed in order to determine whether carbon derived from specific molecules is ultimately stabilised in soil.

Mineralisation of lignin C, i.e. its ultimate oxidation as CO_2, has been studied for several decades through ^{14}C labelling of extracted or synthetic lignin. These incubation studies with synthetic lignin

* Corresponding author. Tel.: +47 92 26 36 08.
E-mail address: daniel.rasse@bioforsk.no (D.P. Rasse).
[1] Present address: INRGREF, Institut National de Recherche en Génie Rural Eaux et Forêts, Tunis, Tunisie.

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(dehydrogenation polymer: DHP) showed a substantial mineralisation of the lignin molecule in soil (Haider and Kladivko, 1980; Martin et al., 1982). Microbial assimilation of lignin C was low (Kassim et al., 1982; Martin et al., 1980). This low assimilation was explained by the fact that microorganisms do not use lignin as a C source and need other easily available substrates (Kirk et al., 1976). During mineralisation, some of the lignin carbon may be dissolved in water (Hofrichter et al., 1999; Steffen et al., 2000; Tuomela et al., 2002). Ultimately, a substantial part of the DHP-lignin was reported to be incorporated into operationally-defined humic substances (Haider et al., 1977; Martin et al., 1980). However, these studies could not determine whether lignin-derived C remains in soil under the form of undecomposed lignin molecules or under newly formed substances.

The objective of the present research was to provide the first comprehensive picture of the fate of lignin-derived C across its transformations processes: (1) C remaining under undecomposed lignin molecules, (2) C in newly formed humic substances, i.e. no longer identifiable as lignin-polymer C, (3) C in microbial biomass, (4) C mineralised as CO_2, and (5) dissolved organic C. To achieve this objective, we designed an incubation experiment with ^{13}C-labelled lignin where both elementary and molecular techniques can be applied.

2. Material and methods

2.1. Soil samples

Agricultural soil was sampled from the experimental field "Les Closeaux" located near the Chateau de Versailles in France (Dignac et al., 2005). Soil texture is loamy with a carbon content of 14.6 g kg^{-1}. Soil samples were collected at 0–25 cm depth from a plot cropped with wheat, sieved at 5 mm and pre-incubated (20 °C and 80% of the maximum water holding capacity) during 3 weeks. This sampling depth corresponds to the ploughing depth and was used to mimic the effect of residue incorporation by the plough.

2.2. ^{13}C labelling of plant material and lignin extraction

Maize plants were grown in a ^{13}C enriched atmosphere in a growth chamber for 1 month. This labelling resulted in a $\delta^{13}C$ signature of 366‰ for maize stems and 350‰ for maize leafs. Thereafter, lignin was isolated from the maize plants according to the method developed by Pew (1957) as modified by Lapierre et al. (1986) to obtain ^{13}C-labelled Maize Milled Enzymatic Lignins (^{13}C-MMEL). Briefly, this natural lignin is obtained after milling and solvent extraction of the plant material. The purity degree of lignin was improved through an enzymatic attack, which removed cellulose intimately associated with the lignin. Thereafter, the lignin was solubilised in a dioxane–water mixture (1:9) before being precipitated with Na_2SO_4 (2%) and freeze-dried. The ^{13}C-MMEL was depleted in ^{13}C as compared to the bulk plant. It displayed an isotopic excess of 0.35% corresponding to a $\delta^{13}C$ ratio of 318 ± 1‰. Analysis of ^{13}C-MMEL by ^{13}C CPMAS NMR spectroscopy indicated the presence of 13% polysaccharide C.

2.3. Incubation of lignin and soil

In sealed glass bottles (120 ml) we mixed 30 g of soil and 30 mg of ^{13}C-MMEL (50 mg $C_{13C\text{-}MMEL}$ g^{-1} SOC) which are noted: amended samples. Twenty-one bottles were incubated in darkness at 20 °C and 80% of moisture during 44 weeks. Additionally, twenty-one non-amended samples, containing soil without ^{13}C-MMEL were incubated under the same conditions. At each measurement date (1, 2, 4, 8, 16, 32 and 44 weeks), three bottles of the amended and the control soil were removed. Half of the samples were air dried at 40 °C and ground.

2.4. Carbon mineralisation

Carbon mineralisation was estimated at 20 different dates during the 44 weeks by sampling of bottle air and measurement of the accumulated CO_2 with a gas chromatograph equipped with a thermal conductivity detector. The ^{13}C isotopic abundance of the evolved CO_2 ($\delta^{13}C_{CO2}$) was measured with a gas chromatograph (Hewlett Packard 5890) coupled to an isotope ratio mass spectrometer (Micromass-GVI Optima). After CO_2 measurements, the bottles were ventilated with synthetic air (19% O_2, 81% N_2) in order to avoid internal CO_2 concentration exceeding 2%, according to Fontaine et al. (2004). The soil moisture was readjusted by adding deionised water.

2.5. Soil microbial biomass

Soil microbial biomass C was measured by the fumigation-extraction method (Vance et al., 1987) for 5 g of each soil sample. Briefly, this method consists of chloroform fumigation of the soil during 16 h followed by K_2SO_4 0.05 M extraction of the fumigated sample and a control sample without fumigation. The K_2SO_4 extracts were frozen and lyophilised, and their C content and $\delta^{13}C$ signature were determined on the CO_2 evolved from a CHN autoanalyser (CHN NA 1500, Carlo Erba) coupled with an isotopic ratio mass spectrometer (VG Sira 10) (Girardin and Mariotti, 1991). Afterwards the chloroform-labile C was calculated as difference and the microbial biomass C estimated by multiplication with 2.64 (Vance et al., 1987). In order to study the incorporation of ^{13}C-MMEL into the soil microbial biomass, we measured the $\delta^{13}C$ value of the microbial biomass in both non-amended and amended samples. Two sources of microbial biomass C could then be differentiated in the amended samples: original soil organic matter and ^{13}C-MMEL. We could therefore compute the microbial growth yield efficiency (GYE) for lignin, which is defined as the proportion of lignin C assimilated into the microbial biomass as compared to the total amount decomposed by the biomass (Thiet et al., 2006). In our case, we computed it as:

$$GYE = \Delta Cmic_{13C-MMEL} / (\Delta Cmic_{13C-MMEL} + \Delta CO_{2_{13C-MMEL}}) \quad (1)$$

where $Cmic_{13C\text{-}MMEL}$ is the microbial C coming from the ^{13}C-MMEL, and $CO_{2_{13C-MMEL}}$ is the emitted CO_2-C coming from the mineralisation of the ^{13}C-MMEL; the Δ symbol indicates that these are differences between two dates.

2.6. Dissolved organic matter

The lignin-derived carbon soluble in water was extracted after agitation of 10 g of soil with 25 ml water during 24 hours followed by centrifugation at 30,000 × g for 10 min and filtration at 1.6 μm. The solution was frozen and freeze dried before analysis.

2.7. Calculation of carbon derived from ^{13}C-MMEL

For each fraction (mineralised, microbial, dissolved in water, MMEL lignin and remaining in soil) the carbon derived from ^{13}C-MMEL was calculated using isotopic mass balance:

$$\left(C_{f_{MMEL}}\right)_A = \frac{\left(C_f\right)_A \cdot \left[\left(\delta^{13}C_f\right)_A - \delta^{13}C_{f_{NA}}\right]}{\left[\left(\delta^{13}C_{MMEL}\right)_A - \delta^{13}C_{f_{NA}}\right]} \quad (2)$$

where $(C_f)_A$ and $(\delta^{13}C_f)_A$ are the total carbon fraction content (mg C g^{-1} SOC) and its ^{13}C isotopic ratio respectively in amended soil,