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Phospholipid ^{13}C stable isotopic probing during decomposition of wheat residues

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ABSTRACT

Disentangling the kinetics of the soil microbial community succession, which is simultaneously driven by newly added plant materials and extant soil organic matter (SOM), can enrich our knowledge on microbial carbon (C) utilization patterns under residue amendment. This understanding might be useful to predict the rapid responses of specific microbial functional groups and develop strategies for balancing the terrestrial C budget. Therefore, our objective was to characterize and estimate the parameters of the microbial community dynamics profiled by phospholipid fatty acids (PLFA) from ^{13}C-labeled wheat residues and SOM. We conducted a 21-day microcosm study using two different arable systems (conventional tillage, CT; no-till, NT) amended with three types of ^{13}C-labeled wheat residues (grains, leaves and roots). The abundances and isotopic fractions of $^{13}CO_2$ flux and ^{13}C-labeled PLFA were measured via gas trace isotope ratio mass spectrometry (IRMS) and gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS), respectively. A double exponential model was used to describe the synthesis-degradation kinetics of PLFA from different microbial origins. We found that the PLFA formation generally reaches its maximal abundance within 7 days (except for PLFA from actinomycetes). The SOM- and wheat residue-derived C fluxes, as well as their PLFA profiles, were inconsistently impacted by the residue quality or the tillage regime over the incubation period. Specifically, the abundances of residue-derived CO_2 and PLFAs significantly decreased in the following order: grains > leaves > roots. However, those abundances derived from SOM were the lowest with the leaf residue treatments. Residue-derived PLFA patterns were highly influenced by fungi and G^- bacteria, while G^+ bacterial and actinomycete PLFAs were preferentially linked to extant SOM mineralization. Compared to the residue-derived counterparts, the SOM-derived microbes were characterized by higher G^+/G^- bacteria and cy17:0/C16:1ω7c ratios, as well as lower fungi/bacteria PLFA ratios. Such distinction between residue and SOM was also evidenced by the contrasting tillage effects on C mineralization and the ratios of cy17:0/C16:1ω7c and fungal/G^- bacterial PLFA. Our study provides evidence with important implications for adapting the microbial-mediated processes of soil C management through residue quality control.

1. Introduction

The dynamics of microbial communities responsible for soil organic matter (SOM) metabolism (Waldrop and Firestone, 2004) are dependent on the quality of organic substrates (Kuzyakov, 2010; Potthast et al., 2010). The two primary natural resources are plant residues and native SOM pools (Kramer and Gleixner, 2006). Modeling the C flows in soil microbial communities can aid in the prediction of rapid responses and strategies of specific functional groups for balancing the global terrestrial C budgets (Schmidt et al., 2011).

Large proportions of exogenous plant residues can be quickly incorporated into soils within days or weeks (Vanlauwe et al., 2005; Brant et al., 2006; Duong et al., 2009; Rubino et al., 2010; Marschner et al., 2011). This immobilized fraction mainly contributes to the soil labile C pool, which accounts for up to 15% of the total SOM (Gleixner et al., 1999) with a turnover time of 0.1–1.5 years (Gleixner et al., 2002; Lal, 2004). In some cases, residues with low lignin and high N contents can lead to a fast

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decay of plant residues (Rubino et al., 2007) because lignin is rich in complex polymers (Yu et al., 2009), while highly available N-rich materials promote a rapid microbial growth (Semenov et al., 2012).

During the residue decomposition, the bacterial and fungal abundance might peak (Rousk and Bååth, 2007; Marschner et al., 2011) and turn over rapidly (Fließbach et al., 2000; Konopka et al., 2002). Fungi and gram-negative bacteria (G^- bacteria) are reported to utilize fresh and easily available substrates, while actinomycetes and gram-positive bacteria (G^+ bacteria), as well as certain fungal groups, can enter the complex decomposition process later (Lu et al., 2004; Moore-Kucera and Dick, 2008; Fontaine et al., 2011; Marschner et al., 2011; Herman et al., 2012). Some fungi, G^+ bacteria and actinomycetes are said to be more efficient with recalcitrant SOM mineralization than G^- bacteria (Waldrop and Firestone, 2004; Fontaine et al., 2011) because resistant compounds are more readily degraded by their exoenzymes (Brant et al., 2006).

Measurements of specific microbial biomarkers in combination with isotopic labeling techniques have been extensively used to study the dynamics of microbial communities and to trace related C-cycling pathways during the metabolism of particular substrates (Waldrop and Firestone, 2004; Madsen, 2006; Rinnan and Bååth, 2009; Cowie et al., 2010). One of the most common biomarkers are the phospholipid fatty acids (PLFA; Liang et al., 2008) because ^{13}C-labeled substrate incorporation into particular microbial groups can be sensitively detected through compound-specific isotope analysis of ^{13}C PLFA (Watzinger, 2015; Yao et al., 2015).

This study estimates the synthesis-degradation succession kinetics of 4 microbial groups (fungi, actinomycetes, G^+ bacteria, and G^- bacteria) through the PLFA analysis and assesses their particular metabolic characteristics using exogenous ^{13}C-wheat residues and endogenous SOM. Our objective was to understand the developmental dynamics of these components of microbial community in utilizing plant residues of different qualities under a variety of soil tillage systems.

2. Materials and methods

2.1. Field experiment and soil sampling

Soil samples were collected in Maulde, Belgium (50°37′N, 3°34′E). The annual precipitation average was 780 mm. The mean minimum and maximum temperature were 6.3 and 13.5 °C, respectively. The soil type was affiliated to Luvisol (FAO, 2006). Conventional tillage (CT) had been carried out over 100 years before the soil samples were taken. In 1995, reduced tillage (0–10 cm of top soil is harrowed) was applied instead of CT. In 2006, 1/3 of the studied field was converted once again to CT and another 1/3 to no-till (NT; direct seeding without any soil disturbance). CT and NT soils were randomly collected using a 2-cm diameter soil auger on November 9, 2010. Soil samples from areas under the same tillage treatments were thoroughly mixed together. Plant litter and other visible residues were removed by hand. Fresh soils (<2 mm) were pre-incubated for 5 days at the target incubation temperature and moisture prior to the microcosm set-up.

2.2. Lab incubation study

The incubation experiment had a completely randomized block design with 3 replications and comprised the following 6 treatments: NG (no-till with grain residue), NL (no-till with leaf residue), NR (no-till with root residue), CG (conventional tillage with grain residue), CL (conventional tillage with leaf residue), and CR (conventional tillage with root residue). Fresh soils (15 g oven-dry weight) were weighed into vials and, then, thoroughly mixed with milled plant materials (<250 μm) at a proportion of 6 mg residue C g^{-1} dry soil. The ^{13}C-labeled wheat residues were obtained by growing wheat plants under continuous labeling with $^{13}CO_2$ (2 atom% excess; Denef and Six, 2006). Roots, leaves and grains were separated based on the following characteristics: (1) 714.1, 632.3 and 651.7 of $\delta^{13}C$ (‰) signatures; (2) 30.3, 40.8 and 44.9 of C%; (3) 32.4, 31.8 and 11.6 of C/N ratios; and (4) 6.4, 3.5 and 0.7 of lignin/N ratios (Bai et al., 2013). The amended soils were aerobically incubated in the darkness at 24 °C and 20% (w/w) soil moisture. Soil and gas subsamples were separately taken at 0, 9, 24, 45 hours and 3, 5, 10 and 21 days. Soil subsamples were frozen immediately and then lyophilized and stored at −20 °C prior to the PLFA analysis. Gas subsamples were collected from the top of closely sealed glass jars with microcosms and trapped in 10-ml vacuum glass vials for the CO_2 measurement.

2.3. Soil and gas analyses

Briefly, the concentration of CO_2 was measured by a gas chromatograph (Shimadzu 14B, Japan), and its isotopic composition was determined through a trace gas preparation unit (ANCA-TGII, SerCon, UK) coupled to an isotope ratio mass spectrometer (IRMS; 20–20, SerCon, UK). The calculation of the respiration rate and residue-derived fraction of CO_2 was described in detail in our previous study (Bai et al., 2013).

Six grams of lyophilized soil were extracted with phosphate buffer, chloroform and methanol (0.9:1:2, v:v:v), and the PLFA fraction was purified by a solid phase extraction (SPE) column. After methylation, the fatty acid methyl esters (FAME) were measured by capillary gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS; GC-C/DeltaPLUS XP Thermo Scientific) via a GC/C III interface, and a total of 22 PLFAs were quantified (Denef et al., 2009). The fatty acid nomenclature was based on the total number of C atoms and the number of double bonds, followed by the position of double bond from the methyl end of the molecule. The prefixes a- and i-indicated anteiso- and iso-branching, respectively (Denef et al., 2009; Potthast et al., 2010). We considered C18:2ω6,9c and C18:1ω9c as fungal origins (Bååth, 2003; Waldrop and Firestone, 2004; Kaiser et al., 2010; Potthast et al., 2010; Dungait et al., 2011), which were indicated as fung-PLFA, despite the fact C18:1ω9 is risky for fungal biomarker (Frostegård et al., 2011). G^+ bacterial PLFAs were iC14:0, iC15:0, aC15:0, iC16:0, iC17:0 and aC17:0 (Bünemann et al., 2004; McMahon et al., 2005; Potthast et al., 2010; Dungait et al., 2011) and were named as G^+-PLFA; G^- bacterial PLFAs included C16:1ω7c, C18:1ω7c, cy17:0 and cy19:0 (Bünemann et al., 2004; Potthast et al., 2010; Dungait et al., 2011; Rinkes et al., 2011) and were named as G^--PLFA; 10MeC16:0 was used to indicate actinomycetes (Potthast et al., 2010) and was named as acti-PLFA; the cy17:0/C16:1ω7c ratio was regarded as a proxy for nutritional or environmental stress (Kieft et al., 1994; Petersen et al., 2002).

A flow diagram in Figure S1 shows the strategy for parameterizing SOM- and residue-derived PLFAs turnover kinetics by a double exponential model and the descriptions of Eqs. (1)–(5).

In brief, the ^{13}C-residue-derived PLFA was calculated as follows:

$$\%\mathrm{PLFA}_{R,t} = \left(\frac{\delta^{13}\mathrm{C}_{\mathrm{PLFA},t} - \delta^{13}\mathrm{C}_{\mathrm{PLFA},0}}{\delta^{13}\mathrm{C}_{R,0} - \delta^{13}\mathrm{C}_{\mathrm{SOM},0}}\right) \times 100 \quad (1)$$

where $\%\mathrm{PLFA}_{R,t}$ is the fraction of residue-derived part of the individual PLFA at time t; $\delta^{13}\mathrm{C}_{\mathrm{PLFA},t}$ and $\delta^{13}\mathrm{C}_{\mathrm{PLFA},0}$ are the $\delta^{13}C$ of the individual PLFA at time t and at the beginning of the measurement, respectively; $\delta^{13}\mathrm{C}_{R,0}$ and $\delta^{13}\mathrm{C}_{\mathrm{SOM},0}$ are the $\delta^{13}C$ of the added residues and native SOM at the beginning of the measurement, respectively. The amounts of residue-derived PLFA (PLFA_R) and