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Carbon flow from litter through soil microorganisms: From incorporation rates to mean residence times in bacteria and fungi

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ABSTRACT

Resource quality and availability modify the microbial contribution to soil organic matter turnover and formation. We created a microbial hotspot at the soil-litter interface in a microcosm experiment to better understand and integrate specific microbial habitats into C turnover models. Reciprocal transplantation of ^{13}C and ^{12}C litter on top of soil cores allowed us to follow C flow into specific members of the microbial food web (bacteria and fungi) and to calculate the turnover times of litter-derived C in these microorganisms at three different stages of maize litter decomposition; early stage (0–4 days), intermediate stage (5–12 days) and later stage (29–36 days).

Litter age influenced the incorporation rate of C into bacteria and fungi and subsequent turnover in phospholipid fatty acid (PLFA) biomarkers. When fresh litter was applied, both fungi and bacteria were able to assimilate labile litter C in the early stage of decomposition, while lower substrate quality in the intermediate stage of decomposition promoted fungal utilization. Utilization of complex litter C sources was minor in both fungi and bacteria in the later stage of decomposition. Different bacterial substrate utilization strategies were reflected by either a decline of the isotopic signal after exchange of ^{13}C by ^{12}C litter or by storage and/or reuse of previously released microbial ^{13}C. The mean residence time of C in the fungal PLFA 18:2ω6,9 was estimated from 46 to 32 days, which is the same or shorter time than that of bacterial PLFAs. This highlights the role of fungi in rapid turnover processes of plant residues, with implications for implementation of bacterial and fungal processes into C turnover models.

1. Introduction

Soil organic carbon (SOC) is the largest active carbon (C) pool in terrestrial ecosystems. Historically it was thought that most SOC originates directly from plant residues, but it is now accepted that plant-derived C primarily enters the SOC pool indirectly via soil microorganisms (Kögel-Knabner, 2002; Liang and Balser, 2011; Miltner et al., 2011). Living microorganisms represent less than 5% of total SOC (Dalal, 1998), but these organisms account for a rapid and iterative decomposition process of C pools in soils. By contrast, the contribution of non-living microbial necromass to the formation of SOC is estimated to be 50–80% (Liang and Balser, 2011; Simpson et al., 2007). This microbially-derived C is assumed to be chemically or structurally complex with reduced decomposability (Moore et al., 2005; Six et al., 2006) leading to higher persistence in soil (Simpson et al., 2007; Kindler et al., 2009; Gleixner, 2013). Due to the heterogeneity of soil, less than 5% of the available soil space contains microbial hotspots (Nannipieri et al., 2003), but SOC decomposition processes in these hotspots are 10–100 times higher than in the bulk soil (Kuzyakov and Blagodatskaya, 2015).

The detritusphere, a microbial habitat which includes the litter layer and the soil influenced by litter, harbors a tremendous number of microorganisms which perform ecologically important biogeochemical processes, such as the decomposition and turnover of plant litter C (Schimel and Schaeffer, 2012; Gleixner, 2013). In these microbial hotspots, accelerated process rates and intensive interactions between C pools occur in a small soil volume (Kuzyakov and Blagodatskaya, 2015), which is restricted to 3–4 mm adjacent to the litter layer (Gaillard et al., 1999; Poll et al., 2008). Gaillard et al. (2003) demonstrated the importance of detritusphere decomposition processes with 5–11 fold higher mineralization rates in the adjacent soil of plant residues in comparison to uninfluenced bulk soil mineralization processes.

Historically, it was thought that resource partitioning between bacteria and fungi in the degradation process of complex substrates

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was an important driver of soil microbial diversity (Zhou et al., 2002). A recent study by Kramer et al. (2016), in a decomposition experiment using different plant C resources, provided evidence that a clear separation between bacterial usage of labile C and fungal usage of more complex C sources did not occur. This discrepancy has fueled an ongoing debate on the contributions of distinct microbial groups to different stages of the decomposition process (Strickland and Rousk, 2010).

Despite the important role of microorganisms in formation and degradation of SOC, internal bacterial and fungal C turnover processes have rarely been reported in the literature. Prokaryotic bacterial and eukaryotic fungal cells vary substantially in their chemical composition (Lengeler et al., 1999) and thus likely influence the decomposition rates of bacterial and fungal necromass. Kindler et al. (2009) separated the C introduced to the soil by cells of the Gram-negative bacterium *Escherichia coli* into fast and slow pools with half-lives of 7 days and 1.3 years. In contrast, fungal biomass C appears to be more persistent in soil, as shown by a half-life of 11.3 days for the easily degradable C pool and 3.8 years for the stable C pool (Schweigert et al., 2015), which has led to the assumption that fungal biomass contributes more to stable SOC than bacteria (Moore et al., 2005; Jastrow et al., 2007; Strickland and Rousk, 2010). Knowledge of group specific C turnover times is an important pre-requisite for accurate C modelling of agricultural management effects on soil C stocks (e.g. RothC model (Jenkinson et al., 1990)) and on pesticide degradation (e.g. PECCAD (Pagel et al., 2014)), but the role of microorganisms is rarely included in most of the models (Manzoni and Porporato, 2009). Within these models, exponential decay functions have been widely used to calculate C turnover times of newly introduced plant resources. They are often valid, however, only for early stages of litter decomposition and cannot be used to accurately fit decay processes from later decomposition phases (Berg and McClaugherty, 2003), because they assume constant C pool sizes (steady state) and proportional C fluxes (first order kinetics) during decomposition (Studer et al., 2014). However, the detritusphere is described as a continuum ranging from intact plant cell material to progressively decaying plant residues (Dungait et al., 2012), which makes it challenging to model and predict temporal small-scale turnover processes during litter decomposition.

Our aims were (i) to quantify the utilization of litter-derived C by bacteria and fungi in the detritusphere and (ii) to assess subsequent C turnover in microbial C pools as a function of residue age by using highly ^{13}C labeled maize litter placed on top of soil cores in a 60 day microcosm experiment. A reciprocal transplantation of labeled (^{13}C) and unlabeled (^{12}C) maize litter permitted quantification of the fate of the ^{13}C in soil organic C (SOC), extractable organic C (EOC), microbial biomass (C_{mic}), and different phospholipid fatty acid (PLFA) molecules; the last as biomarkers for bacteria and fungi. We hypothesized that (i) both bacteria and fungi use labile C from fresh plant residues; (ii) fungi use more complex C sources in the later stages of decomposition due both to their capacity to grow towards a substrate and their wider range of extracellular enzymatic capabilities as compared to bacteria (de Boer et al., 2005); and (iii) the turnover of C is faster in bacteria than in fungi due to the shorter life spans of prokaryotic bacterial cells and longer life spans of eukaryotic fungal cells (Rousk and Bååth, 2011).

2. Materials and methods

2.1. Soil and plant residues

Soil was taken from an experiment on an arable field near Göttingen (Germany, 51°33′N, 91°53′E; 158 m a.s.l.) in May 2014. The dominant soil types on the field were Luvisol and Cambisol with partially stagnic properties (IUSS Working Group WRB, 2007). For a detailed description of the field experiment see Kramer et al. (2012). Samples were taken from the topsoil (0–10 cm) of wheat plots with a known long-term C3 cropping history, at least 30 years, resulting in a soil $\delta^{13}C$ value of −27.3‰. Clay, silt and sand fractions were 7.0, 87.2 and 5.8%, respectively. Total C and N were 12.4 g kg^{-1} and 1.3 g kg^{-1} and pH was 6.0 (Kramer et al., 2012).

For the incubation experiment, senescent maize leaves with different ^{13}C labels (1.2 atom% ^{13}C and 92 atom% ^{13}C, IsoLife, Wageningen, The Netherlands) were used. In the following text, maize leaves with low ^{13}C content will be named "^{12}C litter" and maize leaves with high ^{13}C content "^{13}C litter". Air-dried maize leaves were cut into small pieces (approx. 3 mm) and stored at −20 °C until the start of the experiment.

2.2. Microcosms

Fresh soil was sieved (<2 mm), homogenized and used to fill cylinders (diameter = 5.6 cm, height = 4 cm). Each core contained 45 g air-dried soil which was compacted to a height of 1.5 cm, resulting in a bulk density of 1.2 g cm^{-3}. Soils were saturated with 0.01 M $CaCl_2$ solution, placed on ceramic plates and adjusted to a matric potential of −63 hPa. We used $CaCl_2$ solution to avoid dispersion of clay. For acclimatization to incubation conditions, the cylinders were placed in microcosms (glass containers, 500 ml) and pre-incubated at 20 °C for three days.

At the start of the experiment, 0.3 g maize leaves (equal to approx. 250 μg C g^{-1} soil) per cylinder were rewetted with 2 ml 0.01 M $CaCl_2$ solution, placed on the surface and slightly pressed onto the soil to ensure adequate contact between soil and litter. A mesh (500 μm, polyvinyl chloride) was placed between soil and litter to facilitate the transplantation of maize residues between soil cores during the experiment. The microcosms were incubated for 60 days at 20 °C. Water content was re-adjusted with 0.01 M $CaCl_2$ solution two times (on days 32 and 44) during the experiment.

2.3. Experimental design

The experimental approach consisted of three pulses of ^{13}C labelling representing litter of three ages (early, intermediate and later stage of litter decomposition). ^{13}C and ^{12}C litter was reciprocally transplanted between treatments to guarantee short and distinct labeling phases of the soil by maize litter of different ages (Fig. 1).

The following labeling periods were set up: (i) fresh ^{13}C litter, from 0 to 4 days (early stage); (ii) four day old ^{13}C litter, from 5 to 12 days (intermediate stage); and (iii) 28 day old ^{13}C litter, from 29 to 36 days (later stage). Furthermore, a ^{12}C (iv) and ^{13}C (v) control with continuous presence of ^{12}C and ^{13}C litter and a treatment without litter (vi) were also incubated over the duration of the experiment. At the end of each labeling period, ^{13}C litter was replaced by ^{12}C litter of the same age in order to follow the subsequent ^{13}C turnover in different soil and microbial pools while keeping boundary conditions of this highly active microhabitat as constant as possible. Separate microcosms were sampled 0, 4, 8, 16 and 24 days after the labeling period. We accounted for the fact that transplantation of litter on days 4, 12, and 28 disrupted the fungal hyphae between the litter and the soil (Frey et al., 2003) by raising the mesh on top of all soil cores on these days to exclude an artificial treatment effect (Frey et al., 2003). Soils of the ^{12}C control treatment were destructively sampled after 0, 4, 8, 12, 16, 20, 28, 36, 40, 44, 52, and 60 days. Continuously labeled ^{13}C cores and cores without maize litter were established for respiration measurements during the experiment and harvested after 60 days. Each treatment was replicated four times at each sampling date.