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How do microbial communities in top- and subsoil respond to root litter addition under field conditions?

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ABSTRACT

Contrasting microbial community composition and activity at different soil depths may affect root litter decomposition. These effects have up to now been investigated mainly in laboratory studies, which may not be able to take into account complex *in situ* conditions. Our study aimed to analyze the composition and activity of microbial communities after addition of ^{13}C labeled wheat root litter to a loamy soil under grassland at 30, 60 and 90 cm depths, during a three-year field experiment. We investigated the dynamics of bacterial and fungal abundances and community structures by DNA genotyping and pyrosequencing of 16S and 18S rDNAs. The genetic structures of bacterial and fungal communities were evaluated by automated ribosomal intergenetic spacer analysis. The functions of these communities were analysed by determination of extracellular enzyme activities and viable microbial communities involved in ^{13}C labeled organic matter decomposition studied by ^{13}C PLFAs.

The abundance of fungal and bacterial communities (16S and 18S rDNAs and PLFA) and the potential activities of enzymes involved in the C- and N-cycles were significantly higher at the top 30 cm compared with deeper soil throughout the experiment. Both were stimulated by fresh litter input. A trend to decreasing bacterial and fungal richness was noted after root litter addition at 30 cm, while richness of bacteria at 90 cm and those of fungi at 60 and 90 cm increased. Moreover, root litter addition caused a reduction of the Shannon Weaver Diversity index and a shift in microbial community structure at all three depths, which was more pronounced for bacteria at 30 and 60 cm and for fungi at 90 cm. The changes during litter degradation resulted in similar dynamics of most enzyme activities at all depths. Chitinase activity was enhanced after 29 months compared to initial conditions indicating the availability of high amounts of microbial detritus. The degrading microbial community as assessed by ^{13}C PLFA showed similar temporal dynamics at all three depths. Fungal contribution to this community decreased during later stages of litter degradation, while the contribution of Gram+ bacteria increased. We conclude that litter addition led to convergence of microbial communities of top- and subsoil through stimulation of copiotrophic populations. Soil microbial community structures are thus connected with the amount of fresh litter input. Enzyme activities and ^{13}C PLFA reflect to some extent the changes occurring during degradation, i.e. exhaustion of fresh plant material and accumulation of detritus.

1. Introduction

Heterotrophic microbial community abundance and composition is closely related to plant growth, especially in rhizosphere and detritusphere. It is assumed that the highest microbial abundance,

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species richness and diversity is concentrated in the uppermost soil as a consequence of high litter input and rhizodeposition, whereas these parameters are declining with depth in accordance with substrate availability (Jumpponen et al., 2010; Oehl et al., 2005; Rosling et al., 2003; Zajicek et al., 1986). In addition, the composition of bacterial communities is stratified depending on root vicinity: whereas Gram negative bacteria (Gram−) are dominant in topsoil and the rhizosphere, Gram positive bacteria (Gram+) dominate in subsoil (Blume et al., 2002). This leads to a complete differentiation of the structure of bacterial communities in top- and subsoil (Eilers et al., 2012).

In subsoil, occurrence of carbon is scarce and dominated by stabilised organic matter compounds (Rumpel and Kögel-Knabner, 2011). Therefore, microbial biomass and activity are not homogenously distributed but concentrated near locations with C input, such as rhizosphere and pores (Bundt et al., 2001; Chabbi et al., 2009; Nunan et al., 2003; Kautz et al., 2013), leading to development of hotspots of microbial activity (Nunan et al., 2003; Kuzyakov and Blagodatskaya, 2015). The separation of fresh organic matter and degraders increases with depth and may be the major factor responsible for the slow turnover rate of soil organic matter in deep soil horizons (Holden and Fierer, 2005; Hafner et al., 2014).

Due to these particularities, microbial communities in subsoils may function differently and may have specific responses to land-use or climate changes compared with topsoil communities (Smith et al., 2015). For example, oxidative enzyme activities increase with soil depth (Uksa et al., 2015), while hydrolytic enzyme activities decrease, most probably because both types of enzymes bind to different physical fractions (hydrolytic enzymes to particulate organic matter and oxidative enzymes to clay minerals) (Kramer et al., 2012). Subsoil microbial communities are energy limited (Fontaine et al., 2007), and their community composition could shape enzyme activities, which could be an important control of organic matter decomposition (Wild et al., 2015), in a similar way as in topsoils. In response to contrasting microbial activities, the mechanisms of organic matter stabilization seem to affect different compounds in subsoil as compared to topsoil (Rumpel et al., 2012, 2015).

Global change and ecosystem disturbance may change belowground carbon allocations and water availability (Smith et al., 2016). To understand the changes occurring upon disturbance, it is important to investigate how the subsoil microbial populations respond to plant litter input under *in situ* conditions. The fate of fresh litter input due to disturbance or vegetation change and its effect on subsoil microbial communities and their activities is poorly understood. Nearly all studies on the response of subsoil microbial communities to substrate addition were based on laboratory incubations (e.g. Fierer et al., 2003; Fontaine et al., 2007; Naisse et al., 2015), leaving many open questions about the impact of contrasting conditions at different soil depths occurring *in situ*. In general, the former studies indicated that substrate addition to subsoil stimulates microbial activities depending on substrate quality and nutrient availability. Moreover, accessibility of organic matter (OM) may be a major determining factor for OM decomposition in subsoils (Salomé et al., 2010).

In a recent field incubation with ^{13}C labeled wheat litter aiming to investigate root degradation at three soil depths, we evidenced similar quantities of degraded litter after 36 months, despite contrasting decomposition dynamics (Sanaullah et al., 2011). Indeed, while root litter in topsoil followed exponential decay, at 60 and 90 cm, root litter decay began after a lag phase. Moreover, changes with time in soil organic matter (SOM) bulk chemical composition were horizon dependent (Baumann et al., 2013a). These observations suggest contrasting microbial functioning at different soil depths. However, up to know, it is unknown how microbial communities at depths responded to wheat root addition and which strategy they used to overcome the initial lag phase: they may have changed their community structure or just their activities. Community structures could have converged at different soil depths directly after root litter addition and subsequently separated again, when easily decomposable material became exhausted.

To answer these questions, in the present study, samples from the incubation experiment were analysed for the abundance and activity of microbial communities by several biochemical and genetic tools. We used molecular tools such as soil DNA extraction, DNA fingerprinting with automated ribosomic intergenetic spacer analyses (ARISA) and pyrosequencing of 16S and 18S rDNA genes in order to study general microbial response to root litter addition (Ranjard et al., 2006). Moreover, we used compound specific isotope analyses of phospholipids (PLFA) to analyze the specific changes in the litter degrading community during decomposition at different soil depths (Frostegård et al., 2011). The response of microbial activity to root litter addition at different soil depths was studied by enzyme activities. The aims of this study were to investigate (1) if root litter addition caused increases in similar microbial groups at all soil depths, (2) if root litter additions stimulated similar enzyme activities at all soil depths but at different times (3) to determine if root litter addition causes similar shifts in microbial community structures at all three soil depths. We hypothesized that microbial community composition and functions before root litter addition are contrasting in top- and subsoil in agreement with former studies (Eilers et al., 2012; Ekelund et al., 2001) and that microbial community composition and functions of subsoil are strongly altered upon root litter addition.

2. Material and methods

2.1. Experimental set up

The field experiment was set up at Lusignan in the south-west of France (46°25′12,91″ N; 0°07′29,35″ E) in 2006. The site had been under ley cropping systems since more than 50 years. Soil type at the site is Cambisol with a loamy texture. The details about the experimental setup are described by Sanaullah et al. (2011). Briefly, the soils were excavated with a corer from 30, 60 and 90 cm, mixed with ^{13}C labeled wheat roots and buried again at the same depth under grassland vegetation. To this end, soil-root mixtures were exposed in litterbags (10 × 10 cm, mesh size 100 μm) containing a mixture of 2 g ^{13}C labeled root material (dried at 40 °C, <1 cm pieces) and 100 g native loamy textured soil from each depth. Wheat root material was labeled continuously with ^{13}C ($\delta^{13}C = 1744 \pm 13‰$) by growing wheat hydroponically in a growth chamber under 2 atom% ^{13}C-CO_2 atmosphere for 16 weeks. For analysis, three litterbags of each depth were excavated after 6, 12, 20, 29 and 36 months of incubation. The samples were taken back to the laboratory and stored at −20 °C before analyses.

2.2. Microbial biomass and structure

Microbial biomass is indicated as the amount of microbial DNA, which has been shown to be a robust, fast and easy way to quantify the microbial soil pool (e.g. Fornasier et al., 2014; Marstorp and Witter, 1999). For microbial DNA extraction we used 1 g of soil and the ISO-10063 procedure (Petric et al., 2011), which had been slightly modified by Plassart et al. (2012) to include a mechanical lysis step, using fastPrep®-24 instead of the recommended mini bead-beater cell disruptor. DNA concentrations of crude extracts were determined by electrophoresis in a 1% agarose gel using a calf thymus DNA standard curve (Dequiedt et al., 2011). After