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Seasonal variation in functional properties of microbial communities in beech forest soil

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ABSTRACT

Substrate quality and the availability of nutrients are major factors controlling microbial decomposition processes in soils. Seasonal alteration in resource availability, which is driven by plants via belowground C allocation, nutrient uptake and litter fall, also exerts effects on soil microbial community composition. Here we investigate if seasonal and experimentally induced changes in microbial community composition lead to alterations in functional properties of microbial communities and thus microbial processes. Beech forest soils characterized by three distinct microbial communities (winter and summer community, and summer community from a tree girdling plot, in which belowground carbon allocation was interrupted) were incubated with different ^{13}C-labeled substrates with or without inorganic N supply and analyzed for substrate use and various microbial processes. Our results clearly demonstrate that the three investigated microbial communities differed in their functional response to addition of various substrates. The winter communities revealed a higher capacity for degradation of complex C substrates (cellulose, plant cell walls) than the summer communities, indicated by enhanced cellulase activities and reduced mineralization of soil organic matter. In contrast, utilization of labile C sources (glucose) was lower in winter than in summer, demonstrating that summer and winter community were adapted to the availability of different substrates. The saprotrophic community established in girdled plots exhibited a significantly higher utilization of complex C substrates than the more plant root associated community in control plots if additional nitrogen was provided. In this study we were able to demonstrate experimentally that variation in resource availability as well as seasonality in temperate forest soils cause a seasonal variation in functional properties of soil microorganisms, which is due to shifts in community structure and physiological adaptations of microbial communities to altered resource supply.

1. Introduction

Microbial decomposition of soil organic matter (SOM) plays a key role in global C and N cycling (Davidson and Janssens, 2006). It is well known that microbial decomposition processes are controlled, amongst other factors, by substrate quality (e.g. lignin content) and the availability of labile C and nutrients (Chapin et al., 2002; Schmidt et al., 2011). Resource availability influences decomposition processes via effects on microbial physiology, e.g. production of extracellular enzyme activities. Microbial production of extracellular enzymes is stimulated by substrate supply or if available nutrients or C are scarce (Allison and Vitousek, 2005; Hernandez and Hobbie, 2010; Olander and Vitousek, 2000). Enhanced availability of labile C substrates may also increase the decomposition of recalcitrant SOM ('priming effect'), which is ascribed to either microbial activation by the labile C source or enhanced degradation of SOM for the acquisition of limiting nutrients (Blagodatskaya and Kuzyakov, 2008; Fontaine et al., 2011).

Apart from changing the physiology of microbial communities, alterations in resource availability may also influence microbial processes indirectly. As different species of microbes differ in substrate use efficiency and biomass composition and thus demand for C and nutrients (Degens, 1999; Gusewell and Gessner, 2009), changes in resource supply have been shown to induce shifts in

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microbial community composition (Eilers et al., 2010; Fierer et al., 2012; Griffiths et al., 1999; Waldrop et al., 2004). Changes in microbial community composition may in turn strongly affect microbial processes since certain classes of enzymes are produced by specific groups of microorganisms. This is especially true for phylogenetically 'narrow' processes, i.e. processes performed by a relatively small number of specialized species, such as polyphenol degradation, while a higher functional redundancy between different microbial communities may be found for processes performed by a broad array of soil microorganisms, such as C mineralization or protein depolymerization (Balser and Firestone, 2005; Schimel et al., 2005).

While the relation between microbial community composition and function has already been demonstrated in several studies comparing functional properties of microbial communities from different ecosystems (Balser and Firestone, 2005; Brant et al., 2006; Paterson et al., 2011; Strickland et al., 2009; Waldrop and Firestone, 2004), few studies exist investigating the effects of microbial community changes at a single site, e.g. community shifts following increased or decreased plant inputs, on microbial functions (Brant et al., 2006; Paterson et al., 2011; Wickings et al., 2011). It is still not fully understood if and how changes in microbial community composition resulting from seasonal and experimental induced variation in resource availability affect the functional properties of microbial communities, a topic which is especially important in the light of ongoing global change.

In temperate forest ecosystems a seasonal pattern of microbial processes has been observed which is related to a seasonal variation in availability of different substrates, as well as variation in soil temperature and moisture (Kaiser et al., 2011, 2010b). The seasonal variation in resource availability is mainly driven by plants via belowground C exudation and nutrient uptake during the growing season and litter fall in autumn. These seasonal changes in resource availability have also been shown to induce shifts in microbial community structure (Kaiser et al., 2011, 2010b).

This study aimed at elucidating whether changes in the composition of soil microbial communities related to seasonal and experimentally induced variation in resource availability lead to altered functional properties of these microbial communities. We hypothesized (1) that distinct microbial communities that are adapted to different substrates vary in their physiological capacities and in their functional response to addition of various organic substrates and (2) that the functional response of different microbial communities to addition of C substrates is influenced by microbial nutrient limitation.

In order to test these hypotheses we performed an incubation experiment with soils from a beech forest study site (Kaiser et al., 2010b) characterized by three different microbial communities. We chose winter and summer microbial communities as we assumed microbial adaptation to high availability of labile C in summer and to more recalcitrant substrates (litter) in winter. In summer we also collected soils from a tree girdling plot in which belowground carbon allocation had been interrupted which promoted the establishment of a more saprotrophic community. Collecting soils from a single site ensured that soils were comparable in soil properties. We incubated the different soils with a range of labile and complex substrates (glucose, protein, microbial cell walls, cellulose and plant cell walls) and analyzed changes in various microbial processes and pools of labile C and N in response to substrate addition and experimentally enhanced inorganic N supply. By using ^{13}C labeled substrates we were also able to directly monitor microbial substrate utilization and analyze how the various amendments led to changes in the utilization of soil organic matter (i.e. priming).

2. Material and methods

2.1. Origin of soil

The soil for the incubation experiment originated from a 65-year-old beech forest (*Fagus sylvatica*) about 40 km southwest of Vienna (48°07′ N 16°03′ E, 510 m a.s.l.). Soils were classified as Dystric Cambisols over flysh (pH in $CaCl_2$ between 4.5 and 5.1) with a mean organic carbon content of 7.45% and nitrogen content of 0.48% in the A horizon. The study site and experimental setup in the field was described in detail previously (Kaiser et al., 2010b), microbial communities were characterized by Kaiser et al. (2010b) and Rasche et al. (2010). Girdling of beech trees had been performed in May 2006 by removal of the bark over 10 cm sections around the circumference of the stems. Soils for the incubation experiment were collected in February 2008 (winter community) and in June 2008 (summer community and community from girdling plots). 4 subsamples of mineral soil (5 cm depth, A-horizon) were collected from each of 6 replicate plots in winter and summer, respectively, and from 6 replicate girdled plots in summer. Soils from replicate plots were pooled, sieved (5 mm) and stored at 4 °C (winter) and 12 °C (summer) until the start of the incubation experiment. Half of the winter soil was transferred to 12 °C for equilibration 3 days before the incubation.

2.2. Substrates

Five ^{13}C-labeled substrates differing in complexity and C and N content were used for the incubation experiment: Glucose, protein, microbial cell walls, cellulose and plant cell walls, containing 20 atom % ^{13}C, except for cellulose (16 atom %) and protein (98 atom %). Glucose (99 atom % ^{13}C, from Sigma) and cellulose (97 atom % ^{13}C, from IsoLifeBV) were diluted with the respective unlabeled substances, algal protein extract (98 atom % ^{13}C, from Sigma) was applied undiluted.

^{13}C-labeled microbial cell walls were prepared as follows: Two bacterial species (*Pectobacterium carotovorum* and *Verrucomicrobium spinosum*) and one fungal species (*Aspergillus nidulans*) were grown on ^{13}C-glucose (20 atom % ^{13}C). Growth conditions were described by Keiblinger et al. (2010). Microbial biomass was dried and then resuspended in NaCl-solution. After mechanical destruction of cell walls by ultrasonic treatment and bead beating, residues were repeatedly extracted with NaCl-solution, water, methanol/chloroform (5:3), hexane and pure water to remove all labile cell constituents. The remaining residues were dried, homogenized (ball mill) and stored frozen.

^{13}C-labeled plant cell walls were prepared as follows: ^{13}C-labeled wheat roots (IsoLiveBV, U-60402) and unlabeled, dried wheat roots were finely ground and homogenized in a ball mill. The material was then incubated with α-amylase solution to remove starch (Richter et al., 2009) and further extracted repeatedly with methanol/chloroform/water (12:5:3) to remove other labile substances.

2.3. Experimental setup

The respective substrate (1 mg substrate g^{-1} soil of glucose and protein, 4 mg g^{-1} soil of the other substrates) was amended to each soil in a dry form. A subset of the summer soils (from control and girdling plots) amended with either cellulose or plant cell walls, was also amended with inorganic N (3 mg NH_4NO_3 g^{-1} soil). We added N to these treatments in order to test the effects of increased N availability on the degradation of C substrates (one of them lignin containing), assuming microbial N limitation in summer.

Incubation of soils (22 g) was performed in a microcosm system with 5 replicates for each substrate and soil (Inselsbacher et al.,