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Seasonal differences in tree species' influence on soil microbial communities

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ABSTRACT

The linkage between tree diversity and the soil food web in temperate deciduous forest ecosystems remains uncertain. Using microbial phospholipid fatty acids (PLFAs), we analyzed the effect of tree species composition on microbial communities from topsoil collected in Hainich National Park, Germany. Previous results had shown minimal direct effects of tree species on the microbial community in autumn, most likely due to low plant activity and high nutrient and energy input from litterfall. However, microbial composition was affected indirectly through an influence of tree species on soil pH. In this study, we analyzed PLFA profiles in early summer and compared them with the results from autumn sampling. We hypothesized that plant-based traits would have stronger direct effects on the abundance and structure of the microbial community during the photosynthetically active period. The results showed that the soil microbial community differed more markedly between the tree diversity levels in early summer than in autumn. The acidifying character of the decaying beech litter strongly influenced the soil pH values and structured the soil microbial community indirectly in early summer as it had in autumn. However, the measured differences in the microbial composition in early summer could be attributed primarily to litter quality. This direct influence of plant traits appeared to be eclipsed in autumn because of the high nutrient supply from fresh litter input. Following litter decomposition in the topsoil, however, litter-based plant traits emerged as a factor structuring the soil microbial community in early summer. Our results suggest that the PLFAs i14:0 and i15:0, indicative of Gram-positive bacteria, are strongly involved in decomposition processes and may be promoted by readily available nutrients. Furthermore, our results indicate that a dense root network in association with arbuscular mycorrhizal fungi strongly supported microbial growth in the more diverse forest stands. High proportions of arbuscular mycorrhizal fungi (PLFA 16:1ω5), root-associated microorganisms (PLFAs 16:1ω9, 16:1ω7, 17:1ω8 and 18:1ω7) and bacterial grazers (PLFA 20:5) characterized the microbial community in early summer on these study plots. We conclude that microbial communities are strongly influenced by abiotic controls. However, seasonal differences in litter decomposition rates and root activity should be considered in the analysis of the effects of tree diversity or species on soil microbial communities.

1. Introduction

Soil microorganisms are important drivers of soil processes, and they play a key role in the decomposition of recent plant material (Bardgett et al., 2005). Because they rely on organic carbon (C) for growth, they are affected by any change in C input or C loss in soils (Zak et al., 2003). In forest ecosystems, C input is derived primarily from the decomposition of organic matter, such as leaf and root litter, plant exudates, woody plant debris and animal remains. The contribution of these components varies substantially depending on the tree species present (Gleixner et al., 2005), and root exudates, in particular, vary in quality and quantity among tree species (Grayston et al., 1997; Calvaruso et al., 2011). Additionally, litter quality measures, such as the amount of nutrients and tissue structure, as well as the relative proportions of C and N compounds of different decomposability, such as protein and lignin, vary between broadleaf tree species (Jacob et al., 2009; Gessner et al., 2010).

The total amount of organic matter input is highest in the topsoil. Consequently, this is the soil stratum in which the largest observed effects of tree species on soil chemical properties are found (Augusto et al., 2003; Hagen-Thorn et al., 2004; Thoms et al.,

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2010). In deciduous forests, the soils under *Fagus sylvatica* show lower soil pH values, lower base saturation and higher C/N ratios compared to forest soils under mixed deciduous forests consisting of *Fagus*, *Fraxinus*, *Tilia* and other deciduous tree species (Guckland et al., 2009; Langenbruch et al., 2012). Additionally, both the quantity and quality of litter affect both the predators and the decomposer macrofauna (Weland, 2009). Studies that have investigated the relationships between plant species diversity and soil microbial communities using PLFA profiles in grasslands (Habekost et al., 2008; Breulmann et al., 2012) and in forests (Hackl et al., 2005; Merilä et al., 2010; Brockett et al., 2012; Wu et al., 2012; Myers et al., 2001) have underlined the importance of the quantity and quality of organic resources as well as abiotic factors such as soil pH, soil texture and soil moisture for the soil microbial community.

The life cycle of microbes in temperate broadleaf forests in Central Europe is strongly affected by the seasons through changes in biotic and abiotic factors. In early March, the vegetation starts to produce shoots, roots and leaves from C reserves, followed by a photosynthetically active period beginning typically in April to May. The growth period ends with litterfall in autumn, representing an excellent food input for the decomposer community in the topsoil. During winter, deciduous trees are generally inactive, and decomposition processes are also slow because of the decelerating effect of low temperatures on soil microbial metabolism. Soil temperatures increase in spring, and soil microbes accelerate their activity in conjunction with the beginning of the vegetation period (Kauri, 1982). In summer, the loss of precipitation can produce extremely dry soil conditions that again inhibit soil microbial activity (Moore-Kucera and Dick, 2008). Typically, studies in mature beech forests in Europe show two phases of increased microbial growth over the seasons with maxima in spring and autumn (Kauri, 1982; Kaiser et al., 2010). Unfortunately, studies considering the impact of tree species on soil microorganisms through seasonal cycles are rare (Collignon et al., 2011; Brant et al., 2006). For this reason, we have limited insight into seasonal effects on the influence of tree species on soil microorganisms. We expect differences to emerge during the active vegetation period, when trees might influence soil microorganisms more directly through root exudates and litter decomposition. As a function of litter quality, differences during the first year of decomposition can strongly alter the amounts of nutrients that are released into the soil and that can then be absorbed by microorganisms (Prescott et al., 2000). Meinen et al. (2009a) also reported different seasonal patterns in fine-root necromass in deciduous forest stands differing in tree species composition, with the highest fine root-necromass found in June and the lowest in January. Strong seasonality in the abundance of earthworm populations in mixed forest sites has also been demonstrated and has been attributed to differences in litter decomposition and fluctuations in the soil carbon pool over the year (Cesarz et al., 2007).

In a previous study in Hainich National Park in Central Germany, we found few direct effects of tree features on the soil microbial community after extraction of PLFAs from soil samples following litterfall in autumn (Thoms et al., 2010). The group of arbuscular mycorrhizal fungi (AM fungi) was found in significantly higher concentrations on the plots with the highest tree species diversity. These plots were characterized by higher abundances of maple and ash, whose roots form symbioses with AM fungi. In the present study, we analyzed PLFA profiles from the same plots approximately 7 months after litterfall in early summer and compared all individual PLFAs separately with the autumn PLFA profiles. We hypothesize that a) differences in the microbial biomass and community structure between different tree diversity levels in early summer are greater than those in autumn and that b) direct plant-based traits have a stronger impact on the soil microbial community in early summer than in autumn.

2. Materials and methods

2.1. Study site

The study was conducted in Hainich National Park, a deciduous forest in Thuringia, Central Germany. The unique land use history of Hainich National Park has created a species-rich temperate broadleaf forest that offers the opportunity to study the role of forest diversity in a variety of forest use types in species-poor to species-rich forest patches (Leuschner et al., 2009). In 2005, 12 study plots (50 m × 50 m each) representing three tree diversity levels (DL 1–3) with four replicates per level were established. A maximum distance of 5 km between the plots ensured that they were comparable in terms of homogenous climate and soil conditions (Guckland et al., 2009). Four pure beech forest (DL 1), four beech-ash-linden forest (DL 2) and four beech-ash-linden-hornbeam-maple forest (DL 3) plots form the diversity gradient (a map is shown in Guckland et al., 2009), comprising primarily the tree species *F. sylvatica* L., *Fraxinus excelsior* L., *Tilia* species (*Tilia cordata* Mill., *T. platyphyllos* Scop.), *Carpinus betulus* L. and *Acer* species (*Acer pseudoplatanus* L., *A. platanoides* L., *A. campestre* L.). All plots approached natural conditions without distinct anthropogenic impact (e.g., silvicultural management) over the past several decades. The principal plot characteristics, including stem densities, litter amounts, litter nutrients, macrofauna and soil properties, are summarized in Appendix 1.

2.2. Soil sampling

On May 31 and June 1, 2006, soil samples were collected during a rainy period from all 12 plots using three randomly chosen transects of 30 m × 1 m within the plots. We took 12 samples per plot from soil depths of 0–5 cm of the mineral horizon (Ah horizon) with a split tube (Eijkelkamp, Giesbeek, Netherlands) 5.3 cm in diameter according to the sampling procedure previously used in November 2005 (Thoms et al., 2010). The samples were collected randomly at a minimum distance of 4 m and were mixed to obtain one composite sample per plot. After transport to the laboratory, all samples were immediately sieved <2 mm to remove visible stones, animals, roots and plant material prior to lipid extraction. The water content was quantified using a subsample dried to 105 °C. Soil pH was measured in 1 M KCl solution. For C and N analyses, another subsample was air dried, ball milled and analyzed in an automated element analyzer (Elementaranalysator varioMAX, Elementar Analysensysteme GmbH, Hanau, Germany).

2.3. PLFA analysis

A total of 100 g of fresh soil was extracted according to the method of Bligh and Dyer (1959) and Zelles and Bai (1993). Soil lipids were extracted using a mixture of chloroform, methanol and phosphate buffer (1:2:0.8 v/v/v). Phospholipids were isolated on silica columns and hydrolyzed and methylated using a methanolic KOH solution. Fatty acid methyl esters (FAME) were separated into saturated, polyunsaturated and monounsaturated fatty acids using aminopropyl-modified and silver-impregnated SPE columns. The samples were quantified with a GC-AED System (GC: HP 6890 Series, AED: G 2350 A, Agilent Technologies, Wilmington, USA) using a BPX 70 column (50 m × 0.32 mm I.D., 0.25 μm film thickness) in the split mode (10:1). Helium was used as a carrier gas at a flow rate of 1.3 ml min^{-1}. The temperature program started at 100 °C (for 1 min). Thereafter, the temperature was raised to 135 °C at a rate of