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Effects of plant-symbiotic relationships on the living soil microbial community and microbial necromass in a long-term agro-ecosystem

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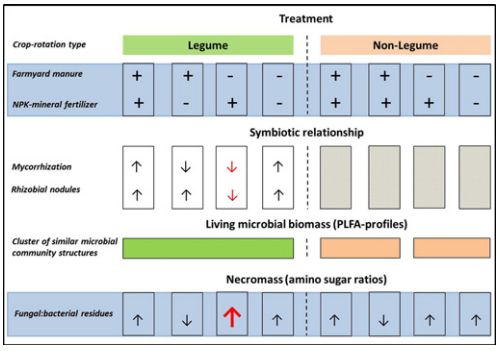
HIGHLIGHTS

- We extracted PLFA and amino sugars from six fertilization treatments under two crops.
- Both, microbial bio- and necromass compositions were shaped by manure addition.
- Short-term effects on microbial biomass result from mineral fertilizers.
- Strength of mineral fertilizer effects depends on crop type and plant-symbionts.
- Turnover of microbial necromass may depend on N fertility.

GRAPHICAL ABSTRACT



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ABSTRACT

We examined the impact of arbuscular mycorrhizal fungi and rhizobia on the living microbial community and microbial necromass under different long-term fertilization treatments at the long-term Static Fertilization Experiment Bad Lauchstädt (Germany). Phospholipid fatty acids (PLFA) and amino sugars plus muramic acid, were used as biomarkers for soil microbial bio- and necromass, respectively, and analyzed from six treatments imposed on two crop rotations, varying only in the inclusion/non-inclusion of a legume. Treatments included: two levels of only farmyard manure (FYM), only mineral fertilizer (NPK), the combined application of both fertilizer types and a non-fertilized control. PLFA profiles differed clearly between the investigated crop rotations and were significantly related to labile C, mineral N, and soil pH. This emphasizes the role of carbon, and of mycorrhizal and rhizobial symbioses, as driver for changes in the microbial community composition due to effects on the living conditions in soil. We found some evidence that legume associated symbiosis with arbuscular mycorrhizal fungi and rhizobia act as a buffer, reducing the impact of varying inputs of mineral nutrients on the decomposer community. While our results support former findings that living microbial populations vary within short-term periods and are reflective of a given crop grown in a given year, soil necromass composition indicates longer term changes across the two crop rotation types, mainly shaped by fertilizer related effects on the community composition and C turnover. However, there was some evidence that specifically the presence of a legume, affects the soil necromass composition not only over the whole crop rotation but even in the short-term.

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1. Introduction

Proper management of agricultural soils can contribute to the mitigation of atmospheric CO_2 increases both by reducing respiratory C losses from soil and by sequestering photosynthetically fixed CO_2 (Smith et al., 2014). These processes are mainly driven by soil microorganisms, which play a central role for the functioning of terrestrial ecosystems, encompassing much more than simply decomposition of organic matter or cycling of mineral nutrients (Nannipieri and Badalucco, 2003). Almost all organic material which enters the soil passes through the pool of living microbes, where it gets degraded, transformed, or incorporated into fungal or bacterial cells (Paterson et al., 2009). It is estimated that up to 80% of soil organic carbon (SOC) may be derived from microbial cellular components, highlighting the significance of the soil microbial community as source of soil organic matter (SOM) genesis and long-term C sequestration (Liang et al., 2011). It is not fully understood, however, how specific management practices affect the sequestration, or in turn, the mineralization of microbial residues in soils. Some of these open questions might be answered by a direct comparison between the living microbial biomass and long-term sequestered necromass.

Since most soil microorganisms are not cultivable, culture independent methods relying on biochemical indicators, such as lipids or amino sugars, are frequently used to analyze microbial communities (Frostegård and Bååth, 1996; Liang and Balser, 2012). Phospholipid derived fatty acids (PLFA) are structural components of all cellular membranes, constantly synthesized during microbial growth, and experiencing a rapid turnover in the soil (White et al., 1979; Zelles, 1999). Thus, PLFA's can be applied as an indicator for the living microbial biomass and for the current structure of the microbial community. If neutral lipid fatty acids (NLFA) are also considered, it is possible to have additional information about the physiological state of fungi, since NLFA mainly derive from triacylglycerols, which are storage products of eukaryotic cells (Bååth, 2003).

In contrast to PLFAs, amino sugars (AS) and muramic acid (MurA) are largely stabilized in the soil and are assumed to represent mainly microbial necromass (Glaser et al., 2004). While MurA occurs exclusively in the peptidoglycane layer of bacterial cell walls, glucosamine (GluN) is the basic component of fungal chitin and is present to a lesser extent in bacterial cell walls. Only negligible quantities of GluN in soil have been assigned to sources not related to fungal or bacterial biomass, such as skeletons of arthropods, earthworm gut lining, nematode egg shells, mollusk polysaccharides, or snail gelatine (Amelung, 2001; Chantigny et al., 1997). Galactosamine (GalN) is a frequent component of bacterial capsular- or exopolysaccharides but also found in significant amounts in fungi (Glaser et al., 2004). Little is known about the biological function of mannosamine (ManN), which also seems to be derived mainly from bacteria (Kenne and Lindburg, 1983; Rüde et al., 1962). In the past, the ratios of GluN/MurA or GluN/GalN have been used to estimate the contribution of fungal and bacterial residues into the SOM pool (Said-Pullicino et al., 2007; Solomon et al., 2001).

Previous studies examining the role of microorganisms in SOM dynamics have either focused only on structural and functional changes of the living microbial community or on shifts in the bacterial and fungal contributions to SOM. Based on the need of understanding SOM dynamics for sustainable food production, a large proportion of this research has been performed in agricultural soils. The type and application rate of fertilizers as well as the plant species and plant-microbe interactions have all been found to affect the biomass, activity and structure of the microbial community (Fließbach et al., 2007; Lambers et al., 2009; Ngosong et al., 2010).

Compared to the great number of PLFA-based studies, the accumulation and turnover of microbial residues in agricultural soils has been researched less intensively. Long-term cropping has been found to reduce total AS contents, which was explained by microbial substrate limitation due to cultivation (Zhang et al., 1998). In this context, a preferential degradation of bacterial derived AS was assumed, resulting in a higher fungal contribution (Zhang et al., 1999). Amelung et al. (2001) even demonstrated that the amount of C and N sequestered in the microbial necromass can be manipulated by changing the input of labile C and N into soil. Finally, evidence on crop- and crop rotation related effects on amounts of total and individual amino sugars in soil was reported recently by Zhang et al. (2014).

To our knowledge, there are only a handful of studies in which the living (PLFA) and non-living (AS) biomass has been measured simultaneously in relation to each other (Appuhn and Joergensen, 2006; Liang et al., 2008; Liang et al., 2015; Zhang et al., 2013), while the impact of fertilization, crop, or symbiotic relationships of crops with rhizobia or AMF in agricultural soils has not been considered. Such work, however, would improve our knowledge about the production and stabilization of amino sugars and thus could have the potential to increase our understanding of the microbial mediation of long-term dynamics of soil organic C and N.

From the background of very long-term, likely steady state conditions at the Static Fertilization Experiment Bad Lauchstädt (SFEBL) we compared the abundance and composition of the living microbial community with the amount of microbial residues by determining both PLFA and AS. In addition to fertilization treatments we considered plant-microbe interactions as influencing factors by sampling two crop rotations that vary only in the inclusion or exclusion of alfalfa (*Medicago sativa*) every 7th and 8th year having a dual symbiosis (AMF and rhizobia) which has a huge impact on the rhizosphere in terms of N and C exudates. We examined changes in crops and cropping history from two perspectives using these different tools: first we examined the effects of long-term fertilization treatments on the living, active microbial community associated with the currently grown crop (alfalfa or sugar beet) using lipid analysis, AMF colonization, and rhizobia nodulation rates. Second, we examined the effects of fertilization and cropping history using the amino sugar microbial necromass pool, assumed to change more slowly over time and reflect longer term patters associated with agricultural management.

2. Materials and methods

2.1. Study site description and sampling

This study took place at the long-term Static Fertilization Experiment Bad Lauchstädt (SFEBL), Sachsen-Anhalt (Germany), which was initiated in 1902 (Körschens and Pfefferkorn, 1998). The soil is a Haplic Chernozem (FAO) (USDA: Mollisol) consisting of 21% clay, 67.8% silt and 11.2% sand. The mean annual temperature and precipitation are 8.8 °C and 480 mm, respectively (Körschens, 2002). The Static Fertilization Experiment Bad Lauchstädt was laid out in a split plot design with the main-plot factor FYM (three levels: no FYM, 20 t FYM ha^{-1} 2 $years^{-1}$, 30 t FYM ha^{-1} 2 $years^{-1}$) and mineral fertilization as a sub-plot factor (6 levels: no, PK, N, NK, NP, NPK) which are all realised in each main plot. The experiment is further stratified in 5 different cropping strips. Four of the cropping strips represent different phases (a different crop on each strip, each year) of a 4 crop rotation (sugar beet, spring barley, potatoes, and winter wheat). In addition, legumes have been included in the crop rotation exclusively on the 5th strip of the experiment since 1924, replacing sugar beet and spring barley every 7th and 8th year.

Since 1970 alfalfa (*Medicago sativa*) has been the only legume species cultivated on this strip. Mineral fertilization (NPK) varies annually in adaption to the nutrient demand of each crop (60 to 170 kg ha^{-1} $year^{-1}$ N, 12 to 60 kg ha^{-1} $year^{-1}$ P, 50 to 230 kg ha^{-1} $year^{-1}$ K). Application of farmyard manure takes place every second year with the cultivation of root crops (potatoes, sugar beet). Alfalfa follows winter wheat in the rotation, receives farmyard manure before seed drilling, and only receives PK in the mineral fertilized treatments.