



Carbon input and crop-related changes in microbial biomarker levels strongly affect the turnover and composition of soil organic carbon



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ABSTRACT

It is increasingly recognized that a detailed understanding of the impacts of land use on soil carbon pools and microbially mediated carbon dynamics is required in order to accurately describe terrestrial carbon budgets and improve soil carbon retention. Toward this understanding, we analyzed the levels of biomarkers including phospholipid fatty acids, amino acids, monosaccharides, amino sugars, and several indicators of labile and stabilized carbon in soil samples from a long-term agricultural field experiment. Our results imply that the composition of soil organic carbon (SOC) strongly depends on both the applied fertilization regime and the cultivated crop. In addition, our approach allowed us to identify possible mechanisms of microbial growth and contributions to soil carbon storage under different long-term agricultural management regimes.

Amino acids and monosaccharides were quantitatively the most dominant biomarkers and their levels correlated strongly positively with microbial biomass. The relative contributions of the studied biomarkers to the total SOC varied only slightly among the treatments except in cases of extreme fertilization and without any fertilizer. In case of extreme fertilization and with alfalfa as crop type, we found evidence for accumulation of microbially derived monosaccharides and amino acids within the labile OC pool, probably resulting from soil C saturation. Interestingly, we also found an accumulation of microbially derived monosaccharides and amino acids in completely unfertilized plots, which we assumed to be caused by the smaller pore space volume and subsequent oxygen limitation for microbial growth. Mineral fertilization also had substantial effects on soil organic N when applied to plots containing alfalfa, a leguminous plant. Our results demonstrate that over-fertilization, fertilizer type, and the cultivated crop type can have major impacts on the turnover and composition of soil organic carbon, and should be considered when assessing management effects on soil C dynamics.

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1. Introduction

Terrestrial ecosystems represent the largest global pool of organic carbon (Batjes, 1998; Janzen, 2005). There is an increasing need to understand the cycling and storage potential of organic carbon in terrestrial ecosystems to mitigate climate change and improve sustainability, especially in agriculturally managed systems since these represent around 40% of all land on earth (Smith

et al., 2008). Efficient management of carbon and nitrogen flows will be required to reduce CO₂, CH₄, and N₂O emissions due to agriculture and to increase carbon stocks in agricultural ecosystems (IPCC, 2007). However, it is difficult to evaluate the efficiency of a specific management strategy if one cannot account for all of the factors that may affect the dynamics of C and N in the soil and their interactions with one-another. One such factor is the use of organic and inorganic fertilizers, which directly or indirectly increase the input of C into the soil and change the availability of nutrients, the soil structure, and the abiotic conditions in the soil environment (Campbell et al., 1991; Marschner et al., 2003). The crop type can also influence soil organic carbon (SOC) turnover due to the

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different physiological and morphological characteristics of different plant species. Important characteristics can include root architecture, nutrient demand, water use efficiency, and the quantity and composition of root exudate (Dakora and Phillips, 2002; Jastrow et al., 2007; Marschner, 2012). Due to the close interactions between plants and soil microorganisms, plant–microbe interactions and the growth, activity and composition of crop-specific microbial communities have profound effects on C and N dynamics. A well-known example of this is that legumes are often used in agriculture to improve the availability of N and other mineral nutrients based on their ability to form symbioses with N-fixing bacteria and arbuscular mycorrhizal fungi (Paustian et al., 1997a; Wilson et al., 2009; Lal, 2010).

Investigations of soil properties using pyrolytic techniques (Py-FIMS, Py-GC-MS) have demonstrated that fertilization and crop rotation affect the SOC composition by altering C and N dynamics within or between several organic carbon pools (Schmidt et al., 2000; Leinweber et al., 2008). The organic carbon (OC) content of non-protected or labile carbon pools is affected directly by the supply of new nutrients and microbial growth, and can be rapidly degraded (Wander, 2004). Management-induced effects on the availability of C, N and P or the oxygen content, pH or temperature of the soil can change the rates of microbial metabolism and decomposition, which can result in quantitative and qualitative changes in the labile OC pool (Tiedje et al., 1984; Knorr et al., 2005; Davidson and Janssens, 2006; Curiel Yuste et al., 2007; Rousk et al., 2009; Hartman and Richardson, 2013). Since rates of OC production and utilization may compensate each other, variations in contents of total labile OC may not be measurable. Even though the total labile OC content may not change, the decomposition and incorporation of plant derived labile OC into microbial biomass may cause a shift in the proportions of OC from different sources, resulting in an altered composition of SOC.

Conversely, OC in protected carbon pools is more resistant to depletion and such pools exhibit low turnover rates. Factors that impede the decomposition of organic constituents include formation of stable complex chemical structures, physical protection by occlusion into micro-aggregates, and chemical protection by interaction with minerals and metal ions (Sollins et al., 1996; Lützow et al., 2006). Microorganisms can enhance the proportion of OC in the protected pool by producing complex organic compounds and promoting aggregate formation around fungal hyphae or bacterial exopolysaccharides. While estimates of total soil organic C are generally based on the size of the protected or stabilized OC pool, most C models predict that SOC levels increase linearly with C input, which has been confirmed for a large number of long-term agricultural field experiments (Paustian et al., 1997b; Huggins et al., 1998; Kong et al., 2005). However, the protective capacity of the soil may be limited, especially if it was originally rich in OC, resulting in C saturation, or in other words, a limit to observed linear responses despite further increase of C input (Hassink, 1997; Hassink et al., 1997; Six et al., 2002; Wiesmeier et al., 2014). It is currently not entirely clear how saturation of the protected pool affects OC turnover within the non-protected OC pool. In addition, little is known about how microbial mediation of OC stabilization and cycling are influenced by the impact of fertilization on plant–microbe interactions. To address these questions, it is necessary to consider physical, chemical and microbiological factors simultaneously.

Biomarkers such as phospholipid fatty acids (PLFA) and amino sugars (AS) have been used extensively to study environmental impacts on living microbial communities and the accumulation of microbial residues, respectively (Bossio et al., 1998; Guggenberger et al., 1999; Amelung et al., 2001; Amelung, 2003; Jangid et al., 2008). Although monosaccharides (MS) and amino acids (AA) are

ubiquitous in the biosphere, they can also be used as biomarkers for microbially derived C and N to some extent. For instance, proportions of bacterial to plant derived monosaccharides can be reflected by the ratios of C6:C5 (mannose + galactose)/(xylose + arabinose) and deoxyC6:C5 (rhamnose + fucose)/(xylose + arabinose) (Murayama, 1984; Oades, 1984). For amino acids, meso-Diaminopimelic acid as well as D-enantiomers of alanine and glutamic acid build up the peptidoglycane layer of bacterial cell walls and have been used to indicate bacterial residues (Grant and West, 1986; Amelung, 2001; Amelung and Zhang, 2001). However, it is important to recall that MS and AA account for a significant proportion of both microbial biomass C and SOC and TN (Neidhardt et al., 1990; Senwo and Tabatabai, 1998; Friedel and Scheller, 2002; Martens et al., 2004). At present, little is known about the mechanisms that are responsible for the stabilization or destabilization of the biomarkers mentioned above, and the factors that govern these mechanisms are similarly challenging to understand.

Our aim in this study was to investigate the effects of long-term fertilization on C and N dynamics under different crop types, and the resulting impacts on SOC composition. This was done using an integrated approach in which chemical and physical parameters were monitored along with four separate biomarkers: PLFA, AS, MS, and AA. We hypothesized that high C inputs due to the long-term addition of organic and/or mineral fertilizer would enhance the stabilization of AA, MS and AS in the soil and increase the contribution of C from the selected biomarkers to the total SOC. We also expected the crop type to influence SOC composition by affecting OC decomposition rates, via plant-induced changes in the availability of labile OC and mineral nutrients, interactions with associated microbial communities, and changes in soil structure.

2. Materials and methods

2.1. Study site description and sampling

The experimental basis for the study was the Static Fertilization Experiment Bad Lauchstädt (SFEBL), Sachsen-Anhalt (Germany). This long-term experiment was initiated in 1902 in order to investigate the effects of organic and mineral fertilization on crop yield, crop quality, and soil fertility. The experiment's soil type is a Haplic Chernozem (FAO) (USDA: Mollisol) consisting of 21.0% clay, 67.8% silt and 11.2% sand. The mean annual temperature and precipitation at the site are 8.8 °C and 480 mm, respectively (Körschens, 2002). A comprehensive description of the experimental site and treatments has been given by Körschens (2002). The crop rotation is sugar beet, spring barley, potatoes, and winter wheat. On one of the six strips of the experiment, legumes have been included in the rotation since 1924, replacing sugar beet and spring barley every 7th and 8th year. Since 1970 alfalfa (*Medicago sativa* L.) has been the only legume species cultivated during this 7th and 8th year legume rotation. The level of mineral fertilization (NPK) depends on the crop that is being cultivated and the amount of farmyard manure (FYM) that is applied, and ranges from 60 to 170 kg ha⁻¹ yr⁻¹ N, 12 to 60 kg ha⁻¹ yr⁻¹ P, and 50 to 230 kg ha⁻¹ yr⁻¹ K. Farmyard manure is applied every second year during the cultivation of root crops (i.e. potatoes or sugar beet). When alfalfa is cultivated, it generally follows winter wheat and receives farmyard manure and only PK in the mineral fertilized treatments before seed drilling. The treatment effects on soil organic carbon and nitrogen as well as on crop yields are proved on the basis of repeated soil samplings (annually or biannually since the 70th of the last century) and annual measurements of crop yields and nutrient uptake by the crops and documented in a range

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