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Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland

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ABSTRACT

Soil microbial carbon use efficiency (CUE), defined as the ratio of organic C allocated to growth over organic C taken up, strongly affects soil carbon (C) cycling. Despite the importance of the microbial CUE for the terrestrial C cycle, very little is known about how it is affected by nutrient availability. Therefore, we studied microbial CUE and microbial biomass turnover time in soils of a long-term fertilization experiment in a temperate grassland comprising five treatments (control, PK, NK, NP, NPK). Microbial CUE and the turnover of microbial biomass were determined using a novel substrate-independent method based on incorporation of ^{18}O from labeled water into microbial DNA. Microbial respiration was 28–37% smaller in all three N treatments (NK, NP, and NPK) compared to the control, whereas the PK treatment did not affect microbial respiration. N-fertilization decreased microbial C uptake, while the microbial growth rate was not affected. Microbial CUE ranged between 0.31 and 0.45, and was 1.3- to 1.4-fold higher in the N-fertilized soils than in the control. The turnover time ranged between 80 and 113 days and was not significantly affected by fertilization. Net primary production (NPP) and the abundance of legumes differed strongly across the treatments, and the fungal:bacterial ratio was very low in all treatments. Structural equation modeling revealed that microbial CUE was exclusively controlled by N fertilization and that neither the abundance of legumes (as a proxy for the quality of the organic matter inputs) nor NPP (as a proxy for C inputs) had an effect on microbial CUE. Our results show that N fertilization did not only decrease microbial respiration, but also microbial C uptake, indicating that less C was intracellularly processed in the N fertilized soils. The reason for reduced C uptake and increased CUE in the N-fertilization treatments is likely an inhibition of oxidative enzymes involved in the degradation of aromatic compounds by N in combination with a reduced energy requirement for microbial N acquisition in the fertilized soils. In conclusion, the study shows that N availability can control soil C cycling by affecting microbial CUE, while plant community-mediated changes in organic matter inputs and P and K availability played no important role for C partitioning of the microbial community in this temperate grassland.

1. Introduction

Soil microorganisms strongly affect processing of organic carbon (C) in soil (Schmidt et al., 2011). The ratio of organic C allocated to growth over organic C taken up by the microbial community composition is termed microbial C use efficiency (CUE), and is an important synthetic representation of the microbial community metabolism (Manzoni et al., 2012; Sinsabaugh et al., 2013). Despite its importance for C cycling, it is still not well understood, which factors shape microbial CUE.

Theoretically, microbial CUE is restricted to 0.88 by

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thermodynamics (Gommers et al., 1988), meaning that not more than 88% of the C that microorganisms take up can be invested into growth, because microbes need at least 12% of it for energy production by respiration to maintain their biomass. Yet, microbial CUE in soil is thought to hardly ever reach the theoretical maximum, because environmental conditions usually request more than minimum energy investment for biomass maintenance and growth (Manzoni et al., 2012; Sinsabaugh et al., 2013). Among the factors that require additional investment of energy and C is an unfavorable stoichiometry of the available elements that force microorganisms to allocate larger proportions of C and energy into C or nutrient acquisition (Manzoni et al., 2012; Sinsabaugh et al., 2013). Moreover, nutrient limitation under conditions of high C:nutrient ratios can trigger overflow respiration, causing low microbial CUE. Hence, the stoichiometry of available elements in soil is assumed to affect microbial CUE.

The soil microbial biomass has a relatively well constrained C:N:P ratio, ranging between 60:7:1 and 42:6:1 on a global average (Cleveland and Liptzin, 2007; Xu et al., 2013). When feeding on substrate with a stoichiometry unfavorable for the maintenance of the microbial biomass stoichiometry, microorganisms have to allocate more energy into the acquisition of missing elements, for example by exoenzymes, in order to be able to maintain their biomass. Thus, microorganisms feeding on substrate with an unfavorable stoichiometry likely partition more C taken up to respiration than microorganisms feeding on substrate that matches their nutritional requirements, which leads to a decrease in CUE (Manzoni et al., 2012; Sinsabaugh et al., 2013). Manzoni et al. (2010) developed a model of C and N mineralization during litter decomposition, which calculates microbial CUE. For litter with an initial C:N ratio of 50–70, microbial CUE was predicted to be about half the maximum CUE achieved with low C:N ratio. In accordance with this conceptual approach, it has been shown empirically, that the bacterial growth efficiency was higher in fertilized agricultural soils than in non-fertilized forest soils (Lee and Schmidt, 2014). The availability of C and nutrients may also affect the turnover of microbial biomass (Cheng, 2009). However, little is known about the turnover time of the microbial biomass, which is in part due to a lack of suitable methods to access the microbial biomass turnover time in soil.

Besides stoichiometry of available elements, microbial CUE and the turnover time of soil microbial biomass might both be affected by concentration and quality of the organic C. The degradation of complex compounds requires many different extracellular enzymes whose production is energy and N consuming. For this reason the CUE of microbial communities decomposing complex compounds should be reduced (Ågren and Bosatta, 1987). Further, different substrates require different metabolic pathways for C assimilation, which results in a different respiration rate per unit C assimilated (Gommers et al., 1988; van Hees et al., 2005). Thus, the microbial CUE might be affected by organic C quality and thus indirectly also by the abundance of plant species, differing in organic C quality. Moreover, microbial community composition might affect CUE since some studies reported a higher CUE of fungi than of bacteria (Lipson et al., 2009; Keiblinger et al., 2010). However, other studies found no evidence for a difference between fungal and bacterial CUE (Six et al., 2006; Thiet et al., 2006).

The available methods for determining microbial CUE in terrestrial ecosystems have been criticized (see Sinsabaugh et al., 2013 and references therein). In most studies of terrestrial ecosystems, CUE has been estimated by the microbial incorporation and respiration of labeled C from specific ^{13}C-labeled substrates (Frey et al., 2013). However, this approach confounds microbial C use efficiency with the use efficiency of a specific substrate, as shown, for example, by the finding that different substrates lead to very different CUE estimates (Frey et al., 2013). To overcome these issues we used a novel approach based on the incorporation of ^{18}O from water into DNA during growth. Since genomic DNA is only synthesized when cells are dividing, the incorporation of the ^{18}O-label into DNA can be used to calculate the microbial growth rate (Schwartz, 2007; Blazewicz and Schwartz, 2011). Using a linear relationship between the microbial DNA and the microbial biomass C (Anderson and Martens, 2013; Spohn et al., 2016; Appendix 1), the increase in microbial biomass C over time can be calculated based on the increase in ^{18}O-DNA. By taking into account the microbial growth rate and the basal respiration rate, microbial CUE can be estimated. Additionally, this approach can also be used to assess the turnover time of microbial biomass in soil by dividing the microbial biomass by the microbial growth rate derived from the incorporation of ^{18}O into DNA.

We used the novel ^{18}O-based method to determine microbial CUE and the turnover of microbial biomass in a long-term fertilization experiment in a temperate grassland in Austria. We hypothesized, first, that nitrogen (N) fertilization increases microbial CUE because the addition of N allows microorganisms to allocate less C to N acquisition and more C to growth. Second, we hypothesized that phosphorus (P) and potassium (K) do not affect microbial CUE because they are not critical for microbial nutrition in temperate grasslands. Third, we hypothesized that the turnover time of microbial biomass is decreased by N fertilization, because it reduces the C costs for nutrient acquisition.

2. Material and methods

2.1. Field experiment and vegetation analyses

The fertilization experiment studied here is conducted in a grassland at the agricultural research station in Gumpenstein, Austria (49°29′37″N, 14°06′10″E). The site is located 710 m above s.l., the mean annual temperature is 7.0 °C, and the mean annual precipitation is 1000 mm. The soil type is a Cambisol formed from various crystalline rocks, and the soil has a silty loamy texture. The fertilization experiment has been set up in 1961, and has been constantly maintained ever since (Pötsch et al., 2013). The fertilization experiment comprises the control (no fertilizer application), a full NPK treatment with nitrogen (N), phosphorus (P), and potassium (K), and three treatments, in which one element is missing for a full NPK treatment (PK, NK, NP). N is added at a rate of 120 kg ha^{-1} yr^{-1} in all treatments, P is added at 120 kg P_2O_5 ha^{-1} yr^{-1}, and K is added at a rate of 240 kg K_2O ha^{-1} yr^{-1}. One third of the total N rate is applied at the beginning of the growing season, one third right after the first cut and one third after the second cut, whereas the full rate of P and K is applied in autumn. All treatments are three times replicated in a split-plot design with a plot size of each 3 × 5 m, and a space between the plots of 0.5 m. All plots are mown three times a year, and the mown biomass is collected and divided into legume and non-legume biomass. Based on the three aboveground biomass harvests, the annual net primary production (NPP) is calculated.

2.2. Sampling and sample preparation

Soil samples of three replicated plots of the above mentioned treatments were collected in May 2015 before the first cut of the year. Seven samples per plot were taken from the upper 7 cm of the soil in each plot by a soil auger with an inner diameter of 1.2 cm. The samples were taken on two diagonal transects in each plot excluding the outer 25 cm of the plot. All samples of one plot were pooled to obtain one mixed sample per plot. The samples were transferred to the lab at the University of Vienna on the day of the sampling and stored at 15 °C. On the following day, the soils were sieved (<2 mm)