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Relevance of substrate quality and nutrients for microbial C-turnover in top- and subsoil of a Dystric Cambisol

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ABSTRACT

The generally high apparent 14C age of soil organic carbon (SOC) in subsoils suggests a high stability against microbial degradation. However, the SOC decomposition in subsoils may also be limited by easily available substrates and nutrients, such as N and P. In topsoils, priming effects in response to substrate additions are largely determined by substrate quality which also differently affects the microbial community, while the knowledge for deep soil layers is very scarce. In order to gain further insight into processes controlling SOC decomposition in subsoils, we conducted a laboratory incubation experiment for 105 days to investigate the impact of different substrates and mineral N and P on SOC mineralization in a Dystric Cambisol sampled at 2–12, 35–65 and 135–165 cm. We studied the impacts of 14C-labeled citric, vanillic and palmitic acid and of N or P alone and in combination with two of the substrates on changes in SOC mineralization. The choice of substrates was based on their nominal oxidation state of carbon, which reflects the energy yield and the biogeochemical reactivity of a compound. Further, the impact of the treatments on the activity of six extracellular enzymes involved in C-, N-, P- and S-acquisition, of peroxidase and phenoloxidase and of the intracellular dehydrogenase was investigated to clarify if substrate qualities and nutrients differently affect the decomposition potential and nutrient demand of the microbial community. Our results show that microbial metabolism is limited by N in both subsoil layers, although the upper subsoil (35–65 cm) may have become P limited during the later stage of incubation. Even after 105 days, C-cycling enzymes and dehydrogenase activity were highly elevated in the lower subsoil (135–165 cm) in response to N additions which indicates a sustained higher decomposition potential and activity of the microbial community once the N-limitation is overcome. In the upper subsoil (35–65 cm), we found high amounts of labile C, indicating a high proportion of fast cycling SOC. Furthermore, all added substrates induced negative priming effects, while positive priming effects were only induced with N addition which was related to N limiting metabolism. The suppressed SOC mineralization likely occurred because the present microbial community was adapted to more labile carbon compounds, resulting in preferential substrate utilization. In the lower subsoil (135–165 cm), real positive priming was induced by vanillic acid and palmitic acid, while citric acid had no effect on SOC mineralization. This clearly reflects that substrate quality matters for inducing positive priming in this subsoil. Altogether, this study evidently shows that in consequence of altered substrate and N input deep SOC storage is destabilized in forest soils.

1. Introduction

In recent years, interest in subsoils has greatly gained attention since subsoil C seems to be more vulnerable to degradation than expected (e.g. Fierer et al., 2003). High apparent ^{14}C ages of soil organic carbon (SOC) in subsoils (Jenkinson et al., 2008; Kögel-Knabner et al., 2008) suggest that subsoil organic matter (SOM) is highly stable and thus cannot greatly contribute to the global C-cycle. However, several studies showed that subsoil C turnover can be strongly accelerated by environmental and management factors (Fierer et al., 2003; Fontaine et al., 2007; Salomé et al., 2010).

Subsoil C decomposition is assumed to be limited by easily available substrates (e.g. Heitkötter et al. (2017); Karhu et al. (2016)) and by the availability of N and P for microbial metabolism (Fierer et al., 2003; Tian et al., 2016). However, additions of different C-sources, such as cellulose (Fontaine et al., 2007), sugars (Jia et al., 2017; Karhu et al., 2016; Salomé et al., 2010; Zhang et al., 2015), organic acid (Heitkötter et al., 2017), synthetic root exudates (de Graaff et al., 2014), leaf litter (Wang et al., 2014) or root litter (Wordell-Dietrich et al., 2016), to subsoils induced positive priming (i.e. increased SOC decomposition (Kuzyakov et al., 2000)) or did not affect C turnover. As suggested by Fontaine et al. (2003), complex compounds should induce positive

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priming effects rather than simple substrates because only SOM-decomposing K-strategists can utilize these compounds for growth and thus are not in competition with r-strategists. For topsoils, substrate quality can differently affect SOC-mineralization as reported by Brant et al. (2006) and Jagadamma et al. (2014). While additions of sugars induced low positive priming effects, more complex substrates such as phenol, oxalate, cinnamic and stearic acid promoted higher losses of SOC (Brant et al., 2006; Jagadamma et al., 2014). However, to our knowledge it is still unclear how different substrate qualities affect C-turnover in subsoils.

Apart from substrate quality, the availability of nutrients can control the extent of priming in soils (Blagodatskaya et al., 2007; Chen et al., 2014; Nottingham et al., 2015). Priming effects tend to decrease through combined addition of N and C in topsoils as reviewed by Blagodatskaya and Kuzyakov (2008). According to the "N-mining theory" (Moorhead and Sinsabaugh, 2006), when nitrogen is not sufficiently available SOM has to be mineralized by microorganisms to mine N. Vice versa, SOC mineralization is predicted to decline with greater N availability and easily available substrates are preferred for utilization by microorganisms (i.e. preferential substrate utilization), resulting in weak or negative priming effects (i.e. suppressed SOC decomposition) (Blagodatskaya et al., 2007; Blagodatskaya and Kuzyakov, 2008). However, some studies also reported increased priming effects after adding C and N or N-containing easily available substrates, such as alanine, to soils (e.g. Hamer and Marschner, 2005; (Chen et al., 2014; Tian et al., 2016). Tian et al. (2016) observed that additions of glucose in combination with N reduced the priming effect by 45% in comparison to pure glucose addition in topsoil but increased the priming effect by 18% in subsoil. Furthermore, it was suggested that N in combination with sucrose increased the contribution of r-strategists to the priming effect, while pure sucrose induced higher contribution of K-strategists to the priming effect (Chen et al., 2014). Adding P in combination with C to soil had either no effect on the amount of mineralized SOC compared to pure C additions (Hartley et al., 2010; Nottingham et al., 2015) or the priming effect was reduced (Hartley et al., 2010). Higher priming in soils amended with only C substrate compared to weaker priming after P addition may be assigned to increasing SOC mineralization to meet the demand for P (Hartley et al., 2010). Consequently, adding C in combination with N or P has the potential to identify if primed SOM is mineralized by microorganisms to acquire N or/and P in soils, which has not been investigated for subsoils.

Linking enzyme activities with SOC decomposition after substrate addition revealed interesting insights into substrate limitation and enzymatic responses in subsoils in a former study, indicating that microorganisms in subsoil became highly P-limited after adding citric acid (Heitkötter et al., 2017). Soil microorganisms excrete hydrolytic and oxidative enzymes to catalyze the degradation of SOM, such as cellulose by β-glucosidase, hemicellulose by β-xylosidase, chitin by chitinase and lignin by phenol- and peroxidase (Burns et al., 2013; Gooday, 1990; Pérez et al., 2002; Sinsabaugh, 2010), thus reflecting the decomposition potential of the microbial community. Further, enzyme activities involved in the acquisition of nutrients, such as phosphatase mineralizing P from nucleic acids, phospholipids and other ester phosphates (Olander and Vitousek, 2000; Sinsabaugh et al., 2008), sulfatase hydrolyzing ester sulfates (Whalen and Warman, 1996) and aminopeptidase and chitinase degrading organic N compounds (Sinsabaugh et al., 2008), reflect the demand for nutrients. Intracellular dehydrogenase activity can be used as a proxy for general oxidative metabolism of microorganisms and are only found in living systems, indicating active microbial biomass (Burns et al., 2013; Kandeler, 2007; Trevors, 1984). Thus, intracellular dehydrogenase is a good indicator for real positive priming (i.e. microbial growth followed by increased SOC decomposition (Blagodatskaya and Kuzyakov, 2008)).

Citric acid, vanillic acid and palmitic acid were selected as substrates for this experiment. The choice was based on the nominal oxidation state of carbon (NOSC) of the substrate. The NOSC can be related to the Gibbs energies of the oxidation half reaction and thus reflects the energy yield and the biogeochemical reactivity of a compound (LaRowe and van Cappellen, 2011). This parameter was already successfully used in several ecological studies to describe organic matter and substrate qualities (Nunan et al., 2015; Riedel et al., 2012; Roth et al., 2015). The NOSC of used substrates in our incubation study was calculated according to LaRowe and van Cappellen (2011). The NOSC of citric acid, vanillic acid and palmitic acid is + 1.00, 0.00 and − 1.75, respectively, indicating that C in citric acid is partly oxidized on average, while C in palmitic acid is reduced. This reflects that the energy yield of C increases following: citric acid < vanillic acid < palmitic acid per carbon atom, since fewer electrons are available for transfer per carbon atom with increasing oxidation state of the substrate. Consequently, a higher NOSC also reflects a lower activation energy investment for microorganisms.

Our study aimed at influencing SOC mineralization and soil microbial community composition by adding the different substrates in combination with N or P to different soil depths to investigate:

i) How substrates of decreasing qualities affect C-turnover in subsoils
ii) How N or P determine the priming effect in subsoils and
iii) How substrate qualities and nutrients differently alter subsoil's microbial activity, nutrient demand and the decomposition potential of the microbial community in the long term as estimated by enzymatic activities.

2. Material and methods

2.1. Soil sampling

In January 2016, a Dystric Cambisol (IUSS Working Group WRB, 2014) was sampled in the Grinderwald (52° 34′ 22″ N, 9° 18′ 51″ E), Lower Saxony, Germany, in 2–12 cm, 35–65 cm and 135–165 cm depth. The study site is under a beech (*Fagus sylvatica* L.) forest stand and is characterized by glaciofluviatile sandy deposits from the Saale glaciation (Bundesanstalt für Bodenforschung, 1973). After sampling, the soils were sieved (< 2 mm) and subsequently stored for two weeks at 4 °C before the incubation experiment started.

2.2. Experimental design

A laboratory incubation study was conducted for 15 weeks at 20 °C. For each depth, 10 treatments with three replicates were set: I) soil only, II) N addition, III) P addition, IV) citric acid, V) citric acid + N, VI) citric acid + P, VII) vanillic acid, VIII) vanillic acid + N, IX) vanillic acid + P, X) palmitic acid. Carbon was added at a rate of 13.3 μg C mg^{-1} SOC (Hamer and Marschner, 2005), N as NH_4NO_3 at a rate of 110 mg N kg^{-1} dry soil (Chen et al., 2014) and P as Ca $(H_2PO_4)_2{\cdot}H_2O$ (triple superphosphate) at a rate of 11 mg P kg^{-1} dry soil.

To differentiate between mineralized SOC and substrate C we used ^{14}C labeled citric acid [1,5-^{14}C] and palmitic acid [$^{14}C(U)$] purchased from Perkin Elmer, Waltham, USA, and vanillic acid [1-^{14}C] purchased from Biotrend, Cologne, Germany. ^{14}C labeled substrates were mixed with respective unlabeled substrates to obtain the required concentrations and desired radioactivity of 20 kBq under the assumption that ^{14}C- and unlabeled substrates are degraded in the same way. The added specific activity was 1.3, 6.7 and 50.1 Bq μg^{-1} substrate C in 2–12, 35–65 and 135–165 cm depth, respectively. Since vanillic acid and palmitic acid are insoluble in H_2O, we used ethanol (EtOH) for vanillic acid and hot ethanol for palmitic acid as solvent.

For the incubation, 50 g and 75 g (dry basis) top- and subsoils, respectively, were weighed into 100 ml polyethylene flasks and wetted to 40% water holding capacity. Then, the samples were placed in airtight vessels and pre-incubated for one week at 20 °C. After pre-incubation, top- and subsoil samples received respective compound solutions and H_2O to obtain 50% of water holding capacity. Since vanillic acid and palmitic acid were dissolved in EtOH, all treatments received 2.36 μg C-ETOH μg^{-1} substrate C (related to depth-specific substrate additions) with equivalent additions to the controls to exclude EtOH effects between treatments. The samples were incubated for 15 weeks at 20 °C in a respirometer (prw electronics, Berlin, Germany).