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Patterns of nitrogen and citric acid induced changes in C-turnover and enzyme activities are different in topsoil and subsoils of a sandy Cambisol

Julian Heitkötter *, Jana Niebuhr, Stefanie Heinze, Bernd Marschner

Department of Geography, Chair for Soil Science and Soil Ecology, Ruhr-Universität Bochum, Universitätsstr, 150, 44801 Bochum, Germany

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ABSTRACT

Studies on factors controlling C-stability in subsoils are very scarce. Recent results suggest a lack of labile C substrates and N limitations in subsoils as a reason for suppressed C-turnover. The catalytic activity of soil enzymes plays an important role for the decomposition of organic matter in soils and can be a powerful tool to shed further light on substrate and N-limitation as a hypothesized controlling mechanism for C-stability in subsoils. Therefore, we studied the impacts of ^{14}C-labelled citric acid and of NH_4NO_3 on changes in soil organic carbon (SOC)-mineralization and enzyme activities of dehydrogenase and 9 extracellular enzymes involved in C-, N-, P- and S-cycle. For this approach, we sampled a sandy Cambisol at three different depths (2–12, 35–65 and 135–165 cm) and conducted a laboratory incubation experiment for 63 days at 10 °C. N-addition reduced SOC-mineralization in the topsoil layer by 43%, while no N-effect was observed in both subsoil layers. In the topsoil samples, dehydrogenase-activity also decreased after the incubation with N additions. Further, the activity of extracellular enzymes involved in P- and N-cycling was differently affected in top- and subsoils, indicating that microorganisms in different soil depths have different demands for N or P after adding inorganic N. Additions of citric acid increased SOC mineralization by about 1.9- and 2.2-fold in the upper (35–65 cm) and lower subsoil (135–165 cm) samples, but only by about 32% in the topsoil samples (2-12 cm). The observed priming effect in the topsoil samples was not accompanied by an increased enzyme activity which indicates "apparent priming". In contrast, priming effects in both subsoil layers were rated as "real priming" indicated by increased enzyme activities and continuously higher SOC-mineralization rates throughout the incubation compared to the controls.

1. Introduction

Subsoils (below 30 cm) often contain >50% of soil organic carbon (SOC) stocks (Batjes, 1996; Jobbágy and Jackson, 2000) and thus can be considered as an important sink of CO_2. Radiocarbon ages of SOC are found to increase with depth (Fontaine et al., 2007; Jenkinson et al., 2008; Kögel-Knabner et al., 2008), indicating higher residence time of C in subsoils. It remains still unclear which mechanisms control C-turnover in subsoils, which is crucial to assess the role of this C-pool in the global C-cycle.

Recent results suggest a lack of fresh C supply in subsoils (e.g. Fontaine et al., 2007; Wang et al., 2014), resulting in depressed SOC-mineralization. This means that microbial activity is limited by lack of easily degradable substrates, such as sugars, organic acids or amino acids (Hamer and Marschner, 2005). Availability and inputs of easily degradable substrates likely decrease with soil depths, since inputs from rhizodeposition, microbial, root and animal cell decomposition or dissolved organic matter are also expected to decline with depth (Dakora and Phillips, 2002; Kuzyakov, 2010; Neff and Asner, 2001).

Easily degradable substrates can be rapidly incorporated and used by soil microorganisms for growth and enzyme production inducing co-metabolic decomposition of soil organic matter, which is known as "real priming effect" (Blagodatskaya and Kuzyakov, 2008; Kuzyakov et al., 2000). Experiments with additions of either cellulose (Fontaine et al., 2007), sugars (Karhu et al., 2016; Salomé et al., 2010; Zhang et al., 2015), synthetic root exudates (Graaff et al., 2014) or leaf litter (Wang et al., 2014) in subsoils showed no consistent impact on SOC-mineralization. For instance, Fontaine et al. (2007) and Zhang et al. (2015) showed that carbon supply increased the mineralization of SOC in subsoil. In detail, about 2600 year old carbon was mineralized with additions of cellulose, while microbes in the treatment without C-addition mostly decomposed recent organic matter (Fontaine et al., 2007). Zhang et al. (2015) detected greater changes in labile and recalcitrant structures of organic matter during incubation with glucose additions using ^{13}C NMR. These results provide evidence that microbial activity in subsoils is limited by fresh C-supply. On the other hand, some studies showed that C-additions did not induce positive priming in subsoils (Salomé et al., 2010) or the priming effect was very small in subsoils

* Corresponding author.
E-mail address: Julian.Heitkoetter@rub.de (J. Heitkötter).

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compared to topsoils (Graaff et al., 2014). However, in most studies only one subsoil layer is considered, making it difficult to transfer the results from one depth to the whole subsoil.

N-limitation in subsoil is another conceivable factor promoting C-stability through insufficient N-supply for the synthesis of enzymes, which are necessary to mineralize SOC (Fierer et al., 2003). Atmospheric nitrogen deposition to terrestrial ecosystems is predicted to increase in the future in consequence of human activity (Galloway et al., 2003), a factor, that was shown to enhance SOC-mineralization with soil depth (Fierer et al., 2003). Although nitrogen is an important parameter influencing organic matter turnover, studies on N-limitation in subsoils are very scarce.

The catalytic activity of soil enzymes plays an important role for the decomposition of organic matter in soils, thus providing microorganisms and plants with nutrients and energy. Enzymes from different nutrient cycles can give general information about microbial activity but further reflect the needs and the decomposition potential of the microorganisms in soil (Burns et al., 2013; Caldwell, 2005). Changes in enzyme activities after substrate or nutrient additions are well documented for topsoils (Blagodatskaya and Kuzyakov, 2008; Chen et al., 2014; Olander and Vitousek, 2000) but no study is known for subsoils. Thus, investigating enzyme activities in combination with C– or N-additions will shed further light on substrate and N-limitation in subsoils as an assumed controlling mechanism for C-stability.

The aim of this study was to evaluate if SOC-mineralization in the subsoils (35–65 cm and 135–165 cm) of a sandy Cambisol under European beech (*Fagus sylvatica L.*) can be enhanced with citric acid- or N-additions. Further, the analysis of enzyme-activities from C-, N-, S- and P-cycle after substrate- and N-additions should provide deeper knowledge of the decomposition potential and needs of microorganisms in subsoils. Citric acid was chosen as a model substance due to high responses on respiration in subsoils in previous experiments (Niebuhr et al., unpublished) and based on its natural occurrence in root exudates (Dakora and Phillips, 2002).

We hypothesized that

i) citric acid and N increase SOC-mineralization in both subsoils, and
ii) will stimulate enzyme production in both subsoil layers, changing the decomposition potential of the microbial community.

2. Material and methods

2.1. Soil sampling and preparation

Soil samples were taken in the Grinderwald (52°34′22″N, 9°18′51″E), located 40 km north-west of Hannover, Lower Saxony, Germany. Three pits within a distance of about 50 m were sampled in 2–12 cm, 35–65 cm and 135–165 cm depth. After sampling, the soils were sieved (<2 mm) and stored at 4 °C. The investigated soil, a Dystric Cambisol (IUSS Working Group WRB, 2014), developed on glaciofluviatile sandy deposits from the Saale glaciation (Bundesanstalt für Bodenforschung, 1973). The beech (*Fagus sylvatica L.*) forest stand was established in 1916 on the study site.

2.2. Physico-chemical soil analysis

The pH of soil samples were measured in 0.01 M $CaCl_2$ (1:2.5, w/v) after 1 h of equilibration with a glass electrode. Carbon and nitrogen were analyzed by dry combustion using a vario El Cube elemental analyzer (Elementar Analysesysteme GmbH, Hanau, Germany). Water-extractable organic carbon (WEOC) was obtained by shaking 10 g soil with 40 ml 0.05 M K_2SO_4 for 30 min. Subsequently, the soil suspensions were vacuum filtered (0.45 μm). Total organic carbon in the solutions was determined using a Dimatoc 2000 (DIMATEC Analysentechnik GmbH, Essen, Germany). Soil texture was analyzed after SOC removal with H_2O_2 by laser diffraction using an Analysette 22 MicroTec plus with a wet dispersion unit (Fritsch GmbH, Idar-Oberstein, Germany).

2.3. Experimental design and incubation procedure

A two factorial (soil depth, substrate/N-addition) laboratory incubation study was conducted for 9 weeks at 10 °C, which is close to the mean soil temperature measured in the Grinderwald. For each pit and depth, three treatments with three experimental replicates were set: Control, Soil + NH_4NO_3 (110 mg N kg^{-1} dry soil (Chen et al., 2014)) and Soil + Citric acid [1,5-^{14}C] (Perkin Elmer, Waltham, USA) at a rate of 13.3 μg C mg^{-1} SOC (Hamer and Marschner, 2005). For the incubation, 30 g and 50 g (dry basis) homogenized top- and subsoils, respectively, were weighed into 100 ml polyethylene flasks and wetted to 40% water holding capacity. Subsequently, the samples were placed in airtight vessels and pre-incubated for one week at 10 °C. ^{14}C-labelled citric acid was mixed with unlabeled citric acid and diluted in H_2O to obtain the required concentrations under the assumption that ^{14}C- and unlabeled citric acid are degraded in the same way. The added radioactivity was 8 kBq for each citric acid treatment. After pre-incubation, top- and subsoil samples received 1.5 ml and 2.5 ml, respectively, citric acid or NH_4NO_3 solution. Same volumes were given to the controls as H_2O. The samples were incubated for 9 weeks at 10 °C in a temperature controlled respirometer (prw electronics, Berlin, Germany). CO_2 was automatically measured every 4 h by determining changes in electrical conductivity in 10 ml 0.08 M and 0.06 M KOH for top- and subsoils, respectively, placed inside the vessels. The amount of ^{14}C–citric acid derived CO_2 was determined on day 2, 7, 9, 11, 14, 21, 28, 35, 42, 49, 56 and 63 by using liquid scintillation counting. A 1 ml subsample of KOH was mixed with 6 ml scintillation cocktail (Ultima Gold, Perkin Elmer, Waltham, USA) and measured with a Tri-Carb 2800TR (Perkin Elmer, Waltham, USA). After sampling, the KOH was renewed.

2.4. Analysis of ^{14}C in microbial biomass and in water-extractable organic carbon

Chloroform fumigation-extraction method (Vance et al., 1987) was used to determine the ^{14}C-activity in microbial biomass (C_{mic}) in samples amended with ^{14}C-labelled citric acid. 10 g moist soil was fumigated for 24 h at room temperature with chloroform. Fumigated and non-fumigated samples were shaken for 30 min with 40 ml 0.05 M K_2SO_4 and then filtered. 2 ml of the filtrates were mixed with 12 ml scintillation cocktail (Ultima Gold, Perkin Elmer, Waltham, USA) and the ^{14}C–activity was measured by liquid scintillation counting. Water-extractable ^{14}C was considered as the radioactivity in the extracts of non-fumigated samples, while ^{14}C in microbial biomass was calculated by subtracting the ^{14}C–activity of non-fumigated samples from respective fumigated samples using a kEC value of 0.45 (Wu et al., 1990). ^{14}C in C_{mic} and WEOC is presented as % of initial ^{14}C-input.

2.5. Extracellular enzymes

Extracellular enzyme activities involved in the C-, N-, P- and S-cycle were analyzed using a fluorescence multiplate assay according to Marx et al. (2001). Following methylumbelliferyl-labelled substrates were used to determine enzymes involved in the C-cycle: 4-methylumbelliferyl-α-D-glucoside (α-glucosidase (α-glu)), 4-methylumbelliferyl-β-D-xylopyranoside (β-xylosidase (β-xyl)), 4-methylumbelliferyl-β-D-glucoside (β-glucosidase (β-glu)), and 4-methylumbelliferyl-β-D-cellobioside (β-cellobiosidase (β-cello)). The enzymes involved in the P-cycle were measured by 4-methylumbelliferyl phosphate disodium salt (acid phosphatase (pho)) and the S-cycle involved enzymes were determined with 4-methylumbelliferyl sulfate potassium salt (sulfatase (sul)). Enzymes involved in the N-cycle were measured using following amido-methylcoumarin (AMC)-labelled substrates: L-tyrosine-7-amido-4-