



## Controlling factors for the stability of subsoil carbon in a Dystric Cambisol



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### ARTICLE INFO

#### Article history:

Received 18 December 2015

Received in revised form 18 August 2016

Accepted 29 August 2016

Available online 6 September 2016

#### Keywords:

Subsoil

<sup>13</sup>C

Carbon dynamics

Substrate limitation

Temperature sensitivity

### ABSTRACT

Subsoils store >50 % of the total global soil organic carbon (SOC), and low SOC content and high mean residence times indicate that subsoils have the potential to sequester additional C on the long-term. Nevertheless, the mechanisms controlling the turnover of SOC in subsoils are poorly understood. The aim of this study was to assess the impact of temperature and substrate limitation on subsoil SOC turnover and evaluate the stability of additional C inputs in subsoils.

In a 63-day microcosm incubation experiment, CO<sub>2</sub> production of undisturbed soil samples from topsoil and two subsoil depth increments was measured at two different temperatures (10 °C and 20 °C). Additionally, <sup>13</sup>C labeled root litter was added to the different samples and measurements of the isotopic signature of the respired CO<sub>2</sub> allowed a differentiation between SOC mineralization and root mineralization. The CO<sub>2</sub> production per unit soil mass was lower in deep subsoil than in the topsoil, but the CO<sub>2</sub> production per unit SOC (specific mineralization) was three times higher in the deepest subsoil than in topsoil. This depth gradient of specific mineralization in undisturbed samples indicates that deep subsoil contained relatively more labile SOC than the topsoil. The temperature sensitivity of SOC mineralization expressed as Q<sub>10-q</sub> decreased from around 3 to around 1 with increasing soil depth. In contrast, the mineralization of the added root material was solely determined by the recalcitrance of the added roots as indicated by a similar Q<sub>10-q</sub> through all three soil depths.

Contrary to the SOC mineralization of undisturbed samples, significantly more added root litter was mineralized in the samples from the upper horizons than in the deepest subsoil samples, revealing a non-linear relationship between mineralization of added C and the SOC content. Thus, the distance between substrate units, as indicated by the SOC content, may be key factor for subsoil SOC dynamics. Moreover, root addition caused no positive priming effects in subsoil horizons indicating that enhanced C inputs to the subsoil can increase the SOC content and tap the unused C storage potential of subsoils.

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### 1. Introduction

With an estimated global carbon stock of 1500–2000 Pg in the first meter, soils contain the largest terrestrial organic carbon (C) pool. For surface soils, the mechanisms controlling soil organic carbon (SOC) turnover have been thoroughly investigated (Flessa et al., 2008; Sollins et al., 1996). Studies on subsoil C dynamics are scarce, although >50 % of SOC stocks are stored in deeper soil horizons (Batjes, 1996; Jobbágy and Jackson, 2000). In contrast to topsoils, subsoils are characterized by low C content and high radiocarbon ages (Rethemeyer et al., 2005; Torn et al., 1997), indicating high C stability. However, little is known about the mechanisms controlling SOC turnover in subsoils. The transferability of results obtained for surface soils to deeper soil horizons is limited because SOC in deeper soil layers is exposed to different environmental conditions (e.g., more constant temperature and

moisture regime, lower O<sub>2</sub> availability and higher CO<sub>2</sub> concentration), which may influence the turnover of SOC (Rumpel and Kögel-Knabner, 2011).

Carbon inputs in subsoils by roots and dissolved organic matter differ in quality and quantity from C inputs in topsoils (Kaiser and Guggenberger, 2000; Rasse et al., 2005). Thus, SOC stability in subsoils is highly, likely due to selective preservation of substrate with lower quality (Rumpel, 2004). In addition, it has been found that the stabilization of SOC in subsoils is controlled by the availability of fresh substrate (Fontaine et al., 2007; Marschner et al., 2008). The input of an easily available energy source may trigger the decomposition of old SOC which is known as priming. Therefore, additional C inputs in deeper soil horizons may lead to a destabilization of native SOC instead of C accumulation. However, only a few studies exist on the priming effects in subsoils and their findings are contradictory (Fontaine et al., 2007; Salomé et al., 2010). Thus, the effect of additional C inputs to subsoils on the mineralization of native SOC remains unclear. However, subsoils may have the potential to store additional C (Lorenz and Lal, 2005; Rumpel, 2014).

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Next to the quality and quantity of C inputs, environmental factors such as temperature influence the SOC decomposition (Kirschbaum, 1995). Similar or even higher response of SOC decomposition to temperature changes were found for subsoil SOC compared to topsoil SOC (for a review, see von Lütow and Kögel-Knabner, 2009). According to the Arrhenius equation, reactants with higher activation energies (low reactive and more recalcitrant SOC) have higher temperature sensitivities compared to labile and less stabilized SOC (Davidson and Janssens, 2006). Thus, it has been assumed that the difference in temperature sensitivity of SOC mineralization between subsoil and topsoil was due to the increase in recalcitrance of SOC with increasing soil depth. However, recent findings indicate that SOC mineralization in subsoils has a lower temperature sensitivity than SOC mineralization in topsoils and that the temperature sensitivity is determined by substrate availability (Davidson and Janssens, 2006; Gillabel et al., 2010). Consequently, if the temperature sensitivity in subsoils is controlled by substrate availability, additional C inputs may increase the temperature sensitivity of SOC mineralization in subsoils. However, there is a lack of experimental evidence for such effects.

In this study we investigated the influence of temperature and substrate limitation on the SOC mineralization in topsoil and subsoil samples for a sandy forest soil. Therefore, we incubated undisturbed samples and disturbed samples with and without additional C ( $^{13}\text{C}$  labeled roots) at 10 °C and 20 °C. The  $\text{CO}_2$  production of undisturbed samples will reveal the SOC stability in topsoil and subsoil under the influence of possible limitations due to low SOC content, spatial segregation and SOC protection due to aggregation or mineral-association. The addition of  $^{13}\text{C}$  labeled roots allows to differentiate between  $\text{CO}_2$  production from the added roots and SOC mineralization. This in turn will provide on the one hand, information of the stability of additional C inputs in topsoil and subsoil. And on the other hand, the comparison of the SOC mineralization with the control samples will reveal priming effects on the native SOC mineralization in topsoil and subsoil. In addition, the two different incubation temperatures will show the temperature response of SOC mineralization.

We hypothesized (i) that SOC will be more stable in subsoils than in topsoils, (ii) that temperature sensitivity of SOC mineralization increases with soil depth, (iii) that additional C substrate will be mineralized faster in topsoils than in subsoils and (iv) that the C addition to subsoils will enhance the mineralization of native SOC because of priming effects.

## 2. Materials and methods

### 2.1. Site description

Soil samples were taken in the Grödenwald, 35 km north-west of Hanover, Germany (52°34'22"N, 9°18'49"E). The vegetation at the site is dominated by common beech (*Fagus sylvatica*) established in the forest in 1916, and the soil is characterized as a Dystric Cambisol (IUSS Working Group WRB, 2014) developed on Pleistocene fluvial and aeolian sandy deposits from the Saale-glaciation. The site is located around 100 m above sea level with a mean annual temperature and mean annual precipitation of 9.7 °C and 762 mm (1981–2010), respectively.

### 2.2. Soil sampling and sample preparation

Undisturbed and disturbed soil samples were taken from three different soil depths, 2–12 cm (in the following referred to as topsoil), 30–60 cm (subsoil<sub>30</sub>) and 130–160 cm (subsoil<sub>130</sub>). The soil samples were collected in September 2013 with four field replicates. To account for the low SOC content and the heterogeneous distribution of SOC, especially in the subsoil, large soil cores were taken using a soil corer with cylinder inlets (height of 18 cm for topsoils and 40 cm for subsoils, diameter of 14.4 cm). These cores represented the undisturbed samples.

The disturbed soil material was obtained from the same soil depth increments. Samples were stored at 6 °C until start of the incubation. The disturbed soil sample was sieved through 2 mm, air dried and stored until use. Table 1 contains the general soil parameters of the topsoil and subsoil samples.

### 2.3. Experimental design

The  $\text{CO}_2$  production of soil samples from topsoil, subsoil<sub>30</sub> and subsoil<sub>130</sub> were measured in a 63-day incubation study. The hypotheses were tested in a 3 × 2 × 3 factorial design, whereby three different depths were incubated at two temperatures (10 °C and 20 °C) with the following three treatments.

- i.) **Undisturbed:** Undisturbed soil samples
- ii.) **Root addition:** Disturbed soil samples with addition of  $^{13}\text{C}$ -labeled root litter
- iii.) **Control:** Disturbed soil without addition of  $^{13}\text{C}$ -labeled root litter.

For the incubation experiment the samples were filled into plastic cylinders with a diameter of 14.4 cm and a height of 18 cm for topsoil samples and 40 cm for subsoil samples. The cylinder was closed with lids on the top and the bottom (in the following referred to as microcosm), top lids had an air inlet and outlet port. The microcosms of the root addition treatment were filled with 2.4 kg (topsoil) to 7.8 kg (subsoil) dry matter homogenized and sieved soil and mixed with 3.8 g of  $^{13}\text{C}$ -labeled and ground ash roots ( $\delta^{13}\text{C}$  of 151 ‰) at a bulk density of 1.4 (topsoil) to 1.6 (subsoil) g cm<sup>-3</sup>, corresponding to the soil samples of the undisturbed treatment. The labeled roots originated from young trees grown in a greenhouse under a  $^{13}\text{CO}_2$ -enriched atmosphere ( $\delta^{13}\text{C}$  300 ‰) for two years and thus are homogeneously labeled. Each microcosm had a headspace volume of around 1 l. Water was added to adjust 60 % of the water holding capacity. The control microcosms were prepared in the same way but without the admixture of  $^{13}\text{C}$  labeled roots. The soil columns of the undisturbed treatment were placed on a suction plate and were irrigated until saturation was reached. Thereafter, water was removed through the suction plate until 60 % of water holding capacity was reached. A leak test was performed for each microcosm by slightly increasing the air pressure in the microcosms. During the incubation all microcosms were flushed with  $\text{CO}_2$  free synthetic air (20 %  $\text{O}_2$  and 80 %  $\text{N}_2$ ) using a constant flow rate of 10 ml min<sup>-1</sup>. The C mineralization was determined by measuring the  $\text{CO}_2$  production in the microcosm headspace on 14 sampling days (1, 2, 3, 4, 5, 7, 9, 11, 14, 17, 20, 25, 30, 63). At each sampling day, the gas flow to the microcosms was stopped and the headspace was sampled twice according to the closed chamber principle. It was not possible to determine  $\text{CO}_2$  production in flow through mode due to the extremely low C content of the subsoil samples. The gas samples were taken with a syringe at the top lid of the microcosm and filled into evacuated vials (20 ml). Sampling was performed twice per sampling day in order to determine the  $\text{CO}_2$  production via the  $\text{CO}_2$  accumulation in the headspace. For the control and root addition treatment, an additional gas sample was taken during the second sampling for stable isotope analysis and filled into evacuated 12-ml gas vials (Labco Exetainer, Labco Limited, Lampeter, UK). The gas flow through the microcosms was restored after the sampling.

### 2.4. Gas and soil analysis

The  $\text{CO}_2$  concentration was analyzed by gas chromatography (Shimadzu GC-2014, Kyoto, Japan) modified according to Loftfield et al. (1997) and Agilent 7890A (GC, Agilent Technologies, Santa Clara,

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