Stability of soil organic carbon in the subsoil

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ABSTRACT

Soils contain the largest carbon (C) pool of the global terrestrial carbon cycle and can act as sources or sinks for CO_2. Although, more than 50 % of the global soil organic carbon (SOC) stocks are stored in subsoils (> 30 cm deep) and the high mean residence time of subsoil organic carbon (OC) indicates that SOC in subsoils is more stable than in topsoils (< 30 cm deep), there is a lack of knowledge on the mechanisms controlling the turnover of SOC in subsoils. In addition, the decreasing SOC content with soil depth also indicates that subsoils may have the potential to sequester additional C and therefore contribute to climate mitigation. Thus, understanding the C dynamics in subsoils are essential to predict the vulnerability of SOC stocks to land-use or climate change and to assess the C sequestration potential of the world soils. The objectives of this thesis were to quantify *in situ* CO_2 production and to identify the sources for CO_2 production in the subsoil, in a two-year field monitoring (Article 1). Further, the temperature sensitivity of organic matter decomposition in the subsoil and the influence of substrate limitation on SOC mineralization were investigated in a laboratory incubation experiment (Article 2) and the stability of additional C inputs into the subsoil was examined in a laboratory and a field incubation (Article 2 and 3). Lastly, the influence of different environmental conditions along a soil profile on the organic carbon decomposition were examined during a field incubation (Article 3).

Field monitoring in a Dystric Cambisol in a Northern German beech forest showed that the annual CO_2 production in the subsoil accounted for 10 % of total soil respiration. Further, isotopic data suggest that CO_2 in the subsoil mainly originated from root respiration and the mineralization in the rhizosphere. Hence, the subsoil contains a large labile C pool, which contributes to the annual soil respiration, despite the high ^{14}C age of the bulk SOC. The laboratory incubation pointed out that the temperature sensitivity of SOC decomposition decreases with soil depth, which implies that SOC recalcitrance is not the main stabilization mechanisms in the subsoil. In addition, the decreasing temperature response of soil respiration with depth indicates that losses of subsoil SOC due to climate change might be even lower than previously assumed. The addition of root litter into the topsoil and the subsoil did not enhanced the mineralization of native SOC. Moreover, root litter was more stable in the subsoil environment as in the topsoil environment, which can be explained by the low and the heterogeneous C inputs into the subsoil. The higher C stability in the subsoil underlines the large C-sequestration potential of the subsoil and climate change mitigation research should also include the deeper soil horizons.

ZUSAMMENFASSUNG

Der größte Kohlenstoffvorrat des globalen terrestrischen Kohlenstoffkreislaufes ist in Böden gespeichert. Mehr als 50 % diese Kohlenstoffvorrates ist in Unterböden (> 30 cm Tiefe) gespeichert, jedoch ist wenig über die Mechanismen, welche die Kohlenstoffdynamik in Unterböden steuern, bekannt. Ungeachtet der enormen Mengen an gespeichertem Kohlenstoff in tieferen Bodenhorizonten deutet die hohe mittlere Verweilzeit des organischen Kohlenstoffs und die geringe Konzentration darauf hin, dass Unterböden zusätzlichen Kohlenstoff langfristig speichern und daher relevant für den Klimaschutz sein können. Ein umfassendes Verständnis der Kohlenstoffdynamik in Unterböden ist somit essentiell, um zum einen das Risiko der Freisetzung von gespeichertem Kohlenstoff durch Landnutzungs- und Klimawandel abzuschätzen und zum anderen das Speicherpotential bewerten zu können. Im Rahmen dieser Dissertation wurde die CO_2-Produktion im Unterboden quantifiziert und Quellen der CO_2-Produltion identifiziert (Artikel 1). Des Weiteren wurde in Laborversuchen die Temperatursensitivität und der Einfluss der Substratverfügbarkeit auf die Kohlenstoffmineralisation im Unterboden bestimmt (Artikel 2). Der Einfluss verschiedener Umweltbedingungen in Ober- und Unterböden auf die Stabilität von zusätzlichen Kohlenstoffeinträgen wurde in Labor und Feldversuchen untersucht (Artikel 2 und 3).

Kontinuierlichen CO_2 Messungen in einer podsolierten Braunerde in einem norddeutschen Buchenwald zeigte, dass die jährliche CO_2 Produktion im Unterboden 10 % der gesamten Bodenatmung ausmacht. Die Isotopendaten der Bodenluft deuten darauf hin, dass das CO_2 im Unterboden hauptsächlich aus jungen Kohlenstoffquellen wie Wurzelatmung und mikrobieller Atmung in der Rhizosphäre stammt. Folglich besitzen Unterböden trotz des hohen ^{14}C-Alters einen labilen Kohlenstoffpool, welcher signifikant zur jährlichen Bodenatmung beiträgt. Die Temperatursensitivität der Mineralisation nahm mit der Tiefe ab. Dies deutet daraufhin, dass die Änderungen der Kohlenstoffmineralisation im Unterboden aufgrund des Klimawandels geringer ausfallen könnten als bisher angenommen. Die Erhöhung der Substratverfügbarkeit durch die Zugabe von Wurzelstreu hatte keinen Einfluss auf die Mineralisation der vorhanden organischen Substanz. Die geringen und heterogenen Kohlenstoffeinträge in den Unterboden mit dem Sickerwasser, führten zu einem geringeren Abbau der eingebrachten Wurzeln im Unterboden. Die höhere Stabilität von organischer Substanz mit zunehmender Tiefe unterstreicht das enorme Speicherpotential von Kohlenstoff im Unterboden.

KEYWORDS

Soil organic carbon, subsoil, C dynamics, substrate limitation, environmental constraints, ^{13}C

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ABBREVIATIONS

C	Carbon
CO_2	Carbon dioxide
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
MAOM	Mineral-associated organic matter
OC	Organic carbon
OM	Organic matter
SOC	Soil organic carbon
SOM	Soil organic matter
$\delta^{13}C$	Isotopic ratio of stable isotopes ^{13}C:^{12}C in parts per thousand [‰]

CHAPTER 1 INTRODUCTION

1.1 Subsoils in the carbon cycle

In the global carbon (C) cycle, soil constitutes the largest active terrestrial C reservoir, storing more C than the atmosphere and the biomass combined. The C pool in the first two meters of world soils is estimated with up to 2400 Gt of C (Batjes, 1996, 2014). About 3-5 % of the soil organic carbon is highly dynamic and the C input and the C output of soils represent major fluxes in the global C cycle. The carbon dioxide (CO_2) efflux from soils is about 10-15 larger than anthropogenic CO_2 emissions (Ciais et al., 2013; Schlesinger and Andrews, 2000). The CO_2 emissions of soils originates from the autotrophic respiration (plant roots and mycorrhizae) and the heterotrophic respiration from the decomposition of soil organic carbon by soil microorganisms. Therefore, even small changes in the environmental conditions could markedly influence the C cycling in soils and affect atmospheric CO_2 concentrations. Global warming will enhance the decomposition of soil organic carbon (SOC) by decomposers (Bond-Lamberty and Thomson, 2010; Raich and Potter, 1995) resulting in higher CO_2 efflux from soils and decreasing SOC stocks. Although, soils store large amounts of C, human activities as land-use and land-use change already caused a significant loss of global SOC stocks (Ciais et al., 2013; Houghton, 2003; Houghton et al., 2012). Therefore, understanding the processes and the mechanisms controlling C stabilization and turnover in soils is a prerequisite to predict future C gains or losses of soils. In the past decades, soil C dynamics were broadly investigated and the identified mechanisms controlling stabilization and mineralization of SOC were incorporated into climate change models. Many studies also have focused on the potential to increase C stocks in soils to counteract increasing CO_2 emissions to the atmosphere.

However, the majority of studies investigating the C dynamics and the C sequestration in soils were restricted to the first 30 cm of the soil (the topsoil), even though more than 50 % of the global SOC stocks are stored in the subsoil, at depths deeper than 30 cm (Batjes, 1996, 2014; Hiederer and Köchy, 2012; Jobbágy and Jackson, 2000). Further, C models which simulate changes in SOC stocks following land-use or environmental changes only include the topsoil and ignore the subsoil. Compared to the topsoil, the SOC pool in the subsoil is characterized by a higher radiocarbon age and longer turnover times (Mathieu et al., 2015; Rethemeyer et al., 2005; Torn et al., 1997), which may led to the assumption that C dynamics in subsoils play a minor role in the annual global C cycle. Despite of the high ^{14}C age of the bulk soil, there is growing evidence that the C turnover in subsoils contributes on much shorter time scales. For example, Gaudinski et al. (2000) reported for a temper-

ate forest that about one third of total soil CO_2 efflux was produced in the subsoil (below the A horizon). Fierer et al. (2005) found similar results in grassland soils in California, where the CO_2 production in the subsoil contributed up to 50 % to total soil respiration. Although this indicates that the CO_2 production in subsoils is relevant for total soil respiration, little is known about the processes and the mechanisms determining the CO_2 production in subsoils, making it almost impossible to simulate the response of CO_2 production in subsoils to environmental changes.

Furthermore, the decomposition of SOC by decomposers is affected by environmental factors, such as soil temperature, soil moisture, oxygen concentration, C and nutrient availability. With increasing soil depth these factors changes leading to the assumption that decomposition processes differ between the topsoil and the subsoil. In contrast, despite the large SOC stocks stored in the subsoil, the C content is low indicating that subsoils may have an unexploited potential to sequester additional C in soils (Lorenz and Lal, 2005). In consequence, there is an urgent need for a better understanding of the C dynamics in subsoils to predict the vulnerability of SOC stocks in the whole soil to global changes and to evaluate the C sequestration potential of subsoils.

1.2 Stability of subsoil C

Although, there is a lack of data on C fluxes and C turnover in subsoils, several explanations were given for the high apparent ^{14}C age and the indicated stability of soil organic matter (SOM) in subsoils. One is the selective preservation of lower quality organic matter during the decomposition process (Lomander et al., 1998; Rumpel, 2004; Sollins et al., 1996). Therefore, SOM in subsoils consists mainly of recalcitrant compounds which accumulate during the decomposition of fresh C inputs and the transport through the soil. These compounds represent the end-products of decomposition which can not be used by microorganisms to maintain their energy demand. Moreover, the increasing stable isotope ratio $\delta^{13}C$ and the decreasing C/N ratio of SOM in the subsoils indicate a higher contribution of microbial-derived and microbial processed organic matter (OM) (Liang and Balser, 2008; Rumpel, 2004; Rumpel and Kögel-Knabner, 2011). In addition, dissolved organic matter (DOM) shows the same behavior of a higher contribution of microbial derived compounds with depth (Kaiser et al., 2002; Kaiser and Kalbitz, 2012). It was shown that long-term stabilization of OM in soil can not only be explained due to the recalcitrance of OM (Marschner et al., 2008; Schmidt et al., 2011). Therefore, the stability of SOM is also controlled by abiotic and biotic soil conditions (von Lützow et al., 2006; Sollins et al., 1996).

One important stabilization mechanism is the formation of organo-mineral compounds, due to sorption or co-precipitation of OM on mineral surfaces (clay minerals, metal ions) (Kleber et al., 2015; von Lützow et al., 2006; Sollins et al., 1996). The association of OM with mineral surfaces may be more important in subsoils, due to a higher availability of unsaturated mineral surfaces as compared to topsoils (Kaiser and Guggenberger, 2003; Rasse et al., 2005). This is also indicated by an increasing contribution of OC associated with mineral surfaces on the bulk SOC with soil depth (Eusterhues et al., 2007; Lorenz et al., 2011). The mineral-associated OM (MAOM) is also characterized by slow turnover rates (Kleber et al., 2015) and therefore contribute to the long-term C storage.

The stability of SOM is also controlled by its accessibility to decomposers and the availability of fresh C inputs. Therefore, factors which enhance or reduce the availability and accessibility of SOM to soil microorganisms determine the stability of SOC (Dungait et al., 2012; Holden and Fierer, 2005; von Lützow et al., 2006). Several studies showed that the availability of an energy source for soil microorganisms plays an important role for the stabilization of SOC (Fontaine et al., 2004, 2007; Marschner et al., 2008). Therefore, as the C inputs from roots, root exudates and DOM decrease with depth (Michalzik et al., 2001; Tückmantel et al., 2017), soil microorganisms are facing more and more energetic constraints in deeper soil layers. Hence, soil microorganisms change to a dormant state, which requires less energy for metabolism and the decomposition of SOM is reduced (Joergensen and Wichern, 2018). In consequence, C turnover in subsoils is hampered and SOM remains stable. In turn, the input of an easily available energy source may remove energy limitations for microorganisms and trigger the decomposition of ancient SOM (Fontaine et al., 2004, 2007). This effect is known as priming and usually described as short-term changes in SOM turnover (Bingeman et al., 1953; Kuzyakov et al., 2000). Priming effects have been well investigated for topsoils (Blagodatskaya and Kuzyakov, 2008; Hamer and Marschner, 2005; Kuzyakov et al., 2000), but not for subsoils. In fact, only a few studies assessed priming effects in subsoils with contrasting findings (Fierer et al., 2003; Fontaine et al., 2007; Karhu et al., 2016; Salomé et al., 2010). Thus, to evaluate the C sequestration potential of subsoils more research is needed to answer the questions whether additional C inputs into subsoils may stimulate the decomposition of old SOM and to which extent. It also remains unclear how stable are additional C inputs.

Next to the availability of fresh C inputs, stability of SOM is also linked due to a limited access for microorganisms. The physical protection of OM due to aggregation is well known as a stabilization mechanisms for SOM. The occlusion of OM reduces the access for microorganisms and enzyme and restricts the diffusion of enzymes, soluble OM and oxygen (von Lützow et al., 2006; Sollins et al., 1996), which in turn limit the decomposition of occluded OM. However, due to the decreasing

density and activity of the soil biota and roots with depth the aggregate formation and stability of occluded OM might be different between topsoils and subsoils (Rumpel and Kögel-Knabner, 2011). Although, little is known about the role of aggregate formation for SOM stability in subsoils, it is assumed that the spatial separation between decomposers and OM is an important mechanism controlling the stability of SOM in subsoils. Moreover, the environmental conditions in subsoils have a strong influence on the availability of SOM for microorganisms. Decreasing SOC contents and microbial biomass with soil depth can simply reduce the likelihood for microorganisms and their enzymes to encounter with SOM (Don et al., 2013; Ekschmitt et al., 2005, 2008). Further, subsoils are characterized by a higher spatial heterogeneity of SOM distribution as compared to topsoils, due to a greater importance of preferential flow paths, root and bioturbation as C inputs (Bundt et al., 2001; von Lützow et al., 2006). These areas may represent hotspots of SOM turnover in subsoils, which are characterized by younger SOC with a wider C/N ratio, a higher microbial activity as compared to surrounding bulk soil (Chabbi et al., 2009; Hagedorn and Bundt, 2002). However, with depth the contribution of hotspots to the whole matrix decreases and leaving a large part of the soil matrix sparsely populated that OM may can persist on the long-term (Bundt et al., 2001). This is important beacause the transport between OM and soil microorganisms in subsoils is mainly driven by slow and small-scale (µm) diffusion processes (Schimel et al., 2011; Xiang et al., 2008). Therefore, fresh substrate may not be transported outside those hotspots and in consequence decomposers in the surrounding soil matrix became substrate limited and even potentially degradable OM can persist (Heitkötter and Marschner, 2018; Schjønning et al., 2003). The importance of spatial separation between decomposers and substrate as stabilization mechanism in subsoils was also indicated by incubation studies, which observed similar or even higher SOC mineralization rates of subsoil samples as compared to topsoil samples (Agnelli et al., 2004; Fierer et al., 2003; Salomé et al., 2010). Before the incubation, samples were usually sieved and mixed which may removed the spatial separation of decomposers and substrate for the subsoil samples.

In summary, the stability and the high ^{14}C age of subsoil OC may be explained more due to the spatial separation of decomposers and substrate and the small and spatial heterogeneous C inputs, which lead to energy and nutrient limitations in the subsoil.

However, even though SOC in the subsoils seems stable, there is also evidence that subsoils contain an active C pool, which contributes to the short-term C cycle in soils (Baisden and Parfitt, 2007; Davidson and Trumbore, 1995; Drewitt et al., 2005) and should not be neglected in C models. Moreover, as the SOM stabilization mechanisms may differ between topsoil and subsoil also the response of SOM decomposition to temperature or land-use change might be different. It is expected

that global warming will accelerate the decomposition of SOM leading to higher C fluxes from soils to the atmosphere. In C models the temperature sensitivity of SOM decomposition is based on the Arrhenius equation, were reactants with higher activation energies such as more recalcitrant SOM have higher temperature sensitivities as compared to more labile SOM. Therefore, it was assumed that the temperature sensitivity of SOM decomposition is higher in subsoils, due to a larger proportion of recalcitrant OM as compared to topsoils. However, research so far has shown that the temperature sensitivity of subsoil OM decomposition can be higher, similar or lower as compared to topsoil OM decomposition (Davidson and Janssens, 2006; Fierer et al., 2003; Hicks Pries et al., 2017b; von Lützow and Kögel-Knabner, 2009), showing that there is still a lack of consensus on the temperature sensitivity of SOM decomposition in deeper soil horizons. The higher temperature sensitivity in subsoils as in topsoils was explained due to an increase in SOM recalcitrance with depth, but as outlined above the high stability of OM in subsoils can not only be explained by recalcitrance. In addition, it was pointed out that the intrinsic temperature sensitivity of SOM may be influenced and obscured by environmental factors e.g. physical protection of OM within aggregates and substrate availability (Davidson and Janssens, 2006; Gillabel et al., 2010). Thus, the lower observed temperature sensitivity in subsoils may be due to the lower SOC content, lower C inputs or a higher degree of physical protection within aggregates. Therefore, if the temperature sensitivity in subsoils is influenced by e.g. substrate availability, additional OC inputs in subsoils as part of C sequestration measures may increase the temperature sensitivity of subsoil OM decomposition. However, so far there is lack of experiments and data for such effects.

In summary, even though subsoils play an active role in the global C cycle as revealed by high observed CO_2 production rates in subsoils (Davidson and Trumbore, 1995; Fierer et al., 2005; Gaudinski et al., 2000), it is not clear whether CO_2 in subsoils originates from the mineralization of ancient SOC or the mineralization of young C sources such as DOM inputs or root inputs. Moreover, information on the contribution of CO_2 production in subsoils to total soil respiration is scare. In addition, the temperature sensitivity of subsoil OM decomposition remains unclear, due to the unknown effect of environmental constraints in subsoils. Further, some laboratory incubation studies were able to show that the decomposition processes in subsoils are hampered due to the unfavorable environment conditions in the subsoil (Fierer et al., 2003; Salomé et al., 2010; Xiang et al., 2008), but there is still a lack of experimental evidence for the influences of environmental constraints on C turnover in the subsoil under field conditions. One aim of this thesis is to improve the knowledge of the C dynamics in subsoils by *in situ* determination of the CO_2 production in different soil depth in combination with isotopic measurements. Further, this thesis also aims to examine the mechanisms

controlling the stability of SOC in subsoils e.g. substrate availability and spatial separation as well as environmental limitation (temperature, moisture, oxygen) in laboratory and field incubation experiments.

1.3 Hypotheses

H1: The subsoil contains a labile C pool, which is a relevant part of the annual CO_2 efflux from the soil to the atmosphere.

H2: The CO_2 in the subsoils is derived from recent C sources, due to the mineralization of root-derived C or autotrophic respiration, while the mineralization of old C will hardly contribute to the CO_2 production

H3: The temperature sensitivity of OC mineralization is higher in the subsoil than in the topsoil if OM recalcitrance is the main stabilization mechanism in the subsoil. If temperature sensitivity in the subsoils are obscured by spatial separation and substrate availability, the temperature sensitivity of OC mineralization in disturbed subsoil samples with added substrate should be higher than in undisturbed samples.

H4: The increasing stability of SOM with soil depth is controlled by the availability of substrate for decomposers. The low C input into subsoils results in substrate limitations for decomposers. Further, the low SOC content in subsoils increases the spatial separation between decomposers and substrate, which also reduce substrate availability.

1.4 Thesis outline

This thesis focuses on a better understanding of the mechanisms regulating C turnover in the subsoil. The first section (Chapter 2) is devoted to the identification of sources for C turnover in the subsoil as well as to the quantification of CO_2 production in the subsoil. The following section investigates the mechanisms and factors controlling SOC mineralization in the subsoil in a laboratory study (Chapter 3) and a field study (Chapter 4). The last section (Chapter 5) summarizes the major findings of this thesis and gives an outlook.

Vertical partitioning of CO_2 production in a beech forest (Chapter 2)

The first study aims to quantify and separate the CO_2 production in topsoil and subsoil horizons. Therefore, a two-years field monitoring of soil temperature, soil moisture and CO_2 concentration up to 150 cm depth was carried out in three soil profiles in a beech forest. In addition, stable isotope labeling experiment was established to trace the fate of fresh litter inputs into the soil and to quantify the contribution to CO_2 production in different soil depths. This was supported by radiocarbon measurements of the soil atmosphere down to 150 cm to evaluate the contribution of old SOC to CO_2 production in the subsoil. This study contribute to hypothesis H1 and H2

Controlling factors for the stability of subsoil carbon in a Dystric Cambisol (Chapter 3)

The second study addresses the influence of temperature and substrate limitation on the C mineralization in the topsoil and the subsoil for a sandy forest soil. Therefore, the CO_2 production of soil samples from a topsoil horizon and two subsoil horizons were determined in a laboratory incubation experiment. Samples were incubated at 10 °C and 20 °C to assess the temperature effect on C mineralization. Further, the addition of ^{13}C labeled roots allowed to evaluate the impact of substrate limitation on SOC mineralization in the subsoil. This study contributes to H3 and H4

Environmental constraints limit C decomposition in the subsoil (Chapter 4)

The objectives of this study are to investigate the impact of changing environmental conditions with soil depth on OC decomposition in the field. Therefore, an *in situ* incubation experiment with topsoil and subsoil samples was carried out. The addition of ^{13}C labeled litter allowed to follow decomposition processes depending on environmental conditions. This study contribute to hypothesis H4.

CHAPTER 2 VERTICAL PARTITIONING OF CO_2 PRODUCTION IN A BEECH FOREST

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Abstract

Large amounts of total organic carbon are temporarily stored in soils, which makes soil respiration one of the major sources of terrestrial CO_2 fluxes within the global carbon cycle. More than half of global soil organic carbon (SOC) is stored in subsoils (below 30 cm), which represent a significant C pool . Although several studies and models have investigated soil respiration, little is known about the quantitative contribution of subsoils to total soil respiration or about the sources of CO_2 production in subsoils. In a two-year field study in a European beech forest in northern Germany, vertical CO_2 concentration profiles were continuously measured at three locations and CO_2 production was quantified in the topsoil and the subsoil. To determine the contribution of fresh litter-derived C to CO_2 production in the three soil profiles, an isotopic labeling experiment using ^{13}C-enriched leaf litter was performed. Additionally, radiocarbon measurements of CO_2 in the soil atmosphere were used to obtain information about the age of the C source in CO_2 production. At the study site, it was found that 90 % of total soil respiration was produced in the first 30 cm of the soil profile where 53 % of the SOC stock is stored. Freshly labeled litter inputs in the form of dissolved organic matter were only a minor source for CO_2 production below a depth of 10 cm. In the first two months after litter application, fresh litter-derived C contributed on average 1 % at 10 cm depth and 0.1 % at 150 cm depth to CO_2 in the soil profile. Thereafter, its contribution was less than 0.3 % and 0.05 % at 10 cm and 150 cm depths respectively. Furthermore CO_2 in the soil profile had the same modern radiocarbon signature at all depths, indicating that CO_2 in the subsoil originated from young C sources, despite a radiocarbon age bulk SOC in the subsoil. This suggests that fresh C inputs in subsoils in the form of roots and root exudates are rapidly respired and that other subsoil SOC seems to be relatively stable. The field labeling experiment also revealed a downward diffusion of $^{13}CO_2$ in the soil profile against the total CO_2 gradient. This isotopic dependency should be taken into account when using labeled ^{13}C and ^{14}C isotope data as an age proxy for CO_2 sources in the soil.

2.1 Introduction

Soils are the world's largest terrestrial organic carbon (C) pool, with an estimated global C stock of about 2400 Gt in first two metres of the world's soils (Batjes, 2014). The CO_2 efflux from soils, known as soil respiration, is the second largest flux component in the global C cycle (Bond-Lamberty and Thomson, 2010; Raich and Potter, 1995) and can be divided into autotrophic respiration, due to roots and mycorrhizae, and heterotrophic respiration, due to the mineralization of soil organic carbon (SOC) by decomposers. Global warming is expected to increase soil respiration by boosting the microbial decomposition of SOC (Bond-Lamberty et al., 2018; Hashimoto et al., 2015) and by greater root respiration (Schindlbacher et al., 2009; Suseela and Dukes, 2013). Although most of the CO_2 is produced in topsoils (< 30 cm), a significant amount of CO_2 is produced in the subsoil (> 30 cm) (Davidson and Trumbore, 1995; Drewitt et al., 2005; Fierer et al., 2005; Jassal et al., 2005). Despite the fact that more than 50 % of global SOC stocks are stored in subsoils (Batjes, 2014; Jobbágy and Jackson, 2000), little is known about the amount and sources of CO_2 production in subsoils. Moreover, the mechanisms controlling CO_2 production in subsoils are still not fully understood. High apparent radiocarbon (^{14}C) ages of SOC in subsoils (Rethemeyer et al., 2005; Torn et al., 1997) lead to an assumption of a high stability of C and a low turnover in subsoils. However, laboratory incubations of subsoil samples show similar mineralization rates of SOC in both subsoils and topsoils (Agnelli et al., 2004; Salomé et al., 2010; Wordell-Dietrich et al., 2017), suggesting that subsoils also contain a labile fraction that should be taken into account as a source for soil respiration.

A range of studies have been conducted on CO_2 production in soils, but most of them have focused on spatial variations in temperature, water content and substrate supply (Borken et al., 2002; Davidson et al., 1998; Fang and Moncrieff, 2001), but ignoring the vertical partitioning of CO_2 production in the whole soil profile which is essential for understanding soil C dynamics. One reason for this might be the measurement methods used to quantify sources and fluxes in the soil profile. Total CO_2 production can easily be measured at the soil surface with an open-bottom chamber, whereas vertical monitoring of CO_2 production needs determination of CO_2 concentrations at several soil depths in order to estimate CO_2 production, i.e. using the gradient method first described by de Jong, E., Schappert (1972). Basically, the CO_2 flux between two depths can be calculated using the effective gas diffusion coefficient and the CO_2 gradient between the two depths. Recently, the development of low-cost sensors for temperature, soil moisture and CO_2 concentration has allowed greater use of the gradient method (Jassal et al., 2005; Maier and Schack-Kirchner, 2014; Pingintha et al.,

2010; Tang et al., 2005). This method can help quantify CO_2 production in the entire soil profile, which is essential for an improved quantitative understanding of whole soil C dynamics including the important contribution made by subsoil. To date there have only been a few studies that have continuously determined CO_2 production in the whole soil profile *in situ* over a longer timescale (Goffin et al., 2014; Moyes and Bowling, 2012).

In the present study, the vertical distribution of CO_2 concentration was measured and CO_2 production rates calculated over a two-year period in a Dystric Cambisol in a temperate beech forest. The objectives of this study were 1) to quantify the contribution of CO_2 production in subsoils to total soil CO_2 production, and 2) to identify sources of CO_2 production along the soil profile using sources partitioning via isotopic data (^{13}C and ^{14}C). It was hypothesized that the majority of CO_2 in subsoils originates from young C sources and not from mineralization of old SOC.

2.2 Materials and methods

2.2.1 Site description and subsoil observatories

The study site is located in a beech forest (Grinderwald) 35 km northwest of Hannover, Germany (52°34´22´´N, 9°18´49´´E). The vegetation is dominated by common beech trees (*Fagus sylvatica*) that were planted 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 precipitation of 9.7 °C and 762 mm (Deutscher Wetterdienst, Nienburg, 1981–2010) respectively. The soil texture of the site is mainly composed of the sand fraction with contents varying from 60 % (< 30 cm) to 90 % (> 120 cm), with SOC contents of 11.5 g kg^{-1} down to (10 cm) 0.4 g kg^{-1} (185 cm) (Heinze et al., 2018; Leinemann et al., 2016).

In July 2013, three subsoil observatories were installed using a stainless steel lysimeter vessel (1.6 m diameter and 2 m height) driven 2 m deep into the soil (Figure 2.1a). Once the vessel had been inserted, the soil inside the containment was excavated by hand and undisturbed soil cores (5.7 cm inner diameter, 4.0 cm height) taken with five replicates at depths of 10, 30, 50, 90 and 150 cm from each subsoil observatory for soil diffusivity measurements. In addition, undisturbed soil samples in the observatories were taken to estimate fine root density. Thus six samples were taken from the forest floor and six samples from each of the upper mineral soil layers (0–10 cm, 10–20 cm, 20–40 cm) using a soil corer (3.5 cm diameter), and three samples were taken from each depth increment of the lower profile (40–200 cm depth) at 20 cm depth intervals using a steel cylinder (12.3 cm diameter and 20 cm height). In the laboratory, the samples were gently washed over sieves of 0.25 mm mesh size to separate the roots from adhering soil particles. Under the stereo microscope, the rootlets were separated into live (biomass) and dead (necromass) roots, and subsequently into fine (< 2 mm in diameter) and coarse roots (> 2 mm in diameter). All live and dead root samples were dried at 70 °C for 48 h and weighed.

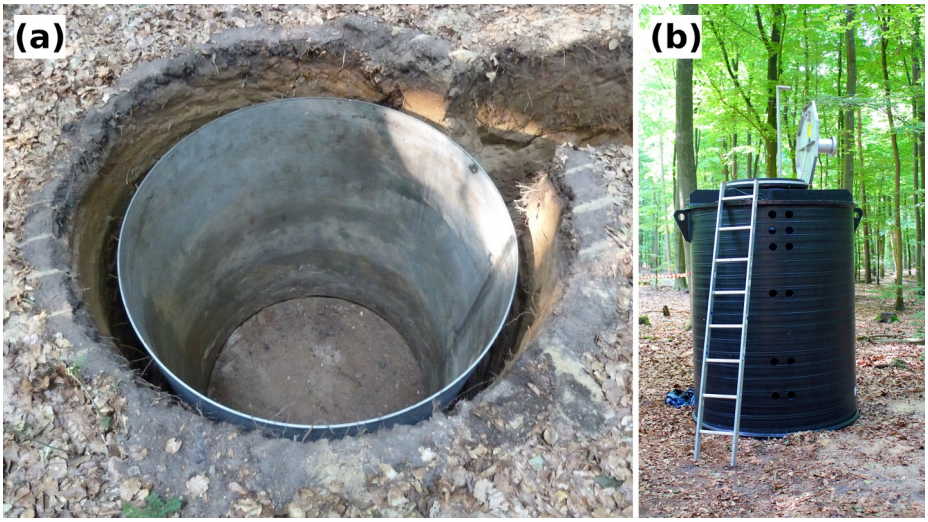

Figure 2.1: Photographs of (a) the used lysimeter vessels to drill the hole for the subsoil observatories and (b) the used polyethylene shaft as subsoil observatory.

After the lysimeter vessel was removed, a polyethylene shaft (1.5 m in diameter and 2.1 m height) was placed in the soil (Figure 2.1b), referred to here as the subsoil observatory. The gap (5 cm) between the subsoil observatory and the surrounding undisturbed soil was refilled. The observatories where installed close to one other, with a maximum distance of 30 m between them.

To monitor the temperature and volumetric water content, combined temperature and moisture sensors (UMP-1,Umwelt-Geräte-Technik GmbH, Germany) were installed at depths of 10, 30, 50, 90 and 150 cm with a horizontal distance of 100 cm from the wall of the subsoil observatories (Figure 2.2a). Measurements were taken every 15 minutes and stored on a data logger inside the subsoil observatory. The CO_2 concentration in the soil air was monitored by solid-state infrared gas sensors (GMP221, Vaisala Oyi, Finland) with a measuring range of 0–10 % CO_2. To protect the PTFE membrane of the CO_2 sensor from damage while being placed in the soil, the sensor was coated with an additional PTFE foil (616.13 P, FIBERFLON, Turkey), to allow gaseous diffusion and prevent water infiltration. The CO_2 concentration was measured every three hours to reduce power consumption. The CO_2 sensors were turned on 15 minutes before the measurement itself due to their warm-up time. In addition, PTFE suction cups (25 mm diameter, 60 mm length) for soil air sampling with stainless steel tubing (2 mm inner diameter) (ecoTech Umwelt-Meßsysteme GmbH, Germany) were installed adjacent to the CO_2 sensors. The gas samplers and CO_2 sensors were installed at the same depths as the temperature and moisture sensors. The horizontal distance of the gas samplers and CO_2 sensors from the subsoil observatory wall increased from 40 cm to 100 cm with increasing soil depth (Figure 2.2a).

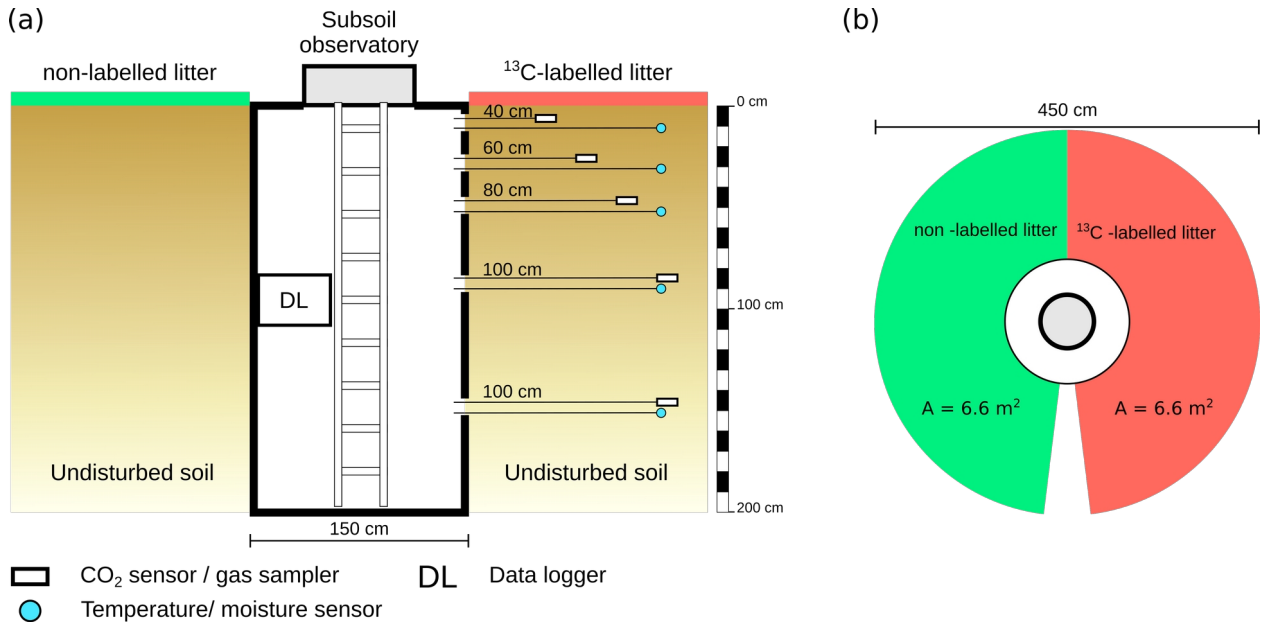

Figure 2.2: Schematic overview of the subsoil observatories, the installed sensors and the labeling experiment, (a) side view of the subsoil observatory and (b) top view of the labeled and control area

2.2.2 Gas sampling and measurements

Soil respiration

The surface CO_2 efflux was measured using the closed-chamber method. Thirty PVC collars with a diameter of 10.4 cm and a height of 10 cm were installed 5 cm deep in the soil around the three subsoil observatories. The organic layer of 15 collars was removed in order to be able to distinguish between mineral soil respiration and total soil respiration. Soil respiration was measured with the EGM-3 SRC-1 soil respiration chamber (PP-Systems, USA) and the LI-6400-09 soil chamber (LI-COR Inc., USA). The measurement system was changed due to technical problems with the EGM-3 system, however a comparison between the two systems revealed only minor differences. Each collar was measured three times per sampling day from March 2014 to March 2016, with sampling ranging from once a month to once a week. Annual soil respiration was derived from linear interpolation of measured CO_2 fluxes from the collars. Furthermore, soil respiration was modeled by fitting an Arrhenius-type model (Eq. 2.1), introduced by Lloyd and Taylor (1994) and using soil temperature data from 10 cm depth, and the measured CO_2 fluxes:

$$F_0 = a \times \mathrm{e}^{\left(\frac{E_0}{T + 273.2 - T_0} \times \frac{T - 10}{283.2 - T_0}\right)} \qquad (2.1)$$

where F_0 is soil respiration [µmol m^{-2} s^{-1}], a, E_0 and T_0 are fitted model parameters, and T is the soil temperature at 10 cm depth [°C].

$^{13}CO_2$ sampling and measurement

In addition to continuous CO_2 concentration monitoring, two gas samples per depth and subsoil observatory were taken at the end of the stainless steel tubing from the suction cups with a syringe and filled into 12-mL evacuated gas vials (Labco Exetainer, Labco Limited, UK). The sampling started in May 2014 with an interval of between once a month and once a week. The CO_2 concentration in the soil gas samples was analyzed by gas chromatography (Agilent 7890A, Agilent Technologies, USA). The $\delta^{13}C$ values of the CO_2 samples were measured by an isotope ratio mass spectrometer (Delta Plus with GP interface and GC-Box, Thermo Fisher Scientific, Germany) connected to a PAL autosampler (CTC Analytics, Switzerland). The ^{13}C results are expressed in parts per thousand (‰) relative to the international standard Vienna Pee Dee Belemnite (VPDB).

$^{14}CO_2$ sampling and measurement

Soil gas samples for radiocarbon analysis were taken in October and December 2014 in subsoil observatories 1 and 3. The CO_2 was sampled using a self-made molecular sieve cartridge as described in Wotte et al. (2017). Briefly, each stainless steel cartridge was filled with 500 mg zeolite type 13X (40/60 mesh, Charge 5634, IVA Analysetechnik GmbH & Co KG, Germany), which is used as an adsorbent for CO_2. The molecular sieve cartridges were connected to the installed gas samplers. The soil atmosphere of the corresponding depth was then pumped with an airflow of 7 mL min^{-1} over a desiccant (Drierite, W. A.Hammond Drierite Company, USA) to the molecular sieve cartridge for 40 minutes to trap the CO_2 on the molecular sieve. Surface samples were taken from a respiration chamber (30 cm diameter) (Gaudinski et al., 2000). The atmospheric CO_2 inside the chamber was removed prior to sampling by circulating an airflow of ≈ 1.5 L min^{-1} from the chamber through a column filled with soda lime until the equivalent of 2-3 chamber volumes had been passed over the soda lime. Thereafter, the airflow was run over a desiccant and the molecular sieve cartridge for 10 minutes to collect the CO_2 sample.

In the laboratory, the adsorbed CO_2 was released from the molecular sieve cartridge by heating the molecular sieve under vacuum (Wotte et al., 2017). The released CO_2 was purified cryogenically and sealed in a glass tube. The radiocarbon (^{14}C) analysis was directly performed on the CO_2 with the gas ion source of the mini carbon dating system (MICADAS, Ionplus,Switzerland) at ETH Zurich

(Ruff et al., 2010). The ^{14}C concentrations are reported as fraction modern carbon ($F^{14}C$), whereby $F^{14}C$ values less than one denote that the majority of the C was fixed before the nuclear bomb tests in the 1960s, while values greater than one indicate C fixation after the bomb tests.

2.2.3 Labeling experiment

To trace the fate of fresh litter inputs in the soil and their contribution to the CO_2 released from different soil horizons, a ^{13}C labeling experiment was performed. In January 2015, the leaf litter layer around the subsoil observatories was removed and replaced with a homogeneous mixture of 237 g ^{13}C labeled and 1575 g non-labeled young beech litter, which is equal to a litter input of 250 g m^{-2}. The labeled litter was distributed on a semi-circular area (6.6 m^2) around the subsoil observatories (Figure 2.2b). The labeled litter originated from young beech trees grown in a greenhouse in a $^{13}CO_2$ -enriched atmosphere. The mixture of labeled and non-labeled litter had an average $\delta^{13}C$ value of 1241 ‰ for subsoil observatory 1 (OB1) and a $\delta^{13}C$ value of 1880 ‰ for subsoil observatories 2 (OB2) and 3 (OB3).

2.2.4 Diffusivity measurements

Gas transport along the soil profile is determined by the diffusivity of the soil. The diffusivity of the soil was determined at depths of 10, 30, 50, 90 and 150 cm, with five undisturbed core sample replicates per depth and per observatory. To account for different water contents, the undisturbed soil cores (5.7 cm diameter, 4.0 cm height) were adjusted in the laboratory at different matrix potentials (-30, -60 and -300 hPa) to cover a wide range of soil moisture. After moisture adjustment, the soil cores were attached to a diffusion chamber as described in Böttcher et al. (2011). The diffusion chamber was flushed with nitrogen to initially establish a gas gradient between the chamber and the top of the sample as an atmospheric boundary condition. The increase in oxygen inside the ventilated chamber was measured over time with an oxygen dipping probe (DP-PSt3-L2.5-St10-YOP, PreSens-Precision Sensing GmbH, Germany). Diffusivity and tortuosity factors (τ) were calculated with an inverse diffusion model (Schwen and Böttcher, 2013).

2.2.5 Data analysis

Gradient method

This method is based on the assumption that molecular diffusion is the main gas transport in the soil atmosphere. Therefore gas fluxes, e.g. CO_2 fluxes in a soil profile, can be calculated from the CO_2 concentration gradient and the effective gas diffusion coefficient in the specific soil layer of interest. In order to account for temperature and pressure dependencies of the CO_2 sensors, the CO_2 concentrations were corrected with a compensation algorithm for the GMP221 (Eq. S2.1) provided by the manufacturer (pers. comm. Niklas Piiroinen, Vaisala Oyi, Finland). For the flux calculation, CO_2 volume concentrations were converted to CO_2 mole concentrations (Eq. 2.2):

$$C = \frac{C_v \times p}{R \times T} \tag{2.2}$$

where C is the CO_2 mole concentration [µmol m^{-3}], C_v is the CO_2 volume fraction [µmol mol^{-1}], p is the atmospheric pressure in [Pa], R is the universal gas constant [8.3144 J K^{-1} mol^{-1}] and T is the soil temperature in [K] measured by temperature sensors at the corresponding soil depths. The CO_2 flux of a soil layer was calculated using Fick's first law (Eq. 2.3)

$$F = -D_s \times \frac{dC}{dz} \tag{2.3}$$

where F is the diffusive CO_2 flux [µmol m^{-2} s^{-1}], D s is the effective diffusivity in the soil atmosphere [m^2 s^{-1}] determined as described below, C is the CO_2 concentration [µmol m^{-3}] and z is the depth [m]. The equation is based on the assumption that 1) molecular diffusion is the dominating transport process in the soil atmosphere and other transport mechanisms - i.e. convective CO_2 transport due to air pressure gradients or diffusion in the soil, and convective transport with soil water are negligible and 2) gas transport is one-dimensional (e.g., de Jong, E., Schappert, 1972; Maier and Schack-Kirchner, 2014). The effective diffusivity D_s was calculated with Eq. (2.4):

$$D_s = D_0 \times \tau \tag{2.4}$$

where D_0 is the CO_2 diffusivity in free air. The pressure and temperature effect on D_0 were taken into account by:

$$D_0 = D_{a0} \times \left(\frac{p_0}{p}\right) \times \left(\frac{T}{T_0}\right)^{1.75} \tag{2.5}$$

where D_{a0} is a reference value of D_0 at standard conditions (1.47×10^{-5} $m^2 s^{-1}$ at T_0 293.15 K and p_0 1.013×10^5 Pa) (Jones, 1994)(Jones, 1994). The dimensionless tortuosity factor τ at each depth was modeled as a function of the air-filled pore space ε for each soil depth. The model was derived from a power function fit from laboratory diffusion experiments (see above) on the undisturbed soil cores. To account for the non-uniform vertical distribution of soil water content in the soil profile, D_s was estimated as the harmonic average between the two measurement depths (Pingintha et al., 2010; Turcu et al., 2005):

$$D_s = \frac{\Delta z_1 + \Delta z_2}{\frac{\Delta z_1}{D_{sz1}} + \frac{\Delta z_2}{D_{sz2}}} \tag{2.6}$$

where $\Delta z_{1,2}$ [m] is the thickness of the corresponding soil layer and $D_{sz1,2}$ is the effective diffusivity of the respective soil layer. Finally, assuming a constant flux between measured CO_2 at depth z_i and z_{i+1}, the CO_2 flux (F_i) was calculated by combining Eq. (2.2-2.6):

$$F_i = \left(\frac{\Delta z_i + \Delta z_{i+1}}{\frac{\Delta z_i}{D_{szi}} + \frac{\Delta z_{i+1}}{D_{szi+1}}} \right) \times \left(\frac{C_{i+1} - C_i}{z_{i+1} - z_i} \right) \tag{2.7}$$

where F_i is the CO_2 flux [μmol $m^{-2} s^{-1}$] at the upper boundary (z_i) between depth z_i and z_{i+1} [m]. To calculate soil respiration (F_0) at the surface with the gradient method, a CO_2 concentration of 400 μmol mol^{-1} at the soil surface and a constant D_s for the first 10 cm were assumed.

CO_2 production

The CO_2 production (P_i) in a soil layer was calculated as the difference between the flux (F_i) leaving the specific soil layer at the upper boundary (z_i) and the input flux (F_{i+1}) at the lower boundary (z_{i+1}) of the specific soil layer. Therefore, P_i had the unit of a flux [μmol $m^{-2} s^{-1}$] (similar approach was done by e.g., Davidson et al., 2006; Fierer et al., 2005; Gaudinski et al., 2000; Hashimoto et al., 2007.

$$P_i = F_i - F_{i+1} \tag{2.8}$$

Total soil respiration was calculated as the sum of CO_2 production in all soil layers. Equation (2.8) is based on the assumption of steady-state diffusion. Steady-state conditions for CO_2 concentration and volumetric water content were mostly given, except during a few heavy rain events where steady-

state conditions were not met due to changing water contents in the profiles. Most soils exhibit increasing CO_2 concentrations with increasing soil depth. Therefore, CO_2 production is mostly positive with upward CO_2 fluxes. However, if the CO_2 concentration in a soil layer is greater than in the layers below, the calculated CO_2 production in the layers below can become negative (downward directed). Hence in the present study no CO_2 production was assumed when the calculated CO_2 production in a soil layer was negative. This approach was based on the assumption that there are no relevant CO_2 sinks in the soil profile. Furthermore, negative CO_2 production is considered as CO_2 storage, which will be released if the CO_2 concentration gradient or diffusion conditions change. In OB1 negative CO_2 production values were calculated in the first year at 30-50 cm depth (331 out of 365) and at 50-90 cm depth (359 out of 365). In the second year negative values also occurred in OB1 at 30-50 cm depth (8 out of 308) and at 50-90 cm depth (182 out of 308)

Isotopic composition of CO_2

To determine the contribution of the labeled leaf litter to CO_2 in the soil atmosphere we used the isotopic mixing equation (Eq. 2.9):

$$L = 1 - \left(\frac{\delta^{13}C_M - \delta^{13}C_L}{\delta^{13}C_B - \delta^{13}C_L} \right) \tag{2.9}$$

where $\delta^{13}C_M$ is the isotopic signature of the gas sample, $\delta^{13}C_L$ is the isotopic signature of the labeled leaf litter (1241 ‰ for OB1 and 1880 ‰ for OB2 and OB3) and $\delta^{13}C_B$ is the average isotopic signature of the soil atmosphere for each observatory and depth before the labeled leaf litter was applied, assuming there was no change. The litter-derived CO_2 flux was calculated by multiplying the amount of litter-derived C (L) with the CO_2 flux of the respective soil layer. Afterwards, litter-derived CO_2 production was determined according to Eq. (2.8). The absolute $^{13}CO_2$ concentration was calculated with isotopic signature of the soil atmosphere and $^{13}CO_2$ fluxes were calculated using Eq. (2.2-2.7). To account for different effective diffusivities of $^{12}CO_2$ and $^{13}CO_2$ the effective diffusivity D_s for $^{13}CO_2$ was adjusted according to (Cerling et al. (1991):

$$D_s = {}^{12}D_s = 1.0044 \times {}^{13}D_s \tag{2.10}$$

where it is assumed that D_s is equivalent to $^{12}D_s$ due to the fact that about 99 % of total CO_2 is $^{12}CO_2$.

2.2.6 Statistical analysis

A Monte Carlo simulation was generated to determine the influence of measurement uncertainties of the sensors, which were used for calculation of CO_2 fluxes and CO_2 production rates. It was assumed that each measurement error was normally distributed. The standard deviation was equal to measurement accuracy, which was obtained from the corresponding manual. The distributions of CO_2, volumetric water content and temperature measurements were used for 1000 Monte Carlo simulations. Unless stated otherwise, the error bars in the final results represent the standard deviation of these simulations. All analyses were performed in R (version 3.3.2) for Linux (R Core Team, 2017).

2.3 Results

2.3.1 Temperature, water content and CO_2 concentration in the profile

Soil temperature showed a distinct seasonality down to 150 cm, with the maximum and the minimum temperatures delayed with increasing soil depth (Figure 2.3a). The minimum soil temperature was 0.3 °C and 4.0 °C in January 2016 at 10 cm and 150 cm depths respectively. The maximum temperature was measured in July in the uppermost layer (16.6 °C) and in August in the deepest layer (14.4 °C). The annual amplitude of soil temperature decreased from 16.3 °C at 10 cm to 10.4 °C at 150 cm. However, mean annual values showed no significant decline with soil depth and were 8.4 °C and 8.3 °C at 10 cm and 150 cm respectively during the two years of observation. Variations in the mean soil temperatures between the three observatories were < 1 °C at all depths (Figure S3.1).

The volumetric water contents also showed seasonal variations at all depths (Figure 2.3b), with depletion during the summer. The minimum of volumetric water content at 10 cm was reached in August (10 %), whereas the minimum at 150 cm was observed two months later in October (6 %). The water reservoir of the soil profile was refilled during the autumn and winter, reaching maximum values at 10 cm (23 %) and 150 cm (22 %) in April (Figure 2.3b), which were delayed by 14 days in the deepest layer. In OB1 and OB3, the mean volumetric water content decreased with increasing soil depth. Only in OB2 did the mean water content increase at 150 cm (Figure S3.2). The water content showed a greater variation between the three observatories than soil temperature (Figure S3.2).

The CO_2 concentration in the soil pores followed a similar seasonality as soil temperature (Figure 2.3c), with a maximum during the summer and a minimum during the winter and early spring. The same behavior was observed for both investigated years, while the values were higher during the first summer. The CO_2 concentration in the uppermost layer ranged from 1,000 to 35,000 µmol mol^{-1} and thus was in a similar range of results for the deepest layer with 7,500 to 35,000 µmol mol^{-1}. However, values were highly variable between the observatories, with OB2 and OB3 showing an increasing CO_2 concentration with greater soil depth, whereas OB1 yielded the highest CO_2 concentrations at 30 to 50 cm depth.

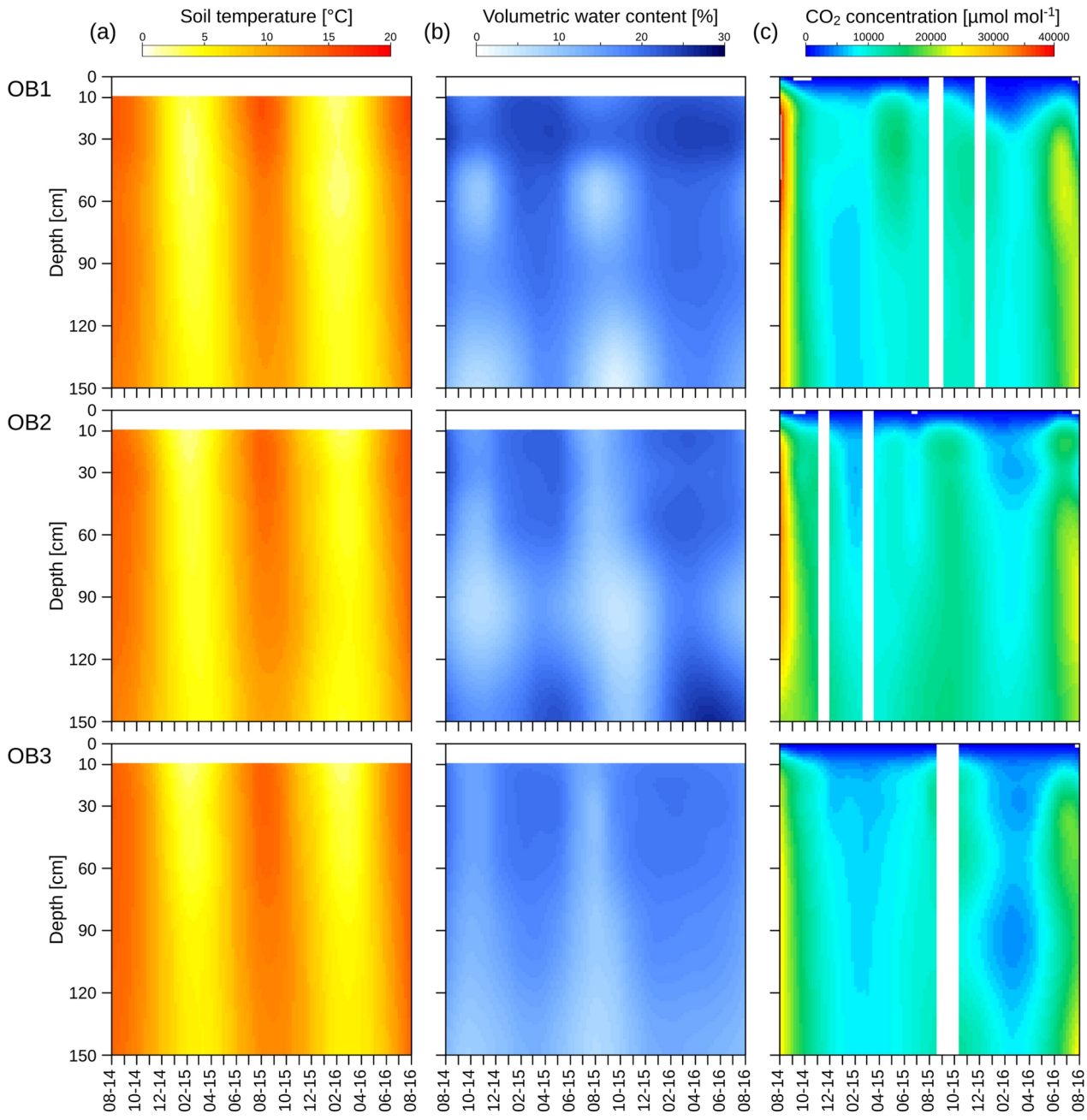

Figure 2.3: Soil profile measurements of temperature (a), volumetric water content (b) and CO_2 concentration for the three observatories (OB). White bars represent periods without measurements

2.3.2 Soil respiration

The mean annual mineral (without the organic layer) soil respiration determined with chamber measurements for the three observatories was 776 ± 193 g C m^{-2} yr^{-1}, with a small variability between the observatories (Table 2.1). The mineral soil respiration modeled with the Lloyd-Taylor function

gave similar results for the same period. In contrast, soil respiration determined with the gradient method showed a high variability between the observatories, but was in the range of the directly measured respiration, except for OB1. This variability can be explained by the higher water content at OB1 and consequently the lower diffusion coefficient. The average diffusion coefficient at OB1 at 10 cm was less than half that at OB2 and OB3. The organic layer increased total respiration by 13 % and 25 % respectively for the Lloyd-Taylor model and chamber measurements (Table 1). For all the methods and in all the observatories, soil respiration correlated well with soil temperature and soil moisture. The highest fluxes were measured when soil temperature (10 cm) was highest and water content (10 cm) was low (Figure 2.3 and Figure 2.4).

Table 2.1: Total soil respiration from August 2014 to August 2015 in [g C m^{-2} yr^{-1}] with and without the organic layer for the three observatories derived from soil surface measurements with linear interpolation (Chamber), modeled with a Lloyd-Taylor function and derived from the gradient method based on CO_2 measurements along the soil profile for one year. Means and standard deviations.

Observatory	Without organic layer			With organic layer	
	Chamber	Llyod-Taylor	Gradient method	Chamber	Llyod-Taylor
OB1	699 (180)	778	469 (2)	923 (70)	990
OB2	804 (211)	780	847 (4)	860 (273)	816
OB3	824 (204)	916	1012 (4)	1120 (349)	980
Mean	776 (193)	825 (79)	776 (278)	967 (266)	929 (98)

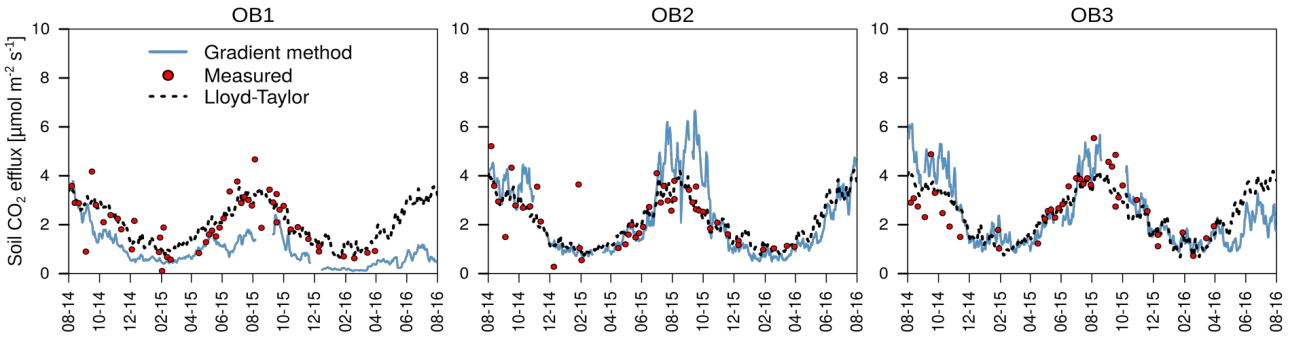

Figure 2.4: Mean daily soil respiration determined with the gradient method, measured with chambers and modeled with a Lloyd-Taylor function for the observatories (OB)

2.3.3 Vertical CO_2 production

The mean CO_2 production rates decreased from 1.4 µmol m^{-2} s^{-1} in the uppermost layer (0–10 cm depth) to 0.03 µmol m^{-2} s^{-1} in the deepest layer (50–90 cm depth) (Figure 2.5). The CO_2 production followed the same seasonality as soil temperature and CO_2 concentration, with the highest

productions rates occurring during the summer and the lowest during the winter months in all soil layers. This seasonal variation was greatest in the top two layers of the soil (0–10, 10–30 cm) (Figure 2.5a-d).

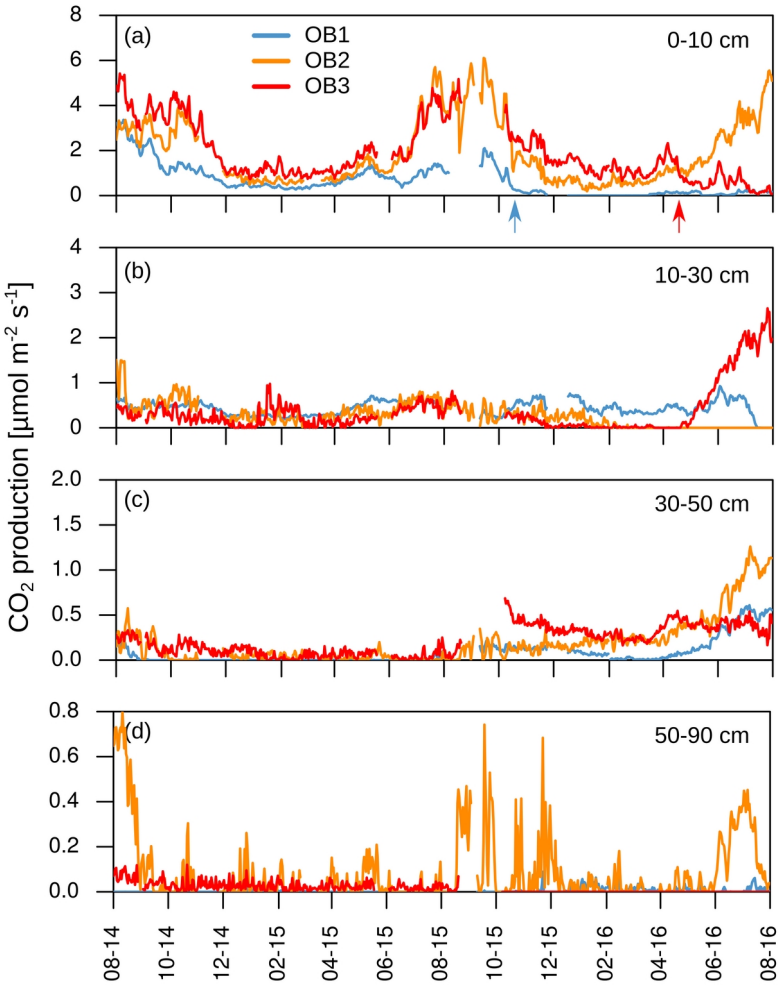

Figure 2.5: Daily mean CO_2 production in each soil layer (a)-(d). Arrows indicate disturbance due to bioturbation of voles close to the CO_2 sensors in 10 cm depth (OB1 and OB3), which created macropores and changed diffusivity.

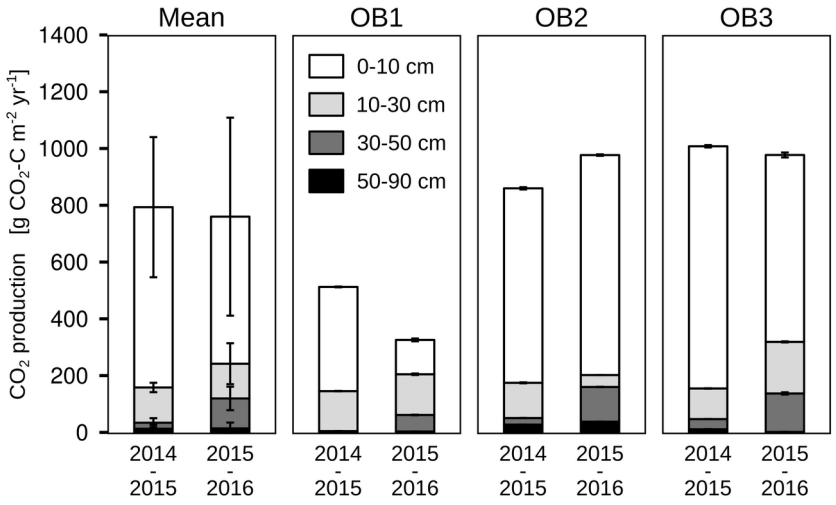

Figure 2.6: Cumulative CO_2 production for each soil layer, observatory (OB) and year of observation. Error bars represent standard deviation.

About 70 ± 17 % of total soil respiration was produced in the first 10 cm of the soil profile where 21 % of the SOC stock (0–1.5 m) was stored. The CO_2 production at 10 to 30 cm accounted for 20 ± 14 % of total soil respiration during the year, and 32 % of the SOC was located in this depth increment. The subsoil (> 30 cm) accounted for 10 ± 9 % of total CO_2 production, with 47 % of the SOC stock stored in the subsoil. The mean total CO_2 production showed no significant differences between the two years. The variation in total annual CO_2 production was greater between the three observatories (326–1,008 g CO_2-C m^{-2} yr^{-1}) than between the two studied years (Figure 2.6). However, the CO_2 production in the different soil layers showed considerable changes with time: it increased by 500 % in the subsoil from 30 to 50 cm in the second year, which increased the contribution of subsoil CO_2 production from 4 % to 16 % of total CO_2 production. This increase was observed in all three observatories. In contrast, the CO_2 production in the first 10 cm in OB1 and OB3 showed a decline from the first to the second year, which was probably caused by methodological variations and does not represent a real decrease in respiration activity since bioturbation of animals (e.g. voles) might have had a strong influence on diffusivity (Figure 2.5a). Voles created macropores, therefore the CO_2 gradient approach was not applicable. This was also indicated by a sudden and rapid drop of CO_2 production between 0 and 10 cm in OB1 (October 2015) (Figure 2.5a). To take the different SOC contents of each soil layer into account, the cumulative CO_2 production was normalized to the SOC stock of the respective layer (Figure 2.7). The specific CO_2 production

decreased from 322 g CO_2-C kg^{-1} SOC yr^{-1} in the first 10 cm to 9 g CO_2-C kg^{-1} SOC yr^{-1} at 50 to 90 cm. It should be noted that the proportion of autotrophic respiration in the total CO_2 production could not be quantified.

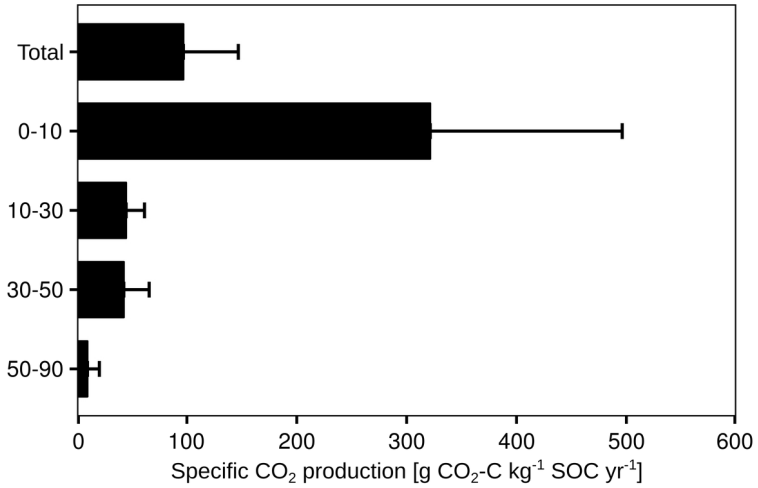

Figure 2.7: Annual specific CO_2 production for the total CO_2 efflux. Mean (n=3) and standard deviation.

2.3.4 Sources of CO_2 production

Contribution of fresh litter

The isotopic signature of soil CO_2 ($\delta^{13}CO_2$) in the observatories before the start of labeling experiment ranged from -25.4 ‰ to -21.8 ‰, with no significant differences between soil depths (Figure 2.8a). The labeling experiment was conducted to assess the fate of fresh litter added on top of the organic layer into different C fractions (e.g. SOC and DOC) including soil CO_2. Six days after the application of the ^{13}C-labeled leaf litter, CO_2 was already enriched in litter-derived C down to 90 cm depth in all the observatories. The isotopic signature ranged from 70 ‰ at 10 cm depth to -19 ‰ at 90 cm depth (Figure 2.8b). Thus, the maximum contribution of litter-derived C to total CO_2 was 5 % at 10 cm depth six days after the litter replacement (Figure 2.8c). At 90 cm, the maximum amount of litter-derived CO_2 was 0.6 % two weeks after the beginning of the labeling experiment (Figure 2.8c). In addition, minor peaks with up to 0.8 % of CO_2 derived from the labeled litter were observed at all depths after rain events within the first six months of litter application. The average contribution of litter-derived CO_2 decreased with time and reached a range of 2.5 % to 0.2 % at 10 cm depth from

January 2015 to July 2016. The total amount of labeled litter-derived C to the CO_2 production below 10 cm was 408 mg C m^{-2} (± 329) (Figure 2.9), which accounted for 0.18 % of total CO_2 production below 10 cm depth.

Assuming that diffusion is the main transport process of CO_2 in the soil atmosphere, the CO_2 flux between two soil layers can be calculated for each C isotope separately. As mentioned, a positive flux indicates release of CO_2 from mineralization or root respiration in the respective soil layer. A negative flux in turn represents downward diffusion of CO_2 from the layer above. Due the high ^{13}C enrichment of the applied litter, negative $^{13}CO_2$ fluxes can indicate a downward diffusion of litter-derived CO_2 from the soil layer above (Figure 2.10). On average for the three observatories, 20 out of 41 sampling had negative $^{13}CO_2$ fluxes below 90 cm depth, indicating a downward movement of labeled litter-derived CO_2. Further, OB2 and OB3 had positive $^{13}CO_2$ fluxes between 10 to 90 cm, indicating a transport of labeled litter-derived C down the soil profile as dissolved organic carbon (DOC) and mineralization of this DOC. While, the observed ^{13}C enrichment in CO_2 in OB1 below 30 cm depth might also be influenced by diffusion of labeled litter-derived $^{13}CO_2$ from the soil layer above (10 to 30 cm).

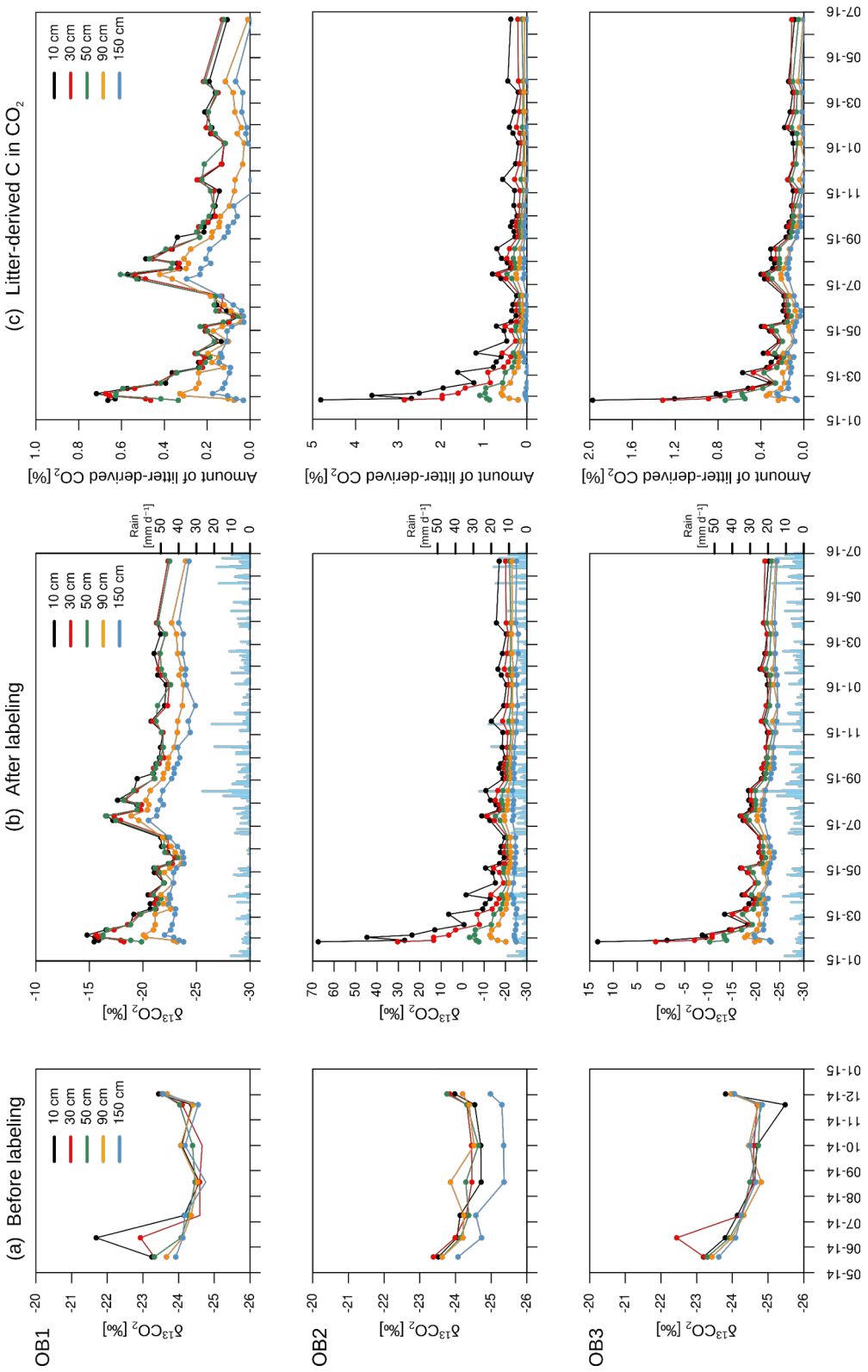

Figure 2.8: Isotopic signature of CO_2 at each depth and observatory (OB) before the addition of the labeled litter (a) and after labeled litter addition (b) with daily precipitation data (blue bars). The relative amount of litter-derived CO_2 on total CO_2 in each depth and observatory (c). Please note the different y-axis ranges for (b) and (c).

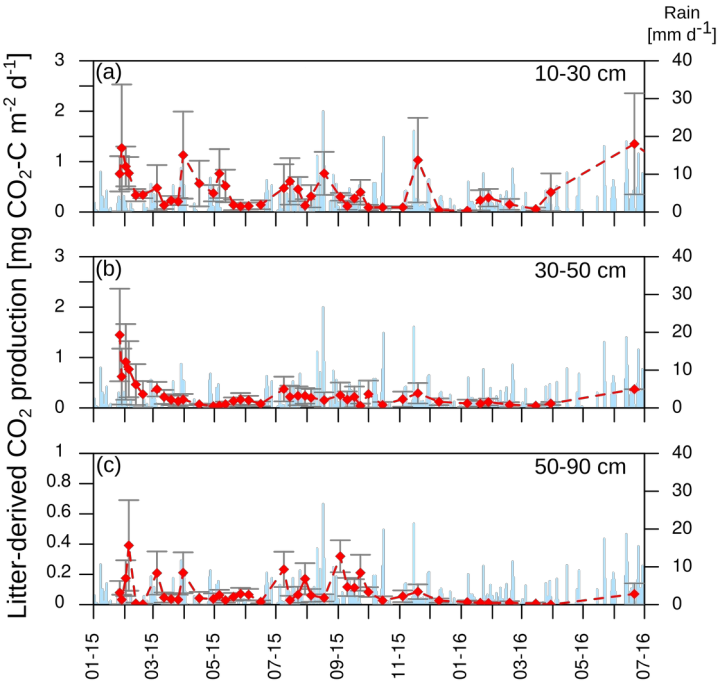

Figure 2.9: Litter-derived CO_2 production in each soil layer (a)-(c). Mean (n=3) and standard error

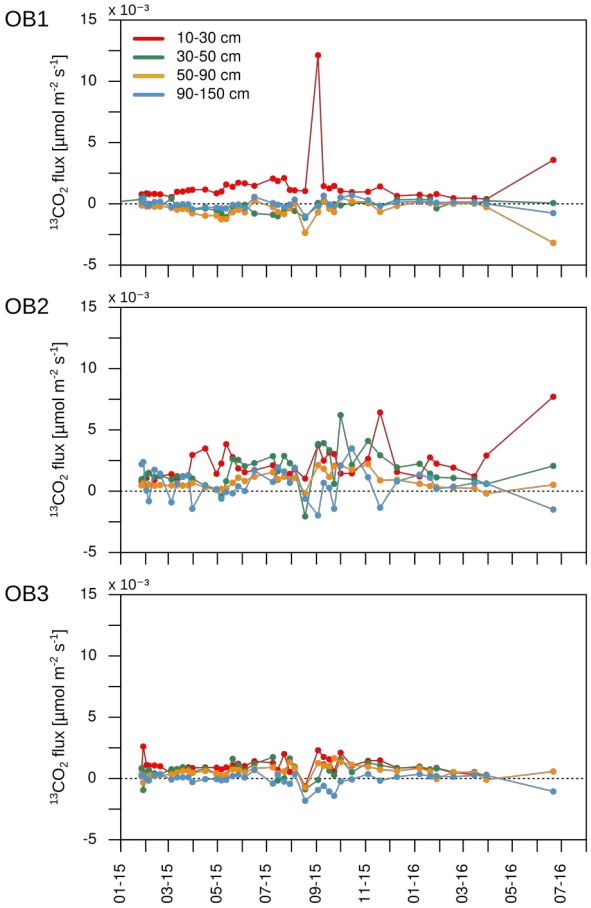

Figure 2.10: $^{13}CO_2$ fluxes for each observatory. Negative fluxes represents diffusion of $^{13}CO_2$ from the soil layer above.

Contribution of old C

The radiocarbon content of the bulk SOC decreased strongly with increasing soil depth from close to atmospheric values ($F^{14}C$ 0.99) at 10 cm to an apparent age of about 3460 years BP ($F^{14}C$ 0.65) at 110 cm depth (Figure 2.11, grey triangles). In contrast, the ^{14}C concentrations of the CO_2 in the soil atmosphere were relatively constant throughout the soil profile and for both samplings, with values in the range of 1.03–1.07 $F^{14}C$ and thus derive mainly from the post-bomb period (Figure 2.11, black dots). This indicates a young source of CO_2 production. Consequently "old" subsoil SOC was not detected as a significant source of CO_2 production.

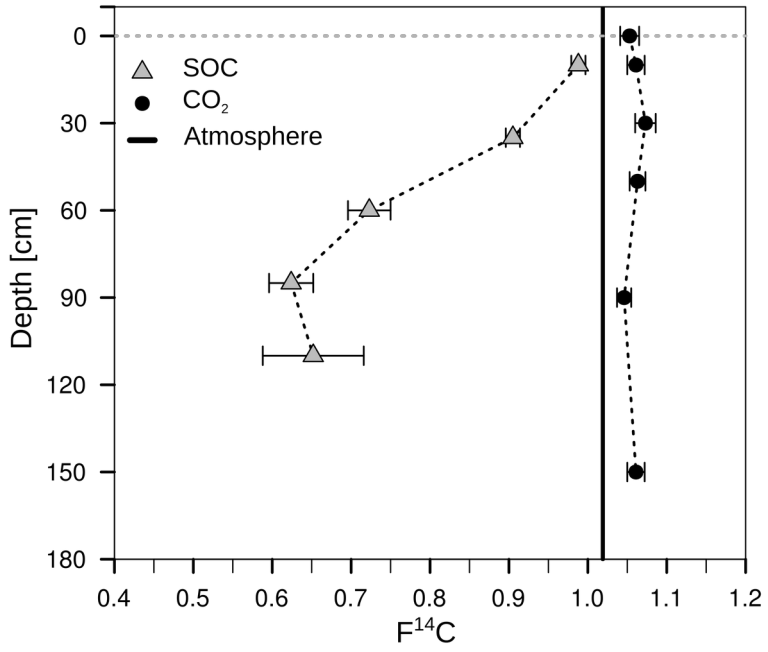

Figure 2.11: Mean ^{14}C concentration ($F^{14}C$) of bulk SOC (grey triangles; data from Angst et al. (2016) and CO_2 in the soil atmosphere (black dots). The solid black lines represents the annual average $F^{14}C$ value in the atmosphere from 2014 measured at the Jungfraujoch alpine research station, Switzerland (Levin and Hammer, pers. communication).

2.4 Discussion

2.4.1 Temperature, water content and CO_2 concentration in the profile

In all three subsoil observatories, increasing CO_2 concentrations with depth were observed. This has also been reported by other studies (Davidson et al., 2006; Drewitt et al., 2005; Fierer et al., 2005; Hashimoto et al., 2007; Moyes and Bowling, 2012). However, the increase was not continuous down to 150 cm depth. Higher CO_2 concentrations were observed between 30 cm and 50 cm depth, indicating a higher CO_2 production at this depth increment, which can be linked to the root distribution in the subsoil observatories (Figure 2.12). About 82 % of the fine root biomass and necromass were found to be located between 0 and 50 cm, and 18 % at the 30 to 50 cm depth. Therefore, the contribution of autotrophic respiration to CO_2 production and the mineralization of dead roots were greater at these depths than in the deep subsoil (> 50 cm). The CO_2 concentration in the soil pores is also controlled by abiotic factors such as effective diffusivity (D_s). The average effective diffusivity (D_s) at 10 cm was about 40 % lower than at 30 cm. Consequently CO_2 accumulated in the soil pores below 10 cm depth due to the lower diffusion of CO_2 between the soil surface and 10 cm depth. The effective diffusivity was mainly controlled by soil water content, which reduced it. For example, the high CO_2 concentration in August 2014 (up to 40,000 µmol mol^{-1}) compared to August 2015 (up to 20,000 µmol mol^{-1}) (Figure 2.3c) can be explained by the higher volumetric water content in 2014 in all profiles. The high water content was related to more precipitation in July 2014 (120 mm) than in July 2015 (47 mm) and to less precipitation in August in both years (49 and 95 mm). Additionally, evapotranspiration was greater in August 2015 than in August 2014 due to a higher mean air temperature (18 °C and 15 °C).

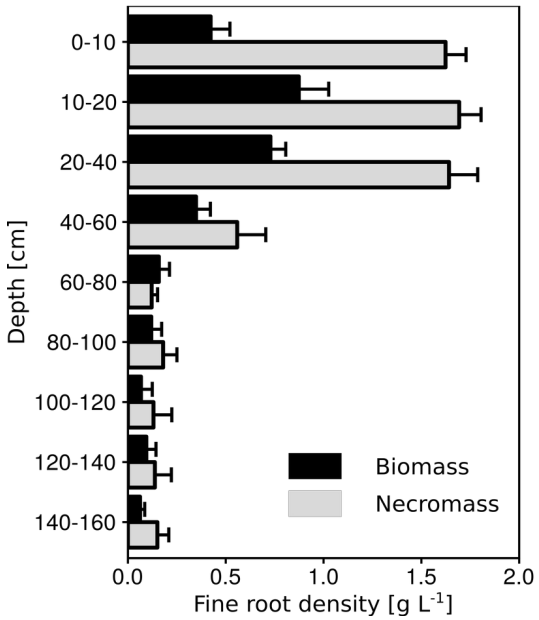

Figure 2.12: Mean fine root density for biomass and necromass of the subsoil observatories. Error bars represent standard error.

2.4.2 Soil respiration

The annual mean total respiration determined using the gradient method corresponded well with the results of the closed chamber measurements, indicating that the gradient method resulted in realistic flux estimations (Table 2.1, Figure 2.4). This is in line with the results reported by other studies (Baldocchi et al., 2006; Liang et al., 2004; Tang et al., 2003). The differences in soil respiration between the methods can be attributed to the different spatial resolution of the corresponding measurements. The chamber measurements were based on five spatial replicates for each subsoil observatory, covering a total measurement area of 1274 cm^2. Therefore chamber measurements accounted for spatial variability in water content and soil CO_2 concentrations below the chamber, whereas the gradient method was based on one profile measurement for CO_2 and water content at each of the three observatories. Large differences in total respiration rates of up to 200 % were found between the three observatories with the gradient method. Both methods have advantages and disadvantages for determining total soil respiration. The gradient method does not alter the soil atmosphere CO_2 gradient and is continuous and less time-consuming than chamber measurements, but it is vulnerable to the spatial heterogeneity of the soil structure, moisture content around the sensors and to changes in diffusivity, e.g. due to bioturbation. For example, the higher soil respiration determined with the gradient method at OB2 and OB3 in summer (Figure 2.4) is linked to lower soil moisture

measured in 10 cm depth (Figure 2.3b) and to higher total soil porosity (51 % OB2, 49 % OB3 vs. 46 % OB1). In consequence, the effective diffusivity (Eq. 2.4) is higher, resulting in higher fluxes. Further, the lower soil respiration of OB1 and OB3 in the second year determined with the gradient method was related to bioturbation of voles, which increased the diffusivity around the CO_2 sensors and leading to a lower CO_2 concentration in 10 cm depth, which in turn led to an underestimation of total soil respiration (Figure 2.4) by the gradient method.

Removing the organic layer in the soil collars was supposed to determine the contribution of CO_2 production in the organic layer to total soil respiration. Since the organic layer was only removed in the soil collars and not around the soil collars, it must be noted that the contribution of the organic layer to total soil respiration might be underestimated with the used method. However, the results are in line with findings from litter manipulation experiments, which reported a contribution of 9 % to 37 % of the organic layer to total soil respiration (Bowden et al., 1993; Kim et al., 2005; Nadelhoffer et al., 2004; Sulzman et al., 2005).

2.4.3 Vertical CO_2 production

The vertically partitioned CO_2 flux revealed that more than 90 % of total CO_2 efflux was produced in the topsoil (< 30 cm). These results correspond well with other studies which have found that more than 70 % of total CO_2 efflux in temperate forests is produced in the upper 30 cm of the soil profile (Davidson et al., 2006; Fierer et al., 2005; Hashimoto et al., 2007; Jassal et al., 2005; Moyes and Bowling, 2012). Nevertheless, only 53 % of the SOC stock is stored in the first 30 cm, indicating that subsoil SOC on the site of the present study may have a slower turnover than topsoil SOC. This is supported by the low ^{14}C concentrations in SOC below 30 cm. However, the higher CO_2 production in the topsoil can be also related to greater fine root biomass and necromass density (Figure 2.12), which may serve as an indicator of autotrophic respiration and heterotrophic respiration in the rhizosphere. Even if the current study is unable to distinguish between autotrophic and heterotrophic respiration, the importance of autotrophic respiration to total soil respiration was shown in a large scale girdling experiment by Högberg et al. (2001). They reported that autotrophic respiration accounted for up to 54 % on total soil respiration. In consequence, autotrophic respiration should be higher in the topsoil than in the subsoil, due to the decreasing root bio- and necromass with increasing soil depth (Figure 2.12).

It is remarkable that the CO_2 production at 30 to 50 cm increased from 23 g C m^{-2} yr^{-1} in the first year to 118 g C m^{-2} yr^{-1} in the second year of the study (Figure 2.6). This can be explained in part by more precipitation in the second year (621 mm) than in the first year (409 mm), inducing less

water-limiting conditions for plants and microbial activity. As a result, the mean volumetric water content was higher in the second year (18 % compared to 16 %) at 50 cm depth, which gave better conditions for the mineralization of SOC by microorganisms (Cook et al., 1985; Moyano et al., 2012). Furthermore, the greater precipitation increased the input of DOC into the subsoil on the site of the present study, which is supported by the study of (Leinemann et al., 2016) who investigated DOC fluxes in subsoil observatories for more than 60 weeks. They found a positive correlation between DOC fluxes, precipitation and water fluxes at 10, 50 and 150 cm depths. Furthermore, they showed that DOC fluxes declined by 92 % between a depth of 10 cm and 50 cm, which was attributed to mineral adsorption and microbial respiration of DOC (Leinemann et al., 2016).

2.4.4 Sources of CO_2 production

Young litter derived CO_2

In this study, a unique labeling approach was used to estimate the contribution of aboveground litter to CO_2 production along a soil profile by applying stable isotope-enriched leaf litter to the soil surface. These results showed that litter-derived C did not significantly contribute to annual CO_2 production below 10 cm depth. Leaf litter is decomposed and washed into the mineral soil as DOC. Within one year, only 0.12 % of total CO_2 production between 10 and 90 cm originated from the labeled leaf litter. Therefore, mineralization of DOC originating from the organic layer was a minor source of CO_2 production in the soil profile below 10 cm. The average DOC flux in the subsoil observatories in the first year was estimated to be 20 g C m^{-2} yr^{-1} at 10 cm depth and 2 g C m^{-2} yr^{-1} at 50 cm depth, indicating a DOC input of 18 g C m^{-2} yr^{-1} into the 10 and 50 cm depth increments (Leinemann et al., 2016). An assumed complete mineralization of this DOC would account for 11 % of CO_2 production at this depth increment. Overall, most of the CO_2 production between a depth of 10 cm and 90 cm must be derived from autotrophic respiration and heterotrophic respiration in the rhizosphere.

Old C derived CO_2

The very similar radiocarbon contents of soil CO_2 produced at different depths, which were 1.06 $F^{14}C$ on average, revealed that ancient SOC components were not a major source of CO_2 production. The results indicate that the CO_2 originated mainly from young (several decades old) C sources, presumably mainly from root respiration, its exudates and DOC. Other studies have found similar results on a grassland site in California down to 230 cm depth (Fierer et al., 2005) and in tem-

perate forests down to 100 cm (Gaudinski et al., 2000; Hicks Pries et al., 2017b). In addition, Hicks Pries et al. (2017) incubated root-free soil from three depths (15, 50 and 90 cm) and compared the radiocarbon signature of the respired CO_2 with their results from the field. They found that CO_2 from the short-term incubations had the same modern signature as the field measurements, despite the high ^{14}C age of the bulk SOC at 90 cm depth (1000 yr BP) (Hicks Pries et al., 2017b). This supports the findings of the present experiment. Therefore, microbial respiration in temperate subsoils is mainly fed by relatively young C sources fixed less than 60 years ago.

Diffusion effects

A highly ^{13}C-enriched CO_2 source was introduced to the top of a soil profile. Shortly afterwards, an enrichment of ^{13}C was measured in CO_2 along the whole soil profile (Figure 2.8b). However, this enrichment could not only be linked to transport and mineralization of litter-derived C along the soil profile (e.g. DOC in seepage water). The diffusion of $^{13}CO_2$ down the soil profile has also to be taken into account. According to Fick's first law, $^{13}CO_2$ diffuses into the soil profile following the $^{13}CO_2$ gradient independently from the $^{12}CO_2$ gradient. Thus even though the total CO_2 concentration increased with soil depth, meaning an upward diffusion of $^{12}CO_2$, the $^{13}CO_2$ gradient could be the opposite due to ^{13}C-enriched leaf litter leading to a downward diffusion of $^{13}CO_2$. Consequently this could lead to a misinterpretation of the pathways of subsoil $^{13}CO_2$ in tracer experiments. Furthermore, this effect should also be taken into consideration when interpreting $^{14}CO_2$ soil profile measurements as an indicator of the age of the mineralized SOC, as in other field studies (Davidson et al., 2006; Davidson and Trumbore, 1995; Fierer et al., 2005; Gaudinski et al., 2000). Downward diffusion of $^{14}CO_2$ might be an important factor for explaining the observed $^{14}CO_2$ profiles. If this downward diffusion is the case, the $^{14}CO_2$ gradient should not have a continuous decrease with soil depth since the $^{14}CO_2$ gradient is the driving factor for diffusion according to Eq. (2.3). In fact, $^{14}CO_2$ concentration at 30 cm depth in subsoil OB1 was greater than at 50 cm depth (Figure 2.13), which in turn led to a downward diffusion of $^{14}CO_2$ from a depth of 30 cm to 50 cm. This might lead to a rejuvenation of the $^{14}CO_2$ soil profile and to an underestimation of the mineralization of old SOC in subsoils.

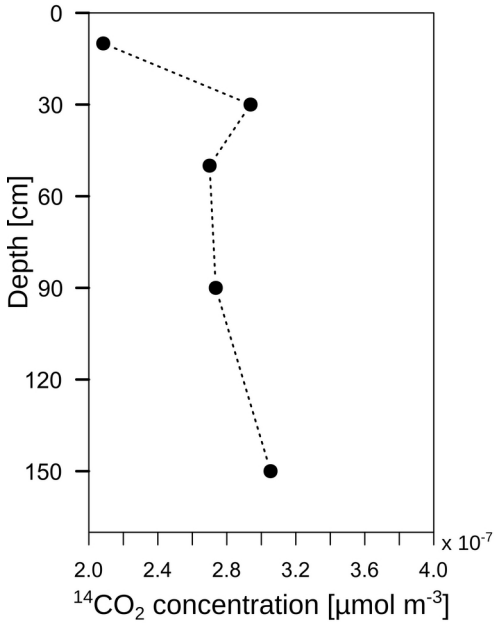

Figure 2.13: Soil air $^{14}CO_2$ concentration in observatory 1 from December 2014.

2.5 Conclusion

The gradient method allowed total soil respiration to be partitioned vertically along a soil profile. Most of the CO_2 (90 %) was produced in the topsoil (< 30 cm). However, the subsoil (> 30 cm), which contained 47 % of SOC stocks, accounted for 10 % of total soil respiration. This can be explained by a larger amount of stable SOC in subsoils as compared to topsoils. However, the modern radiocarbon signature of CO_2 throughout the soil profiles indicated that mainly young carbon sources were being respired from roots and root exudates and autotrophic respiration. The contribution of old SOC to subsoil CO_2 production was too small to significantly alter the ^{14}C concentrations in the soil atmosphere used to identify CO_2 sources. Furthermore, this study showed that the mineralization of fresh litter-derived C only contributed to a small part of total soil respiration, underlining the importance of roots and the rhizosphere for subsoil CO_2 production.

CHAPTER 3 CONTROLLING FACTORS FOR THE STABILITY OF SUBSOIL CARBON IN A DYSTRIC CAMBISOL

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Abstract

Subsoils store than 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_2 production of undisturbed soil samples from topsoil and two subsoil depth increments was measured at two different temperatures (10 °C and 20 °C). Additionally, ^{13}C labeled root litter was added to the different samples and measurements of the isotopic signature of the respired CO_2 allowed a differentiation between SOC mineralization and root mineralization. The CO_2 production per unit soil mass was lower in deep subsoil than in the topsoil, but the CO_2 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 $Q_{10\text{-}q}$, 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 $Q_{10\text{-}q}$ 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.

3.1 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 more than 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_2 availability and higher CO_2 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).

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 Lützow 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}C labeled roots) at 10 °C and 20 °C. The 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}C labeled roots allows to differentiate between 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.

3.2 Materials and methods

3.2.1 Site description

Soil samples were taken in the Grinderwald, 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.

3.2.2 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 ($subsoil_{30}$) and 130–160 cm ($subsoil_{130}$). 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 3.1 contains the general soil parameters of the topsoil and subsoil samples.

Table 3.1: Soil parameters of the topsoil and subsoil samples and the used root litter. Means and standard errors (n = 4).

	C [mg g^{-1}]		N [mg g^{-1}]		C/N		$\delta^{13}C$ [‰]		pH ($CaCl_2$)		Sand [%]		Silt [%]		Clay [%]	
Roots	402.1	(3.4)	11.3	(0.1)	35.5	(0.1)	151.1	(3.6)								
Topsoil	13.3	(0.9)	0.7	(0.06)	19.5	(0.7)	-28.0	(0.05)	3.4	(<0.1)	70.6	(2.4)	25.4	(2.2)	4.0	(2.4)
$Subsoil_{30}$	4.5	(0.7)	0.3	(0.07)	17.1	(1.7)	-26.6	(0.1)	4.2	(<0.1)	70.9	(6.0)	26.1	(5.8)	3.0	(0.3)
$Subsoil_{130}$	0.4	(0.09)	0.04	(0.01)	8.6	(0.8)	-25.8	(0.1)	4.1	(0.1)	91.1	(5.2)	6.6	(4.2)	2.3	(1.1)

3.2.3 Experimental design

The CO_2 production of soil samples from topsoil, subsoil 30 and subsoil 130 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}C-labeled root litter

iii.) Control: Disturbed soil without addition of ^{13}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}C-labeled and ground ash roots ($\delta^{13}C$ of 151 ‰) at a bulk density of 1.4 (topsoil) to 1.6 (subsoil) g cm^{-3} , corresponding to the soil samples of the undisturbed treatment. The labeled roots originated from young trees grown in a greenhouse under a $^{13}CO_2$ -enriched atmosphere ($\delta^{13}C$ 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}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 CO_2 free synthetic air (20 % O_2 and 80 % N_2) using a constant flow rate of 10 mL min^{-1}. The C mineralization was determined by measuring the 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 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 CO_2 production via the CO_2 accumulation in the headspace. For the control and root addition treat-

ment, 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.

3.2.4 Gas and soil analysis

The 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, USA). The CO_2-production [mg CO_2-C d^{-1}] was calculated with Eq. (3.1):

$$CO_2\text{-production} = \left(\frac{CO_2 \times M \times T_n \times V_0 \times d}{V_m \times (T_n + T)} \right) \quad (3.1)$$

where CO_2 is the CO_2 concentration change [ppm h^{-1}] between the two samplings at each sampling day, M is the molar weight of C [g mol^{-1}], T_n is the norm temperature [273.15 K], T is the incubation temperature [°C], V_0 is the volume of the microcosm headspace [m^3], d is the time of one day [24 h] and V_m is the molar volume of an ideal gas [22.4136 L mol^{-1}]. The $\delta^{13}C$ values of the headspace CO_2 were measured by isotope ratio mass spectrometer (Thermo Fisher Scientific MAT 253, Bremen, Germany) connected to a Gasbench II (Thermo Fisher Scientific MAT 253, Bremen, Germany) and a PAL autosampler (CTC Analytics AG,Zwingen, Switzerland). In the root addition treatment, native SOC mineralization [mg CO_2-C d^{-1}] CO_2-SOC was calculated using Eq. (3.2):

$$CO_2\text{-SOC} = CO_2\text{-production} \times \left(\frac{\delta^{13}CO_2 - \delta^{13}C_{root}}{\delta^{13}CO_{2\,control} - \delta^{13}C_{root}} \right) \quad (3.2)$$

where CO_2-production is the amount of the total respired C, $\delta^{13}CO_2$ is the isotopic signature of the respired CO_2, $\delta^{13}C$ roots is the isotopic signature of the added ash roots and $\delta^{13}CO_{2control}$ is the average isotopic signature of the respired CO_2 of the control samples without root addition. By using the isotopic signature of the respired CO_2 for the native SOC we account for fractionation effects of the mineralization. The mineralization of added root material CO_2-root [mg CO_2-C d^{-1}] was obtained with Eq. (3.3).

$$CO_2\text{-root} = CO_2\text{-production} - CO_2\text{-SOC} \quad (3.3)$$

In order to account for the different SOC contents in topsoil and subsoil, specific respiration rates were obtained by dividing CO_2 -production through the initial SOC content [g] or added root C [g]. The C and N contents of the soil and the ash roots (Table 3.1) were measured by dry-combustion

with an elemental analyzer (LECO TruMac, LECO, St. Joseph, USA). The stable isotope ratios of the initial soil and the added roots (Table 3.1) were determined using an isotope ratio mass spectrometer (Delta Plus, Thermo Fisher Scientific, Bremen, Germany) coupled to an elemental analyzer (CE Instruments FLASH EA 1112, Thermo Fisher Scientific, Bremen, Germany).

3.2.5 Temperature sensitivity and priming

Temperature sensitivity of SOC and root mineralization was expressed with the $Q_{10\text{-}q}$ parameter. The common Q_{10} calculation, as the ratio of C respired at the higher temperature to C respired at the lower temperature after a fixed time, ignores the fact that the labile SOC pool is mineralized faster at higher temperatures than at lower temperatures. In consequence, the temperature sensitivity expressed as Q_{10} over a fixed time is confounded due to changes in the source of mineralization (Reichstein et al., 2000). Therefore, we used the $Q_{10\text{-}q}$ method described in Conant et al. (2008). The advantage of this method is that it allows to compare $Q_{10\text{-}q}$ values of different SOC pools with different stability against mineralization (Conant et al., 2008). Due to the incubation time of 63 days in this study, it can be assumed the determined $Q_{10\text{-}q}$ reflects mainly the labile SOC pool that has been mineralized during the incubation. On average 0.2–0.8 % of the SOC was mineralized during the incubation. The $Q_{10\text{-}q}$ values for the SOC and root mineralization were determined with Eq. (3.4)

$$Q_{10-q} = \frac{t_C}{t_W}\left(\frac{10}{T_W - T_C}\right) \tag{3.4}$$

where t_C is the time required to respire 0.1 % of SOC (1.5 % of added root C) at 10 °C (T_C) over t_W the time needed to respire 0.1 % of SOC (1.5 % of added root C) at 20 °C (T_W). The priming effect induced due to root addition was estimated by comparing the specific cumulative SOC mineralization of the control samples with the root addition samples at the end of the incubation. To determine priming effects over time, a dimensionless priming factor P, was calculated with Eq. (3.5)

$$P = \frac{CO_{2root}}{CO_{2\,control}} - 1 \tag{3.5}$$

where CO_{2root} is the daily amount of native SOC respired in the root addition treatment, and $CO_{2control}$ the daily amount of SOC respired from the control sample. A priming factor smaller than zero indicates a lower mineralization of native SOC in the root addition treatment than in the control treatment (negative priming), whereas a priming factor larger than zero indicates the enhanced decomposition of native SOC due to substrate addition and was called positive priming.

3.2.6 Statistical analysis

The normal distribution of the residuals was tested with the Shapiro-Wilk test and the homogeneity of variance was tested with Levenes test. A generalized least square linear model was used for the undisturbed treatment to account for different variances in the different sampling depths. The differences of the cumulative SOC mineralization among different soil depths were verified by a two-way ANOVA of the model with depth and temperature as fixed factors. The differences in temperature sensitivity for each treatment were tested with a one-way ANOVA and depth as fixed factor. To evaluate differences among depths of the specific cumulative root-derived C mineralization, a two-way ANOVA with depth and temperature as fixed factors was used. To test the root addition effect on the cumulative SOC mineralization, a two-way ANOVA was also performed with depth and treatment as fixed factors. As post-hoc test, a pairwise t-test was performed at the significance level of 0.05, p-values were adjusted with Holm correction (Holm, 1979). All statistical analyses were performed with R version 3.2.2 (Fire Safety) for Linux (R Core Team, 2015).

3.3 Results

3.3.1 SOC stability in topsoil and subsoil

Significantly more CO_2 per mass of soil was respired in the topsoil samples than in the subsoil samples. The total C mineralized at 10 °C incubation temperature decreased from 33 ± 8 mg CO_2-C kg^{-1} soil in the topsoil to 10 ± 1 mg CO_2-C kg^{-1} soil in $subsoil_{30}$ to 2 ± 1 mg CO_2-C kg^{-1} soil in $subsoil_{130}$ (Figure 3.1a). The same pattern was also observed at 20 °C but with around 200 % higher CO_2 production in the topsoil and 50 % higher CO_2 production in the $subsoil_{130}$. However, contrary to our hypothesis that SOC is more stable in subsoil than in topsoil, the C mineralization normalized to the SOC content showed that SOC was more stable in topsoil and $subsoil_{30}$ than in $subsoil_{130}$ (Figure 3.1b). In the following the CO_2 production normalized to the SOC content are called specific CO_2 production. $Subsoil_{130}$ respired three times more C per SOC than topsoil and $subsoil_{30}$ samples within 63 days of incubation at 10 °C. There were no significant differences between topsoil and $subsoil_{30}$ samples.

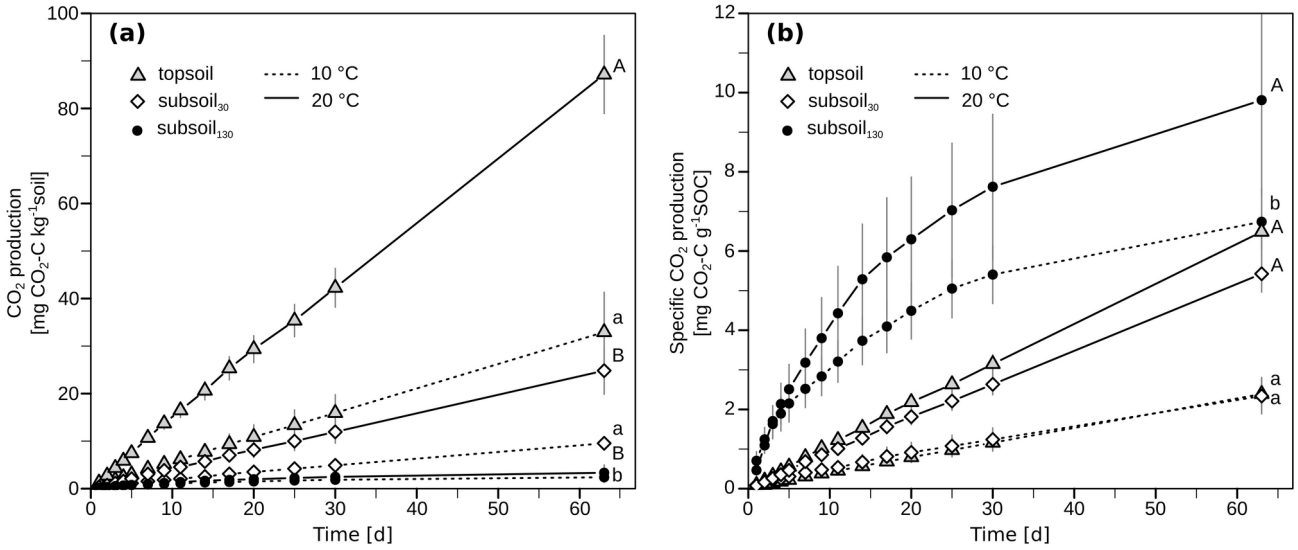

Figure 3.1: Cumulative CO_2 production of SOC in the undisturbed treatment for 10 °C (dashed lines) and 20 °C (solid lines). Values are expressed as (a) mg CO_2-C kg^{-1} dry soil equivalent, (b) mg CO_2-C g^{-1} SOC. Different letters indicate significant differences between the depths at 10 °C. Different capital letters indicates significant differences at the 20 °C. Means and standard errors (n = 4)

The highest daily specific respiration rates were observed in $subsoil_{130}$ (Figure 3.2) and also the highest decrease over the incubation time. In $subsoil_{130}$, the major part of the C mineralization took place early in the experiment. Two thirds of the total respired C in $subsoil_{130}$ was lost within the first

three weeks of incubation, topsoil and subsoil$_{30}$ only lost one third of their total respired C during this time. This trend was found for both temperature treatments. After day 30, the daily specific respiration rate of subsoil$_{130}$ was equal to topsoil and subsoil$_{30}$.

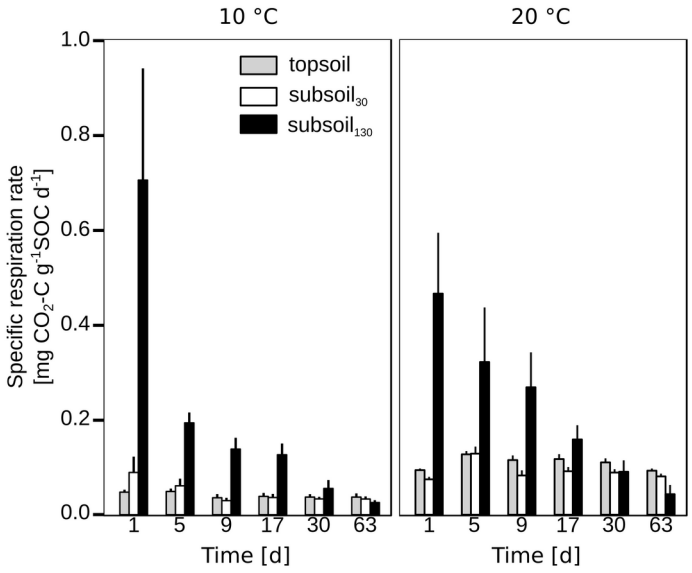

Figure 3.2: Specific daily respiration of SOC in the undisturbed treatment at 10 °C and 20 °C. Values are expressed as mg CO_2-C g^{-1} SOC d^{-1}. Means and standard errors (n= 4).

3.3.2 Temperature sensitivity of SOC and root mineralization

The temperature sensitivity of SOC mineralization decreased significantly from topsoil to subsoil$_{130}$, which contradicts our hypothesis of increasing temperature sensitivity with increasing soil depth. The temperature sensitivity was quantified by $Q_{10\text{-}q}$ values of the SOC, which decreased with increasing soil depth from 3 ± 0.5 in topsoil samples to 1 ± 0.3 in subsoil$_{130}$ samples (Figure 3.3). The decrease of $Q_{10\text{-}q}$ for SOC mineralization was observed for undisturbed samples, as well as for the control and native SOC of the root addition treatment. We also found no impact of a decreased substrate limitation on temperature sensitivity, since the temperature sensitivity of the native SOC mineralization in the root addition treatment was similar to the temperature sensitivity in the control treatment. The $Q_{10\text{-}q}$ was similar for the SOC with and without root addition (Figure 3.3), whereas the added root litter showed no significant differences in temperature sensitivity between the different soil depths.

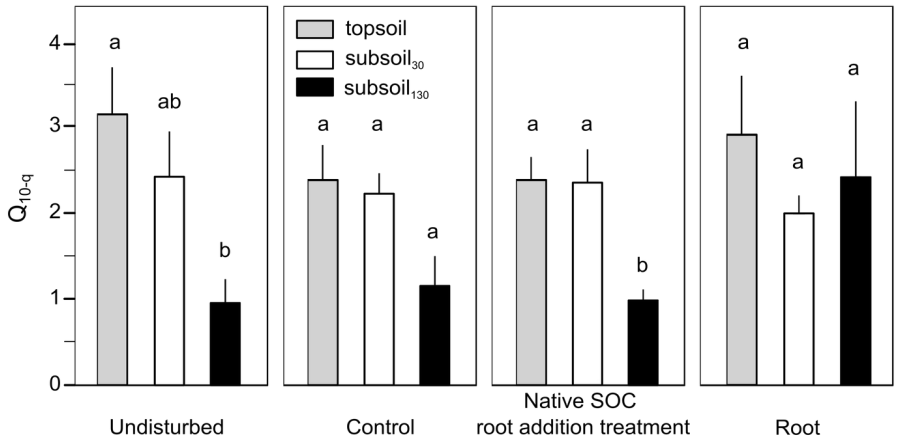

Figure 3.3: Temperature sensitivity (Q_{10-q}) for the topsoil and subsoil samples of the different treatments. Undisturbed, control and native SOC root addition treatment, represent the temperature sensitivity of SOC mineralization. Root represents the temperature sensitivity of the mineralization of the added root litter. Different letters indicate significant differences within each treatment. Means and standard errors (n = 4).

3.3.3 Mineralization of added root carbon in topsoil and subsoil

Significantly more of the added root C was respired in topsoil (7 ± 0.6 %) and subsoil$_{30}$ (6 ± 0.9 %) than in the subsoil$_{130}$ (3 ± 0.6 %) during the two months of the incubation experiment (Figure 3.4). At 20 °C around 30 % more root C was respired compared to the respective 10 °C incubation. However, this temperature effect was not significant.

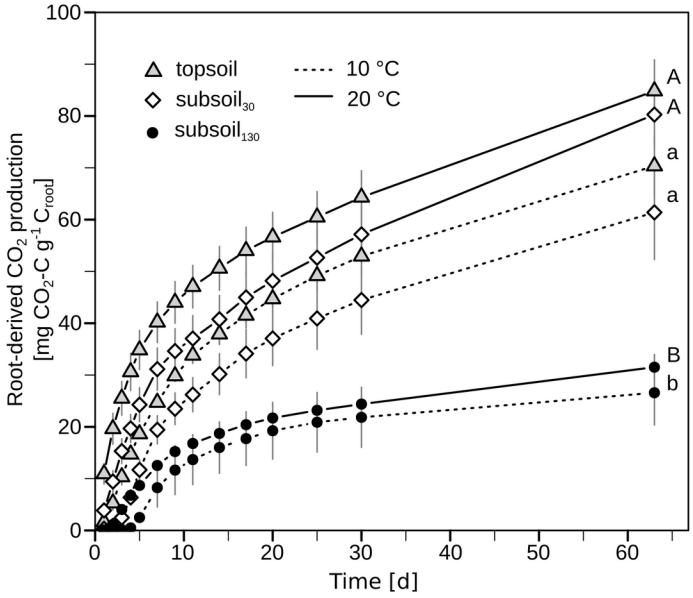

Figure 3.4: Cumulative CO_2 production of the added root litter for 10 °C (dashed lines) and 20 ° C (soil lines). Values are expressed as mg CO_2-C g^{-1} C_{root}. Different letters indicate significant differences between the depths at 10 °C. Different capital letters indicates significant differences at the 20 °C. Means and standard errors (n = 4).

The topsoil samples showed a fast response to root addition in respiration rates. The highest daily respiration rates of the added roots (Figure 3.5) were observed two days after the start of the incubation. In $subsoil_{30}$, the highest daily respiration rates of the added roots were reached at day 4, while there was a lag time of four days until the mineralization of root-derived C started in the deepest samples ($subsoil_{130}$) with maximum respiration rates being reached at day 5. Similar results were found for the 20 °C treatment, but with a two-day shorter lag time in $subsoil_{30}$ and $subsoil_{130}$. We found a non-linear relationship between total mineralized roots and the SOC content of the soil samples. Accordingly, decreasing amounts of roots were mineralized with decreasing SOC content of the surrounding soil, regardless of different microbial communities (Figure 3.6). The added roots were more stable in $subsoil_{130}$ than in topsoil or $subsoil_{30}$.

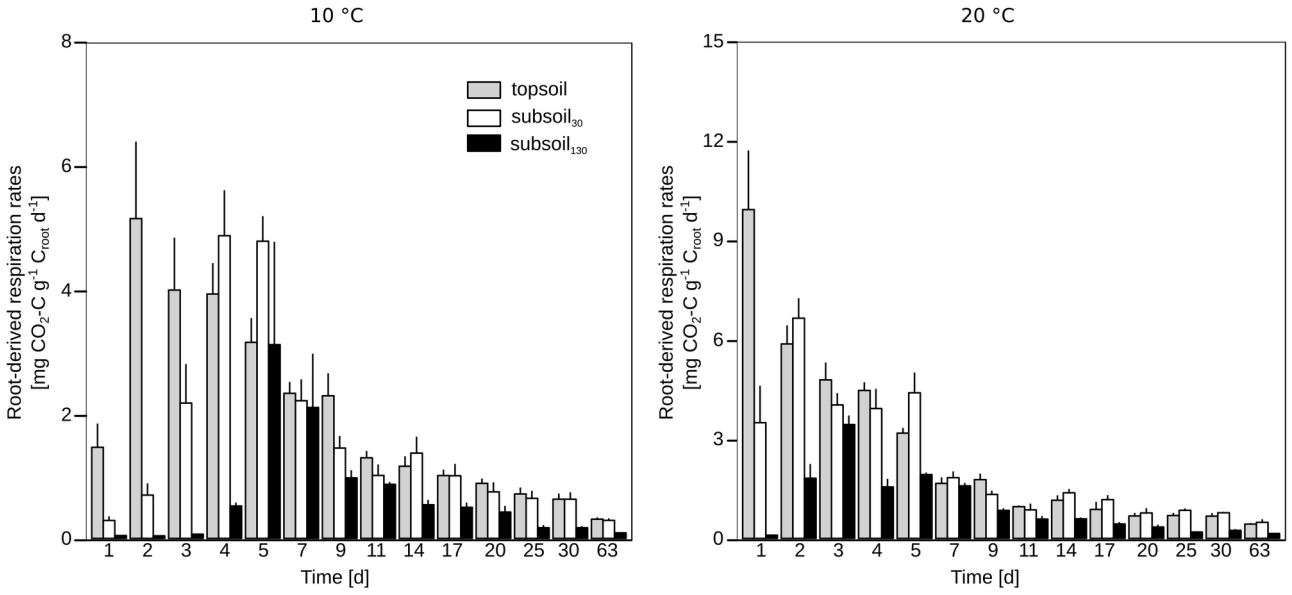

Figure 3.5: Daily respiration of the added root litter at 10 °C and 20 °C. Values are expressed as mg CO_2-C g^{-1} C_{root} d^{-1}. Means and standard errors (n = 4).

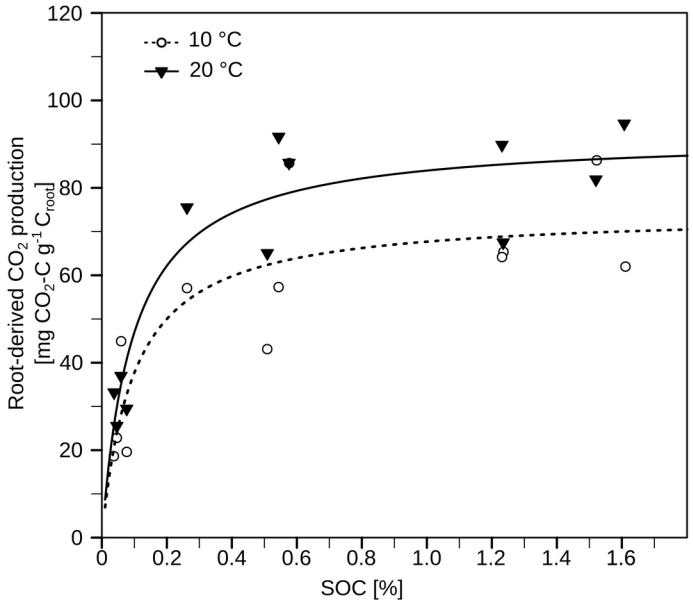

Figure 3.6: Michaelis-Menten function fitted through specific root litter mineralization and SOC content for 10 °C (open symbols and dashed line) and for 20 °C (filled symbols and solid line)

3.3.4 Priming effects in topsoil and subsoil

The addition of ground roots as an additional source of energy for microorganisms had no positive priming effect on the mineralization of the native SOC during the incubation experiment. In contrast, even more SOC was respired in the control samples at the end of the incubation than in the root addition samples for all soil depths, indicating on average a negative priming effect (Figure 3.7). The root addition reduced the native SOC mineralization by 15 ± 5 % in the topsoil and by 20 ± 13 % and 14 ± 20 % in the $subsoil_{30}$ and $subsoil_{130}$, respectively, for the total incubation period. However, the decrease of SOC mineralization after root addition was not significant. Nevertheless, priming effect expressed as ratio between the daily respiration rates of the control and root addition treatment samples changed over the time course of the incubation. During the first few days of the incubation even positive priming was observed (Figure 3.7) which occurred simultaneously to the highest mineralization rates of the added roots (Figure 3.5). Priming was investigated only for the 10 °C treatment, which represents the mean annual temperature of the site.

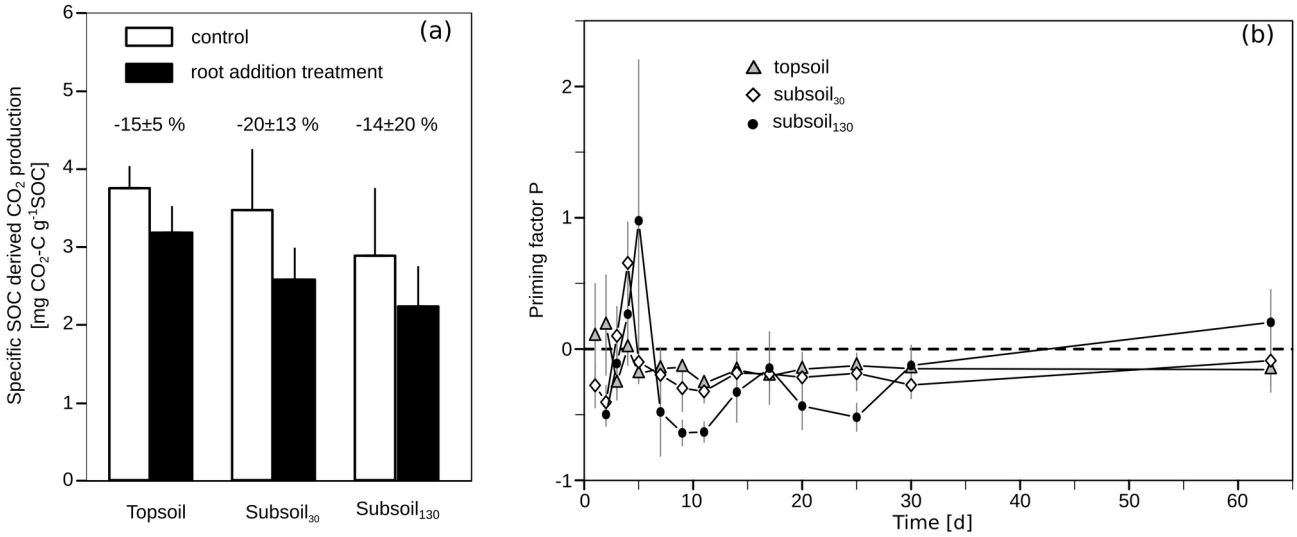

Figure 3.7: Total specific SOC derived CO_2 production (a) for the control and the root addition treatment. Values are expressed as mg CO_2-C g^{-1} SOC. (b) Priming effects expressed as dimensionless priming factor P. Means and standard errors (n = 4).

3.4 Discussion

3.4.1 SOC stability in topsoil and subsoil

Respiration rates decreased with increasing soil depth following the strong depth gradient in SOC. However, per unit SOC higher CO_2 production was observed in $subsoil_{130}$ than in topsoil. This is surprising due to the fact that the mean ^{14}C age of the SOC increases with increasing soil depth, indicating a slow turnover and high stability of subsoil SOC (Rethemeyer et al., 2005; Trumbore, 2000). Due to the incubation time of 63 days, our results only show the dynamic of the labile and fast-cycling C pool. Therefore, these results indicate that the subsoils at our site contain a fast-cycling labile C pool, which comprises a relatively large fraction of the total subsoil SOC as compared to the topsoil. This pool is fed by root litter (roots were visible during the sampling in all depths) and by DOC, which are the main inputs of C to deeper soil horizons (Rumpel and Kögel-Knabner, 2011). Also, other studies found higher CO_2 production per SOC in subsoils than in topsoils (Agnelli et al., 2004; Jörgensen et al., 2002; Lavahun et al., 1996; Salomé et al., 2010). During the incubation experiment, 0.7 % of the SOC in $subsoil_{130}$ was respired, whereas in topsoil and $subsoil_{30}$ only 0.2 % of the total C was respired. Hence, the relative proportion of labile C to total C in each depth at our site was higher in $subsoil_{130}$ than in the topsoil. A similar pattern was observed by Salomé et al. (2010) in an Eutric Cambisol under agriculture use. In their 51-day incubation they found a higher or equal proportion of respired C per SOC in subsoil samples (80–100 cm) as compared to topsoil samples (5–10 cm).

Another explanation for the higher specific respiration in $subsoil_{130}$ might be the lower carbon use efficiency of microorganisms in subsoils. Several studies observed an increasing metabolic quotient (CO_2 production per microbial biomass) with increasing soil depth, whereas the microbial biomass was decreasing with soil depth (Agnelli et al., 2004; Jörgensen et al., 2002; Lavahun et al., 1996). Lavahun et al. (1996) assumed that the substrate in the subsoil is more recalcitrant and microorganisms incorporate less substrate into their biomass, but to meet their energy demands for growth and maintenance, they had to utilize more of the substrate. Therefore, microorganisms mineralize more SOC in subsoil than in topsoil. Also, microbial recycling (Basler et al., 2015) and an increasing degree of transformation of dissolved organic carbon with depth (Kaiser and Kalbitz, 2012) may decrease the carbon use efficiency. Further, the spatial isolation of microorganism and substrate in subsoils my foster anabolism instead of catabolism, thus increase the relative C loss compared to topsoils. In addition, differences in soil texture between topsoil, $subsoil_{30}$ and $subsoil_{130}$ may influence

the size of the stabilized SOC fraction (Flessa et al., 2008; von Lützow et al., 2006). Due to the higher silt content in topsoil and subsoil$_{30}$ as compared to the subsoil$_{130}$ (Table 3.1) more SOC might be physically protected leading to lower specific mineralization rates. However, comparing the four plots at the site with slightly different texture revealed no consistent impact of different clay and silt content on the specific mineralization rates. Thus, the lower carbon use efficiency and the larger proportion of labile SOC may explain the depth gradients of specific SOC mineralization.

3.4.2 Temperature sensitivity

The discussion on the temperature sensitivity based on Q_{10} values of SOC in different soil depths has been controversial (Conant et al., 2008; Fang et al., 2005; Fierer et al., 2003; Karhu et al., 2010; Vanhala et al., 2007; Winkler et al., 1996). A mechanistic explanation for different temperature sensitivities for labile and stabilized SOC is provided by the Arrhenius equation. According to this equation, higher temperature sensitivities were explained by a lower quality (increasing recalcitrance) of the SOC due to a lower reactivity due to higher activation energies (Davidson and Janssens, 2006; von Lützow and Kögel-Knabner, 2009). However, although it is assumed that recalcitrance of SOC increases with increasing soil depth (Rumpel, 2004) we found no increase in $Q_{10\text{-}q}$ values with soil depth, suggesting that quality of SOC does not decrease with soil depth. Our results are in line with results from Gillabel et al. (2010) who also found no higher $Q_{10\text{-}q}$ values in the subsoil compared to the topsoil. Such an increase would be expected if SOC quality alone determined Q_{10} in the subsoil (Bosatta and Ågren, 1999).

Gillabel et al. (2010) concluded that temperature sensitivity of SOC decomposition was determined by both, SOC recalcitrance and physical stabilization of SOC, and that the relationship between $Q_{10\text{-}q}$ and SOC stability depended on which of those stabilization factors was the dominant mechanism leading to greater SOC stability. For the soil investigated in our study, this would mean that substrate recalcitrance is not the dominant factor controlling the SOC temperature sensitivity in subsoils.

In addition, Davidson and Janssens (2006) pointed out that the temperature sensitivity of SOC mineralization, according to Arrhenius kinetics, can be influenced by the substrate availability. They assume that the enzymes for decomposition are separated from SOC, which is more likely to be the case in subsoils than in topsoils. In consequence, an observed temperature sensitivity under substrate limitation can be lower than under conditions without substrate limitations (Davidson and Janssens, 2006). The separation of decomposers and SOC can be altered by the environmental conditions such as physical protection within soil aggregates, chemical protection like adsorption onto the mineral

surface or abiotic conditions like drought, flooding and freezing (Davidson and Janssens, 2006). However, physical protection within aggregates plays no role in the investigated soil. Also chemical protection on clay minerals cannot explain the lower temperature sensitivity in our subsoils, as the clay content in subsoil$_{130}$ was even lower than in the topsoil (Table 3.1). In addition, the abiotic factors in our experiment temperature and soil moisture were not varied during the incubation. Thus, the temperature sensitivity of SOC mineralization in subsoils might be attenuated by other SOC stabilization mechanisms. These mechanisms could be differences in the microbial community between topsoils and subsoils or nutrient limitation for microorganisms (Fierer et al., 2003). However, the mineralization of the added root material showed a similar response to temperature changes in topsoil and subsoil, suggesting that differences in the microbial community nor nutrient limitation had an influence on the temperature sensitivity. The temperature sensitivity of the mineralization of the added root seems to be mainly controlled by their recalcitrance and therefore was similar in all soil depths.

3.4.3 Stability of added root C and priming effects

The soil environment seems to be the major controlling factor for C turnover (Schmidt et al., 2011). We found that the same root litter was decomposed much more slowly when mixed into subsoil material as compared to when mixed into topsoil material. Soil abiotic conditions such as temperature and water content (60 % WHC) cannot explain this finding since they were standardized in our experiment. In addition, the daily respiration rate of the added root litter (Figure 3.5) showed a delay in root mineralization with increasing soil depth with mineralization starting after 4 days in subsoil$_{130}$ as compared to 1 day/h in the topsoil. This indicates that the decomposers and substrate were not in equilibrium in the beginning of the experiment for the subsoil samples. On the one hand, the microbial community in subsoil had to adapt to the new substrate of the added roots. On the other hand, the lower SOC content and the homogeneous distribution of SOC by sieving in the subsoil may lead to spatial segregation of substrate and decomposers. The combination of SOC distribution and low SOC content in subsoil decrease the likelihood of exoenzymes to clip a molecule or a microorganism to encounter with substrate and thus to make it assimilable for microorganism (Ekschmitt et al., 2005). Therefore, the SOC content and the SOC distribution as indicator for the distance between substrate units might be a major factor for SOC turnover in subsoils. Similar results were shown in a study by Don et al. (2013) where compost was incubated with soil at different degrees of mixing and dilution with mineral soil. They found that C mineralization was highest in samples if the compost was not mixed with the mineral soil and lowest if the compost was diluted with mineral soil.

Moreover, the separation between decomposer and substrate has been discussed as a stabilization mechanism process for SOC (Dungait et al., 2012; Kemmitt et al., 2008). Kemmitt et al. (2008) found that the mineralization of C is not controlled by the microbial biomass size and community. Further, the separation between microorganisms and SOC is determined by diffusion, soil pore size and soil pore connectivity (Kuka et al., 2007; Xiang et al., 2008). $Subsoil_{130}$ had a lower silt content and a higher sand content as compared to the other horizons, which reduced the field capacity and therefore also reduced the mobility and the connectivity of microorganisms and exoenzymes. In consequence, $subsoil_{130}$ in this study, with its low SOC content, provided the best conditions to store additional C since added C is turned over with the lowest rates. These results seem to be a contradiction to our findings in the undisturbed samples, where $subsoil_{130}$ showed the highest specific mineralization. This highest mineralization rate points to the fact that the $subsoil_{130}$ comprised a relatively large proportion of labile SOC as compared to the topsoil. However, we added the same amount of root litter to each soil depth, therefore the proportion of labile root C was similar in all depths. In addition, as discussed above, the SOC distribution was changed due to sieving and mixing process which may increase the spatial segregation between decomposers and substrate as compared to the undisturbed samples, despite the increase of SOC content due to root addition.

Additionally, it is interesting that there were no significant differences in root mineralization in topsoil and $subsoil_{30}$ (Figure 3.4), despite the differences in SOC content. This matches the findings of Sanaullah et al. (2011), who found no difference in the mineralization of additional C (added as root litter) in three different depths (30, 60 and 90 cm) after three years of field incubation in a loamy soil on grassland. Beside the different incubation time, the differences in SOC content between our study and those of Sanaullah et al. (2011) may explain the different observed mineralization pattern. The SOC content in the study of Sanaullah et al. (2011) were between 9 and 3 mg g^{-1}, which is similar to our topsoil and $subsoil_{30}$. While, the SOC content in $subsoil_{130}$ in our study was much lower with 0.4 mg g^{-1}. Therefore, we hypothesize that the lower root mineralization in $subsoil_{130}$ can be explained by the non-linear relation (Figure 3.6) between SOC turnover and SOC content (Don et al., 2013) and not as an effect of the short incubation time.

The addition of labile C to our subsoils did not enhance the turnover of the native SOC. This finding is in line with the results from Salomé et al. (2010), who also could not find an enhanced mineralization of native SOC after the addition of glucose to subsoils. Our results support the hypothesis that stability of SOC in subsoils is not controlled by substrate limitations. In contrast, Fontaine et al. (2007) observed a priming effect on subsoil SOC after the addition of labeled cellulose. They concluded that the stability of SOC in subsoils is controlled by the supply of fresh C.

However, on the one hand priming effects are determined by soil properties, especially the pH, with higher priming effects occurring in neutral soil with a pH between 6 and 8 (Blagodatskaya and Kuzyakov, 2008). The addition of an easily available substrate stimulates the microbial activity and enzyme synthesis, whereby the stimulation of microorganisms is higher in neutral soils than in acidic soils (Blagodatskaya and Anderson, 1998). Our subsoil had a pH of 4, while Fontaine et al. (2007) had a pH of 7 in the subsoil. On the other hand, differences in C inputs due to different land use and the related adaption of the microbial communities might also influence priming effects. In our study we used an acidic forest soil which may have received higher C inputs due to deep rooting and DOC transport and the microbial communities were better adapted to the added root material than the agricultural soils and the added cellulose as used by Fontaine et al. (2007). The remarkable consequence of our findings is that the addition of C to forest subsoils did not lead to destabilization of the native SOC during the two month of incubation. Also, additional C from root litter was more stable in the deep subsoil than in topsoil horizons. The main questions that arise from these results are how much C can be added to subsoils until more SOC will be mineralized than stored? Are forest soils more suitable to store additional C in their subsoils than agricultural soils?

3.5 Conclusion

Regardless of the high ^{14}C ages of subsoil SOC, we showed that subsoil contained a larger relative fraction of labile SOC than the topsoil, as revealed by higher specific mineralization at least for the deep subsoil of our site. This indicates on the one hand a more extreme mixture of SOC pools in subsoils, consisting of a labile SOC pool, likely derived by root litter, and a passive C pool with high ^{14}C ages. And on the other hand, the higher specific mineralization of undisturbed samples supports the hypothesis of a lower carbon use efficiency in subsoils.

Temperature sensitivity of SOC mineralization decreased with soil depth. This is an important finding, because it implicates that the SOC mineralization in subsoils will be less affected by temperature changes due to climate change than SOC mineralization in topsoils. It also supports our hypothesis of a larger relative proportion of labile SOC in subsoils, since the mineralization of labile SOC is assumed to be less temperature sensitive.

The SOC stability in subsoils is often related to a lack of an easily available energy source for the microbial community, however increasing the SOC content up to 80 % in our deepest samples did not lead to positive priming of the native SOC mineralization. Furthermore, we found that additional C inputs of roots were more stable in deep subsoil horizon with a low SOC content than in topsoil. Also, the stability of the added C varied between the investigated subsoil horizons, revealing differences within the subsoil, which could be attributed to different SOC contents. Decreasing SOC content in the subsoil cause a larger spatial segregation between decomposers and substrate. Thus, C inputs into subsoil horizons with a low SOC content may be more effective in increasing SOC stocks than topsoil SOC amendments.

CHAPTER 4 ENVIRONMENTAL CONSTRAINTS LIMIT CARBON DECOMPOSITION IN THE SUBSOIL

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Contribution: I analyzed the data, compiled the graphs and the tables and wrote the manuscript. Further, I contributed to the design of the lab work and supervised the master student who did the lab work. This study incorporates the lab work and raw data acquired within the master thesis of Franziska Johannes from the Technische Universität Braunschweig. The field work was conducted by Sebastian Preusser.

Status: Not submitted yet.

Abstract

With increasing soil depth, C stability increases as indicated by a high radiocarbon age, while the soil organic carbon content decreases. This indicates that the subsoil, may have the potential to sequester additional C on the long-term. Even though, the mechanisms determining the decomposition processes in subsoils are poorly understood, even though more than half of global SOC is stored in subsoils. It is assumed that SOC decomposition in subsoils is limited due to unfavorable environmental conditions for microorganisms. In laboratory studies different factors on the SOC decomposition in subsoils have been investigated such as temperature increase, oxygen limitation or substrate availability. However, there is a lack of experimental evidence for the influence of environmental conditions and their interaction on the C decomposition in the subsoil under field conditions. Here we carried out a reciprocal soil transfer experiment of a well characterized Dystric Cambisol under beech forest in order to asses the impact of environmental conditions in the topsoil and the subsoil on decomposition processes. Soil material form the subsoil and the topsoil were placed into microcosms and exposed to a topsoil (5 cm) and two subsoil environments (45 cm and 110 cm) for one year in the field. Further, ^{13}C labeled root litter was added to evaluate the influence of macro and micro environmental conditions on the decomposition. The amount recovered root-derived C in the mineral-associated organic matter fraction after 3 and 12 months was used as an indicator for differences in the decomposition.

After 12 months of field exposure in the deepest subsoil environment the amount of root-derived C in the mineral-associated organic matter was 19 % lower for the topsoil material and 37 % lower for the subsoil material as compared to samples exposed to the topsoil environment. This indicates, that environmental conditions in the subsoil hampered OC decomposition. Despite small differences in soil temperature and oxygen concentration between the topsoil and the subsoil, decomposition in the subsoil environment was mainly substrate limited. The lower C input with seepage water into the subsoil led to slower decomposition of the added root litter as compared to the topsoil horizon. Furthermore, the sandy soil texture in the subsoil resulted in a lower water content and a more heterogeneous distribution of water fluxes, both may increased the spatial separation between decomposers and substrate and could enhanced the prevailing substrate limitation in the subsoil. In addition, after 3 months more root-derived C was recovered in the mineral-associated organic matter if roots were embedded in topsoil material. The higher SOC content in the topsoil material compensated the substrate limiting conditions in the subsoil macro environment on the short-term. However,

without regular supply of fresh substrate via DOC or roots, the easily available substrate in the topsoil material will be consumed and the micro environmental advantage of higher C availability will vanish.

The exposure of subsoil material to the topsoil environment increased the SOC content by 18 % within on year, underlining the large C sequestration potential of subsoils. Therefore, measures to increase soil organic carbon stock should also incorporate the subsoil.

4.1 Introduction

Soil organic carbon (SOC) plays a major role in the global carbon (C) cycle as soils contain the largest active terrestrial carbon reservoir. Thus soils play an important role for climate change, due to the fact that soils can be sources or sinks for atmospheric CO_2. Increasing C content in soils can contribute to a reduction of anthropogenic greenhouse gas emissions (Fuss et al., 2018; Lal, 2016; Paustian et al., 2016). Hence, a lot of studies investigated the effect of different management to increase the SOC stocks (Minasny et al., 2017). These management practices mostly focus on the topsoil, which is usually the first 30 cm of the soil profile. However, it should not be neglected that more than 50 % of the global soil organic carbon (SOC) stocks resides in the subsoil (> 30 cm) (Batjes, 2014; Jobbágy and Jackson, 2000). It is assumed that the low C input and the low C content in subsoils results in unsaturated mineral surfaces with C and therefore having the potential to sequester additional C (Kaiser and Guggenberger, 2003; Rasse et al., 2005; Rumpel and Kögel-Knabner, 2011; Stewart et al., 2008). In addition, the increasing apparent radiocarbon age of OC with soil depth (Rethemeyer et al., 2005; Torn et al., 1997), indicate a higher C stability in subsoils than in topsoils. Therefore, the C sequestration potential of soils might be larger when the whole soil profile is taken into account. However, increasing the OC inputs may result in higher OC stocks, but the fate of OC inputs depends on soil environmental conditions and may differ between topsoils and subsoils. In previous studies, it has been shown that mineralization of OC differs between topsoils and subsoils (Fierer et al., 2003; Heinze et al., 2018; Salomé et al., 2010; Wordell-Dietrich et al., 2017) due to different environmental conditions. However, it still remains unclear how the environmental conditions determine the C stability in the subsoil.

The mineralization of OC by microorganism strongly depends on environmental factors such as temperature, soil moisture and C input. Within a soil profile those factors changes with depth, e.g. the temperature and moisture regime in subsoils are more constant over the year than in topsoils. But, due to the non-linear response of OC mineralization to temperature (Curtin et al., 2012; Lloyd and Taylor, 1994) the annual OC mineralization may be lower in the subsoil than in the topsoil. In addition, the C inputs in form of dissolved organic matter and belowground C inputs (roots and root exudates) decline with depth (Heinze et al., 2018; Michalzik et al., 2001; Tückmantel et al., 2017). The decline is accompanied by a change in quality, leading in subsoils to an accumulation of microbial residues and aged plant-derived compounds with a lower biodegradability (Kaiser and Kalbitz, 2012; Rumpel, 2004; Rumpel et al., 2002). Therefore, stability of subsoil OC is also often linked to a lack of fresh C inputs, as easily available energy source which may trigger microbial decomposition in

subsoils (Fontaine et al., 2007; Marschner et al., 2008). So far, only a few studies assessed the impact of substrate addition (e.g. sugars, cellulose or root litter) on microbial decomposition in subsoils, but with varying results. Some studies found an enhancement of SOC mineralization in subsoils after substrate addition (Fontaine et al., 2007; Karhu et al., 2016; Zhang et al., 2015), while others reported no change in SOC mineralization after substrate addition (Salomé et al., 2010; Wordell-Dietrich et al., 2017). This indicates, that SOC mineralization in subsoils might not only energy limited due to lower C inputs. Furthermore, also the spatial separation between decomposers and substrate may hamper SOC mineralization in subsoils. On the one hand, the accessibility of OC to microorganisms can be limited due to aggregation (Flessa et al., 2008; von Lützow et al., 2006; Sollins et al., 1996). On the other hand it was pointed out that low SOC contents can result in energetic constraints for microorganisms, due to a reduced chance to encounter with the substrate (Don et al., 2013; Ekschmitt et al., 2008). The mechanisms controlling SOC turnover in subsoils are usually investigated in time limited (hours to several weeks) laboratory incubations under controlled conditions with few varying factor, e.g. temperature, moisture or substrate availability. Only a few in situ studies showed that OC decomposition slows with soil depth (Baumann et al., 2013; Gill and Burke, 2002; Hicks Pries et al., 2017a), but the question as to whether and to which extent environmental conditions (e.g. temperature, soil moisture, substrate supply) and their interaction determine OC decomposition in subsoils remains.

In a recent field study, Preusser et al., (2019) could show that the abundance of microbial biomass decreases with decreasing C inputs along a soil profile. But, they also found that microbial communities responded different to C availability and environmental conditions a long the soil profile. While the abundance of fungi was mostly controlled by C availability, the bacterial biomass seemed also controlled by soil moisture. Therefore, if environmental conditions in the subsoil limits the microbial community, this should also be reflected in the decomposition of OM in the subsoil. To disentangle the effect of macro and micro environmental conditions in different soil depth on OC decomposition we carried out a reciprocal soil transfer experiment. In this experiment soil material from a topsoil horizon and a subsoil horizon were *in situ* exposed to different soil depths for one year, reflecting different macro and micro environmental conditions. The exposure of the soil material to different soil depths, changed environmental conditions at the macro scale of centimeters to decimeters. Macro environment refers to abiotic factors such as temperature, soil moisture, root density and C-input. Further, using soil material from a topsoil and a subsoil horizons was supposed to reflect different micro environmental conditions, which were determined by soil properties such as

SOC content, pH or soil texture. The embedding of ^{13}C labeled root litter into different soil materials and the exposure of this material to different soil depths, allowed us to follow the decomposition of root OM depending on macro and micro environmental conditions.

We hypothesized that if the macro environmental conditions in the subsoil limit OC decomposition, the added root litter should decompose slower and the amount of root-derived C found in the mineral-associated organic matter (MAOM) fraction will be lower in samples exposed to a subsoil environment than in samples exposed to a topsoil environment. If micro environmental conditions such as a low SOC content hamper OC decomposition, the amount of root-derived C found in the MAOM will be lower if roots are embedded in subsoil material than if embedded in topsoil material in the same macro environment. Lastly, the exposure of subsoil material to a topsoil environment should reveal the C sequestration potential of the subsoil, due to the formation of MAOM caused by higher C inputs.

4.2 Materials and methods

4.2.1 Site description

The study site is located in a beech forest (Grinderwald), 35 km north-west of Hannover, Germany (52°34′22″N, 9°18′49″E). The vegetation at the site is dominated by common beech (Fagus sylvatica) established 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 precipitation of 9.7°C and 762 mm (1981-2010), respectively (Data from DWD station in Nienburg, Germany). Soil texture is characterized by high sand fraction with varying contents of 60 % (< 30 cm) to 85 % (> 85 cm), with SOC content of 11.5 g kg^{-1} in the topsoil (10 cm) and 0.4 g kg^{-1} in the subsoil (185 cm) (Heinze et al., 2018; Leinemann et al., 2016). The basic soil characteristics are summarized in Table 4.1. Further, the soil temperature and the volumetric water content in several depths were monitored in three subsoil observatories at the study site (Figure 4.1 and Table 4.2, Wordell-Dietrich et al., 2020).

Table 4.1: Soil characteristic of the study site. Mean values from (Heinze et al., 2018) (n = 24 for each depth). Initial OC content of the added root litter, TOP_5 and SUB_{110} samples.

Depth [cm]	pH ($CaCl_2$)	OC [%]	Sand [%]	Silt [%]	Clay [%]
10	3.5	1.15	65.0	31.0	3.0
35	4.2	0.52	61.5	34.5	4.0
60	4.2	0.12	72.1	25.1	2.8
85	4.0	0.04	86.6	11.6	1.7
110	3.9	0.05	84.9	13.1	2.0
135	4.0	0.07	75.5	21.6	3.0
Roots		48.7			
TOP_5		2.84	72.0	28.0	
SUB_{110}		0.11	87.0	13.0	

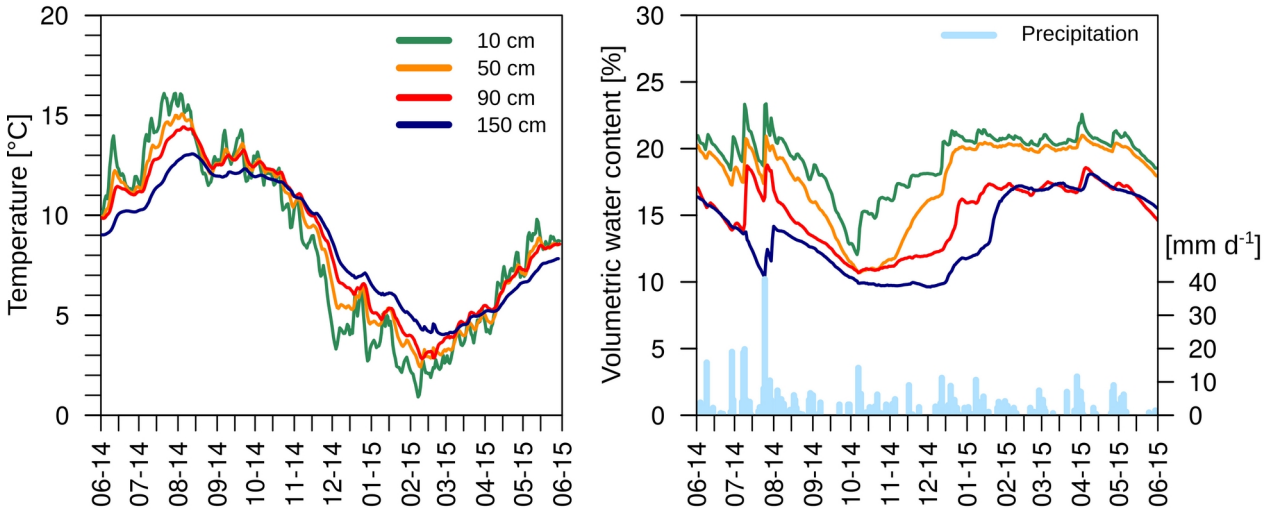

Figure 4.1: Mean daily soil temperature (°C) and volumetric water content (%) in different soil depths at the study site. (n = 3)

Table 4.2: Minimum, maximum and range of soil temperature and volumetric water content in different soil depth of the study site during the exposure of TOP_5 and SUB_{110} samples.

		June 2014 - September 2014			September 2014 - June 2015		
	Depth [cm]	Min	Max	Range	Min	Max	Range
Temperature [°C]	10	10.1	16.1	6.1	0.9	14.3	13.3
	50	9.9	15.1	5.1	2.4	13.6	11.2
	90	9.8	14.4	4.6	2.8	13.3	10.4
	150	9.0	13.1	4.1	4.0	12.3	8.3
Volumetric water content [%]	10	16.8	23.4	6.6	12.1	22.6	10.5
	50	14.3	20.9	6.7	10.7	21.0	10.3
	90	12.5	18.8	6.2	10.7	18.6	7.9
	150	10.5	16.4	5.9	9.6	18.1	8.5
Precipitation [mm]		231.0			277.0		

4.2.2 Experimental design

In a reciprocal soil transfer experiment, we examined the influence of macro and micro environmental on the decomposition of OM along a soil profile. In June 2014, twelve soil pits were excavated using a digger around three mature beech trees (four pits per tree) in a distance of 2.5 m. The soil material from 5 to 10 cm depth from each pit were mixed and sieved (< 2 mm) to one composite sample (in the following referred as TOP_5). The same procedure was used for the subsoil material from 110 to 115 cm depth (SUB_{110}). The two different soil materials represented different micro envi-

ronmental conditions. The samples were filled into microcosms with a diameter of 10.5 cm and a height of 2 cm. To ensure a water percolation into the microcosms, but also adequate separation from the surrounding soil, the microcosms were covered with a 500 µm polyamide mesh on the top and the bottom (Preusser et al., 2019). Microcosms, were exposed in different soil depths (5, 45 and 110 cm), reflecting different macro environmental conditions in the soil profile. To follow decomposition of OM, ^{13}C labeled root litter was added to the microcosms, resulting in two treatments.

i.) **Control**: Soil material without addition of ^{13}C labeled roots

ii.) **Root addition**: Soil material with addition of ^{13}C labeled roots

The microcosms were filled with 242.3 g of TOP_5 material (bulk density of 1.4 g cm^{-3}) and with 277.0 g of SUB_{110} material (bulk density 1.6 g cm^{-3}). For the root addition treatment ^{13}C labeled European beech root litter ($\delta^{13}C$ 8283 ‰) with different diameter (0.5 to 2 mm) was cut into 1-2 cm segments and homogenized. Thereafter, root litter was added with a root density of 10 g L^{-1} and 2 g L^{-1} to TOP_5 (1.73 g) and SUB_{110} samples (0.35 g), respectively. The added amount of roots should reflect natural root biomass in the respective soil depth. The labeled roots originated from young trees grown in a greenhouse under $^{13}CO_2$ enriched atmosphere (9.38 atom % ^{13}C; IsoLife Wageningen, Netherlands). The addition increased the SOC content in the TOP_5 samples by 12 % and in the SUB_{110} samples by 55 %.

The prepared microcosms were placed into the tree facing profile walls of the excavated soil pits in the three different depths (5, 45 and 110 cm). In each depth, a TOP_5 and SUB_{110} sample with and without added roots were randomly incorporated into the soil profile wall. The microcosms in each soil depth had a horizontal offset from the microcosms in other soil depths above or below, to avoid vertical influences (Figure 4.2). The soil pits were refilled by the same soil material of the respective depth. The microcosms were sampled at intervals of three month, in total four samplings. At each sampling date, three soil profiles (one per tree) were selected and all microcosms were removed. However, we used only samples from the first (September 2014) and last sampling (June 2015) in this study. For a more detailed description of the experimental design see Preusser et al., (2019). Soil samples were stored at 0 °C for transport. In the lab, an aliquot of 22 g was removed for size and density fractionation. The aliquots were sieved (< 2 mm), air-dried and stored until use. In total 72 microcosms (2 treatments x 2 soil materials x 3 soil depths x 3 replicates x 2 sampling dates) were analyzed.

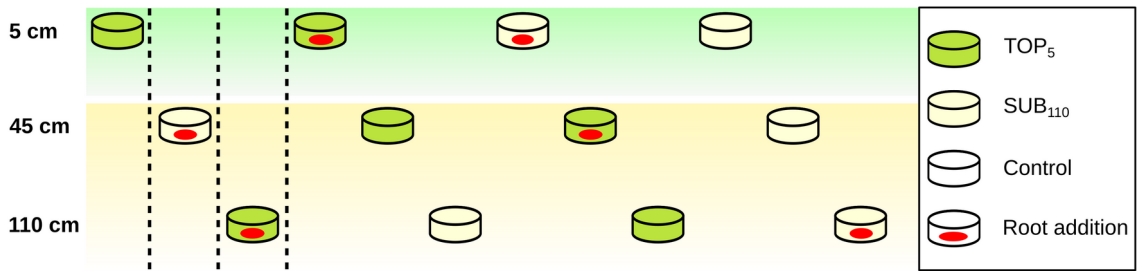

Figure 4.2: Setup of the soil translocation experiment.

4.2.3 Sample analysis

In order to analyze the decomposition and transformation of the labeled root litter, samples were fractionated. A combined size and density fractionation of SOM was performed according to (Cerli et al., 2012; Golchin et al., 1994), where the bulk soil was separated into two light fractions and two heavy fractions. Briefly, first the free particulate organic matter (fPOM) fraction was isolated by suspending 10 g of bulk soil in 40 mL sodium polytungstate (SPT) solution (1.6 g cm^{-3}) (TC Tungsten Compounds, Grub am Forst, Germany) in a centrifuge bottle. The bottle was gently shaken by hand for 2 minutes and then centrifuged for 20 min at 4000 rpm. Thereafter, the supernatant with floating particles was vacuum filtrated through a 0.45 µm cellulose nitrate filter (Sartorius GmbH, Göttingen) and washed with de-ionised water until the electrical conductivity was below 10 µS (usually after 1.5 L). The separated fraction was dried at 50°C. Second, the remaining soil was re-suspended in SPT and dispersed by ultrasound (Branson Digital Sonifier, Model 450-D, Danbury, CT, USA) with an energy input of 60 J mL^{-1} to obtain the occluded particulate organic matter (oPOM) fraction. During ultra sonication the samples were cooled in an ice bath. Subsequently, the samples was centrifuged, vacuum aspirated and dried as for the free particulate organic matter. The remaining heavy fraction was rinsed with de-ionised water and separated into two particle size fractions: sand (> 63 µm) and silt and clay (< 63 µm) by wet sieving and dried at 50 °C. The particles smaller than 63 µm represented the mineral-associated organic matter (MAOM).

Dried (50°C) subsamples of bulk soil and all four fractions were ground and analyzed for C and N content by dry-combustion with an elemental analyzer (LECO, TruMac, St. Joseph, MI, USA). The carbon isotope content of the bulk soil and all fractions were determined using an isotope ratio mass spectrometer (Delta Plus, Thermo Fisher Scientific, Bremen, Germany) coupled to an elemental analyzer (CE Instruments FLASH EA 1112, Thermo Fisher Scienctific, Bremen, Germany) at the lab of the Thünen Institute of Climate-Smart Agriculture.

The average C recovery was 98 % for all samples. However, the C recoveries differed between TOP_5 and SUB_{110} samples. The mean C recovery for TOP_5 samples was 81 % (± 16 %) and 116 % (± 39 %) for SUB_{110} samples. The C recoveries of more than 100 % in the SUB_{110} samples can be attributed to a large variability of fPOM and oPOM in the SUB_{110} samples. This variability and the rather large root pieces used (1-2 cm) resulted in a very large variability of recovered ^{13}C in fPOM and oPOM. Therefore, we did not calculated a complete mass balance of the added roots and used only the MAOM as indicator for root decomposition.

4.2.4 Calculations and statistics

The root-derived C (R) in the MAOM fraction was calculated using the isotopic mixing equation (Eq 4.1):

$$R = 1 - \left(\frac{\delta^{13}C_A - \delta^{13}C_{root}}{\delta^{13}C_{control} - \delta^{13}C_{root}} \right) \quad (4.1)$$

where $\delta^{13}C_A$ is the isotopic signature of the MAOM in the root addition treatment, $\delta^{13}C_{roots}$ is the isotopic signature of the added roots, $\delta^{13}C_{control}$ is the isotopic signature of the MAOM in the control treatment. The effect of different macro and micro environmental conditions on the C content in the bulk soil and the MAOM fraction was evaluated with linear models. In order to do so, a linear model was fitted by restricted maximum likelihood using the generalized least squares function of the nlme package in R (Pinheiro et al., 2019). To identify factors influencing the C content a step wise model reduction was performed as described in (Zuur et al., 2009). In brief, all factors (soil depth and sampling date) and their interactions was used as an initial model, which was compared to a simplified model (omit one factor) using an ANOVA. If models were significant different, the omitted factor had a significant effect on the C content. Further, the effect of different micro environmental conditions (TOP_5 vs. SUB_{110} soil material) on the decomposition of the added roots was tested with same linear model as described before with soil material, soil depth and sampling date and their interactions as fixed factor. A TukeyHSD post-hoc test was performed at the significance level of 0.05 for the final model. Normal distribution of the residuals was tested with a the Shapiro-Wilks test and the homogeneity of variances with Levenes test. All statistical analyses were carried out in R (version 3.6.0) for Linux (R Core Team, 2019).

4.3 Results

4.3.1 Topsoil samples - TOP$_5$

After 12 months of field exposure in different soil environments all TOP$_5$ samples lost in average 13.3 % (± 1.7 %) of the initial SOC regardless of soil depth (Figure 4.3). Further, the differences in the SOC content between TOP$_5$ samples translocated to a subsoil environment and non-translocated TOP$_5$ samples were small and not significant after 12 months, this was found in both treatments (Table S4.1). However, the C quality in TOP$_5$ samples changed differently during the field exposure depending on soil depth, as revealed by size and density fractionation of the control samples (Figure 4.4 and Table 4.3). In general, the fPOM fraction decreased, while the oPOM and MAOM fraction increased. The largest changes between both sampling dates were found in the non-translocated TOP$_5$ samples, where the contribution of fPOM to total SOC decreased from 30 % (± 13 %) to 10 (± 2 %), while the contribution of MAOM to total SOC increased from 53 % (± 7 %) to 68 % (± 15 %). In contrast, TOP$_5$ samples exposed to the deepest subsoil environment (110 cm) showed a lower decrease in the fPOM fraction from 23 % (± 11 %) to 18 % (± 3 %) and also a lower increase in the MAOM fraction (4 %) (Figure 4.4 and Table 4.3).

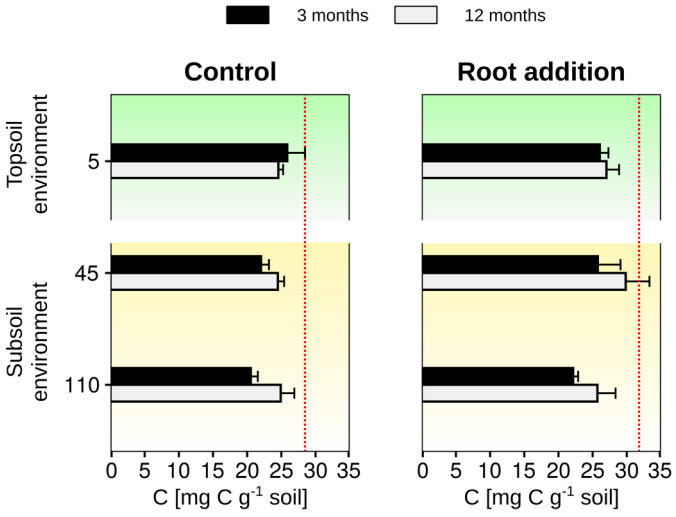

Figure 4.3: Soil organic carbon content of TOP$_5$ samples exposed to different soil environments for the control and the root addition treatment after 3 and 12 months. The dotted red line represents the initial SOC content. Means and standard error (n = 3).

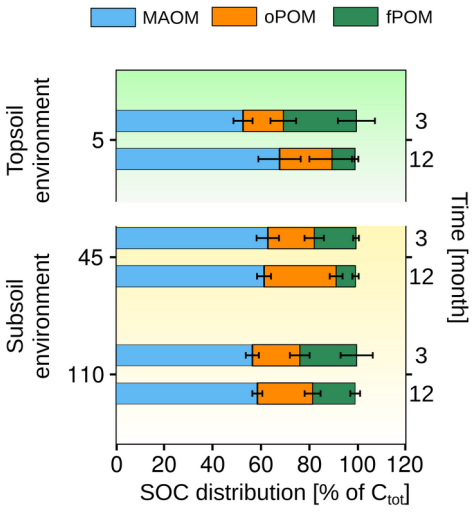

Figure 4.4: Soil organic carbon distribution of TOP$_5$ samples (control treatment) after 3 and 12 months of field exposure in different soil environments separated into the mineral-associated organic matter (MAOM), the occluded particulate organic matter (oPOM) and the free particulate organic matter (fPOM) fraction. Mean and standard error (n= 3).

Table 4.3: Absolute C distribution and change over time in TOP_5 samples of the control treatment in free and occluded organic matter (fPOM, oPOM) and mineral-associated organic matter (MAOM) expressed as mg C g^{-1} dry soil. Mean and standard error (n = 3). Asterisks denote a significant differences between sampling time.

Depth [cm]	Time [months]	fPOM	oPOM [mg C g^{-1} soil]	MAOM
5	3	5.6 (± 1.3)	3.3 (± 1.2)	10.0 (± 1.2)
	12	1.6 (± 0.3)	4.0 (± 1.8)	11.4 (± 0.4)
Change		-3.9*	0.7	1.4
45	3	3.1 (± 0.4)	3.5 (± 0.8)	11.3 (± 0.5)
	12	1.6 (± 0.3)	6.0 (± 0.8)	12.2 (± 0.1)
Change		-1.5	2.5	1.0
110	3	4.2 (± 1.1)	3.6 (± 0.7)	10.3 (± 0.9)
	12	3.2 (± 0.5)	4.1 (± 0.4)	10.6 (± 0.8)
Change		-1.0	0.5	0.4

4.3.2 Subsoil samples - SUB_{110}

The exposure of SUB_{110} samples to a topsoil environment significantly increased the SOC content as compared to SUB_{110} samples in a subsoil environment in both treatments (Figure 4.5 and Table S4.2). Compared to the initial values, the SOC content of SUB_{110} samples (control) in a topsoil environment increased from 1.1 to 1.3 mg C g^{-1} with in one year for the control treatment. This represents a C sequestration rate of 6.2 g C m^{-2} yr^{-1} in a SUB_{110} layer of 2 cm height exposed to a topsoil environment. In contrast, in the root addition treatment the SOC content of SUB_{110} samples in the topsoil environment decreased from initially 1.7 to 1.1 mg C g^{-1} within one year. However, in both treatments the C content in the MAOM fraction was on average 43 % higher in samples exposed to a topsoil environment as compared to samples in a subsoil environment (Figure 4.6). While the bulk and the MAOM C content was different between topsoil and subsoil environment, there was no significant difference between the two subsoil environments (45 and 110 cm soil depth).

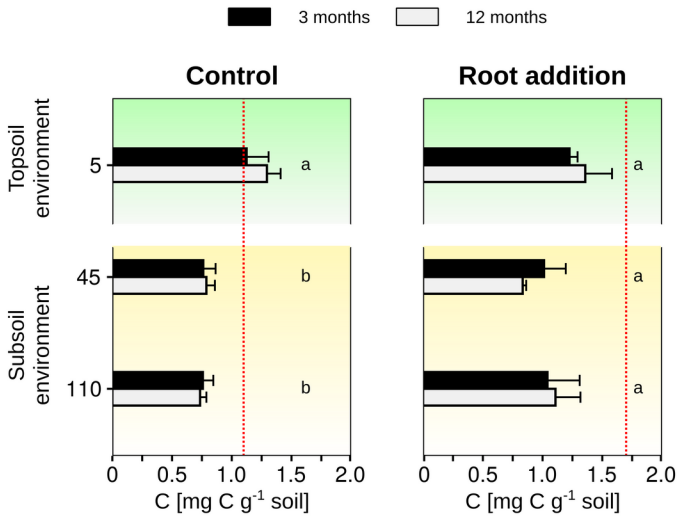

Figure 4.5: Soil organic carbon content of SUB_{110} samples exposed to different soil environments for the control and the root addition treatment after 3 and 12 months. The dotted red line represents the initial SOC content. Different letters denote significant differences between exposure depths. Means and standard error (n = 3).

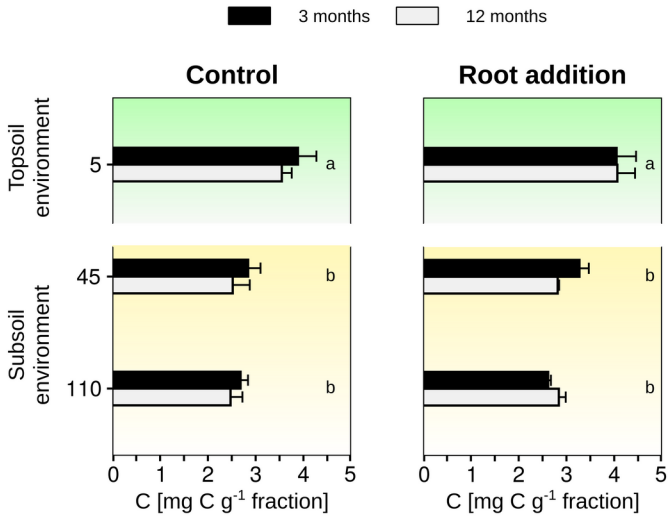

Figure 4.6: Organic carbon content of the mineral-associated organic matter (MAOM) fraction of SUB_{110} samples for the the control and the root addition treatment exposed to different soil environments after 3 and 12 months. Different letters denote significant differences between exposure depths. Means and standard error (n = 3).

4.3.3 Root decomposition

The amount of recovered root-derived C in the MAOM fraction in TOP_5 and SUB_{110} exposed to different soil depth revealed that the decomposition of the added root litter was influenced by the macro environmental conditions in the soil as well as by micro environmental conditions in the sam-

ples. After one year of field exposure 5.6 % (± 0.7 %) of the initial added root litter C was recovered in the MAOM fraction of TOP_5 samples exposed to the topsoil environment. In contrast, in TOP_5 samples exposed to the subsoil environment (110 cm) contained only 4.6 % (± 0.9 %) of the initial root litter C in the MAOM fraction. However, TOP_5 samples in 45 cm had the highest amounts of recovered root-derived C in the MAOM fraction with 6.3 %, but also the highest standard error (± 2.0 %) (Figure 4.7). A similar trend was observed for SUB_{110} samples, more of the added root C was recovered in the MAOM fraction in SUB_{110} samples exposed to the topsoil environment (4.6 ± 0.9 %) than in SUB_{110} samples exposed to the subsoil environment in 45 cm (2.7 ± 0.7 %) and 110 cm depth (3.7 ± 0.8 %) (Figure 4.7). Therefore, after 12 months of field exposure in the deepest subsoil environment (110 cm) the amount of root-derived C in the MAOM fraction were 19 % in TOP_5 and in 37 % SUB_{110} samples lower as compared to their counterparts in the topsoil environment (Figure 4.8). Even though, this differences was not significant it still suggest that the macro environmental conditions in the subsoil limited the decomposition of the added root litter. Moreover, the decomposition was also affected by micro environmental conditions in the samples, as revealed by the lower amounts of recovered root-derived C in the MAOM fraction in SUB_{110} samples than in TOP_5 samples exposed to the same depth. After 12 months SUB_{110} samples contained 57 % (45 cm) and 19 % (110 cm) less of the initial root C in the MAOM fraction than TOP_5 samples in the same subsoil environment depth (Figure 4.9). In contrast, in the topsoil environment the difference between TOP_5 and SUB_{110} samples was only 3 % after 12 months. Results from the linear mixed effect model showed that the amount of recovered root-derived C in the MAOM was slightly more influenced by macro environmental conditions in the soil profile as by the micro environmental conditions in the samples (Table S4.3). However, the difference was not significant.

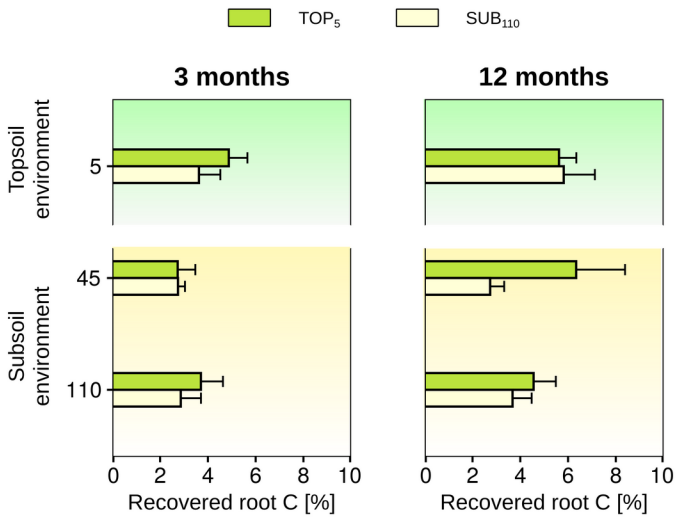

Figure 4.7: Amount of recovered root-derived C in the MAOM fraction of TOP_5 and SUB_{110} samples exposed to different soil environments after 3 and 12 months. Means and standard error (n = 3).

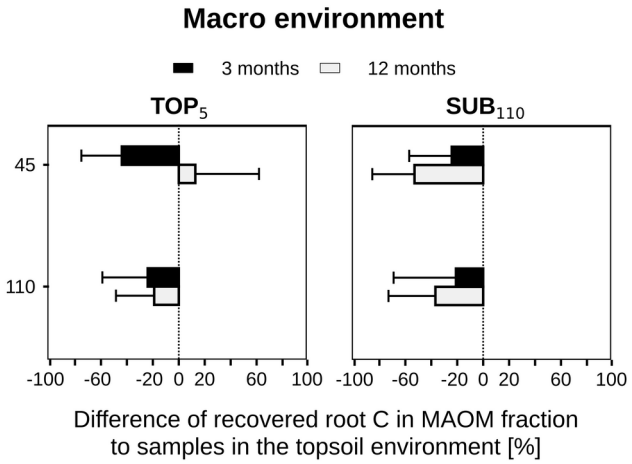

Figure 4.8: Relative difference [%] between topsoil and subsoil environment of recovered root-derived C in the MAOM fraction in TOP_5 and SUB_{110}. Values smaller zero indicate macro environmental limitations on root decomposition in the subsoil environment. Means and standard error (n = 3).

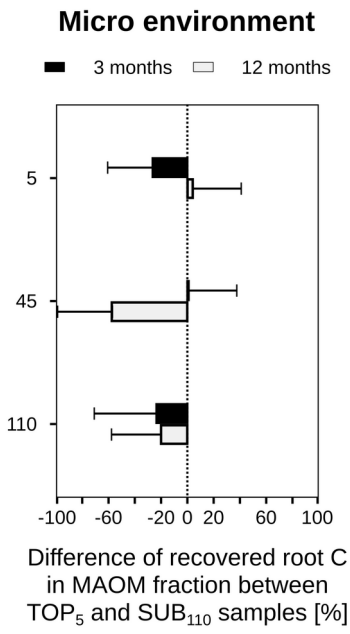

Figure 4.9: Relative difference [%] between TOP_5 and SUB_{110} of recovered root-derived C in the MAOM fraction. Values smaller zero indicate micro environmental limitations in the SUB_{110} samples on root decomposition. Means and standard error (n = 3).

4.4 Discussion

4.4.1 Macro environment limitations

The exposure of TOP_5 and SUB_{110} samples to different soil depths was conducted to reveal the effect of macro environmental conditions in the topsoil and subsoil on the OC decomposition. Although, the total SOC content of TOP_5 samples were not significantly affected by soil depth, the lower amounts of recovered root-derived C in the MAOM fraction in TOP_5 and SUB_{110} samples exposed to a subsoil environment (Figure 4.7) supports the perception of less favorable environmental conditions for microbial decomposition in the subsoil than in the topsoil. Further, the lower loss of fPOM in TOP_5 control samples in the subsoil environment supports the hypothesis of environmental limitations in the subsoil for OC decomposition (Table 4.3). This is in line with findings reported by Hicks Pries et al. (2018), which exposed ^{13}C labeled root litter in three different soil depths (15, 55 and 95 cm) in a coniferous forest. After three years, they recovered more particulate root C in 95 cm depth than in 15 cm. In addition, Gill and Burke (2002) also observed a lower decomposition of buried root litter in 1 m depth than in 10 cm after three years of field exposure. However, the question remains which environmental constraints limit OC decomposition in the subsoil.

At our study site, the macro environment represented by soil temperature, soil moisture, root density and C input differed between topsoil and subsoil. The soil temperature showed differences with depth as the subsoil environment was characterized by a lower temperature amplitude during the experiment (Figure 4.1 and Table 4.2). This may led to a lower OC decomposition in the subsoil environment, due to the non-linear response of temperature on C decomposition (Curtin et al., 2012; Lloyd and Taylor, 1994). However, soil temperature difference between topsoil and subsoil environment (2 °C) was small therefore the differences in decomposition might be also small. Oxygen limitation can be excluded because it was above 18 % in all depths (data not shown). Instead, larger differences were found in the water content between topsoil and subsoil (Figure 4.1 and Table 4.2) which is attributed to the higher sand content in the subsoil (Table 4.1). Water is a major factor for the OC decomposition in the soil. Since it is a transport medium of soluble OC and decomposers and contributes to the translocation of nutrients from the topsoil to the subsoil. In fact, for the study site it was shown that the dissolved organic carbon (DOC) fluxes decline with increasing soil depth from 20 g C m^{-2} yr^{-1} at 10 cm depth to 2 and 1.2 g C m^{-2} yr^{-1} at 50 and 150 cm depth (Leinemann et al., 2016). Furthermore, the root biomass (Heinze et al., 2018; Wordell-Dietrich et al., 2020) and the root exudations (Tückmantel et al., 2017) decreases with increasing soil depth, underlining substrate limi-

tation for microbial decomposers in the subsoil environment at the study site. The microbial biomass of TOP_5 samples exposed to the subsoil environment was 20 % lower as compared to samples in the topsoil environment as reported in the study from Preusser et al. (2019). Similar effects of substrate limitation for the OC decomposition in subsoils were reported in laboratory incubation experiments (Fontaine et al., 2007; Heitkötter and Marschner, 2018).

In addition, Heitkötter and Marschner (2018) showed that SOC mineralization in the subsoil from the study site is limited to some hotspots such as preferential flow paths or rooting channels, but they also found that SOC outside of those hotspots was mineralized after substrate addition (glucose). This indicates, if there are regions with SOC in the subsoil which are disrupted from regular water fluxes, this may also lead to a substrate limitation for decomposers. For the study site it was shown that the spatial heterogeneity of water fluxes were higher in the subsoil than in topsoil (Leinemann et al., 2016), suggesting that the C input into the subsoil environment were not evenly distributed. In consequence, microbial decomposers in the subsoil environment at the study site are substrate limited due to the low amounts of C input via seepage water and the spatial separation due to the sandy soil texture.

4.4.2 Micro environment limitations

Next to the macro environmental conditions in the subsoil, the OC decomposition was also affect by micro environmental conditions inside the used samples, as revealed by the higher amounts of recovered root-derived C in TOP_5 samples than in SUB_{110} samples exposed to the same subsoil environment (Figure 4.7 and Figure 4.9). Soil microorganism might be less substrate limited in TOP_5 samples than in SUB_{110} samples, due to the higher SOC content of TOP_5 samples (Table 4.1). This is in line with Preusser et al. (2019) who found also higher response of the microbial biomass to root addition in SUB_{110} samples than in TOP_5 samples exposed to the same subsoil environment, supporting the hypothesis of substrate limitation in SUB_{110} samples. However, this micro environmental advantage of higher substrate availability for decomposers in TOP_5 samples may have only a short-term effect. If the easily degradable OC in TOP_5 samples are consumed, decomposers will be substrate limited again because of the low C inputs via seepage water in the subsoil. This is in line with results from incubation studies which found a higher mineralization of an added substrate in topsoil samples as compared to subsoil samples (Salomé et al., 2010; Wordell-Dietrich et al., 2017). However, with increasing incubation time the mineralization rates of the added substrate decreases, indicating substrate limitation.

In addition, results from laboratory incubation studies also indicated that OC decomposition might be also controlled the by spatial separation of decomposers and substrate (Don et al., 2013; Salomé et al., 2010; Wordell-Dietrich et al., 2017). Therefore, the lower SOC content in SUB_{110} samples may reduced the probability for microorganisms and exoenzyms to encounter with the added roots or substrate as compared to C rich TOP_5 samples (Ekschmitt et al., 2008). This may also explain the observed loss of SOC in SUB_{110} samples exposed to the subsoil environment after 3 months (Figure 4.5), as the sample preparation (sieving and mixing) led to better contact between substrate and decomposers. However, this disturbance effect was only short term, as indicated by the small differences of SOC content between 3 and 12 months of SUB_{110} samples exposed to the subsoil environment (Figure 4.5).

Moreover, the diffusion of soluble C is the main transport mechanisms between substrate and decomposers in the subsoil environment (Schimel et al., 2011; Xiang et al., 2008). Hence, if the diffusion pathways are missing or disrupted, e.g. due to a lower water content, the OC decomposition should also be reduced due to lower substrate supply (Schjønning et al., 2003). In consequence, the higher sand and lower water content in SUB_{110} samples (Table S4.4) may also limited root decomposition as compared to TOP_5 samples exposed to the same subsoil environment.

Overall, the micro environmental limitations for root decomposition were more pronounced in the substrate limited subsoil environment. While the high C input and the more homogeneous water flux in the topsoil environment may compensate these micro environmental effects, indicated by only minor differences in the amount of recovered root-derived C in the MAOM fraction in TOP_5 and SUB_{110} samples exposed to the topsoil environment (Figure 4.7 and Figure 4.9). Even though, the higher SOC content in TOP_5 samples influenced root litter decomposition in the subsoil environment, the macro environmental conditions (low C input and spatial separation) in the subsoil environment will limit decomposition processes on the long-term at the study site.

4.4.3 Implications for soil C sequestration in the whole soil profile

The translocation of the C poor SUB_{110} samples to the topsoil environment also revealed the C sequestration potential of the subsoil. In fact, the higher C input in the topsoil environment lead to the formation of MAOM in SUB_{110} (Figure 4.6) as compared to SUB_{110} samples exposed to the subsoil environment, supporting the hypothesis that additional C can be stored on unsaturated mineral surface in the subsoil. The observed C sequestration rate of 6.2 g C m^{-2} yr^{-1} would represent an increase of total SOC stocks (0-185 cm) by 0.13 %. However, it must be kept in mind that a subsoil layer of 2 cm thickness only was exposed to the topsoil environment. A 20 cm thick subsoil layer

with a bulk density of 1.6 g cm^{-3} could theoretically sequester 0.6 Mg C ha^{-1} yr^{-1} at our study site. Even if our experiments lasted only one year, it can be assumed that C sequestration in the translocated subsoil material would last longer, as similar C sequestration rates were observed in other studies. For example Alcántara et al. (2016) investigated deep ploughed agricultural soil in northwestern Germany. By deep ploughing, C poor subsoil from 55 to 90 cm depth where translocated to the soil surface. They reported an average C sequestration rate of 0.4 Mg C ha^{-1} yr^{-1} in the first 30 cm of the deep ploughed soil profile. A similar study by Schiedung et al. (2019) investigated changes in SOC stocks after deep soil flipping of pastureland in New Zealand. They reported a mean SOC sequestration rate of 1.2 Mg C ha^{-1} yr^{-1} for flipped soils in the first 15 cm of the soil, 20 years after soil flipping. Underlining, the enormous potential of C storage in soils.

Therefore, C storage actions such as afforestation (Bastin et al., 2019) should also include considerations to increase the SOC in the subsoil. However, methods to translocate C into subsoils as deep ploughing or deep soil flipping are not appropriate for all sites. In the light of the enormous C storage potential in subsoils, there is an urgent need for more research and method development to increase the C content in subsoils. For example, a modified method as used by (Kalks et al., 2020) were a C solution can be directly injected into the subsoils. In dry periods additional C (e.g. slurry or manure) may be directly injected via small tubes (5-10 mm in diameter) to a depth of 1m. However, that would require long term monitoring approaches in the field to measure C dynamics, particularly recording DOC and nitrogen exports to the waterbodies. The usage of a ^{13}C label would allow a better source identification of C in DOC, CO_2 and SOC.

4.5 Conclusion

The *in situ* exposure of topsoil and subsoil material to different soil depths in combination with stable isotope labeling revealed that the macro environmental conditions in the subsoil are less favorable for microbial decomposition leading to a slower decomposition of root litter and particulate organic matter. The OC decomposition in the subsoil at our study site was mainly controlled by the availability of substrate as an energy source for decomposers. The low C input with seepage water into the subsoil horizons and the heterogeneous distribution of water fluxes due to the sandy soil texture leading to substrate limiting conditions in the subsoil environment. Further, the exposure of topsoil material with a high SOC content to the subsoil environment could not compensate for the substrate limiting conditions on the long-term. In contrast, the exposure of C poor subsoil material in the topsoil environment revealed the large C sequestration potential in the subsoil. Therefore, measures to increase C stocks in soils should also include subsoils, even though there is currently a lack of methods to increase SOC stocks in subsoils.

CHAPTER 5 SYNTHESIS & CONCLUSION

5.1 Summary of main results

Table 5.1: Overview of the objectives and the main results for each chapter.

Chapter	Objectives	Results
Vertical partitioning of CO_2 production in a beech forest (Field monitoring of CO_2 concentration in the soil profile and field labeling with ^{13}C enriched litter)	1. Quantification of CO_2 production in the subsoil 2. Identification of sources for CO_2 production along the soil profile	1. 10 % of the annual CO_2 production was produced in the subsoil (> 30 cm). 2. Neither dissolved fresh leaf litter nor old SOC contributed significantly to the CO_2 production in subsoils **CO_2 in subsoils originates from young C sources such as autotrohpic respiration and heterotrpohic respiration in the rhizosphere**
Controlling factors for the stability of subsoil carbon in a Dystric Cambisol (Laboratory incubation experiment)	1. Assessing the impact of temperature and substrate availability on SOC mineralization in the subsoil 2. Determining the stability of additional C inputs in the subsoil.	1. Temperature sensitivity of SOC mineralization decreases with soil depth. Higher substrate availability did not increase native SOC mineralization in the subsoil. 2. Lower mineralization of added dead roots in the subsoil than in the topsoil **SOC mineralization in subsoils might be less vulnerable to climate change. Additional C inputs in subsoils may not destabilize old SOC.**
Environmental constraints limit carbon decomposition in the subsoil (Reciprocal soil transfer experiment)	1. Examining the influence of environmental conditions in a soil profile on the OC mineralization 2. Estimating the C sequestration potential in subsoils due to the formation of MAOM	1. Long-term stability of OC in the subsoil was controlled by the low and spatial heterogeneous C inputs. Higher SOC content increased OC decomposition in the subsoil environment only on a short-term basis 2. Higher C inputs in the subsoil, increased the SOC content by 18 % mainly due to the formation of MAOM. **The unfavorable environmental conditions in the subsoil as the low and heterogeneous C inputs limit the mineralization of OC. Therefore, subsoils provide a great potential for long-term C sequestration.**

5.2 CO_2 production in the subsoil

Although the ^{14}C age of bulk SOC increases with soil depth, indicating mean residence times of several thousand years (Angst et al., 2016), the subsoil also comprises a C pool with much shorter turnover rates. The results of the two year field monitoring of CO_2 concentrations in five depths down to 150 cm in the three subsoil observatories at the Grinderwald study site (Chapter 2) showed that a significant amount of total soil respiration originated from CO_2 production in the subsoils (Figure 2.6). The subsoil (> 30 cm) accounted for 10 % of the annual soil respiration, which is less than the reported 20-50 % found in the few other studies on CO_2 production in subsoils. (Davidson et al., 2006; Davidson and Trumbore, 1995; Fierer et al., 2005; Jassal et al., 2005). The lower values reported in this study might be partly explained due to the depth specific measurements and not soil horizon specific measurements. The subsoil horizons (B) at the study site starts at 10 cm depth (Leinemann et al., 2016, Supplement 1), therefore the average proportion of CO_2 production in the subsoil to total CO_2 production at the study site was 26 %. In addition, the differences in climate (temperate, tropical, Mediterranean) and vegetation cover (grassland, forest) may also explain the wide range of the observed CO_2 production in subsoils.

Furthermore, the laboratory incubation (Chapter 3, Figure 3.1), showed that the SOC mineralization per unit SOC was similar or even higher in the subsoil samples than in the topsoil samples. Thus, subsoils contain a labile C pool that contributes to the annual CO_2 efflux from soils, confirming hypothesis **H1**.

Moreover, the $^{14}CO_2$ data revealed that CO_2 in the subsoil originated mainly from recent C sources (Figure 2.11). This is similar to the few other studies (Fierer et al., 2005; Gaudinski et al., 2000), which measured $^{14}CO_2$ in the subsoil. In addition, the ^{13}C labeling experiment showed that annual litter-derived C inputs were only a minor source for CO_2 production below 10 cm. Further, C inputs via DOC below 10 cm depth at the study site were estimated with 18 g m^{-2} yr^{-1} (Leinemann et al., 2016), while the annual CO_2 production below 10 cm depth is about ten time larger (200 gC m^{-2} yr^{-1}, Figure 2.6). Thus, CO_2 in subsoil must originate from other C sources such as autotrophic respiration and heterotrophic respiration in the rhizosphere, as root-derived C seems to be the major C source in the subsoil at the study site, which confirms hypothesis **H2**.

5.3 Temperature sensitivity of the subsoil

The laboratory incubation experiment with undisturbed topsoil and subsoil samples (Chapter 3) showed a decreasing temperature sensitivity of SOC mineralization with soil depth (Figure 3.3). Thus the hypothesis **H3** of a higher temperature sensitivity of SOC mineralization in subsoils due to a higher recalcitrance of subsoil OM than of topsoil OM cannot be confirmed. As pointed out by Davidson and Janssens, (2006) the intrinsic temperature sensitivity of SOM may be attenuated due to environmental constraints such as limited access to fresh substrate for decomposers, low SOC content or aggregation. Therefore, the low SOC content and the low C inputs in the subsoil may obscure the temperature sensitivity of SOC mineralization in the subsoil. However, neither the addition of root material, nor the disturbance due to sieving, affected the temperature sensitivity of SOC mineralization in the subsoil. Therefore, the attenuating effect of substrate limitation or physical protection on the temperature sensitivity in the subsoils could not be confirmed. Further, the lower temperature sensitivity in the subsoil may also supports the hypothesis **H1** of a labile C pool in the subsoil, as the mineralization of labile SOC is assumed to be less temperature sensitive.

Nevertheless, as the temperature sensitivity of subsoils are usually investigated in laboratory incubations under controlled environmental conditions with varying results (Conen et al., 2008; Fang et al., 2005; Fierer et al., 2003; Karhu et al., 2010; Winkler et al., 1996), the question of how climate change will affect SOC mineralization in subsoils remains open. In a unique two year field warming experiment where the whole soil was warmed down to 100 cm depth, Hicks Pries et al. (2017b) found that the temperature sensitivity of CO_2 production was similar in topsoil and subsoil horizons. This indicates, that SOC mineralization in subsoils and topsoils may respond similar to climate change in temperate and warm climates. In contrast, in higher latitudes where temperature is the limiting factor for decomposition e.g. permafrost regions, the temperature response of soils and subsoils will be higher (Carey et al., 2016; Tang et al., 2019). However, field and laboratory data on the temperature response of SOC mineralization in subsoils is rare, which make it hard to incorporate C dynamics in subsoils in global C models.

In conclusion, SOC mineralization in the subsoil showed no higher temperature sensitivity than in the topsoil. Further, temperature sensitivity in the studied subsoil was not obscured by other stabilization mechanisms such as substrate limitation or aggregation. This implicates, that SOC mineralization in the subsoil might be less affected by climate change as previously assumed.

5.4 Carbon stability in subsoils is controlled by substrate availability

The high stability of subsoil OC and the high ^{14}C ages were also explained due the lack of an easily available energy source for soil microorganisms (Fontaine et al., 2004, 2007; Marschner et al., 2008). The lower C inputs into the subsoil due to DOM, roots and roots exudates results in substrate limitations for decomposers to meet their energy demand (Joergensen and Wichern, 2018). Hence, the addition of OC to subsoils may stimulate the decomposition of native SOC, which is known as priming. However, research so far on priming in subsoils are limited and findings are inconsistent (Fierer et al., 2003; Fontaine et al., 2007; Karhu et al., 2016; Salomé et al., 2010).

The incubation experiment of Chapter 3, revealed that the addition of root litter to topsoil and subsoil horizons did not enhance the native SOC mineralization (Figure 3.7), which fits to findings of Salomé et al. (2010). In contrast, others reported positive priming effects in subsoils after glucose addition (Fontaine et al., 2007; Karhu et al., 2016). The different results of priming observed in the incubation studies, might be explained due to the differences in soil properties, for example higher positive priming effects were observed in soils with a pH between 6 and 8 (Blagodatskaya and Kuzyakov, 2008). The soil used in this thesis had a pH of 4, while the other studies reported a pH of 7 (Fontaine et al., 2007; Salomé et al., 2010). In addition, the subsoil used in this thesis is characterized by a much lower SOC content (0.4 mg C g^{-1}, Table 3.1) as compared to Fontaine et al. (2007) (23.3 mg C g^{-1}), which may limited the probability for microorganisms or exoenzymes to encounter with SOC (Don et al., 2013; Ekschmitt et al., 2008), due to a larger spatial separation. This is also indicated by the lower mineralization of the added roots in the C poor subsoil as compared to the topsoil (Figure 3.4). A similar pattern was found in the reciprocal soil transfer experiment of Chapter 4, where topsoil and subsoil material with and without ^{13}C labeled root litter were exposed to different depths, reflecting different soil environmental conditions. In the topsoil samples exposed to the subsoil environment a higher amount of root-derived C was recovered in the MAOM fraction as in subsoil samples exposed to the same soil environment (Figure 4.7). This implies that more of the added root C was mineralized in the topsoil samples than in the subsoil samples, which is in line with the results from the laboratory incubation. One explanation might be that decomposers are less limited on labile substrate in the topsoil samples as in the subsoil samples, due to a higher SOC content. Another explanation might be that diffusion of soluble C is the main transport process for substrate in the subsoil environment and during the laboratory incubation Therefore, the lower sand content (70 %) in the topsoil and the first subsoil horizon (Table 3.1) provided a better connectivity between

decomposers and substrate, which caused a lower spatial separation than in the deep subsoil samples with a higher sand content (90 %). This partly supports the hypothesis of a higher spatial separation in the subsoil than in the topsoil (**H4**)

However, the laboratory incubation also showed that with increasing incubation time the mineralization rates of the added roots decreased in all samples (Figure 3.5), indicating substrate limiting conditions despite the higher SOC content and lower sand content in the topsoil samples. Therefore, the benefit of better micro environmental conditions on OC mineralization might by only a short-term effect and without supply of C inputs OC mineralization is reduced. This assumption is supported by results of the reciprocal soil transfer experiment. Samples with high and low SOC content exposed to the subsoil environment (110 cm depth) contained less root-derived C in the MAOM fractions as their counterparts exposed to the topsoil environment (Figure 4.7), underlining the importance regular substrate supply for SOC decomposition in the subsoil environment. In addition, the comparison of the topsoil samples exposed to 5 cm and 110 cm depth showed a lower loss in 110 cm in the fPOM fraction (Figure 4.4, Table 4.3), which is seen as the most labile and easily degradable OC fraction in soils (von Lützow et al., 2007). So far, only a few studies investigated the decomposition of OM in subsoils under field conditions. However, the results presented in this thesis are in line with findings from Hicks Pries et al. (2018) and Gill and Burke (2002) who exposed root litter to topsoil and subsoil horizons and after three years they recovered more root C in the subsoil horizon as in the topsoil horizon.

For the study site it was shown that the C inputs via DOC strongly decline with soil depth and are more heterogeneous (Leinemann et al., 2016). Also, the root biomass (Heinze et al., 2018; Wordell-Dietrich et al., 2020) and the root exudations (Tückmantel et al., 2017) decreased with increasing depth. This supports the hypothesis **H4** that decomposition in the subsoil is substrate limited due to lower C inputs. In conclusion, the laboratory incubation revealed that micro environmental differences in SOC content and soil texture controlled OC decomposition on short-term, while macro environmental conditions such as C input via DOC or root distribution in the soil profile control the long-term stability of subsoil SOC. In consequence, the high stability of subsoil OC is controlled by the amount and distribution of regular C inputs. Therefore, subsoils may have the greatest potential for long-term C sequestration due to the low C inputs.

5.5 Implications

Based on current estimates about 60 Gt of C was emitted from soils to the atmosphere between 1959 to 2010 due to the land conversion and agricultural use (Lal, 2016). This implicates that soils have a C deficit and can store additional C. Thus, soil C sequestration is a good and cost effective measure for climate change mitigation (Fuss et al., 2018). Research so far mainly focused on increasing C stocks in topsoils (<30 cm) (Minasny et al., 2017) but ignoring the large volume of the subsoil (> 30 cm) for C sequestration. However, a few studies already showed that natural or anthropogenic C burial due to land slides, erosion or deep ploughing can significantly sequester large amount of C in the long-term (Alcántara et al., 2016, 2017; Berhe et al., 2007; Quinton et al., 2010; Schiedung et al., 2019). Therefore, C sequestration measures and research should also include the subsoils.

The advantages of C sequestration in subsoils versus C sequestration in topsoils are first the slower mineralization of OC in the subsoil, which may results in long-term storage. Secondly, as C sequestration mainly focus on degraded or agricultural soil, the soil volume for soil C sequestration is limited. However, if the subsoils are included the volume is much larger. Further, also other soils might be taken into consideration e.g. forest soils. Lastly, the C saturation concept proposed by Stewart et al. (2007, 2008) assumed that, the further a soil is from its carbon saturation level, the higher is the efficiency of C sequestration. This is also supported by findings from the reciprocal soil transfer experiment (Chapter 4), the translocation of C poor subsoil material to a topsoil environment increased the SOC content of the subsoil material by 18 % within one year. In consequence, the C sequestration efficiency should be much higher in subsoil due to the low SOC content as compared to topsoils.

Unfortunately, the number of methods to increases SOC in deeper soil horizons is limited and methods which disturb the whole soil such as deep ploughing will not work in established forest. In turn, deep ploughing might be used as site preparation for afforestation. Therefore, future soil C research should also focus on method development to increases SOC stocks in subsoils with lower disturbance of the soil.

As found in this thesis, the stability of OC in the studied forest subsoil is determined by the low C inputs. However, further research must show if the results found on this specific study site are transferable to other soil type and climatic regions. Further, if the stability of SOC in subsoils is controlled by C inputs, the risk of SOC losses due to changes in the C inputs should also be assessed.

5.6 Conclusion

Although subsoils store more than 50 % of global SOC stocks, the knowledge on the mechanisms controlling the turnover of such a large C pool is limited. Thus, predicting changes in subsoil OC stocks following global change is almost impossible without understanding the C dynamics in deeper soil horizons. This is even more complicated, as the contribution of subsoils to total soil respiration is unclear. In spite of the increasing mean residence times of SOC with depth, it was shown that the subsoil accounted for 10 % of the annual CO_2 production. Moreover, the modern radio carbon signature of CO_2 in the subsoil (Chapter 2) and the higher specific mineralization rates in the deep subsoil observed in the laboratory incubation experiment (Chapter 3) revealed that mainly recent C sources were being respired, likely root-derived C.

In addition, the temperature sensitivity decreased with soil depth, even under elevated substrate availability. This implicates, that SOC mineralization in the subsoil might be less affected by climate change as previously assumed. Further, the lower temperature sensitivity in the subsoil also supports the hypothesis that subsoils contain a labile C pool, since the mineralization of labile SOC is assumed to be less temperature sensitive.

The lack of fresh substrate as an available energy source for decomposers is given as an explanation for the high stability of subsoil OC. However, in the laboratory incubation the addition of root litter to the subsoil as an available energy source did not enhance the mineralization of native subsoil OC. This is an important finding, because it implicates that additional C inputs to subsoils as part of C sequestration measures may not affect the mineralization of stable and ancient subsoil OC. Furthermore, during the laboratory incubation added roots were more stable if mixed in subsoil material with a low SOC and a high sand content than if mixed into C rich topsoil material. This suggests that decomposers are facing a greater spatial separation from the substrate in the subsoil than in the topsoil. However, the *in situ* exposure of topsoil and subsoil samples with added root litter to a topsoil and a subsoil environment indicated that long-term C stability in the subsoil environment was controlled by the distribution and the quantity of C inputs and less due to micro environmental differences such as the SOC content.

In consequence, heterogeneous and low C inputs into the subsoil, result in substrate limiting conditions for decomposers, which explains the high stability of OC in subsoils.

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SUPPLEMENT CHAPTER 2

Compensation algorithm of dependence of pressure and temperature for GMP221 sensors (Eq. S2.1):

$$c_{[i+1]} = c_1 - k_{p1}[c_i] \times \left(\frac{p-1013}{1013}\right)^2 - k_{p2}[c_i] \times \left(\frac{p-1013}{1013}\right) \times p - k_{t1}[c_i] \times \left(\frac{T-25}{25}\right)^3 - k_{t2}[c_i] \times \left(\frac{T-25}{25}\right)^2 - 16320 \times \left(-(k_{t3}[c_i])^2 + k_{t3}[c_i]\right) \times \left(\frac{T-25}{25}\right) \quad \text{(Eq. S2.1)}$$

where i ∈ {1,2,3,4}, $c_{(i+1)}$ [ppm] is the compensated CO_2 reading in the iteration process, c_1 is the uncompensated reading in [ppm], p is the pressure in [hPa], T is the temperature in [°], and k_{p1}, k_{p2}, k_{t1}, k_{t2} and k_{t3} are empirical derived functions.

$$k_{p1}[c_i] = A_{p1} \times c_i^4 + B_{p1} \times c_i^3 + C_{p1} \times c_i^2 + D_{p1} \times c_i \quad \text{(Eq. S2.2)}$$

$$k_{p2}[c_i] = A_{p2} \times c_i^3 + B_{p2} \times c_i^2 + C_{p2} \times c_i \quad \text{(Eq. S2.3)}$$

$$k_{t1}[c_i] = A_{t1} \times c_i^3 + B_{t1} \times c_i^2 + C_{t1} \times c_i + D_{t1} \quad \text{(Eq. S2.4)}$$

$$k_{t2}[c_i] = A_{t2} \times c_i^2 + B_{t2} \times c_i \quad \text{(Eq. S2.5)}$$

$$k_{t3}[c_i] = A_{t3} \times c_i^3 + B_{t3} \times c_i^2 + C_{t3} \times c_i \quad \text{(Eq. S2.6)}$$

where c_i is the CO_2 concentration in [%] and A, B, C, D are empirical derived constants (Table S2.1).

Table S2.1: Empirical derived constants for temperature and pressure compensation.

A_{p1} =	0.97501	A_{p2} =	-9.3269E-3	A_{t1} =	0.046481	A_{t2} =	-3.0166	A_{t3} =	8.3600E-5
B_{p1} =	-54.1519	B_{p2} =	0.14345	B_{t1} =	-1.02280	B_{t2} =	-8.8421	B_{t3} =	-2.4199E-3
C_{p1} =	479.778	C_{p2} =	15.7164	C_{t1} =	-37.4433			C_{t3} =	0.066814
D_{p1} =	-11362.8			D_{t1} =	-49.000				

The compensated reading was calculated in an iterative process. In the first iteration loop (i=1), c_2 was calculated from Eq. S2.1 by using c_1 for Eq. S2.2-Eq. S2.6. The obtained c_2 was then used in the following loop and so on. The iteration stops at the last c_5, which was the temperature and pressure corrected reading.

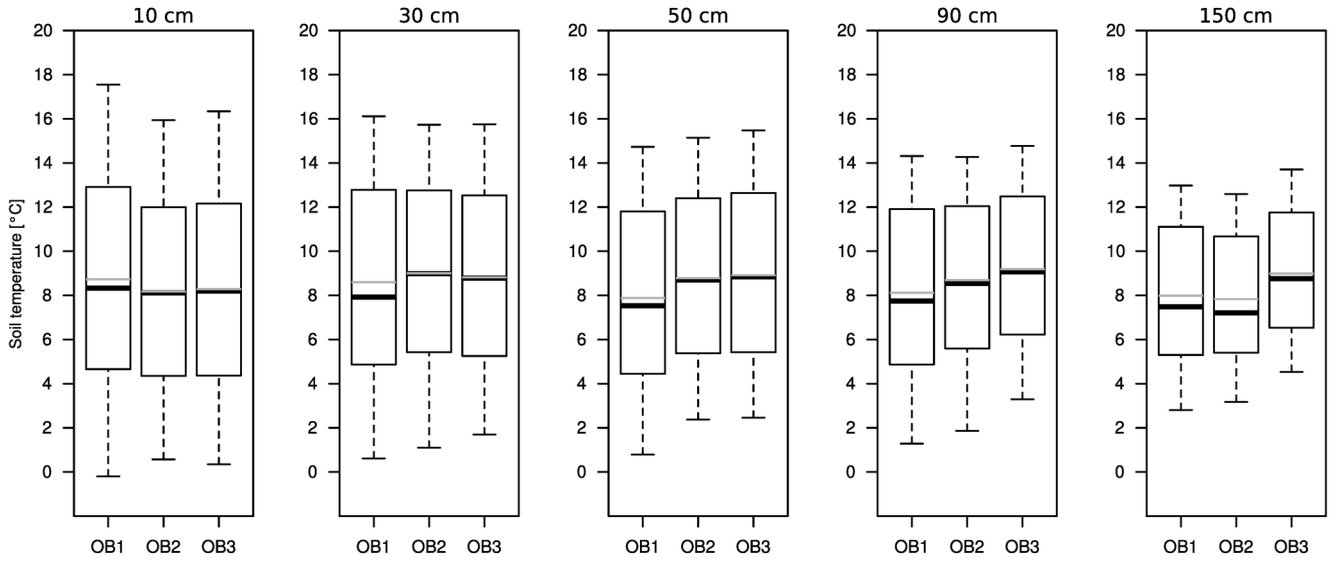

Figure S2.1: Box-whisker-plot of soil temperature for each soil depth and observatory (OB). Medians and means are shown as black and grey lines respectively.

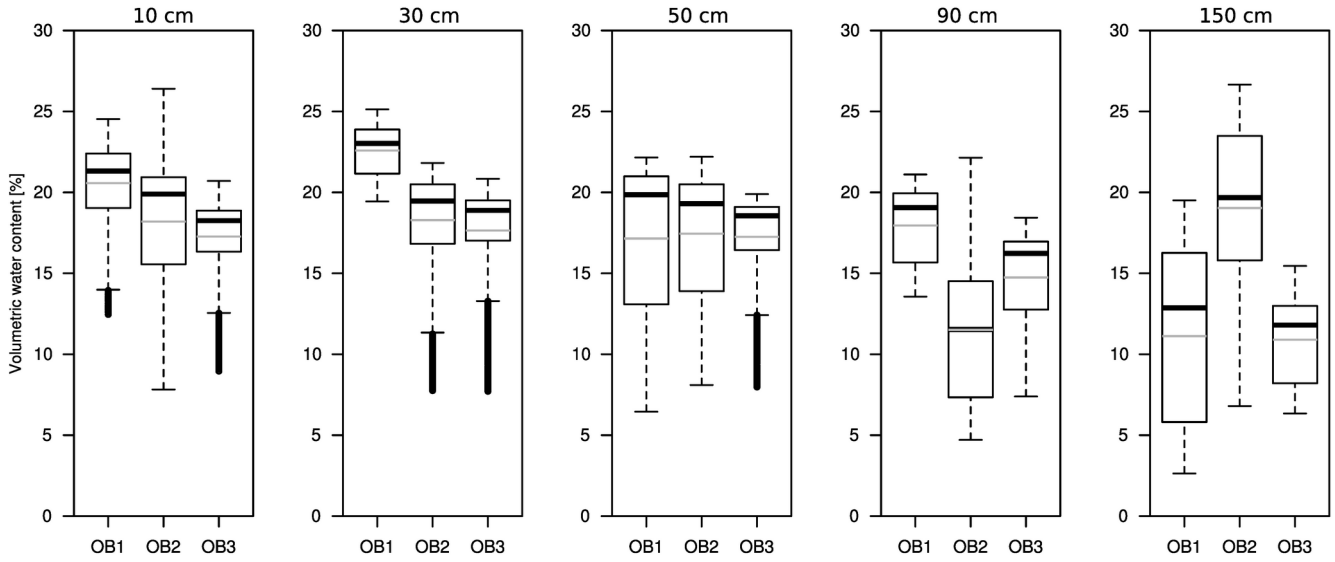

Figure S2.2: Box-whisker-plot of volumetric water content for each soil depth and observatory (OB). Medians and means are shown as black and grey lines respectively.

SUPPLEMENT CHAPTER 4

Table S4.1: Model outputs for the assessment of the effect of soil depth and sampling time on the C content in the bulk soil and the MAOM fraction for the TOP_5 samples for both treatments.

TOP_5 - Bulk OC [mg C g^{-1} soil]									
Control					Root addition				
	Model: OC ~ Depth + Time					Model: OC ~ Depth + Time			
	Value	Std.Error	t-value	P-value		Value	Std.Error	t-value	P-value
Intercept (5 cm | 3 months)	24.29	1.33	18.23	< 0.01	Intercept (5 cm | 3 months)	25.10	1.88	13.37	< 0.01
45 cm	-1.96	1.63	-1.20	0.25	45 cm	1.27	2.30	0.55	0.59
110 cm	-2.50	1.63	-1.53	0.15	110 cm	-2.61	2.30	-1.13	0.28
12 months	1.84	1.33	1.38	0.19	12 months	2.87	1.88	1.53	0.15

TOP_5 - MAOM OC [mg C g^{-1} soil]									
Control					Root addition				
	Model: OC ~ Time					Model: OC ~ Depth + Time			
	Value	Std.Error	t-value	P-value		Value	Std.Error	t-value	P-value
Intercept (3 months)	41.18	1.39	29.53	< 0.01	Intercept (5 cm | 3 months)	40.18	1.69	23.77	< 0.01
12 months	3.16	1.97	1.60	0.13	45 cm	2.08	2.07	1.01	0.33
					110 cm	-0.49	2.07	-0.24	0.81
					12 months	4.26	1.69	2.52	0.02

Table S4.2: Model outputs for the assessment of the effect of soil depth and root addition on the C content in the bulk samples and the MAOM fraction for the SUB_{110} samples.

SUB_{110} - Bulk OC [mg C g^{-1} soil]									
Control					Root addition				
	Model: OC ~ Depth					Model: OC ~ Depth			
	Value	Std.Error	t-value	p-value		Value	Std.Error	t-value	p-value
Intercept (5 cm)	1.21	0.07	16.45	< 0.01	Intercept (5 cm)	1.24	0.06	19.71	< 0.01
45 cm	-0.43	0.10	-4.18	< 0.01	45 cm	-0.40	0.07	-5.78	< 0.01
110 cm	-0.46	0.10	-4.18	< 0.01	110 cm	-0.16	0.12	-0.97	0.35

SUB_{110} - MAOM OC [mg C g^{-1} soil]									
Control					Root addition				
	Model: OC ~ Depth					Model: OC ~ Depth			
	Value	Std.Error	t-value	p-value		Value	Std.Error	t-value	p-value
Intercept (5 cm)	3.72	0.19	19.60	< 0.01	Intercept (5 cm)	4.06	0.24	16.55	< 0.01
45 cm	-1.04	0.27	-3.86	< 0.01	45 cm	-1.24	0.25	-5.01	< 0.01
110 cm	-1.14	0.27	-4.24	< 0.01	110 cm	-1.42	0.25	-5.64	< 0.01

Table S4.3: Model outputs for the assessment of the effect of soil depth, soil material (TOP_5 and SUB_{110}) and sampling time on the amount of recovered root-derived C in the MAOM fraction.

Recovered root-derived C in MAOM				
	Model: Root ~ Depth + Origin + Time			
	Value	Std.Error	t-value	p-value
Intercept (5 cm | SUB_{110} | 3 months)	3.79	0.67	5.68	<0.01
45	-1.36	0.70	-1.95	0.06
110	-1.30	0.70	-1.86	0.07
TOP_5	1.04	0.56	1.83	0.08
12 months	1.35	0.56	2.40	0.02

Table S4.4: Water content (%) of TOP_5 and SUB_{110} samples after sampling (3 and 12 months). Means and standard error (n = 6).

	Water content (%)			
Depth	TOP_5		SUB_{110}	
[cm]	3 months	12 months	3 months	12 months
5	14.0 (± 1.2)	9.7 (± 0.5)	3.6 (± 0.1)	2.4 (± 0.1)
45	11.5 (± 1.1)	11.0 (± 0.7)	2.7 (± 0.2)	2.4 (± 0.2)
110	17.8 (± 0.8)	16.7 (± 0.4)	4.4 (± 0.1)	4.3 (± 0.1)

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