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Factors and mechanisms controlling soil organic carbon turnover in subsoils of agricultural sites

Dissertation

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by

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Preface

This thesis was prepared within the Research Training Group "Regulation of soil organic matter and nutrient turnover in organic agriculture" (Graduiertenkolleg 1397/3) and funded by the Deutsche Forschungsgemeinschaft (DFG). The thesis is submitted to the Faculty of Organic Agricultural Sciences to fulfil the requirements for the degree "Doktor der Naturwissenschaften" (Dr. rer. nat.). The dissertation is based on three papers as first author, which are published in or submitted to international refereed journals. The manuscripts are included in chapters 2, 3 and 4. Chapter 1 comprises a general introduction to the research topic as well as the objectives this thesis was based on. Chapter 5 contains the overall conclusions and is followed by an outlook on future research needs in chapter 6. Supplementary materials are found in chapter 8.

The following papers are included in this thesis:

Chapter 2:

Struecker, J., Joergensen, R.G., 2015. Microorganisms and their substrate utilization patterns in topsoil and subsoil layers of two silt loams, differing in soil organic C accumulation due to colluvial processes. Soil Biology and Biochemistry 91, 310-317

Chapter 3:

Struecker, J., Dyckmans, J., Joergensen, R.G., 2016: Amaranth decomposition under elevated CO_2 concentrations – comparing topsoils with C-poor and C-rich subsoils. Soil Biology and Biochemistry (under revision)

Chapter 4:

Struecker, J., Kaiser, M., Dyckmans, J., Joergensen, R.G., 2016. 4. Maize root decomposition in subsoil horizons of two silt loams differing in soil organic C accumulation due to colluvial processes. Geoderma (submitted)

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List of abbreviations

α	Significance level
AMB	Microbial biomass C activated by glucose amendment
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
C	Carbon
C3 plant	Plant with C3 pathway for carbon fixation in photosynthesis
C4 plant	Plant with C4 pathway for carbon fixation in photosynthesis
Cam	Colluvic Cambisol
CFE	Chloroform fumigation extraction
$CHCl_3$	Chloroform
CLPP	Community level physiological profile
CO_2	Carbon dioxide
CS	Cambisol subsoil
CT	Cambisol topsoil
CV	Coefficient of variation
DFG	Deutsche Forschungsgemeinschaft
Fig	Figure
flF	Free light fraction
fPOM	Free particulate organic matter
GalN	Galactosamine
GlcN	Glucosamine
HCl	Hydrochloric acid
HCO_3^-	Hydrogencarbonate

HF	Heavy fraction
HPLC	High performance liquid chromatography
K_2SO_4	Potassium sulphate
k_{EC}, k_{EN}	Extractable portion of total C, and N from microbial biomass
LD	Linear discriminant function
LS	Luvisol subsoil
LT	Luvisol topsoil
Luv	Haplic Luvisol
ManN	Mannosamine
MBC	Microbial biomass carbon
MRT	Mean residence time
MSIR	Multi substrate induced respiration
MurN	Muramic acid
n	Number of samples
N	Total nitrogen
N_2	Nitrogen (gaseous)
O_2	Oxygen
OC	Organic carbon
olF	Occluded light fraction
p	Probability value for significance
PLFA	Phospholipid fatty acids
qCO_2	Metabolic quotient
r	Correlation coefficient
SOC	Soil organic carbon

SOM	Soil organic matter
SPT	Sodium polytungstate
Vol.%	Volume percent
WHC	Water holding capacity
WRB	World reference base for soils
$\delta^{13}C$	$^{13}C/^{12}C$ ratio expressed relative to the VPDB standard
$\delta^{15}N$	$^{14}N/^{15}N$ ratio expressed relative to the air standard

Summary

Two-third of the terrestrial C is stored in soils, and more than 50% of soil organic C (SOC) is stored in subsoils from 30 – 100 cm. Hence, subsoil is important as a source or sink for CO_2 in the global carbon cycle. Although SOC contents in subsoil are considerably smaller than in topsoil, the SOC stocks in subsoil are of significant size compared to other terrestrial C-pools. Even in shallow soils, which contain only small amounts of SOC below the organic horizon, most of the stable organic carbon (OC) is stored in subsoil, as several studies have shown that subsoil OC is of a higher average age than OC in topsoils. In terms of climate change research it would be desirable to avoid mineralization of this old subsoil SOC and to promote SOC storage in subsoil. However, there is still a lack of knowledge regarding the mechanisms of C sequestration and C turnover in subsoil.

Three main factors are discussed, which separately or in combination with each other possibly reduce carbon turnover rates in subsoil: Resource limitation, changes in the microbial community, and changes in gas conditions.

Resource limitation can be subdivided into three aspects: a) Limited substrate quality, b) Limited fresh organic matter input, and c) Limited access to substrate. Limited substrate quality means that the organic material does not provide enough energy to cover the energy demand for substrate degradation. This point is closely related to the second aspect, the limited input of fresh organic matter into subsoil, as fresh organic matter would supply enough energy for a co-degradation of fresh and old SOC. The third aspect, the limited access to substrate can be either caused by the physical separation of substrate and microorganisms or the physico-chemical stabilization of SOC. These conditions make it hard for microorganisms to access the substrate.

The relationships between SOC stocks and composition and functional diversity of the microbial community in subsoil have not been thoroughly investigated. Therefore it is widely

unknown in how far changes in the microbial community could also play a major role regarding the carbon sequestration mechanisms in subsoil. However, the structure of the microbial community seems to strongly influence SOC dynamics and in reverse, quantity and quality of the available substrates are major factors affecting the microbial community. As SOC characteristics are supposed to change with depth, composition and functional diversity of the microbial community probably also change with depth, which again alters SOC sequestration processes.

Changes in gas conditions towards higher CO_2 and lower O_2 concentrations compared to atmospheric conditions might have negative impacts on the microbial community. It was proven that bacteria are relatively robust to changes in the gas conditions in terms of elevated CO_2 while the fungal community was affected by changes in atmospheric gas conditions. But by now there are only assumptions and few experiments on the influence of the different gas conditions in subsoil.

The experiments conducted in this study focused on two neighbouring arable sites, with depth profiles differing in SOC stocks: One Colluvic Cambisol with high SOC contents (8-12 g kg$^-$ ^{1}) throughout the profile and one Haplic Luvisol with low SOC contents (3-4 g kg^{-1}) below 30 cm depth. The SOC contents developed naturally over the past 800 years due to erosion and deposition of surface soil material. This offers the opportunity to investigate the extent to which natural C availability or other subsoil specific conditions influence C turnover, while all other environmental conditions on the two sites are equal.

The first experiment was designed to gain more knowledge regarding the microbial community and its influence on carbon sequestration in subsoil. Soil samples were taken at four different depths (0-15, 40-50, 50-60, and 75-85 cm) on the two neighbouring arable sites. Microbial biomass C (MBC) was determined using CFE, to identify depth gradients of microbial biomass C in relation to the natural C availability. The amino sugars muramic acid and glucosamine were

measured as indicators for bacterial and fungal residues and ergosterol was determined as a marker for saprotrophic fungi, to quantify changes in the in the microbial community composition. Multi-substrate-induced-respiration (MSIR) was used to identify shifts in functional diversity of the microbial community along the profiles. Discriminant analysis of respiration values obtained from the 17 substrates used in the MSIR revealed that substrate use in subsoil differed significantly from that in topsoil and also differed highly between the two subsoils, indicating a strong influence of resource limitations on microbial substrate use. Amino sugar analysis and the ratio of ergosterol to microbial biomass C showed that fungal dominance decreased with depth. The results clearly demonstrated that not only the fungi to bacteria ratio but also substrate use of the microbial community changed with depth according to substrate availability.

The second experiment was an incubation experiment, which was carried out for 176 days at 22 °C, using elevated CO_2 concentrations (4 and 8%) with and without the addition of amaranth, a C4 plant. We determined the effects of these treatments on soil organic C (SOC), microbial biomass C and fungal ergosterol. Soil samples were taken from topsoil and subsoil of the two neighbouring arable sites. Soils lost between 26 and 38% SOC during the incubation, without effects of elevated CO_2 concentrations. Amaranth-derived C losses were generally stronger in the Colluvic Cambisol (7.5 mg g^{-1}), especially at elevated CO_2 concentrations, than in the Haplic Luvisol (7.0 mg g^{-1}), without clear differences between topsoils and subsoils. Amaranth addition caused negative priming effects on SOC mineralization in the subsoils, which were similar in the Cambisol (11%) and Luvisol (14%). Elevated CO_2 concentrations had no general effects on microbial biomass C or ergosterol with and without amaranth addition. However, the contribution of amaranth-derived microbial biomass C to total microbial biomass C was significantly ($\alpha = 0.1$) reduced at elevated CO_2 concentrations (Topsoil: 31%; Subsoil: 54%). This leads to the conclusion that elevated CO_2 and reduced O_2 concentration alter the metabolism of microorganisms but not

the degradation of added plant residues is general. The fact that in the substrate amended samples there was less autochthonous SOC degraded suggests a negative priming effect after the addition of a relatively complex substrate. The microbial community can probably retrieve energy and nutrients more easily from the plant residues than from autochthonous SOC.

The third experiment was a field experiment carried out for two years. Mesh bags containing original soil material (C3 plant ^{13}C signature) and maize root residues (C4 plant) were buried at three different depths (35, 45, and 65 cm) at the two neighbouring arable sites. The recovery of the soilbags took place 12, 18, and 24 months after burial. We determined the effects of these treatments on SOC, density fractions, and MBC. The mean residence time for maize-derived C was similar at all depths and both sites (403 d). Microbial biomass C increased to a similar extent (2.5 fold) from the initial value to maximum value. This increase relied largely on the added maize root residues as about 50% of the MBC was maize-derived after two years. However, there were clear differences visible in terms of the substrate use efficiency, which decreased with depth and was lower in the Haplic Luvisol than in the Colluvic Cambisol. Hence freshly added plant material is highly accessible to microorganisms in subsoil and therefore equally degraded at both sites and depths, but its metabolic use was determined by the legacy of soil properties.

These findings provide strong evidence that resource availability from autochthonous SOM as well as from added plant residues have a strong influence on the microbial community and its use of different substrates. However, under all of the applied conditions there was no evidence that complex substrates, i.e. plant residues, are less degraded in subsoil than in topsoil, irrespective of the autochthonous resource stocks.

Zusammenfassung

Zwei Drittel der terrestrischen Kohlenstoffvorräte sind in den Böden der Welt gespeichert. Mehr als 50% dieser organischen Kohlenstoffvorräte im Boden sind in Unterböden zwischen 30 und 100 cm gespeichert. Demnach können Unterböden eine wichtige Rolle, als Quelle oder Speicher von CO_2, im globalen Kohlenstoffkreislauf spielen. Obwohl die C Gehalte im Unterboden deutlich geringer sind als im Oberboden, sind die Kohlenstoffvorräte im Unterboden, verglichen mit anderen terrestrischen C-Pools von bedeutender Größe. Sogar für flachgründige Böden, die nur geringe Kohlenstoffgehalte unterhalb des organischen Horizontes aufweisen, wurde in einigen Studien gezeigt, dass der Großteil des stabilen organischen Kohlenstoffs im Unterboden gespeichert wird, da der organische Kohlenstoff im Unterboden ein deutlich höheres Durchschnittsalter aufweist als im Oberboden. Bezogen auf die Forschung zum Klimawandel, wäre es wünschenswert die Mineralisierung des im Unterboden gespeicherten Kohlenstoffs zu vermeiden und weitere Speicherung von organischem Kohlenstoff im Unterboden zu fördern. Allerdings bestehen nachwievor große Wissenslücken die Mechanismen betreffend, die Kohlenstoff Speicherung oder Umsatz im Unterboden steuern.

Derzeit werden drei Hauptfaktoren diskutiert, die entweder alleine oder in Kombination miteinander, die Umsatzraten für organischen Kohlenstoff im Unterboden möglicherweise reduzieren: Begrenzte Ressourcenverfügbarkeit, Veränderungen in der mikrobiellen Gemeinschaft und eine veränderte Bodengaszusammensetzung im Unterboden.

Der Faktor "Begrenzte Ressourcenverfügbarkeit" kann in drei Unterpunkte unterteilt werden: a) Verminderte Substratqualität, b) Verminderter Input von frischer organischer Substanz und c) Begrenzter Zugang zum Substrat. Verminderte Substratqualität bedeutet in diesem Zusammenhang, dass das verfügbare organische Material nicht so viel Energie liefert, wie für seinen Abbau benötigt wird. Dieser Punkt ist eng mit dem zweiten Punkt, dem verminderten Input

von frischer organischer Substanz verknüpft, da frische organische Substanz einen Überschuss an Energie liefert, der auch den Abbau von energiearmer organischer Substanz ermöglichen würde. Der dritte Punkte, der begrenzte Zugang zum Substrat, kann sowohl durch die räumliche Trennung von Mikroorganismen und Substrat als auch die physico-chemische Stabilisierung der organischen Substanz an der Bodenmatrix hervorgerufen werden. In beiden Fällen ist der Zugang zum Substrat für die Mikroorganismen erheblich erschwert.

Veränderungen in der mikrobiellen Gemeinschaft könnten ebenfalls eine entscheidende Rolle spielen in Bezug auf die Mechanismen, die die Kohlenstoffspeicherung im Unterboden steuern. Die Beziehung zwischen Kohlenstoffvorräten, Zusammensetzung und funktioneller Diversität der mikrobiellen Gemeinschaft wurden bis dato nicht eingehend untersucht. Die Struktur der mikrobiellen Gemeinschaft scheint die Kohlenstoffdynamik im Boden stark zu beeinflussen, während umgekehrt Quantität und Qualität der organischen Bodensubstanz die Zusammensetzung der mikrobiellen Gemeinschaft stark beeinflussen. Da angenommen werden kann, dass die Eigenschaften der organischen Bodensubstanz sich mit der Tiefe verändern, ist es sehr wahrscheinlich, dass sich auch die Zusammensetzung und die funktionelle Diversität der mikrobiellen Gemeinschaft mit der Tiefe verändern. Das könnte wiederum einen Einfluss auf den Prozess der Kohlenstoff-Stabilisierung im Unterboden haben.

Veränderungen in der Bodengaszusammensetzung hin zu erhöhten CO_2 und verminderten O_2 Gehalten, im Vergleich zur Atmosphärenluft, könnten einen negativen Einfluss auf die mikrobielle Gemeinschaft haben. Es wurde zwar nachgewiesen, dass Bakterien sehr robust auf Veränderungen der Gaszusammensetzung reagieren, wohingegen bei Pilzen ein negativer Einfluss von O_2 Mangel beobachtet wurde. Allerdings gibt es bislang nur wenige Annahmen und Studien, die sich auf den Einfluss von veränderten Bodengasverhältnissen im Unterboden auf die mikrobielle Gemeinschaft beziehen.

Die Experimente im Rahmen dieser Studie wurden auf zwei benachbarten landwirtschaftlich genutzten Flächen durchgeführt, deren Tiefenprofile sich in den organischen Kohlenstoffgehalten unterscheiden. Bei der ersten Fläche handelt es sich um ein Kolluvium über einer verschütteten Schwarzerde, das im gesamten Profil hohe organische Kohlenstoffgehalte aufweist (8-12 g kg^{-1}). Bei der anderen Fläche handelt es sich um eine Parabraunerde, die unterhalb von 30 cm geringe organische Kohlenstoffgehalte aufweist (3-4 g kg^{-1}).

Die organischen Kohlenstoffgehalte auf den beiden Flächen haben sich in den vergangenen 800 Jahren durch Erosion und Deposition natürlich entwickelt. Diese Konstellation ermöglicht es zu untersuchen, inwiefern die Verfügbarkeit von organischem Kohlenstoff oder andere Unterboden-spezifischen Bedingungen den Kohlenstoffumsatz beeinflussen, da die anderen Umweltbedingungen für die beiden Flächen als gleich zu betrachten sind.

Das erste Experiment diente dazu mehr Wissen über die mikrobielle Gemeinschaft im Unterboden und ihren Einfluss auf die Kohlenstoffspeicherung zu generieren. Die untersuchten Bodenproben wurden in vier Tiefen (0-15, 40-50, 50-60, and 75-85 cm) auf den beiden Flächen genommen. Mikrobieller Kohlenstoff wurde mit Hilfe der CFE-Methode bestimmt, um Tiefengradienten der mikrobiellen Biomasse in Bezug zu den Gehalten organsicher Substanz zu setzen. Die Aminozucker Muraminsäure und Glucosamin wurden als Indikatoren für bakterielle und pilzliche Residuen gemessen. Desweiteren wurde Ergosterol als Marker für saprotrophe Pilze bestimmt. Diese Werte dienen dazu Veränderungen in der mikrobiellen Gemeinschaft zu quantifizieren. Die Multi-Substrat-induzierte-Respirations Methode kam zum Einsatz, um Veränderungen in der funktionellen Diversität der mikrobiellen Gemeinschaft zu identifizieren. Die Diskriminanzanalyse der von 17 Substraten hervorgerufenen Respirationswerte hat ergeben, dass die Substratnutzung sich sowohl zwischen Ober- und Unterboden als auch zwischen den beiden Unterböden stark unterscheidet. Das deutet auf einen starken Einfluss der begrenzten

Nährstoffressourcen auf die Substratnutzung der mikrobiellen Gemeinschaft hin. Die Analysen von Aminozuckern und Ergosterol haben gezeigt, dass die pilzliche Dominanz in der mikrobiellen Gemeinschaft mit der Tiefe abnimmt. Damit wird nicht nur das Pilz-Bakterien-Verhältnis sondern auch die Substratnutzung der mikrobiellen Gemeinschaft durch die veränderte Nährstoffverfügbarkeit in der Tiefe bestimmt.

Das zweite Experiment war ein Inkubationsexperiment, bei dem Bodenproben mit und ohne Zugabe von Amaranthstreu (C4 Pflanze) für 176 Tage bei 22°C und erhöhten CO_2 Konzentrationen inkubiert wurden. Es wurde der Einfluss dieser Behandlungen auf organischen Kohlenstoff, mikrobiellen Kohlenstoff und Ergosterol bestimmt. Die untersuchten Proben stammten aus dem Ober- und Unterboden der beiden untersuchten Flächen. Der proben haben während der Inkubation zwischen 26 and 38% des ursprünglichen organischen Kohlenstoffs verloren. Die Verluste von Amaranth-C waren generell im Kolluvium (7,5 mg g-1) größer als in der Parabraunerde (7,0 mg g-1), besonders unter erhöhten CO_2 Konzentrationen. Unterschiede zwischen Ober- und Unterböden wurden hier nicht festgestellt. Die Zugabe von Amaranthstreu hat im Unterboden von beiden Flächen einen ähnlichen negativen Priming Effekt hervorgerufen (Kolluvium 11%, Parabraunerde 14%). Die erhöhten CO_2 Konzentrationen hatten keinen generellen Einfluss auf den mikrobiellen Kohlenstoff oder auf Ergosterol, unabhängig von der Amaranth-Zugabe. Allerdings war der Amaranth-C Anteil am mikrobiellen Kohlenstoff bei erhöhten CO_2 Konzentrationen signifikant geringer ($\alpha < 0,1$) (Oberboden: 31%; Unterboden: 54%). Das führt zu der Schlussfolgerung, dass erhöhte CO2 und geringere O2 Konzentrationen zwar einen Einfluss auf den Metabolismus der Mikroorganismen haben, aber nicht auf den Abbau von Pflanzenmaterial generell. Die Tatsache, dass in den Unterbodenproben mit Amaranth-Zugabe weniger bodenbürtiger organischer Kohlenstoff abgebaut wurde, deutet auf einen negativen Priming Effekt nach der Zugabe eines relativ komplexen Substrates hin. Dieser wird wahrscheinlich dadurch

hervorgerufen, dass die Mikroorganismen Energie und Nährstoffe leichter aus dem Pflanzenmaterial gewinnen können und dieses daher bevorzugt abbauen.

Das dritte Experiment war ein Feldversuch über zwei Jahre. Netzbeutel mit original Bodenmaterial (C3 Pflanzen ^{13}C Signatur) und Maiswurzeln wurden (C4 Pflanze) wurden in drei Tiefen (35, 45, und 65 cm) auf den beiden Flächen vergraben. Nach 12, 18 und 24 Monaten wurden die Beutel wieder ausgegraben. Anschließen wurde der Einfluss der Behandlungen auf organischen Kohlenstoff, Dichtefraktionen und mikrobiellen Kohlenstoff bestimmt. Die mittlere Verweilzeit für Mais-C war auf beiden Flächen und in allen Tiefen ähnlich (403 d). Auch der Anstieg von mikrobiellem C von Startwert zum Maximalwert war überall ähnlich (2,5-fach). Dieser Anstieg wurde zu großen Teilen durch die Maiszugabe hervorgerufen, da nach 2 Jahren mehr als 50% des mikrobiellen Kohlenstoffs maisbürtig waren. Allerdings gab es deutliche Unterschiede bezogen auf die Substratnutzungseffizienz, die mit der Tiefe abgenommen hat und in der Parabraunerde generell geringer war als im Kolluvium. Demnach ist das hinzugefügte Pflanzenmaterial für die Mikroorganismen gut zugänglich und wird dementsprechend auch auf beiden Flächen gleichermaßen gut abgebaut. Die Art der Nutzung im Metabolismus der Mikroorganismen wird allerdings von den Eigenschaften des Bodens beeinflusst.

Diese Ergebnisse liefern deutliche Hinweise, dass die Nährstoffverfügbarkeit sowohl aus bodenbürtiger organischer Substanz als auch aus hinzugefügtem Pflanzenmaterial einen starken Einfluss auf die mikrobielle Gemeinschaft und deren Nutzung verschiedener Substrate hat. Allerdings hat keines der Experimente Hinweise darauf geliefert, dass komplexe Substrate wie Pflanzenstreu im Unterboden weniger stark abgebaut werden als im Oberboden, unabhängig von den natürlichen organischen Kohlenstoffgehalten im Boden.

1.   General introduction

1.1.   SOC in subsoil

Two-third of the terrestrial C is stored in soils, which makes soil an important reservoir in the global carbon cycle (Batjes, 1996). This reservoir can either be source or sink for CO_2 (Rumpel et al., 2002; Rumpel and Kögel-Knabner, 2011), which is of increasing interest due to climate change concerns (Bailey et al., 2002). In terms of climate change research it would be desirable to avoid mineralization of SOC and to promote its storage. The fact that more than 50% of soil organic C (SOC) is stored in subsoils at 30-100 cm (Batjes, 1996; Lal and Kimble, 1997), has directed the scientific focus also towards subsoil. In natural landscapes all mineral soil horizons are considered as subsoil. On arable sites subsoil begins below cultivation depth (Schimel and Schaefer, 2012). Although SOC contents in subsoil are considerably smaller than in topsoil, the SOC stocks in subsoil are of significant size compared to other terrestrial C-pools (Rumpel et al., 2002). Even in shallow soils, which contain only small amounts of SOC below the organic topsoil horizon, most of the stable OC is stored in subsoil (Lorenz & Lal, 2011), as several studies have shown that subsoil OC is of a higher average age than OC in topsoils (Rumpel et al., 2002; Rumpel and Kögel-Knabner, 2011). This leads to the assumption that the mineralization rate of organic compounds in subsoil is lower than in topsoil. This makes subsoil a sink for carbon. Hence creating conditions, which avoid mineralisation from this pool and promote further carbon sequestration in subsoil, is desirable within mitigation strategies for climate change.

However, there is still a lack of knowledge regarding the mechanisms of C sequestration and C turnover in subsoil (Sanaullah et al., 2011; Cotrufo et al., 2013). Most models for SOC storage and turnover assume that mechanisms controlling C dynamics are the same in topsoil and subsoil (e.g. Jenkinson and Coleman, 2008), although there are strong indications that environmental conditions in subsoil are different from those in topsoil, e.g. less variation in

temperature, and reduced nutrient availability (von Lützow et al., 2006), which might lead to reduced substrate mineralization. But next to these abiotic factors changes in biotic factors, e.g. reduced soil microbial biomass, changes in the microbial community composition, and reduced input of plant biomass (Fierer et al., 2003) might also be of particular importance.

Furthermore the soil genesis has to be taken into account as the soil forming processes have a strong impact on the soil organic matter composition in subsoil (Rumpel et al., 2002). This was also confirmed in a meta-study by Kirkby et al. (2011), which has proven that the recent cultivation of sites is negligible compared with properties related to soil genesis in terms of subsoil carbon stocks .

1.2. Factors controlling carbon sequestration in subsoil

Three main factors are discussed, which separately or in combination with each other possibly reduce carbon turnover rates in subsoil.

1.2.1. Resource limitation

The first factor „Resource limitation" can be subdivided into three aspects: a) Limited substrate quality, b) Limited fresh organic matter input, and c) Limited access to substrate. Limited substrate quality means that the organic matter in subsoil is energetically not attractive for the microbial community, as the organic material does not provide enough energy to cover the energy demand for the production of enzymes, which are necessary for substrate degradation (Salome et al., 2010; Rumpel and Kögel-Knabner, 2011; Dungait et al., 2012; Cotrufo et al., 2013). This point is closely related to the second aspect, the limited input of fresh organic matter into subsoil, as fresh organic matter would supply energy rich compounds to degrade more recalcitrant compounds as well (Fontaine et al., 2007). In terms of possible future land use changes towards deeper rooting

1. General introduction

plants for C storage purposes the effects of substrate addition to subsoil have to be investigated to clarify if the added plant substrate is transferred to stable SOM fractions or if it causes a mineralisation of previously stabilized SOC due to priming effects (Fontaine et al., 2007). This would be an adverse effect to the desired increase in SOC sequestration.

The third aspect, the limited access to substrate can be either caused by the physical separation of substrate and microorganisms (Joergensen and Potthoff, 2005; Rumpel and Kögel-Knabner, 2011) or the physico-chemical stabilization of SOC. The physical separation of microorganisms and substrate is caused by the lack of anthropogenic disturbance and limited bioturbation through roots. This leads to reduced mixing of the soil and small pore sizes. These conditions make it hard for microorganisms to access substrate, which is not in their nearest environment (Bachmann et al., 2008). Furthermore, the percentage of SOC, which is either occluded in aggregates or bound to the mineral surfaces, increases in comparison with the topsoil. Both are hard to access for microorganisms and are considered as stabilized (Lavahun et al., 1996; Chabbi et al., 2009; Schimel and Schaefer, 2012; Schrumpf et al., 2013). The degree of stabilization depends on the underlying mechanism. Physico-chemical interactions with the soil matrix are almost irreversible and stabilize organic matter effectively over long periods of time. The physical protection occurs through occlusion in soil aggregates. This stabilization mechanism is a little less effective and stabilizes organic matter for shorter periods (Cotrufo et al. 2013).

1.2.2. Changes in the microbial community

The relationships between SOC stocks and composition and functional diversity of the microbial community in subsoil have not been thoroughly investigated. Therefore it is widely unknown in how far changes in the microbial community could also play a major role regarding the carbon sequestration mechanisms in subsoil. However, it is proven that soil microorganisms influence C dynamics in two ways: (1) decomposition of organic matter input and (2) production of residues, which are an important component of SOC (Kögel-Knabner, 2002). The structure of the microbial community seems to strongly influence SOC dynamics (Schmidt et al., 2011), as it has been repeatedly suggested that fungal dominance increases the SOC sequestration potential due to a higher C utilization efficiency (Adu and Oades, 1978; Jastrow et al., 2007). In reverse, quantity and quality of the available substrates are major factors affecting the microbial community (Fierer et al., 2003). As SOC characteristics are supposed to change with depth, composition and functional diversity of the microbial community probably also change with depth, which again alters SOC sequestration processes (Schmidt et al., 2011).

1.2.3. Changes in gas conditions

It is assumed that CO_2 concentrations in subsoil are higher than under atmospheric conditions and O_2 concentrations correspondingly lower (Salome et al., 2010). It was proven that bacteria are relatively robust to changes in the gas conditions in terms of elevated CO_2 while the fungal community was affected by changes in atmospheric gas conditions (Hayden et al., 2012) as for example laccases need oxygen for substrate degradation, which is limited in subsoil (Ekschmitt et al., 2008). But by now there are only assumptions and few experiments (Salome et al., 2010; Hayden et al., 2012) on the influence of the different gas conditions in subsoil.

1.3.   Gas conditions in subsoil – Preliminary experiments

As there are only assumptions and few experiments (Salome et al., 2010; Hayden et al., 2012) on the gas conditions in subsoil, we conducted a pre-experiment to measure subsoil gas concentration for CO_2 and O_2 on two sites in three different subsoil depths (45, 65, and 85 cm) in three replications.

The idea was to take the samples with a mobile probe (Fig 1.1a) as well as with stationary probes (Fig. 1.1c). The mobile probe should enable us to detect the spatial variability of the subsoil gas conditions on one sampling date. This sampling technique has the advantage that there is no persistent macro pore created. The disadvantage is the handling of the probe during the sampling. If the soil has high silt and clay contents soil gets stuck in the inlet of the probe (Fig. 1.1b) and the air can not be pumped through the probe. The stationary probes were installed to detect the temporal variability in the different depths over the vegetation period. The advantage of this sampling technique is that the gas concentrations are able to equilibrate in the reservoir of the probe over two weeks, which should lead to representative samples for the subsoil around the probe. The disadvantage is the inevitable creation of a macro pore, which might cause a mixing of subsoil air with topsoil and surface air.
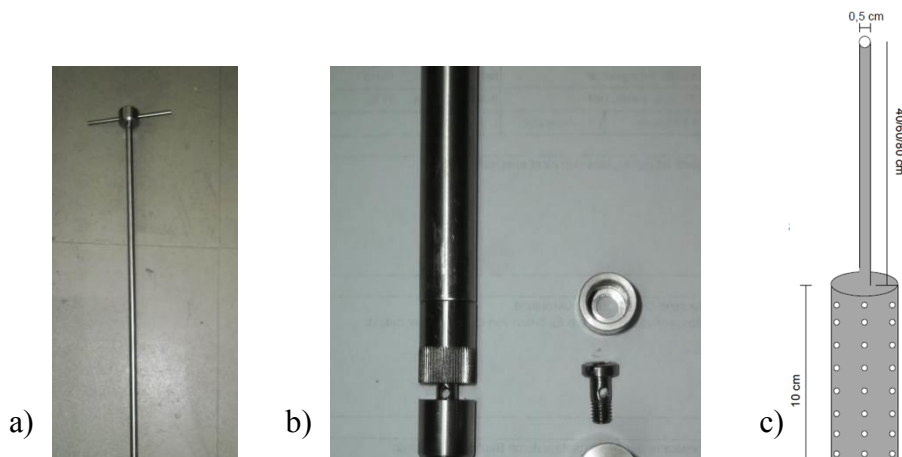
Figure 1.1: Gas probing equipment a) Mobile gas probe b) Inlet of the mobile probe c) Schematic sketch of the stationary probes

1. General introduction

Samples from both probe types were taken every second week from March to August 2014 with evacuated glass tubes. The samples were analysed for CO_2 and O_2 concentrations using an automated gas chromatograph (Shimadzu) with an electron capture detector (CO_2) and a flame ionization detector (O_2) according to Loftfield et al. (1997). From sampling on the field until the analysis in the laboratory the samples were stored in water filled buckets (Fig. 1.2), to avoid gas exchange due to the expected high CO_2 concentration gradient between subsoil air and ambient air. This problem had become evident during some pre-test with a standard gas-mixture containing 5 Vol% CO_2.

Figure 1.2: Water filled transportation bucket for gas sampling tubes

Due to the high silt and clay contents on both sampling sites the measurement with the mobile probe did not provide reasonable results. Hence the sampling with this probe was omitted. The results of the stationary probe showed a high spatial and temporal variability. However, it was proven, that subsoil gas conditions differ strongly from ambient gas conditions (Table 1.1). The average of all measurements for CO_2 was 4.0 Vol% and 18.3 Vol% for O_2 and the extreme value for CO_2 was 8.2 Vol% and 12.8 Vol% for O_2. Therefore we decided to use gas mixtures with the following composition for the planned incubation experiment under subsoil gas conditions: subsoil average: 4 Vol% CO_2, 17 Vol% O_2, and 79 Vol% N_2, and subsoil extreme: 8 Vol% CO_2, 13 Vol% O_2, and 79 Vol% N_2.

1. General introduction

Table 1.1: Example of subsoil gas samples taken from the stationary probes on the 31st July 2014.

Depth [cm]	Replicate	Site 1		Site 2	
		CO_2 [Vol%]	O_2 [Vol%]	CO_2 [Vol%]	O_2 [Vol%]
45	1	4,7	23,04	7,1	18,05
65	1	6.7	19.1	7.4	16.3
85	1	6.5	17.8	5.4	20.2
45	2	8.2	16.3	7.2	17.1
65	2	4.6	18.7	5.3	19.4
85	2	7.7	15.3	5.6	19.7
45	3	5.3	19.2	3.6	22.3
65	3	5.3	18.6	5.3	19.0
85	3	6.2	18.7	3.5	18.9

1.4. Objectives of this work

With regards to the current knowledge on the carbon dynamics in subsoil, there have been only few studies investigating the biotic and abiotic factors controlling carbon sequestration in subsoil in relation to the site specific natural carbon contents. Therefore we decided to investigate two depth profiles with different SOC stocks in all our experiments. In one profile, SOC contents show a strong depth gradient (0-85 cm), while the other profile has similar SOC contents throughout the entire profile (0-85 cm). The SOC contents developed naturally over the past 800 years due to erosion and deposition of surface soil material. This offers the opportunity to investigate the extent to which natural C availability (Fierer et al., 2003) or other subsoil specific conditions (Salome et al., 2010) influence biomass, composition and functional diversity of the microbial community, and the degradation of freshly added plant material. Therefore we planned three experiments to address this topic.

1.4.1. Objective of the first experiment: Determination of the microbial biomass, composition and functional diversity of microbial communities in two depth profiles of arable land.

Replicated field samples were taken from both sites in four different depths (5-15, 40-50, 50-60, and 75-85 cm). All samples were analysed for microbial biomass C to gain insights if the microbial biomass decreases with depth, which could be related to lower C mineralization rates. The choice of sites gives additional insights regarding the relationship between C contents and the microbial biomass C. Fungal and bacterial residue C were measured to determine the fungal C to bacterial C ratio. This provides insights if the fungal dominance which is observed in topsoils decreases with depth and if the changes are influenced by the natural C contents on the sites. The multi-substrate-induced-respiration (MSIR) method was applied to characterize the functional diversity of the microbial community in relation to depth and C contents. Additionally, we wanted to determine whether MSIR method is suitable for discriminating between microbial communities in topsoil and subsoil by substrate use and whether it is even possible to characterise the microbial community composition with this method. These investigations should allow us to draw conclusions regarding the question of whether C limitation is the major factor controlling the microbial community in subsoil (Fierer et al., 2003) or whether other environmental factors (Salome et al., 2010) might be even more important.

1.4.2. Objective of the second experiment: Investigation of the influence of subsoil gas conditions on SOC and plant residue decomposition

A long-term incubation experiment with subsoil and topsoil samples from the two experimental sites was carried out, using different CO_2 and O_2 concentration levels with and without amaranth (*Amaranthus hypochondriacus* L) addition, a C4 plant with higher $\delta^{13}C$ values than the autochthonous SOC. This allows monitoring amaranth-derived SOC and microbial

biomass C under incubation conditions without possibility to measure soil CO_2 evolution. This should provide insights if SOC and plant residue decomposition are reduced under elevated CO_2 and reduced O_2 conditions. This could be caused by negative effects on fungi (Ekschmitt et al., 2008; Hayden et al., 2012), as both sites are fungal dominated and fungi are the main decomposers of fresh plant material (Keiblinger et al., 2010). The experiment should provide additional insights on the question if the addition of plant substrate causes enhanced autochthonous SOC mineralization through a positive priming effect (Fontaine et al., 2007; Kuzyakov, 2010) under the different applied gas conditions. The choice of two sites, which strongly differ in autochthonous SOC contents in subsoil, offers the opportunity to investigate the extent to which natural C availability (Fierer et al., 2003) or other subsoil specific conditions (Salome et al., 2010) control C turnover as all other environmental conditions on the two sites are equal.

1.4.3. Objective of the third experiment: Determination of the fate and pathways of added plant residues under field conditions

We have buried mesh-bags containing a mixture of soil and maize roots (Fig. 1.3) in three different subsoil depths (35, 45, and 65 cm) in the field on both sites. The bags were recovered at three sampling dates (12, 18, and 24 months) in three replicates per site and depths. In terms of possible future land use changes towards deeper rooting plants for C storage purposes the effects of substrate addition to subsoil have to be investigated to clarify if the added plant substrate is transferred to stable SOM fractions or if it causes a mineralisation of previously stabilized SOC due to priming effects (Fontaine et al., 2007). This would be an adverse effect to the desired increase in SOC sequestration. These effects have been investigated mostly in laboratory experiments (Kuzyakov, 2010) where not only substrate was added but also other environmental factors (e.g. gas conditions, temperature, moisture) have been changed. This makes it difficult to

transfer the results from these experiments to the field or ecosystem scale. Degradation and fate of the maize roots were not only analysed by the incorporation into the microbial biomass but also by density fractionation, which gives additional information on the stabilization of SOC (Schrumpf et al., 2013). This offers the opportunity to identify site and depth specific differences in the decomposition of maize roots and to elucidate the pathways of plant residues towards stabilized SOC in relation to those site and depth specific properties.

Figure 1.3: Burial of the soilbags in June 2013

2. Microorganisms and their substrate utilization patterns in topsoil and subsoil layers of two silt loams, differing in soil organic C accumulation due to colluvial processes

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2. Microorganisms and their substrate utilization patterns in topsoil and subsoil

ABSTRACT

In order to gain more knowledge regarding the microbial community and its influence on carbon sequestration in subsoil, two depth profiles with different natural soil organic carbon (SOC) contents were sampled. This makes it possible to investigate the extent to which natural SOC availability or other subsoil specific conditions influence the composition and the functional diversity of the microbial community and in return how the microbial community composition affects SOC sequestration under these conditions. Soil samples were taken at four different depths on two neighbouring arable sites; one Colluvic Cambisol with high SOC contents (8-12 g kg^{-1}) throughout the profile and one Haplic Luvisol with low SOC contents (3-4 g kg^{-1}) below 30 cm depth. Multi-substrate-induced-respiration (MSIR) was used to identify shifts in functional diversity of the microbial community along the profiles. The amino sugars muramic acid and glucosamine were measured as indicators for bacterial and fungal residues and ergosterol was determined as a marker for saprotrophic fungi. Discriminant analysis of respiration values obtained from the 17 substrates used in the MSIR revealed that substrate use in subsoil differed significantly from that in topsoil and also differed highly between the two subsoils. Amino sugar analysis and the ratio of ergosterol to microbial biomass C showed that fungal dominance decreased with depth. MSIR clearly demonstrated that not only the fungi to bacteria ratio but also substrate use of the microbial community changed with depth according to substrate availability.

Keywords: amino sugars; fungi to bacteria ratio; microbial biomass; multi-substrate-induced-respiration; SOC; soil profile

2. Microorganisms and their substrate utilization patterns in topsoil and subsoil

2.1. Introduction

More than 50% of soil organic C (SOC) is stored in subsoils from $30 - 100$ cm (Batjes, 1996; Lal and Kimble, 1997). Hence, subsoil is important as a source or sink for CO_2 in the global carbon cycle (Rumpel et al., 2002; Rumpel and Kögel-Knabner, 2011). In terms of climate change research it would be desirable to avoid mineralization of subsoil SOC and to promote SOC storage in subsoil. However, there is still a lack of knowledge regarding the mechanisms of C sequestration and C turnover in subsoil (Sanaullah et al., 2011; Cotrufo et al., 2013). Most models for SOC storage and turnover assume that mechanisms controlling C dynamics are the same in topsoil and subsoil (e.g. Jenkinson and Coleman, 2008), although there are strong indications that environmental conditions in subsoil are different from those in topsoil, e.g. temperature, pH, and nutrient availability (von Lützow et al., 2006).

Some processes, like physical separation of substrate and microorganisms or priming effects through substrate input, have received attention in previous studies (Fontaine et al., 2007; Salome et al., 2010; Sanaullah et al., 2011). In contrast, the relationships between SOC stocks, composition and functional diversity of the microbial community in subsoil have not been thoroughly investigated. Soil microorganisms influence C dynamics in two ways: (1) decomposition of organic matter input and (2) production of residues, which are an important component of SOC (Kögel-Knabner, 2002). The structure of the microbial community seems to strongly influence SOC dynamics (Schmidt et al., 2011), as it has been repeatedly suggested that fungal dominance increases the SOC sequestration potential due to a higher C utilization efficiency (Adu and Oades, 1978; Jastrow et al., 2007). In reverse, quantity and quality of the available substrates are major factors affecting the microbial community (Fierer et al., 2003). As SOC characteristics are supposed to change with depth, composition and functional diversity of the

microbial community probably also change with depth, which again alters SOC sequestration processes (Schmidt et al., 2011).

Two depth profiles with different SOC stocks were sampled in order to increase knowledge on the microbial community in subsoil. In one profile, SOC contents show a strong depth gradient (0-85 cm) (Table 2.1, Fig. 2.1a), while the other profile has similar SOC contents throughout the entire profile (0-85 cm) (Table 2.1, Fig. 2.1a). The SOC contents developed naturally over the past 800 years due to erosion and deposition of surface soil material. This offers the opportunity to investigate the extent to which natural C availability (Fierer et al., 2003) or other subsoil specific conditions (Salome et al., 2010) influence biomass, composition and functional diversity of the microbial community, while all other environmental conditions on the two sites are equal.

The aim of this study was to determine the microbial biomass, composition and functional diversity of microbial communities in two depth profiles of arable land. We wanted to determine whether the multi-substrate-induced-respiration (MSIR) method is suitable for discriminating between microbial communities in topsoil and subsoil by substrate use and whether it is even possible to characterize the microbial community composition with this method. The choice of sites with different depth gradients for SOC should also allow us to draw conclusions regarding the question of whether C limitation is the major factor controlling the microbial community or whether other environmental factors might be even more important.

2. Microorganisms and their substrate utilization patterns in topsoil and subsoil

2.2. Material and methods

2.2.1. Site description and field sampling

Soil samples were taken from two arable fields at the Hessian State Manor of Frankenhausen, northern Hessia, Germany (51°24' N; 9°25' E), the experimental farm of Kassel University. The area is characterized by a mean annual temperature of 9.3 °C and a mean annual precipitation of 687 mm. The soils of site I (referred to as Cambisol or Cam) can be characterized as a Colluvic Cambisol according to the WRB (FAO, 2014). The Colluvic horizon of the Colluvic Cambisol covers the original soil surface of a Chernozem by about 70 cm, resulting in an Ap / M / fAh sequence. The soils of site II (referred to as Luvisol or Luv) can be classified as a Haplic Luvisol according to the WRB (FAO, 2014), although the Al horizon was eroded. This results in an Ap / Bt sequence. The soils of the two sites have been developed on loess and are within a distance of 300 to 400 m from each other, which means that environmental conditions are equivalent on both sites, although they have different SOC profiles due to erosion and deposition. Land use at both sites was also similar for at least 400 years, when both sites were used as grassland first and then as cropland since early 20th century (Troßbach, 2000). Soil characteristics are shown in Table 1.

Both sites were sampled on a 10 m × 15 m area in April 2013. Soil samples were taken at four different depths: 5-15 cm (1), 40-50 cm (2), 50-60 cm (3), 75-85 cm (4), with 5 randomized field replicates (n = 40). Depths were chosen to avoid mixing of the natural soil horizons at all sampling points. The samples were sieved < 2 mm and stored at 4°C. Soil pH was measured in a soil to water ratio of 1 to 2.5. Total C and total N were determined after combustion using a Vario MAX elemental analyser (Elementar, Hanau Germany). As the samples did not contain any carbonate, total C equals SOC.

2. Microorganisms and their substrate utilization patterns in topsoil and subsoil

Table 2.1: Different physico-chemical parameters for both sampling sites and all depths. Letters indicate significant differences between depths (capital letters Cambisol, small letters Luvisol, significance level $p < 0.05$).

Depth [cm]	Bulk density [g cm^{-3}]	Water content [%]	Sand [%]	Silt [%]	Clay [%]	pH	SOC [mg g^{-1}]		Total N [mg g^{-1}]		C/N	
Colluvic Cambisol												
5-10	1.50	19.5	2	79	19	7.7	12.79	A	1.18	A	10.8	B
40-50	1.53	21.3	2	77	21	7.9	7.95	C	0.71	B	11.1	B
50-60	1.58	23.6	1	76	23	7.9	8.27	BC	0.74	B	11.2	B
75-85	1.52	24.1	1	69	30	7.9	10.11	B	0.71	B	14.3	A
Haplic Luvisol												
5-10	1.59	20.2	2	78	20	7.4	11.52	a	1.09	a	10.5	
40-50	1.62	20.8	2	70	29	7.4	4.24	b	0.37	b	11.5	
50-60	1.63	20.1	2	70	28	7.6	3.69	b	0.32	b	11.6	
75-85	1.61	19.4	1	73	25	7.6	2.84	c	0.25	b	11.6	
CV [±%]						2.5	6.8		8.1		4.7	

CV = mean coefficient of variation between replicate samples within site and depth (n = 5)

2.2.2. Soil microbial properties

Fumigated (24 h with ethanol-free CHCl$_3$ at 25 °C) and non-fumigated soil samples (10 g) were extracted with 40 ml of 0.5 M K$_2$SO$_4$ by 30 min horizontal shaking at 200 rev min^{-1} and

filtered (V3, Munktel & Filtrak, Bärenstein, Germany) to measure extractable microbial biomass C and N (Brookes et al., 1985; Vance et al., 1987). Simultaneous determination of organic C and total N in the extracts was conducted after catalytic high temperature combustion, using a multi N/C 2100S automatic analyser (Analytik Jena, Jena, Germany). Microbial biomass C was calculated as E_C/k_{EC}, with E_C = (organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990). Microbial biomass N was calculated as E_N/k_{EN}, with E_N = (total N extracted from fumigated soil) – (total N extracted from non-fumigated soil) and k_{EN} = 0.54 (Brookes et al., 1985; Joergensen and Mueller, 1996).

The fungal cell membrane component ergosterol was extracted from moist soil with 100 ml ethanol (Djajakirana et al., 1996). Sample size (fresh weight) was dependent on the sampling depth: 2 g for depth 1, 5 g for depth 2, 6 g for depth 3, and 7 g for depth 4. The amount of ergosterol was determined by reversed-phase HPLC using 100 % methanol as the mobile phase and detected at 282 nm.

The amino sugars muramic acid (MurN), mannosamine (ManN), galactosamine (GalN) and glucosamine (GlcN) were determined according to Appuhn et al. (2004) as described by Indorf et al. (2011). Dried samples (105°C) of 0.5 g soil were weighed into 20 ml test tubes, mixed with 10 ml 6 M HCl and hydrolysed for 6 h at 105 °C. The HCl was removed by a rotary evaporator; the residue was dissolved in water and centrifuged. After derivatization with *ortho*-phthaldialdehyde, the fluorometric emission of the amino sugars was measured at 445 nm after excitation at 330 nm. The HPLC system consisted of a Dionex (Germering, Germany) P 580 gradient pump, a Dionex Ultimate WPS-3000TSL analytical autosampler with in-line split-loop injection and thermostat and a Dionex RF 2000 fluorescence detector. From the measured amino sugars, bacterial C, fungal C and microbial residue C were calculated according to Appuhn and Joergensen, (2006) and Engelking et al. (2007).

2.2.3. Functional diversity by CLPP

The community level physiological profiles (CLPP) were determined by the MSIR approach using the MicroResp^{TM} method (Campbell et al., 2003). The soil was adjusted to a water holding capacity of 50% and pre-incubated for 5 d in the dark at 25 °C, prior to CLPP analysis. 300 mg of moist soil were added to each well of a 1.1 ml deep-well microtiter plate (Nunc, Langenselbold, Germany), before the aqueous solutions of the different C sources were applied and the wells were sealed with a CO_2 trap.

The physiological profiles were determined by applying distilled water (for basal respiration), 8 amino acids (γ-aminobutyric acid, L-leucine, alanine, arginine, L-asparagine L-aspartic acid, L-glutamine, and L-serine), 1 amino sugar (N-acetyl-glucosamine), 2 neutral sugars (arabinose and d-glucose), and 6 carboxylic acids (malic acid, citric acid, α-ketoglutaric acid, quinic acid, malonic acid, and L+tartaric acid). These substrates represent root exudates (Campbell et al., 1997; Stevenson et al., 2004; Andersen et al., 2013) or microbial products (Amelung et al., 2001; Nehls et al., 2001; Meyer et al., 2008) and have been used in previous studies (Campbell et al., 2003; Stevenson et al., 2004; Wakelin et al., 2008; Sradnick et al., 2013), providing good discrimination (Stevenson et al., 2004). Substrates were added with a C concentration of 10% of SOC to avoid demonstrated inhibitive effects of higher concentrations (Lerch et al. 2013). Because of lower solubility L-aspartic acid and L-asparagine were applied at lower concentrations for samples C1-C4 and L1. The colorimetric CO_2 trap was produced according to Campbell et al. (2003). The colour of the CO_2 trap was measured immediately before sealing and after 6 h of incubation (25 °C) at 572 nm (FLUOstar, BMG, Offenburg, Germany). The calibration of the CO_2 trap is described in Sradnick et al. (2013).

In addition to the CLPPs, values for microbial activity (basal respiration) and microbial biomass C activated by glucose amendment (AMB) (Kaiser et al., 1992) were obtained from this

method. The metabolic quotient qCO_2 (Anderson and Domsch, 1990) was calculated as the ratio of basal respiration rates to microbial biomass C.

2.2.4. Statistics

All statistical analyses were performed in R (R Development Core Team, 2009). Differences between the sampling depths were determined using *ezanova* for repeated measurements. Results were only considered if there was no significant sphericity. If the ANOVA indicated significant differences, it was followed by a pairwise t-test with Bonferroni correction. Correlations were calculated using Spearman's correlation coefficient as the data are not normally distributed. For the CLPPs, discriminant analysis was used to identify classification success according to substrate use. The values used in the discriminant analysis and the other figures on MSIR were standardized for overall microbial activity of the sample as similarly suggested by Garland (1997).

2.3. Results

2.3.1. Depth gradients for physico-chemical parameters

Soil pH and bulk density did not show a depth gradient and did not differ between the sites (Table 1). In the Luvisol, the silt content decreased with depth at the expense of the clay content. In the Cambisol, soil texture remained similar in the Ap and M horizons, but also showed decreasing silt and increasing clay contents in the relict fAh horizon.

In the Luvisol, SOC significantly ($p < 0.01$) decreased with depth to lowest contents at 75-85 cm. In the Cambisol, SOC decreased to lowest contents at 40-50 cm depth, followed by a continuous increase until 75-85 cm. At the three subsoil layers, the SOC content of Cambisol was 2 to 4 times higher than that of the respective Luvisol subsoil layers (Table 2.1, Fig. 2.1a). Total N

followed SOC with an average C/N ratio of 11.2, except the 75-85 cm layer of the Cambisol, where this ratio was significantly ($p < 0.01$) wider (Table 2.1). Hence SOC and total N were highly correlated for all samples ($r = 0.97$, $p < 0.01$, $n = 40$), although the correlation became much weaker when focusing on the Cambisol subsoil depths ($r = 0.55$, $p = 0.03$, $n = 15$)

2.3.2. Depth gradients of microbial indices

Microbial biomass decreased from topsoil to 75-85 cm by about 80% in both soil types, with topsoil values of 210 µg g^{-1} in the Cambisol and 150 µg g^{-1} in the Luvisol (Fig. 2.1b). The strongest decrease (70%) was visible from topsoil to first subsoil sampling depth, with a much weaker decrease afterwards. Microbial biomass C contents were slightly higher in all layers of the Cambisol than in the Luvisol. Microbial biomass N contents also significantly ($p < 0.01$) decreased with depth (90% decrease), but they were approximately two times higher in the Cambisol than in the Luvisol subsoil layers (Table 2.2). The microbial biomass C/N ratio was identical in the topsoil layers of both soil types, but showed a stronger increase in the Luvisol. The correlation between total N and microbial biomass N ($r = 0.90$, $p < 0.01$, $n = 40$) was stronger than that between SOC and microbial biomass C ($r = 0.75$, $p < 0.01$, $n = 40$). These correlations also differed between topsoils ($n = 20$) and subsoils ($n = 15$). With total N and microbial biomass N ($r = 0.73$, $p < 0.01$), showing a stronger correlation than SOC and microbial biomass C ($r = 0.59$, $p < 0.01$) in the Cambisol and the opposite in the Luvisol (total N to microbial biomass N: $r = 0.84$, $p < 0.01$; SOC to microbial biomass C: $r = 0.90$, $p < 0.01$). In subsoil (depths 2-4) the correlations became weaker. In the Cambisol subsoil, there was no correlation between SOC and microbial biomass C ($r = 0.04$) and a weak but not significant correlation between total N and microbial biomass N ($r = 0.34$, $p = 0.2$). In the Luvisol subsoil, there were still good correlations between SOC and microbial biomass

C (r = 0.78, p < 0.01) and total N and microbial biomass N (r = 0.61, p = 0.02), which were, however, weaker than in the whole profile.
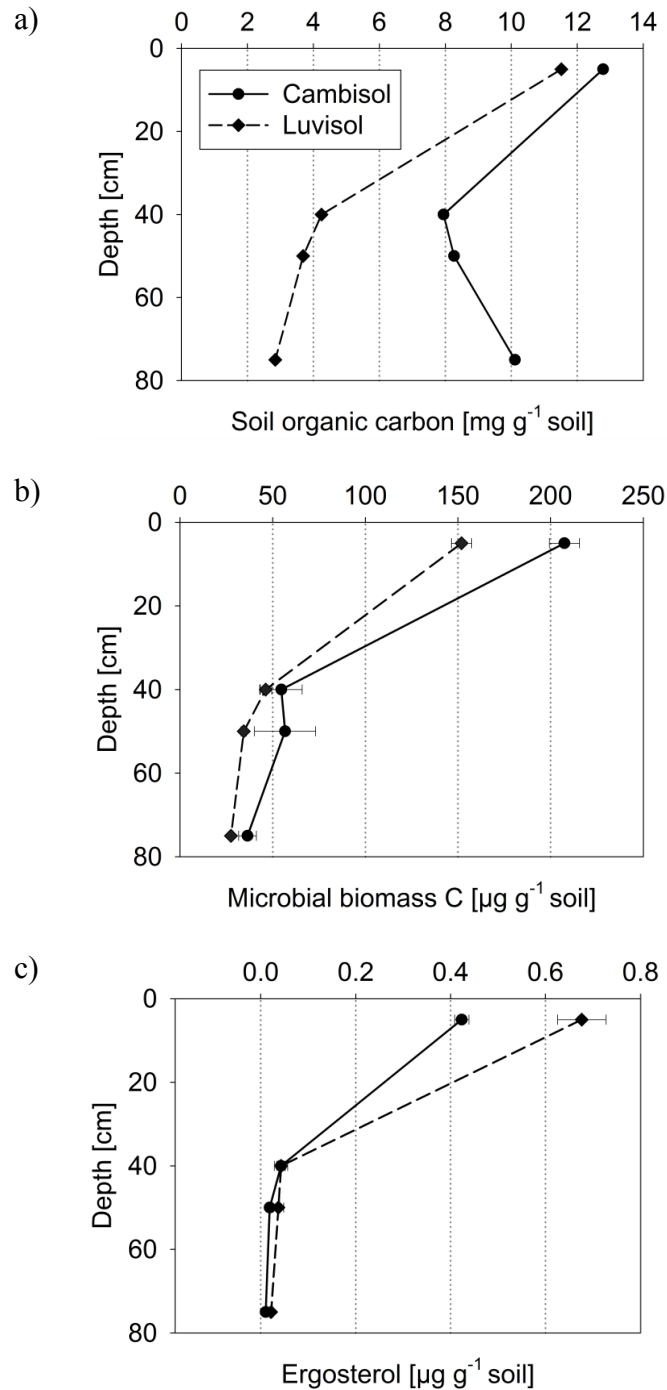
Figure 2.1: Depth gradients of soil chemical and microbial parameters: a) SOC, b) microbial biomass C, c) ergosterol (saprotrophic fungi); error bars show one standard error (n = 5).

2. Microorganisms and their substrate utilization patterns in topsoil and subsoil

In the Luvisol topsoil, the mean ergosterol content was 0.68 µg g⁻¹ soil, which exceeded that of the Cambisol by 35% (0.42 µg g⁻¹) (Fig. 2.1c). In both soil types, ergosterol declined significantly ($p < 0.01$) to a mean content of 0.04 µg g⁻¹ at 40-50 cm, followed by a small further decline to minimum values of 0.01 (Cam) and 0.02 (Luv). In both soil types, the contribution of ergosterol to microbial biomass C declined by 85% with depth, in the Luvisol from 4.4 to 0.8 mg g⁻¹, and in the Cambisol from 2.0 to 0.3 mg g⁻¹ (Fig. 2.2a).

Table 2.2: Basal respiration, microbial biomass carbon and nitrogen for both sampling sites and all depths. Letters indicate significant differences between depths (capital letters Cambisol, small letters Luvisol, significance level $p < 0.05$).

Depth [cm]	Basal Respiration [µg CO$_2$-C g⁻¹ soil d⁻¹]	Microbial biomass C [µg g⁻¹ soil]		N [µg g⁻¹ soil]		C/N
Colluvic Cambisol						
5-10	15.3	207.5	A	59.1	A	3.5
40-50	11.4	54.7	B	12.9	B	4.7
50-60	12.3	56.7	B	10.8	B	4.9
75-85	11.8	36.4	B	6.7	B	5.4
Haplic Luvisol						
5-10	18.1	151.9	a	45.2	a	3.5
40-50	10.1	46.2	b	6.3	b	8.2
50-60	10.5	34.4	bc	4.6	b	7.5
75-85	9.2	27.3	c	3.2	b	11.0
CV [±%]	19	24		27		30

CV = mean coefficient of variation between replicate samples within site and depth (n = 5)
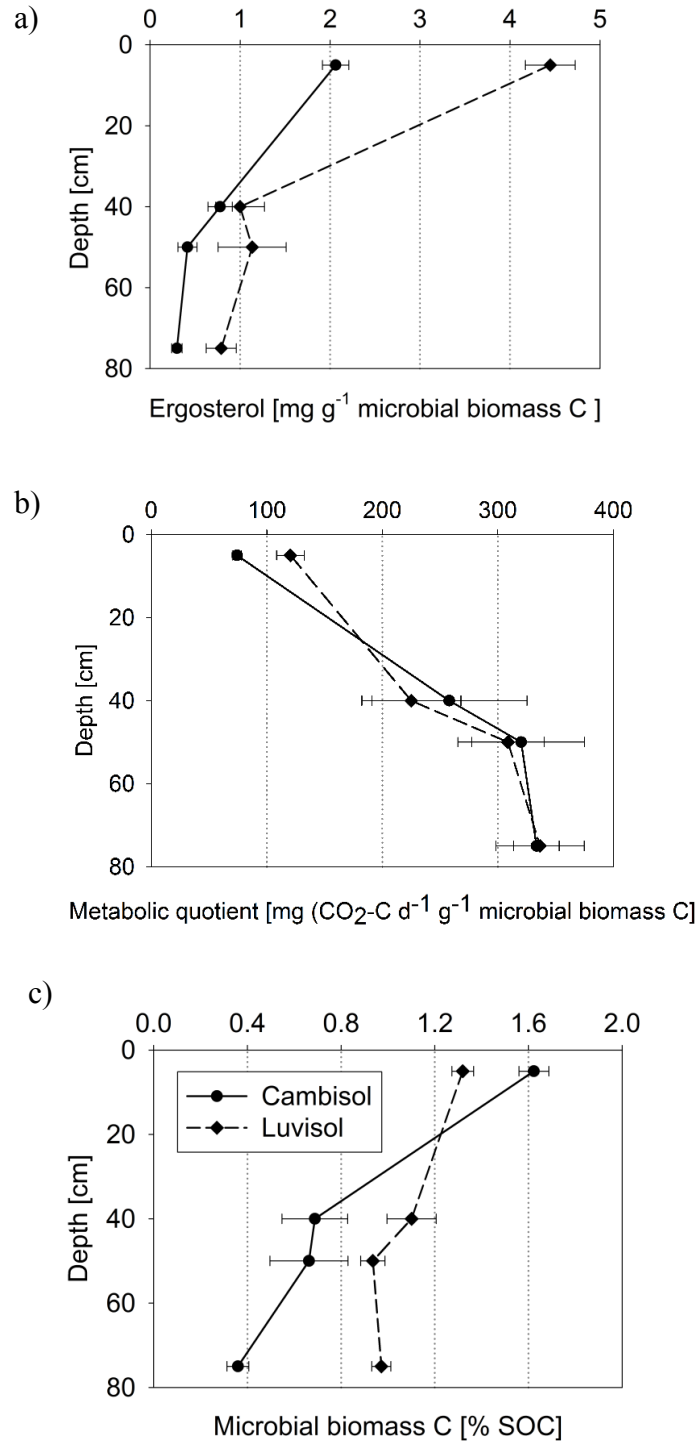
Figure 2.2: Depth gradients of microbial indices: a) contribution of ergosterol to microbial biomass C, b) metabolic quotient qCO$_2$, c) microbial biomass C as percentage of SOC; error bars show one standard error (n = 5).

2. Microorganisms and their substrate utilization patterns in topsoil and subsoil

Basal respiration did not significantly ($\alpha = 0.05$) decline with depth and did not differ between the soil types (Table 2.2). Consequently, the metabolic quotient $q\text{CO}_2$ showed a nearly linear increase (Cam: $r = 0.74$, $p < 0.01$, $n = 15$; Luv: $r = 0.77$, $p < 0.01$, $n = 15$) with depth from 75 in the Cambisol topsoil and 120 in the Luvisol topsoil to more than 300 mg CO_2-C g^{-1} microbial biomass C d^{-1} at 50-60 cm and a smaller increase towards 75-85 cm in both soil types, without any significant ($\alpha = 0.05$) difference between the two soil types (Fig. 2.2b). The contribution of microbial biomass C to SOC decreased from 1.6% in the topsoil to 0.4% at 75-85 cm in the Cambisol and from 1.3 to 1.0 % in the Luvisol (Fig. 2.2c).

In both soil types, fungal C contents significantly ($p < 0.01$) decreased with depth by 70% at 75-80 cm (Fig. 2.3a). Fungal C contents were roughly 0.5 mg g^{-1} soil higher in topsoil and 1 mg g^{-1} soil higher in all subsoil layers of the Cambisol than in the Luvisol. In the Luvisol, bacterial C declined linearly by 45% with depth from the topsoil to 75-85 cm, whereas bacterial C varied without a significant ($\alpha = 0.05$) depth gradient around 1.7 mg g^{-1} soil in the Cambisol (Fig. 2.3b). The fungal C to bacterial C ratio decreased with depth from 3.3 in the topsoil to 1.8 at 75-85 cm, with a tendency to be lower in the Cambisol (Fig. 2.3c). In both soil types, the contribution of microbial residue C to SOC varied around 50% in the topsoil, increased to 65% at 40-50 cm, remained roughly constant down to 75-85 cm in the Luvisol and linearly declined to 35% at 75-85 cm in the Cambisol (Fig. 2.3d).

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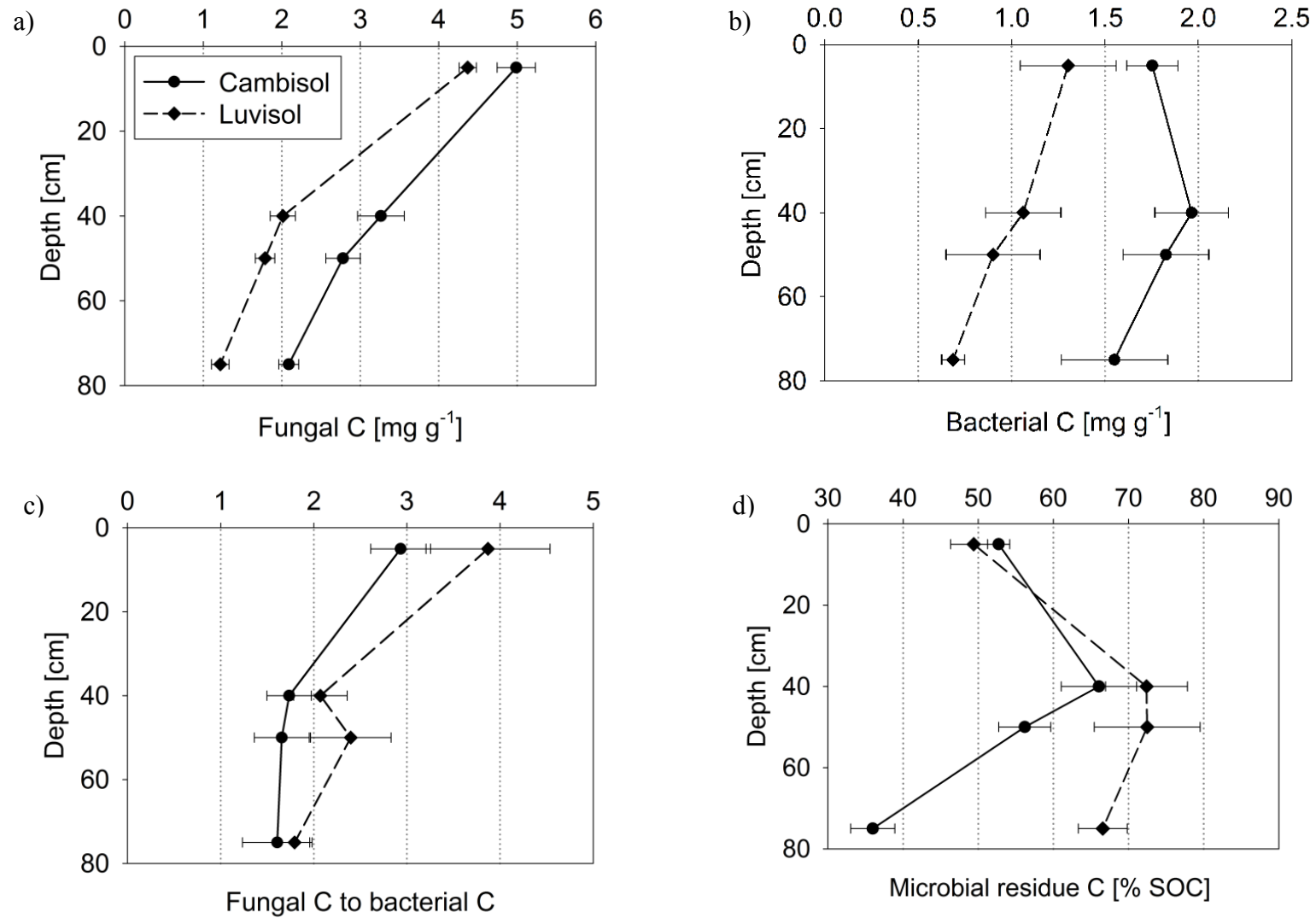
Figure 2.3: Depth gradients for microbial residue indices. a) fungal C, b) bacterial C, c) fungal C to bacterial C ratio, d) microbial residue C as percentage of SOC; error bars show one standard error (n = 5).

2.3.3. Microbial functional diversity by CLPP

Linear discriminant analysis showed that substrate use differed between depths and sites (Fig. 2.4). The first linear discriminant function (LD 1) distinguished between depths, showing topsoil layers in the negative range and subsoil layers in the positive range. The second linear discriminant function (LD 2) differentiated between the soil types, showing Cambisol samples in the positive range and Luvisol samples in the negative range. LD 1 (78%) and LD2 (17%) explained more than 95% of variance and were both significant (Wilks-Lambda $p \leq 0.05$).
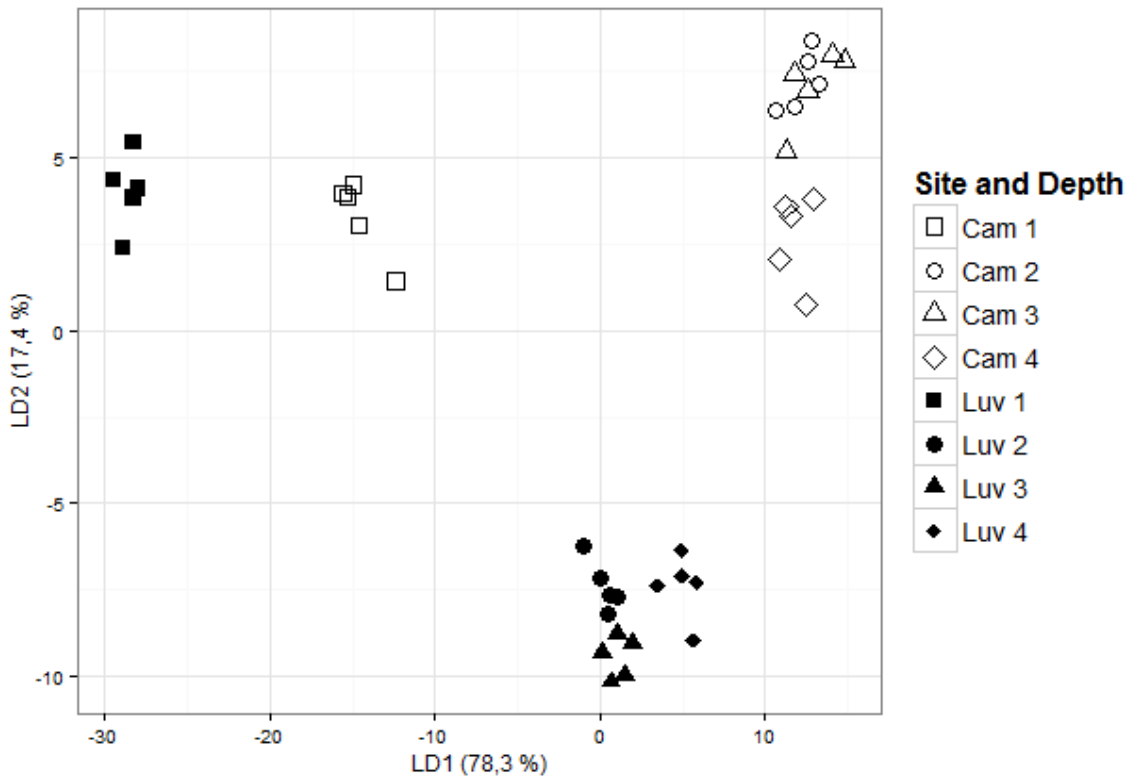
Figure 2.4: Linear discriminant analysis of MSIR values from 17 different substrates standardised on microbial activity for both sites (Cambisol = Cam; Luvisol = Luv) and four sampling depths (1 = 5–10 cm; 2 = 40–50 cm; 3 = 50–60 cm; 4 = 75–85 cm).

Large differences between topsoil and subsoil were shown by sugars and amino acids, especially alanine, L-asparagine, and L-serine (Fig. 2.5bc, see also Sup. 1 for individual values). Large differences between the soil types were shown by arginine, which exhibited higher

2. Microorganisms and their substrate utilization patterns in topsoil and subsoil

respiration rates at all depths of the Luvisol, and the carboxylic acids (Fig. 2.5a, Sup. 1), especially citric acid, α-ketoglutaric acid, malonic acid, and L+tartaric acid, which exhibited high respiration rates at all depths of the Cambisol and low rates in the subsoil samples of the Luvisol.
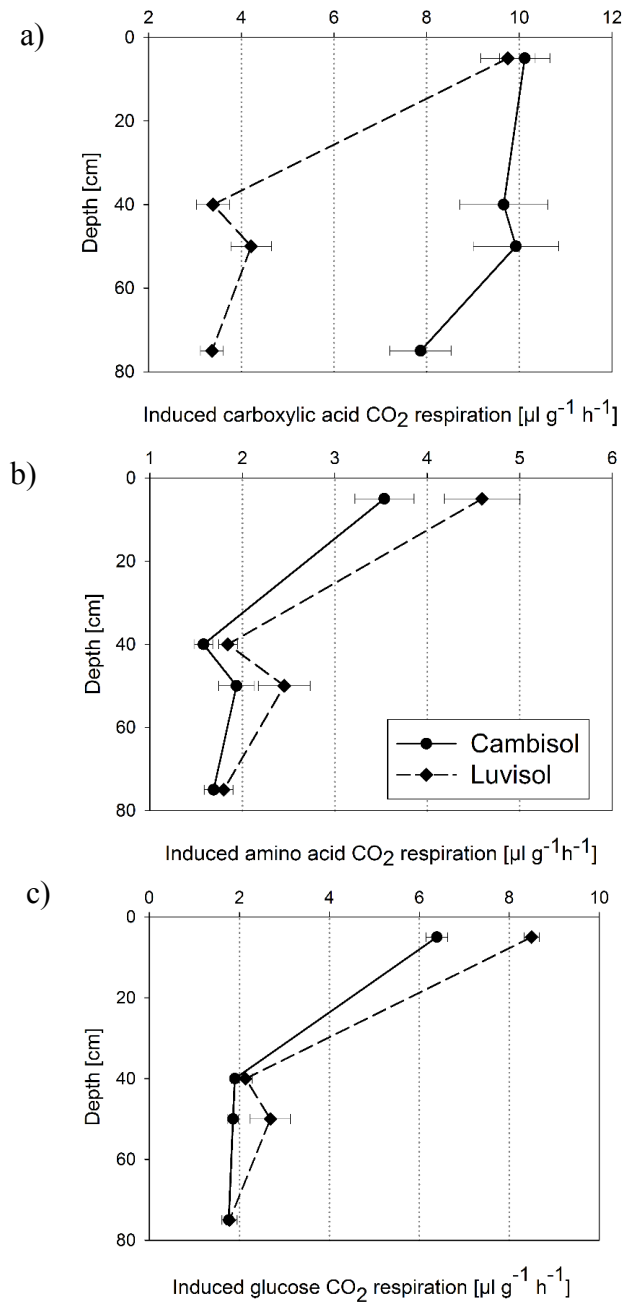
Figure 2.5: Mean CO_2 respiration [$\mu l\ g^{-1}\ h^{-1}$] for a) carboxylic acids (mean of 6 carboxylic acids), b) amino acids (mean of 8 amino acids), and c) glucose; error bars show one standard error (n = 5).

2.4. Discussion

2.4.1. The microbial community in subsoil

Although the Cambisol contains more SOC throughout the entire profile than the Luvisol, the depth gradients of microbial biomass C and ergosterol contents were similar in both soil types. This suggests that not only the microbial biomass of topsoil but also that of subsoil layers strongly depends on the input of fresh plant material, i.e. root residues (Rasse et al., 2005) and rhizodeposition (Wichern et al., 2008; Pausch et al., 2013) and not only on whole SOC contents. This is also supported by the fact that there is no correlation between SOC and microbial biomass C in the Cambisol subsoil. The importance of fresh plant material input is especially true for soil fungi, as indicated by the strong decreases in the ergosterol to microbial biomass C ratio and the fungal C to bacterial C ratio with depth. This is in line with Sradnick et al. (2014) using the same methods and with Fierer et al. (2003). Fungi are strong producers of exo-enzymes for decomposing recalcitrant plant material, such as cellulose and lignin as well as soil organic matter (Keiblinger et al., 2012). However, they seem to need a certain amount of fresh substrates for decomposing these substances.

The low contribution of microbial biomass C to SOC in the deeper layers of the Cambisol, especially in the former A horizon of the covered Chernozem, indicates that the organic matter accumulated is not readily available to soil microorganisms (Anderson and Domsch, 1989). The differences in the contribution of microbial residues to SOC down the profiles indicate some interesting differences between the layers but also between the soil types. The strong increase in the contribution of microbial residue C to SOC from 50% in the topsoil to 70% at 40-50 cm indicates the ongoing transformation of plant residues to microbial residues during prolonged microbial decomposition, as repeatedly suggested by others (Boström et al., 2007; Liang and Balser, 2008; Rumpel and Kögel-Knabner, 2011). A value of 70%, which was roughly maintained

down to 75-85 cm depth in the Luvisol, seems to be the maximum obtainable under the environmental conditions of the experimental site. The very low contribution of microbial residues to SOC at 75-85 cm depth in the Cambisol, falling below 40% of SOC, indicates the disproportionate presence of less decomposed plant material in the covered former A-horizon. This is in line with the significantly higher C/N ratio in this layer, usually used as an indication of the accumulation of less decomposed plant remains (Jenkinson et al., 2008; Rumpel and Kögel-Knabner, 2011). One reason might be that the covered A horizon of the former Chernozem was vegetated with a beech (*Fagus sylvatica* L.) forest (potential natural vegetation), before being transformed into arable land in the early Medieval period (Troßbach, 2000). A history of forestation has already been suggested by Sradnick et al. (2014) as an explanation for the high contribution of plant residues in combination with high C/N ratios in subsoil layers, as plant material with high C/N ratios and higher lignin contents, like tree residues, are less decomposable than e.g. green manure (Silver and Miya, 2001, Hassan et al., 2014).

The even lower level of fungal dominance in the Cambisol might also be evidence that bacteria in the Cambisol benefit from the more abundant and evenly distributed C and N, as suggested by Hendrix et al. (1986) and Holland and Coleman (1987). Therefore, N availability seems to have a stronger influence on the microbial community composition in subsoil than C availability (Kaiser et al., 1992), as bacteria are more competitive for easily available N components than fungi (Rinnan and Bååth, 2009, Geisseler et al., 2010).

The importance of substrate availability depending on the different substrate requirements of the microbial community is also shown in the correlations between microbial biomass C and N and SOC and total N. There is no correlation between SOC and microbial biomass in the Cambisol subsoil, which indicates that the amount of available SOC would be sufficient for microbial growth. There is also only a weak and non-significant correlation between total N and microbial biomass

N, so there seems to be a requirement for more N to increase microbial growth. This may be supported by the weak correlation between total N and SOC in the Cambisol subsoil. Usually the correlation between SOC and total N is relatively high, as in the Luvisol, but total N shows a stronger decrease than SOC in the Cambisol profile. This may indicate a higher demand for N than for C in the microbial community, but other factors are probably limiting for microbial growth, as this correlation is also rather weak. In the Luvisol subsoil, more SOC and N are clearly required to increase microbial growth, as both are significantly correlated with microbial biomass C and N.

The matter of resource availability has already been suggested by Fierer et al. (2003) as an important factor controlling changes in the microbial community in depth profiles. This is strongly supported by our findings, but in cases like our Cambisol, where SOC is not limiting and N only to a small degree, there must be other factors limiting microbial growth.

2.4.2. Microbial functional diversity in subsoil

The functional diversity of the microbial community significantly differed between the topsoil and subsoil layers as well as between the subsoil layers of the two soil types. To understand the reasons for this differentiation, it is important to take a closer look at the reaction to distinct substrate groups and even single substrates that were added. The highest respiration rates were measured after adding carboxylic acids to both soil types at all depths. This is due to the fact that microorganisms preferably catabolise carboxylic acids as long as there are other substrates available for anabolism (Gunina et al., 2014). The high respiration rates for carboxylic acids in the Cambisol subsoil layers indicate that there were enough other substrates available for anabolism. The opposite occurred in the Luvisol subsoil layers. The respiration rates for carboxylic acids strongly decreased in these layers in comparison with the topsoil layers. The similar microbial biomass in all subsoil layers leads to the assumption that the microorganisms in the Luvisol subsoil

have an increased C demand (Apostel et al., 2013). This stimulates the incorporation of carboxylic acids into biomass, although carboxylic acids are energetically less favourable than amino acids to form, e.g. PLFAs (Gunina et al., 2014). The high respiration rates for the carboxylic acids in the Cambisol subsoil suggest that there is not necessarily an elevated C demand, contrasting the view of Goldfarb et al. (2011). The differentiation in the microbial functional diversity between the two soil types seems to be partially controlled by substrate availability.

The respiration values for glucose are highest in the topsoil and decrease strongly with depth. This indicates that the anabolic demand for substrate is much higher in subsoil than in topsoil (Gunina et al, 2014). The highest glucose-induced respiration rates were measured in the Luvisol topsoil. The glucose respiration rates were generally well correlated ($r = 0.76$, $p < 0.01$) with the ergosterol content, as fungi need glucose for the production of unsaturated triacylglycerols as storage metabolites (Lundberg et al., 2001). This synthesis is accompanied by CO_2 release.

In the subsoil layers of both soil types, amino acid-induced respiration rates were significantly lower than in the topsoil, indicating an increasing N-limitation at lower depths. Amino acids are rapidly and efficiently incorporated as intact molecules into the microbial biomass by the direct uptake route, especially at wider C/N-ratios (Geisseler et al., 2010). Requirements of microbial metabolism determine at a later stage whether the amino acids are oxidized for energy production or used for protein synthesis or other metabolic products (Gunina et al., 2014). The entrance point into the metabolic cycle of the cell is also important for the further pathway and how fast amino acids are oxidized after incorporation. Amino acids entering between glycolysis and citric acid cycle, e.g. alanine, are oxidized later than amino acids, which enter directly into the citric acid cycle, e.g. glutamine (Gunina et al., 2014). This was reflected by the respiration rates, which were generally higher for glutamine and other amino acids, containing three or more oxygen atoms, than for alanine and other amino acids, containing only two oxygen atoms. The amino acid-induced

respiration rates were two times higher in the topsoil than in the subsoil layers of both soil types. This indicates that the subsoil layers generally were N-depleted, so that the microorganisms have to use amino acids for anabolic processes.

The tendency towards lower amino acid-induced respiration in the Cambisol subsoil despite higher total N contents can be explained by the higher presence of bacteria in the Cambisol subsoil layers in comparison with those of the Luvisol. Bacteria have more amino acid membrane transporters than fungi (Geisseler et al., 2010). Consequently, bacteria are more competitive in the uptake of amino acids, which they preferably anabolize as long as other energy sources are available to match their catabolic demands (Gunina et al., 2014).

2.5. Conclusions

Resource availability is an important factor for controlling size and functional diversity of the soil microbial community as well as the ratio of fungi to bacteria. Our results indicate that C limitation is only the major controlling factor in terms of microbial community size in subsoils with low SOC stocks, while other factors limit microbial growth in subsoils with high C stocks. The decrease in fungal dominance with depth may also be explained by resource availability, as fungi suffer from a lack of fresh plant input in both soil types. The C and N stocks also control the reaction of microorganisms to substrate addition. The MSIR method differentiates precisely between topsoil and subsoil layers according to the differences in N demands and between subsoils according to SOC demands. Therefore, the selection of substrates in relation to their metabolism in microorganisms is crucial to retrieve maximum information on the microbial community composition and substrate requirements. Hence, SOC and N availability are major factors controlling size, composition and substrate use of the microbial community, but the result from the

Cambisol provides evidence that there must be other factors, e.g. lack of other nutrients, that limit microbial growth in subsoil.

3. Amaranth decomposition under elevated CO₂ concentrations – comparing topsoils with C-poor and C-rich subsoils

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ABSTRACT

To investigate factors and mechanisms controlling C sequestration in subsoil, an incubation experiment was carried out for 176 days at 22 °C, using elevated CO_2 concentrations (4 and 8%) with and without the addition of amaranth, a C4 plant. We determined the effects of these treatments on soil organic C (SOC), microbial biomass C and fungal ergosterol. Soil samples were taken from topsoil (5-15 cm) and subsoil (75-85 cm) on two neighbouring arable sites; one being a Colluvic Cambisol with high SOC contents in the subsoil, the other a Haplic Luvisol with low SOC contents below 30 cm depth. Soils lost between 26 and 38% SOC during the incubation, without effects of elevated CO_2 concentrations. Amaranth-derived C losses were generally stronger in the Colluvic Cambisol (7.5 mg g^{-1}), especially at elevated CO_2 concentrations, than in the Haplic Luvisol (7.0 mg g^{-1}), without clear differences between topsoils and subsoils. Amaranth addition caused negative priming effects on SOC mineralization in the subsoils, which were similar in the Cambisol (11%) and Luvisol (14%). Elevated CO_2 concentrations had no general effects on microbial biomass or ergosterol with and without amaranth addition. However, the contribution of amaranth-derived microbial biomass C to total microbial biomass C was significantly ($\alpha = 0.1$) reduced at elevated CO_2 concentrations (Topsoil: 31%; Subsoil: 54%).

Keywords: C-turnover, decomposition, gas conditions, microbial biomass, priming effect, subsoil

3. Amaranth decomposition under elevated CO₂ concentrations

3.1. Introduction

Subsoil organic carbon accounts for more than 50% of the world's soil organic carbon (SOC) stocks (Batjes, 1996). This subsoil OC is of a higher average age than that in topsoils (Rumpel et al., 2002; Rumpel and Kögel-Knabner, 2011), which leads to the assumption that the mineralization rate of organic compounds in subsoil is lower than in topsoil. This makes subsoil a sink for carbon, which directs attention within the climate change debate towards subsoil (Bailey et al., 2002). Creating conditions, that avoid mineralisation from this pool and promote further carbon sequestration in subsoil is desirable as part of mitigation strategies for climate change. However, there are still some knowledge gaps regarding the factors and mechanisms of carbon stabilization below the A-horizon (Sanaullah et al., 2011; Cotrufo et al., 2013).

Three main factors are discussed, which separately or in combination have the potential to reduce carbon turnover rates in subsoil. The first factor is the physical separation of substrate and microorganisms (Joergensen and Potthoff, 2005; Rumpel and Kögel-Knabner, 2011). The lack of anthropogenic disturbance and limited bioturbation by roots leads to reduced mixing of the soil and small pore sizes. These conditions make it hard for microorganisms to access substrate, that is not in their nearest environment (Bachmann et al., 2008). Furthermore, the percentage of SOC, that is either occluded in aggregates or chemically bound to the mineral surfaces, increases in comparison with the topsoil. Both are hard to access for microorganisms and are considered as stabilized (Schrumpf et al., 2013).

The second factor is the limited input of fresh organic substrate into subsoil (Fontaine et al., 2007). Subsoil organic matter no longer contains labile compounds, which makes the substrate more recalcitrant and therefore metabolically less attractive for microorganisms (Salome et al., 2010; Rumpel and Kögel-Knabner, 2011; Dungait et al., 2012; Cotrufo et al., 2013).

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The third factor is related to the gas conditions in subsoil. It is assumed that CO_2 concentrations in subsoil are higher than under atmospheric conditions and O_2 concentrations correspondingly lower (Salome et al., 2010). It has been proven that bacteria are relatively robust to changes in the gas conditions in terms of elevated CO_2 while the fungal community was affected by changes in atmospheric gas conditions (Hayden et al., 2012). For example laccases need oxygen for substrate degradation, which is limited in subsoil (Ekschmitt et al., 2008). Factors one and two are widely accepted as being part of the mechanisms that control carbon turnover in subsoil, while there are only assumptions and few experiments (Salome et al., 2010; Hayden et al., 2012) on the influence of the different gas conditions in subsoil.

To address this knowledge gap, a long-term incubation experiment was carried out, using different CO_2 concentration levels with and without the addition of amaranth (*Amaranthus hypochondriacus* L), a C4 plant with higher $\delta^{13}C$ values than the autochthonous SOC. This makes it possible to monitor amaranth-derived SOC and microbial biomass C under incubation conditions without the possibility of measuring soil CO_2 evolution. Topsoil and subsoil samples were taken from two arable sites with different subsoil OC contents (Struecker and Joergensen, 2015). This difference has been developed naturally over the past 800 years due to erosion and deposition of surface soil material. It provides the opportunity to investigate the extent to which natural C availability (Fierer et al., 2003) or other subsoil specific conditions (Salome et al., 2010) control C turnover and the microbial community, as all other environmental conditions on the two sites are the same (Struecker and Joergensen, 2015).

The aim was to investigate the following three hypotheses: (1) Elevated CO_2 concentrations generally reduce microbial mineralization of SOC, due to fungal dominance of the microbial community in the investigated soils (Struecker and Joergensen, 2015). (2) Elevated CO_2 concentrations especially reduce the decomposition of fresh plant residues, due to specific negative

effects on fungi (Ekschmitt et al., 2008; Hayden et al., 2012), the main decomposers of plant residues (Keiblinger et al., 2010). (3) Elevated CO_2 concentrations have strongest effects in the Haplic Luvisol subsoil, due to a strong C and N limited microbial community with a stronger fungal dominance in comparison with the Colluvic Cambisol (Struecker and Joergensen, 2015).

3.2. Material and methods

3.2.1. Site description and field sampling

Soil samples were taken from two arable fields at the Hessian State Manor of Frankenhausen, northern Hessia, Germany (51°24' N; 9°25' E), the experimental farm of Kassel University. The area is characterized by a mean annual air temperature of 9.3 °C and a mean annual precipitation of 687 mm. The soils of site I (referred to as Cambisol) can be characterized as a Colluvic Cambisol according to the WRB (FAO, 2014). The Colluvic horizon of the Colluvic Cambisol covers the original soil surface of a Chernozem by about 70 cm, resulting in an Ap / M / fAh sequence. The soils of site II (referred to as Luvisol) can be classified as a Haplic Luvisol according to the WRB (FAO, 2014), although the Al horizon was eroded. This results in an Ap / Bt sequence. The soils of the two sites have developed on loess and are within a distance of 400 m from each other, which means that environmental conditions are equivalent on both sites, although they have different SOC profiles due to erosion and deposition (Struecker and Joergensen, 2015). Land use at both sites was also similar for at least 400 years, when both sites were used as grassland first and then as cropland since the early 20th century (Troßbach, 2000).

Both sites were sampled in August 2014. Soil samples were taken at two depths, 5-15 cm for the topsoil and 75-85 cm for the subsoil. These depths were chosen to avoid a mixing of the natural soil horizons. All samples were stored at 4°C until they were filled into the incubation jars and further treated.

3.2.2. Evaluation of subsoil gas conditions

To get an idea of the gas conditions in subsoil under field conditions we conducted a pre-experiment in which we installed stationary gas probes at three depths (45, 65 and 85 cm) on two arable fields. These probes were sampled from March to August 2014 every second week. We measured average CO_2 concentrations of 4 Vol%, which is about 100 times higher than atmospheric conditions. Average O_2 concentrations were correspondingly lower (17 Vol%). The extreme values were 8 Vol% for CO_2 and 13 Vol% for O_2.

3.2.3. Incubation procedure

The incubation experiment comprised six different treatments for two soil types and two depths, which equals 24 sample types with three laboratory replicates for each type (72 incubation samples). Additional control samples with and without amaranth addition were prepared for both soil types and depths, which were analysed after the pre-incubation to receive start values for the different measures for all treatments.

All samples were sieved to < 2 mm. 150 g of the samples were filled into incubation jars of 1500 mL volume and the water content was adjusted to 50% water holding capacity. For the amaranth (*Amaranthus hypochondriacus* L. var. 'Neuer Typ') treatments, 4 g of dried and shredded amaranth were added, which equals a C addition of 10.7 mg g^{-1} and an N addition of 0.6 mg g^{-1}. Amaranth was taken from a field experiment carried out in 2008 (Heitkamp et al., 2012) and air-dried before storage. Both incubation samples and control samples were pre-incubated for 19 days at 22 °C.

During the incubation experiment, the elevated gas concentration samples were flushed with the equivalent gas mixtures (Medium: 4 Vol% CO_2, 17 Vol% O_2, 79 Vol% N_2; Extreme: 8

Vol% CO_2, 13 Vol% O_2, 79 Vol% N_2) (Westfalen, Münster, Germany) weekly to maintain a constant gas concentration within the incubations jars. The concentrations were checked on a regular basis and stayed in the target range (gas mixture -+1 %) of the average and extreme subsoil gas condition measured in the pre-experiment throughout the incubation.

3.2.4. Free particulate matter (fPOM) extraction

fPOM was extracted using the first step (flF extraction) of the density fractionation scheme of John et al. (2005) as modified by Cerli et al. (2012), Griepentrog and Schmidt (2013), and Kaiser and Berhe (2014). Soil of 10 g dry weight was dispersed in 50 ml of a 1.6 g cm^{-3} sodium polytungstate (SPT) solution. After one hour, the sample was centrifuged at 4000 g for 30 min. After another 30 min, the fPOM was decanted on a filter and washed with 1.5 L of distilled water.

3.2.5. Analysis of soil properties

All soil samples were analysed for soil microbial biomass C and N using the fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). Fumigated (24 h with ethanol-free $CHCl_3$ at 25 °C) and non-fumigated soil samples of 10 g were extracted with 40 ml of 0.05 M K_2SO_4 (Potthoff et al., 2003) by 30 min horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). Organic C was determined in the extracts using a multi N/C 2100S automatic analyser (Analytik Jena AG, Germany). Microbial biomass C was calculated as E_C/k_{EC}, with E_C = (organic C extracted from fumigated soil) − (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990).

For the determination of ^{13}C, 20 ml aliquots of 0.05 M K_2SO_4 extracts of fumigated and non-fumigated samples were freeze dried for about 3 days and were analysed by isotope ratio mass

spectrometry (Delta V Advantage, Thermo Electron, Bremen, Germany interfaced to an elemental analyser Flash 2000, Thermo Fisher Scientific, Cambridge, UK).

The fungal cell membrane component ergosterol was extracted from moist 2 g topsoil and 8 g subsoil with 100 ml ethanol (Djajakirana et al., 1996). Then, ergosterol was determined by reverse phase HPLC using 100 % methanol as the mobile phase and detected at a wavelength of 282 nm.

In fPOM and the bulk soil samples, total C and N as well as $\delta^{13}C$ and $\delta^{15}N$ were measured by isotope ratio mass spectrometry. As the samples did not contain any carbonate, total C equals soil organic C (SOC).

3.2.6. Calculations and statistics

Isotope values are expressed in delta notation relative to VPDB and air for ^{13}C and ^{15}N, respectively. The amount of substrate derived microbial biomass (MB-^{13}C) was calculated by the following equation (Potthoff et al., 2003):

$$MB\text{-}^{13}C\ (\%) = \frac{(^{13}C\text{-}Atm.\,excess_{\text{fum}} \times C_{\text{fum}}) - (^{13}C\text{-}Atm.\,excess_{\text{nfum}} \times C_{\text{nfum}})}{(C_{\text{fum}} - C_{\text{nfum}})} \times 100$$

where C_{fum} and C_{nfum} represent the mass of C (mg g^{-1}) extracted from the fumigated and non-fumigated samples, respectively, and ^{13}C-Atm.excess$_{\text{fum}}$ and ^{13}C-Atm.excess$_{\text{nfum}}$ represent the corresponding ^{13}C atom% excess values calculated from the amaranth amended and un-amended treatments. The fraction of amaranth-derived C (f$_{\text{amaranth-C}}$) was calculated for each individual replicate of all treatments from the $\delta^{13}C$ data according to a two pool-mixing model with the following equation:

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$$f_{\text{amranth-C}} = \frac{\delta^{13}C_{\text{sample}} - \delta^{13}C_{\text{control}}}{\delta^{13}C_{\text{amaranth}} - \delta^{13}C_{\text{control}}}$$

where $\delta^{13}C_{\text{sample}}$ represents the $\delta^{13}C$ value of SOC in the mixed sample (amaranth and autochthonous SOC), microbial biomass C or fPOM; $\delta^{13}C_{\text{control}}$ is the average $\delta^{13}C$ value of the non-amended control samples and $\delta^{13}C_{\text{amaranth}}$ is the $\delta^{13}C$ of the amaranth residues.

To determine possible priming effects, the amount of autochthonous C in the respective fractions of amaranth-amended samples was obtained by subtracting the amaranth-derived C from the total amount of C. This was compared with the SOC content of the non-amended control samples. Significantly lower values of autochthonous C in the amended sample in comparison with SOC in the control sample would indicate a priming effect.

Isotopic fractionation during microbial decomposition processes remains a controversial issue and is often suggested to be negligible and of little importance (Ehleringer et al., 2000; Ekblad et al., 2002). Also, Collins et al. (2000) found no evidence of isotopic discrimination after incubating maize residues for 50 days. We therefore assume that no fractionation occurred during the incubation process (see also Rochette et al., 1999) as the added substrate did not show an enrichment in heavy isotopes comparing start and end of the incubation.

The data was tested for homogeneity of variances using the Levene test and for normal distribution of the residues using the Shapiro-Wilk test, accompanied by a graphical assessment of histograms and qq-plots. The analysis of the main effects, site, gas, and substrate, and their interactions, on different measures was conducted with an analysis of variance which should be robust against some violations of normal distribution of residues, as homogeneity of variances was always ensured and the number of samples was relatively large. The three different gas treatments were considered as repeated measures in the analysis of variance, using *ezANOVA*. To account for

sphericity of the data, Mauchly's test for sphericity was applied and followe by a Greenhouse-Geisser correction. The Kruskal-Wallis test was used to determine differences between groups and treatments. This more conservative and non-parametric test was chosen, as the residues of several variables in various treatments were not normally distributed. Correlations for the gas treatments were calculated using Spearman's correlation coefficient for the same reason. All statistical analyses were performed in R (R Development Core Team, 2010).

3.3. Results

3.3.1. Development of control samples

All samples lost large amounts of SOC during the incubation (Fig. 3.1a). These losses were significantly lower ($p < 0.01$) in the Cambisol topsoil (-26%) than in the subsoil (-38%), whereas the SOC losses did no differ between the Luvisol topsoil and subsoil (-28%). Microbial biomass decreased significantly in both topsoils ($p < 0.01$), but remained nearly constant in the subsoil (Fig. 3.2a). SOC and microbial biomass C decreased to a similar extent in topsoils (27%), in subsoils the SOC loss was markedly stronger (CS: 38%; LS: 28%) than the decrease of microbial biomass C (< 10%). The values for SOC and MBC at the end of the incubation in the control samples were significantly determined by sampling site for topsoil and subsoil (Table 3.1).

The ergosterol content generally decreased significantly ($p < 0.01$) during incubation (Fig. 3.3a). In the two topsoils, also the contribution of ergosterol to microbial biomass C decreased significantly ($p < 0.01$) within 6 months (Fig. 3.3c), whereas this decrease was not significant in the subsoil.

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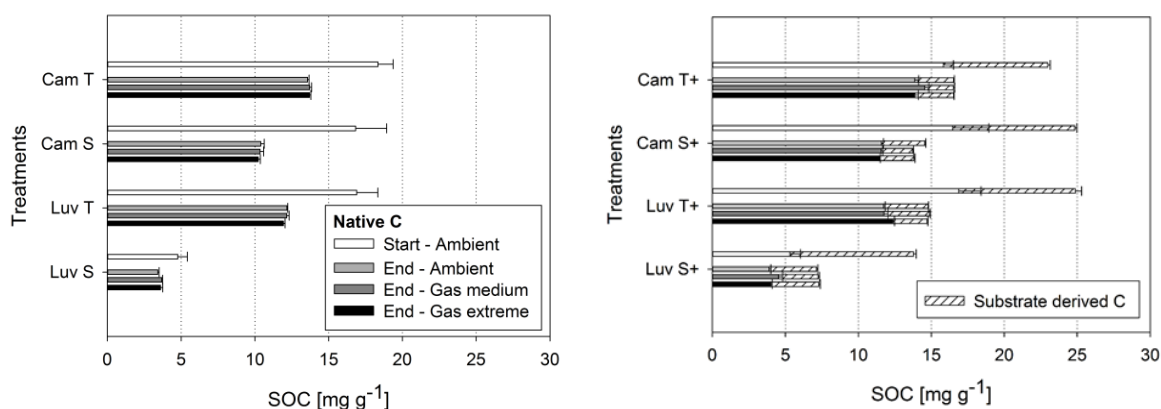
Figure 3.1: Reaction of SOC to different gas conditions with and without substrate addition a) Start and end values SOC [mg g^{-1}] for samples without substrate addition b) Start and end values SOC [mg g^{-1}] for samples with substrate addition (Cam = Cambisol, Luv = Luvisol, T = Topsoil, S = Subsoil, + = labelled substrate added at beginning of incubation). Bars show arithmetic means (n=3), error bars show one standard error.

Table 3.1: Main effects on autochthonous SOC, MBC, and ergosterol, and probability values for the 3-way ANOVA, using site, substrate and gas treatments as factors. The different gas treatments were considered as repeated measures.

	SOC (autochthonous) (mg g^{-1} soil)		MBC (autochthonous) (µg g^{-1} soil)		Ergosterol (µg g^{-1} soil)	
	Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil
Cambisol	13.9	10.9	204	73	0.43	0.22
Luvisol	12.0	3.9	160	30	0.46	0.39
Ambient	12.8	7.3	175	34	0.50	0.26
Medium	13.0	7.5	187	52	0.41	0.29
Extreme	13.0	7.3	185	69	0.43	0.37
No substrate	12.9	6.9	199	49	0.28	0.01
Amaranth	13.0	7.9	165	55	0.61	0.60
Probability values						
Site	<0.01	<0.01	<0.01	<0.01	n.s.	0.09
Gas	n.s.	n.s.	n.s.	0.03*	n.s.	n.s.
Substrate	n.s.	<0.01	0.02	n.s.	<0.01	<0.01
Site × gas	n.s.	n.s.	n.s.	n.s.	0.06	n.s.
Site × substrate	n.s.	n.s.	n.s.	0.04	<0.01	0.09
Gas × substrate	n.s.	n.s.	0.02*	0.06*	0.06	n.s.
Site × gas × substrate	n.s.	n.s.	n.s.	<0.01*	0.07	n.s.
CV [± %]	8	49	23	77	48	133

* p-values after Greenhouse-Geisser correction; CV = mean coefficient of variation between replicate samples within site and depth (n = 3); n.s. = not significant

44
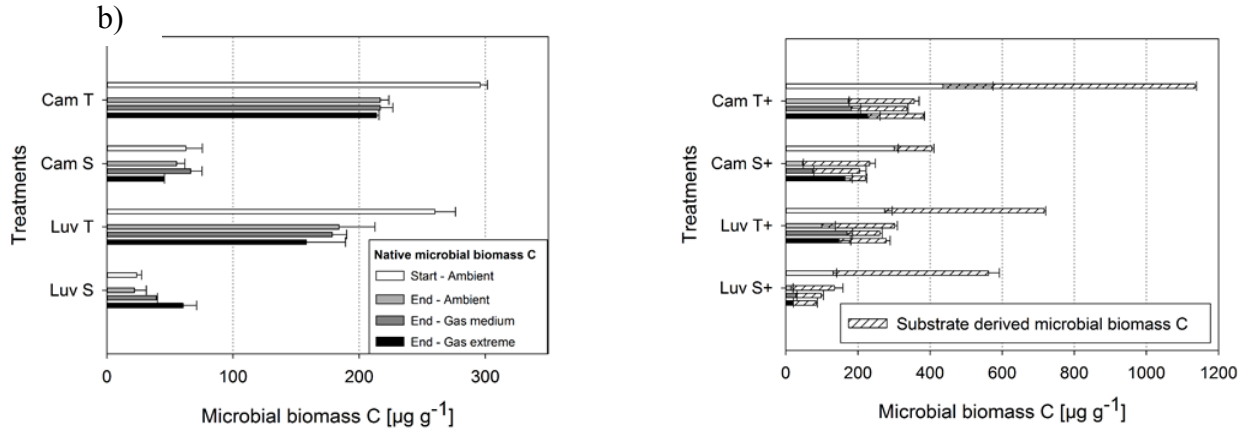
Figure 3.2: Reaction of microbial biomass C to different gas conditions with and without substrate addition a) Start and end values microbial biomass C [µg g⁻¹] for samples without substrate addition b) Start and end values microbial biomass C [µg g⁻¹] divided into native and substrate derived for samples with substrate addition (Cam = Cambisol, Luv = Luvisol, T = Topsoil, S = Subsoil, + = labelled substrate added at beginning of incubation). Bars show arithmetic means (n=3), error bars show one standard error.

3.3.2. Effects of amaranth addition

The amendment of amaranth had a strong and highly significant ($p < 0.01$) effect on the development of autochthonous SOC in subsoil, autochthonous MBC in topsoil and ergosterol in topsoil and subsoil during the incubation (Table 3.1).

During the 19-day pre-incubation, roughly 28% of the initially added amaranth derived C were already lost towards the start ("day zero") of the incubation. During the 176-day incubation, roughly 65% of the "day zero" contents were additionally lost, i.e. amaranth-derived C was on average lost at a doubled rate in comparison with autochthonous SOC (Fig. 3.1). About 2.5 mg amaranth-derived C g⁻¹ soil remained in the Cambisol, whereas 3.0 mg g⁻¹ soil remained in the Luvisol, without significant differences between topsoil and subsoil in both cases. Amaranth addition did not affect the loss of autochthonous SOC in the topsoil, but significantly ($p < 0.01$) reduced these losses in the subsoil (Table 3.1, Fig. 3.4).

3. Amaranth decomposition under elevated CO₂ concentrations
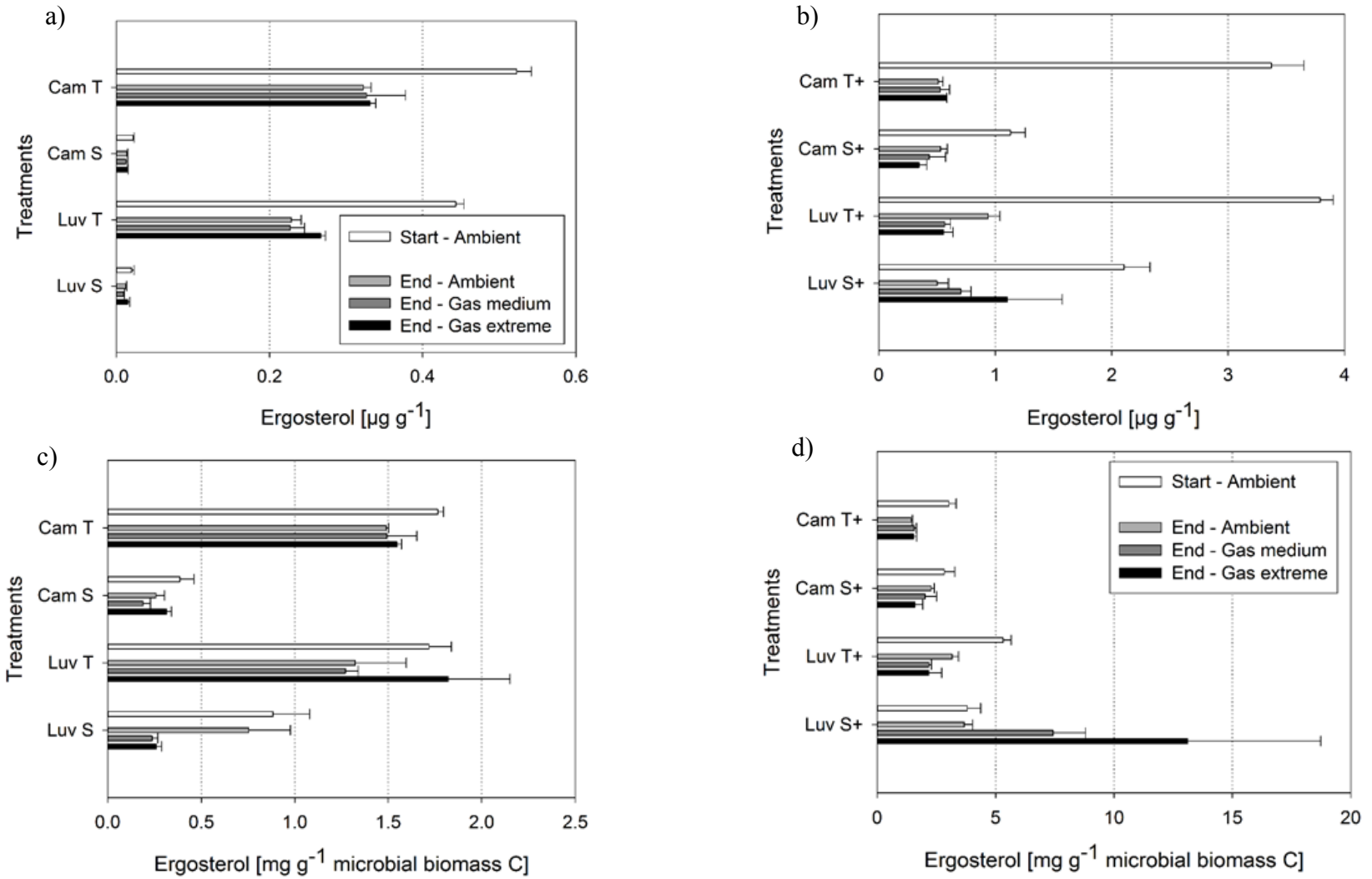
Figure 3.3: Reaction of ergosterol and the proportion of ergosterol in microbial biomass C to different gas conditions with and without substrate addition a) Start and end values ergosterol [μg g^{-1}] for samples without substrate addition b) Start and end values ergosterol [μg g^{-1}] for samples with substrate addition c) Start and end values ergosterol [mg g^{-1} microbial biomass C] for samples without substrate addition d) Start and end values ergosterol [mg g^{-1} microbial biomass C] for samples with substrate addition (Cam = Cambisol, Luv = Luvisol, T = Topsoil, S = Subsoil, + = labelled substrate added at beginning of incubation). Bars show arithmetic means (n=3), error bars show one standard error.

3. Amaranth decomposition under elevated CO₂ concentrations
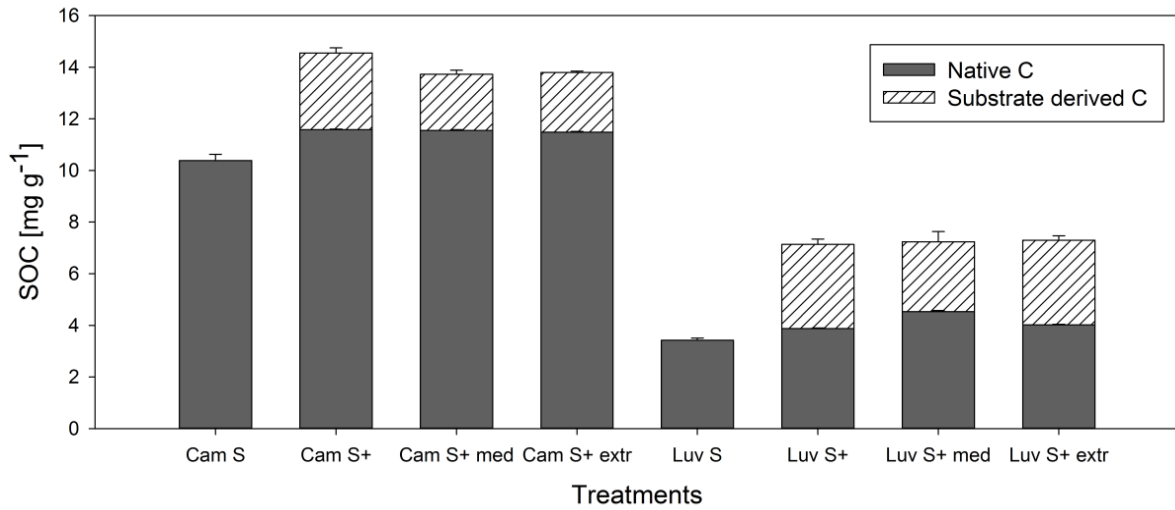
Figure 3.4: Proportion of native C and labelled C in subsoil samples at the end of the incubation experiment (Cam = Cambisol, Luv = Luvisol, + = labelled substrate added at beginning of incubation). Bars show arithmetic means (n=3).

Table 3.2: Average C/N ratio of fPOM at start and end of incubation compared with fresh amaranth: C/N = 18.9

	fPOM start	fPOM end
Cam Topsoil	66.1	18.1
Cam Subsoil	61.5	14.8
Luv Topsoil	49.4	16.7
Luv Subsoil	69.5	20.4
CV [± %]	14.3	15.6

CV = mean coefficient of variation between replicate

samples within site and depth (n = 3)

# 3. Amaranth decomposition under elevated CO_2 concentrations

Amaranth addition increased microbial biomass C in all samples after pre-incubation, which remained significant ($p < 0.01$) until the end of incubation, i.e. +10-20% in the topsoil and + 250-300% in the subsoil (Fig. 3.2). After pre-incubation, about 60% of the microbial biomass C were amaranth-derived in the two topsoils, whereas only 30% were amaranth-derived in the Cambisol subsoil and more than 75% in the Luvisol subsoil. Except for the Cambisol topsoil, the proportion of amaranth-derived microbial biomass C increased during the incubation, while total microbial biomass C decreased (Fig. 3.2b). Amaranth addition caused a stronger increase in microbial biomass than in SOC, but the increase in ergosterol was even stronger at 6 to 8-fold in the topsoil and 50- (LS) to 100-fold (CS) in the subsoil (Fig. 3.3b) compared to the control treatments. This increase considerably declined until the end of incubation but still exceeded the values before substrate addition, except in the Cambisol topsoil (Fig. 3.3). Consequently, the contribution of ergosterol to microbial biomass C generally increased during pre-incubation (Fig. 3.3cd) and decreased again, except in the Luvisol subsoil where it remained stable (Fig. 3.3d).

Amaranth addition increased the C/N ratio of free particulate matter (fPOM) during pre-incubation to 60, which was markedly above the C/N of 19 in fresh amaranth. Then, the C/N ratio in fPOM decreased again towards 17 until the end of incubation (Table 3.2).

3.3.3. Effects of elevated CO_2 concentrations

Elevated CO_2 concentrations (medium and extreme) did not affect SOC losses (Tables 3.1 and 3.3, Fig. 3.1). The same was also generally true for autochthonous microbial biomass C in topsoil (Fig. 3.2a) and ergosterol in subsoil due to high variability (Fig. 3.3a), although in the Luvisol subsoil elevated CO_2 concentrations significantly ($p < 0.05$) increased ergosterol contents (Fig. 3.3a). In subsoils autochthonous MBC significantly increased with elevated CO_2 concentrations, while amaranth derived MBC significantly decreased (Tables 3.1 and 3.3).

3. Amaranth decomposition under elevated CO₂ concentrations

Ergosterol in topsoils showed a decreasing trend with elevated CO_2, which was also affected by the interaction of sampling site and gas treatment (Table 3.1). In the non-amended samples elevated CO_2 concentration slightly increased the contribution of ergosterol to microbial biomass C in the Cambisol topsoil and strongly decreased it in the Luvisol subsoil (Fig. 3.3c). Both effects were weakly significant ($p < 0.10$).

Table 3.3: Table 3: Main effects on amaranth derived SOC, and MBC, and probability values for the 2-way ANOVA, using site, and gas treatments as factors. The different gas treatments were considered as repeated measures.

	SOC (amaranth derived) (mg g⁻¹ soil)		MBC (amaranth derived) (µg g⁻¹ soil)	
	Topsoil	Subsoil	Topsoil	Subsoil
Cambisol	2.44	2.48	164	129
Luvisol	2.85	3.07	143	86
Ambient	2.86	3.11	193	154
Medium	2.55	2.43	122	101
Extreme	2.51	2.80	146	67
Probability values				
Site	n.s.	<0.01	n.s.	n.s.
Gas	n.s.	n.s.	0.08*	0.07*
Site × gas	n.s.	n.s.	n.s.	n.s.
CV [± %]	27	23	32	56

* p-values after Greenhouse-Geisser correction; CV = mean coefficient of variation between replicate samples within site and depth (n = 3); n.s. = not significant

Elevated CO_2 concentrations did not affect autochthonous- and amaranth-derived SOC losses (Fig. 3.1, Tables 3.1 and 3.3). In contrast, there was a clear trend ($p < 0.10$) towards 25-55% less amaranth-derived C incorporation into microbial biomass C at elevated CO_2 concentrations (Table 3.3). There was also no systematic influence of elevated CO_2 concentrations on the ergosterol content in the non-amended Cambisol samples. In the non-amended Luvisol topsoil, an

increased CO_2 concentration caused a significant (r = -0.67; p < 0.05) decrease in ergosterol (Fig. 3.3b), whereas there was no significant increase in the Luvisol subsoil detected (Fig. 3.3b).

3.4. Discussion

The stronger decrease in ergosterol in comparison with microbial biomass C and SOC during the incubation without amaranth indicates that saprotrophic fungi suffer more from the lack of C input than the rest of the microbial community (Rasse et al., 2005; Wichern et al., 2008; Keiblinger et al., 2012; Pausch et al., 2013). In the topsoil, nearly 30% of SOC and microbial biomass C were lost during 176 day of incubation at 22 °C and 50% WHC. These high losses indicate that optimum conditions were created for the decomposition of SOC in the topsoil. The higher SOC losses in the subsoil in combination with the absence of microbial biomass C losses indicate that microorganisms are able to anabolize SOC under artificial laboratory conditions in contrast to the field situation. However, the spatial variability is probably much higher in the subsoil than in the topsoil (Jörgensen et al., 2002). This problem is intensified by methodological constraints of the CFE method, where the error accumulates on the difference between fumigated and non-fumigated samples (Joergensen and Olfs, 1998). The high coefficient of variation in the current subsoil samples indicates the importance of these two problems at deep soil depth.

In the amaranth-amended samples, the autochthonous SOC contents in subsoil declined less over the 6 months than in the control samples. This suggests a negative priming effect and contrasts the results others (Fontaine et al., 2007, Guenet et al., 2012), who observed an additional CO_2 release from autochthonous SOC after substrate addition. However, the current results are in line with Dalenberg and Jager (1989), who found negative priming effects after addition of more complex substrates, e.g. wheat straw and sewage sludge, to topsoil samples. In these cases, the microbial community can probably retrieve energy and nutrients more easily from the plant

residues than from autochthonous SOC (Kramer and Gleixner, 2008). However, type and amount of substrate might change priming effects, as they often last longer than the substrate itself remains in soil. For example, exo-enzymes excreted in the presence of substrates might still be able to degrade SOC in the long-term (Kuzyakov, 2010). Furthermore in the field, there is usually a combination of water-soluble compounds and plant residues. The water-soluble compounds can reach micro-pores and are more easily accessible for microorganisms whereas plant residues are less mobile, but longer lasting.

The strong increase in the C/N ratio in the fPOM, consisting to more than 90% of amaranth residues, after pre-incubation in comparison with the initial plant material indicates that the microbial community takes up large amounts of amaranth-derived N. This increase in the C/N ratio has been previously observed during the initial decomposition of N-rich substrates under N-limited conditions (Ågren et al., 2013). The initial increase in C/N ratio of the fPOM fraction was followed by a decrease in C and an increase in N contents. This results in decreased C/N ratios, typical for the N accumulation in decomposing plant material (Moore et al., 2006). However, the C/N ratios of the fPOM fraction remain above autochthonous bulk soil values for the Cambisol topsoil and for the Luvisol topsoil and subsoil. This indicates the low degradation state of the fPOM material (Moore et al., 2006; Sanaullah et al., 2011). For the Cambisol subsoil, the C/N ratio of the autochthonous bulk soil and the fPOM are rather similar. This supports the view that the contribution of microbial residue C to SOC is relatively small in the Cambisol subsoil (Struecker and Joergensen, 2015) and that an increased C/N ratio indicates a larger proportion of less degraded plant residues (John et al., 2005; Jenkinson et al., 2008).

Microbial biomass C showed an extreme increase in comparison with the control samples after pre-incubation in all amended sample types. Hence microbial biomass C and total N increased strongly and to a similar extent due to substrate addition, whereas the increase in SOC was lower.

The large proportion of amaranth derived C in the microbial biomass at the beginning of the incubation shows that also amaranth-derived C was anabolised by the microbial community. However, the lower proportion of amaranth derived C in the microbial biomass of the Cambisol subsoil in comparison with the Luvisol subsoil indicates a co-metabolism of added and autochthonous C in the Cambisol subsoil. This might be caused by the higher ratio of autochthonous SOC to microbial biomass C, facilitating the access of autochthonous organic matter.

The positive effects of amaranth addition on ergosterol generally remain until the end, although the contribution of fungi to the microbial biomass decreased. The positive amaranth effects on fungal biomass were more pronounced in the Luvisol subsoil, where Struecker and Joergensen (2015) found an especially strong C and N limitation in different soil samples from the same sites, using community-level physiological profiling with the multi-substrate induced respiration approach. This supports the view of others that the weaker fungal dominance in subsoil is caused by limited fresh substrate in subsoil (Fierer et al., 2003; Sradnick et al., 2012; Struecker and Joergensen, 2015).

Elevated CO_2, which is combined with reduced O_2 concentrations, generally had no effects on microbial biomass C without amaranth addition and only minor effects with amaranth addition, i.e. a reduced incorporation of amaranth-derived C into microbial biomass C. These absence of gas effects might be explained by the fact that there were no anoxic conditions in the incubation jars, as there were even in the extreme treatment always more than 10 Vol% oxygen present (Salome et al., 2010). This seems to be sufficient for most microorganisms. The minor effects on substrate incorporation in the amended samples might be explained by the negative effects of elevated CO_2 concentrations on saprotrophic fungi (Ekschmitt et al., 2008; Hayden et al., 2012), which suffer from O_2 limited conditions, as for example laccases, which need oxygen for substrate degradation

(Ekschmitt et al., 2008). This view is in line with the reduced ergosterol contents in the topsoil at elevated CO_2 concentrations. In the subsoil, the extremely low and highly variable ergosterol contents reduced the significance of treatment effects and made it impossible to detect significant differences between the sites, especially in the absence of amaranth. However, the values for ergosterol were significantly better explained using mixed models, that considered the gas treatments than in models without them, leading to weakly significant interactions between site, substrate, and gas treatment in topsoil. One possible explanation might be the fixation of CO_2 by bacteria, which was observed by Miltner et al. (2005). Another explanation could be the production of HCO_3^- lowering the pH of the sample, which may increase the SOC availability to the subsoil microbial community.

3.5. Conclusion

Elevated CO_2 concentrations at 4 or 8% did not affect the mineralization of SOC in the topsoils, but not in the subsoils either. Amaranth-derived C losses were generally stronger in the Colluvic Cambisol, especially at elevated CO_2 concentrations, than in the Haplic Luvisol, without clear differences between topsoils and subsoils. Hence hypotheses 1 and 3 have to be rejected. Amaranth addition caused negative priming effects on SOC mineralization only in the subsoils. Elevated CO_2 concentrations had no general effects on microbial biomass and ergosterol with and without amaranth addition. But the contribution of amaranth-derived microbial biomass C to total microbial biomass C was significantly reduced at elevated CO_2 concentrations, indicating a reduced substrate use efficiency, which confirms hypothesis 2 to some extent. Although this result need further confirmation, probably at even higher CO_2 than 8%, doubts were confirmed that the gas composition has strong effects on C mineralization as long as oxygen is present.

4.   Maize root decomposition in subsoil horizons of two silt loams differing in soil organic C accumulation due to colluvial processes

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4. Maize root decomposition in subsoil horizons

ABSTRACT

To analyse mechanisms controlling sequestration of organic C in subsoil, a field experiment was carried out for two years. Mesh bags containing original soil material and maize root residues (C4 plant) were buried in three different depths (35 cm, 45 cm, and 65 cm) of two neighbouring arable sites and were sampled after 12, 18 and 24 months. The sites were a Colluvic Cambisol with high soil organic carbon (SOC) contents in the subsoil, the other a Haplic Luvisol with low SOC contents below 30 cm depth. We determined the effects of the site, depths, and time on bulk SOC, organic C associated with soil density fractions, and microbial biomass C (MBC) in the soilbags. The MBC increased to a similar extent (2.5 fold) from the initial value to its maximum. This increase relied largely on the added maize root residues, as about 50% of the MBC was maize-derived after two years. However, we detected distinct differences in the substrate use for anabolism compared to catabolism, which decreased with depth and was lower in the Haplic Luvisol than in the Colluvic Cambisol. Freshly added plant material seems to be highly accessible to microorganisms in subsoil, but its metabolic use was determined by the soil properties of the two sites. The addition of plant residues also had an impact on aggregation dynamics resulting in an almost complete replacement of formally aggregate occluded material (i.e., occluded light fraction) by maize derived material after 24 months.

Keywords: C turnover, decomposition, density fractionation, field experiment, microbial biomass, subsoil

4. Maize root decomposition in subsoil horizons

1. Introduction

Two-thirds of the terrestrial C is stored in soils, which makes soil an important reservoir in the global carbon cycle (Batjes, 1996). This reservoir can either be a source or sink for CO_2 (Rumpel et al., 2002; Rumpel and Kögel-Knabner, 2011), which is of increasing interest due to climate change concerns (Bailey et al., 2002). The fact that more than 50% of soil organic C (SOC) is stored at a depth of 30-100 cm (Batjes, 1996; Lal and Kimble, 1997) has directed particular scientific attention towards subsoil. However, there is still limited knowledge regarding the mechanisms that control C sequestration and turnover in subsoil (Sanaullah et al., 2011; Cotrufo et al., 2013).

The high ^{14}C-based mean age found for organic compounds in subsoil (Rumpel et al., 2002; Rumpel and Kögel-Knabner, 2011) leads to the assumption that their mineralization rate is slower than in topsoil. There are strong indications that environmental conditions in subsoil are different from those in topsoil, such as less variation in temperature and reduced nutrient availability (von Lützow et al., 2006), which might lead to reduced substrate mineralization. These environmental factors also have an influence on the microbial community in subsoil, which usually shows smaller biomass and is less fungal dominated compared to topsoil (Fierer at al., 2003, Struecker and Joergensen, 2015). These changes in the microbial community and its functional diversity are presumably among the various factors controlling C sequestration in subsoil.

Another important factor is the limited input of fresh organic matter into subsoil (Fontaine et al., 2007). Only root-derived plant residues play a significant role in subsoils (Rumpel et al., 2002), which contain less labile and therefore more easily degradable compounds than shoot-derived residues (Rasse et al., 2005). Other C inputs in subsoil originate from soluble substrates (dissolved organic C) percolating through the soil profile (von Lützow et al., 2006; Rumpel and Kögel-Knabner, 2011).

4. Maize root decomposition in subsoil horizons

In terms of potential future land use changes towards deeper rooting plants to accumulate organic C in subsoil, the fate of the added plant substrate has to be clarified. Concerning this matter, it is largely unknown to what extent the added substrate is stabilized against microbial decomposition or contributes to the mineralization of stabilized SOC due to priming effects (Fontaine et al., 2007). The latter would be an adverse effect to the desired increase in SOC sequestration. The effects of substrate addition to subsoil have been investigated mostly in laboratory experiments (Kuzyakov, 2010) where not only substrate was added but also other environmental factors (e.g., gas conditions, temperature, moisture) were modified. This impedes the transfer of the results from such experiments to the field or ecosystem scale and underscores the need for more field experiments to analyse the decomposition of plant residues under subsoil conditions. One option is the burial of soilbags in the field (Sanaullah et al., 2011) that mimic hot spots of microbial activity (Schrumpf et al., 2013), being similar to naturally occurring hot spots along preferential flow pathways or rooting zones (Chabbi et al., 2009).

Degradation and fate of the maize roots can be analysed by their incorporation into the microbial biomass but also by density fractionation, providing additional information on the stabilization of SOC (Schrumpf et al., 2013). The free light fraction (flF) and occluded light fraction (olF) consist mainly of organic debris present either free and easily available for microorganisms in the soil matrix (flF) or occluded within aggregates (olF) (Golchin et al., 1994; Cerli et al., 2012). In contrast to the flF, the olF consists of smaller and slightly decomposed organic particles, which are better protected against microbial degradation by occlusion in aggregates. Therefore, the turnover times of olF are considered to be longer than those of the flF (Schrumpf et al., 2013). The C in the heavy fraction (HF) is bound to the mineral fraction of the soil matrix (e.g. John et al., 2005) and, therefore, is considered to be stabilized against decomposition with turnover times of decades to centuries (Schrumpf et al., 2013).

4. Maize root decomposition in subsoil horizons

The aim of this study was to test the following hypotheses regarding the fate and pathways of freshly added residues from maize roots as a function of soil depth and resource availability under field conditions: (1a) The mean residence times of maize root residues increase with depth and (1b) are lower in the Colluvic Cambisol than in the Haplic Luvisol, due to the higher microbial biomass in the former. (2a) Substrate incorporation into microbial biomass decreases with depth and (2b) is higher in the Colluvic Cambisol than in the Haplic Luvisol, due to higher resource limitations in the Luvisol (Struecker and Joergensen, 2015). (3) The maize residues will remain mostly in the flF, due to limited microbial degradation.

2. Material and methods

2.1. Site

Soil was sampled from two arable fields at the Hessian State Manor of Frankenhausen, northern Hessia, Germany (51°24' N; 9°25' E), the experimental farm of the University of Kassel. The area is characterized by a mean annual air temperature of 9.3 °C and a mean annual precipitation of 687 mm. The soils of site I (referred to as Cambisol or Cam) can be characterized as a Colluvic Cambisol according to the WRB (FAO, 2014). The Colluvic horizon of the Colluvic Cambisol covers the original soil surface of a Chernozem by about 70 cm, resulting in an Ap / M / fAh sequence. The soils of site II (referred to as Luvisol or Luv) can be classified as a Haplic Luvisol according to the WRB (FAO, 2014), although the Al horizon was eroded. This results in an Ap / Bt sequence. The soils of the two sites have been developed on loess and are within a distance of 400 m from each other, which means that climatic conditions are equivalent on both sites, although they have different SOC profiles due to erosion and deposition. Land use at both sites was also similar for at least 400 years, during which time both sites were used as grassland

first and then as cropland since the early 20th century (Troßbach, 2000). Soil characteristics are shown in Table 1.

2.2. Soilbag experiment

Samples from both sites were taken at three different subsoil depths (35, 45, and 65 cm) in April 2013. The autochthonous, naturally occurring SOC contents and isotopic signatures unaffected by the added maize roots were determined from these samples. Afterwards each sample was mixed with 1.5 weight-percent of dried and shredded maize root residues (1-2 mm), which equals a C addition of 4.2 mg g^{-1} and an N addition of 0.06 mg g^{-1}. The mixture was filled into 5 × 5 cm mesh bags (mesh size: 100 µm). This mesh size prevents losses from the bags but allows access by microorganisms. In June 2013, the bags were buried on their original field sites at their original depths with 9 field replicates. They were recovered after 12 (t1), 18 (t2), and 24 (t3) months with 3 field replicates per depth. Before burial the soil samples were sieved to allow homogeneous mixing with the maize roots. Afterwards they were stored field moist at 4°C until they were buried. The sampled soilbags were also stored field moist at 4°C. At the end of the experiment (t3) the soil surrounding the bags in a distance of 15 cm diameter was also sampled, to investigate the effects of the buried soil/root mixture on the characteristics of the soil material in close vicinity to the buried samples. These samples were sieved < 2 mm and also stored field-moist at 4°C.

2.3. Microbial biomass C

Soil microbial biomass C (MBC) was analysed by fumigation-extraction (Vance et al., 1987). Fumigated (24 h with ethanol-free CHCl$_3$ at 25 °C) and non-fumigated soil (10 g) was extracted with 40 ml of 0.05 M K$_2$SO$_4$ (Potthoff et al., 2003) by 30 min horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). Organic C in the

extracts was determined using a multi N/C 2100S automatic analyser (Analytik Jena AG, Jena, Germany). MBC was calculated as E_C/k_{EC}, with E_C = (organic C extracted from fumigated soil) - (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990).

For the determination of ^{13}C, 20 ml aliquots of 0.05 M K_2SO_4 extracts of fumigated and non-fumigated samples were freeze dried for about 3 days and were analysed by isotope ratio mass spectrometry (Delta V Advantage, Thermo Electron, Bremen, Germany interfaced to an elemental analyser Flash 2000, Thermo Fisher Scientific, Cambridge, UK).

2.4. Density fractionation

Density fractionation was conducted with control and soilbag samples using the method of John et al. (2005) as modified by Cerli et al. (2012), Griepentrog and Schmidt (2013), and Kaiser and Berhe (2014). We conducted a series of pre-tests to define the most suitable experimental setting with respect to the density cut offs used to separate the flF and the olF and the amount of ultrasonic energy applied to disperse aggregates before the olF was separated (Cerli et al., 2012). Soil of 10 g dry weight was dispersed in 50 ml of a 1.6 g cm^{-3} sodium polytungstate (SPT) solution. After one h, the sample was centrifuged at 4000 g for 30 min. After another 30 min, the free light fraction (flF) was decanted on a filter and washed with 1.5 L of distilled water. The solid residue was mixed with 50 ml of a 1.6 g cm^{-3} SPT again and dispersed with an ultrasonic probe at 300 J cm^{-3}. After one h, the sample was centrifuged again at 4000 g for 30 min. After another 30 min for stabilization, the occluded light fraction (olF) was decanted on a filter and rinsed with 1.5 L of distilled water. The residual heavy fraction (HF) was also washed with distilled water until the conductivity of the washing water was below 50 μS cm^{-1}.

In the three density fractions as in the bulk soil samples, total C and N as well as $\delta^{13}C$ were measured using isotopic ratio mass spectrometry (Delta Plus, Finnigan MAT, Bremen Germany)

with interface (Conflo III, Finnigan MAT, Bremen, Germany) and elemental analyser (NA1110, CE-Instruments, Rodano Milan, Italy). As the samples did not contain any carbonate, total C equals soil organic C (SOC).

2.5. Calculations and statistics

The amount of maize-derived C in the microbial biomass ($^{13}C_{MB}$) was calculated by the following equation (Potthoff et al., 2003):

$$^{13}C_{MB}\ (\%) = \frac{(^{13}C\text{-}Atm.\,excess_{\text{fum}} \times C_{\text{fum}}) - (^{13}C\text{-}Atm.\,excess_{\text{nfum}} \times C_{\text{nfum}})}{(C_{\text{fum}} - C_{\text{nfum}})} \times 100$$

where C_{fum} and C_{nfum} represent the mass of C (mg g^{-1}) extracted from the fumigated and non-fumigated soil, respectively, and ^{13}C-Atm.excess$_{\text{fum}}$ and ^{13}C-Atm.excess$_{\text{nfum}}$ represent the corresponding ^{13}C Atom % excess values calculated from the maize amended and control treatments. The fraction of maize-derived C ($f_{\text{maize-C}}$) was calculated for each individual replicate of all treatments and the fractions derived from the density fractionation from the isotope data according to a two pool-mixing model with the following equation:

$$f_{\text{maize-C}} = \frac{\delta^{13}C_{\text{sample}} - \delta^{13}C_{\text{control}}}{\delta^{13}C_{\text{maize}} - \delta^{13}C_{\text{control}}}$$

where $\delta^{13}C_{\text{sample}}$ represents the $\delta^{13}C$ value of SOC, MBC and the density fractions (fIF, oIF, HF); $\delta^{13}C_{\text{control}}$ is the average $\delta^{13}C$ value of the non-amended control samples and $\delta^{13}C_{\text{maize}}$ is the $\delta^{13}C$ of the maize residues. In accordance with Collins et al. (2000), Ehleringer et al. (2000) and Ekblad et al. (2002), we assumed that no isotopic fractionation occurred during the experimental period, as the added substrate did not show an enrichment in heavy isotopes when comparing the start and end of the experiment.

4. Maize root decomposition in subsoil horizons

Assuming a first order decay kinetic (Balesdent and Mariotti, 1996), the degradation can be described by the following equation:

$$SO^{13}C_{ti} = SO^{13}C_{t0} \times e^{-kt} \text{ or } -k = ln\ (SO^{13}C_{ti} / SO^{13}Ct0) / t$$

where $SO^{13}C$ equals the maize-derived C content at the sampling date ti, $SO^{13}C_{t0}$ the maize-derived C content before burial, k the decay constant, and t the time between burial and sampling date. Then, mean residence time (MRT) of the maize root residues is 1/k.

The data were tested for homogeneity of variances, using the Levene test and for normal distribution of residues, using the Shapiro-Wilk test, accompanied by a graphical assessment of histograms and qq-plots. We used Student's t-test for pairwise comparisons, because the data for SOM fractions and density fractions were normally distributed. The test for significance of the main effects site, depth, and time as well as their interactions on different measures was conducted with an analysis of variance, where time and depth were considered as repeated measures and site as independent factor, applying a mixed effects model approach using *ezANOVA*. To account for sphericity of the data, Mauchly's test for sphericity was applied and followed by a Greenhouse-Geisser correction. All statistical analyses were performed by R (R Development Core Team, 2010).

3. Results

3.1. SOC and total N

The addition of 1.5% maize root residues contributed 30-40% to SOC in the soilbags (Table 4.1). As maize root residues have a wider C/N ratio (64) than autochthonous SOC (10), N did not strongly increase after substrate addition.

Table 4.1: Contents of soil organic carbon (SOC), total nitrogen (N) and microbial biomass C (MBC) as well as the SOC/total N ratio without roots; contents of maize-derived SOC (SOC$_m$) and total N, with maize roots for both sites and all depths at the beginning of the experiment.

Depth (cm)	SOC - roots (mg g^{-1} soil)	SOC$_m$ + roots	Total N - roots (mg g^{-1} soil)	Total N + roots	SOC/total N - roots	MBC - roots (µg g^{-1} soil)
Cambisol						
35	14.4	5.9	1.5	1.6	9.6	216
45	10.9	7.6	1.1	1.2	10.0	133
65	9.0	3.3	0.9	1.0	9.7	83
Luvisol						
35	10.8	6.2	1.2	1.3	9.4	161
45	6.9	5.9	0.65	0.72	10.5	49
65	5.4	3.9	0.52	0.57	10.3	39

The content of maize-derived C significantly decreased during the experiment, without site or depth effects (Table 4.2). After 12 months, maize-derived SOC in the bulk sample was generally less than 50% of the initial value (Fig. 4.1a), which equals an average mean residence time (MRT) of 355 days for maize root residues for both sites and all depths. After 24 months, maize-derived SOC in the bulk sample was always mostly degraded, resulting in an even lower estimated MRT of 291 days. Autochthonous SOC inside the soilbags increased over 24 months, without showing significant site × depth, site × time or time × depth interactions (Table 4.2, Fig. 4.1a). Total N showed a slight decrease at 35cm, but remained stable at all other depths (Fig. 4.1b).

4. Maize root decomposition in subsoil horizons

Table 4.2: Main effects of site, depth, and sampling time on autochthonous soil organic C (SOC_a), maize-derived SOC (SOC_m), autochthonous microbial biomass C (MBC_a), maize-derived MBC (MBC_m), and total N. The factors time and depth were considered as repeated measures. The values given for the factor steps of the main effects are the mean value of all results obtained from this factor step irrespective of the other main effects and factor steps.

Main effects	SOC_a	SOC_m	MBC_a	MBC_m	Total N
	(mg g^{-1} soil)		(µg g^{-1} soil)		(mg g^{-1} soil)
Cambisol	17.4	1.15	156	116	1.16
Luvisol	13.6	1.13	105	81	0.80
35 cm	19.0	1.02	214	132	1.29
45 cm	15.0	1.19	91	83	0.90
65 cm	12.5	1.22	85	80	0.75
12 months	13.3	1.61	176	91	0.97
18 months	14.5	1.42	95	82	0.97
24 months	18.6	0.40	121	123	0.98
Site	0.03	n.s.	0.04	0.01	<0.01
Depth	0.02*	n.s.	0.01*	<0.01*	<0.01*
Time	<0.01*	<0.01*	<0.01*	0.05*	n.s.
Site × depth	n.s.	n.s.	n.s.	n.s.	0.04*
Site × time	n.s.	0.08*	0.04*	0.07	n.s.
Time × depth	n.s.	n.s.	0.04*	0.10	n.s.
Site × depth × time	n.s.	n.s.	0.08*	n.s.	n.s.
CV (± %)	34	71	72	54	30

* p-values after Greenhouse-Geisser correction

CV = mean coefficient of variation between replicate samples within site and depth (n = 3)
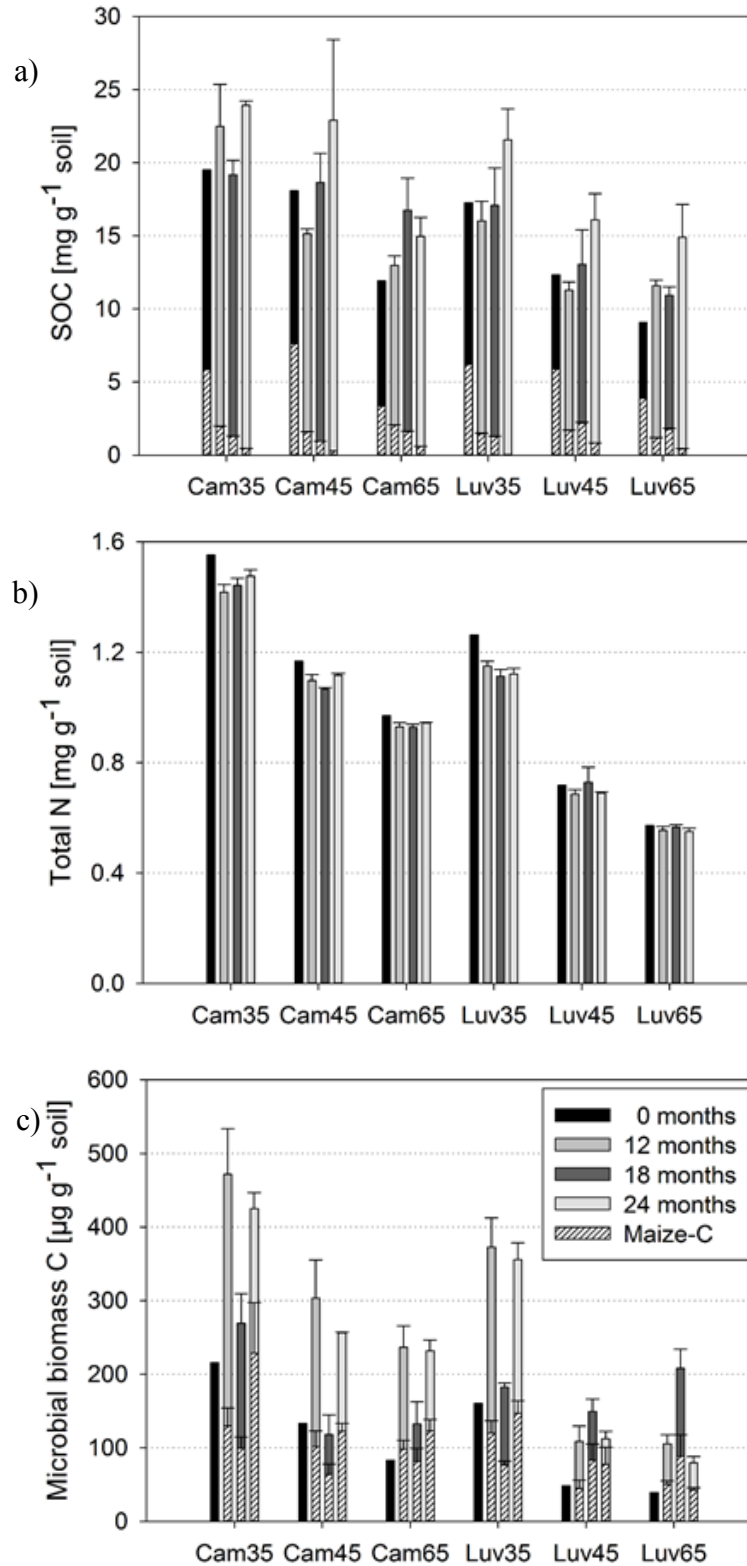
Figure 4.1: Contents of the autochthonous a) soil organic carbon (SOC), b) total nitrogen (N), and c) microbial biomass C (MBC) and the proportions of the maize-derived organic C for SOC (a) and MBC (c) at the four sampling dates for the three soil depths of the two sites (soil depth: 35 cm (35), 45 cm (45), 65 cm (65); sites: Cambisol (Cam) and Luvisol (Luv). Error bars show one standard errors from the three field replicates.

3.2. Density fractions

Initially, all soil samples (without residue addition) contained no or negligible amounts of the free light fraction (flF). Hence, almost all flF recovered after root residue addition was maize-derived (Table 4.3). Before substrate addition, the Cambisol samples contained less occluded light fraction (olF) at 35 cm, than at 45 and 65 cm, while the Luvisol contained generally less olF, but with a contrasting distribution (Table 4.3). Regarding the contribution of each fraction to SOC, the HF contained by far the largest C amounts, despite the low C contents (0.3-1.0%) compared with flF or olF (35-45%), even after substrate addition (Fig. 4.2). HF-C increased almost always over the 24 months, accompanied by a decrease in flF-C, whereas the proportion olF-C remained within a similar range throughout the experiment.

Table 4.3: Amounts of free light fraction (flF), occluded light fraction (olF), and heavy fraction (HF) with and without maize roots at the beginning of the experiment.

Depth	flF		olF	HF
	- roots	+ roots	- roots	- roots
(cm)	$(mg\ g^{-1}\ soil)$		$(mg\ g^{-1}\ soil)$	$(mg\ g^{-1}\ soil)$
Cambisol				
35	0.1	13.9	6.5	979
45	0.0	12.3	11.0	984
65	0.0	12.4	10.0	983
Luvisol				
35	0.2	12.6	4.5	981
45	0.1	14.1	2.0	984
65	0.0	9.9	2.0	987
CV (± %)	123	12	64	0

CV = mean coefficient of variation between replicate samples within site and depth (n = 3)
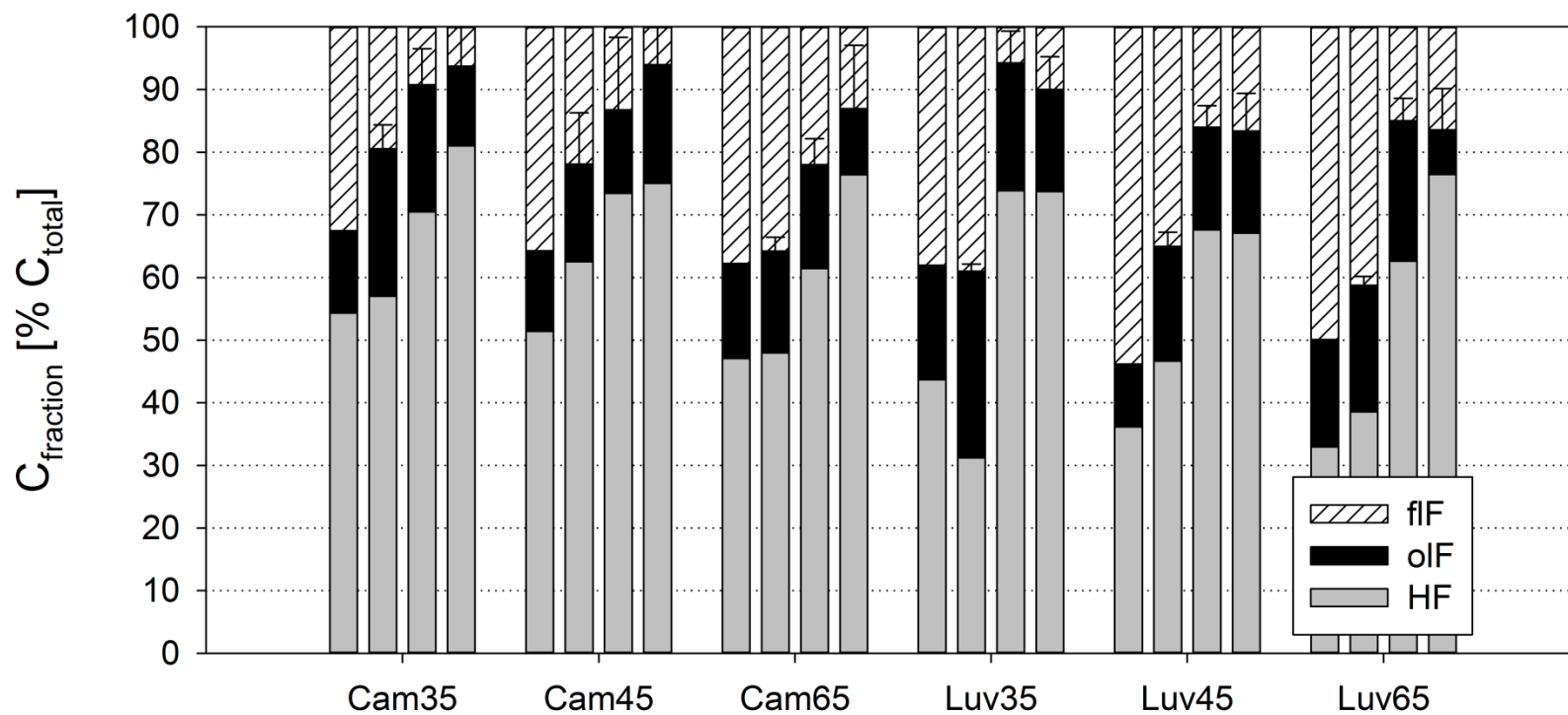
Figure 4.2: Contributions of organic C associated with the three density fractions to the bulk soil organic C after 0, 12, 18, and 24 months (bars per group from left to right) for the three soil depths of the two sites (soil depth: 35 cm (35), 45 cm (45), 65 cm (65); sites: Cambisol (Cam) and Luvisol (Luv)). Error bars show one standard errors from the three field replicates.

4. Maize root decomposition in subsoil horizons

The C/N ratio of the maize root residues (after mixing: maize root residues = flF) strongly increased from 64 to 80-115 after mixing the maize root residues into the soil (Fig. 4.3a). After 12 months, the C/N ratio significantly ($p < 0.01$) decreased again below the initial values of the maize root residues (Fig. 4.3a). The olF C/N ratio decreased within the first 12 months, but showed a significant ($p < 0.01$) increase within the next 12 months, exceeding the starting values (Fig. 4.3b). The HF C/N ratio remained roughly stable despite some variation (Fig. 4.3c).

In the Cambisol, the recovered maize root residues did not decrease within the first 12 months, followed by a decrease to similar values at all depths, despite some depth-specific temporal variations (Fig. 4.4a). In the Luvisol, the recovered maize root residues already decreased within the first 12 months, followed by a further decrease in the next six months to stable values until the end (Fig. 4.4a). In the Cambisol, the proportion of maize-derived olF-C continuously increased until 18 months (5-15%), followed by an even stronger increase (50-65%) until the end, so that 70-90% of olF-C were maize-derived (Fig. 4.4b). In the Luvisol, there was no continuous increase until 18 months at 35 and 45 cm depth, but also a strong increase (60%) from 18 to 24 months. At 65 cm depth, the Luvisol samples showed the same development as the Cambisol samples, so more than 95% of olF-C was maize-derived after 24 months (Fig. 4.4b). The proportion of maize-derived C in HF-C did not show a clear pattern (Fig.4.4c).

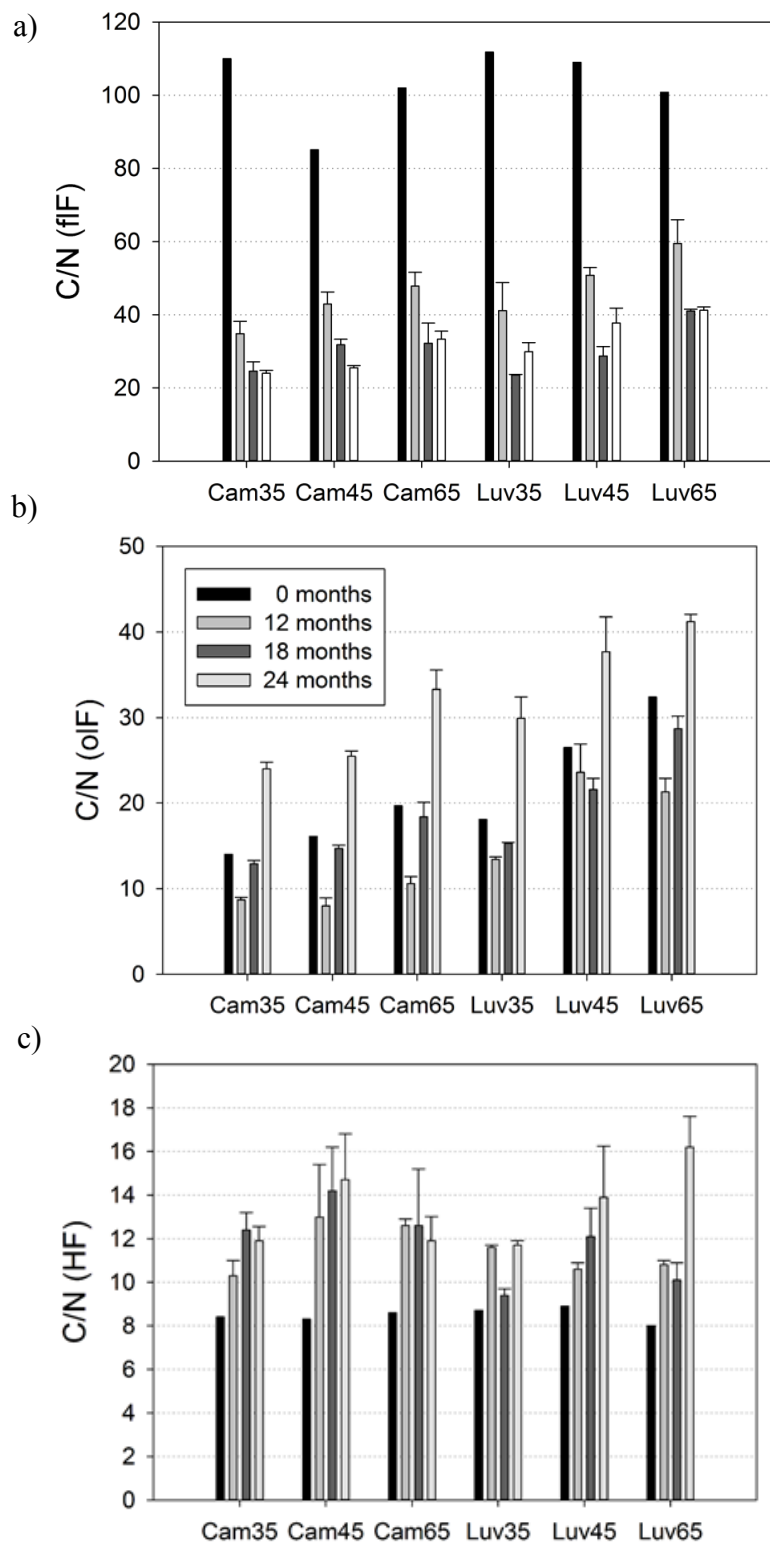# 4. Maize root decomposition in subsoil horizons

Figure 4.3: C to N ratios of the different density fractions during the experiment. a) free light fraction (flF), b) occluded light fraction (olF), c) heavy fraction (HF) for the three soil depths of the two sites (soil depth: 35 cm (35), 45 cm (45), 65 cm (65); sites: Cambisol (Cam) and Luvisol (Luv). Error bars show one standard errors from the three field replicates.

4. Maize root decomposition in subsoil horizons
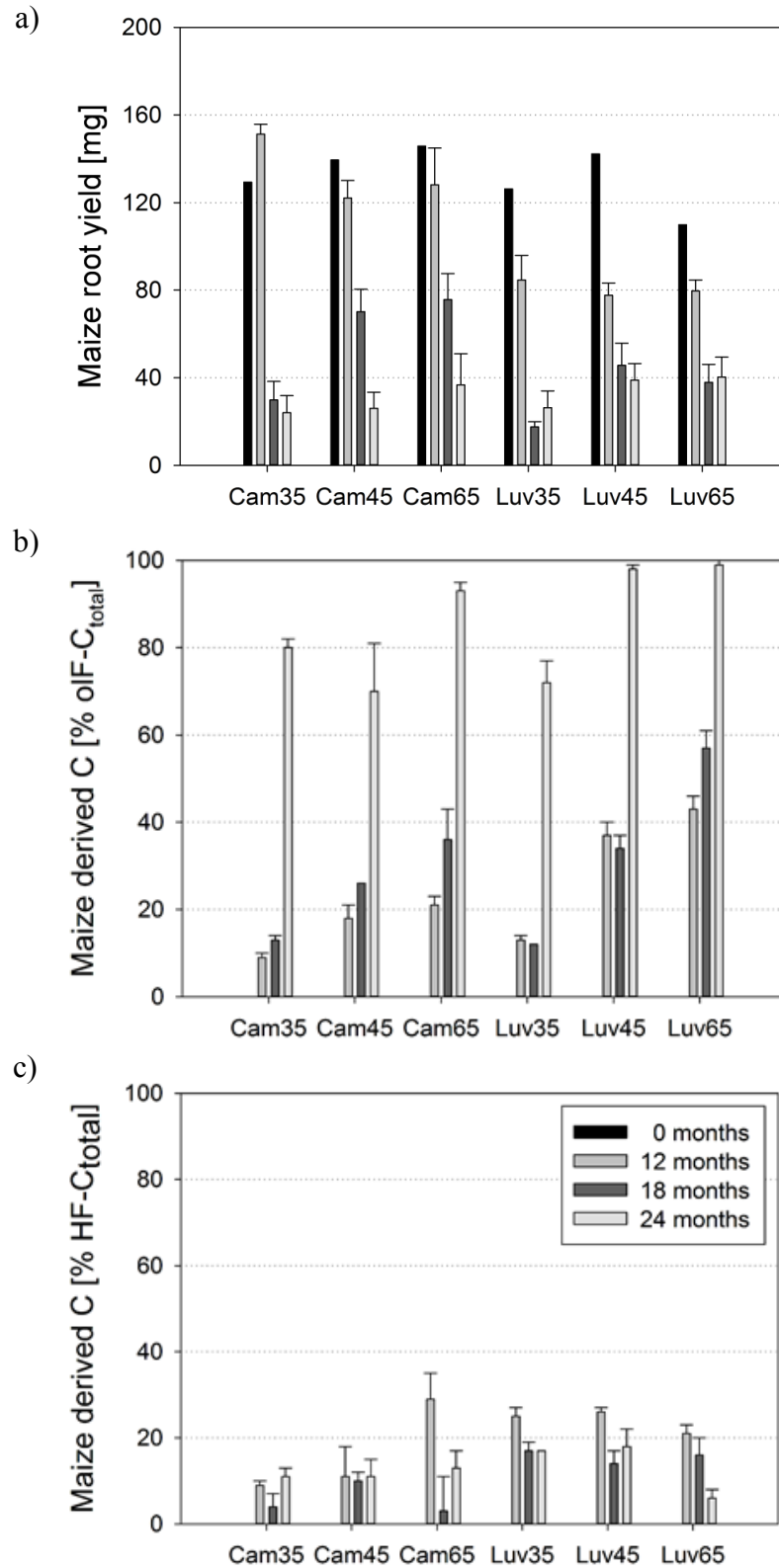

Figure 4.4: a) Amount of maize roots recovered in free light fraction (flF), b) maize-derived C in the occluded light fraction (olF), c) maize-derived C in the heavy fraction (HF) for the three soil depths of the two sites (soil depth: 35 cm (35), 45 cm (45), 65 cm (65); sites: Cambisol (Cam) and Luvisol (Luv). Error bars show one standard errors from the three field replicates.

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3.3. Microbial biomass C

Total MBC increased within the first 12 months in all samples (Fig. 4.1c). All Cambisol samples and the 35 cm depth of the Luvisol (Luv35) showed the lowest content of MBC after burial in November 2014, while Luv45 and Luv65 reached their maximum after burial at the same time. In all samples, MBC was higher after 24 months than the initial values, but decreased compared to June 2014. This results in an average turnover time of 624 days for MBC, with the faster turnover in the Cambisol samples (Cam35 = 418 d; Cam45 = 445 d; Cam65 = 582 d) than in the Luvisol samples (Luv35 = 694 d; Luv45 = 796 d; Luv65 = 808 d). However, both sites show a depth gradient with lower turnover times at lower depths.

Maize-derived MBC increased from June 2013 to June 2014 in all samples and the proportion of maize-derived MBC compared with autochthonous MBC was even higher in November 2014 and June 2015 than in June 2014 (Fig. 4.1c). After 24 months, the neighbouring soil contained 11 to 50 $\mu g\ g^{-1}$ soil maize-derived MBC, with tendencies to be higher in the Cambisol and some decline with depth (Table 4.4).

Table 4.4: Contents of maize-derived microbial biomass C (MBC_m) in soil surrounding the soil-bags after 2 years of exposure.

Depth (cm)	MBC_m ($\mu g\ g^{-1}$ soil)
Cambisol	
35	50
45	43
65	18
Luvisol	
35	16
45	16
65	11
CV (± %)	24

CV = mean coefficient of variation between replicate samples within site and depth (n = 3)

4. Discussion

4.1. SOC and total N

The highly variable increase in SOC in the soilbags was caused by an increase in autochthonous SOC. We observed large amounts of fresh roots growing directly around the bags, which were presumably accompanied by the growth of hyphae from arbuscular mycorrhizal fungi into the bags. The enhanced root growth was probably supported by the large pores, which were inevitably created during burial of the soilbags and the increased nutrient availability due to higher microbial activity.

The decrease in maize derived C was highly variable within the replicates of the different sample types suggesting a high spatial heterogeneity for microbial decomposition in subsoil (Jörgensen et al., 2002; Chabbi et al., 2009). However, the means for the three replicates were similar for all sample types with mean residence times of about 300 days for all three depths at both sites. This suggests that the decomposition kinetics of plant residues depend rather on very distinct conditions than on more general factors like depth or autochthonous SOC contents.

The effect of the soilbags on the surrounding soil after 24 months differed between the 35 cm and the deeper layers. While the SOC contents decreased at 35 cm depth, those at 45 and 65 cm depth increased. The increase in the deeper layers suggests that substrate addition did not cause enhanced SOC losses in these layers. Hence, there is no evidence of a positive priming effect in the deeper subsoil layers. The decrease in SOC at 35 cm depth was probably supported by enhanced additional substrate input through rhizodeposition. As root exudates contain soluble and easily degradable substrate, they might have provided energy for decomposition of older SOC. Therefore it appears to be crucial to differentiate between different subsoil conditions (e.g., root abundance or resource availability) when assessing the impact of treatments (e.g., substrate addition).

A slight decrease in total N was only visible at 35 cm depth at both sites. This decrease was probably caused by the competition for N between microorganisms and plants (Geisseler et al., 2010) in this still densely rooted subsoil horizon (Jackson et al., 1996). Therefore, N was presumably largely taken up by the actual vegetation, (Geisseler et al., 2010). In the deeper layers, there were fewer actual roots, that could take up N from the soil. Hence, no decrease in total N was detected.

4.2. Density fractions

The strong increase in the C/N ratio in the flF between mixing of soil and maize root residues and the analysis for t_0 (7 days) in comparison with the fresh maize root residues indicates that the microbial community takes up large contents of N from the plant material immediately, as the flF consists to more than 90% of maize root residues. This increase in the C/N ratio is typical for the initial decomposition of N-rich substrate in N-limited environments (Ågren et al., 2013). Afterwards, the C/N ratio in the flF decreases continuously, following the increase in total N (from 0.40 % to 1.17 %) in the flF within the first 18 months of burial, which is typical for the N accumulation in decomposing litter (Moore et al., 2006). Within the last 6 months of burial, C/N ratios remained constant or showed a slight increase again, suggesting that a critical C/N ratio was reached, which had been followed by mobilization of N (Ågren et al., 2013). The decrease of the C/N ratio was caused by the ongoing microbial degradation of the plant material, but the values in the flF remain well above SOM values, indicating the low degradation state of flF material (Moore et al., 2006; Sanaullah et al., 2011).

In contrast, the C/N ratio of the olF decreased within the first 12 months of burial. Due to the addition of easily available plant material, the microbial community might have been enabled to access this in aggregates occluded material via excretion of exo-enzymes, causing further

degradation of the organic material (Kuzyakov et al., 2010). The later increase in the C/N ratio of the olF was probably caused by the occlusion of less degraded maize derived compounds. This is supported by the extreme shift from autochthonous C to maize-derived C between 18 and 24 months of burial, which suggests that 75-95% of the olF were maize-derived after 24 months. These findings indicate that freshly added plant residues replace the formally occluded material rather quickly in subsoil. They also show that aggregation becomes a highly dynamic process after the addition of plant residues to subsoil. These stimulated aggregation processes due to higher contents of fresh plant residues have already been observed for topsoils (Wang et al., 2013). This enhanced dynamic in aggregation processes causes the exposure of material to degradation that was protected before. On the one hand, the protection of the added plant residues from degradation is beneficial in terms of reduced C mineralization, but on the other hand the fate of the formally stabilized and now exposed material is unknown. Therefore, it remains difficult to assess the overall effects of plant residue additions in terms of enhanced C sequestration. However, the increase of SOC in the soilbags provides some evidence that the addition of maize roots has no adverse effects regarding C storage.

The increase in the C/N ratio of the HF shows a high variability. However, the proportion of maize-derived C in the HF remains relatively small and does not explain the whole increase in HF-C. Therefore, the increase in HF-C relies to a certain extent on autochthonous SOC, which would suggest an enhanced autochthonous SOC stabilization in the HF during the experiment. Diochon et al. (2016) found an enhanced stabilization of microbial decomposition by-products in the mineral fraction during an incubation experiment with plant residues. The increase in MBC, the apparent decomposition of maize residues by the microorganisms, the mixture of maize-derived and autochthonous SOC in the HF, as well as the smaller C/N ratios in the HF provide some evidence that the stabilized C in the HF in our experiment is also of microbial origin. Presumably

the organic matter formerly protected in aggregates as olF, which was replaced by maize-derived components, was further metabolically processed and thereby partially stabilized in the HF (Cotrufo et al., 2013).

Before substrate addition, Luv35 contained more olF than Luv45 and Luv65. As the total C contents in the Luvisol also decrease with depth, the decrease in olF follows this pattern. The Cambisol samples show a contrasting pattern, with Cam35 containing less olF than Cam45 and Cam65. As the olF consists mainly of small, slightly degraded plant material particles (Wagai et al., 2009; Schrumpf et al., 2013), which are occluded in aggregates, this pattern is also reflected in a decreasing proportion of SOC metabolically processed by microorganisms in total SOC (Struecker and Joergensen 2015). The reason for this depth distribution in the Cambisol is probably the ongoing deposition of eroded material containing weakly degraded plant material (Berhe et al., 2008; Berhe, 2012). This organic matter from the eroded material becomes occluded in aggregates after deposition (Wang et al. 2014) and is therefore protected from further degradation as long as disturbance is kept to a minimum (Berhe et al., 2012).

4.3. Microbial biomass C

The increasing proportion of maize-derived MBC proves that the microbial community utilizes the added maize root residues for biomass formation, with the amount of maize-derived C which is incorporated into microbial biomass being higher in the Cambisol and declining with depth. However, as only a small proportion of the maize-derived C which was lost from the soilbags was incorporated, the largest amount was presumably respired as described by Rubino et al. (2012) for laboratory incubations. But material of the maize root residues added were also lost from the soilbags due to leaching of soluble compounds (Murphy et al., 2000; Chantigny, 2003) or hyphal transfer via soil fungi (Poll et al., 2008; Rottmann et al., 2010), as maize-derived C was detected

in the microbial biomass of the surrounding soil at both sites and all depths. Luv45 and Luv65 differed from all other sampling points in terms of MBC development, reaching the highest contents after 18 months. These two sampling points also differ strongly from the other sampling sites in their autochthonous C and N contents, which are lower than at all other sites and they also had the smallest initial amounts of MBC, which indicates that microbial growth might be resource limited (Fierer et al., 2003; Struecker and Joergensen, 2015). Hence the substrate was presumably used for catabolism first to cover energy demands. Afterwards, the proportion of anabolism increased.

The increase in autochthonous MBC after 24 months provides some evidence that additional autochthonous SOC could be used by soil microorganisms, which was not accessible before due to resource limitations. The decrease in maize-derived C and the increase in autochthonous SOC support the view that soil microorganisms preferably degrade fresh substrate compared with autochthonous SOC. The latter is presumably energetically less attractive or no contains labile compounds (Salome et al. 2010; Rumpel and Kögel-Knabner 2011; Dungait et al. 2012; Cotrufo et al. 2013). The fact that at those sampling points, containing higher amounts of autochthonous C, no more than 60% of MBC were maize-derived at any sampling date, indicates a co-use of this C for anabolism, while the strong initial losses of maize-derived C indicate a preferential use of substrate-derived C for catabolism.

5. Conclusions

Maize root residues were similarly decomposed in the soilbags added at all depths and both sites. Hence, the initial differences in MBC, which remain visible throughout the experiment, had no effect on the loss rates. However, these differences became apparent when looking at substrate incorporation, which was lower in soil samples with lower MBC contents, i.e. in the Luvisol in

comparison with the Cambisol and at 45 and 65 cm depth in comparison with 35 cm depth. This means that the maize root residues were catabolised to a larger extent at sampling points with smaller autochthonous resource stocks, compared to those with larger autochthonous resource stocks. The accessibility of the maize root residues for soil microorganisms was also reflected by the decreasing C/N ratio of the material that remained in the flF, indicating an enhanced degradation of the added maize residues. The addition of maize roots also had an impact on material that was formally protected within aggregates as this material was almost completely replaced by maize derived material. But, despite this release of protected material and the energy input through of fresh plant material there was no evidence to suggest enhanced microbial decomposition of autochthonous SOC within and outside the soilbags in the subsoil. Therefore, the addition of relatively complex substrates, e.g. plant residues, seems not to enhance CO_2 emissions from the studied subsoils caused by a positive priming effect.

Acknowledgements

5.   General conclusions

The research presented above was able to provide some novel insights regarding the factors and mechanisms controlling the turnover of autochthonous and substrate derived C in subsoils of arable land. The choice of sites, one profile, where SOC contents show a strong depth gradient (0-85 cm), and another profile, which has similar SOC contents throughout the entire profile, provided additional insights to which extent these SOC contents, which developed naturally over the past 800 years, due to erosion and deposition of surface soil material, govern C turnover due to differences in C availability.

(1) Resource availability is an important factor for controlling the size of the soil microbial community as well as the ratio of fungi to bacteria. Our results indicate that C limitation is only the major controlling factor in terms of microbial community size in subsoils with low SOC stocks, while other factors limit microbial growth in subsoils with high C stocks. The decrease in fungal dominance with depth may also be explained by resource availability, as fungi suffer from a lack of fresh plant input in both soil types. The C and N stocks also control functional diversity of the microbial community. The MSIR method differentiates precisely between topsoil and subsoil layers according to the differences in N demands and between subsoils according to SOC demands. Therefore, the selection of substrates in relation to their metabolism in microorganisms is crucial to retrieve maximum information on the microbial community composition and substrate requirements using the MSIR method. Hence, SOC and N availability are major factors controlling size, composition and substrate use of the microbial community.

(2) Elevated CO_2 concentrations at 4 or 8% did not affect the mineralization of autochthonous SOC in the topsoils, but also not in the subsoils. Amaranth-derived C losses were generally stronger in the Colluvic Cambisol, especially at elevated CO_2 concentrations, than in the Haplic Luvisol, without clear differences between topsoils and subsoils. Elevated CO_2

concentrations had no general effects on microbial biomass C and ergosterol with and without amaranth addition. But the contribution of amaranth-derived microbial biomass C to total microbial biomass C was significantly reduced at elevated CO_2 concentrations, indicating reduced substrate use efficiency. Hence doubts were confirmed that the gas composition has strong effects on C mineralization as long as any oxygen is present.

(3) Amaranth addition caused negative priming effects on SOC mineralization in the subsoils. In the amaranth-amended samples, the autochthonous SOC contents in subsoil declined less over the 6 months than in the control samples. This suggests a negative priming effect after the addition of a relatively complex substrate. The microbial community can probably retrieve energy and nutrients more easily from the plant residues than from autochthonous SOC.

(4) Maize root residues were similarly decomposed in the soilbags added at all depths and both sites. Hence, the initial differences in MBC, which remain visible throughout the experiment, had no effect on the loss rates. However, these differences became apparent looking at the substrate use efficiencies, which were lower in soil samples with lower MBC contents, i.e. in the Luvisol in comparison with the Cambisol and at 45 and 65 cm depth in comparison with 35 cm depth. This means that the maize root residues were catabolised to a larger extent.

Summarizing these findings we found strong evidence that resource availability from autochthonous SOM as well as from added plant residues have a strong influence on the microbial community and its use of different substrates. However, under all of the applied conditions there was no evidence that complex substrates, i.e. plant residues, are less degraded in subsoil than in topsoil, irrespective of the autochthonous resource stocks.

6.   Future research needs

Considering the conclusions we were able to draw from our experiments, some further research needs arose, which should be addressed either to confirm previous findings or to gain a better understanding of C sequestration in subsoil. First of all more sites, which differ in their natural SOC contents, but are rather similar in terms of other environmental conditions should be investigated to confirm the findings of our study. Ideally the samples should represent several levels of SOC stocks.

In order to finally out rule the gas conditions in subsoil as a major governing factor, experiments in an O_2-free atmosphere could be conducted. There are some assumptions that smaller pores contain so little O_2, due to reduced gas conductivity with smaller pore diameters, that it becomes critical for the microbial community (Berisso et al., 2012; Berisso et al., 2013). Regarding anoxic conditions in subsoil water filled pores or water films reducing the diffusion O_2 might also play a role in C turnover (Young and Ritz, 2000). Therefore it might provide some additional insight, if subsoil samples with and without substrate addition were incubated at different distinct water contents or water filled pore spaces. To maintain the integrity of the soil structure, which might be crucial in terms of pore sizes and aeration of the sample, more experiments using undisturbed soil cores, would be desirable.

As several studies regarding priming effects in subsoil provided contrasting results (Dalenberger & Jager, 1989; Fontaine et al., 2007; Guenet et al., 2012), this topic also needs some additional research. Therefore subsoil samples should be incubated with substrates of varying complexity and different amounts of these substrates. Furthermore several sampling dates during the time of the incubation could provide some insights regarding changes of the effect in relation to the remaining amount of substrate.

7. Supplementary material

Supplement 1: CO_2 respiration [$\mu l\ g^{-1}\ h^{-1}$] values for substrates used in the MSIR which provided good discrimination either between depths or between sites

	Amino acids				Sugars		Carboxylic acids			
Depth	Alanine	L-asparagine	L-serine	Arginine	Glucose	Arabinose	Citric acid	α-keto-glutaric acid	Malonic acid	L+tartaric acid
[cm]	CO_2 respiration [$\mu l\ g^{-1}\ h^{-1}$]									
Colluvic Cambisol										
5-10	3.41	5.84	4.58	0.82	6.39	3.82	12.11	14.67	6.87	7.61
40-50	1.52	1.99	2.14	0.62	1.90	1.79	13.94	18.11	7.22	6.92
50-60	1.68	2.47	2.54	0.53	1.86	1.89	14.77	17.47	6.59	7.97
75-85	1.69	2.01	2.15	0.56	1.76	1.71	10.44	13.89	5.30	6.84
Haplic Luvisol										
5-10	4.03	8.81	5.74	2.02	8.49	4.87	12.46	13.83	5.35	6.24
40-50	1.59	2.57	1.86	1.39	2.14	1.73	2.59	5.66	1.76	2.17
50-60	2.42	3.07	1.78	1.29	2.69	1.72	3.85	5.26	3.16	2.60
75-85	1.88	2.27	1.87	0.99	1.78	1.62	3.52	4.78	2.18	2.94
CV* [%]	15.8	18.0	11.9	20.2	15.0	11.8	22.7	14.0	24.5	15.3

* mean coefficient of variation between replicate samples within site and depth (n=5)

8.   Acknowledgements

9.   References

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