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University of Hohenheim
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Prof. Dr. Ellen Kandeler

Soil microbial assimilation and turnover of carbon depend on resource quality and availability

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Karolin Müller
Saalfeld (Saale)

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Examination commitee:

Supervisor and Reviewer: Prof. Dr. Ellen Kandeler

Co-Reviewer: Prof. Dr. Liliane Rueß

Additional Examiner: Prof. Dr. Andreas Fangmeier

Head of Committee: Jun.-Prof. Dr. Andrea Knierim

Dean: Prof. Dr. Ralf Vögele

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1 Summary

The decomposition of soil organic carbon (SOC), which is predominantly performed by soil microorganisms, is an important process in global carbon (C) cycling. Despite the importance of microbial activity to the global C budget, the effects of resource quality and availability on soil microorganisms are little understood. Therefore, soil microbial biogeochemistry is still highly uncertain in earth system models. In general, C enters the soil either belowground via the root system or aboveground via shoot litter. Most of this plant-derived C enters the soil organic C pool via incorporation into soil microorganisms, but the subsequent fate of C is rarely reported. This thesis was designed to improve understanding of the roles of bacteria and fungi as foundational members of the soil food web. It includes their contributions to C transfer through the soil system and to higher trophic levels of arable ecosystems.

The study presented in Chapter 5 used a field experiment established in 2009 to investigate C flow at three soil depths over five consecutive years after a C3 to C4 crop exchange. Root-derived C (belowground pathway) was introduced by the cropping of maize plants, whereas shoot-derived C (aboveground pathway) was introduced by application of shoot litter to the soil surface. The proportion of maize-derived C varied between the different soil pools with lower incorporation into SOC and EOC (extractable organic C) and higher incorporation ratios of maize C into microbial groups. Although root-C input was three times higher than shoot-C input, similar relative amounts of maize-C were found in microorganisms. A simultaneous supply of both plant resources had an additive effect on the C input in almost all investigated C pools in topsoil. Both root and shoot C were transferred to a depth of 70 cm. At all three depths, fungi utilized the provided maize C to a greater extent than did either Gram-positive or Gram-negative bacteria.

Fungal biomass was labeled with maize-C to 78% after the fifth vegetation period, indicating preferential utilization of litter-derived C by saprotrophic fungi. Since fungal biomass is low in comparison to bacteria, the overall contribution of fungi remains unclear with respect to the decomposition of plant material.

The second study (Chapter 6) investigated, in a microcosm experiment, the effects of decreasing resource quality on microorganisms during plant residue decomposition at the soil-litter interface. Reciprocal transplantation of labeled ^{13}C and unlabeled ^{12}C maize litter to the surface of soil cores allowed us to follow C transfer and subsequent C turnover from residues into microbial biomass of fundamental members (bacteria and fungi) of the detritivore food web during three stages of the litter decomposition process. Quality (i.e. age) of the maize litter influenced C incorporation into bacteria and fungi. Labile C from freshly introduced litter was incorporated by both groups of microorganisms, whereas saprotrophic fungi additionally used complex C in the intermediate stage of decomposition. Bacteria responded differentially to the introduced litter; either by turnover of litter C in their phospholipid fatty acids (PLFAs) over time, or by storage and/or reuse of previous microbially released C. Saprotrophic fungi, however, showed a distinct litter C turnover in the 18:2ω6,9 PLFA. The mean residence time of C in the fungal biomass was 32 to 46 days; the same or shorter time than in bacterial PLFAs.

In the third study, presented in Chapter 7, another field experiment was conducted to distinguish herbivore- from detritus-based food chain members over two consecutive years. Three treatments were established: maize as crop plant, maize shoot litter application, and fallow without C input. This provided root-derived C, shoot-derived C, and autochthonous organic matter, respectively, as the main C resource. The altered C supply due to plant removal had less severe effects on the micro-food web structure than expected. Bacteria and fungi showed a marked resilience to changed C availability. In the first growing season, nematode abundance under plant cultivation was similar to that under litter and fallow conditions. After the second harvest, the abundance of detritivore food chain members increased, reflecting the decomposition of root residues. Small but significant effects of the treatments were

restricted to the topsoil for nematodes and to the rooted zone for microorganisms. Results of this experiment suggest considerable micro-food web resilience to altered C and nutrient availability, and indicate that organic matter from previous vegetation periods was successfully utilized to overcome C deprivation.

In conclusion, this thesis provides new insights into microbially mediated decomposition processes at different time scales and at different soil depths. Stable isotope probing combined with biomarker analysis enabled us to study C fluxes between biotic and soil C pools to separate the contributions of bacteria and fungi to soil C cycling. These results can be used as a basis for an empirical model of C flow through the entire soil food web.

2 Zusammenfassung

Der Abbau von organischer Bodensubstanz (OBS) ist ein wichtiger Prozess des globalen Kohlenstoffkreislaufes (C-Kreislaufes), der im Wesentlichen von Bodenmikroorganismen durchgeführt wird. Trotz der großen Bedeutung der mikrobiellen Aktivität für die globale C-Bilanz, ist der Einfluss von Substratqualität und -verfügbarkeit auf Bodenmikroorganismen wenig erforscht. Die mikrobielle Biogeochemie ist deswegen in Erdsystemmodellen nur unzureichend implementiert. Generell wird der C der OBS unterirdisch über das Wurzelsystem oder oberirdisch durch Streu zugeführt. Ein Großteil des pflanzenbürtigen C wird indirekt durch den Einbau in Mikroorganismen der OBS zugeführt, aber über das darauffolgende Schicksal von C in der mikrobiellen Gemeinschaft ist wenig bekannt. Ziel der vorliegenden Arbeit war es, dass Wissen über die Rolle von Bakterien und Pilzen als fundamentale Bestandteile des Bodennahrungsnetzes in Agrarökosystemen zu erweitern. Das beinhaltet sowohl ihre Beteiligung am C-Transfer durch das Bodensystem, als auch in höhere trophische Stufen des Nahrungsnetzes.

Die im 5. Kapitel präsentierte Studie nutzte ein im Jahr 2009 angelegtes Feldexperiment um den C-Eintrag in drei Bodentiefen nach einem C3-C4 Pflanzenwechsel in fünf aufeinanderfolgenden Jahren zu untersuchen. Hierbei wurde durch den Anbau von Maispflanzen wurzelbürtiger C (unterirdischer C-Kanal), sowie durch die Applikation von Maisstreu auf die Bodenoberfläche sproßbürtiger C (oberirdischer C-Kanal) in den Boden eingetragen. Der prozentuale Anteil von maisbürtigem C variierte in den Bodenpools: geringere Einträge fanden sich in der OBS und im extrahierbaren organischen C (EOC) und höhere Einträge in den mikrobiellen Gruppen. Unabhängig vom Substrat wurde der gleiche relative Anteil an Mais-C in die Bodenpools eingebaut, obwohl dreimal so viel wurzelbürtiger C wie sproßbürtiger

C zur Verfügung stand. Eine Versorgung mit beiden Pflanzensubstraten zeigte im Oberboden einen additiven Effekt in fast allen untersuchten Bodenpools. Sowohl wurzel- als auch sproßbürtiger C wurde bis in eine Tiefe von 70 cm transferiert. In allen drei Tiefen assimilierten Pilze die angebotenen C-Substrate im größeren Umfang als Gram-positive und Gram-negative Bakterien. Nach der fünften Wachstumsperiode konnte ein Einbau von bis zu 78 % Mais-C in die pilzliche Biomasse nachgewiesen werden, was auf verstärkte Nutzung des Mais-C durch saprotrophe Pilze hindeutet. Da die pilzliche Biomasse im Gegensatz zur bakteriellen Biomasse gering ist, bleibt die tatsächliche Bedeutung der Pilze am Abbauprozess von Pflanzenmaterial allerdings unklar.

Die zweite Studie (Kapitel 6) untersuchte den Einfluss abnehmender Substratqualität auf Bodenmikroorganismen an der Boden-Streu Grenzschicht in einem Mikrokosmenexperiment. Ein reziproker Austausch von markierter ^{13}C und unmarkierter ^{12}C Maisstreuauflage auf Bodenkerne erlaubte es, den C-Eintrag von Pflanzenresten in die Hauptakteure (Bakterien und Pilze) des detritivoren Nahrungsnetzes und den darauffolgenden C-Umsatz in der mikrobiellen Biomasse zu drei unterschiedlichen Zeiten während des Streuabbaus zu bestimmen. Die Qualität (d. h. das Alter) der Maisstreu beeinflusste hierbei die Aufnahme von C in Bakterien und Pilze. Einfach verfügbarer C aus kürzlich eingetragener Streu wurde von beiden Mikroorganismengruppen genutzt, während saprotrophe Pilze zusätzlich noch komplexeres C-Substrat im fortgeschrittenen Abbaustadium nutzten. Bakterien reagierten unterschiedlich auf die angebotene Streu, indem sie entweder den Streu-C in ihren Phospholipidfettsäuren (PLFAs) über die Zeit umsetzten, oder es dauerhaft speicherten und/oder zusätzlich noch mikrobiell freigesetzten C aufnahmen. Saprotrophe Pilze zeigten einen deutlichen Abbau von Mais-C in der 18:2ω6,9 PLFA. Die mittlere Verweildauer von C in der pilzlichen Biomasse betrug 32 bis 46 Tage und wurde genauso schnell oder sogar schneller als in den bakteriellen PLFAs umgesetzt.

Für die in Kapitel 7 präsentierte Studie wurde ein weiteres Feldexperiment genutzt, um die Mitglieder der Herbivoren- und Detritivorennahrungskette über zwei auf-

einanderfolgende Jahre zu untersuchen. Drei Behandlungen wurden etabliert um wurzelbürtiges, sproßbürtiges und autochthones organisches Material als verfügbares C-Substrat zur Verfügung zu stellen: Anbau von Maispflanzen, Ausbringen von Maisstreu und Brachflächen. Entgegen den Erwartungen zeigte die veränderte C-Versorgung durch Entfernen der Ackerpflanze nur wenig Einfluss auf die mikrobielle Gemeinschaftsstruktur des Bodennahrungsnetzes. Bakterien und Pilze zeigten eine ausgeprägte Anpassungsfähigkeit an die geringere C-Verfügbarkeit. In der ersten Vegetationsperiode war die Nematodenabundanz unter Pflanzenkultivierung vergleichbar mit denen der Streu- und Brachflächen. Nach der zweiten Ernte wurden hingegen die Mitglieder der Detritivorennahrungskette durch den Abbau von Wurzelbiomasse gefördert. Die wenigen, aber signifikanten, Effekte der Behandlung waren auf einzelne Mitglieder der Nematodengemeinschaft im Oberboden und für Bakterien und Pilze zusätzlich noch auf die Wurzelzone beschränkt. Die Ergebnisse dieser Studie zeigen eine ausgeprägte Widerstandsfähigkeit des Bodenmikronahrungsnetzes auf Substrat- und Nährstoffverfügbarkeit und deuten darauf hin, dass älteres organisches Material genutzt wird, um C-Mangel auszugleichen.

Zusammenfassend hat die vorliegende Arbeit dazu beigetragen neue Erkenntnisse zu mikrobiellen Abbauprozessen mit unterschiedlicher zeitlicher und räumlicher Auflösung zu erlangen. Die Nutzung von stabilen Isotopen in Kombination mit PLFA-Biomarkeranalysen ermöglichte es, den C-Fluss zwischen abiotischen und mikrobiellen C-Pools im Boden zu untersuchen und den Anteil von Bakterien und Pilzen am C-Umsatz im Boden zu bestimmen. Die vorliegenden Ergebnisse können als Basis für ein empirisches Modell des C-Flusses durch das gesamte Bodennahrungsnetz genutzt werden.

3 General Introduction

3.1 Carbon cycle

Globally, soil organic carbon (SOC) stocks comprise the largest active terrestrial carbon (C) reservoir, storing more than 2300 Pg C within the top three meters of the soil profile (Jobbagy & Jackson 2000; Jansson et al. 2010). This is more C than in the atmospheric (760 Pg) and biotic pools (560 Pg) combined (Lal 2004). Historically it has been assumed that SOC stocks are inert and resilient over hundreds of years (Martel & Paul 1974; Jenkinson et al. 1992) and that they rely on the balance between input and output of C within the soil (Schulze & Freibauer 2005), but recent studies have shown that SOC stocks are more susceptible to climate and land use changes than expected (Davidson & Janssens 2006). Bellamy et al. (2005) estimated, as an effect of climate change, an annual loss of 13 million tons of C per year in the upper 15 cm of soil for the whole of England and Wales; this is equivalent to 8% of the United Kingdom's total CO_2 emission in 1990. These losses may alter the functions of SOC as essential for storage of plant nutrients, substrate supply for soil organisms, water capacity and quality improvement, soil stability, and as a sink for atmospheric CO_2 (Lal 2004).

Plant biomass is the main component of belowground C stocks. Plants remove approximately 120 Pg C per year from the atmosphere through photosynthesis (Beer et al. 2010); 50% of this fixed C is released back to the atmosphere by autotrophic respiration, while the other 50% is translocated to the soil surface and belowground (Schimel et al. 1994; Grace & Rayment 2000) and available as a C source for soil microorganisms (Gougoulias et al. 2014). Sequestration, mineralization, methane emission, erosion, and transport of dissolved and particulate C (Lal 2004) alter the C

balance and therefore determine the loss or accumulation of soil C stocks. However, many of these factors controlling input and output of C are still poorly understood, especially the role of microorganisms' involvement in processing plant-derived C in the soil. Soil microorganisms, in their tremendous quantity and diversity, are responsible for degradation of almost all organic substances in soil (Schulze & Freibauer 2005). Consequently, SOC stocks depend strongly on microbial activity, which in turn is affected by environmental factors including soil texture, moisture, temperature and pH (Brüggemann et al. 2011). As a pre-requisite for future modeling of SOC loss or accumulation processes and its response to changing environmental conditions, it is essential to disentangle the effects of resource availability and quality on the underlying members of the soil food web and their C transfer through the soil system and into higher trophic levels of the soil food web.

3.2 Plant resources in arable ecosystems

Currently, approximately 40% of terrestrial land area is used for agriculture and this will increase in the near future (Foley et al. 2005; Tilman et al. 2011). Agroecosystems are characterized by distinct environmental conditions: frequent disturbance as a result of tillage and seeding, mechanical compaction due to farm machinery, and application of herbicides, insecticides and fertilizers (Larsen et al. 2004). Additionally, varying availability of C sources and absence of a permanent litter layer influence the abundance and diversity of belowground food web members (Postma-Blaauw et al. 2005). There are two major pathways of C introduction to soil: belowground C input via the plant root system, and aboveground via shoot and litter C input. Both pathways introduce C sources of different quantities and complexity to the soil (Gougoulias et al. 2014), and depend on plant species and agricultural management practices (Clapp et al. 2000; Lorenz & Lal 2005).

The belowground pathway provides C derived from exudates and dead root biomass, which is known as rhizodeposition (Gougoulias et al. 2014). Exudates are continuously released by living roots during the growth period and consist mostly

of low molecular weight compounds such as sugars, sugar alcohols, amino acids, and organic acids, but also more complex secondary metabolites, all of which are deposited to the root zone of the soil profile (Kögel-Knabner 2002). Microorganisms in deeper soil layers can also profit from this root-derived C supply (Grayston et al. 2001; Moore-Kucera & Dick 2008). The aboveground pathway introduces senescent shoot and leaf litter resources to the soil surface, predominantly at the end of the growing season. Most of the aboveground plant biomass is harvested as crop, but, for example, corn stover can remain on the soil surface. The effect of aboveground litter on soil organisms is mostly limited to the surface and topsoil, but can be translocated to deeper zones due to tillage, earthworm activity, desciccation cracks (Natsch et al. 1996), or as dissolved organic C by infiltrating rainfall (Marschner & Bredow 2002). Consequently, the soil C cycle in arable soils depends more on belowground C inputs than on the aboveground C input (Drigo et al. 2010).

Low molecular weight compounds can be rapidly utilized by microbes (Gunina & Kuzyakov 2015), whereas complex structural compounds of plant cells, such as lignin, cellulose, and hemicellulose must be depolymerized by the activity of specific enzymes before the C can be taken up by bacteria or fungi (Wallenstein & Weintraub 2008). The persistence of plant tissue residues to the decomposition material which supplies organic matter (OM) to the soil is affected mainly by its lignin content or C:N ratio (Melillo et al. 2002; Berg & McClaugherty 2003). Soil organic matter (SOM) is composed of a continuum ranging from fresh to progressively decaying plant, microbial, and soil faunal detritus as well as exudates (Dungait et al. 2012). The plethora of compounds in SOM leads to huge differences in C residence times: from 1-2 years in an active pool, which consists of active microorganisms and polysaccharides; to over 15-100 years in a slow pool, containing lignified tissue, waxes and polyphenols; to 500-1000 years in a passive pool, containing humic substances, clay-organic matter complexes, and biochar (Dungait et al. 2012). Recent findings suggest that a significant proportion of SOM consists of microbial necromass (i.e. microbial residues) (Kögel-Knabner 2002; Cotrufo et al. 2013). These include chitinous cell wall compounds from fungi (Nakas & Klein 1979;

Moore et al. 2005; Six et al. 2006), which are chemically complex and selectively avoided as C sources in the decomposition process. It is expected that processes similar to those which govern plant residue decomposition are responsible for the breakdown of microbial necromass (Crowther et al. 2015).

The application of fresh plant material enhances the decomposition of older SOM sources, the so-called priming effect (Fontaine et al. 2007), which has short-term effects on the activity of soil microorganisms (Stockmann et al. 2013). Additionally, ploughing can induce SOM decomposition, due to greater disturbance and breakup of aggregates, bringing formerly non-accessible sources into contact with soil microorganisms. Moreover, climatic conditions (e.g dry/wet or freeze/thaw cycles) can affect decomposition and increase mobilization of dissolved or particulate OM to deeper soil layers (Kalbitz et al. 2000; Totsche et al. 2007). Although quantity and quality of substrates decrease with depth (Blume et al. 2002; Bausenwein et al. 2008), their linkage to minerals, rather than their chemical recalcitrance, may be a major factor enhancing the stability of SOC (Bol et al. 2009; Dungait et al. 2012). The physical environment and restricted supply of fresh OM in deeper soil layers increases the spatial proximity of substrates to their degrading microorganisms; this seems to play a crucial role in the degradation of C stocks in subsoils (Salomé et al. 2010).

3.3 Decomposer microorganisms

Soils harbor a tremendous quantity and diversity of soil microorganisms connected by complex interactions (Hättenschwiler et al. 2005), which are directly or indirectly involved in the decomposition of SOM (Bardgett et al. 2005). The heterogeneity of C incorporation into soil creates microbial hotspots such as the rhizosphere (soil influenced by plant roots), or detritusphere (soil influenced by plant litter), both of which are characterized by high process rates; these rely on the supply of labile C input (Kuzyakov & Blagodatskaya 2015). For example, the detritusphere is restricted to only 3-4 mm below the litter layer (Gaillard et al. 1999; Poll et al. 2008),

but bacteria and fungi show 5 to 11 fold higher C rates compared to uninfluenced bulk soil (Gaillard et al. 2003).

Ekschmitt et al. (2008) estimated the microbial contribution to overall biotic decomposition activity in soil to be 85-90%, whereas faunal activity contributes only 10-15%. Bacteria and fungi play key roles as first level consumers of plant-derived C and form the basis of the soil food web. The C transfer through these organisms is commonly known as the bacterial and fungal energy channel (Wardle 2002). Historically it was thought that the bacterial energy channel favored easily available C compounds which could be immediately incorporated into bacterial biomass and therefore promote rapid growth and rapid turnover of C and nutrients (Holtkamp et al. 2008; Ingwersen et al. 2008). In contrast, the fungal-dominated energy channel was considered to be a slow C and nutrient cycle (Joergensen & Wichern 2008), characterized by long fungal cell generation times (Högberg et al. 2010), and higher persistence to microbial decomposition (Guggenberger et al. 1999). Indeed, former studies in forest (Moore-Kucera & Dick 2008) and grassland (Denef et al. 2007; Denef et al. 2009) systems highlighted the prominent role of fungi as degraders of complex C sources in the decomposition process due to both their high extracellular enzymatic activity and their capacity for hyphal growth, facilitating active exploration of new resources. These abilities give fungi a selective advantage over immobile bacteria, which rely on spatially proximate C and nutrient supplies (de Boer et al. 2005).

Recent studies have provided evidence that such clear resource partitioning - labile C utilization by bacteria and complex C utilization by fungi - is less distinct. The significant contribution of bacteria to mineralization of complex substrates, as well as fungal utilization of labile C in the early stages of litter decomposition (Bastian et al. 2009; España et al. 2011; Kramer et al. 2016), have fueled the ongoing debate on the roles of specific groups of soil microorganisms in litter decomposition (Strickland & Rousk 2010; Rousk & Frey 2015). Resource partitioning is considered a major driver of soil microbial diversity (Zhou et al. 2002) with implications for both primary decomposers (Goldfarb et al. 2011) and higher trophic levels in soil

food webs; e.g. soil nematodes (Yeates et al. 1993; Leroy et al. 2009; Ferris 2010). With highly diverse food preferences that include plant residues, fungal hyphae, bacteria, and eukaryotes, nematodes fulfill a central role in top-down regulation of fundamental groups of the soil food web (Yeates et al. 1993; Ferris 2010). Because of their quick responses to changes in availability and quality of resources, nematode abundance and diversity can be used to investigate the C decomposition pathway (Ruess 2003).

3.4 Stable isotope probing

Recently, stable isotope probing (SIP) has become a common approach for the study of C fluxes within and between the atmosphere – plant – soil reservoirs (Bowling et al. 2008). In field experiments, natural abundance of ^{13}C in the atmosphere (1.1 atom%) is often used to introduce the ^{13}C label into soil by switching a cropping system from C3 to C4 plants (Steinbeiss et al. 2008; Kramer et al. 2012). The distinct $^{13}C/\ ^{12}C$ ratio of plants as a consequence of isotopic fractionation during photosynthesis makes it possible to trace the fate of plant-derived C in the soil (Peterson & Fry 1987) using differences in the isotopic signatures of C3 (-24 to -33‰) and C4 (-10 to -20‰) plant material (Tcherkez et al. 2011). Pulse labeling with ^{13}C enriched CO_2 is also used to label single plants at field scales or in pot experiments, and then to trace the subsequent C transfer into rhizosphere microorganisms and higher trophic levels of the soil food web (Pausch et al. 2016; Scheunemann et al. 2016). In addition to natural abundance C isotopes, commercially available highly enriched ^{13}C compounds (i.e. glucose as labile C source or complex plant material), are often used in decomposition studies to estimate groups of microorganisms involved in the decay of plant-derived C (Eichorst & Kuske 2012; Kramer et al. 2016). Investigations of C fluxes via plants into the soil system have largely been restricted to the microbial community in topsoil; *in situ* C incorporation into microorganisms in deeper soil layers have been less often reported.

SIP combined with phospholipid fatty acid (PLFA) analysis is often used to separate C flow into different soil microorganisms (Butler et al. 2003; Treonis et al. 2004; Paterson et al. 2008). PLFAs can be used as proxies for total viable microbial biomass in soils (Frostegård et al. 1991), because rapid degradation of PLFAs occurs directly after cell death (Zelles et al. 1992; Drenovsky et al. 2004). Furthermore, group-specific PLFAs provide insights into the soil microbial community because terminally branched fatty acids are specifically associated with, variously, Gram-positive bacteria (i15:0, i16.0 etc.), cyclopropyl saturated fatty acids (cy17:0, cy19:0) or monounsaturated fatty acids (16:1ω7) to Gram-negative bacteria, and the polyunsaturated PLFA 18:2ω6,9 to saprotrophic fungi (Ruess & Chamberlain 2010; Willers et al. 2015). The latter PLFA separates decomposer fungi from arbuscular mycorrhizal fungi, which is represented by 16:1ω5 (Willers et al. 2015). Analyzing these PLFAs with isotope-ratio mass spectrometry makes it possible to link microbial activity with functional processes in the environment (Boschker & Middelburg 2002; Sims 2008).

4 Study Objectives and Hypotheses

The present thesis was conducted as part of the DFG research unit FOR 918 "*Carbon flow in belowground food webs assessed by isotope tracers*". The overall aim of the research unit was to follow carbon (C) flow through abiotic and biotic compartments in a terrestrial ecosystem. This was done using isotope tracers to assess the fluxes of C from inputs into the soil, to the outputs, which involved identification of C dynamics within and between different trophic levels of the entire soil food web. C transfer within the soil food web included fundamental decomposer organisms (bacteria and fungi), nematodes as key group of the micro-food web, as well as meso- and macrofaunal predators in an arable ecosystem. This thesis elucidated the effects of resource quality and availability on bacteria and fungi as fundamental members of the soil food web. This aim was addressed in three different studies.

The first study (Chapter 5) used a field experiment, established in 2009, to investigate the long-term effects of resource quality and availability on topsoil and subsoil microorganisms over five consecutive years. Naturally abundant ^{13}C from C4 maize was introduced to the soil; belowground via maize roots; and aboveground via maize litter, on a soil that had previously been planted with C3 wheat. Four different treatments were applied to follow the maize C in the soil at the end of each vegetation period in September: a corn maize treatment with C supplied belowground and aboveground; a fodder maize treatment with belowground C input alone; a wheat treatment with maize shoot litter addition; and a wheat treatment without maize C addition, which served as control. This study quantified C incorporation of root- and shoot-derived maize C into soil organic C (SOC), extractable organic C (EOC), total microbial biomass (C_{mic}), and bacteria and

fungi at three different soil depths; topsoil (0-10 cm), lower rooted zone (40-50 cm) root-free zone (60-70 cm). The following hypotheses were investigated:

- The incorporation of plant-derived C into different soil C pools in topsoil (SOC, EOC, C_{mic}, Gram-positive bacteria, Gram-negative bacteria, and fungi) depends on C origin.
- Shoot litter application fosters the incorporation of maize-derived C into the microbial pools due to higher total C input.
- Root-derived C is more important than shoot-derived C for subsoil microbial decomposer organisms due to spatial proximity to microbes.

The second study (Chapter 6) was conducted as a microcosm experiment with C3 soil (from the wheat plots in Study 1) and highly ^{13}C labeled maize litter applied to the surface of soil cores. A reciprocal transplantation of ^{13}C litter with ^{12}C litter of the same quality made it possible to quantify litter-derived C in detritusphere microorganisms and their subsequent C turnover at three stages of litter decay: early stage (0-4 days), intermediate stage (5-12 days), and later stage (29-36 days) of decomposition. At each stage, the mean residence times of SOC, EOC, C_{mic}, and bacterial and fungal phospholipid fatty acids in different soil C pools were calculated. The following hypotheses were tested:

- Both bacteria and fungi use labile C from fresh plant residues.
- Fungi use, in addition, more complex C sources in the later stages of decomposition, due to their ability to grow towards a substrate and to their wide range of extracellular activity.
- The turnover of C is faster in bacterial than in fungal microorganisms as a consequence of the shorter life spans of prokaryotic bacterial cells and longer life spans of eukaryotic fungal cells.

The third study (Chapter 7) investigated, in a field experiment established in 2012, the root- and detritus-based food chains in the micro-food web of an arable soil.

To separate the micro-food web into rhizosphere, detritusphere, and bulk soil, three treatments were applied: maize plant treatment alone, organic amendment treatment by maize litter application, and a bare soil treatment by removal of all plants. This provided roots, litter and autochthonous organic matter (OM) as the main C sources. The aim was to determine the effects of plant removal over two consecutive years in July (highest root exudation), September (highest plant residue input), and December (highest transport of OM) on soil microorganisms and on nematode fauna at three soil depths (as with the first study). The following hypotheses were investigated in order to characterize the food web structure:

- The rhizosphere food web is highly structured with both herbivore and detrital food chain components due to the availability of C resources (rhizodeposits, root litter C) of different complexities.
- The detritusphere is moderately structured by detrital food chain components due solely to the availability of complex shoot litter.
- The bulk soil is less structured with fallow soil assemblages due to the exclusion of fresh plant C input.

5 Carbon transfer from maize roots and litter into bacteria and fungi depends on soil depth and time

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Karolin Müller[1], Susanne Kramer[2], Heike Haslwimmer[1], Sven Marhan[1], Nicole Scheunemann[3], Olaf Butenschoen[3], Stefan Scheu[3], Ellen Kandeler[1]

[1] Institute of Soil Science and Land Evaluation, Soil Biology, University of Hohenheim, Emil-Wolff-Straße 27, 70599 Stuttgart, Germany

[2] Institute of Vegetable and Ornamental Crops Großbeeren, Theodor-Echtermeyer-Weg 1, 14979 Großbeeren, Germany

[3] J.F. Blumenbach Institute of Zoology and Anthropology, Georg August University Göttingen, Berliner Straße 28, 37073 Göttingen, Germany

5.1 Abstract

Plant-derived carbon (C) transfer to soil is one of the important factors controlling the size and structure of the belowground microbial community. The present study quantifies this plant-derived C incorporation into abiotic and biotic C pools in top- and subsoil in an arable field over five years. Stable isotope analysis was used to determine the incorporation of maize root and shoot litter C into soil organic C (SOC), extractable organic C (EOC), total microbial biomass (C_{mic}), ergosterol and phospholipid fatty acids (PLFAs). The following treatments were investigated: corn maize (CM), providing root- and shoot-derived C (without corncobs), fodder maize (FM), providing only root-derived C, and wheat plus maize shoot litter amendment (WL), providing only shoot-derived maize C. Wheat plants (W) without maize litter amendment served as control. Soil samples were taken each September directly before harvest from 2009 to 2013. During the experiment, the maize-derived C signal increased in SOC, EOC, C_{mic}, ergosterol, bacterial and fungal PLFAs in the topsoil (0-10 cm). Although total maize shoot C input was threefold lower than maize root C input, similar relative amounts of maize C derived from shoots and roots were incorporated into the different C pools in the WL and the FM treatments, indicating the importance of shoot-derived C sources for microorganisms in the topsoil. An additive effect of both C sources was found in the CM treatment with almost twice as much maize-derived C in the respective pools. Furthermore, the proportion of maize-derived C varied between the different pools with lower incorporation into the total SOC (17%) and total EOC (24%) pools and higher incorporation ratios of maize C into PLFAs of different microbial groups (29% in Gram-positive (Gr^+) bacterial PLFA-C, 44% in Gram-negative (Gr^-) bacterial PLFA-C, 69% in fungal PLFA-C and 78% in ergosterol) in the CM treatment in topsoil after five years. After the third and fifth vegetation periods, we also detected maize-derived C in the rooted zone (40-50 cm depth) and the root-free zone (60-70 cm depth). The maize-derived C incorporation was lower in subsoil C pools in comparison to topsoil C pools. In the root-free zone, the maize-derived C was found to be 2% in total SOC, 28% in total EOC, 9% in Gr^+ bacterial PLFA-C, 20% in

Gr^- bacterial PLFA-C and 53% in fungal PLFA-C. Saprotrophic fungi incorporated maize-derived C in all soil depths to a greater degree than Gr^+ and Gr^- bacteria, indicating the importance of saprotrophic fungi in this agro-ecosystem.

5.2 Introduction

Soils contain a tremendous number of microorganisms and as primary decomposers they transform carbon (C) within the soil via decomposition, polymerization and immobilization of organic matter (Jastrow et al. 2007; Paterson et al. 2008). Generally, there are two main pathways of plant C input into the soil: first, via rhizodeposition by living plants and the decomposition of root litter after plant senescence; second, by incorporation of plant shoot material and its leachates (Gougoulias et al. 2014). Both pathways introduce C compounds of varying complexity into the soil, ranging from low molecular weight compounds, such as amino acids, sugars and peptides, to recalcitrant high molecular weight compounds, such as cellulose, hemicellulose, lignin or proteins (Brüggemann et al. 2011). During the early stage of plant residue decomposition, easily available and water-soluble C compounds are released into the soil (Bastian et al. 2009; Poll et al. 2010). After the depletion of these substrates, more complex plant-derived compounds are used and this is associated with more intensive interactions between microorganisms (Dilly et al. 2004; Fioretto et al. 2005). Previous studies have shown that labile and recalcitrant plant compounds are utilized by distinct microbial communities (Paterson et al. 2008). Gram-positive (Gr^+) bacteria use both easily available and recalcitrant compounds whereas Gram-negative (Gr^-) bacteria preferentially process low molecular weight compounds (Waldrop & Firestone 2004; Kramer & Gleixner 2006; Holtkamp et al. 2008). Saprotrophic fungi produce a wide range of extracellular enzymes, allowing decomposition of the recalcitrant ligno-cellulose matrix that other organisms are unable to degrade (de Boer et al. 2005).

The use of stable isotopes in combination with biomarker molecule analyses such as phospholipid fatty acids (PLFA) or ergosterol enables the determination of C incorporation into different groups of microorganisms (Butler et al. 2003; Treonis

et al. 2004; Paterson et al. 2008). However, most research on C incorporation into soil microorganisms has been done as short-term laboratory incubations (Marx et al. 2007; Semenov et al. 2012; Yao et al. 2012) or within-season field studies (Treonis et al. 2004; Tavi et al. 2013). A ^{13}C pulse labeling experiment with maize plants showed that 20% of the C assimilated by plants was transferred into belowground C pools and the isotope label occurred in both bacteria and fungi only two days after the ^{13}C pulse labeling (Pausch & Kuzyakov 2012). Furthermore, the authors showed that root-derived C was incorporated into the soil food web mainly via saprotrophic fungi rather than bacteria. In a longer term study, Kramer et al. (2012) found that ergosterol consisted of up to 76% maize-derived C after the second vegetation period, indicating an important role of saprotrophic fungi in plant C degradation.

C fluxes and the contribution of microorganisms are often investigated only for the topsoil (tilled layer), while C inputs into deeper soil horizons receive less attention. Generally, microbial abundance and diversity declines with increasing depth due to lower nutrient and C supply as well as larger separation of microbes from substrates (Salomé et al. 2010). Kramer & Gleixner (2008) reported a shift in the isotopic signature of different C pools in two soil profiles to a depth of 60 cm at one time point 40 years after a vegetation change from C3 to C4 crops. In this study, the contribution of C4-derived C to the soil organic matter (SOM) C in 40-60 cm was only 3%. However, the incorporation of maize C into PLFA biomarkers was 34%, suggesting faster turnover of microbial biomass C compared to bulk SOM C in subsoil.

To the best of our knowledge, a continuous determination of root- and shoot-derived C incorporation into different groups of soil microorganisms in an agriculturally managed ecosystem has not yet been reported for time periods longer than two years. Furthermore, the C incorporation into the subsoil microbial community is entirely lacking for a continuous time series. Therefore, a field experiment was set up on arable land in 2009 to determine the incorporation of root- and shoot-derived maize C into soil organic C (SOC), extractable organic C (EOC), microbial biomass

(C_{mic}), Gr^+ bacteria, Gr^- bacteria and fungi in the topsoil (0-10 cm) as well as into two subsoil layers, one representing the lower rooting zone (40-50 cm), the other the almost root-free zone (60-70 cm).

We hypothesized that (i) the incorporation of plant-derived C in the different C pools in topsoil (SOC, EOC, C_{mic}, Gr^+ bacteria, Gr^- bacteria and fungi) depends on C origin, (ii) shoot litter application fosters the incorporation of maize-derived C in the microbial pools due to higher total C input, and (iii) root-derived C is more important than shoot-derived C for the subsoil microbial decomposer organisms due to spatial proximity.

5.3 Material and Methods

Study Site

The experiment was established on an agricultural field located in Holtensen (51°33'N, 9°53'O, 158 m a.s.l.) north-north-west of the city of Göttingen (Lower Saxony, Germany). The site has a temperate climate with mean air temperature of 7.9 °C and mean annual precipitation of 720 mm (Fig. S5.1). According to IUSS (2007), the dominant soil types are Cambisols (Braunerden, KA 5 (2005)) and Luvisols (Parabraunerden, KA 5 (2005)) with partially stagnic properties (Pseudogley, KA 5 (2005)). Due to long-term agricultural use, two plough layers at 20 and 30 cm depths were found and a strong compaction in and below the second plough layer (below 30 cm) occurs with a bulk density of 1.6 g cm^{-3} (Table S5.2). Before the start of the experiment, the isotopic signature of SOC in the Ap horizon was -27.4 $\pm$ 0.01 ‰ and the isotopic ^{13}C value increased with depth (-25.5 $\pm$ 0.03‰ at 60-70 cm depth). More detailed information about the soil properties are given in Table S5.2 and in Kramer et al. (2012).

The experiment was set up in 2009 with maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) in a strip design of two rows. Ten plots (24 x 24 m) were arranged in a factorial design within each row. Four different treatments with four replicates per

treatment were established, differing in the source of maize C input. The corn maize (CM) treatment introduced the $\delta^{13}C$ signal belowground by rhizodeposition during the growing season and decomposition of dead root material after harvest and aboveground by maize litter decomposition. In the fodder maize (FM) treatment the $\delta^{13}C$ signal derived only from belowground input via roots and rhizodeposition, because aboveground shoot biomass was removed after harvest. Maize litter was applied to wheat plots (WL) to introduce the C4 signal into the soil via aboveground maize shoot decomposition only. Wheat plots (W) were used as reference to maintain habitat function for soil organisms without changing the isotopic signature. To establish the CM and FM treatments, hybrid maize "Ronaldino" (34 kg ha^{-1}) was sown in 2009, "Fernandez" in 2010 (26 kg ha^{-1}) and "Cordisco" from 2011 to 2013 (2011: 21 kg ha^{-1}, 2012: 21 kg ha^{-1}, 2013: 23 kg ha^{-1}). In the WL and W plots winter wheat "Julius" (224 kg ha^{-1}) was grown in 2009 and spring wheat "Melon" (2010: 224 kg ha^{-1}; 2011: 180 kg ha^{-1}; 2012: 204 kg ha^{-1} and 2013: 189 kg ha^{-1}) was sown in the following four vegetation periods. For fertilization and herbicide application see tables S5.3 and S5.4. To establish the CM and WL treatments, maize shoots (without corncobs) were chopped into 1 cm^2 pieces, dried, and applied at 0.8 kg dry weight m^{-2} (equivalent to 0.35 kg C m^{-2}) annually in autumn on half of both maize and wheat plots. In April of each year, all experimental plots were tilled with a chisel plough to a depth of 12 cm.

Soil sampling

Soil samples were taken yearly in September from 2009 to 2013 shortly before maize harvest. Ten soil cores (diam. 2.5 cm) were taken down to 70 cm depth randomly from each plot between the maize rows. Three soil horizons were investigated: topsoil (0-10 cm) within the plough layer, rooted subsoil below the plough layer (40-50 cm) and the deep almost root-free subsoil (60-70 cm). Samples from each layer per plot were mixed and homogenized by hand. Samples were transported in a cooling box to the laboratory and stored at 4 °C until sieving (approximately one week). After sieving ($<$2 mm) and removal of plant particles, the soil samples

were stored at -28 °C until further analysis. Soil water content was determined gravimetrically after drying for 3 days at 60 °C. All presented data are related to soil dry weight.

Soil organic carbon (SOC) and ^{13}C-SOC

For SOC determination, 3 g bulk soil were dried at 60 °C for 72 h and subsequently ground in a ball mill. To remove carbonates, subsamples of 300 mg were taken and treated with 200 µl hydrochloric acid (HCl, 1M), dried at 60 °C for another 24 h and homogenized using mortar and pestle. Subsamples of 20-50 mg from each sample were weighed into tin capsules and analyzed for total C content as well as $\delta^{13}C$ content using a coupled system of elemental analyzer (NA 1110, CE-Instruments, Rodano, Milan, Italy) and isotope ratio mass spectrometer (Delta plus, Finnigan MAT, Bremen, Germany). Acetanilide (C_8H_9NO, Merck, Darmstadt, Germany) was used for internal control. $\delta^{13}C$ was measured against V-PDB standard. Isotope natural abundance was expressed by the delta notation

$$\delta^{13}C = \frac{R_{sample} - R_{standard}}{R_{standard}} \cdot 1000$$

with R_{sample} and $R_{standard}$ referring to the $\delta^{13}C$ content in samples and standard, respectively (Reineking et al. 1993).

Microbial biomass carbon (C_{mic}) and $^{13}C_{mic}$

Microbial biomass C (C_{mic}) was estimated with the chloroform-fumigation-extraction method (CFE) according to Vance et al. (1987). Briefly, 10 g of soil was fumigated with ethanol-free chloroform for 24 h to release the microbial biomass C. After removing the chloroform, 40 ml of 0.025 M K_2SO_4 solution was added, shaken for 30 min on a horizontal shaker at 250 rev min^{-1} and centrifuged for 30 min at 4420 x g. Organic C in the clear supernatant was measured with a TOC-TN_b Analyzer Multi-N/C 2100S (Analytik Jena, Jena, Germany). Inorganic C was removed by

adding 1 M HCl to the extracted samples before measurement (Pausch et al. 2012). Since only visible roots were removed prior to fumigation, it cannot be fully excluded that fine root-derived C slightly contributed to chloroform labile C (Mueller et al. 1992).

A second subsample of 10 g soil was treated similarly but without fumigation to determine the amount of extractable organic carbon (EOC). To determine the C_{mic} content, C content of non-fumigated samples was subtracted from the C content of the fumigated samples. The k_{EC} factor of 0.45 was used for estimation of C_{mic} (Joergensen 1996).

For $\delta^{13}C$ determination in C_{mic}, 10 ml of fumigated and non-fumigated extracts were evaporated at 60 °C in a rotatory evaporator (RVC 2-25, Martin Christ, Osterode am Harz, Germany). The residues were ground and weighed into tin capsules with a minimum of 10 µg C per sample (Marhan et al. 2010). $\delta^{13}C$ was analyzed by elemental analyzer (Euro EA 3000, EuroVector, Milan, Italy) coupled with an isotope ratio mass spectrometer (IRMS, Delta Plus XP, Thermo Finnigan MAT, Bremen, Germany). For calculation of the $\delta^{13}C$ in C_{mic} the following equation was used

$$\delta^{13}C_{mic} = \frac{c_{nf} \cdot \delta_{nf} - c_f \cdot \delta_f}{c_{nf} - c_n}$$

where c_{nf} and c_f are the extracted organic C contents of the non-fumigated and fumigated samples and δ_{nf} and δ_f are the respective $\delta^{13}C$ values.

Ergosterol and ^{13}C-ergosterol

Ergosterol as a biomarker for saprotrophic fungi was extracted using the method of Djajakirana et al. (1996). Two g of soil were dispersed in 25 ml ethanol, shaken for 30 min at 250 rev min^{-1} on a horizontal shaker and subsequently centrifuged for 30 min at 4420 x g. For topsoil 10 ml, and for subsoil 20 ml of the extracts were evaporated at 50 °C in a vacuum rotary evaporator (RVC 2-25, Martin Christ, Osterode am Harz, Germany). The residues were dissolved in 1 ml methanol and transferred into 2 ml brown glass HPLC vials using cellulose-acetate filters (0.45 µm; Sartorius Stedim

Biotech GmbH, Göttingen, Germany). Ergosterol concentration in the extracts was quantitatively determined by high-performance liquid chromatography (HPLC) analysis (Beckmann Coulter, System Gold 125, Fullerton, CA, USA). For this, a 250 mm x 4.6 mm Spherisorb ODS II 5 µm column was used with a mobile phase of pure methanol, a flow rate of 1 ml min^{-1}, and a detection wavelength of 282 nm (Beckmann Coulter, System old 166 UV-detector, Fullerton, CA, USA).

For determination of ^{13}C in ergosterol, 15 g soil from topsoil were treated according to Kramer et al. (2012). Each sample was homogenized in an ultrasonic bath, saponified for 30 min and filtered. The extracts were mixed with 100 ml H_2O_{deion} and 80 ml petroleum ether and separated into two phases by shaking. The upper phase was saved and the lower phase was rinsed again with 50 ml petroleum ether. The upper phases were pooled and 20 µl of ethylene glycol was added and evaporated to near dryness with a rotator evaporator at 300-500 mbar at 40 °C. The residues were mixed with 2 ml of methanol/water (95/5, v/v) and transferred into brown glass HPLC vials. Concentration and cleaning of the extracts was performed using a Varian preparative HPLC with a Varian Pro-Star 210 pump and a 701 Fraction Collector (Varian Medical Systems, Darmstadt, Germany) equipped with a Nucleosil 120 C4 (250 mm x 16 mm) column (Macherey-Nagel, Oensingen, Switzerland). The eluent was methanol/water (95/5, v/v) with a flow rate of 6 ml min^{-1}. From each sample, 1 ml was injected and the ergosterol fraction was collected, evaporated under nitrogen at 60 °C, dissolved in 70 µl iso-octane and transferred to a brown glass vial. The isotopic C composition of ergosterol was measured on a GC-C-IRMS system. The system consisted of a gas chromatograph (6890 series, Agilent Technologies, Santa Clara, CA, USA) coupled with a gas chromatography combustion III Interphase (Thermo Finnigan, Waltham, MA, USA) and a Delta Plus XP mass spectrometer (Thermo Finnigan MAT, Bremen, Germany). An Rtx-5 column (30 m x 0.25 mm, film thickness of 0.25 µm) was used with helium as carrier gas (flow rate 1.5 ml min^{-1}). The combustion and reduction reactors had temperatures of 940 °C and 640 °C, respectively. The GC program was set as follows: initial temperature was 160 °C for 1 min, temperature was increased to

270 °C with a rate of 5 °C min^{-1} followed by a rate of 2 °C min^{-1} to 300 °C, then held for 10 min. The injector temperature was 280 °C.

Phospholipid fatty acid (PLFA) analysis and ^{13}C-PLFA

PLFAs were extracted using the procedure described by Frostegård et al. (1993). Lipids of 2 x 4 g for topsoil and 2 x 10 g for subsoil samples were extracted with a Bligh & Dyer solution (chloroform, methanol, citrate buffer (pH 4); 1:2:0.8; v/v/v) and further separated into glycolipids, neutral lipids and phospholipid fatty acids using silica acid SPE cartridges (Bond Elut SI, 500 mg, 3 ml, Agilent Technologies Inc, Santa Clara, CA, USA). The two replicates of each sample were combined into one column before fractionation. Methanolysis of PLFAs was done with 0.2 M methanolic KOH as described by Ruess et al. (2007). The fatty acid methyl esters (FAMEs) were dissolved in iso-octane and measured on an AutoSystem XL gas chromatograph (Perkin-Elmer Corporation, Norwalk, CT, USA) equipped with a HP-5 capillary column (cross-linked 5% phenyl methyl siloxane; 50 m x 0.2 mm, film thickness of 0.33 µm) and a flame ionization detector. The temperature program began at 70 °C for 2 min, increased by 30 °C min^{-1} to 160 °C, then by 3 °C min^{-1} to 280 °C and held for 15 min. The injection temperature was 260 °C. Helium was used as carrier gas.

The PLFAs i15:0, a15:0, i16:0 and i17:0 are predominantly found in Gram-positive (Gr^+) bacteria and cy17:0 in Gram-negative (Gr^-) bacteria (Frostegård et al. 1993; Zelles 1999). 18:2ω6,9 was used as a biomarker for saprotrophic fungi (Frostegård & Bååth 1996).

For determination of $\delta^{13}C$, the PLFA samples were fractionated with Ag^+-SPE cartridges (6 ml, Supelco, Palo Alto, CA, USA) according to Kramer et al. (2008). Before use, cartridges were conditioned with acetone and n-hexane. A stepwise elution with 6 ml n-hexane containing increasing amounts of acetone (99:1, v/v; 96:4, v/v; 90:10, v/v and 0:100, v/v) separated FAMEs into four different fractions. The first fraction contained saturated FAMEs, the second and third fractions

monoenoic trans and cis FAMEs and the fourth fraction dienoic FAMEs. The first and fourth fractions were kept and evaporated to dryness by means of N_2 and re-dissolved in 70 µl iso-octane. A gas chromatograph (6890 series, Agilent Technologies, Santa Clara, CA, USA) equipped with a VS-23MS capillary column (Varian Medical Systems, Palo Alto, CA, USA; 30 m x 250 µm, film thickness of 0.25 µm) was coupled with a gas chromatography-combustion III Interphase (Thermo Finnigan, Waltham, MA, USA) to a Delta Plus XP mass spectrometer (Thermo Finnigan MAT, Bremen, Germany) to determine the $\delta^{13}C$ values of the FAMEs. The oven temperature was 290 °C and the injector temperature 250 °C. The temperature program started at 80 °C for 2 min, increased by 10 °C min^{-1} to 140 °C, further increased by 4 °C min^{-1} to 240 °C and held for 5 min. The $\delta^{13}C$ values of all FAMEs were corrected for the addition of a methyl group by using a mass balance equation (Denef et al. 2007). The $\delta^{13}C$ value of methanol used for methylation was -40.23‰.

Calculation of maize-derived C

For the calculation of relative amounts of maize-derived C in SOC, C_{mic}, ergosterol and PLFAs the following model was used

$$\%C_{maize} = \frac{\delta_{sample} - \delta_{reference}}{\delta_{maize} - \delta_{wheat}} \cdot 100$$

where δ_{sample} is the $\delta^{13}C$ value of the respective sample, $\delta_{reference}$ is the $\delta^{13}C$ mean value of reference plots (with wheat plants only) and δ_{maize} is the $\delta^{13}C$ value of the maize residues. An average $\delta^{13}C$ value of maize material (-13.01‰) was used for calculation of maize C (Kramer et al. 2012). To assess the relative amount of maize C in C_{mic}, ergosterol and PLFAs, the average $\delta^{13}SOC$ values of the wheat field (δ_{wheat}) of -27.1‰ (topsoil), -26.23‰ (rooted zone) and -25.72‰ (root-free zone) were used. For calculation of maize-derived C in SOC a $\delta^{13}C$ value of -28.31‰ for wheat plants was used.

Statistical analyses

Treatment and date effects in the different C pools and the relative incorporation of maize-derived C in these pools were analyzed by repeated measures analysis of variance (rm-ANOVA) with sampling date as repeated factor. In addition, separate ANOVAs with post-hoc tests (Tukey HSD, $p < 0.05$) for comparisons of means were performed for each sampling date. Best fitted transformation (log or reciprocal transformation) was used to improve homogeneity of variance (tested by Levene's test). For statistical analyses the software STATISTICA 6.0 (Tulsa, OK, USA) was used. All errors are reported as standard error of the means (SEM).

5.4 Results

Maize-derived C in SOC and EOC in the topsoil

The incorporation of maize-derived C in the total SOC pool increased over time with highest relative maize-derived C incorporation of 17% in the CM treatment after the fifth vegetation period (Fig. 5.1a). The incorporation rate of maize-derived C in total SOC was highest in the initial year especially in the CM treatment and the rate of increase was lower in the following years. The FM and WL treatments showed similar increases in relative maize C incorporation but were approximately half as much as in the CM treatment at the end of the experiment (treatment effect: $F_{2, 9} = 11.14$, $p = 0.004$). Total SOC content ranged between 10.3 and 14.7 mg g^{-1} soil with little variation over time (Fig. 5.2a). However, in 2013 the FM treatment had 20% lower absolute SOC amounts in comparison to the WL treatment (treatment effect: $F_{3, 12} = 6.58$, $p = 0.007$). Sampling date affected the total SOC content as well as the relative ^{13}C incorporation into this pool (Table S5.5).

Maize-derived C in the EOC pool fluctuated over time (Fig. 5.1b), but maize-derived C incorporation was highest at all times in the CM treatment (treatment effect:

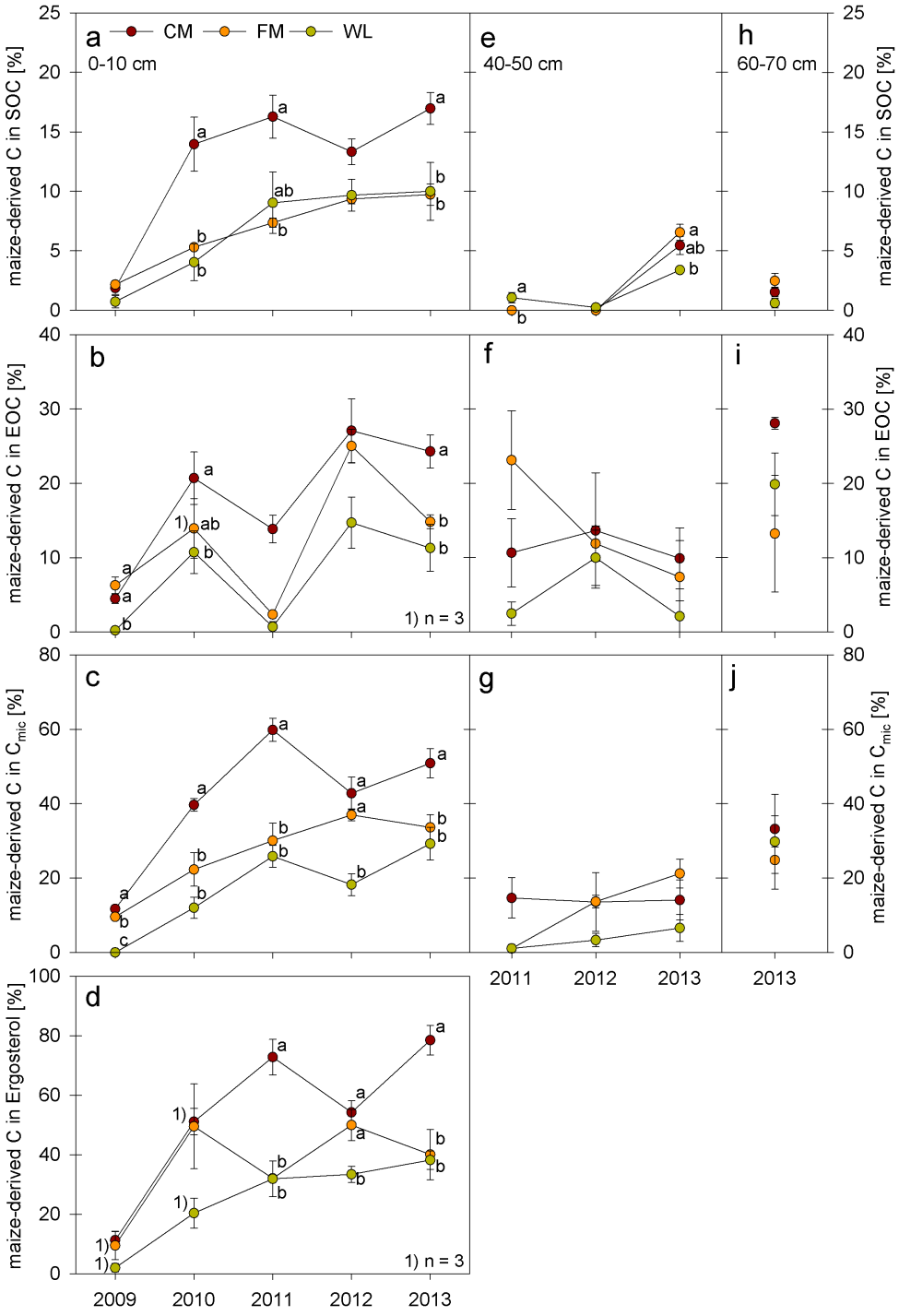

Figure 5.1: Relative incorporation of maize-derived C in SOC, EOC, C_{mic} and ergosterol in 0-10 cm (a, b, c, d), 40-50 cm (e, f, g) and 60-70 cm (h, i, j) depth in corn maize (CM), fodder maize (FM) and wheat plus maize litter (WL) treatment. Different letters indicate statistically significant differences between treatments at this date (Tukey HSD: $p < 0.05$). Error bars indicate standard error (n =4). Results of 2009 and 2010 for SOC, EOC, C_{mic} and ergosterol were published previously in Kramer et al. (2012).

$F_{2, 8} = 14.22$, $p = 0.002$). In 2013, 24% of EOC-C was maize-derived in the CM treatment. The FM and WL treatments showed similar incorporation values in the EOC pool over time, with the exception of 2012. For this year, 25% of the EOC-C was maize-derived C in the FM, whereas in the WL treatment only 15% was maize-derived C. At the end of the experiment, the EOC pools in the FM and WL treatments showed relative maize C incorporation of 14% and 11%, respectively, which is half of the relative maize C incorporation value in the CM treatment. However, the total EOC content varied between 13.8 and 43.3 µg g^{-1} soil with an increase over time (Fig. 5.2b) with typically higher EOC content in the treatments with litter amendment (treatment effect: $F_{3, 12} = 22.67$, $p < 0.001$).

Maize-derived C in the topsoil microbial community

The incorporation of maize-derived C in the microbial biomass (C_{mic}) increased from 2009 to 2011 in each of the treatments (Fig. 5.1c). This trend continued for the FM treatment in 2012 as well, whereas maize-derived C in the CM and WL treatments decreased. In 2013 an opposite trend was found, whereby maize-derived C increased in the treatments with litter amendment and decreased in the FM treatment. At the end of the experiment, the WL and FM treatments showed similar relative amounts of maize-derived C in C_{mic}, with 34% and 29% respectively (treatment effect: $F_{2, 9} = 10.53$, $p = 0.004$). In contrast to the SOC and EOC pools, the highest maize-derived C incorporation in C_{mic} was found in the CM treatment in 2011 with 60%.

The relative incorporation of maize-derived C in biomarker PLFA-C varied among Gr^+ bacteria, Gr^- bacteria and saprotrophic fungi (Fig. 5.3). In 2013, the maize-derived C in Gr^+ bacterial PLFA-C was significantly higher in the CM treatment, at 30%, than in the FM and WL treatments, with 19% and 20%, respectively (treatment effect: $F_{2, 8} = 13.40$, $p = 0.003$). Early in the experiment, the incorporation of maize-C in the Gr^- bacterial PLFA-C showed a tendency similar to that of maize C incorporation in Gr^+ bacteria PLFA-C (Fig. 5.3b). But during the final year, a

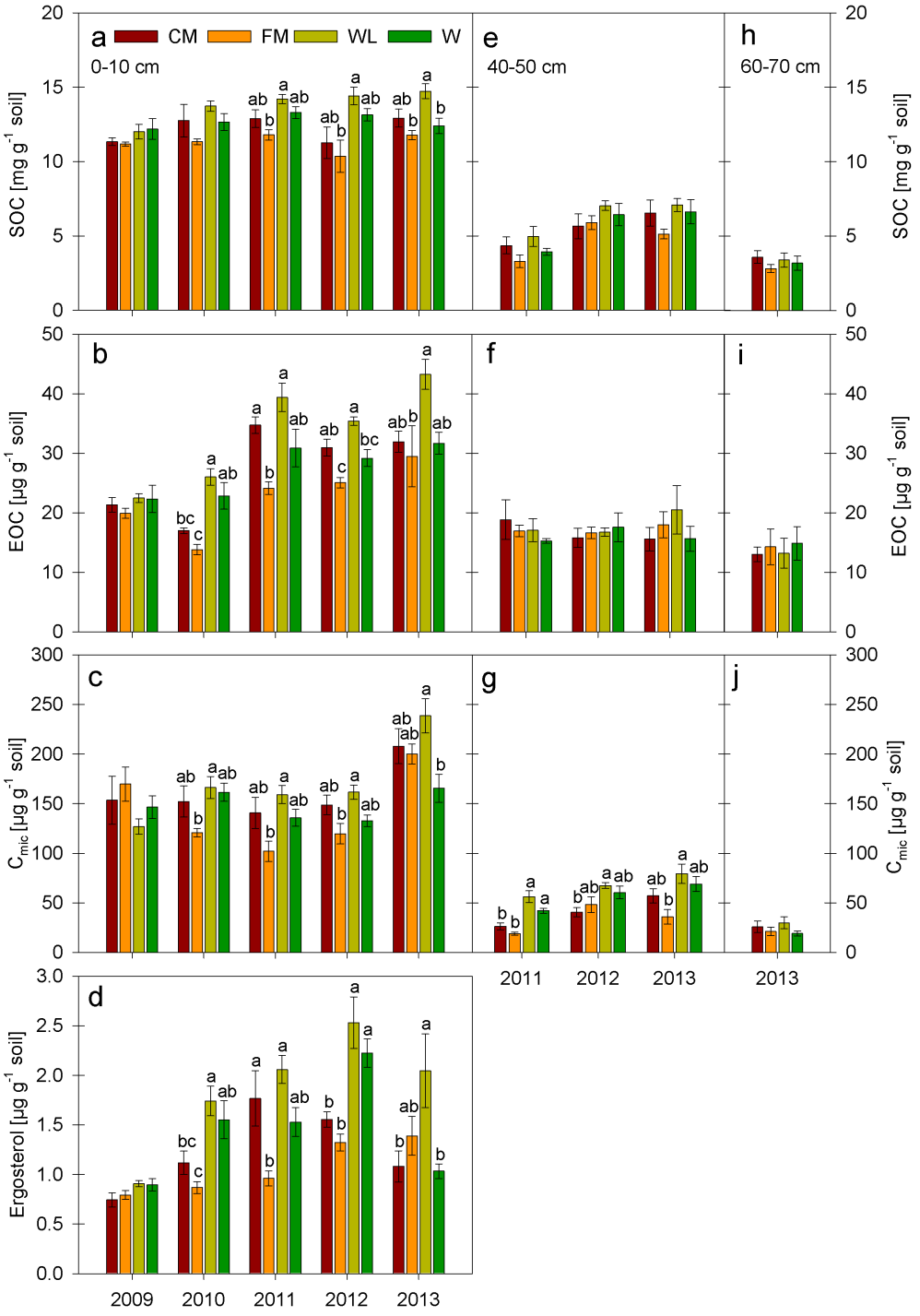

Figure 5.2: Total amount of C in SOC, EOC, C_{mic} and ergosterol in 0-10 cm (a, b, c, d), 40-50 cm (e, f, g) and 60-70 cm (h, i, j) depth in corn maize (CM), fodder maize (FM), wheat plus maize litter (WL) an wheat (W) treatment. Different letters above bars indicate statistically significant differences between treatments at this date (Tukey HSD: $p < 0.05$). Error bars indicate standard error (n =4). Results of 2009 and 2010 were published previously in Kramer et al. (2012).

strong incorporation of maize-derived C in cy17:0 resulted in a higher percentage of ^{13}C signals in Gr^- bacterial PLFA-C compared to Gr^+ bacterial PLFA-C in all treatments. The maize-derived C in Gr^- bacterial PLFA-C was higher in the CM plots, with 44%, than in the FM and WL plots, with 31% and 28%, respectively (treatment effect: $F_{2, 8} = 20.90$, $p < 0.001$). The absolute Gr^+ and Gr^- fatty acid amounts increased in all treatments over time (Fig. 5.4a, b). Additionally, the amount of fatty acids for both bacterial groups was affected by treatment, with higher amounts in plots with litter application (treatment effect: $PLFA_{Gr^+}$: $F_{3, 12} = 59.67$, $p < 0.001$; $PLFA_{Gr^-}$: $F_{3, 12} = 18.02$, $p < 0.001$).

Maize-derived C signals were highest in the fungal biomarker molecules ergosterol (Fig. 5.1d) and PLFA 18:2ω6,9 (Fig. 5.3c). Relative maize-derived C incorporation in ergosterol and 18:2ω6,9 correlated closely for topsoil (Pearson correlation coefficient, $r_p = 0.83$, $p < 0.001$, $n = 52$). In deeper soil layers, ergosterol content was below the detection limit for GC-C-IRMS; furthermore, only 18:2ω6,9 was investigated. After the fifth vegetation period, the fungal PLFA-C was 69% labeled with maize-derived C in the CM treatment whereas ergosterol-C was even more strongly labeled with 78% maize-derived C (treatment effect: $PLFA_{fungi}$: $F_{2, 9} = 29.35$, $p < 0.001$; ergosterol: $F_{2, 6} = 22.36$, $p = 0.002$). The incorporation of solely root- or shoot-derived maize C was equal in the FM and WL treatment in both the fungal PLFA (FM: 33%, WL: 32%) and ergosterol (FM: 40%, WL: 38%). The addition of litter generally enhanced fungal biomass (treatment effect: $PLFA_{fungi}$: $F_{3, 12} = 11.89$, $p < 0.001$; ergosterol: $F_{3, 12} = 17.10$, $p < 0.001$) in both maize and wheat plots (Fig. 5.2d and 5.4c), with the exception of ergosterol content in 2013, which was lower than in the previous year. The effect of sampling date was pronounced in all microbial groups and in the relative maize-derived C incorporation within these pools (Table S5.5).

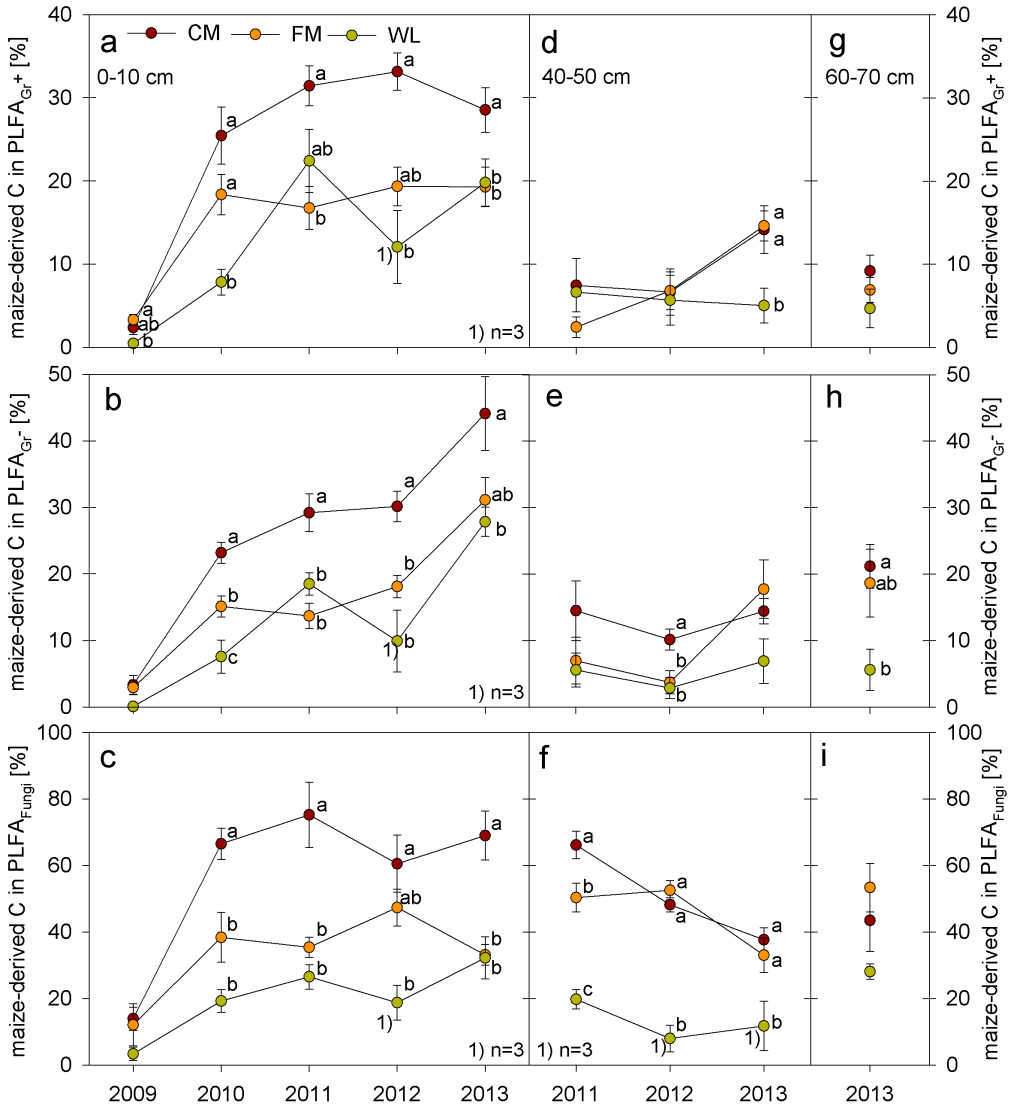

Figure 5.3: Relative incorporation of maize-derived C in gram-positive bacteria ($PLFA_{Gr^+}$), gram-negative bacteria ($PLFA_{Gr^-}$) and fungi in 0-10 cm (a, b, c), 40-50 cm (d, e, f) and 60-70 cm (g, h, i) depth. Different letters indicate statistically significant differences between treatments at this date (Tukey HSD: $p < 0.05$). Error bars indicate standard error (n=4). For legend see Figure 5.1.

Subsoil C and ^{13}C pools

Maize-derived C in total SOC in subsoil (Fig. 5.1e, h) was detected in the rooted zone (40-50 cm depth) first in 2013 with 5.6%, 4.7% and 2.9% in the CM, FM and WL treatments, respectively (treatment effect: $F_{2,\,9} = 2.75$, $p = 0.013$). The absolute SOC content decreased with increasing depth (Fig. 5.2e, h) and treatment

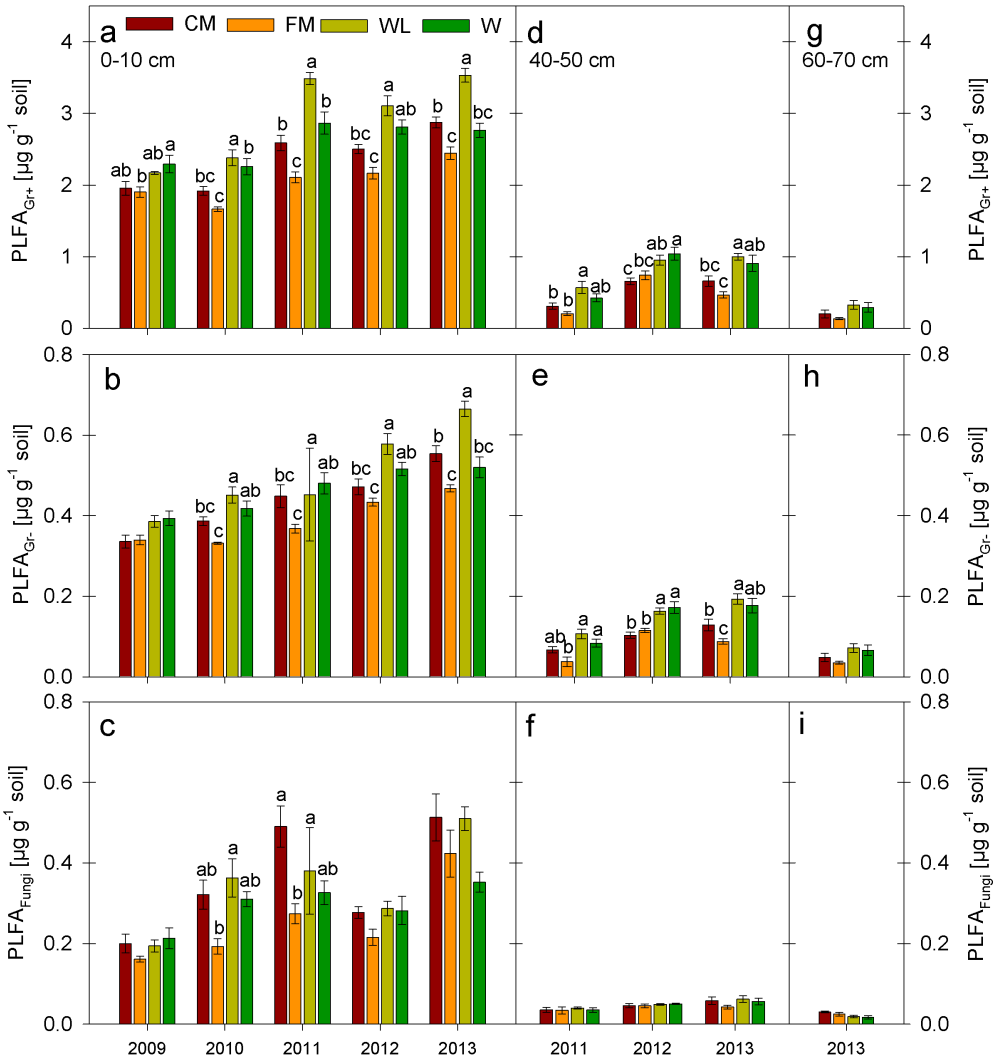

Figure 5.4: Total amount of gram-positive bacteria ($PLFA_{Gr^+}$), gram-negative bacteria ($PLFA_{Gr^-}$) and fungi in 0-10 cm (a, b, c), 40-50 cm (d, e, f) and 60-70 cm (g, h, i) depth. Different letters above bars indicate statistically significant differences between treatments at this date (Tukey HSD: $p < 0.05$). Error bars indicate standard error (n=4). Results of 2009 and 2010 were published previously in Kramer et al. (2013). For legend see Figure 5.2.

did not significantly affect the amount of SOC. In contrast to SOC, maize-derived C was detected in the EOC pool in the rooted zone (Fig. 5.1f) as well as in the root-free zone (60-70 cm depth, Fig. 5.1i). Surprisingly, maize C incorporation was higher in the root-free zone compared to the rooted zone, while the EOC content was similar in all treatments and subsoil depths (Fig. 5.2f, i). The contribution

of maize-derived C in the subsoil EOC pool was highest in the CM treatment in the root-free zone in 2013. However, treatment did not affect the incorporation of maize-derived C in both subsoil depths.

The proportion of maize-derived C in C_{mic} showed the same trend as EOC, with a higher relative contribution of maize-derived C in the root-free zone (Fig. 5.1j) than in the rooted-zone (Fig. 5.1g). The incorporation of maize-derived C into C_{mic} in the root-free zone was 30%, 26% and 21% in the CM, WL and FM treatments, respectively, after five vegetation periods, but the differences were not significant. In the rooted zone, the incorporation of maize-derived C into C_{mic} was affected by treatment with greater maize-derived C incorporation into microorganisms in the maize treatments than in the WL treatment (treatment effect: $F_{2, 9} = 7.53$, $p = 0.014$). Microbial biomass varied between 18 and 69 µg g^{-1} soil in the rooted zone with higher microbial biomass in the wheat treatments (Fig. 5.2g, treatment effect: $F_{2, 9} = 19.49$, $p < 0.001$), while C_{mic} in the deeper root-free zone ranged between 19 and 30 µg g^{-1} soil (Fig. 5.2j) but was unaffected by treatment.

A maize-derived C label was detected in all microbial biomarker PLFAs in both subsoil depths. In 2013 in the rooted zone, Gr^+ and Gr^- bacterial PLFA-C showed similar relative amounts of maize-derived C label, with 14 to 18% in the maize treatments and 5 to 7% in the WL treatment (Fig 5.3d, e). However, variations in maize-derived C incorporation by treatment was only significant in the Gr^- bacterial PLFA ($F_{2, 8} = 8.49$, $p = 0.008$). By contrast, maize-derived C incorporation differed between Gr^+ and Gr^- bacterial PLFAs in the root-free zone. Gr^- bacteria had a higher maize C label of approximately 20% in the maize plant treatments (Fig. 5.3h), whereas Gr^+ bacterial PLFA-C had a maize C label of between 5% and 9% independent of treatment (Fig. 5.3g). The abundances of both Gr^+ (Fig 5.4d, g) and Gr^- bacteria (Fig. 5.4e, h) decreased from 40-50 to 60-70 cm depths. However, abundances of Gr^+ and Gr^- bacteria were affected by treatment in the rooted zone (treatment effect: $PLFA_{Gr^+}$ $F_{3, 12} = 14.14$, $p < 0.001$ and $PLFA_{Gr^-}$ $F_{3, 12} = 18.54$, $p < 0.001$).

As in the topsoil, the incorporation of maize-derived C in subsoil was higher in the fungal PLFA 18:2ω6,9 (Fig. 5.3f) than in the bacterial PLFAs. Maize-derived C in the fungal PLFA was highest in 2011 at 66% in the CM treatment in the rooted zone. The maize C signal in 18:2ω6,9 was significantly higher (treatment effect: $F_{2,\,8} = 35.90$, $p < 0.001$) in the maize treatments than in the WL treatment after five vegetation periods. But in contrast to bacterial PLFAs, the maize-derived C in the fungal PLFA decreased over time in the rooted zone, whereas the abundance of fungi remained stable (Fig. 5.3f, Fig. 5.4f). Treatment affected fungal abundance neither in the rooted (Fig. 5.4f) nor in the root-free zone (Fig. 5.4i). In 2013, the incorporation of maize-derived C in the fungal PLFA was higher in the root-free zone than in the rooted zone in all treatments with 53%, 43% and 28% in the FM, CM and WL treatments, respectively (Fig 5.3i).

Sampling date also affected the absolute C pool sizes, with the exception of EOC in the rooted zone (Figs. 5.2, 5.4). The effect of sampling date was present in the relative incorporation of maize-derived C in total SOC, Gr^+ bacterial PLFA-C and fungal PLFA-C (Table S5.5).

5.5 Discussion

Previous studies of the same field clarified the input of C derived from maize roots, rhizodeposition and litter as prerequisites for understanding the quantity and quality of microbial food resources (Kramer et al. 2012). Root biomass of wheat and maize was measured at the same plots as this experiment at the 0-10 cm depth once in the initial year in July 2009 (Kramer et al. 2012; Pausch et al. 2013), and estimated in the following years. We assume that root biomass and rhizodeposition was similar during each vegetation period. In 2009, wheat root biomass C was found to be much higher (0.47 mg C g^{-1} soil) than that of maize (0.15 mg C g^{-1} soil, excluding crown roots). In the 40-50 cm subsoil depth, root distribution decreased with increasing depth to 0.02 mg C g^{-1} soil for wheat and 0.01 mg C g^{-1} soil for maize plots, suggesting that root-derived C supply in the subsoil was low in each of the

treatments in the rooted zone (Kramer et al. 2013) and negligible in the root-free zone. Wheat was grown on the WL and W plots to maintain the habitat function of the soil without changing its isotopic signature. However, higher amounts of organic C as well as microbial and fungal biomass in the wheat treatments indicated that more C entered the soil under wheat cultivation than under maize cultivation.

The rhizodeposition of maize roots in topsoil was estimated to be 0.37 mg C g^{-1} soil in July 2009 (Pausch et al. 2013). In spring of each year, the crown roots of the maize plants were incorporated into the soil by chisel plough tillage, which introduced an additional 8.0 mg C g^{-1} soil per year. In November of each year, the application of maize shoot litter had supplied 2.54 mg C g^{-1} soil per year on each WL and CM plot (Kramer et al. 2012). Assuming that root biomass and rhizodeposition were equal in each vegetation period, we calculated the total root-derived maize C in the FM treatment as 34.6 mg C g^{-1} soil and the total shoot-derived maize C as 10.2 mg C g^{-1} soil in the WL treatment. In CM plots, which combined both C sources, the total maize-derived C input was calculated as 44.8 mg C g^{-1} soil.

Root- and shoot-derived C incorporation into SOC, EOC, bacteria and fungi of topsoil

The stable isotope approach was used to follow the incorporation of maize C from roots and shoot litter into different abiotic and biotic C pools of soil over a period of five years. A low root-derived C incorporation was detected in SOC at the end of the first vegetation period in the topsoil. Since our soil sampling was performed between plant rows, our study yielded lower C incorporation into the SOC fraction than Balesdent & Balabane (1992) who investigated rhizosphere soil. The increase in maize-derived C in SOC in the second year was caused mainly by tillage (to 12 cm), mixing rhizosphere and bulk soil at the beginning of the second season. During the following years, the incorporation of root-derived maize C increased only slightly, by approximately 9%. Further development of our agro-ecosystem would probably lead to a further increase of maize-derived C in this fraction. This

assumption is supported by results of Kramer & Gleixner (2006) who estimated a relative amount of maize-derived C in the SOC pool of 14% after a period of 23 years. The addition of maize shoot material on the CM plots increased the relative maize-derived C label in SOC up to 17% at our field site, whereas Kramer & Gleixner (2006) found a C incorporation of both root- and shoot-derived maize C of 31% into SOC after 39 years.

The EOC pool represents a more labile fraction of soil organic C and is an important resource for the soil microbial community. In the present study, the total EOC pool increased over time, especially in plots planted with wheat, indicating that a certain amount of EOC was derived from wheat rhizodeposition. Although total root C input was threefold higher than total shoot C input, root-derived C was only slightly higher in the EOC pool in the FM plots at 15%, while shoot-derived C in the WL plots was 11%. This is noteworthy because of differences in the chemical composition of root- and shoot-derived C sources, with a higher proportion of recalcitrant compounds in roots (Amin et al. 2013). They found that maize leaves have a higher soluble C fraction of 27% of dry plant matter, whereas the soluble C fraction of maize roots represents only 16%. Marx et al. (2007) showed that low amounts of C derived from rhizodeposition were incorporated into the EOC pool in the rhizosphere, while in bulk soil rhizodeposit-derived C was present only in C_{mic} and CO_2. Recent research provides evidence that this labile plant C is utilized efficiently by microorganisms and thus becomes the main precursor of stable SOM by promoting aggregation and chemical bonding to minerals (Cotrufo et al. 2013).

We detected a stronger maize signal (up to 60%) in the C_{mic} pool than in the EOC and SOC pools, suggesting that soil microorganisms efficiently used the supplied maize sources. Although pool sizes of root- and shoot-derived maize C were different, similar relative amounts of maize root and shoot C were assimilated into total microbial biomass. Therefore, soil microorganisms used the shoot-derived C to a greater degree than root-derived C, which is in accordance with findings of Puget & Drinkwater (2001) and Rasse et al. (2005). Both studies assumed that root-derived C is more stable than shoot-derived C in soil. Rasse et al. (2005)

calculated that the mean residence time of root-derived C in soils was 2.4 times higher than residence time of shoot-derived C.

The use of PLFA biomarkers made it possible to specify the incorporation of root- and shoot-derived C into bacteria and fungi. In general, fungi incorporated maize-derived C to a greater degree than bacteria. These findings are supported by Rubino et al. (2009), who found a label incorporation of an added C source of 13 to 61% in bacteria and up to 78% in saprotrophic fungi. We found an additive effect of root- and shoot-derived C incorporation (CM treatment) for saprotrophic fungi but not for bacteria. Presumably, this is related to differences in fungal and bacterial growth: fungi are able to grow towards unevenly distributed food sources, whereas bacteria depend on transport of labile C fractions (Poll et al. 2006). High incorporation of root-derived C into Gr^- bacteria has been reported earlier using the PLFA biomarkers 16:1ω7 and 18:1ω7 (Treonis et al. 2004; Denef et al. 2009; Tavi et al. 2013). Although we could not detect these biomarkers by GC-C-IRMS, we found a high maize-derived C incorporation into the Gr^- bacteria biomarker PLFA cy17:0. In general, the relative maize-derived C label of bacteria may not originate exclusively and directly from rhizodeposition or litter decomposition, but also from C transfer from primary to secondary microbial decomposers through trophic interactions or cross-feeding (Seth & Taga 2014). The relative contribution of direct feeding on different resources and indirect feeding (cross-feeding) was investigated in a microcosm experiment using highly labeled single compounds (glucose, cellulose) and complex substrates (maize roots and shoots) (Kramer et al. 2016). The authors concluded that key players from all domains (bacteria, fungi and protists) were involved in substrate decomposition, irrespective of complexity or recalcitrance of C resources.

Since ergosterol is the predominant sterol in cell membranes of saprotrophic fungi (Klamer & Bååth 2004) and is not present in arbuscular mycorrhizal (AM) fungi (Olsson et al. 2003), we assumed that saprotrophic fungi have utilized most of the maize-derived C compared to symbiotic fungi. Our results of the first two vegetation periods indicate that AM colonization of maize roots was low at our experimental

field site (J. Moll, pers. comm.). High incorporation of ^{13}C in saprotrophic fungi has also been detected for an organic arable field and for grassland soils (Butler et al. 2003; Denef et al. 2009; Tavi et al. 2013). Further support comes from a pulse labeling experiment indicating that saprotrophic fungi utilized most of the plant-fixed C as quickly as two days after labeling (Pausch et al. 2016).

In summary, we could partly verify our first hypothesis that the incorporation of plant-derived C in the different C pools in topsoil (SOC, EOC, C_{mic}, Gr^+ bacteria, Gr^- bacteria and fungi) depends on C origin. The relative incorporation value of root- and shoot-derived C was similar in the different soil C pools, likely due to high amounts of more recalcitrant root material and lower amounts of more labile shoot litter material in topsoil. This suggests that the recalcitrance of a substrate was more important for C incorporation into bacteria and fungi than the absolute amount of a substrate. In our second hypothesis, we assumed that shoot litter application fostered the incorporation of maize-derived C in the microbial pools due to higher total C input. An additive effect of root- and shoot-derived C was found in almost all investigated soil C pools in topsoil, which confirmed our second hypothesis.

Contribution of maize-derived C in subsoil

Subsoil is different than topsoil, as evaluated by pedological, environmental and physiochemical properties (Salomé et al. 2010). While types of crops and management practices strongly affect soil C pools in topsoil, the impacts of these factors are largely neglected in subsoil, especially below the plough zone (Kramer et al. 2013). For this reason we assumed the maize C label to be low in the subsoil SOC pool in the early years of the experiment. Even after five years of maize cultivation, maize root-derived C was approximately 5% in the SOC pool. Kramer & Gleixner (2008) showed a maize-derived C incorporation of only 6% in SOC at 20 to 40 cm depth even after 40 years of continuous maize cultivation, suggesting a low contribution of fresh plant material even after decades. The absence of maize roots and their

rhizodeposits in the root-free zone reduced the root-derived C incorporation to approximately 2% in our study. The long turnover time of C in the SOC pool is also supported by data of Kramer & Gleixner (2008) showing only 3% of maize-derived C in SOC after 40 years of continuous maize cultivation. Although shoot litter C was applied on the soil surface and incorporated only to 12 cm depth by chisel plough tillage, maize litter C was also found in the rooted zone after five years of maize cultivation. Earthworm burrows, root channels and desiccation cracks (Natsch et al. 1996) may contribute the mobilization of maize root and shoot litter in the root-free subsoil. As the incorporation of fresh maize C sources in SOC are low and the available old SOC material is protected by mineral interactions in subsoil (Eusterhues et al. 2003), the EOC pool as a mobile fraction of SOC is assumed to be an important resource for soil microorganisms in deeper soil layers (Marschner & Bredow 2002). We found a root-derived C incorporation of 8% into the EOC pool in the rooted zone, while the shoot-derived C incorporation was only 2%. In the root-free zone, the incorporation of maize C into the EOC pool was higher than in the rooted zone due mainly to transport of labile EOC sources from the above soil layers into the root-free soil by seepage water and a low abundance of decomposer organisms using these compounds. However, Kindler et al. (2011) concluded that this vertical flux of fresh plant C represents a significant supply of C for the endogenous microorganisms in deeper soil layers.

Maize-derived C was incorporated not only into the microbial biomass of the topsoil, but also into microbial pools of deeper soil layers. Although more maize-derived C was present in the rooted zone than in the root-free zone, soil microorganisms benefited from maize C especially in the deepest soil layer. Notably, this was independent of the source (either root or shoot). We assumed effective incorporation of the low amount of fresh maize C into microbial biomass irrespective of origin in the root-free soil layer.

In the present experiment, Gr^+ bacteria, Gr^- bacteria and saprotrophic fungi used maize-derived C in deeper soil layers, but the extent of use was group specific. Gr^- bacteria incorporated relatively more maize-derived C in deeper layers than Gr^+

bacteria. This may have been due to either the preferential transport of Gr^- bacteria within the soil profile (Dibbern et al. 2014) or by differences in feeding strategies of the two bacterial groups. Gr^+ bacteria are known to use more recalcitrant C sources derived from SOC, whereas Gr^- bacteria utilize more fresh maize C in the subsoil (Waldrop & Firestone 2004; Kramer & Gleixner 2008). High incorporation of maize-derived C in the 18:2ω6,9 PLFA was also found in both subsoil layers. In the rooted zone, saprotrophic fungi used more root- than shoot-derived C, while C incorporation in the deepest soil layer was independent of origin. The high usage of shoot-derived C in the root-free zone was possibly due to the ability of fungal hyphae to grow toward their substrates and exploit C sources (Miller & Fitzsimons 2011). In summary, fungi in subsoils benefited more from root- and shoot-derived maize than either Gr^- or Gr^+ bacteria. Further, the presence of a C source was more important than the C origin, especially for soil microorganisms in the root-free zone.

5.6 Conclusion

We attempted to quantify the incorporation of maize root- and shoot-derived C into microbial C pools in a soil profile by using stable isotopes under field conditions over several years. In consideration of all of our findings, highest incorporation of root- and shoot-derived C was found in saprotrophic fungi, indicating a key role in processing plant-derived C compounds in topsoil and in subsoil. Notably, in topsoil, similar relative amounts of root- and shoot-derived maize C were incorporated in all investigated C pools although the input of root-derived C was three times higher than the input of shoot-derived C. In the root-free subsoil, the presence of a C source was more important than the origin of C. This study provides unique data spanning several years providing opportunities for future modeling of C transfer through the belowground food web with major implications for C assimilation of bacteria and fungi in agricultural ecosystems.

5.7 Acknowledgements

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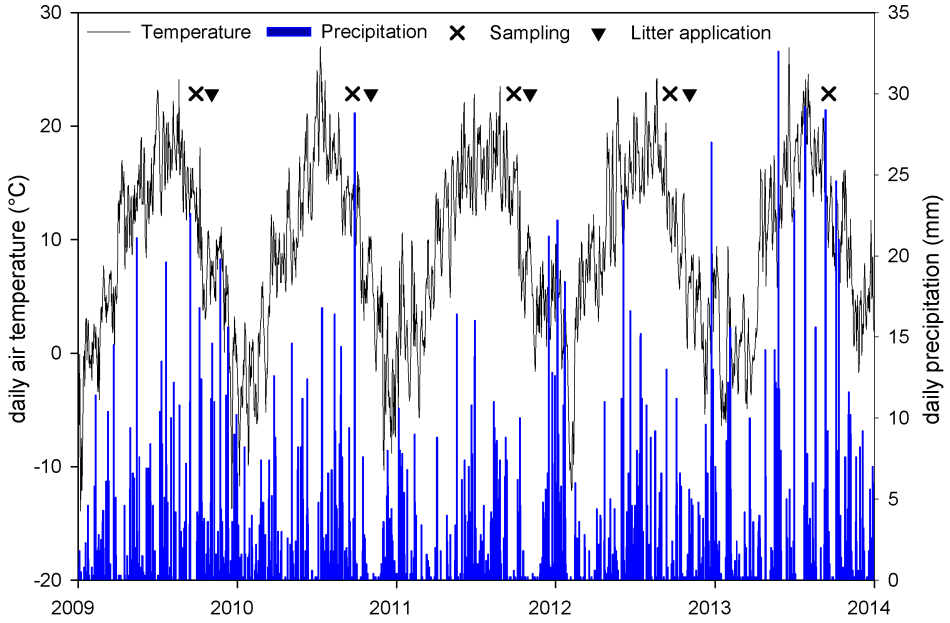
5.8 Supplementary Material



S 5.1: Temperature and precipitation data from the "Wetterstation Göttingen" over the duration of the experiment.

S 5.2: Selected soil properties of the field site modified after Kramer et al. (2012) and Pausch & Kuzyakov (2012).

Horizon*	Depth [cm]	Texture# clay/silt/sand [%] (w/w)	pH ($CaCl_2$)	Bulk density [g cm^{-3}]	SOC [g kg^{-1}]	Total N [g kg^{-1}]	C/N	δ13C [‰]
Ap1	0-25	7.0/87.2/5.8	6.0	1.38	12.4	1.3	9.8	-27.4
A(l)p2	25-37	7.1/87.8/5.0	6.2	1.61	6.9	0.8	9.2	-26.5
Btw1	37-65	7.1/87.7/5.1	6.6	1.55	3.3	0.4	8.9	-26.1
Btw2	> 65	6.8/88.4/4.8	7.0	1.68	1.8	0.3	6.9	-25.5

* Classification according to KA 5 (2005).
# Texture according to the German classification system.

S 5.3: Fertilization practice during the experiment.

Date	Fertilizer	Wheat plots N [kg ha^{-1}]	Maize plots N [kg ha^{-1}]	Maize plots P [kg ha^{-1}]
13.03.2009	Granular SSA	21	21	
03.04.2009	Ammonium nitrate urea solution	50		
20.04.2009	Ammonium nitrate urea solution	50		
22.04.2009	Ammonium nitrate urea solution		122	
23.04.2009	Diammonium phosphate		32	83
25.05.2009	Ammonium nitrate urea solution	40		
03.06.2009	Ammonium nitrate urea solution	40		
14.04.2010	Diammonium phosphate		32	83
20.04.2010	Ammonium nitrate urea solution	61	79	
09.06.2010	Ammonium nitrate urea solution	40		
28.03.2011	Ammonium nitrate urea solution		118	
11.04.2011	Diammonium phosphate		32	83
15.04.2011	Ammonium nitrate urea solution	58		
24.05.2011	Ammonium nitrate urea solution	79		
22.03.2012	Ammonium nitrate urea solution		72	
04.04.2012	Ammonium nitrate urea solution	61		
16.04.2012	Ammonium sulphate		20	
16.04.2012	Diammonium phosphate		19	48
15.05.2012	Ammonium nitrate urea solution	72		
23.04.2013	Ammonium sulphate		20	
23.04.2013	Diammonium phosphate		19	48
24.04.2013	Ammonium nitrate urea solution	72		
25.04.2013	Ammonium nitrate urea solution		76	
11.06.2013	Ammonium nitrate urea solution	72		

S 5.4: Herbicide application during the experiment.

Date	Wheat plots	Maize plots
22.10.2008	Arena C 0.41 l ha^{-1}	Arena C 0.41 l ha^{-1}
03.04.2009	ARTUS 25 g ha^{-1}	
	Attribut 70 g ha^{-1}	
	CCC 720 1 l ha^{-1}	
	Primus 50 ml ha^{-1}	
15.04.2009		Roundup TURBO 1.2 kg ha^{-1}
23.04.2009		Mesurol liquid 0.2 l ha^{-1}
		TMTD 98% Satec 0.1 kg ha^{-1}
25.04.2009	Bravo 500 SC 0.5 l ha^{-1}	
	CCC 720 0.5 l ha^{-1}	
	Input 0.8 l ha^{-1}	
	Moddus 0.15 l ha^{-1}	
19.05.2009	Matador 300 0.6 l ha^{-1}	
	U 46 M-Fluid 1.3 l ha^{-1}	
27.05.2009	CLICK 0.8 l ha^{-1}	
	MILAGRO 0.8 l ha^{-1}	
	Peak 16 g ha^{-1}	
05.06.2009		Buildock 0.3 l ha^{-1}
		Matador 300 0.5 l ha^{-1}
		Taspa 0.3 l ha^{-1}
		Pirimor Granulat 0.2 kg ha^{-1}
14.04.2010		Mesurol liquid 0.2 l ha^{-1}
		TMTD 98% Satec 100 g ha^{-1}
29.04.2010	Biathol 70 g ha^{-1}	
	MCPA Berghoff 1.3 l ha^{-1}	
05.06.2010		CLICK 1 l ha^{-1}
		MILAGRO 0.5 l ha^{-1}
		Peak 14 g ha^{-1}
21.03.2011		Glyphosate 3 l ha^{-1}
19.05.2011		CLICK 1 l ha^{-1}
		MILAGRO 0.5 l ha^{-1}
		Peak 14 g ha^{-1}
23.05.2011	Biathol 50 g ha^{-1}	
	Pointer 20 g ha^{-1}	
	Topik 0.5 l ha^{-1}	
01.06.2011	Input 1 l ha^{-1}	
28.09.2011		Glyphosate 4 l ha^{-1}
09.03.2012	Roundup UltraMax 3 l ha^{-1}	
15.05.2012	Dirigent SX 25 g ha^{-1}	
	PRIMUS 75 ml ha^{-1}	
24.05.2012		Calaris 0.8 l ha^{-1}
		Tomigan 180 0.6 l ha^{-1}
		Dual Gold 0.8 l ha^{-1}
15.06.2012	Input 1 l ha^{-1}	
	Tomigan 180 0.9 l ha^{-1}	
15.04.2013	Glyphosate 4 l ha^{-1}	Glyphosate 4 l ha^{-1}
17.05.2013	PRIMUS 50 ml ha^{-1}	
	GROPPER 25 g ha^{-1}	
06.06.2013		Peak 20 g ha^{-1}
		EFFIGO 0.35 l ha^{-1}
		MILAGRO 0.5 l ha^{-1}
		Terbuthylazin 500 0.85 l ha^{-1}
28.06.2013	Taspa 0.31 l ha^{-1}	
	Shock DOWN 0.11 l ha^{-1}	

S 5.5: Treatment and date effects tested with repeated measures ANOVA for 0-10 cm and 40-50 cm depth and one-way ANOVA for 60-70 cm depth. The table shows F-ratios and P-values in parantheses. T = treatment, D = date, T*D = interaction between treatment and date.

	Parameter	Factor	0-10 cm	40-50 cm	60-70 cm
Absolute C pool sizes	SOC	T	**10.02 (0.001)**	1.66 (0.227)	0.60 (0.621)
		D	**4.08 (0.006)**	**41.51 (< 0.001)**	-
		T*D	1.34 (0.228)	1.05 (0.418)	-
	EOC	T	**22.66 (< 0.001)**	0.37 (0.774)	0.12 (0.944)
		D	**43.15 (< 0.001)**	0.13 (0.878)	-
		T*D	**2.08 (0.037)**	0.72 (0.632)	-
	C_{mic}	T	1.83 (0.194)	**19.49 (< 0.001)**	0.94 (0.451)
		D	**31.08 (< 0.001)**	**16.66 (< 0.001)**	-
		T*D	**4.55 (< 0.001)**	1.28 (0.307)	-
	$PLFA_{Gr+}$	T	**59.67 (< 0.001)**	**14.14 (< 0.001)**	2.71 (0.092)
		D	**74.81 (< 0.001)**	**76.83 (< 0.001)**	-
		T*D	**3.81 (0.001)**	2.41 (0.058)	-
	$PLFA_{Gr-}$	T	**18.01 (< 0.001)**	**18.54 (< 0.001)**	2.89 (0.079)
		D	**103.2 (< 0.001)**	**70.18 (< 0.001)**	-
		T*D	**3.81 (0.001)**	**2.97 (0.026)**	-
	$PLFA_{Fungi}$	T	**11.89 (0.001)**	1.28 (0.327)	2.62 (0.099)
		D	**34.74 (< 0.001)**	**9.16 (0.001)**	-
		T*D	**2.31 (0.022)**	0.48 (0.817)	-
	Ergosterol	T	**17.10 (< 0.001)**	-	-
		D	**25.53 (< 0.001)**	-	-
		T*D	**3.58 (0.001)**	-	-
Relative ^{13}C incorporation	SOC	T	**11.14 (0.004)**	1.70 (0.237)	3.66 (0.069)
		D	**43.59 (< 0.001)**	**179.8 (< 0.001)**	-
		T*D	**3.76 (0.003)**	**10.03 (< 0.001)**	-
	EOC	T	**14.22 (0.002)**	2.44 (0.177)	0.61 (0.580)
		D	**39.31 (< 0.001)**	2.40 (0.127)	-
		T*D	2.06 (0.070)	2.43 (0.096)	-
	C_{mic}	T	**31.82 (< 0.001)**	**7.53 (0.014)**	0.35 (0.717)
		D	**48.05 (< 0.001)**	2.33 (0.129)	-
		T*D	**3.66 (0.005)**	1.26 (0.326)	-
	$PLFA_{Gr+}$	T	**13.40 (0.003)**	1.16 (0.356)	1.36 (0.305)
		D	**49.89 (< 0.001)**	**6.76 (0.006)**	-
		T*D	**5.18 (< 0.001)**	**3.44 (0.030)**	-
	$PLFA_{Gr-}$	T	**20.90 (< 0.001)**	**8.49 (0.008)**	**4.91 (0.036)**
		D	**73.90 (< 0.001)**	3.45 (0.054)	-
		T*D	**3.75 (0.003)**	1.11 (0.384)	-
	$PLFA_{Fungi}$	T	**29.35 (< 0.001)**	**54.03 (< 0.001)**	3.33 (0.083)
		D	**19.38 (< 0.001)**	**17.70 (< 0.001)**	-
		T*D	**3.07 (0.011)**	2.83 (0.065)	-
	Ergosterol	T	**18.77 (0.005)**	-	-
		D	**26.77 (< 0.001)**	-	-
		T*D	**3.89 (0.006)**	-	-

6 Carbon flow from litter through soil microorganisms: From incorporation rates to mean residence times in bacteria and fungi

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Karolin Müller[1], Sven Marhan[1], Ellen Kandeler[1], Christian Poll[1]

[1] Institute of Soil Science and Land Evaluation, Soil Biology, University of Hohenheim, Emil-Wolff-Straße 27, 70599 Stuttgart, Germany

6.1 Abstract

Resource quality and availability modify the microbial contribution to soil organic matter turnover and formation. We created a microbial hotspot at the soil-litter interface in a microcosm experiment to better understand and integrate specific microbial habitats into C turnover models. Reciprocal transplantation of ^{13}C and ^{12}C litter on top of soil cores allowed us to follow C flow into specific members of the microbial food web (bacteria and fungi) and to calculate the turnover times of litter-derived C in these microorganisms at three different stages of maize litter decomposition; early stage (0 to 4 days), intermediate stage (5 to 12 days) and later stage (29 to 36 days).

Litter age influenced the incorporation rate of C into bacteria and fungi and subsequent turnover in phospholipid fatty acid (PLFA) biomarkers. When fresh litter was applied, both fungi and bacteria were able to assimilate labile litter C in the early stage of decomposition, while lower substrate quality in the intermediate stage of decomposition promoted fungal utilization. Utilization of complex litter C sources was minor in both fungi and bacteria in the later stage of decomposition. Different bacterial substrate utilization strategies were reflected by either a decline of the isotopic signal after exchange of ^{13}C by ^{12}C litter or by storage and/or reuse of previously released microbial ^{13}C. The mean residence time of C in the fungal PLFA 18:2ω6,9 was estimated from 46 to 32 days, which is the same or shorter time than that of bacterial PLFAs. This highlights the role of fungi in rapid turnover processes of plant residues, with implications for implementation of bacterial and fungal processes into C turnover models.

6.2 Introduction

Soil organic carbon (SOC) is the largest active carbon (C) pool in terrestrial ecosystems. Historically it was thought that most SOC originates directly from plant residues, but it is now accepted that plant-derived C primarily enters the SOC

pool indirectly via soil microorganisms (Kögel-Knabner 2002; Liang & Balser 2011; Miltner et al. 2011). Living microorganisms represent less than 5% of total SOC (Dalal 1998), but these organisms account for a rapid and iterative decomposition process of C pools in soils. By contrast, the contribution of non-living microbial necromass to the formation of SOC is estimated to be 50-80% (Simpson et al. 2007; Liang & Balser 2011). This microbially-derived C is assumed to be chemically or structurally complex with reduced decomposability (Moore et al. 2005; Six et al. 2006) leading to higher persistence in soil (Simpson et al. 2007; Kindler et al. 2009; Gleixner 2013). Due to the heterogeneity of soil, less than 5% of the available soil space contains microbial hotspots (Nannipieri et al. 2003), but SOC decomposition processes in these hotspots are 10 to 100 times higher than in the bulk soil (Kuzyakov & Blagodatskaya 2015).

The detritusphere, a microbial habitat which includes the litter layer and the soil influenced by litter, harbors a tremendous number of microorganisms which perform ecologically important biogeochemical processes, such as the decomposition and turnover of plant litter C (Schimel & Schaeffer 2012; Gleixner 2013). In these microbial hotspots, accelerated process rates and intensive interactions between C pools occur in a small soil volume (Kuzyakov & Blagodatskaya 2015), which is restricted to 3-4 mm adjacent to the litter layer (Gaillard et al. 1999; Poll et al. 2008). Gaillard et al. (2003) demonstrated the importance of detritusphere decomposition processes with 5 to 11 fold higher mineralization rates in the adjacent soil of plant residues in comparison to uninfluenced bulk soil mineralization processes.

Historically, it was thought that resource partitioning between bacteria and fungi in the degradation process of complex substrates was an important driver of soil microbial diversity (Zhou et al. 2002). A recent study by Kramer et al. (2016), in a decomposition experiment using different plant C resources, provided evidence that a clear separation between bacterial usage of labile C and fungal usage of more complex C sources did not occur. This discrepancy has fueled an ongoing debate on the contributions of distinct microbial groups to different stages of the decomposition process (Strickland & Rousk 2010).

Despite the important role of microorganisms in formation and degradation of SOC, internal bacterial and fungal C turnover processes have rarely been reported in the literature. Prokaryotic bacterial and eukaryotic fungal cells vary substantially in their chemical composition (Lengeler et al. 1999) and thus likely influence the decomposition rates of bacterial and fungal necromass. Kindler et al. (2009) separated the C introduced to the soil by cells of the Gram-negative bacterium *Escherichia coli* into fast and slow pools with half-lives of 7 days and 1.3 years. In contrast, fungal biomass C appears to be more persistent in soil, as shown by a half-life of 11.3 days for the easily degradable C pool and 3.8 years for the stable C pool (Schweigert et al. 2015), which has led to the assumption that fungal biomass contributes more to stable SOC than bacteria (Moore et al. 2005; Jastrow et al. 2007; Strickland & Rousk 2010). Knowledge of group specific C turnover times is an important pre-requisite for accurate C modelling of agricultural management effects on soil C stocks (e.g. RothC model (Jenkinson et al. 1990)) and on pesticide degradation (e.g. PECCAD (Pagel et al. 2014)), but the role of microorganisms is rarely included in most of the models (Manzoni & Porporato 2009). Within these models, exponential decay functions have been widely used to calculate C turnover times of newly introduced plant resources. They are often valid, however, only for early stages of litter decomposition and cannot be used to accurately fit decay processes from later decomposition phases (Berg & McClaugherty 2003), because they assume constant C pool sizes (steady state) and proportional C fluxes (first order kinetics) during decomposition (Studer et al. 2014). However, the detritusphere is described as a continuum ranging from intact plant cell material to progressively decaying plant residues (Dungait et al. 2012), which makes it challenging to model and predict temporal small-scale turnover processes during litter decomposition.

Our aims were (i) to quantify the utilization of litter-derived C by bacteria and fungi in the detritusphere and (ii) to assess subsequent C turnover in microbial C pools as a function of residue age by using highly ^{13}C labeled maize litter placed on top of soil cores in a 60 day microcosm experiment. A reciprocal transplantation

of labeled (^{13}C) and unlabeled (^{12}C) maize litter permitted quantification of the fate of the ^{13}C in soil organic C (SOC), extractable organic C (EOC), microbial biomass (C_{mic}), and different phospholipid fatty acid (PLFA) molecules; the last as biomarkers for bacteria and fungi. We hypothesized that (i) both bacteria and fungi use labile C from fresh plant residues; (ii) fungi use more complex C sources in the later stages of decomposition due both to their capacity to grow towards a substrate and their wider range of extracellular enzymatic capabilities as compared to bacteria (de Boer et al. 2005); and (iii) the turnover of C is faster in bacteria than in fungi due to the shorter life spans of prokaryotic bacterial cells and longer life spans of eukaryotic fungal cells (Rousk & Bååth 2011).

6.3 Materials and Methods

Soil and plant residues

Soil was taken from an experiment on an arable field near Göttingen (Germany, 51°33'N, 91°53'E; 158 m a.s.l.) in May 2014. The dominant soil types on the field were Luvisol and Cambisol with partially stagnic properties (IUSS 2007). For a detailed description of the field experiment see Kramer et al. (2012). Samples were taken from the topsoil (0-10 cm) of wheat plots with a known long-term C3 cropping history, at least 30 years, resulting in a soil $\delta^{13}C$ value of –27.3‰. Clay, silt and sand fractions were 7.0, 87.2 and 5.8%, respectively. Total C and N were 12.4 g kg^{-1} and 1.3 g kg^{-1} and pH was 6.0 (Kramer et al. 2012). For the incubation experiment, senescent maize leaves with different ^{13}C labels (1.2 atom% ^{13}C and 92 atom% ^{13}C, IsoLife, Wageningen, The Netherlands) were used. In the following text, maize leaves with low ^{13}C content will be named "^{12}C litter" and maize leaves with high ^{13}C content "^{13}C litter". Air-dried maize leaves were cut into small pieces (approx. 3 mm) and stored at -20 °C until the start of the experiment.

Microcosms

Fresh soil was sieved (< 2 mm), homogenized and used to fill cylinders (diameter $= 5.6$ cm, height $= 4$ cm). Each core contained 45 g air-dried soil which was compacted to a height of 1.5 cm, resulting in a bulk density of 1.2 g cm^{-3}. Soils were saturated with 0.01 M $CaCl_2$ solution, placed on ceramic plates and adjusted to a matric potential of -63 hPa. We used $CaCl_2$ solution to avoid dispersion of clay. For acclimatization to incubation conditions, the cylinders were placed in microcosms (glass containers, 500 ml) and pre-incubated at 20°C for three days.

At the start of the experiment, 0.3 g maize leaves (equal to approx. 250 µg C g^{-1} soil) per cylinder were rewetted with 2 ml 0.01 M $CaCl_2$ solution, placed on the surface and slightly pressed onto the soil to ensure adequate contact between soil and litter. A mesh (500 µm, polyvinyl chloride) was placed between soil and litter to facilitate the transplantation of maize residues between soil cores during the experiment. The microcosms were incubated for 60 days at 20°C. Water content was re-adjusted with 0.01 M $CaCl_2$ solution two times (on days 32 and 44) during the experiment.

Experimental design

The experimental approach consisted of three pulses of ^{13}C labelling representing litter of three ages (early, intermediate and later stage of litter decomposition). ^{13}C and ^{12}C litter was reciprocally transplanted between treatments to guarantee short and distinct labeling phases of the soil by maize litter of different ages (Fig. 6.1).

The following labeling periods were set up: (i) fresh ^{13}C litter, from 0 to 4 days (early stage); (ii) four day old ^{13}C litter, from 5 to 12 days (intermediate stage); and (iii) 28 day old ^{13}C litter, from 29 to 36 days (later stage). Furthermore, a ^{12}C (iv) and ^{13}C (v) control with continuous presence of ^{12}C and ^{13}C litter and a treatment without litter (vi) were also incubated over the duration of the experiment. At the end of each labeling period, ^{13}C litter was replaced by ^{12}C litter of the same age in

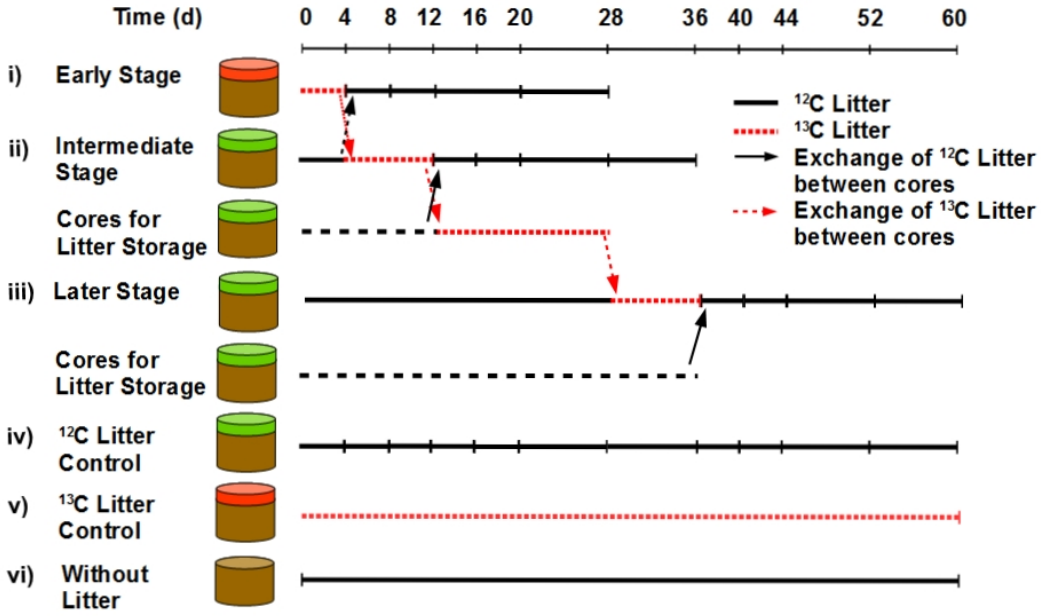

Figure 6.1: Scheme of the reciprocal transplantation of labeled ^{13}C (dotted lines) and unlabeled ^{12}C (solid lines) litter on top of soil cores over a period of 60 days. Dotted lines represent the pulse labeling phases of ^{13}C litter in the respective treatments. The exchange of the litter of the same age was performed among the different treatments, which is shown by the dotted arrows for ^{13}C litter and solid arrows for ^{12}C litter. Dashed lines represent soil cores used only for preparation of enough litter material of a certain decomposition stage. The destructive soil sampling in each treatment is displayed by vertical marks.

order to follow the subsequent ^{13}C turnover in different soil and microbial pools while keeping boundary conditions of this highly active microhabitat as constant as possible. Separate microcosms were sampled 0, 4, 8, 16 and 24 days after the labeling period. We accounted for the fact that transplantation of litter on days 4, 12, and 28 disrupted the fungal hyphae between the litter and the soil (Frey et al. 2003) by raising the mesh on top of all soil cores on these days to exclude an artificial treatment effect. Soils of the ^{12}C control treatment were destructively sampled after 0, 4, 8, 12, 16, 20, 28, 36, 40, 44, 52, and 60 days. Continuously labeled ^{13}C cores and cores without maize litter were established for respiration measurements during the experiment and harvested after 60 days. Each treatment was replicated four times at each sampling date. At each sampling, the mesh with the litter was removed from the soil cylinders. Subsequently, a slice of 3 mm

thickness (approx. 9 g soil) was taken from the top of each core to provide soil samples most strongly influenced by the presence of litter (Poll et al. 2008). Litter and soil samples were immediately frozen after sampling and stored at -28°C until further analyses.

Respiration

Carbon dioxide (CO_2) production was measured at 2 - 4 day intervals during the experiment. Evolved CO_2 was trapped in 1 M NaOH solution and precipitated as $SrCO_3$ with 0.1 M $SrCl_2$. Remaining NaOH was then titrimetrically quantified with 0.1 M HCl. To determine the $\delta^{13}C$ value of CO_2-C, the $SrCO_3$ precipitate was washed with air-free deionized water following the procedure described in Poll et al. (2008). A mass of 0.3 – 0.5 mg of the $SrCO_3$ was weighed into tin capsules and analyzed by elemental analyzer (EA, Euro EA 3000, EuroVector, Milan, Italy) coupled with an isotope ratio mass spectrometer (IRMS, Delta Plus, Thermo Finnigan, Waltham, USA).

Soil organic carbon (SOC), extractable organic C (EOC) and microbial C (C_{mic})

To determine soil organic C (SOC), 0.3 g of soil was dried at 60°C for three days, then ground with a ball mill. A subsample of 15 mg was weighed into a tin capsule and measured for total SOC and isotopic composition as described above. The chloroform-fumigation-extraction method (1.5 g soil extracted with 15 ml 0.025M K_2SO_4 solution) was used to determine microbial biomass carbon (C_{mic}) and $^{13}C_{mic}$ according to Marhan et al. (2010). The fumigation was performed for 24h under vacuum in a desiccator. Microbially bound C was calculated using a k_{EC} factor of 0.45 (Joergensen 1996). To determine the extractable organic C (EOC), samples were treated identically but without fumigation. For isotopic measurement of EOC and C_{mic}, 10 ml aliquots of the supernatant were dried in a vacuum rotary evaporator (RVC 2-25, Martin Christ, Osterode am Harz, Germany) at 60°C. Residue was

ground, 10-15 mg were weighed into tin capsules, and determined as described for SOC. To calculate the $\delta^{13}C$ in C_{mic} the following equation was used

$$\delta^{13}C_{mic} = \frac{c_{nf} \cdot \delta_{nf} - c_f \cdot \delta_f}{c_{nf} - c_n}$$

where c_{nf} and c_f are the corresponding extracted organic C contents (µg C g^{-1} soil) of the non-fumigated and fumigated samples and δ_{nf} and δ_f are the corresponding $\delta^{13}C$ values.

Phospholipid fatty acid (PLFA) analysis

PLFAs were extracted from 4 g soil following the procedure described by Frostegård et al. (1991) with a Bligh & Dyer solution (chloroform, methanol, citrate buffer (pH 4), 1:2:0.8, v/v/v). Lipid fractionation and subsequent estimation of fatty acid methyl esters (FAMEs) were performed according to Kramer et al. (2013). For determination of the $\delta^{13}C$ signature in FAMEs, an HP 6890 Gas Chromatograph (Agilent Inc., USA) coupled via a combustion III Interface (Thermo Finnigan, USA) to a Delta Plus XP mass spectrometer (Thermo Finnigan MAT, Germany) was used according to the procedure described by Müller et al. (2016). In total 30 PLFA peak were detected with GC-FID. After fractionation with Ag^{+}-SPE columns (6ml, Supelco, Palo Alto, USA) we were not able to analyze clear peaks in the 2 and 3 fraction (including monoenoic trans and cis FAMEs), because of either low concentration or weak separation of single fatty acids. The PLFAs i15:0, a15:0, i16:0 and i17:0 were used as biomarkers for gram-positive bacteria, while cy17:0 and 18:2ω6,9 were used as gram-negative bacterial biomarker and fungal biomarker, respectively (Frostegård et al. 1993; Frostegård & Bååth 1996; Zelles 1999). The $\delta^{13}C$ values of all FAMEs were corrected for the addition of a methyl group using a mass balance equation (Denef et al. 2007). The methanol used for methylation had a $\delta^{13}C$ value of -40.23‰.

Calculation of mean residence time (MRT) and statistical analysis

For the calculation of relative amounts of litter-derived ^{13}C in SOC, EOC, C_{mic}, and PLFAs the following equation was used:

$$\%C_{maize} = \frac{\delta_{sample} - \delta^{12}C_{control}}{\delta^{13}C_{litter} - \delta^{12}C_{litter}} \cdot 100$$

where δ_{sample} is the $\delta^{13}C$ value of the respective sample, $\delta^{12}C_{control}$ is the $\delta^{13}C$ value of the ^{12}C control cores, $\delta^{13}C_{litter}$ is the $\delta^{13}C$ value of the ^{13}C maize leaves and $\delta^{12}C_{litter}$ is the $\delta^{13}C$ value of the ^{12}C maize leaves.

Due to heterogeneity of variance, the non-parametric Kruskal-Wallis H test was used. Significance level $\alpha = 0.05$ was applied to test the absolute values of the soil C pools for a treatment effect for each sampling date. All errors were reported as standard errors of the mean (SEM). General nonlinear models (Pinheiro & Bates 2000) were applied to the absolute ^{13}C values using the nlme package (version 3.1.120) in R version 3.1.2 (R Core Team 2014). Mean residence time (MRT) of ^{13}C in the different C pools was obtained by estimating the ^{13}C dilution rate for each of the three labeling treatments using a negative exponential decay function

$$F_t = F_0 \cdot e^{-kt}$$

where F_0 and F_t are the ^{13}C enrichment at time 0 and time t and k is the specific ^{13}C decay rate constant. A double exponential decay function

$$F_t = F_1 \cdot e^{-kt_1} + F_2 \cdot e^{-kt_2}$$

was used to estimate C turnover in the total microbial biomass measured by CFE, to account for differences in the biodegradability of the extracted microbial compounds. MRT (in days) was calculated as the inverse k value. Analysis of variance (ANOVA) was used to test the significance of k in each model.

6.4 Results

C mineralization

The application of maize litter initially increased the respiration rate of soil microorganisms by a factor of approximately 7 in comparison to cores without litter addition (Fig. 6.2a). Over the 60 day period, the isotopic C composition of the added litter had no significant influence on cumulative CO_2 production (Fig. S6.1a). The highest mineralization rate of litter-derived C was detected at the very early stage of litter decomposition at 73.7 $\pm$ 5.4 ng g^{-1} soil h^{-1}, which is equal to 17% of total CO_2-C production in the ^{13}C litter control treatment at day 2 (Fig. 6.2b). Litter-derived C decreased continuously over the first third of the experiment, remaining stable at approximately 9 ng h^{-1} g^{-1} in the ^{13}C control treatment from day 24 until the end of the experiment. After replacing the ^{13}C litter with ^{12}C litter in the respective labeling treatments, ^{12}C litter of the same age was subsequently mineralized and therefore, the ^{13}C label in CO_2 vanished rapidly in all three decomposition phases (Fig. 6.2b).

Nearly 10% of the initially added litter-C was mineralized in the ^{13}C litter control over the 60 days of the experiment (Fig. S6.1b). In the early stage of decomposition, 2.3 $\pm$ 0.2% of the litter C was mineralized within 4 days. The relative value of initial litter C mineralization was slightly higher in the intermediate phase with 2.6 $\pm$ 0.3%, but labeling in the intermediate phase lasted twice as long as in the early stage. During the later phase of decomposition, only 0.5 $\pm$ 0.05% of the introduced litter was mineralized.

^{13}C in SOC, EOC and C_{mic}

The amount of total SOC was stable during the experiment, approximately 11.9 mg g^{-1} soil (Fig. S6.2a). In the early labeling phase, 18.9 µg litter C g^{-1} soil was incorporated into SOC and the incorporation of litter-derived C during the intermediate and later phase labeling was 12 µg g^{-1} soil and 4.7 µg g^{-1} soil,

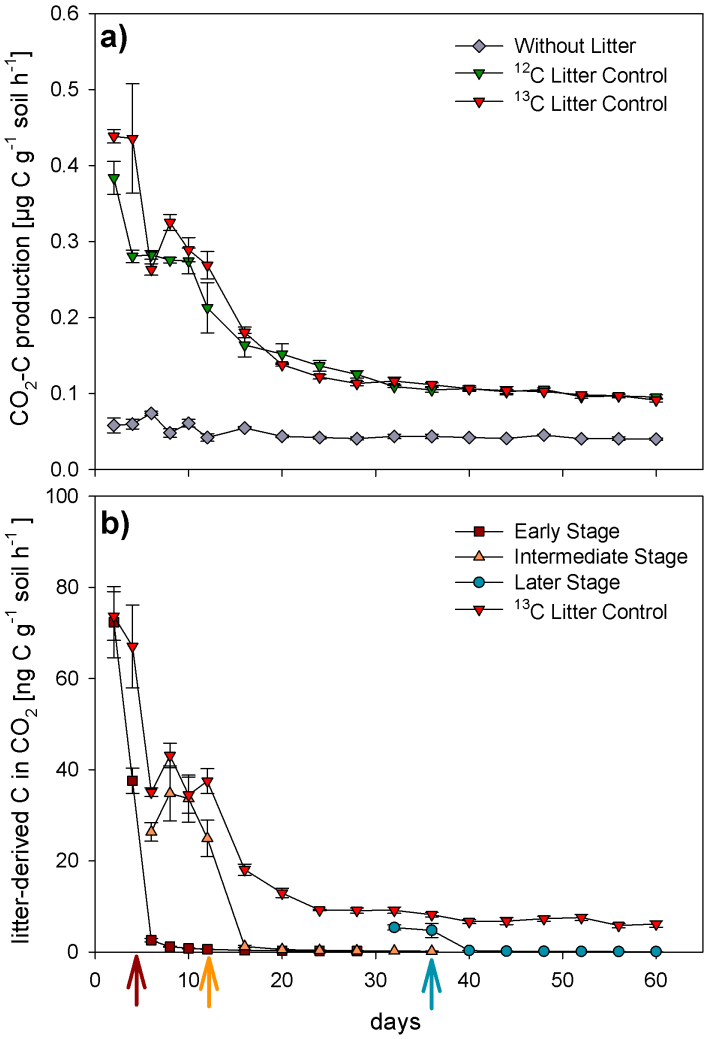

Figure 6.2: (a) Total CO_2-C production and (b) litter-derived C in CO_2 over the experimental period of 60 days. Values are means of four replicates; error bars indicate SEM. Different letters in (a) indicate a statistically significant isotopic effect of CO_2 production by the ^{12}C and ^{13}C litter control treatments (t-test, $p<0.05$). Arrows in (b) indicate exchange of ^{13}C by ^{12}C litter in the respective treatment.

respectively (Fig. S6.2b). The isotopic label remained stable in SOC throughout the 24 days after litter exchange and a MRT of 103 days could be determined only for the early decomposition stage (Table 6.1).

The total EOC pool decreased slightly over time (Fig. S6.3a) with values ranging from 93 $\pm$ 13 µg g^{-1} soil (day 4) to 69 µg $\pm$ 8 µg g^{-1} soil (day 60). The incorporation of litter C into EOC was highest during early decomposition, 1.8 $\pm$ 0.1 µg ^{13}C g^{-1}

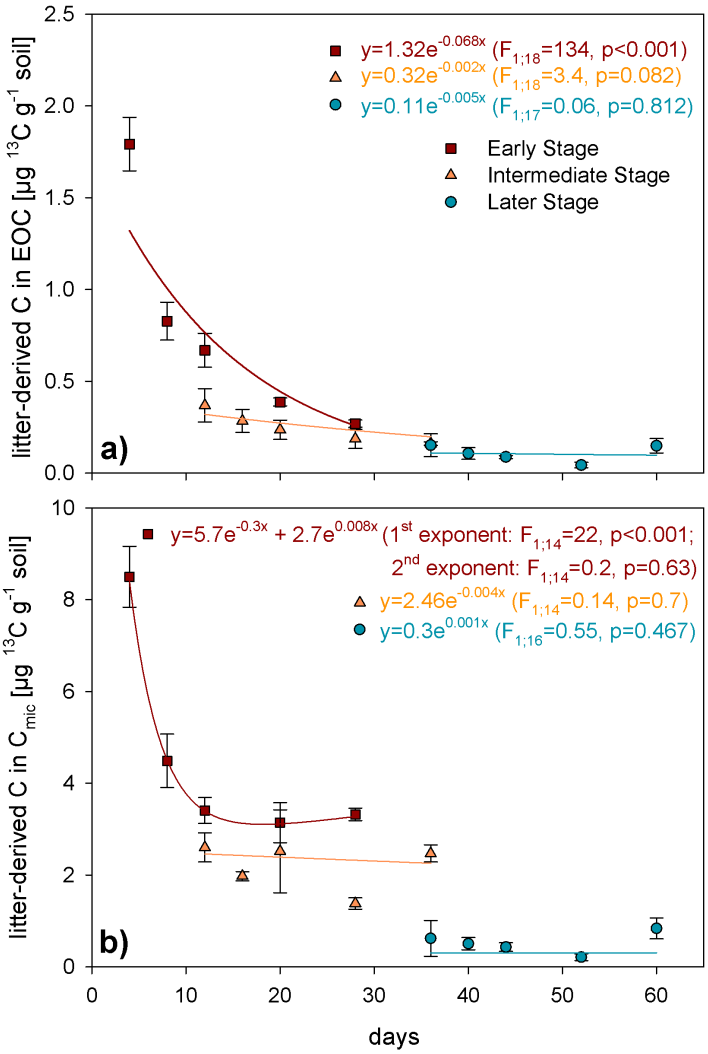

Figure 6.3: (a) Litter-derived C in the extractable organic C (EOC) pool and (b) in the microbial biomass (C_{mic}). Values are means of four replicates; error bars indicate SEM. Colored lines represent the exponential decay function of the respective stages. ANOVA results for each fitted model are given in brackets.

soil directly after pulse labeling; it then rapidly decreased and 85% of the initial ^{13}C was incorporated within 24 days (Fig. 6.3a). The ^{13}C label in EOC during the intermediate and later stages of litter decomposition was considerably lower than in the early stage; 0.4 $\pm$ 0.1 µg ^{13}C g^{-1} soil and 0.2 $\pm$ 0.1 µg ^{13}C g^{-1} soil, respectively. The ^{13}C in the EOC fraction declined by 40% in the intermediate stage within 24 days after litter exchange. In the later stage of decomposition, the introduced low amounts of litter C remained stable, with a loss of 2.1% over 24 days. Therefore,

Table 6.1: Mean residence time (MRT) of different soil C pools and microbial fractions in the detritusphere, calculated as invers *k* value from the fitted exponential decay functions from Figures 2, 3 and S2.

Fraction	Early Stage (d)	Intermediate Stage (d)	Later Stage (d)
SOC	103	-	-
EOC	15	50	200
C_{mic}	3.3 and 125*	250	-
18:2?6,9	46	32	33
cy17:0	41	44	26
i15:0	-	62	-
a15:0	48	83	-
i16:0	100	50	48
i17:0	-	66	-

* from a double exponential model

the calculated MRT increased with decreasing substrate quality, from 15 days in the early stage to 200 days in the later stage of decomposition (Table 6.1).

Total microbial biomass (C_{mic}) ranged from 175 to 244 µg g^{-1} soil and exhibited only minor variation over time, suggesting a steady state across all time points and treatments (Fig. S6.3b). During the early phase of labeling, microorganisms assimilated 8.5 $\pm$ 0.7 µg ^{13}C g^{-1} soil, but within 24 days after litter exchange, the microbially incorporated litter C decreased by 61% (Fig. 6.3b). By using a double exponential model in the early stage of decomposition, MRTs were calculated as 3.3 and 125 days of litter-derived C in C_{mic} (Table 6.1). In the intermediate and later phases, the incorporated litter C was 70% and 93% lower than in the early stage of decomposition (intermediate stage: 2.6 $\pm$ 0.3 µg ^{13}C g^{-1} soil and later stage 0.6 $\pm$ 0.3 µg ^{13}C g^{-1} soil). The MRT of incorporated litter C in the microbial biomass was 250 days in the intermediate stage of decomposition (Table 6.1). The calculation of MRT for the later stage was not possible due to a strong increase in the ^{13}C signal at day 60.

^{13}C in PLFAs

The incorporation pattern of litter-derived C in bacterial PLFAs corresponded to the findings in total microbial biomass, with highest values in the early stage treatment on day 4 and lower litter-derived C incorporation in the intermediate and later stages of decomposition (Fig. 6.4).

The absolute amounts of single bacterial PLFAs were stable over time with only minor variations at the beginning of the experiment (Fig. S6.3c-g). The absolute ^{13}C incorporation in single bacterial PLFAs varied from 3.6 $\pm$ 0.3 ng ^{13}C g^{-1} soil in i17:0 to 16.4 $\pm$ 5.5 ng ^{13}C g^{-1} soil in a15:0 (Fig. 6.4) in the early stage of litter decomposition. Litter C decreased in the gram-negative bacterial PLFA cy17:0 by 22% (Fig. 6.4e) and in the gram-positive bacterial PLFAs a15:0 and i16:0 in the early stage of decomposition by 28 and 46%, respectively (Fig. 6.4a, c). MRTs of C in these PLFAs were 41 days for cy17:0, 48 days for a15:0 and 100 days for i16:0 (Table 6.1). In contrast, during the early stage of litter decomposition, the gram-positive bacterial PLFAs i15:0 (Fig. 6.4b) and i17:0 (Fig. 6.4d) were lower in ^{13}C enrichment immediately after transplantation; their ^{13}C labels increased over time.

In the intermediate and later phases, incorporated litter C was lower than in the early phase in all investigated bacterial PLFAs (Fig. 6.4), but a distinct decline in ^{13}C content occurred after litter transplantation, and ranged from 25% to 47% of the initial ^{13}C label in the intermediate stage and 16% to 63% in the later stage. MRTs of C in the gram-negative bacterial PLFA cy17:0 in the intermediate and later stages of decomposition were 44 and 26 days, respectively. These were lower than MRTs of C in the gram-positive bacterial PLFAs of the same labeling phases, which ranged from 62 to 83 days in the intermediate stage and in i16:0 was 48 days in the later stage (Table 6.1).

In contrast to the bacterial PLFAs, the fungal PLFA 18:2ω6,9 (Fig. 6.4f) had highest ^{13}C values during the intermediate stage (22.9 $\pm$ 10.2 ng ^{13}C g^{-1} soil). After litter exchange, a rapid decrease in ^{13}C content was observed in the fungal PLFA in the

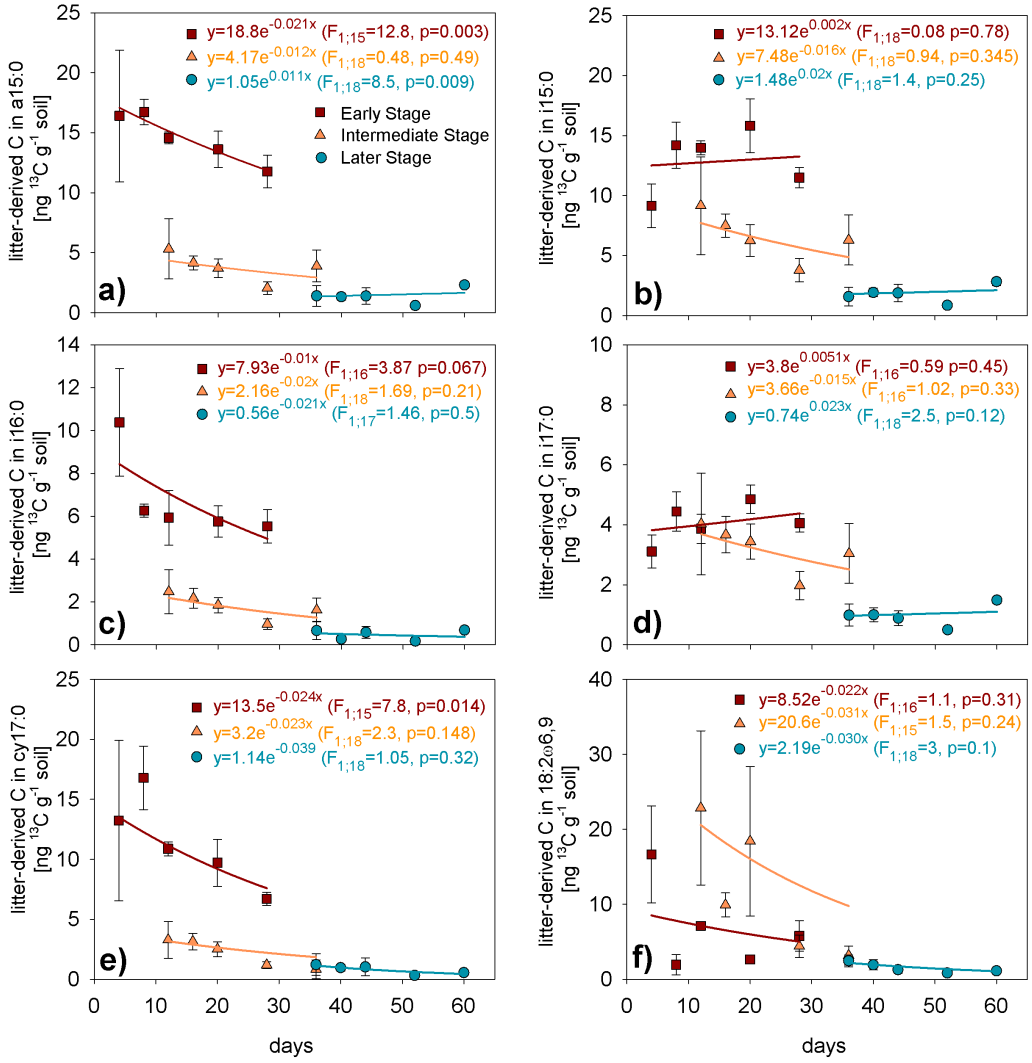

Figure 6.4: Litter-derived C in different PLFA biomarkers: Gram-positive bacterial biomarker (a) a15:0, (b) i15:0, (c) i16:0, (d) i17:0, (e) gram-negative bacterial biomarker cy17:0, and (f) saprotrophic fungal biomarker 18:2ω6,9. For legend see Fig. 6.3.

early and intermediate stages of decomposition. In comparison to high incorporation in the early and intermediate stages, the fungal PLFA had a lower ^{13}C value of only 2.5 $\pm$ 0.8 ng ^{13}C g^{-1} soil in the later stage. The decreases in litter-derived C in the fungal PLFA were 65% and 68% in the early and intermediate stages, similar over the 24 days after litter exchange, and less pronounced in the later stage at only 38%. MRTs were in the same range as calculated for bacteria; 46 days in the early stage, 32 days in the intermediate, and 33 days in the later decomposition

stage (Table 6.1). The abundance of the fungal PLFA was in the range of 0.3 to 0.8 µg g^{-1} soil and showed no pronounced changes over the experimental period (Fig. S6.3h).

6.5 Discussion

Incorporation of litter derived-C into soil microorganisms

We studied C flow through the microbial community to better understand C dynamics at the soil-litter interface, the detritusphere. In general, the incorporation of maize litter into different microbial and chemical soil C pools was driven by the quality/age of added litter material. Microbial activity increased immediately with the addition of fresh litter, as seen by the 7 fold higher respiration rate in the detritusphere as compared to uninfluenced bulk soil. The isotopic composition of maize litter did not significantly influence its mineralization, as similar rates of litter-induced respiration were found for the ^{12}C and ^{13}C litter treatments during the experiment. This rate was a prerequisite for the calculation of mean residence times (MRTs) in different microbial fractions. However, the low ^{13}C content in CO_2 (17% of total CO_2-C) and C_{mic} (4.4% of total C_{mic}-C) provided evidence that older, autochthonous C sources were additionally mineralized to the extent of 139 µg C g^{-1} soil over the 60 days,, indicating a positive priming effect resulting from the fresh litter-derived C supply (Bradford et al. 2008).

The applied litter introduced approximately 2% C of the autochthonous SOC content to the soil system, comparable to the annual amount of fresh C entering the soil in the long-term field experiment in the same soil (Kramer et al. 2012; Müller et al. 2016). Contrary to our expectations, the presence of a new C source did not stimulate microbial growth over time, although plant-derived C was metabolized by bacteria and fungi, shown by the rapid change in the isotopic signatures of the respective microbial biomarker molecules. Furthermore, the low ^{13}C content in the

EOC pool (and the rapid subsequent depletion of litter-derived ^{13}C within this pool) suggests high utilization of the labile fraction of the litter.

The observed temporal pattern of C incorporation into soil microorganisms during litter decomposition is in agreement with the conceptual model of Berg & Matzner (1997), who found a rapid initial decline in soluble substrates followed by slow decomposition of more complex cell wall polymers; this could be seen in our study in the decline of litter C incorporation into microbial compounds as litter quality decreased (Fig. 6.3). In the intermediate decomposition stage (days 5 to 12), fungi incorporated more litter-derived C than in the early decomposition stage (days 0 to 4), underscoring the ability of fungi to utilize not only easily available but also more complex C resources for assimilation and energy supply. In the later decomposition stage (days 29 to 36) mineralization and assimilation of litter derived-C was much lower, presumably due to the reduced decomposability and greater complexity of the remaining C source, i.e. its lower substrate quality (de Boer et al. 2005; Bertrand et al. 2006).

In our study, all identified PLFAs showed a distinct ^{13}C signal after 4 days of litter decomposition, with higher relative values in the fungal PLFA 18:2ω6,9 than in all investigated bacterial PLFAs. Kramer et al. (2016) were among the first to demonstrate simultaneous and overlapping substrate usage by bacteria and fungi in the early stage of litter decomposition. The PLFA 18:2ω6,9 is a preferred biomarker for saprotrophic fungi (Ruess & Chamberlain 2010), but it has recently also been found in plant cells (Kaiser et al. 2015). However, previous studies from the same soil indicate that the plant-derived 18:2ω6,9 makes only a minor contribution to the fungal pool (Kramer et al. 2016; Müller et al. 2016).

Fresh litter-derived C was incorporated into total bacterial PLFA-C by 0.5 to 1.3% in single bacterial biomarkers after 4 days. We assumed that water-extractable litter C was transferred to the adjacent detritusphere (top 3 mm of the soil core) as an effect of rewetting the litter at the start of the experiment, which may have accelerated C access and utilization, especially for less mobile bacteria. Amin et al. (2013) estimated that this soluble C fraction of maize residues could be as high

as 26%, and could be leached into the adjacent soil where it would be available to soil microbes and could influence their enzymatic activity (Poll et al. 2008). Kramer et al. (2016) found that *Bacteriodetes* and *Gammaproteobacteria*-related species are predominantly involved in the maize leaf decomposition in our soil, while Padmanabhan et al. (2003) and Schellenberger et al. (2010) found *Actinobacteria* to be the bacterial key group in the utilization of easy available C in a number of soils. However, there is evidence that some members of the fungal community are also consumers of labile C sources: de Boer et al. (2005) and Poll et al. (2010) described *Mortierellaceae* and *Mucor spp*. as pioneer colonizers of fresh plant litter and dominant in the utilization of labile C sources in the early stage of litter decomposition. Kramer et al. (2016) used rRNA-SIP techniques to demonstrate that only a few members of the soil fungal community (*Chaetomium* and *Fusarium spp*.) contributed to the early use and incorporation of litter-derived C. Since we used the same soils as Kramer et al. (2016), we expected a similar fungal community to contribute to the early usage of litter-derived C in the present experiment.

Previous studies have shown that a high C/N ratio enhances C incorporation into soil via the fungal food chain (Frey et al. 2003; Moll et al. 2015). Our experimental approach with reciprocal transplantation of labeled and unlabeled litter was somewhat destructive to the hyphal network growing in the litter layer, and therefore manipulated the C transfer in the soil. It has been reported that disruption of hyphal connections at the soil-litter interface decreases the decomposition rates of residues, especially for N-poor litter (Chigineva et al. 2011). But we assumed a fast resettlement of the substrate by fungal hyphae after the litter exchange. The substantial role of fungi in the decomposition process is indicated by its high incorporation of C during the early and intermediate stages of litter decomposition, presumably due to hyphal exploration and exploitation of more complex substrates. But distinctly different assimilation patterns between bacteria and fungi of C sources differing in complexity were not apparent. Our findings are in line with findings from Arcand et al. (2016), showing a prominent role of fungi in later stages of the decomposition. With these findings, we can confirm our first and second

hypotheses; (i) both fungi and bacteria utilized labile C sources in the beginning of the decomposition process, and (ii) fungi are capable of exploiting complex C resources in aged litter when labile litter C is depleted.

Our total PLFA results showed a stable fungal-to-bacterial ratio as well as a gram-positive-to-gram-negative bacterial ratio (data not shown), but we are aware that microbial community composition may change over time, with a succession from generalists (r strategists) to specialists (K strategists), especially in the intermediate and later decomposition stages, as a result of changing substrate quality and availability (Frankland 1998; Marschner et al. 2011; Kramer et al. 2016).

Internal microbial C turnover in the detritusphere

The chloroform-fumigation-extraction (CFE) method extracts C mainly from the cytoplasm of microbial cells and does not extract the cell wall fraction (Jenkinson et al. 1976) due to its irreversible adsorption to soil particles (Chenu et al. 2002). CFE extracts contain metabolic products such as sugars, carboxylic and amino acids, and polymers such as DNA and RNA, which differ in their stability and biodegradability (Apostel et al. 2015). A recent study by Malik et al. (2015) highlighted the role of bacteria in microbial hotspots with an investigation of ^{13}C enrichment in DNA and RNA molecules directly after a pulse labeling pot experiment with the herb *Chenopodium ambrosioides*. They found the highest values of ^{13}C per gram soil in PLFAs, but the relative ^{13}C incorporation was higher in DNA and RNA immediately after the pulse. A rapid decrease was then observed, resulting in rapid turnover times of 1.3 and 0.8 days in bacterial DNA and RNA in comparison to slower turnover times of 42 days for bacterial PLFAs. They assumed that PLFA biomarkers are less suitable for determining rapid C fluxes and suggested the use of nucleic acid biomarkers to disentangle the microbial contribution to decomposition processes and SOM genesis. To account for the rapid turnover of DNA and RNA pools and the slower turnover of structural cell wall compounds, we applied a double exponential model representing a rapidly utilized C pool and a second, slower

decomposable C pool in the early stage of litter decomposition. The MRT of C in C_{mic} in the early stage of decomposition was calculated at 3.3 and 125 days for the pools of rapid and slow internal C turnover, respectively (Table 6.1). This is in line with calculations from Gunina & Kuzyakov (2015), who determined, in a meta-analysis, MRTs of 1.25 days for the rapidly decomposing C pool and 250 days for the slower C pool turnover by analyzing the fate of a glucose amendment in soil microorganisms. Additional support for the double exponential model is given by Studer et al. (2014), who calculated an internal C turnover of 6 days for the decay of fresh C in rhizosphere microorganisms, and Cheshire (1979), who recovered 15-20% of ^{14}C labeled glucose in soil microbial residues 3 years after application. We were not able to fit a suitable double exponential model to total C_{mic} for the intermediate and later decomposition stages. The approximately 200 μg g^{-1} soil pool size of total C_{mic} may have been too large for reliable detection of small changes in the isotopic signature (1.1% and 0.3% ^{13}C incorporation in total C_{mic} in the intermediate and later decomposition stages, respectively). It is also possible that during intermediate and later stages of decomposition, litter C supply was not sufficient for balanced microbial growth (i.e. the synthesis of a broad spectrum of compounds for growth) with the utilized C instead stored in a single pool of storage compounds (Nguyen & Guckert 2001). Lemanski & Scheu (2014) demonstrated that once incorporated, glucose-derived ^{13}C in bacterial PLFAs did not significantly decrease over 52 weeks in a grassland soil, suggesting either strong storage of C or continuous uptake of labelled C residues formed after the glucose input. Therefore, we used a single exponential model to calculate MRT in the intermediate stage of 250 days (Table 6.1), which is in line with Gunina & Kuzyakov (2015).

It has been suggested that storage of C in biomass is more persistent when mediated by fungi and more labile when utilized by bacteria, mainly as a result of differences in their lifespans (Bååth 1998; Rousk & Bååth 2011). Field data have indicated that bacterial biomass turns over 2-3 times during a growing season, while fungi regenerate only 75% of their biomass over the same time period (Moore et al. 2005). The MRT of C in the fungal PLFA 18:2ω6,9 was estimated at 46 days at

the beginning of the experiment and became shorter in the intermediate and later stages, at 32 and 33 days, respectively (Table 6.1). Surprisingly, these turnover times were in the same or shorter in range than the C turnover in bacterial PLFAs. This result seems notable due to the fact that eukaryotic fungal cells are larger and more complex than prokaryotic bacterial cells and therefore have a smaller surface to volume ratio. Thus, a measurable response of enrichment and turnover of ^{13}C in fungal cell components should take longer (Moore et al. 2005). Therefore, we must reject our third hypothesis that bacterial C turnover is faster than fungal turnover. In a long-term field experiment using the same soil, Müller et al. (2016) found a relative contribution of 78% of plant-derived C in the fungal PLFA after the fifth vegetation period, suggesting an effective assimilation of plant-derived C and/or a long retention of incorporated C into the fungal biomass. Our new findings challenge the previous views of residue C dynamics, which suggested longer retention of C in fungal biomass in comparison to bacterial biomass.

The MRT of C in different bacterial PLFAs ranged from 26 to 100 days (Table 6.1). Variability in the calculated MRTs was high, indicating various life strategies for bacteria when substrate is available. For example cy17:0, a representative biomarker for Gram-negative bacteria, showed an incorporation of labile litter C in the early stage of decomposition and a subsequent rapid ^{13}C decrease while biomass remained constant. This result suggests an additional utilization of more complex C derived from the ^{12}C substrate after litter exchange. By contrast, the i15:0 and i17:0 PLFAs showed an increase in litter-derived C, although labeled litter was replaced by ^{12}C litter of the same quality in the early stage of decomposition. This may be explained by the lack of availability of complex C sources for metabolic usage, as well as effective catabolic and recycling systems of formerly metabolized C released by microorganisms. Additionally, the increase in the maize C signal provides evidence of indirect C assimilation due to cross-feeding between trophically related microorganisms (Manefield et al. 2002; DeRito et al. 2005) and should be taken into account when interpreting MRTs for microorganisms.

We found an increase in the isotopic label in nearly every C pool between the fourth and fifth sampling points in the intermediate and later stages of decomposition. This may be explained by a re-translocation of litter C from bulk soil to the detritusphere, mediated by dying fungi as an effect of species succession in the fungal community from generalists to specialists. Fungal hyphae are considered as "highways" for C and nutrient transport (Boddy 1993; Cairney 2005), mainly from actively growing parts of hyphae to the base of fungi, which act as sinks (Cairney 1992). This could serve to transfer some litter C to the bulk soil and serve as a source for *K*-strategists which appear during the decomposition process (Poll et al. 2010). As a consequence, re-translocation of C and nutrients from the base of the fungal system to the surface and litter layers could occur. This bi-directional C and nutrient translocation has been described by others (Frey et al. 2003; Boberg et al. 2014; Guhr et al. 2016). These small-scale translocation, recycling, and cross-feeding processes create an effective internal "soil microbial loop" (Malik et al. 2015) which stores some of the incorporated litter C in soil microbial pools over longer times. This is supported by the observed litter-derived C incorporation into the total SOC pool (Fig. S6.2). We found a slow decrease in ^{13}C in the early stage of decomposition, followed by steady state conditions in the intermediate and later phases, hampering the calculation of MRTs. Further investigation is needed to better characterize microbial C dynamics in soil hotspots. Special attention should be paid to the proportions of C in different cell components such as nucleic acids, proteins, and cell envelopes, and to the turnover of C within these structures, to better evaluate the contributions of bacteria and fungi to soil C cycling.

6.6 Conclusion

Microbial utilization and subsequent release of organic C are major processes contributing to microbially regulated SOC formation. This study investigated C flow through the microbial community in a microbially active hotspot of the soil, the detritusphere. Microbial hotspots are particularly important for C and

nutrient turnover in soils; implementing specific parameterizations into C turnover models may improve their predictive power. Our data show that microbially driven C turnover in the detritusphere is a highly dynamic process, which depends on succession within the microbial community and on the consecutive availability from the litter layer of substrates of changing quality and quantity. We have evidence that fungi play an important role in plant residue decomposition, through greater utilization of easily available and more complex litter-derived C than bacteria. In contrast to the literature, fungal PLFA-C showed a rapid turnover, while bacterial PLFA behaved differently, with the potential for enormous impact on soil genesis. We observed either a clear dilution of the ^{13}C signal after labelling or a strong storage and/or reuse of previously released microbial C, reflecting various bacterial substrate utilization strategies. Our results contribute to the basic knowledge needed for future modelling approaches by identifying MRTs of different soil microbial communities.

6.7 Acknowledgements

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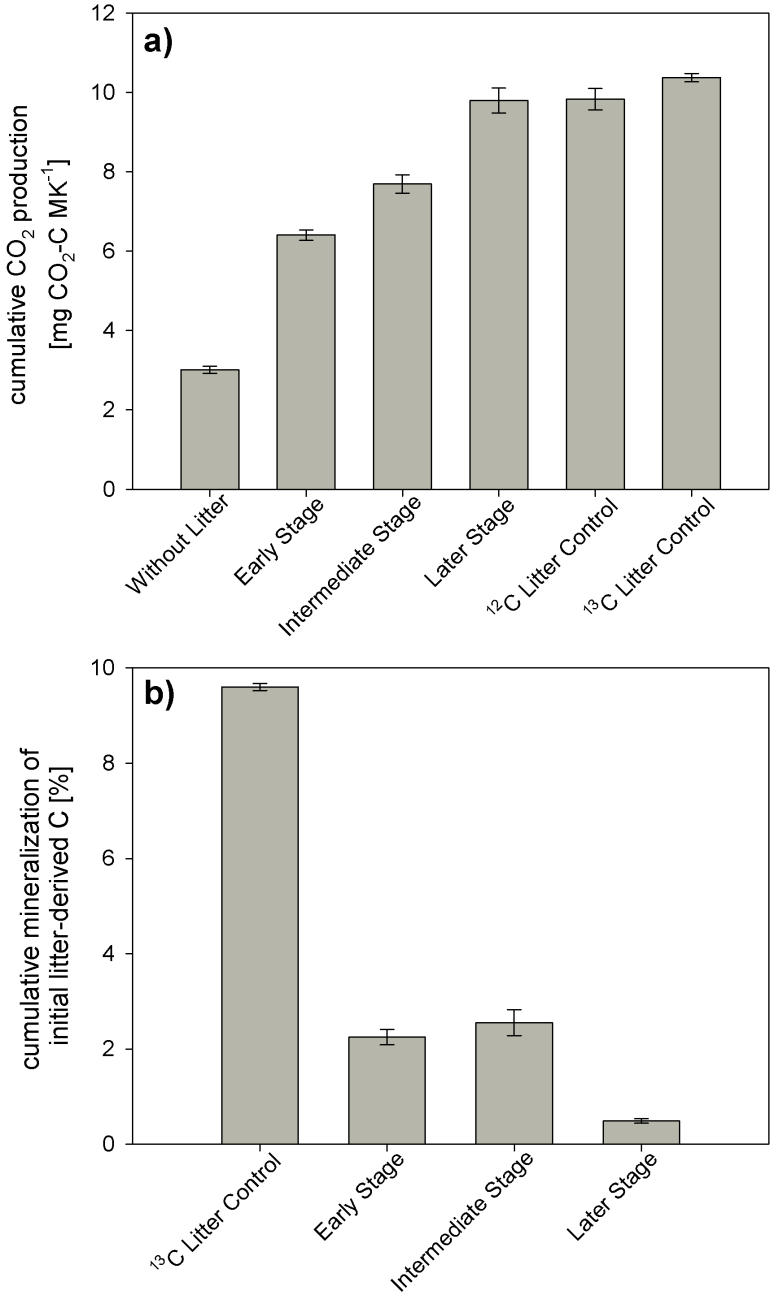
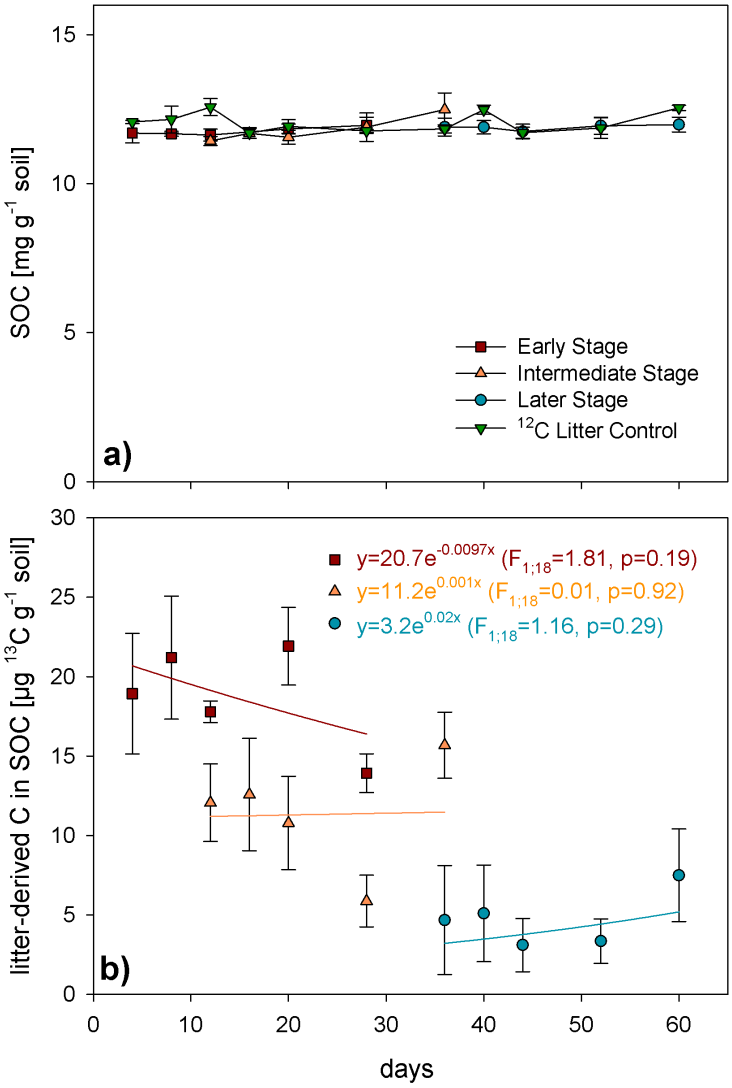
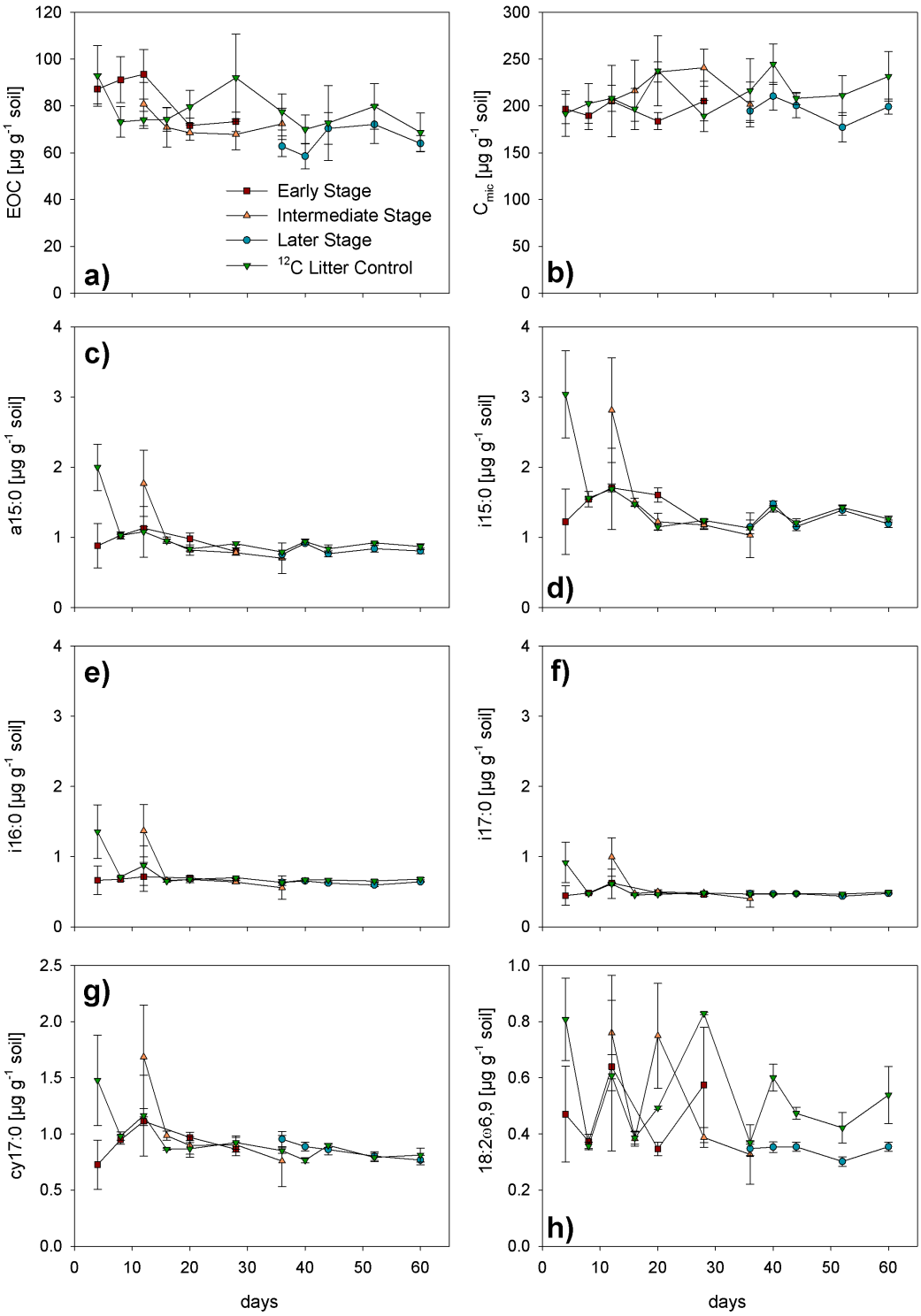
6.8 Supplementary Material



S 6.1: (a) Cumulative CO_2-C production after 60 days of incubation, and (b) cumulative mineralization of initial litter-derived C in the ^{13}C Litter Control treatment and during the three labeling phases. Early stage and intermediate stages ended after 28 and 36 days, respectively, while all other treatments ended after 60 days. Values are means of four replicates, error bars indicate SEM.



S 6.2: (a) Total soil organic C (SOC) pool over the experimental period of 60 days, and (b) litter-derived C in SOC. Values are means of four replicates; error bars indicate SEM. Colored lines represent the exponential decay function of the respective stages. ANOVA results for each fitted model are given in brackets.



S 6.3: Total soil C pools over the experimental period of 60 days: (a) EOC, (b) C_{mic}, Gram-positive bacterial biomarker (c) a15:0, (d) i15:0, (e) i16:0, (f) i17:0, (g) gram-negative bacterial biomarker cy17:0 and (h) saprotrophic fungal biomarker 18:2ω6,9. Values are means of four replicate; error bars indicate SEM.

7 Disentangling the root- and detritus-based food chain in the micro-food web of an arable soil by plant removal

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Olena Glavatska[1], Karolin Müller[2], Olaf Butenschoen[3], Andreas Schmalwasser[4], Ellen Kandeler[2], Stefan Scheu[3], Kai U. Totsche[4], Liliane Rueß[1]

[1] Institute of Biology, Ecology Group, Humboldt-Universität zu Berlin, Philippstr. 13, 10115 Berlin, Germany

[2] Institute of Soil Science and Land Evaluation, Soil Biology Department, University of Hohenheim, 70593 Stuttgart, Germany

[3] J.F. Blumenbach Institute of Zoology and Anthropology, Georg August University Göttingen, Berliner Str. 28, 37073 Göttingen, Germany

[4] Institute of Geosciences, Hydrogeology Section, Friedrich Schiller University of Jena, Burgweg 11, 07749 Jena, Germany

7.1 Abstract

Soil food web structure and function is primarily determined by the major basal resources, which are living plant tissue, root exudates and dead organic matter. A field experiment was performed to disentangle the interlinkage of the root- and detritus-based soil food chains. An arable site was cropped either with maize, amended with maize shoot litter or remained bare soil, representing food webs depending on roots, aboveground litter and soil organic matter as predominant resource, respectively. The soil micro-food web, i.e. microorganisms and nematodes, was investigated in two successive years along a depth transect. The community composition of nematodes was used as model to determine the changes in the rhizosphere, detritusphere and bulk soil food web. In the first growing season the impact of treatments on the soil micro-food web was minor.

In the second year plant-feeding nematodes increased under maize, whereas after harvest the Channel Index assigned promotion of the detritivore food chain, reflecting decomposition of root residues. The amendment with litter did not foster microorganisms, instead biomass of Gram-positive and Gram-negative bacteria as well as that of fungi declined in the rooted zone. Likely higher grazing pressure by nematodes reduced microbial standing crop as bacterial and fungal feeders increased. However, populations at higher trophic levels were not promoted, indicating limited flux of litter resources along the food chain.

After two years of bare soil microbial biomass and nematode density remained stable, pointing to soil organic matter-based resources that allow bridging periods with deprivation. Nematode communities were dominated by opportunistic taxa that are competitive at moderate resource supply. In sum, removal of plants from the system had less severe effects than expected, suggesting considerable food web resilience to the disruption of both the root and detrital carbon channel, pointing to a legacy of organic matter resources in arable soils.

7.2 Introduction

Soil organisms, their community structure and function in the food web, play a key role in soil carbon dynamics. However, while the turnover of total amounts and individual fractions of soil carbon are well investigated (Pendall & King 2007; Marschner et al. 2008; Todorovic et al. 2010), less is known about the biotic diversity and interactions in soil food webs that determine major carbon and energy pathways (Gessner et al. 2010; Nielsen et al. 2011; Crotty et al. 2014). Recent studies suggest that mineralisation and sequestration of carbon is shaped by the diversity within (horizontal diversity) and across (vertical diversity) trophic levels, and that the driving mechanisms are broadly the same across ecosystems (Gessner et al. 2010). This calls for experiments under field conditions to improve current food web models with empirical data and to disentangle the relationship between food web structure and ecosystem function (de Ruiter et al. 2005).

Resource quality and availability, i.e. the horizontal diversity at the base of the food web, has an important impact on soil carbon flow (Gessner et al. 2010; Crotty et al. 2014). Soils comprise two major food chains, either root or detritus based (Scheu et al. 2005). In the root-based food chain the predominant carbon sources are living plant tissue and root exudates. In the detritus-based food chain dead organic matter fuels either the bacterial or fungal carbon channel, depending on labile or recalcitrant resources, respectively (Ruess & Ferris 2004). These differences in quality and accessibility of plant-derived substrates result in three major soil carbon and energy pathways based on roots, bacteria, and fungi (Moore & de Ruiter 2002; Scheu et al. 2005). Particularly in arable systems the internal belowground carbon cycle is shaped by root-derived resources, due to the regular harvest of crop, and the variation in rhizodeposits depending on growing season (Drigo et al. 2010).

Soil nematodes use an exceptionally wide range of resources and form functional groups at each trophic level of the food web (Yeates et al. 1993), thereby holding a central position in both bottom-up and top-down controlled processes (Yeates 2001; Ferris 2010). Although some plant parasites cause disease, most taxa beneficially

affect soil processes, such as nutrient mineralisation by bacteria (Bardgett et al. 1999; Yeates et al. 2009; Neher 2010). In addition, nematode assemblages react quickly to shifts in availability and quality of exogenous resources (Duncan et al. 2007; Leroy et al. 2009). Due to these characteristics, nematodes are widely applied indicators for soil carbon pathways (Ruess 2003; Ruess & Ferris 2004). Moreover, nematode life strategy and trophic composition reflect general food web conditions, expressed by the Enrichment and Structure Index calculated based on nematode family composition (Ferris et al. 2001). In arable land, the nematode faunal analysis concept was employed to indicate organic enrichment (Ferris & Bongers 2006) as well as to assign changes in plant resources related to crop type, season or management practice (Ferris & Matute 2003; Leroy et al. 2009; Ugarte et al. 2013). Additionally, the effectiveness of fallow as method to control plant-parasitic nematodes and to restore soil properties has been investigated (Pate et al. 2000; Villenave et al. 2001; Háněl 2003). However, the impact of fallow or bare soil on soil food webs was rarely assessed (Sánchez-Moreno et al. 2006).

To disentangle the root- and detritus-based food chain, a field experiment was performed on an arable land. By removal of plants the root channel was eliminated, and by organic amendment the detritus channel was fostered. Three treatments were established: plant (*Zea mays*) with removing stalks at harvest, application of maize shoot litter, and bare soil, providing roots, litter and autochthonous organic matter as predominant resources, respectively. These experimental manipulations separated the micro-food web into rhizosphere (maize plant), detritusphere (maize litter) and bulk soil (bare soil) habitats. While the rhizosphere food web comprises both the herbivore and the detritivore food chain, the detritusphere and the bulk soil food webs lack the herbivore chain, with bulk soil assemblages additionally facing deficiency of recent resource input.

Sampling at the field site was performed during two successive years, in July (high root exudation), September (plant residue input), and December (transport of organic matter), covering the major seasonal resource changes within a crop cycle. Microbial biomass and nematode fauna were investigated along a depth gradient

from the topsoil (0–10 cm) to the rooted zone (40–50 cm) to root free soil (60–70 cm) (Kramer et al. 2012). We hypothesise that removing the plant from the system, i.e. the root based carbon and energy channel, will result in a loss in connectivity and complexity of soil food webs. Based on the predominant resources available the manipulations were expected to result in the following food web conditions: i) rhizosphere – highly structured with both herbivore and detrital food chain present, ii) detritusphere – moderately structured with detrital food chain solely, and iii) bulk soil – low structure of assemblages inhabiting bare soil.

7.3 Materials and Methods

Field site and agriculture management

The experiment was conducted on an arable field near Göttingen (Germany), a region with a mean annual precipitation of 720 mm and mean air temperature of 7.9°C. The dominant soil types at the site are Cambisols and Luvisols, with a pH ($CaCl_2$) of 6.0, a bulk density of 1.4 g cm^{-3}, an organic C and a total N content of 12.4 and 1.3 g kg^{-1}, respectively. More details on physical and chemical soil properties are given in Kramer et al. (2012). The field work at Reinshof Experimental Farm has been permitted by the University of Göttingen.

During the spring (April 2012), the non-selective herbicide Round-up (Glyphosate: 4 l ha^{-1}) was applied to the field followed by tillage of the land using a chisel plough to a depth of 12 cm. The maize cultivar Codosco (I.G. Pflanzenzucht GmbH, München, Germany) was then sown at a density of 11.5 grains m^{-2}. Inorganic N fertilizer (ammonium nitrate urea solution: 76 kg N ha^{-1}, ammonium sulphate: 20 kg N ha^{-1}) and NP fertilizer (diammonium phosphate: 19 kg N ha^{-1}, 111 kg P ha^{-1}) were applied shortly prior to and after sowing of the maize seeds to improve plant growth. During the growing season the plots received a combination of herbicides: Peak: 20 g ha^{-1} (750 g kg^{-1} prosulforon, for control of broad leaved weeds in forage and grain maize), EFFIGO: 0.35 l ha^{-1} (267 g l^{-1} clopyralid + 67 g l^{-1} picloram,

a post-emergence herbicide to control dicotyledonous weeds), MILAGRO: 0.50 l ha^{-1} (240 g l^{-1} nicosulfuron, for the control of grass in forage and grain maize), Terbuthylazin 500: 0.85 l ha^{-1} (a selective residual triazine herbicide for pre and post emergent weed control in maize). At harvest in September 2012, the corncobs were removed by hand and maize plants were cut to a height of 10 cm above soil surface. Maize stalks were then shredded to particle sizes of <1 cm^2 and air-dried to obtain maize litter.

Again, in the following spring (April 2013) Glyphosate (4 l ha^{-1}; N-phosphonomethyl-glycine, a post-emergence, non-selective, foliar herbicide) was used to kill the layer of weeds that had developed. Three weeks later, the soil was tilled to a depth of 12 cm and the maize cultivar Codosco planted at a density of 8.5 grains m^{-2} and fertilized according to the practice in 2012. The same herbicides were applied, with slight variations accounting for the emergence of weeds, in the following combinations: Terbuthylazin 500: 0.85 l ha^{-1}, MILAGRO: 0.50 l ha^{-1}, Peak: 20 g ha^{-1}, EFFIGO: 0.35 l ha^{-1}. The crop was harvested in September (2013) and all crop residues removed from the experimental field site.

Treatments

In May 2012, a total of 12 experimental plots (size 5 x 5 m) were established on the field in two adjacent rows separated by a 5 m buffer stripe within and 2 m buffer strips between rows. Three treatments were assigned to the plots on the basis of the different resource qualities for the soil food web: plant (maize as crop), litter (application of maize shoot litter) and bare soil, each with four replicates. In the plant treatment, which constituted all the major food web resources, there is a supply of carbon via living root tissues and rhizodeposition carbon during the growing period and after the harvest decaying roots continue to provide belowground carbon. Litter and bare soil treatments were established by allowing maize plants to grow for three weeks after sowing and then removing the plants by hand from the plots. Therafter, for the litter treatment the resource for the detritus-based

food web was enhanced by amendment with shredded maize shoot litter applied to the soil surface at an amount of 0.8 kg dry weight m^{-2} (equivalent to 0.35 kg C m^{-2}), resembling the shoot biomass of maize crop. In the bare soil treatment the food web did not receive any recent plant resources and was therefore dependent on autochthonous soil organic matter.

Litter and bare soil treatment plots were covered by nets (AGROFLOR Kunststoff GmbH, Wolfurt, Austria) to adjust for differences in light intensity and temperature between planted and unplanted plots, during maize growing period. Shading was set up at a level representing mean leaf area index (LAI) of plants during the vegetation period, which was compared randomly by a lux meter across plots. In addition, weeding of all plots was performed at regular intervals across seasons to maintain experimental treatments and to prevent plant carbon input by weeds.

Soil water regime

In August 2012, six EnviroSCAN water content profile probes (Sentek Inc., Sidney, Australia) were installed in three plots that cover each treatment with two replicates to determine soil moisture conditions at the field site. In the plant plots, the first sensor was installed directly in a crop row and the second between the rows. The volumetric water content of the soil was determined at four depths (0–10, 10–20, 20–30 and 40–50 cm) from August 2012 until June 2013, thereafter measurements were aborted due to the theft of data loggers. Twelve tension-controlled lysimeters (UMS GmbH, München, Germany) were installed in six plots at two depths to monitor water and organic matter fluxes below the plough layer (30 cm) and below the main rooting zone (60 cm). Seepage water samples were taken every fortnight from August 2012 until April 2014 (Dibbern et al. 2014).

A compensation of water uptake by the plants via conventional irrigation was initially intended but turned out not to be feasible due to the very variable temporal and spatial soil moisture distribution at planted plots. Relevant differences in soil water content with maize presence occurred only in autumn, where predominantly

the subsoil below the plough layer was dryer, whereas in summer the planted plots were wetter in topsoil and rooted zone (Table S7.1). Soil water contents of the different treatments converged in winter with those under bare soil at all plots.

Sampling

Soil samples were collected during two successive vegetation periods of 2012 and 2013, in July, September, and December. Samples were taken at three different depths: plough layer (0–10 cm), rooted zone (40–50 cm) and root free zone (60–70 cm). From each plot eight samples were taken with a soil corer (diam. 2.5 cm), bulked and gently mixed by hand. Subsamples of about 50 g fresh weights each were taken for analysis of microorganisms and nematodes. An additional 30 g fresh weight was used for determination of the actual soil water content at sampling date. For each treatment and soil depth four replicates were collected resulting in a total of 216 samples during the two seasons.

Microorganisms

Phospholipid fatty acids (PLFAs) were extracted from 2 x 4 g and 2 x 10 g of each topsoil and subsoil samples, respectively, using a Bligh and Dyer solution (chloroform, methanol, citrate buffer (pH 4), 1:2:0.8, v/v/v) as described by Frostegård et al. (1993). Fractionation into glycolipid, neutral lipid and phospholipid fatty acids was performed using silica acid columns (Bond Elut SI, 500 mg, 3 ml, Agilent Technologies Inc, Santa Clara, USA). The two replicates of each sample were combined into one column before fractionation. To transform PLFAs into fatty acid methyl esters (FAMEs), an alkaline methanolysis was done as described by Ruess et al. (2007).

The FAMEs were measured with an AutoSystem XL gas chromatograph (Perkin-Elmer Corporation, Norwalk, CT, USA) equipped with a capillary column HP-5 (50 m $\times$ 0.2 mm, film thickness of 0.33 µm) and a flame ionization detector. Helium

was used as carrier gas. The temperature program started with 70°C for 2 min, increased with 30°C min^{-1} to 160°C, further 3°C min^{-1} to 280°C and held for 15 min. The injection temperature was 260°C.

The total amount of soil PLFAs was used as proxy for the total microbial biomass. The share of Gram-positive bacteria was assigned by their specific marker PLFAs i15:0, a15:0, i16:0 and i17:0, and that of Gram-negative bacteria by the markers cy17:0 and cy19:0, whereas 18:2ω6,9 was used for saprotrophic fungi (Frostegård et al. 1993; Ruess & Chamberlain 2010). Please note that total soil PLFAs comprise, besides microorganisms, also fatty acids derived from other organisms and tissue. However, the pool size of bacteria and fungi in soil PLFAs is much higher compared e.g. plants and animals, as the latter predominantly contribute fatty acids to the soil neutral lipid fraction (Ruess & Chamberlain 2010).

Nematode fauna

Nematodes were extracted from 50 g of soil per sample using a modified Baermann method according to Ruess (1995). The extraction was at room temperature for 24 h (approx. 18°C), followed by a heating regime with successive 5°C steps for 6 h, from 20°C to 45°C. Nematodes were fixed in 4% cold formaldehyde solution. For each sample the total number of nematodes was counted under a light microscope at 63×magnification, and then 10% of the total individuals per sample were determined to genus level at 630×magnification.

As a scale of soil ecosystem conditions nematode faunal analysis was applied without considering the dauerlarvae (non-active stage) of Rhabditidae. This concept is based on nematode life history strategy and distinguishes families into colonizers (c) and persisters (p) as extremes on a scale from 1 to 5, respectively (Bongers 1990). To determine the major carbon channels, food web enrichment and structure the following indices were assigned: the ratio of fungal to bacterial feeder (F/B) (Freckman & Ettema 1993) and the Enrichment (EI), Structure (SI), Basal (BI) and Channel Index (CI) according to Ferris et al. (2001). The F/B ratio mirrors

changes in nematode microbial resources and the *CI* determines the dominance of the fungal or bacterial energy channel in the soil food web. The *EI* provides information on nutrient availability and soil fertility, while the *SI* informs about the stability and structure of the soil food web and the regeneration stage after a disturbance. The following equations were used:

$$EI = \frac{100 \cdot e}{e + b}$$

$$SI = \frac{100 \cdot s}{s + b}$$

$$BI = \frac{100 \cdot b}{e + s + b}$$

$$CI = \frac{100 \cdot 0.8Fu_2}{(3.2Ba_1) + (0.8Fu_2)}$$

where *e* is the enrichment, *b* the basal, and *s* the structure component. Fu_2 represents fungal feeders with c-p-classification 2, Ba_1 bacterial feeders with c-p-classification 1.

Statistical analysis

The effects of major resource, time and soil depth on nematode communities (i.e. density, taxa, indices) and on PLFA amounts of total microorganisms, bacteria, and fungi, were analysed by analysis of variance (ANOVA). Microbial data were log transformed, and data sets that achieved normal distribution were subjected to further analyses. As both microbial biomass and nematode density decreased significantly with depth, and moreover soil horizons along the profile are dependent, each soil layer was investigated separately. A repeated-measures ANOVA for each vegetation period with the factors season (S) and treatment (T) was performed. Differences between means with one sampling date were inspected using Tukey's Honestly Significant Difference (HSD) test. Analysis was performed using STATISTICA 9.1 for Windows (StatSoft, Hamburg).

The Spearman's rank-order correlation was employed to assign the association between nematodes and biotic (microorganisms) and abiotic variables (soil moisture). This nonparametric correlation was employed, as data for soil water content did not achieve normal distribution even after transformation. The Spearman's correlation determines the strength and direction of the relationship between two variables, here nematode trophic groups and microbial diet, and nematode density and actual soil water content at sampling.

7.4 Results

Biomass of microorganisms

Based on the marker PLFAs detected, Gram-positive bacteria were the dominant microbial group across treatments and depth (Fig. 7.1). Treatment effects on the different microbial groups occurred predominantly in the second vegetation period and were restricted to the topsoil and the rooted zone. In the topsoil the total microbial biomass increased in plant and litter plots compared to bare soil plots in autumn 2012 ($F_{2,9} = 4.797$, $p = 0.043$). Plant presence further enhanced the biomass of Gram-positive bacteria in autumn ($F_{2,9} = 4.919$, $p = 0.040$), and of Gram-negative bacteria in autumn ($F_{2,9} = 6.080$, $p = 0.025$) and winter ($F_{2,9} = 12.028$, $p = 0.004$), in comparison to litter and bare soil plots in 2013. In contrast, in litter amended plots there was a tendency for fungal biomass to increase during summer ($F_{2,9} = 3.907$, $p = 0.066$) and winter ($F_{2,9} = 4.438$, $p = 0.051$) as compared to the bare soil and plant plots.

During the first vegetation period no changes were detected in the rooted zone, whereas during the second year, the biomass of total microorganisms, fungi, Gram-positive and Gram-negative bacterial were affected by treatments in winter ($PLFA_{total}$: $F_{2,9} = 8.120$, $p = 0.012$; $PLFA_{fungi}$: $F_{2,9} = 12.982$, $p = 0.003$; $PLFA_{Gr+}$: $F_{2,9} = 6.870$, $p = 0.018$; $PLFA_{Gr-}$: $F_{2,9} = 7.234$, $p = 0.016$), with plant plots displaying highest, while litter amended and bare soil plots lowest and intermediate

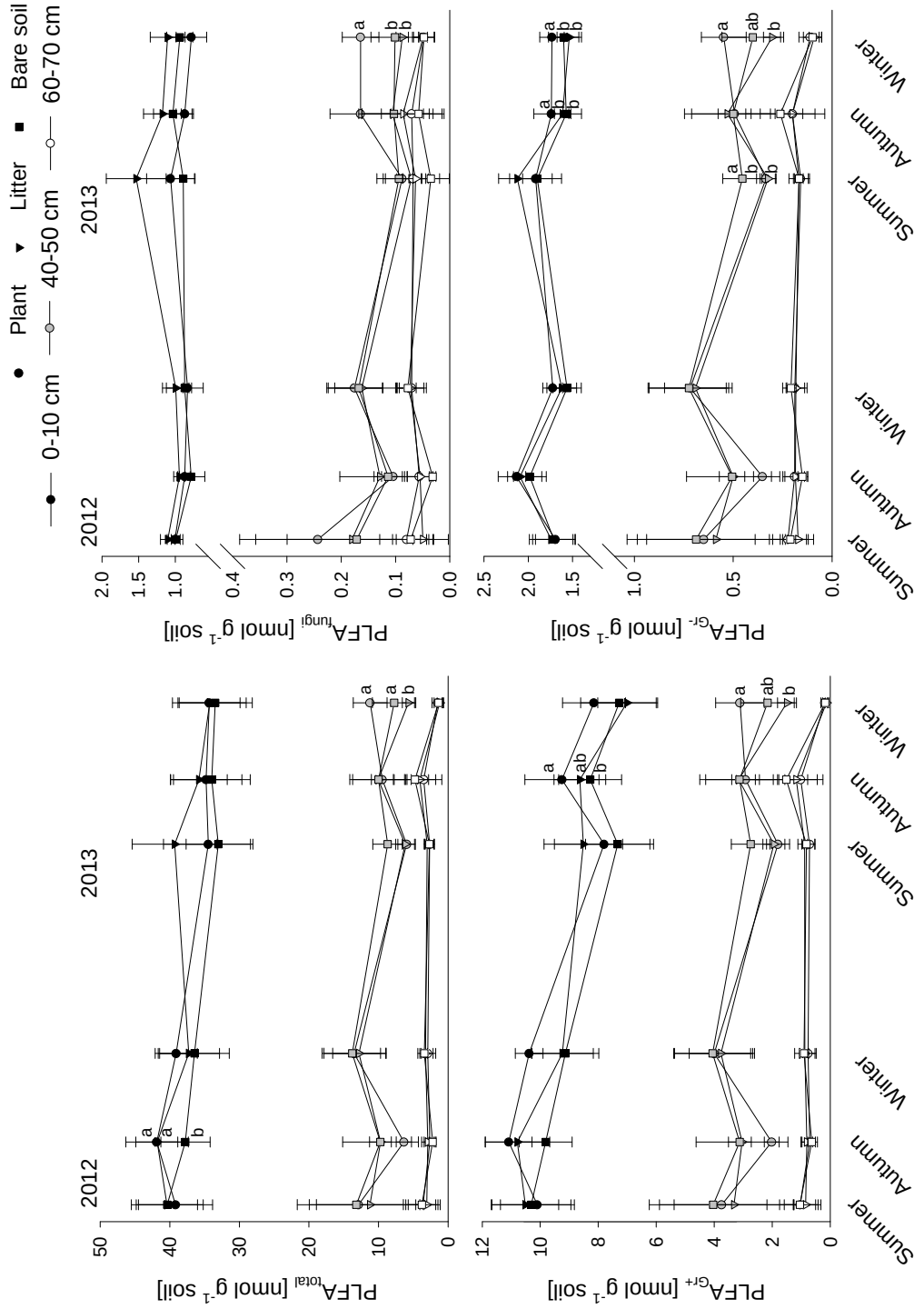

Figure 7.1: Biomass of microbial groups determined as soil phospholipid fatty acids (PLFAs in nmol g^{-1} dry weight soil $\pm$ SD) in soil cropped with maize (plant), amended with maize shoot litter (litter) or bare soil, in topsoil (0-10 cm), rooted zone (40-50 cm), and root free zone (60-70 cm) in the years 2012 and 2013. Given is the biomass for total microbial assemblages ($PLFA_{total}$), Gram-positive bacteria ($PLFA_{GR+}$), Gram-negative bacteria ($PLFA_{GR-}$) and fungi ($PLFA_{fungi}$). Values within a sampling date with the same or no letters are not significantly different according to Tukey´s HSD test at $p < 0.05$.

values, respectively. The Gram-negative bacteria also responded earlier in the season (summer: $F_{2,9} = 4.559$, $p = 0.048$) with highest biomass at bare soil plots.

Nematode density and community structure

Significant effects of the different treatments on nematode population density were restricted to the topsoil (Fig. 7.2). The amendment with litter fostered the density in summer and autumn across both years (ANOVA, $p < 0.05$). The absence of plants had no negative impact on nematode density and more nematodes were detected under bare soil compared to plant plots in autumn 2013 (Tukey´s HSD test, $p < 0.05$). Comparing the abundance of frequent bacterial feeders with regular occurrence across seasons in the topsoil revealed quite different patterns (Fig. 7.3). *Cephalobus* was facilitated by litter predominantly during the first year (autumn 2012: $F_{2,9} = 5.88$, $p = 0.023$, winter 2013: $F_{2,9} = 5.91$, $p = 0.023$), whereas the related genus *Acrobeloides* showed an opposite density distribution (summer 2013: $F_{2,9} = 4.27$, $p = 0.049$, autumn 2013: $F_{2,9} = 4{,}87$, $p = 0.037$). The abundance of *Eucephalobus* decreased over the experimental period with a positive impact of litter amendment in winter 2013 ($F_{2,9} = 7.18$, $p = 0.01$), whereas *Alaimus* proliferated under bare soil at both winter samplings (winter 2012: $F_{2,9} = 11$, $p = 0.004$, winter 2013: $F_{2,9} = 12.95$, $p = 0.002$; Fig. 7.4). *Eumonhystera*, a genus feeding mainly on bacteria but also ingesting unicellular eukaryotes, had its highest density in litter and bare soil plots in autumn of the first year, however, a significant treatment effect was only visible during winter 2013 under litter compared to plant treatment plots ($F_{2,9} = 6.13$, $p = 0.021$, Fig. 7.4). A similar pattern occurred for the root-feeding *Malenchus* (autumn 2012: $F_{2,9} = 5.53$, $p = 0.027$). Among fungal feeders *Aphelenchoides* showed a variable distribution pattern with positive response to litter in the first (autumn 2012: $F_{2,9} = 6.21$, $p = 0.025$) as well as to the bare soil treatment in the second season (autumn 2013: $F_{2,9} = 5.02$, $p = 0.03$), whereas plant presence promoted the occurrence of *Aphelenchus* with time, which was significant in winter 2013 ($F_{2,9} = 11.64$, $p = 0.003$).

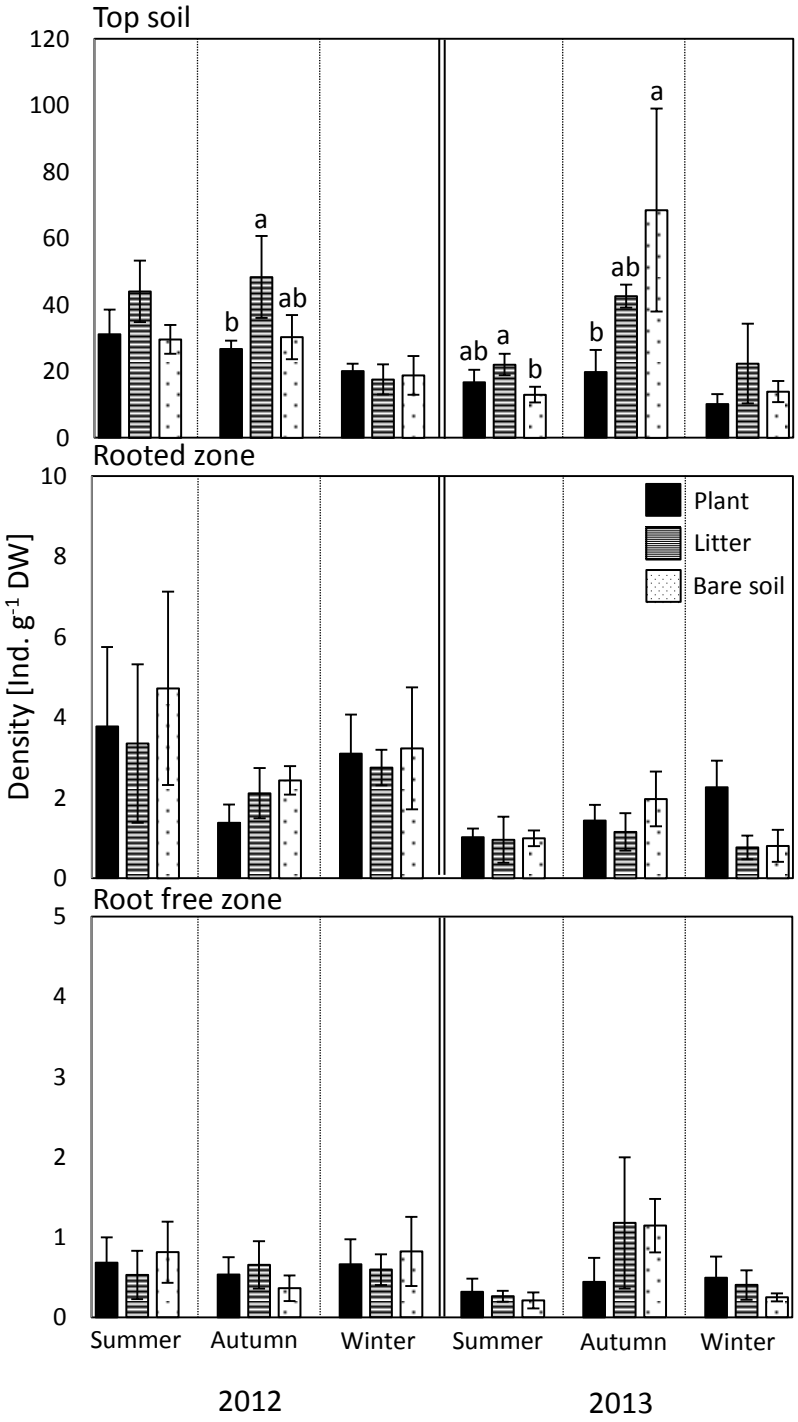

Figure 7.2: Density (Individuals g^{-1} dry weight soil $\pm$ SD) of nematodes in soil cropped with maize (plant), amended with maize shoot litter (litter) or bare soil, in topsoil (0-10 cm), rooted zone (40-50 cm), and root free zone (60-70 cm) in the years 2012 and 2013. Values within a sampling date with the same or no letters are not significantly different according to Tukey´s HSD test at $p < 0.05$.

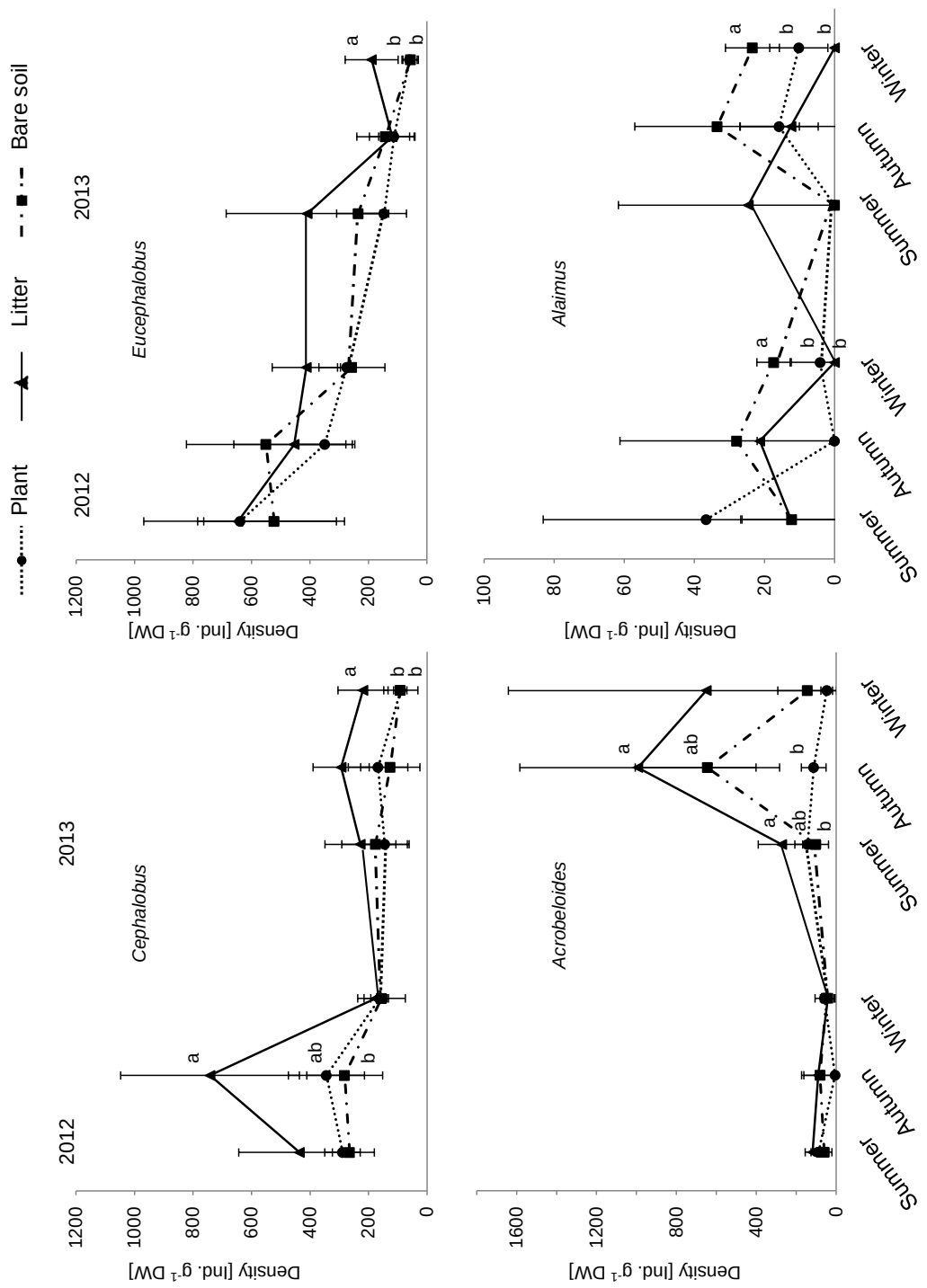

Figure 7.3: Occurrence of the bacterial-feeding taxa *Cephalobus*, *Eucephalobus*, *Acrobeloides* and *Alaimus* in soil cropped with maize (plant), amended with maize shoot litter (litter) or bare soil. Abundance (individuals 100 g^{-1} dry weight soil $\pm$ SD) is given in topsoil (0-10 cm), rooted zone (40-40 cm), and root free zone (60-70 cm) in the years 2012 and 2013. Values within a sampling date with the same or no letters are not significantly different according to Tukey´s HSD test at $p < 0.05$.

Nematode trophic structure and faunal indices

The major trophic groups of nematodes across treatments were bacterial feeders with an average relative abundance of 56%, followed by fungal (23%) and plant (20%) feeders in the upper soil layer (Fig. 7.3). Omnivores and predators were scarce with proportions below 1.1%. While no significant differences were detected during the first plant growth period, in the second season the proportion of plant feeders strongly declined in the absence of a plant (summer: $F_{2,9} = 4.19$, $p = 0.05$, autumn: $F_{2,9} = 8.29$, $p = 0.009$ and winter: $F_{2,9} = 5.03$, $p = 0.034$), whereas the proportion of bacterial feeders was distinctly higher in litter and bare soil plots in the topsoil (winter: $F_{2,9} = 14{,}29$, $p = 0.0016$). The occurrence of nematode trophic groups was related to the biomass of microorganisms at the base of the food web (S7.2 Table). In particular, bacterial feeders showed positive correlations ($p < 0.04$) to Gram-positive and Gram-negative bacteria across seasons (except winter 2012 and summer 2013), however this was restricted to the rooted zone in 40–50 cm depth (S7.2A and S7.2B Tables). A similar pattern occured for plant feeders and bacteria ($p < 0.04$, not summer and autumn 2013), with additional positive relations between these groups at 60–70 cm depth during summer 2012 and autumn 2013. In contrast, the frequency of fungal feeders was not linked to fungi (S7.2C Table). For higher trophic level nematodes, omnivores showed positive correlations in the topsoil (winter 2013, $p < 0.04$) and rooted zone (summer and autumn 2012, $p < 0.01$) with all microbial groups, whereas for predators this relationship was weak with only once showing a negative correlation to Gram-negative bacteria (autumn 2012, $R = -0.72$, $p = 0.01$; (S7.2A-S7.2C Table).

In the first plant growing season no distinct changes in micro-food web conditions were assigned by nematode faunal analyses. In the second vegetation period the average Enrichment Index (*EI*) in the upper soil layer was 58, indicating a moderate to good nutrient availability (Table 7.1). The *EI* was higher at the plant compared to litter plots, pointing to nitrogen enrichment during the growing season (summer 2013, Tukey's HSD test, $p < 0.05$). The Structure Index (*SI*), ranging from zero to 30, indicated disturbance and very low food web complexity, but no treatment

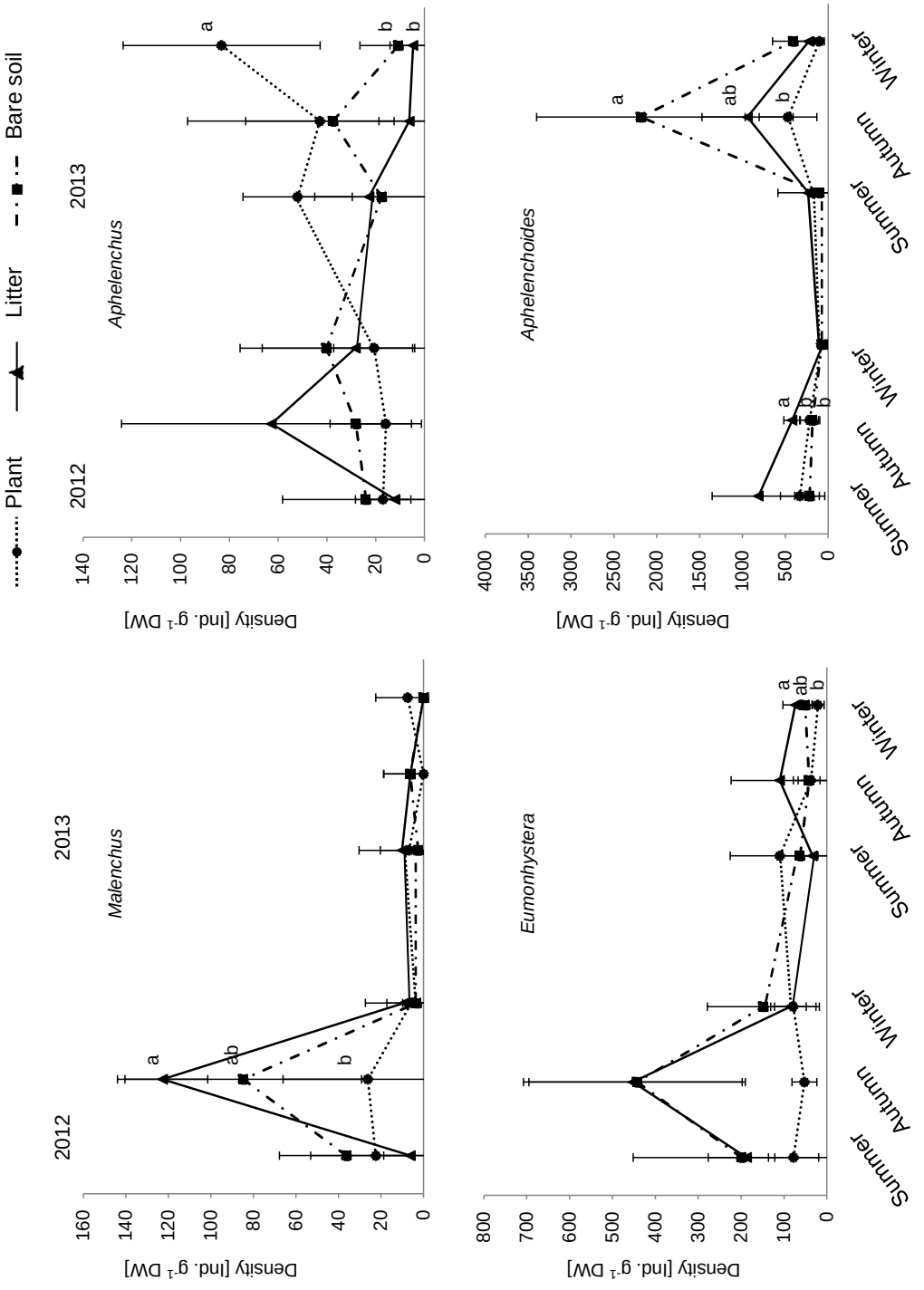

Figure 7.4: Occurrence of the root-feeding *Malenchus*, the bacteria and unicellular eukaryote feeding *Eumonhystera*, and the fungal-feeding *Aphelenchus* and *Aphelenchoides* in soil cropped with maize (plant), amended with maize shoot litter (litter) or bare soil. Abundance (individuals 100 g^{-1} dry weight soil $\pm$ SD) is given in topsoil (0-10 cm), rooted zone (40-50 cm), and root free zone (60-70 cm) in the years 2012 and 2013. Values within a sampling date with the same or no letters are not significantly different according to Tukey´s HSD test at $p < 0.05$.

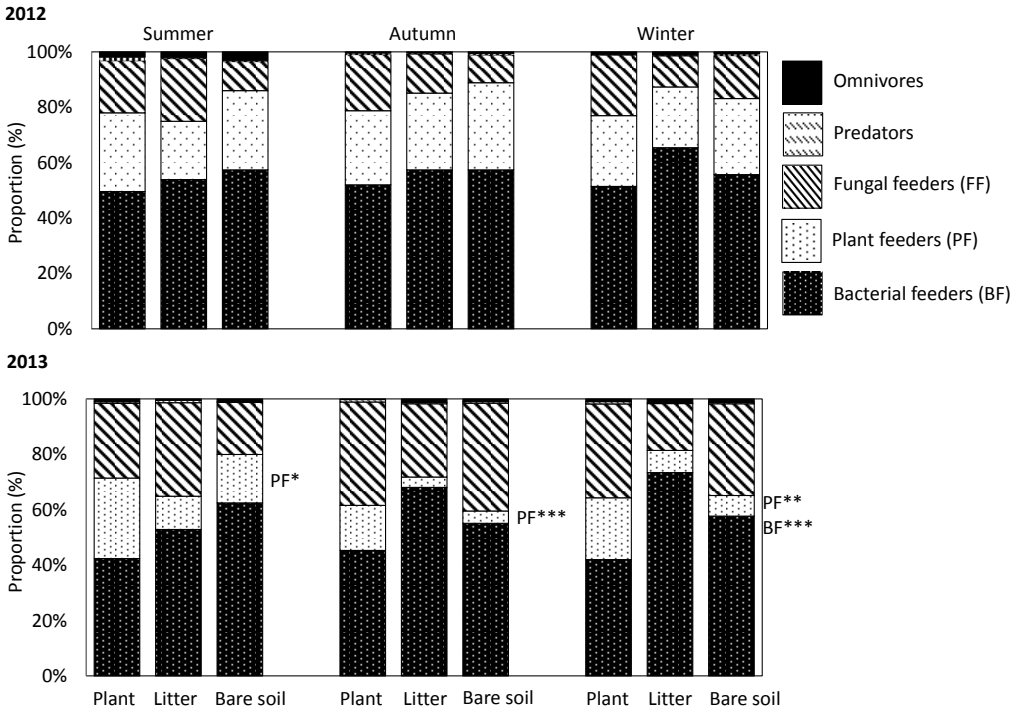

Figure 7.5: Proportion of nematode trophic groups (% ± SD) in soil cropped with maize (plant), amended with maize shoot litter (litter) or bare soil in topsoil (0-10 cm) in the years 2012 and 2013. ANOVA with the factors season (S) and treatment (T), significant effects are indicated by *, **, *** at $p < 0.05$, 0.01, 0.001.

effects. In contrast, the Basal Index (*BI*) was affected by plant presence and increased in the rooted zone in summer and winter, whereas it decreased in the topsoil during the summer (Tukey's HSD test, $p < 0.05$). The Channel Index (*CI*) with an average below 50 indicated a carbon flow mainly through the bacterial channel, with the highest values at the plant plots in summer and winter (Tukey's HSD test, $p < 0.05$).

Table 7.1: Nematode food web conditions (community indices ± SD) at plots cropped with maize (plant), amended with maize shoot litter (litter) or bare soil, in topsoil (0-10 cm), rooted zone (40-50 cm) and root free zone (60-70 cm) after two vegetation periods. Presented are the *EI* – enrichment index, *SI* – Structure Index, *BI* – Basal Index, and *CI* – Channel Index. ANOVA with the factors season (S) and treatment (T) with *, **, *** as $P <$ 0.05, 0.01, 0.001. Values within a row and sampling date with the same or no letters are not significantly different according to Tukey´s HSD test at $P < 0.05$.

Depth	Indices	Summer			Autumn			Winter			ANOVA
		Plant	Litter	Bare soil	Plant	Litter	Bare soil	Plant	Litter	Bare soil	
0-10 cm	EI	53 ± 6**a**	41 ± 6**b**	45 ± 1**ab**	59 ± 9	69 ± 8	77 ± 11	58 ± 5	59 ± 15	68 ± 10	S***, SxT*
	SI	11 ± 4	11 ± 9	6 ± 5	7 ± 6	11 ± 2	13 ± 12	17 ± 9	7 ± 12	22 ± 6	
	BI	45 ± 5**b**	55 ± 5**a**	53 ± 1**ab**	40 ± 9	30 ± 8	22 ± 11	39 ± 4	40 ± 16	29 ± 9	S***, SxT*
	CI	43 ± 15	56 ± 25	30 ± 19	43 ± 25	18 ± 6	24 ± 15	40 ± 9a	18 ± 9b	26 ± 12**ab**	S*
40-50 cm	EI	40 ± 10	51 ± 11	58 ± 7	57 ± 6	67 ± 10	63 ± 5	55 ± 9**b**	77 ± 11**a**	61 ± 10**ab**	S***, T*
	SI	7 ± 13	13 ± 15	0	0	10 ± 9	7 ± 8	6 ± 8	30 ± 36	11 ± 13	T*
	BI	57 ± 10**a**	44 ± 5**ab**	42 ± 7**b**	43 ± 6	31 ± 9	36 ± 6	44 ± 10**a**	22 ± 12**b**	37 ± 11**ab**	S**, T*
	CI	88 ± 15**a**	63 ± 27**ab**	45 ± 13**b**	47 ± 24	34 ± 13	43 ± 13	57 ± 18**a**	17 ± 8**b**	33 ± 17**ab**	S***, T*
60-70 cm	EI	61 ± 12	63 ± 32	39 ± 20	75 ± 12	76 ± 10	76 ± 11	52 ± 22	57 ± 9	55 ± 8	S*
	SI	0	0	0	0	28 ± 29	10 ± 12	10 ± 20	10 ± 20	0	
	BI	39 ± 12	37 ± 32	61 ± 20	25 ± 12	22 ± 11	23 ± 11	42 ± 13	39 ± 2	45 ± 8	S**
	CI	60 ± 35	52 ± 55	71 ± 34	24 ± 18	21 ± 12	18 ± 8	69 ± 38	22 ± 16	56 ± 31	S*

7.5 Discussion

Rhizosphere food web

The rhizosphere associated food web comprises both, the herbivore and detritivore food chain, and therefore represent a hotspot of species interactions (Kerry 2000; Bais et al. 2006; Uren 2007). The presence of maize plants had a positive impact on microorganisms, and total biomass increased in the first, and that of all groups (total, Gram-positive and Gram-negative bacteria, fungi), in the second season. These effects occurred in autumn and winter, yet were not apparent in the root free zone. Previous studies at the experimental field site estimated that maize allocated 0.30 kg C m^{-2} as root-C belowground (Pausch et al. 2013). During fallow periods primary decomposers obviously rely on such root carbon of the harvested crop. This is supported by the increase in the fungal decomposition channel assigned by the higher *CI* in nematode communities, indicating usage of recalcitrant (e.g. dead roots) resources.

A growing plant sustains energy flux belowground via both, the primary production-based herbivore and the decomposition-based detrital food chain. As in microorganisms (see above), this dual resource supply likely fosters nematode population development in comparison to the litter and bare soil treatments. However, nematode numbers at plant plots were generally similar to those under bare soil in the topsoil, and no positive plant impact in the rooted and root-free zone was observed. This could be attributed to the reduced soil moisture due to water uptake by plants. Both Görres et al. (1998) and Alphei & Coenen (1999) observed a distinct effect of soil water content on nematodes density. During the experimental period the seasonal average of the soil moisture showed only small differences, ranging between 24 to 32% (volumetric water content) across all treatments and seasons (S7.1 Table). In summer, the planted soil was slightly wetter in the topsoil and rooted zone, whereas in autumn it was dryer. However, this had not negative impact on nematode density, which corresponds to Griffiths et al. (2003) showing that

variations sufficient to affect plant growth had only minor impact on the nematode community.

Another reason for the lack in a general promotion of the micro-food web by maize presence could be its low root biomass with 0.08 to 0.2 mg C g^{-1} soil only (Pausch et al. 2013). From 2009 to 2012 the arable field site was cultivated by wheat, with twice as much root biomass in the topsoil (Kramer et al. 2012), beneficially affecting microbial as well as meso- and macrofauna communities as compared to maize (Kramer et al. 2013; Moll et al. 2015; Scheunemann et al. 2015). Among nematodes the decline of the plant-feeding *Malenchus* and the rhizosphere associated bacterial-feeding *Eucephalobus* (Dmowska & Ilieva 1995) with time also points to maize as a poor host. However, in the second season the density of plant feeders was highest at plants plots, predominantly in summer, which coincides with the maximum development stage of maize roots (Pausch et al. 2013). Overall, the herbivore food chain was clearly promoted under maize crop compared to litter and bare soil plots.

As herbivore and detritivore food chains merge at higher trophic levels, plant presence can support a more diverse food web with a considerable build-up of higher trophic levels (Heijboer et al. 2017). However, in the investigated arable soil, the density of predators and omnivores was low, also reflected by the low *SI*, pointing to a disturbed and basal food web across all treatments (Thoden et al. 2011). Thus, the enhanced resource entry into the micro-food web at plant plots was not mirrored by higher trophic levels, suggesting that the energy flux along the food chain was generally low. Correspondingly, Zhang et al. (2015) reported that bottom-up effects of vegetation on plant-feeding nematodes were weakly correlated with predator occurrence. In sum, the structure of the rhizosphere micro-food web at the plant plots, although characterized by high resource availability, was set by the predominance of bottom-up effects to lower trophic levels but this did not result in more connectivity, i.e. energy flux and biomass build up, to higher trophic levels.

Detritusphere food web

Compared to rhizosphere communities, food webs in bulk soil, based on detrital resources only, were assumed to be less complex. By adding maize litter the resource supply to the detritus based food chain was enhanced by a rate of 0.35 kg C m^{-2}. In previous studies at the experimental field similar amendment fostered microorganisms, in particular fungi and the fungal decomposition channel, yet these plots were cultivated with maize or wheat during the vegetation period (Scharroba et al. 2012; Kramer et al. 2013; Moll et al. 2015). In the present experiment with the root channel excluded, application of maize litter did not alter total microbial biomass significantly as compared to the bare soil treatment, except once in autumn of the first season in topsoil. These findings highlight the importance of the linkage between the herbivore and detritivore food chain, i.e. food web connectivity and structure, for the energy transfer within the entire food web.

During the second year the biomass of all microbial groups was generally lowest in the rooted zone of the litter plots. In contrast, the nematode density was high, reflecting the input of organic matter, which is reported to increases total numbers of nematodes (Ferris et al. 1996; Ferris & Bongers 2006; Tao et al. 2009). This points to enhanced nematode grazing as regulating agent for microbial biomass, and is supported by the positive correlation of bacterial biomass and bacterial feeders. The amendment of soil with recalcitrant plant residues generally results in enhanced carbon incorporation into the fungal food chain (Frey et al. 2003; Moll et al. 2015). However, enhanced carbon flux in the fungal decomposition channel was not assigned by the *CI* at litter plots. The maize shoot litter applied was rich in nitrogen (C to N ratio 18.3); furthermore maize litter contains large amounts of easily decomposable carbohydrates (O-alkyl-C as 79% of shoot biomass) (Helfrich et al. 2006). This may explain the observed high activity of the bacterial channel in the detritusphere food web, which was most apparent during winter across soil depths. At that season bacterial populations were mobilized and transported along the soil depth profile at the experimental arable field (Dibbern et al. 2014).

Within nematode communities, plant-feeders showed considerable resilience to the absence of plants, with negative effects only in the second season. They were mainly represented by the family Tylenchidae, taxa generally assigned as plant parasites (Yeates et al. 1993) or "plant associated" (McSorley 1997; McSorley 1999), but fungal feeding was also reported (Freckman & Ettema 1993). This points to alternate food choice and the ability to switch diet if root resources become limiting. Among bacterial feeders the opportunistic genera *Cephalobus* and *Acrobeloides* were fostered by litter amendment, thereby displaying an opposing trend (Fig. 7.3). While *Cephalobus* achieved its maximum density in autumn 2012 and strongly decreased in 2013, the opposite was true for *Acrobeloides*. Likely, these taxa in the same trophic group negatively affect each other (Postma-Blaauw et al. 2005). Generally, organic amendment such as mulching fosters nematode top predators and omnivores (Wardle et al. 1995; McSorley & Frederick 1999), and comparable effects are also evident in other soil fauna, e.g. Collembola and spiders, in the detritivore food chain (Sereda et al. 2015). However, in the present study no positive impact of litter amendment was apparent at nematode higher trophic levels, rather the micro-food web structure remained disturbed and at a basal stage.

Bulk soil food web

Under crop, i.e. with maize or wheat, the C_{org} content at the experimental field ranged between 10.6 to 13.7 mg g^{-1} soil across two growing seasons (Alphei & Coenen 1999). Leaving such a cropland fallow can lead to reduced soil carbon sequestration (Drijber et al. 2000) and the lack of recent carbon input by crop plants at bare soil plots was expected to result in a decline in decomposer organisms and micro-food web complexity with time (Neher et al. 1995; Sánchez-Moreno et al. 2006). Conform with this hypothesis, the amount of total soil PLFAs in the topsoil was generally lower in bare compared to planted soil, whereas in the rooted zone bare soil plots held an intermediate position, and no treatment effects occurred in the deeper root-free zone. This resilience of microorganisms below the plough layer suggests sufficient resources to sustain considerable biomass. The availability of

maize carbon after one vegetation period was still seven times higher as compared to older carbon sources (Pausch & Kuzyakov 2012), pointing to maize root residues as substrate for microbial decomposition. Correspondingly, Scheunemann et al. (2015) reported that soil arthropods continued to incorporate old C_3-derived organic matter after a switch to the C_4 crop maize. Usage of such "old" SOM by the detritivore food chain in the bulk soil likely is as an important characteristic of arable systems, where regular removal of crop cuts the internal terrestrial C-cycle, with plant carbon only partly returned to the soil as dead organic matter.

Within nematode communities certain taxa were fostered in the bare soil plots, predominantly opportunists such as the bacterial feeder *Acrobeloides* and the fungal feeder *Aphelenchoides*. The presence of these opportunists reflects the replacement of the more diverse nematode fauna under crop plant by few taxa tolerant to a wide range of environmental factors common under fallow (Háněl 2003). On the other hand, the *K*-strategist *Alaimus*, adapted to an undisturbed environment (Bongers 1990), increased at bare soil plots at the end of the second vegetation period. Thus with time the bare soil became a suitable habitat for certain long-lived big nematode taxa able to cope with low resource availability.

7.6 Conclusion

The nematode micro-food web in the investigated arable soil showed marked resilience to the lack of plants, litter amendment or bare soil, and the structure of the rhizosphere, detritusphere and bulk soil food webs was little affected in the first year. In the second vegetation period, bottom-up effects maintained by belowground plant productivity were apparent and the herbivore food chain was fostered. However, neither good resource availability nor presence of both the herbivore and detritivore food chain had positive impact on higher trophic levels of the rhizosphere food web at plant plots. Comparably, only limited flux of the amended organic resources along the food chain occurred at litter plots and the detritusphere food web remained at basal conditions. Even under bare soil, with

no resource input at all, the trophic structure and diversity of the bulk soil food web was only moderately affected. Instead detritivores efficiently exploited organic resources from previous vegetation periods, with the establishment of long-lived *K*-strategists pointing to stable environmental conditions. In sum, the different decomposer food webs in the arable soil were well adapted to changes in carbon availability and major pools. Apparently, the turnover of organic resources from previous vegetation period was sufficient to support the demands of the micro-food web for at least two growing seasons.

7.7 Acknowledgements

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7.8 Supporting Information

S 7.1: Volumetric water content in soil cropped with maize (plant), amended with maize shoot litter (litter) or fallow along the depth profile. Presented are the average values (yearly quarter) in different seasons in 2012 and 2013. Statistical significances are based on two-way ANOVA with the factors season (S) and treatment (T); *** - $P < 0.001$.

	Autumn 2012			**Winter 2012**			**Summer 2013**			
Depth	Plant	Litter	Bare soil	Plant	Litter	Bare soil	Plant	Litter	Bare soil	ANOVA
0-10 cm	27.5 ± 2.3	30.8 ± 1.9	29.5 ± 1.6	31.9 ± 2.9	30.2 ± 1.4	31.6 ± 1.4	29.8 ± 4.6	24.2 ± 4.1	25.7 ± 4.5	T***, S***, TxS***
10-20 cm	25.5 ± 1.0	28.5 ± 0.8	28.5 ± 1.3	28.3 ± 1.8	28.7 ± 1.2	29.3 ± 1.2	31.8 ± 1.4	30.2 ± 1.9	29.7 ± 1.5	T***, S***, TxS***
20-30 cm	27.3 ± 1.8	30.1 ± 0.7	29.0 ± 1.1	29.0 ± 2.1	29.7 ± 0.8	29.4 ± 0.8	35.4 ± 1.2	30.3 ± 1.3	29.6 ± 1.3	T***, S***, TxS***
40-50 cm	25.8 ± 0.5	31.4 ± 0.8	29.1 ± 1.1	27.7 ± 2.2	30.6 ± 0.9	29.2 ± 0.7	33.6 ± 0.9	32.8 ± 1.4	29.6 ± 1.3	T***, S***, TxS***

S 7.2: Non-parametric Spearman correlation between nematode trophic groups and the biomass of different microbial food sources: A – Gram-positive bacteria, B – Gram-negative bacteria, C – fungi. Investigated were the top soil (0-10 cm), rooted zone (40-50 cm) and root free zone (60-70 cm) in two successive years. Significant correlations are marked in bold. – trophic group or diet not present.

A

		Gram-positive bacteria											
Depth	Trophic group	Summer 2012		Autumn 2012		Winter 2012		Summer 2013		Autumn 2013		Winter 2013	
		R	*P*	*R*	*P*	*R*	*P*	*R*	*P*	*R*	*P*	*R*	*P*
0-10 cm	Bacterial feeders	0.46	0.13	-0.1	0.75	0.1	0.76	-0.06	0.85	0.24	0.44	-0.48	0.12
	Plant feeders	0.51	0.09	-0.02	0.95	-0.01	0.97	0.1	0.75	0.53	0.08	0.09	0.78
	Fungal feeders	-0.43	0.16	-0.15	0.63	0.17	0.6	0.2	0.54	0.08	0.81	-0.32	0.31
	Predators	0.09	0.79	-0.71	0.01	0.15	0.64	0.44	0.15	0.31	0.32	-0.25	0.43
	Omnivores	0.42	0.17	-0.03	0.92	0.01	0.98	0.18	0.57	-0.27	0.4	**0.59**	**0.04**
40-50 cm	Bacterial feeders	**0.93**	**0**	**0.59**	**0.04**	0.13	0.7	-0.24	0.44	**0.61**	**0.04**	**0.72**	**0.01**
	Plant feeders	**0.83**	**0**	**0.76**	**0**	**0.6**	**0.04**	0.2	0.54	0.43	0.16	**0.73**	**0.01**
	Fungal feeders	0.55	0.06	0.4	0.2	0.3	0.34	0.04	0.9	0.48	0.11	**0.8**	**0**
	Predators	0.41	0.18	-	-	-0.13	0.68	-0.22	0.5	0.48	0.11	-	-
	Omnivores	**0.76**	**0**	0.7	0.01	0.34	0.28	-	-	0.15	0.64	-	-
60-70 cm	Bacterial feeders	0.47	0.12	-0.18	0.57	0.57	0.05	-0.01	0.98	0.34	0.28	0.1	0.75
	Plant feeders	0.5	0.1	0.41	0.18	0.2	0.53	0.3	0.35	**0.59**	**0.04**	0.02	0.94
	Fungal feeders	0.38	0.23	0.06	0.85	0.1	0.76	-0.38	0.23	0.1	0.75	0.21	0.51
	Predators	-	-	-	-	0.53	0.07	-	-	-0.31	0.33	-	-
	Omnivores	-0.04	0.89	-0.22	0.5	-0.18	0.57	-	-	-0.28	0.39	0.04	0.89

B

		Gram-negative bacteria											
Depth	Trophic group	Summer 2012		Autumn 2012		Winter 2012		Summer 2013		Autumn 2013		Winter 2013	
		R	*P*	*R*	*P*	*R*	*P*	*R*	*P*	*R*	*P*	*R*	*P*
0-10 cm	Bacterial feeders	0.47	0.12	-0.21	0.51	0.08	0.81	-0.03	0.91	0.18	0.57	-0.5	0.1
	Plant feeders	0.47	0.12	-0.03	0.93	0.01	0.97	0.15	0.65	0.57	0.05	0.06	0.86
	Fungal feeders	-0.4	0.2	-0.19	0.56	0.32	0.31	0.15	0.63	0.06	0.86	-0.17	0.6
	Predators	0.12	0.72	**-0.72**	**0.01**	0.12	0.71	0.44	0.15	0.16	0.62	-0.09	0.77
	Omnivores	0.3	0.34	0.1	0.76	0.25	0.43	0.22	0.48	-0.36	0.25	**0.63**	**0.03**
40-50 cm	Bacterial feeders	**0.94**	**0**	**0.64**	**0.02**	0.08	0.81	-0.16	0.62	**0.61**	**0.04**	**0.77**	**0**
	Plant feeders	**0.86**	**0**	**0.73**	**0.01**	**0.65**	**0.02**	0.41	0.18	0.43	0.16	**0.75**	**0.01**
	Fungal feeders	0.54	0.07	0.31	0.33	0.32	0.31	-0.1	0.76	0.48	0.11	**0.8**	**0**
	Predators	0.41	0.18	-	-	-0.13	0.68	-0.22	0.5	0.48	0.11	-	-
	Omnivores	**0.75**	**0.01**	**0.75**	**0.01**	0.41	0.19	-	-	0.15	0.64	-	-
60-70 cm	Bacterial feeders	0.55	0.07	-0.13	0.68	0.27	0.4	-0.19	0.56	0.41	0.19	0.12	0.71
	Plant feeders	**0.59**	**0.04**	0.41	0.18	0.06	0.86	0.35	0.27	**0.63**	**0.03**	-0.01	0.96
	Fungal feeders	0.5	0.1	0.13	0.68	-0.06	0.86	-0.13	0.7	0.17	0.59	0.31	0.33
	Predators	-	-	-	-	0.46	0.13	-	-	-0.22	0.5	-	-
	Omnivores	-0.13	0.68	-0.13	0.68	-0.33	0.29	-	-	-0.28	0.39	0.22	0.5

C

		Fungi											
Depth	Trophic group	Summer 2012		Autumn 2012		Winter 2012		Summer 2013		Autumn 2013		Winter 2013	
		R	*P*	*R*	*P*	*R*	*P*	*R*	*P*	*R*	*P*	*R*	*P*
0-10 cm	Bacterial feeders	0.45	0.14	0.19	0.56	0.42	0.17	0.21	0.51	**0.73**	**0.01**	0.52	0.08
	Plant feeders	0.34	0.29	0.06	0.86	-0.08	0.8	-0.08	0.81	0.09	0.78	-0.1	0.76
	Fungal feeders	0.1	0.76	0.52	0.08	0.04	0.9	0.51	0.09	0.22	0.5	-0.01	0.98
	Predators	-0.2	0.53	0.31	0.33	-0.21	0.51	0.64	0.02	0.43	0.17	-0.45	0.14
	Omnivores	0.35	0.27	0.18	0.57	0.26	0.41	-0.15	0.63	0.16	0.61	**0.62**	**0.03**
40-50 cm	Bacterial feeders	**0.75**	**0.01**	0.38	0.22	-0.1	0.76	0.15	0.63	**0.59**	**0.04**	**0.69**	**0.01**
	Plant feeders	**0.82**	**0**	0.31	0.32	**0.76**	**0**	0.24	0.44	0.31	0.32	**0.71**	**0.01**
	Fungal feeders	0.22	0.5	0.05	0.88	0.23	0.47	0	1	0.46	0.13	**0.78**	**0**
	Predators	0.2	0.52	-	-	-0.31	0.33	-0.39	0.21	0.39	0.21	-	-
	Omnivores	**0.77**	**0**	**0.83**	**0**	0.26	0.41	-	-	0.09	0.78	-	-
60-70 cm	Bacterial feeders	0.12	0.71	0.22	0.5	0.33	0.3	-0.21	0.51	0.32	0.31	0.33	0.3
	Plant feeders	0.41	0.19	0.29	0.37	0.55	0.06	0.57	0.05	0.49	0.11	0.22	0.48
	Fungal feeders	0.02	0.95	0.05	0.88	0.16	0.62	0.15	0.63	0.25	0.43	0.28	0.38
	Predators	-	-	-	-	0.08	0.82	-	-	-0.31	0.33	-	-
	Omnivores	-0.22	0.5	-0.22	0.5	0.03	0.92	-	-	0.02	0.95	-0.13	0.68

8 General Discussion

This thesis elucidated, in an arable ecosystem, case-specific responses of bacteria and fungi to plant resources at different temporal and spatial scales, and their contributions to C transfer from plants to the belowground soil food web. C input manipulation studies were performed first, to investigate the *in situ* long-term effects of root- and shoot-derived sources on fundamental underlying members of the soil food web down a soil profile over five consecutive years (Chapter 5); second, to clarify bacterial and fungal contributions to C turnover processes in soil microhabitats (Chapter 6); and third, to quantify *in situ* effects of a diminished C supply on microorganisms and nematodes as key members of the soil micro-food web (Chapter 7).

8.1 Effects of resource quality and availability on soil microorganisms

Plant resources are the major compounds supplying belowground C pools, but their incorporation into those pools depends mainly on the quality rather than the availability of substrates. Our comparison of plant residues of different complexities (roots vs. shoots) provides evidence that the labile C pool of residues is the fraction which is most utilized by soil microorganisms. This was seen in a long-term field experiment, in which the manipulation of C input via roots and shoots resulted in the same relative amounts of plant C in soil microorganisms (Chapter 5), and during the decomposition process at the soil-litter interface (Chapter 6). The low proportion of ^{13}C in the soil EOC pool demonstrated that labile plant-derived C was utilized by microorganisms, as seen in higher ^{13}C values in C_{mic} and different microbial

PLFAs (Fig. 5.1, 5.3). The presence of two plant resources in the long-term field experiment doubled the C incorporation into microbial biomass, indicating that the microbial biomass benefited from the additional amount of labile C (Fig. 5.2, 5.4).

Kramer et al. (2012) concluded, over two consecutive years of the same field trial, that root-derived C is more important than shoot-derived C. This excluded crown root C, however, which is the major C source in the belowground pathway (Pausch & Kuzyakov 2012). It was not taken into account due to its limited decomposition within the time frame of the study. This might be different over a longer time scale; then, the influence of slow growing specialists (especially in the fungal community), which exploit these substrates and start the decomposition process, could alter the importance of total root C as a source for microorganisms. This suggests that the recalcitrance of substrate determined the C utilization by bacteria and fungi more than the absolute quantity of the substrate. This is in line with findings from Rasse et al. (2005), who determined that the mean residence time of root-derived C in soil is 2.4 times higher than that of shoot-derived C due to a higher proportion of lignified compounds in the secondary cell walls of roots than in shoots (Amin et al. 2013).

Furthermore, it is noteworthy that the removal of plants had less severe effects on microbial biomass than expected (Chapter 7). In the first year of plant removal, the structure of the micro-food web remained similar to that of treatments with plants and litter amendments, and in the second year as well only minor effects on microbial biomass were seen (Fig. 7.1). This was likely due to utilization of plant C from previous years, as wheat had been cultivated on the field site. Decomposition of the extended root systems of wheat plants in comparison to maize roots (Kramer et al. 2013) was likely the reason for the observed lesser effect of plant removal on members of the micro-food web. This legacy effect may have obscured the effect of plant resources, leading decomposer organisms to utilize autochthonous C to maintain microbial pool size and function in the soil profile.

Subsoil microbial communities rely on transport processes from the surface or from the rooted zone to deeper soil layers. Although microbial C pools were very small

below the plough zone (Fig. 5.4, 7.1), Kramer et al. (2013) showed that the specific enzyme activity (enzyme activity per microbial biomass) of oxidative enzymes was higher in deeper soil layers, indicating specialized microbial communities (Scharroba et al. 2012; Moll et al. 2016) and/or greater enzyme production due to lack of spatial proximity of microorganisms to their C sources. Since PLFA-SIP could be applied to subsoil samples, we were able to demonstrate that subsoil microorganisms in both the rooted and the root-free zones metabolized a distinct quantity of plant C into their biomass (Fig. 5.3). The maize-C label was found in up to 53% of the saprotrophic PLFA 18:2ω6,9 at 70 cm depth in 2013. Surprisingly, shoot-derived C was also detected in subsoil microorganisms, which indicates either a translocation of dissolved and particulate plant material (Barkle et al. 1999) or a transport of topsoil microorganisms after certain weather conditions (heavy rainfall or snowmelt) (Dibbern et al. 2014). The decrease in the ^{13}C label in the rooted zone indicates that possibly more maize C was translocated belowground in 2011 than in the following two years, and/or the turnover of the translocated C might have been higher than the supply of fresh C from topsoil in 2012 and 2013. These results provide new insights into subsoil microbial processes and contributes to current research on microbial decomposition processes in deeper soil layers (Preusser et al. 2017).

8.2 Soil microbial carbon utilization, turnover and transfer to higher trophic levels

Resource partitioning during decomposition processes is presumed to be the major driver of soil microbial diversity (Zhou et al. 2002), but results of this thesis challenge the premise of a clear separation of substrate-dependent C utilization by distinct groups of microorganisms. Members of both the bacterial and fungal communities were involved in the initial decomposition process, when labile C sources were present, but as labile C sources became depleted, fungi were able to acquire C from complex compounds (Fig. 6.4f). Surprisingly, the relative plant-derived C values

in both the short-term microcosm experiment (3.4% in fungi and 0.7 to 1.8% in bacteria after 4 days of decomposition) and in the long-term field experiment were always higher in the fungal biomass than in the bacterial biomass (Fig. 5.3). This indicates that saprotrophic fungi are a key group in the decomposition of plant-derived C sources. Fungi have a selective advantage because they are able to exploit new C sources through their hyphal growth. Since they are able to translocate C and nutrients via their hyphal systems over distances >1m (Jennings 1987; Cairney 2005), they have the capacity to succeed in areas characterized by prolonged C depletion. This seems to be especially important for the fungal community in deeper soil layers where C quality is low and accessibility to new C resources is restricted.

Bacteria, however, rely on the availability of labile substrates in close proximity to their cells, leading to the formation of small-scale microbial hotspots with enhanced microbial activity (Kuzyakov & Blagodatskaya 2015) such as the rhizosphere (Pausch et al. 2016) and the detritusphere (Chapter 6). In our study, bacteria incorporated relatively less plant C into their respective biomass than fungi. This could have been due to the huge quantity of inactive or dormant bacteria in soil, which led to dilution of the ^{13}C value, and might have resulted in an underestimation of the bacterial contribution to the decomposition process. The differences in absolute pool sizes of bacterial and fungal PLFA-C may have indicated the importance of the fungal energy channel in spite of lower fungal than bacterial biomass. Our results are in line with findings from Pausch et al. (2016), who showed that C transfer to higher trophic levels in the rhizosphere was performed mainly by highly plant-C enriched saprotrophic fungi 2 days after a ^{13}C-CO_2 pulse labeling of maize plants. This utilization of recently deposited plant C by saprotrophic fungi was even more rapid than the utilization of these substrates by mycorrhizal fungi, as shown by Hannula et al. (2012). This demonstrates the central role of saprotrophic fungi in processing plant root- and shoot-derived C and their contribution to the genesis of SOM.

High incorporation of plant C into fungal biomass as compared to bacteria over time in the long-term experiment raised the following questions: (i) do fungi have higher C use efficiencies (CUE) than bacteria? and/or (ii) do they have the capacity to store C longer in their biomass than bacteria? The use of PLFAs during maize litter decomposition revealed that the mean residence time of C in fungal biomass was in the same range or shorter than in bacterial biomass (Table 6.1). This finding contradicts the historical assumption of slow C turnover via the fungal community. High incorporation rates of plant-derived C into fungal biomass may result from higher CUE of saprotrophic fungi for plant material in comparison to bacterial CUE for these substrates. Overall, we observed that mineralization of litter decreased with age (i.e. quality, Fig. 6.2) and thus also affected the incorporation of C into bacterial and fungal biomass, especially in the later stage of litter decomposition (Fig. 6.4). While both bacteria and fungi used labile C sources in the early stage of decomposition, utilization of complex C sources was dominated by fungi. This overlapping substrate utilization in the early stage of decomposition was also found by Kramer et al. (2016), in a microcosm experiment using C substrates of varying complexities. The authors demonstrated, with RNA-SIP, that only a few bacterial and fungal members of the soil micro-food web were involved in degradation of complex root and shoot plant material. But once introduced into the soil, the plant-derived C remained in the SOC pool (Fig. 6.2), with a long mean residence time (Table 6.1) and therefore contributed to the formation of SOC stocks. This complicates the determination of turnover in micro-food webs, because cross-feeding within and between different groups of microorganism ("microbial loop", Chapter 6) as well as top-down regulation by higher trophic levels (Chapter 7, Pausch et al. (2016) and Scheunemann et al. (2016)) in the soil food web can occur simultaneously.

8.3 Conclusion and outlook

This thesis used stable isotope probing combined with biomarker analysis to study C fluxes between different biotic and soil C pools as a pre-requisite for modeling C dynamics in arable fields. We provided evidence that absolute C pool size alone does not indicate the contributions of groups of microorganisms to the decomposition of plant roots and shoots, which is instead strongly influenced by the quality of the substrate. As a consequence, the availability of complex resources may play a crucial role during periods of C starvation or in deeper soil layers, after labile C sources have been consumed by soil microbes.

Furthermore, this thesis highlights the central role of saprotrophic fungi in the soil microbial food web. Although fungal abundance was low in our investigated soil, their high relative incorporation of plant C demonstrated the potential of fungally-mediated C transfer to the soil. An overlapping substrate utilization of labile C from bacteria and fungi was shown, with subsequent C turnover in fungi at least as rapid as in bacteria. Additionally, cross-feeding between members of the same trophic level as well as top-down regulation from higher trophic levels influenced C flow into different members of the soil food web.

The use of stable isotopes provided a reliable way to follow C fluxes from plant resources through the overall microbial community. Fresh plant resources could have a strong priming effect (as seen in the microcosm study in Chapter 6), which altered the availability of older SOM-derived sources. Application of the stable isotope ^{18}O can be used in future studies in combination with ^{13}C labeled substrates to separate plant C utilizers in microbial hotspots from the larger numbers of inactive microbes and other active microbial SOM utilizers (Hungate et al. 2015). Since this ^{18}O-SIP approach can be combined with molecular techniques as well, source-specific consumers can be identified in the heterogeneous soil environment. Therefore, microbial responses to the quality and availability of plant resources and to their regulation by higher trophic levels of the soil food web remain worthy of future research.

Although numerous models for soil C cycling are available, e.g. the PECCARD model for the degradation of pesticides (Pagel et al. 2014), or RothC for the long-term fate of C sources (Jenkinson et al. 1990), C turnover in specific microbial compartments as well as feeding interactions are rarely included (Manzoni & Porporato 2009). This hampers the precision of earth system models, especially in the context of climate and land use changes. The data gathered here; on microbial abundance, microbial resource utilization, as well as C turnover in their respective biomass; can be implemented in a predictive C flux model. In combination with trophic interactions, examined within the framework of the FOR 918 research unit, a highly-resolved empirical food web model can be established.

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Publications and Presentations

Peer-reviewed Journal Articles

Müller, K., Kramer, S. Haslwimmer, H., Marhan, S., Scheunemann, N., Butenschoen, O., Scheu, S. & Kandeler, E. (2016). Carbon transfer from maize roots and litter into bacteria and fungi depends on soil depth and time. *Soil Biology and Biochemistry 93*, 79-89.

Müller, K., Marhan, S., Kandeler, E. & Poll, C. (2017). Carbon flow from litter through the soil microbial community in the detritusphere: From carbon incorporation rates to mean carbon residence times of bacteria and fungi. *Soil Biology and Biochemistry 115C*, 187-196.

Glavatska, O., **Müller, K.**, Butenschoen, O., Schmalwasser, A., Kandeler, E., Scheu, S., Totsche, K.U. Ruess, L. (2017). Disentangling the root- and detritus-based food chain in the micro-food web of an arable soil by plant removal, *PLoS ONE 12 (7)*, e0180264.

Oral Presentations

Müller, K., Haslwimmer, H., Marhan, S. & Kandeler, E. (2015). Carbon flow via the herbivore and detritivore food chain in an arable ecosystem. *Meeting of the Commission III of the German Soil Science Society 2015*, Bremen, Germany.

Müller, K., Marhan, S., Poll, C. & Kandeler, E. (2015). Mikrobieller Kohlenstoffumsatz während des Aubbaus von Maisstreu - ein Mikrokosmenexperiment. *Annual Meeting of the German Soil Science Society 2015*, Munich, Germany.

Kandeler, E., **Müller, K.**, Kramer, S. & Marhan, S. (2015). Microbial colonisation and resource partitioning in agricultural soils, Ecology of Soil Microorganisms 2015, Prague, Czech Republic.

Poster Presentations

Müller, K., Haslwimmer, H., Kramer, S., Marhan, S. & Kandeler, E. (2013). Langzeit-Kohlenstoffeintrag in die mikrobielle Gemeinschaft einer Agrarfläche. *German Soil Science Society 2013*, Rostock, Germany.

Müller, K., Haslwimmer, H., Marhan, S. & Kandeler, E. (2013). Carbon flow via the herbivore and detritivore food chain in an arable ecosystem. *Annual Meeting of the Ecological Society of Germany, Austria and Switzerland 2013*, Postdam, Germany.

Müller, K., Marhan, S., Poll, C. & Kandeler, E. (2015). Turnover of microbial carbon in the detritusphere. *Ecology of Soil Microorganisms 2015*, Prague, Czech Republic.

Müller, K., Marhan, S., Poll, C. & Kandeler, E. (2016). Turnover of microbial carbon in the detritusphere, *Soil Food Webs: Linking Structure, Energy Flux and Function*, Berlin, Germany.

Müller, K., Marhan, S., Poll, C. & Kandeler, E. (2016). Turnover of microbial carbon in microbial hot spots. *SOM_{mic}: Microbial Contribution and Impact on Soil Organic Matter, Structure and Genesis*, Leipzig, Germany.

Curriculum Vitae

Personal Information

Name	Karolin Müller
Birth Date	9. November 1987
Birth City	Saalfeld (Saale), Germany
Marital Status	Unmarried

Studies

2012–2017	PhD Student Institute of Soil Science and Land Evaluation, Soil Biology Group, University of Hohenheim, Stuttgart, Germany Funding by the Deutsche Forschungsgemeinschaft (DFG) FOR 918 "Carbon flow in belowground food webs assessed by isotope tracers" Thesis: "Soil microbial carbon assimilation and turnover of carbon depend on resource quality and availability"
2006–2012	Studies of Biology, University of Rostock, Germany Focus: Aquatic Ecology, Microbiology, Genetics and Environmental Law Thesis: "Phosphatlimitierung und Phosphataufnahme im Cyanobakterienplankton der Darß-Zingster Boddenkette"

School Education

1999–2006	Higher Education Entrance Qualification (Abitur) Gymnasium "Am Weißen Turm", Pößneck, Germany

Professional Experience

since 09/2017	Project Coordinator EIT Food Institute of Food Science and Biotechnology, Food Physics and Meat Science Group, University of Hohenheim, Stuttgart, Germany
since 01/2017	Scientific Assistant Institute of Soil Science and Land Evaluation, Soil Biology Group, University of Hohenheim, Stuttgart, Germany
01/2016–06/2016	Scientific Assistant Institute of Soil Science and Land Evaluation, Soil Biology Group, University of Hohenheim, Stuttgart, Germany
08/2011–09/2011	Student Assistant Leibniz Institute for Baltic Sea Research, Warnemünde, Germany
03/2009–06/2009	Student Assistant Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

Other Activities

07/2008	Internship "Village de Tortue",Gonfaron, France
04/2016–07/2016	Training Certified Quality and Environmental Management Representative for ISO 9001 and 14001 Fellbach, Germany

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Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation selbstständig angefertigt, nur die angegebenen Quellen und Hilfsmittel benutzt und inhaltlich oder wörtlich übernommene Stellen als solche gekennzeichnet habe. Ich habe noch keinen weiteren Promotionsversuch unternommen.

Filderstadt, 23. November 2017 Karolin Müller