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Effects of Resource Availability and Quality on Soil Microorganisms and their Carbon Assimilation

Dissertation

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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.

Susanne Kramer

Stuttgart, den 06.11.2013 Susanne Kramer

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1 Summary

Soil microorganisms play a pivotal role in decomposition processes and therefore influence nutrient cycling and ecosystem function. Availability and quality of resources determines activity, growth and identity of substrate users. In agricultural systems, availability of resources is dependent on, for example, crop type, management, season, and depth. At depth substrate availability and microbial biomass decrease. However, there remain gaps in our understanding of C turnover in subsoil and how processes in the topsoil may influence abundance, activity, and function of microorganisms in deeper soil layers. With respect to substrate quality it is thought that bacteria are the dominant users of high quality substrates and more labile components whereas fungi are more important for the degradation of low quality and more recalcitrant substrates (i.e. cellulose, lignin). Therefore, this thesis was designed to increase our understanding of C turnover and the influence of both availability and quality of substrates on microorganisms in an agricultural soil.

In the first and second studies, a recently established C3-C4 plant exchange field experiment was used to investigate the C flow from belowground (root) and aboveground (shoot litter) resources into the belowground food web. Maize plants were cultivated to introduce a C4 signal into the soil both by plant growth (belowground / root channel) and also by applying shoot litter (aboveground litter channel). To separate C flow from the shoot litter versus the root channel, maize litter was applied on wheat cultivated plots, while on half of the maize planted plots no maize litter was returned. Wheat cultivated plots without additional maize litter application served as a reference for the calculation of incorporated maize-C into different soil pools. Soil samplings took place in two consecutive years in summer, autumn and winter. Three depths were considered (0-10 cm: topsoil, 40-50 cm: rooted zone beneath the plough layer, 60-70 cm: unrooted zone). In the third study a microcosm experiment with substrates of different recalcitrance and complexity was carried out to identify primary decomposers of different plant litter materials (leaves and roots) during early stages of decomposition (duration of 32 days) and to follow the C flow into the next higher trophic level (protozoa).

The first study showed that the time of sampling strongly influenced the abundances of bacterial and fungal phospholipid fatty acids (PLFAs) as well as of enzyme activities at every depth. High bacterial and fungal PLFA abundances and enzyme activities in the profiles in winter indicated enhanced substrate availability and transient flow conditions after an increase

in soil water content and freeze/ thaw cycles. Increased substrate availability in the wheat plots due to higher wheat than maize root biomass (and therefore higher rhizodeposition) resulted in higher extractable organic C (EOC), and greater abundances of bacterial and fungal PLFAs, mainly in topsoil but also in the rooted zone beneath the plough layer (40-50 cm). Enzyme activities were less influenced by crop type in the topsoil but more frequently at 40-50 cm. Litter amendment increased bacterial and fungal PLFA abundances and most enzyme activities in the topsoil only. These effects of enhanced substrate availability were still evident about almost one year after litter amendment. In the 60-70 cm soil depth, effects could be observed only on rare occasions. Reduced substrate availability in deeper soil layers decreased bacterial and fungal PLFA abundances and hydrolytic enzyme activities. But these patterns were not observed for oxidative enzymes. Since oxidative enzymes are preferably bound on mineral surfaces, their stabilization is likely enhanced in deeper soil layers. Increased pH at depth, which was closer to the optimum of these enzymes, could also have been a reason for the differences in patterns of oxidative enzymes. The higher specific enzyme activities (enzyme activity per total microbial biomass) of both, hydrolytic and oxidative enzymes, in deeper soil layers than in topsoil indicated that the particular conditions in subsoil (lower substrate availability and therefore greater spatial separation of enzymes and substrate) may have changed the productivity and efficiency of microorganisms or the microbial community itself. Further, a general enhanced stabilization of enzymes at depth could have also contributed to these findings.

The second study showed that the recently introduced C from the two resources (root vs. shoot litter) was detectable after only a few months in the different pools and that it accumulated over time. Surprisingly, irrespective of the resource, there were no differences in the absolute or relative amounts of the incorporated C between the two resources (above- and belowground) in spite of the fact that the input of root derived C was calculated as 5 times lower than aboveground litter derived C. This indicates that root derived C was assimilated by microorganisms to a considerable degree and was incorporated in different soil pools to a relatively greater extent than was aboveground litter. The additive effect of both resources (root and shoot litter) indicates that presence or availability of both of these resources did not result in any preference or synergistic effect. The highest incorporation of recent C was found in the fungal biomarker ergosterol, indicating high and rapid assimilation of rhizodeposits as well as aboveground litter-derived C by fungi.

The third study showed that substrate quality influenced decomposition rates as well as substrate users and that all investigated groups (bacteria, fungi, protists) were involved in the degradation of the substrates. As the most labile substrate, glucose was mineralized most rapidly and assimilated in greater relative amounts than the other substrates. Cellulose mineralization and assimilation was slightly delayed, possibly because microbes had to produce enzymes in order to decompose it. At the end of the incubation (after 32 days) the mineralized amounts of glucose and cellulose were similar, whereas leaves and roots were mineralized in less amounts. The mineralization and assimilation of roots was lowest, confirming its low resource quality. Key glucose users were *Actinobacteria* (*Arthrobacter* spp., *Humicoccus* spp.) and *Proteobacteria* (*Oxalobacteraceae*, *Pseudomonas* spp.), the yeast *Cryptococcus* spp., and the plant pathogen *Phytium* spp.. The glucose users were distinct from the cellulose, leaf, and root users with the exception of *Flavobacterium* and *Phytium* spp.. Key cellulose users belonged to the bacterial phyla *Proteobacteria* (*Cellvibrio* spp.), *Bacteroidetes* (*Flavobacterium* spp.) and *Actinobacteria* (*Streptomycetaceae*, *Kitasatospora* spp.). Key fungal cellulose users were *Chaetomium* and *Geomyces* species. Most cellulose users were also identified as leaf and root consumers, indicating the importance of cellulose as a substrate from plant materials. Surprisingly, although the mineralization and assimilation of roots was lower than that of leaves, the same key taxa used both plant materials, indicating that the quality of complex substrates affects the decomposition rate but not the primary degrading taxa. Involvement of myxobacteria as well as protozoa suggests secondary trophic labeling already during early stages of decomposition.

In conclusion, this thesis extends our understanding of decomposition of different resources, C turnover and the identity of specific microorganisms directly involved in these processes, which is an important basis for improvement of current decomposition and food web models.

2 Zusammenfassung

Bodenmikroorganismen spielen eine zentrale Rolle im Abbau von organischer Substanz und beeinflussen somit Nährstoffkreisläufe und die Funktion von Ökosystemen. Die Verfügbarkeit und Qualität von Ressourcen bestimmt die Aktivität, das Wachstum und die Identität der entsprechenden Substratnutzer. In Agrarökosystemen ist die Verfügbarkeit von Ressourcen zum Beispiel abhängig von Kulturpflanzenart, Bodenbearbeitung, Jahreszeit und Bodentiefe. In der Tiefe ist die Substratverfügbarkeit und somit auch die mikrobielle Biomasse verringert. Aber es ist immer noch wenig bekannt über den C-Umsatz im Unterboden und wie und ob Prozesse im Oberboden die Abundanz, Aktivität und Funktion von Mikroorganismen im Unterboden beeinflussen. In Bezug auf die Substratqualität wird davon ausgegangen, dass Bakterien vor allem am Abbau hoch qualitativer und leicht abzubauender Substrate beteiligt sind, während Pilze eine wichtigere Rolle beim Abbau von qualitativ minderwertigeren und schwerer abzubauenden Substraten (z.B. Cellulose, Lignin) spielen. Die vorliegende Arbeit wurde durchgeführt, um das Verständnis des C-Umsatzes und den Einfluss von Verfügbarkeit und Qualität von Substraten auf Mikroorganismen in einer Agrarfläche zu verbessern.

In der ersten und zweiten Studie wurde ein Feldversuch genutzt, der angelegt wurde, um mit Hilfe eines C3-C4 Pflanzenwechsels den C-Fluss von unterirdischen (Wurzel) und oberirdischen (Streu) Ressourcen ins unterirdische Nahrungsnetz zu untersuchen. Maispflanzen wurden angebaut, um das C4-bürtige Signal in den Boden einzuführen. Dies geschah einerseits durch Pflanzenwachstum (unterirdisch / Wurzelkanal) und andererseits durch das Rückführen von Streu auf die Flächen (oberirdischer Streukanal). Um den C-Fluss des Wurzel- und Streu-Kanals zu trennen, wurde Streu auf Flächen aufgebracht, auf denen Weizen angebaut wurde, während auf der Hälfte der Flächen, auf denen Mais angebaut wurde, keine Streu zurückgeführt wurde. Weizenflächen auf denen keine zusätzliche Maisstreu aufgebracht wurde, wurden als Referenz für die Berechnung des C-Eintrags in die unterschiedlichen Bodenpools genutzt. Bodenproben wurden in 2 aufeinanderfolgenden Jahren im Sommer, Herbst und Winter genommen. Es wurden 3 unterschiedliche Bodentiefen betrachtet (0-10 cm: Oberboden, 40-50 cm: durchwurzelte Zone unter dem Pflughorizont, 60-70 cm: nicht durchwurzelte Zone). In der dritten Studie wurde ein Mikrokosmenexperiment mit Substraten unterschiedlicher Rekalzitranz und Komplexität durchgeführt, um primäre Nutzer von unterschiedlichem Pflanzenmaterial (Blätter und Wurzeln) während der frühen

Abbauphase (32 Tage) zu identifizieren und den C-Fluss in eine nächst höhere trophische Ebene (Protozoen) zu untersuchen.

Die erste Studie zeigte, dass der Zeitpunkt der Bodenprobenahme einen großen Einfluss auf die Abundanz von bakteriellen und pilzlichen Phospholipidfettsäuren (PLFAs) in jeder der drei betrachteten Bodentiefen hatte. Die hohe Abundanz von bakteriellen und pilzlichen PLFAs und Enzymaktivitäten vor allem an den Winterterminen deutete auf eine erhöhte Substratverfügbarkeit und verbesserte Fließbedingungen im Bodenprofil, nach einer Erhöhung des Bodenwassergehalts und Frier/Tau-Zyklen, hin. Eine erhöhte Substratverfügbarkeit, wahrscheinlich durch die erhöhte Wurzelbiomasse des Weizens im Vergleich zu Mais und demnach höchstwahrscheinlich auch eine vermehrte Exsudation, führte zu erhöhten Mengen von extrahierbarem organischen C (EOC) und einer höheren Abundanz bakterieller und pilzlicher PLFAs in den Weizenplots. Diese Effekte waren hauptsächlich im Oberboden zu beobachten, seltener auch in der Tiefe bei 40-50 cm. Die Enzymaktivitäten waren im Oberboden nicht so deutlich von der Kulturpflanzenart beeinflusst, dafür aber stärker in 40-50 cm. Das Aufbringen von Streu führte ebenfalls zu einer Erhöhung der bakteriellen und pilzlichen PLFA Abundanzen sowie der Enzymaktivitäten, dies war aber nur im Oberboden zu beobachten. Der positive Effekt durch das Aufbringen der Streu war noch nach fast einem Jahr zu sehen. In der untersten Tiefe (60-70 cm) konnten nur selten Behandlungseffekte verzeichnet werden. Die reduzierte Substratverfügbarkeit in der Tiefe führte zu geringeren bakteriellen und pilzlichen PLFA Abundanzen und reduzierter Aktivität der hydrolytischen Enzyme. Überraschenderweise zeigten die oxidativen Enzyme eine andere Tiefenverteilung. Die Aktivitäten dieser Enzyme blieben in der Tiefe gleich oder waren sogar erhöht. Da diese Enzyme bevorzugt an mineralischen Oberflächen gebunden sind, könnten sie in der Tiefe stärker stabilisiert worden sein als die hydrolytischen Enzyme. Der ansteigende pH-Wert in der Tiefe, der näher an dem optimalen pH-Wert der oxidativen Enzyme liegt, könnte ebenfalls eine Rolle gespielt haben. Die höheren spezifischen Enzymaktivitäten (Enzymaktivität pro mikrobielle Biomasse) von hydrolytischen und oxidativen Enzymen in den unteren Tiefen im Vergleich zum Oberboden können unterschiedliche Gründe haben. Die spezifischen Bedingungen in tieferen Bodenschichten, wie z.B. geringere Substratverfügbarkeit und daher eine wahrscheinlich höhere räumliche Trennung von Mikroorganismen, Enzymen und deren Substraten, könnte zu einer Erhöhung der Produktion und Effizienz der Mikroorganismen geführt haben bzw. zu einem Wechsel der mikrobiellen Gemeinschaft zu generell effizienteren Arten. Außerdem

spielte wahrscheinlich eine grundsätzlich größere Stabilisierung von Enzymen in der Tiefe eine Rolle.

In der zweiten Studie wurde gezeigt, dass der C aus den unterschiedlichen Ressourcen (Wurzeln und Streu) schon nach ein paar Monaten in den unterschiedlichen Pools detektiert wurde und über die Zeit anstieg. Überraschenderweise unterschieden sich die absoluten und relativen C-Mengen der Ressourcen (Wurzel vs. Streu) in den untersuchten Pools nicht, obwohl die zur Verfügung gestandene Menge von wurzelbürtigem C fünffach geringer war als die der Streu. Dies deutet darauf hin, dass wurzelbürtiger C in hohem Maße von Mikroorganismen assimiliert und stärker in die unterschiedlichen Pools eingebaut wurde im Vergleich zu streubürtigem C. Der additive Effekt, der auftrat, wenn beide Ressourcen zur Verfügung standen, deutet darauf hin, dass keine der beiden Ressourcen präferiert genutzt wurden oder synergistisch wirkten. Der höchste relative Einbau von C wurde im pilzlichen Biomarker Ergosterol nachgewiesen, was die hohe und schnelle Assimilation von Rhizodepositen und sowie der Streu durch Pilze belegt.

Die dritte Studie zeigte, dass die Substratqualität die Abbauraten sowie die Substratnutzer beeinflusst. Alle untersuchten Gruppen (Bakterien, Pilze, Protisten) waren am Abbau der Substrate beteiligt. Das labilste Substrat, Glukose, wurde am schnellsten mineralisiert und stärker in die mikrobielle Biomasse aufgenommen als die anderen Substrate. Die Cellulose Mineralisation und Assimilation war etwas verzögert, wahrscheinlich da erst Enzyme produziert werden mussten, um die Cellulose nutzen zu können. Am Ende der Inkubation (nach 32 Tagen) waren die mineralisierten Mengen der Glukose und Cellulose gleich, während die Blätter und Wurzeln in geringerem Ausmaß mineralisiert wurden. Die Mineralisation und Assimilation der Wurzeln war am geringsten, was die geringe Substratqualität widerspiegelt. Die Haupt-Glukose Nutzer waren *Actinobacteria* (*Arthrobacter* spp., *Humicoccus* spp.) und *Proteobacteria* (*Oxalobacteraceae*, *Pseudomonas* spp.), die Hefe *Cryptococcus* spp., and das Pflanzen Pathogen *Phytium* spp.. Die Glukose Nutzer unterschieden sich deutlich zu den anderen Substratnutzern. Nur *Flavobacterium* und *Phytium* spp. waren auch am Abbau der anderen Substrate beteiligt. Die Haupt-Cellulose Nutzer gehörten zu *Proteobacteria* (*Cellvibrio* spp.), *Bacteroidetes* (*Flavobacterium* spp.) and *Actinobacteria* (*Streptomycetaceae, Kitasatospora* spp.). Haupt-Pilz Nutzer waren *Chaetomium* and *Geomyces* Arten. Die meisten Cellulose Nutzer waren auch am Abbau der Pflanzenmaterialien beteiligt. Obwohl die Mineralisation und Assimilation der Wurzeln geringer war als die der Blätter, wurden keine Unterschiede in den substratnutzenden Taxa

festgestellt. Dies deutet darauf hin, dass die Qualität von komplexen Substraten die Abbaurate bestimmt, aber die Nutzer sich erstmal nicht unterscheiden. Die Anreicherung von Myxobakterien und Protozoen deutete auf eine sekundäre Anreicherung durch trophische Verknüpfungen hin.

Diese Arbeit erweitert das Verständnis vom Abbau unterschiedlicher Ressourcen, C Umsatz und der direkten Beteiligung von spezifischen Mikroorganismen, was einen wichtigen Beitrag zur Verbesserung von bisherigen Abbau- und Nahrungsnetz-Modellen leisten kann.

3 General Introduction

3.1 Carbon cycle

Soils comprise the largest terrestrial reservoir for carbon (C) (Chapin III et al. 2009). The total soil C pool is four times higher than the biotic pool, composed of vegetation, and three times higher than the atmospheric pool (Lal 2004). Estimations of soil C stocks in the top 100 cm range from 1500-2000 Pg C (Janzen 2005). About 30 % of earth's land area is used for agriculture and about one-fourth of soil organic matter C is stored in agricultural soils (Nieder & Benbi 2008, Schulze 2006).

Carbon dioxide (CO_2) concentration in the atmosphere has risen from a pre-industrial value of about 280 ppm to 379 ppm in 2005 (IPCC 2007). The global increase in CO_2 concentration is primarily due to burning of fossil fuels. However, land use change, which includes deforestation and land management practices, is another significant factor (IPCC 2007).

There is still uncertainty about how the observed rising atmospheric CO_2 concentration will affect soil C stocks due to resulting temperature increases and changing precipitation patterns. Whether soils will serve as sources or sinks of increasing C, assuming that increased CO_2 concentration will enhance plant growth while higher temperatures will increase decomposition, will depend on the relationship between plant derived C inputs and decomposition (Davidson & Janssens 2006). Agricultural management has the potential to enhance C sequestration in soil and can therefore mitigate CO_2 emissions to some extent. For example, tillage practices like ploughing physically disrupt aggregates and bring hitherto inaccessible organic matter (OM) into contact with microorganisms, increasing soil organic matter (SOM) decomposition. Leaving plant residues or returning them to the soil surface after harvest increases the SOM pool by the amount of plant derived C in the residues and lowers the potential of C loss by erosion (Lal 2004). Application of fresh organic matter (FOM) can also result in increased SOM mineralization, the so called priming effect (Fontaine et al. 2007). However, it is expected that the priming effect is more important as a short-term effect than it is for long-term C-sequestration (Stockmann et al. 2013).

The goal of agricultural soil management under the conditions of a changing climate is to ensure plant growth, to reduce the loss of plant derived C from SOM, and to simultaneously maintain the nutrient cycles needed to sustain or enhance the quality and productivity of soil (Lal 2004). Therefore, to adapt agricultural management in a sustainable way, identification

of the microbial groups involved in microbially mediated soil processes such as decomposition, and their role in the C-cycle in soil, are important (King 2011).

3.2 Decomposition and involved soil organisms

Major routes of C-input into soil are via living plants by rhizodeposition and via dead plant material such shoot litter and roots. In soils a huge consortium of organisms (macrofauna, mesofauna, microfauna and microflora) is directly or indirectly involved in the formation and turnover of SOM by decomposition of plant residues and remineralization of nutrients (Bardgett et al. 2005). The most important group in decomposition is the microflora, due to their biochemical and phylogenetic diversity (Coleman & Crossley 1996). As primary degraders of organic material, both bacteria and fungi play key roles in decomposition and represent an important basis of the food web (Bardgett et al. 2005, deBoer 2005, Berg & McClaugherty 2008).

Traditionally, food webs are separated into herbivore and detrital food chains; the latter is further separated into bacterial and fungal energy channels (Moore et al. 2005). The bacterial channel is believed to process more labile organic matter and dominates in fertile and productive ecosystems, whereas the fungal channel processes dominantly more recalcitrant organic matter and is prevalent in infertile and unproductive ecosystems (Wardle et al. 2004). In addition, the bacterial channel is assumed to be a fast cycle with rapid nutrient and biomass turnover of the food web members. In comparison, the fungal channel is seen as a slow cycle with slow turnover of nutrients and biomass (Moore et al. 2005).

Earlier views on food webs regarded aboveground litter as the main source of decomposer fauna, often ignoring the belowground root system as a significant food source. But recent food web analyses in grasslands and forests have shown the importance of belowground root derived C-input to food webs (Ostle et al. 2007, Pollierer et al. 2007). Further, the structure of the decomposer system can be highly influenced by the quantity and quality of root exudates and litter (Wardle et al. 2004, Krashevska et al. 2012). However, the relative importance of and influence on the food web structure of aboveground vs root derived resources is still largely unknown.

As food webs can be regulated either top-down (predation) or bottom-up (resource quantity and quality) this can have consequences for biotic interactions and on the further C flow through the soil food web (Wardle et al. 2004, Ayres et al. 2009). Therefore, as the basis of

the soil food web, bacteria and fungi can be important bottom-up drivers of the structure of soil food webs by their specific resource utilization during decomposition of organic matter.

3.2.1 Resource availability and its influence on microbial degraders

Resource availability affects microbial biomass and activities (Geisseler et al. 2011). The input and turnover of organic C through both topsoil and subsoil processes is influenced by agricultural management, e.g. soil tillage, crop type, nitrogen (N) amendment, and residue management such as mulching (Clapp et al. 2000, Lorenz & Lal 2005). For example, vegetation dependent factors such as plant species influence the size and composition of microbial communities (Grayston et al. 2001, Moore-Kucera & Dick 2008). Within the soil profile the distribution of roots and the amounts of exudates affect resource availability into deeper soil layers. In mulching systems, plant litter left on fields serves as additional substrate and is likely to affect microbial communities and their function. No-tillage systems with plant litter returned to the soil surface promote fungal growth whereas conventional systems, in which chopped plant resources are distributed back into the soil by ploughing promote the bacterial pathway (Hendrix et al. 1986). The advantage fungi have are their hyphal structures; with these they are able to grow towards aboveground litter or translocate nutrients from the soil into fungal biomass in the litter layer (Holland & Coleman 1987, Frey et al. 2003). Additionally, fungi have greater resistance to the low water potentials that are often a condition close to surface residues (Holland & Coleman 1987).

Resource amounts and types change within seasons due to growth stages of the plants and/or management system, and these in turn affect decomposer communities (Hendrix et al. 1986, Kaiser et al. 2010). During plant growth rhizodeposits such as exudates are released into the soil; after harvest, roots and, depending on management, residual plant litter and roots remain, respectively, on or in the soil. Observed changes in the microbial community over seasons has been attributed to alterations in both C and nutrient availabilities (Smit et al. 2001, Lipson & Schmidt 2004, Schadt et al. 2003). The proportion of fast-growing bacteria was highest in summer and highest species richness has been found in spring and autumn after fertilization and harvest of plants (Smit et al. 2001). It has been shown as well that under snow cover microbial activity was measurable and that the community differed between summer and winter; therefore a change in the function of microorganisms would be expected (Schmidt & Lipson 2004, Schadt et al. 2003).

Seasonal changes in abiotic factors such as temperature, drying/rewetting and freeze/thaw cycles can influence the availability of substrates and therefore strongly influence microbial biomass, community composition and activity; seasonal effects can be even higher than treatment effects (Debosz et al. 1999, Bell et al. 2010). The transport of organic matter through the soil profile is also highly dependent on seasonal weather variations during the year. Drying/wetting, strong precipitation events, or freeze/thaw cycles increase the release of mobile organic dissolved and particulate substances (MOPS) (Majdalani et al. 2008). Depending on the season, these events result in enhanced transport of MOPS (Totsche et al. 2007), DOM (Kalbitz et al. 2000) and colloids (Cheng & Saiers 2009) in the soil profile and therefore availability of substrates at depth as well.

In general, lower substrate availability in deeper soil layers in comparison to topsoil results in decreasing microbial biomass (Blume et al. 2002, Bausenwein et al. 2008, Gelsomino & Azzellino 2011) and activity (Fang & Moncrieff 2005). However, specific activities (activity per microbial biomass) of assimilation and mineralization do not always decrease with depth (Blume et al. 2002, Gelsomino and Azzellino 2011). Observed reduced decomposition in deeper soil layers is additionally due to stabilization of organic matter which is highly associated with clay minerals (Rumpel et al. 2004). Spatial separation between microorganisms and the substrate seems to play a role in subsoil as well (Salomé et al. 2010). Therefore additional mechanisms which reduce the availability of resources must be included when decomposition processes in subsoil are considered. Although deeper soil layers store high amounts of organic C (Jobbagy & Jackson 2000), the contribution of microorganisms to carbon dynamics in subsoils has received far less attention than in topsoils (Rumpel & Kögel-Knabner 2011).

3.2.2 Resource quality and its influence on microbial degraders

Resource quality influences decomposition processes, microbial consumers, and therefore potential C retention in soil. Roots are generally more slowly degraded in comparison to leaves, resulting in higher retention of root derived C in comparison to aboveground litter derived C in SOM (Puget & Drinkwater 2001, Rasse et al. 2005, Kätterer et al. 2011). These effects can be explained by both higher chemical recalcitrance and limited enzymatic access in roots in comparison to leaves. For example, lower amounts of soluble compounds, higher contents of recalcitrant compounds like lignin, tannin, cutin, suberin, and greater

interconnections with phenolic acids within the cell walls restrict enzymatic attack (Abiven et al. 2005, Rasse et al. 2005, Bertrand et al. 2006, Fujii & Takeda 2010).

Rasse et al. (2005) calculated the residence time of shoot and root derived C from 14 studies including field and laboratory experiments. In field experiments with *in situ* root growth the mean residence time of root derived C was 2.4 times higher than of shoot-C. In comparison, in experiments with litter bags in the field and in lab experiments, when root and shoot litter were mixed into the soil, the residence time of root litter was on average only 1.3 times higher than of shoot litter. The authors proposed that *in situ* root growth results in specific stabilization mechanisms, in addition to the general higher chemical recalcitrance of roots. They argued that the specific chemical nature of rhizodeposits leads to high physico-chemical stabilization with minerals; the specific water conditions at the root surfaces as well as the small structures of root hairs and mycorrhizal hyphae promote the physical stabilization of aggregates. The lower contribution of root derived C in litter experiments in comparison to *in situ* experiments is therefore explained by the exclusion of living roots and the specific stabilization mechanisms connected to them (Rasse et al. 2005).

Although it is known that root derived components are stabilized in SOM to a greater extent than aboveground derived material, only a few studies have considered the contribution of root derived C in comparison to aboveground litter in different soil fractions, in the microbial biomass, or different groups of microorganisms (i.e. Balesdent & Balabane 1996, Puget & Drinkwater 2001, Williams et al. 2006). During plant growth, rhizodeposits, composed of water-soluble exudates such as sugars and organic acids and water-insoluble compounds like sloughed off cells, mucilage, and other root debris, are released actively or passively from roots (Grayston et al. 1996, Farrar et al. 2003). Exudates are readily available substrates for microorganisms and are normally quickly mineralized or used for growth (Kuzyakov 2002). Puget & Drinkwater (2001) observed that rhizodeposit-derived C was also biologically immobilized (incorporated into microbial biomass) in soil to a greater relative extent than shoot litter-derived C. The mechanisms behind this are unclear. One possible explanation is that resource inputs which are delivered continuously in low quantities (such as rhizodeposits) are retained to a greater extent than resources which are applied once (such as aboveground litter) (Puget & Drinkwater 2001). This effect was observed by Jans-Hammermeister et al. (1998) who did a microcosm experiment with ^{14}C-glucose. Mineralization of substrate-derived C was lower and storage efficiency was higher in the microbial biomass under a daily addition of glucose as compared to a pulse addition.

It is commonly thought that high quality and more easily decomposable compounds (i.e. exudates) are used mainly by bacteria and low quality SOM (i.e. lignin) degradation is dominated by fungi. For example Paterson et al. (2007) observed that the main decomposers of different plant fractions were defined by the quality of the fraction (labile: mainly by bacteria vs. recalcitrant: mainly by fungi). Further, Elfstrand et al. (2008) showed that root- and shoot litter-derived substrates are processed by different soil organisms. Therefore, the quality of plant C resources determines degrader communities and affects C allocation and sequestration in soil (Eisenhauer et al. 2010, Ladygina & Hedlund 2010).

The contribution of C accumulation in soil by the microbial community itself is dependent on 1) its growth efficiency (the efficiency with which substrates are incorporated in the microbial biomass); 2) the rate at which biomass derived compounds or metabolites are degraded by other organisms, and 3) the degree of protection of their biomass (e.g. from grazing) (Six et al. 2006). There is still a debate about C use efficiencies of different microorganisms and whether or not fungi do have a higher C use efficiency in contrast to bacteria. Fungal cells have a higher C/N ratio than bacteria, which results in a higher C content of fungal as compared to the same amount of bacterial biomass (Six et al. 2006). Additionally cell wall components of fungi are degraded more slowly than bacterial cell walls (Nakas & Klein 1979), and turnover times of fungi are generally longer than those of bacteria (Rousk & Bååth 2011). However, due to their smaller size and their occupation of smaller soil pores bacteria are expected to be better protected against grazing by higher trophic levels (Wardle et al. 1993). In addition, fungi are involved in macroaggregate formation, which protects SOM from decomposition (Bossuyit et al. 2001, Helfrich et al. 2008), which could explain why higher fungal activity has been correlated with higher C contents in soils under different land uses (forest, grassland, agricultural land) and management systems (Bailey et al. 2002). Hence, the authors concluded that C sequestration was higher in fungal dominated systems. Therefore the interaction between resource quality and the resulting selections by resource consumers can determine C accumulation in soil.

3.3 Enzymes

One important function of soil microorganisms is the degradation by extracellular enzymes of insoluble polymers such as, i.e. cellulose, lignin, and chitin into smaller subunits. It has been shown that substrate presence induces respective enzyme synthesis (Suto & Tomita, 2001)

and therefore enzyme activities can be used to yield information about availability and decomposition of particular substrates in soils (Geisseler & Horwath 2009). Whereas hydrolytic enzymes (e.g. β-glucosidase, N-acetyl-β-D-glucosaminidase, xylosidase) are responsible for the decay of organic substrates with faster turnover times like carbohydrates or chitin, oxidative enzymes (e.g. phenol- and peroxidase) have an important function in the degradation of SOM components with slower turnover times (e.g. lignin) (Horwath 2007). For degradation of complex resources a cascade of enzymes is necessary to degrade organic components completely and since one taxon normally does not produce all necessary enzymes a synergistic interaction between different taxa is required (Sinsabaugh et al. 1991).

Besides intracellular enzymes, which remain in the cell, extracellular enzymes occur attached to the outer parts of microbial cells or are released completely and exist as free enzymes either in the soluble phase or associated with minerals and organic components (Burns 1982, Burns & Dick 2002). Factors such as C and N availability and the diffusion rates of enzymes, substrates, and products affect the production of enzymes in soil (Allison et al. 2005, Allison 2005). In addition to substrate availability and the stoichiometry of microbial nutrient demand, the productivity of enzymes also depends on climatic and environmental parameters such as temperature, moisture, and pH (Sinsabaugh et al. 1991, Geisseler et al. 2011). Hydrolytic and oxidative enzyme activities seem to be influenced differently by environmental factors. This was shown by the observed greater dependency on pH of oxidative than of hydrolytic enzymes' activity (Sinsabaugh et al. 2008, Sinsabaugh 2010). For this reason oxidative and hydrolytic enzyme activities are often uncorrelated to each other (Sinsabaugh 2010). Since enzymes' production is cost-intensive their production follows 'ecological rules' meaning that resource gains must be higher than costs (Allison et al. 2005, Allison 2005).

Some enzymes seem to be constitutively produced at a low-level or stabilized on minerals (Burns 1982, Allison et al. 2005, German et al. 2011). When the target substrate is available, enzyme activities increase the amount of microbially available substrates and the expression of respective enzymes is stimulated (Suto & Tomita 2001). Stabilization of enzymes on minerals or humic acids may alter their turnover rate and also their activity status. Allison (2006) observed increased activity of enzymes when they were stabilized by the addition of allophane, but activities of the same enzymes were strongly inhibited by the presence of humic acids.

A way to look at enzyme activities that takes into account the microbial biomass is the specific enzyme activity or enzyme efficiency on a per unit microbial biomass basis. This ratio gives further information about the production and / or stabilization of enzymes (Kandeler & Eder 1993, Taylor et al. 2002, Allison et al. 2007). Changes in efficiencies can be due to differing nutrient demands of microorganisms, higher or lower affinity of enzymes or quality of resources (Allison et al. 2007, Amin et al. 2013) and has been associated with a community change (Allison et al. 2007). However, community changes are not necessarily connected to changes in enzyme activities (Lucas et al. 2007). A higher specific enzyme activity can also be observed due to stabilization of enzymes, in which case it is not clear if the *in-situ* activity is comparable to the measured potential enzyme activity (Wallenstein & Weintraub 2008, Geisseler et al. 2011).

3.4 Use of stable isotope C to determine C flow in soil

The application of stable isotope tracer methods to determine fluxes within and between C pools in soil has increased considerably (Balesdent & Balabane 1992, 1996, Bowling et al. 2008). Transformations of C have been followed by switching from C3 to C4 plants or resources derived from C3 or C4 plants (Steinbeiss et al. 2008, Esperschütz et al. 2009, Nottigham et al. 2009). Additionally, ^{13}C or ^{14}C pulse labeling or continuous labeling has been applied to introduce a distinct C signal into the soil system and to quantify the C incorporation into different soil pools (Kuzyakov & Cheng 2004, Leake et al. 2006, Williams et al. 2006, Werth & Kuzyakov 2008).

The analysis of the incorporation of specific resources into the microbial biomass (i.e. $^{13}C_{mic}$) provides valuable information about the general utilization of these substrates by microorganisms. A higher resolution of microorganisms involved in particular processes can be achieved with compound-specific stable isotope probing (CSIP) which can provide a direct link of abundance and function *in situ* (Sims 2007).

A common marker to estimate fungal biomass in soil is ergosterol, which is the predominant sterol in fungal cell membranes. It is only present in higher fungal phyla, i.e. Basidiomycota, Ascomycota and the majority of Zygomycota, and does not occur in plants (Weete & Gandhi 1997, Klamer & Bååth 2004). Conflicting data regarding the presence of ergosterol in membranes of arbuscular mycorrhizal fungi (AM fungi) have been reported in the literature (Hart & Reader 2002, Olsson et al. 2003).

With nucleic acid based stable isotope probing (NA-SIP) a much higher taxonomic resolution of substrate-utilizing organisms is possible. To separate the labeled and non-labeled nucleic acids by density-gradient centrifugation a high label of RNA or DNA is required which can be achieved only through the application of artificially labeled material (>98 atom%). For this reason, most studies applying NA-SIP are lab experiments. The advantage of using RNA rather than DNA is the higher synthesis rate of RNA, resulting in more efficient labeling; it is dependent only on the activity of the cell and independent of replication (Manefield et al. 2002). The combination of stable isotope probing with new generation (high-throughput) sequencing techniques (i.e. pyrosequencing) also makes it possible to detect less abundant or less active organisms which would have been missed by cloning and could have potentially high importance for an ecosystem (Baldrian et al. 2012, Dohrmann et al. 2013).

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4 Overview of the thesis

In the present thesis, biomarkers of groups of microorganisms (PLFAs of gram+, gram-, general bacteria, fungi) and enzyme activities were used to identify the influence of both substrate availability and quality on members of the microbial community and their function (Study 1). In addition, the stable isotope ^{13}C was used to follow the C flow of differing resources into soil pools such as total organic matter (C_{org}), extractable organic C (EOC), as well as into microorganisms (C_{mic}, ergosterol) (Studies 2 & 3). Nucleic acid stable isotope probing (NA-SIP) was used to identify, at a high taxonomic resolution, the microorganisms involved in degradation of substrates of varying recalcitrance and complexity (Study 3).

In the first study we focused on the question of how crop type (wheat vs. maize), management (litter amendment), and season (two consecutive years: summer, autumn, winter) influenced the abundances of different groups of microorganisms (bacteria, gram+, gram-, and fungi) and their functions (via enzymes involved in C-cycling of labile and more recalcitrant substrates). Different depths in the soil profile (0-10 cm: topsoil, 40-50 cm: beneath the plough layer, and 60-70 cm: unrooted zone) were examined. We expected that crop type, management strategy and season would have more pronounced effects in top- than in subsoil on microbial abundances and enzyme activities. Further, we hypothesized that decreasing substrate availability at depth result in lower abundances and activities but that specific conditions in subsoil result in changes in the physiology of soil microorganisms as indicated by their enzyme production, and that abiotic interactions between microorganisms, substrates, and soil enzymes function as drivers of C dynamics in subsoils.

To quantify C incorporation from belowground (root / rhizodeposit) and aboveground (shoot litter) resources into different soil pools and microorganisms over time (two consecutive years: summer, autumn, winter), topsoil samples from the field experiment were analysed with ^{13}C isotope probing in the second study. We hypothesized that the incorporation of C into soil pools (C_{org}, EOC, C_{mic} and ergosterol) depends on the origin of C resources (root vs. shoot litter) entering the soil.

The specific bacterial and fungal degraders of particular resources, and their contributions and interactions during degradation of organic matter, are largely unexplored. With nucleic acid stable isotope probing (NA-SIP) it is possible to identify substrate assimilating microorganisms at high taxonomic resolution. Therefore, in the third study, a microcosm experiment was carried out with highly ^{13}C labelled materials. We used substrates of varying

recalcitrance and complexity. As complex substrates of different qualities we used maize leaves and roots. To be able to detect the users of either labile or more recalcitrant components of the litter materials we additionally used single substrates which are highly abundant in litter materials as model substances (glucose and cellulose). CO_2 production, incorporation of substrate derived C into CO_2 and into microbial biomass (C_{mic}) were analyzed over time for 32 days. We considered bacteria and fungi as primary decomposers, and protists (*Peronosporomycetes*, protozoa) as further decomposers and as members of the next trophic level. We hypothesized that the complexity and recalcitrance of substrates defines primary consumers across kingdoms, that distinct bacterial and fungal substrate utilization channels may actually not exist, and that the diversity of primary consumers as well as secondary trophic links should increase with substrate complexity.

5 Temporal variation in surface and subsoil abundance and function of the soil microbial community in an arable soil

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Abstract

Many studies of the microbial ecology of agricultural ecosystems focus on surface soils, whereas the impacts of management practice and season on soil microbial community composition and function below the plough zone are largely neglected. Deep soils have a high potential to store carbon; therefore any management driven stimulation or repression of microorganisms in subsoil could impact biogeochemical cycling in agricultural sites. The aim of this study was to understand whether soil management affects microbial communities in the topsoil (0-10 cm), rooted zone beneath the plough layer (40-50 cm), and the unrooted zone (60-70 cm). In a field experiment with different crops [wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.)] and agricultural management strategies (litter amendment) we analysed microbial biomass as phospholipid fatty acids (PLFAs) and enzyme activities involved in the C-cycle (β-glucosidase, N-acetyl-β-D-glucosaminidase, xylosidase, phenol- and peroxidase) across a depth transect over a period of two years. Wheat cultivation resulted in higher bacterial and fungal biomass as well as higher enzyme activities at most sampling dates in comparison to maize cultivated plots, and this effect was visible to 50 cm depth. Litter application increased bacterial and fungal biomass as well as hydrolytic enzyme activities but effects were apparent only in the topsoil. In winter high microbial biomass and enzyme activities were measured in all soil layers, possibly due to increased mobilization and translocation of organic matter into deeper soil. Hydrolytic enzyme activities decreased with depth, whereas oxidative enzyme activities showed no decrease or even an increase with depth. This could have been due to differing sorption mechanisms of hydrolytic and oxidative enzymes. Specific enzyme activities (enzyme activity per microbial biomass) were higher in the deeper layers and possible reasons are discussed.

Introduction

Soils have a great potential to store carbon throughout entire soil profiles (Lorenz and Lal, 2005). The global carbon stock within the first meter of soils has been estimated at 1500-2000 Pg (Janzen, 2005). Although over 50 percent of the global organic carbon pools in soil are found below 30 cm depth (Jobbagy and Jackson, 2000), the contribution of microorganisms to carbon dynamics in subsoils has received far less attention than in topsoils (Rumpel and Kögel-Knabner, 2011). Focusing on top- as well as on subsoils in agricultural ecosystems is important, because soil management (e.g. soil tillage, crop type, N amendment, and residue management such as mulching) may influence not only the input and turnover of organic C (Clapp et al., 2000; Lorenz and Lal, 2005), but also alter subsoil processes related to plant nutrient acquisition (Harrison et al., 2011).

Soil microorganisms play an important role in the formation and turnover of SOM by decomposition of plant residues and remineralization of nutrients (Bardgett et al., 2005). Their size, community composition and function can therefore be used to investigate decomposition and deduce SOM turnover in soil. Soil microbial communities are not uniformly dispersed throughout the soil profile, but reflect patches of available resources, predominantly plant litter and roots. Crop type or management strategy therefore affect community structure and distribution. For example, vegetation dependent factors such as plant species influence the size and composition of microbial communities (Moore-Kucera and Dick, 2008) through amount, availability and quality of exudates, distribution of roots in the soil profile, and through the quality of plant residues. With agricultural amendments such as litter application the additional substrates are likely to affect microbial community and their function throughout the soil profile. Studies in natural ecosystems indicate that increased nutrient availability modifies microbial assemblages not only in topsoils but also in subsoils of the vadose zone (Schütz et al., 2009).

To understand SOM dynamics in top- and subsoils it is also important to take into account abiotic changes in depth. For example, higher absolute amounts of minerals in deeper soil layers can result in higher stabilization potential of organic matter with minerals at depth (Rasse et al., 2005). Also seasonally dependent abiotic factors such as temperature and soil moisture can have a strong influence on biomass and activity of microbes, and seasonal effects can even be higher than treatment effects (Debosz et al., 1999; Bell et al., 2010). Moreover, climatic forcing in the topsoil, e.g. drying/wetting or freezing/thawing cycles, triggers the release of mobile organic dissolved and particulate substances (MOPS)

(Majdalani et al., 2008). Transient flow conditions affecting organic matter further occur as a result of cell death and lysis during drying, disruption of soil structure due to mechanical stress, and harvesting practices which increase fractured plant residues. Overall these multiple factors result in an enhanced transport of MOPS (Totsche et al., 2007), DOM (Kalbitz et al., 2000) and colloids (Cheng and Saiers, 2009) in the soil profile, depending on season.

It is widely thought that substrate quality is lower in subsoil than in topsoil, suggesting that soil organic matter is less degradable at depth. Both substrate pools and microbial biomass generally decline (Blume et al., 2002; Bausenwein et al., 2008; Gelsomino and Azzellino, 2011), and activity also decreases with increasing soil depth (Fang and Moncrieff, 2005). However, in studies where assimilation or mineralization activities were normalized to the size of the microbial biomass, these specific activities showed either similar values within the soil profile or even an increase with depth (Blume et al., 2002; Gelsomino and Azzellino, 2011).

One important function of soil microorganisms is the degradation of insoluble polymers like cellulose, lignin, and chitin into smaller subunits by extracellular enzymes. It has been shown that substrate presence induces respective enzyme synthesis (Suto and Tomita, 2001) and therefore enzyme activities can be used to yield information about availability of particular substrates in soils (Geisseler and Horwath, 2009). Whereas hydrolytic enzymes (e.g. β-glucosidase, N-acetyl-β-D-glucosaminidase, xylosidase) are responsible for the decay of organic substrates with faster turnover times like carbohydrates or chitin, oxidative enzymes (e.g. phenol- and peroxidase) have an important function in the degradation of SOM components with slower turnover times (e.g. lignin) (Horwath, 2007). Specific enzyme activity (enzyme activity per unit microbial biomass) gives further information about the production and / or stabilization of enzymes (Kandeler and Eder, 1993; Taylor et al., 2002; Allison et al., 2007).

The present study investigated the effects of frequently cultivated crop types (maize and wheat) and management (litter and no litter) on microbial community composition (PLFAs), and its function (enzyme activities) at three different depths (topsoil, rooted zone beneath plough layer, unrooted zone) in an arable field over a period of two years.

We hypothesized that crop type, management strategy (litter amendment) and season have more pronounced effects on microbial properties in the top- than in the subsoil, as surface communities are more exposed to mechanical, chemical or vegetation changes. Further, we

expected that not only lower microbial abundance, but also changes in physiology of soil microorganisms (their enzyme production and expression), as well as abiotic interactions between microorganisms, substrates, and soil enzymes may drive C dynamics in subsoils.

Materials and methods

Field site and soil samples

The main field experiment investigating carbon flow in belowground food webs was set up by the University of Göttingen (Lower Saxony, Germany) on arable land (51°33´N, 91°53´E; 158 m a.s.l.) in April 2009 (Kramer et al., 2012). The area has a temperate climate with mean annual precipitation of 720 mm and mean air temperature of 7.9°C. Dominant soil types at the site are Luvisols and Cambisols with partially stagnic properties (IUSS 2007). The clay and sand fractions decrease from 7.0 and 5.8 to 6.8 and 4.8 % (w / w) from the Ap1 to the Bv2 horizon, respectively, whereas the silt fraction increases from 87.2 to 88.4 % (w / w). The soil bulk density of the site increases from 1.38 g cm^{-3} in the Ap1 to 1.68 g cm^{-3} in the Bv2 horizon. The pH_{CaCl2} in the Ap1 to the Bv2 (> 65 cm) horizon increases from 6.0 to 7.0. The C_{org} and total N content decrease from the Ap1 to the Bv2 (> 65 cm) horizon from 11.6 to 1.8 mg g^{-1} dry weight and 1.2 to 0.3 mg g^{-1} dry weight, respectively (for details see Kramer et al., 2012; Pausch and Kuzyakov, 2012).

Four treatments were established which were differentiated by crop type (wheat vs. maize) and management strategy (litter or no litter application). To allow feasible agricultural management a strip design was chosen, with wheat (*Triticum aestivum* L) cultivated in the first (north) and maize (*Zea mays* L.) in the second (south) strip, each strip with 10 plots of 24 x 24 m. Before sowing, soil was tilled with a chisel plough to a depth of 12 cm. In the first vegetation period (2009) winter wheat ("Julius", sown at 224 kg ha^{-1}) and maize ("Ronaldinio", sown at 34 kg ha^{-1}) were grown. In the second period (2010) the varieties used were summer wheat ("Melon", sown at 224 kg ha^{-1}) and hybrid maize ("Fernandez", sown at 26 kg ha^{-1}). Fertilization practice was as follows: on the maize plots ammonium nitrate urea solution (2009: 122.4 kg N ha^{-1}; 2010: 79.2 kg N ha^{-1}) and di-ammonium phosphate (2009 / 2010: 32.4 kg N ha^{-1} and 82.8 kg P ha^{-1}) were applied twice, shortly before and after seeding. The wheat plots received granular NS fertilizer (21.0 kg N ha^{-1}, 24.0 kg S ha^{-1}) in March 2009 and ammonium nitrate urea solution between 39.5 and 61.3 kg N ha^{-1} in April, May and June in both 2009 and 2010. After harvest in early November 2009 chopped maize litter excluding

cobs (0.8 kg m^{-2} dry weight equivalent to 0.35 kg C m^{-2}) was applied on 5 randomly chosen plots from the 10 plots in each strip to establish the Corn Maize (CM) and Wheat + maize Litter (WL) treatments. The other 5 plots of each strip were the plots without litter addition and designated Fodder Maize (FM) and Wheat (W) treatments. In November 2010 the harvested maize litter from this year was applied.

Soil samples were taken with a soil corer to 70 cm depth and separated as follows: topsoil (0-10 cm), rooted zone beneath the plough layer (40-50 cm), and unrooted zone (60-70 cm). Ten soil cores randomly distributed on each plot were taken between plants. The soil from each depth was mixed and homogenized. Soil samples were cooled and transported to the laboratory. Soils were sieved (< 2 mm), water content was gravimetrically determined (105 °C for 24 h), and samples were frozen at -24 °C. All data presented here are expressed on a soil dry weight basis.

Soil samples were collected three times a year; in summer, autumn, and winter. Summer sample collections were July 2009 and 2010 (high root exudation) and autumn collections in September 2009 and 2010 (shortly before maize harvest). Winter sample collections (highest translocation of MOPs) were in December 2009 and in January 2011. The second winter collection was delayed due to heavy snow in December 2010. For more details see Kramer et al. (2012).

Analyses

Extractable organic Carbon (EOC)

Ten g (fresh weight) of soil were extracted with 0.025 M K_2SO_4 solution (1:4, w/v, soil/extractant ratio), shaken for 30 min on a horizontal shaker at 250 rev min^{-1}, and centrifuged for 30 min at 4422 x g. Organic C was measured with a DOC/TN analyzer (Dimatoc 100, Dimatec, Essen, Germany). Addition of 1 M HCl to the extracts of samples from 60-70 cm before measurement removed potentially present small amounts of inorganic C (Pausch and Kuzyakov, 2012).

Phospholipid fatty acid analysis (PLFA)

PLFAs of two replicates of 4 g soil (fresh weight) from each plot were extracted following the description of Frostegård et al. (1991) with Bligh / Dyer solution [chloroform, methanol,

citrate buffer (pH = 4; 1:2:0.8; v/v/v)] and further separated into glyco-, neutral lipids and phospholipid fatty acids with silica acid columns (0.5 g silicic acid, 3 ml; Varian Medical Systems, Palo Alto, California). Before fractionation of lipids the two replicates were combined onto one silica column. Glyco - and neutral lipids were not of interest in this study, thus only the PLFA-fractions were analysed.

To transform the PLFAs into fatty acid methyl esters (FAMEs) a mild alkaline methanolysis of PLFAs was done as described in Frostegård et al. (1991) and Ruess et al. (2007). The resulting PLFA-MEs were measured using an AutoSystem XL gas chromatograph (Perkin Elmer Corporation, Norwalk, CT, USA) equipped with a capillary column HP-5 (crosslinked 5 % phenyl methyl siloxane; 50 m x 0.2 mm, film thickness of 0.33 µm) and a flame ionisation detector. Helium was used as the carrier gas. The injector temperature was 260 °C and that of the detector 280 °C. Initial temperature was 70 °C, held for 2 min, increased to 160 °C by 30 °C min^{-1} and then by 3 °C min^{-1} until 280 °C was reached and held for 15 min.

Identification of FAMEs was based on their retention time assessed with a fatty acid methyl ester- and a bacterial acid methyl ester-mix (Sigma-Aldrich, St. Louis, USA). Quantification was calculated with an added internal fatty acid methyl ester-standard (Sigma-Aldrich, St. Louis, USA) which was added to the samples before methanolysis.

FAMEs were exemplarily identified by GC-MS using a HP 5890 Series II Plus coupled with a 5972 mass selective detector (Hewlett Packard/Agilent, Waldbronn, Germany) equipped with a DB-5MS column (30 m x 0.25 mm x 0.25 µm). Helium 5.0 was used as the carrier gas at 1 ml min^{-1}. The temperature program was set as follows: 50 °C, held for 1 min, increasing by 9 °C min^{-1} to 180 °C, 5 °C min^{-1} to 260 °C and 20 °C min^{-1} to 300 °C and held for 11 min. The injector temperature was set at 250 °C and the transfer line temperature at 300 °C. A mass range of *m/z* 50 – 450 was monitored.

The branched fatty acids i15:0, a15:0, i16:0 and i17:0 were considered as gram positive and the cy17:0 and cy19:0 as gram negative in origin (Zelles, 1999). In addition to these biomarkers, 16:1ω7 was included for the total bacteria calculation (Frostegård and Bååth, 1996). 18:2ω6,9c was assessed as a fungal biomarker (Frostegård and Bååth, 1996; Kaiser et al., 2010a). For calculation of $PLFA_{sum}$ the following fatty acids were summed: specific bacterial, specific fungal, 14:0, 15:0, 16:1ω6, 16:1ω5, 16:0, 17:0, 18:2ω6,9t, 18:1ω9c, 18:3ω3, 18:1ω7, 18:1ω9t, 18:0, 20:4ω6, 20:5ω3, 20:3ω6, 20:2, 20:0, 22:0, 24:0.

Potential enzyme activities

The activities of the enzymes β-glucosidase (EC 3.2.1.21), N-acetyl-β-D-glucosaminidase (EC 3.2.1.52) and β-xylosidase (EC 3.2.1.37) were analysed according to Marx et al. (2001). Substrates linked to fluorescent 4-methylumbelliferone (4-MU), standards and MES-buffer 2-[N-morpholino]ethanesulfonic acid (pH 6.1) were obtained from Sigma Aldrich (St. Louis, USA). After dissolving substrates in dimethyl sulfoxide (DMSO), sterile water was added and aliquots were mixed with autoclaved MES-buffer (0.1 M) to a final concentration of 1 mM. Standards were dissolved in methanol and deionised water was added. Aliquots were mixed with MES-buffer to a final concentration of 10 µM.

One g soil (fresh weight) was mixed with 50 ml sterile water and dispersed with an ultrasonic disaggregator (50 J s^{-1} for 120 s). From this, 50 µl soil suspension, 50 µl buffer and 100 µl of respective substrate were added into microplate wells (PP microplate, black 96 well, Greiner Bio-one GmbH, Frickenhausen, Germany). Standards were added to 50 µl soil suspensions and the respective buffers to get concentrations of 0, 0.5, 1, 2.5, 4 and 6 µM. After pre-incubation (30 min) at 30 °C, measurements were made at 0, 30, 60, 120 and 180 min with a Fluorescence Microplate Reader (FLx800, BioTek Instruments Inc., Winooski, VT, USA) with excitation at 360 nm and emission at 460 nm. Between measurements the microplates were kept in an incubator at 30 °C. Three analytical replicates of all samples were run.

The potential enzyme activities of phenoloxidase and peroxidase were measured spectrophotometrically using tetramethylbenzidin (TMB) as the substrate (Johnsen and Jacobsen, 2008). TMB was dissolved in dimethyl sulfoxide (DMSO) and water to a final concentration of 60 mM. The working solution was made with sodium acetate buffer for a final concentration of 12 mM TMB. For phenoloxidase activity measurement 50 ml sodium acetate buffer (50 mM) (pH 5.0) was added to 0.4 g of soil (fresh weight) and dispersed by an ultrasonic disaggregator (50 J s^{-1} for 120 s). Each sample well contained 200 µl of soil suspension to which 50 µl of the working solution was added. Blank wells contained soil suspension and 50 µl sodium acetate buffer instead of substrate. Negative control wells contained no soil suspension. For peroxidase activity measurement the procedure was the same as described above but in addition 10 µl hydrogene peroxide solution (0.3 %) was added to every well and peroxidase activity was calculated as the difference between measured activity of phenoloxidase (without H_2O_2) and measured activity with H_2O_2. Three analytical replicates of each sample were analyzed. Measurements were made at 0, 6, 9, 12 and 15 min on a microplate reader (ELx808, Absorbance Microplate Reader, BioTek Instruments Inc.,

Winooski, VT, USA) at 630 nm. Between measurements the microplates were kept in an incubator at 25 °C.

Enzyme activities were calculated on the basis of soil dry weight and on the basis of total microbial biomass ($PLFA_{sum}$) to yield information about the specific enzyme activity of the microbial community.

Data analyses

Factorial ANOVA was used to test for effects of crop or litter with sampling dates (at one depth) or depths (at one date) as repeated measures. Separate ANOVAs for each sampling date and every depth were performed when significant results were obtained. Tukey`s Honest Significant Difference (HSD) test for comparison of means was used. Best fitted transformation (log or root 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.

To get information about the differences in microbial abundances and activities between topsoil and subsoil, subsoil-to-topsoil ratios of $PLFA_{sum}$ data, enzyme activities and specific enzyme activities (averaged over all treatments per depth and sampling date) were calculated.

Results

Soil water content and extractable organic C (EOC)

Soil water content generally decreased with soil depth, with the exception of summer 2010 where highest water content was measured at 60-70 cm (Tab. 5.1). At all depths, soil water content showed a strong seasonal effect, with higher water content in winter than in summer and autumn (Tabs. 5.1, S5.1). In the topsoil, water content was affected by crop and litter, whereas in the two deeper layers an effect of litter on the water content was not observable (Tab. 5.1). In many cases, higher water content was measured in wheat than in maize plots (Tab. 5.1).

Extractable organic C (EOC) content decreased significantly with soil depth in all treatments (Tab. 5.1). The date of sampling generally affected the EOC content in the different soil depths (Tab. S5.1). The highest EOC contents were observed in the topsoil in summer 2010 and in the deeper layers two months later, in autumn 2010. In the topsoil, wheat plots often

showed higher EOC content than maize plots (Tabs. 5.1, S5.1). Litter application increased the EOC content in the topsoil especially in the winter samplings and in autumn 2010 (Tabs. 5.1, S5.1) but in general there was no treatment effect detectable in the two deeper layers (Tab. 5.1).

Table 5.1 Soil water content (SWC) and extractable organic C (EOC) with SE in parentheses at different sampling dates and depths. Letters indicate significant effects of treatments (C = crop; L = litter) within depth and date. Depth as repeated measure was significant at every date when not denoted differently. W = Wheat, WL = Wheat + maize Litter, FM = Fodder Maize, CM = Corn Maize. * = P < 0.05; ** = P < 0.01.

		Treatment				
	Depth [cm]	W	WL	FM	CM	ANOVA
SWC [%]						
Summer 09	0-10	23.74 (1.04)	23.72 (1.00)	21.37 (1.21)	22.26 (0.91)	
	40-50	n.d.	n.d.	n.d.	n.d.	
	60-70	13.14 (0.68)	14.56 (0.49)	19.16 (0.15)	19.31 (0.33)	C**
Autumn 09	0-10	19.45 (1.23)	19.95 (0.27)	14.84 (0.79)	14.22 (0.73)	C**
	40-50 #	19.84 (0.64)	20.26 (0.41)	11.88 (0.31)	11.71 (1.26)	C**
	60-70	17.56 (1.17)	18.39 (0.90)	14.29 (0.42)	15.02 (0.29)	C**
Winter 09/10	0-10	27.65 (0.45)	28.54 (0.67)	25.07 (0.39)	27.34 (1.10)	C*,L*
	40-50	23.35 (0.36)	24.01 (0.29)	22.44 (0.31)	22.70 (0.33)	C**
	60-70	22.85 (0.46)	23.24 (0.25)	22.43 (0.47)	23.42 (0.44)	
Summer 10	0-10	11.86 (0.12)	14.44 (0.99)	12.98 (0.79)	13.12 (0.28)	
	40-50	10.50 (0.59)	15.20 (1.54)	15.35 (1.49)	13.99 (1.22)	CxL*
	60-70	13.81 (1.07)	18.14 (0.93)	20.81 (1.75)	18.90 (0.63)	C*,CxL*
Autumn 10	0-10	20.70 (0.72)	21.49 (0.56)	16.50 (0.87)	16.76 (0.89)	C**
	40-50	18.77 (0.69)	19.26 (0.76)	15.41 (0.72)	15.90 (1.25)	C**
	60-70	16.98 (0.95)	17.28 (0.94)	16.61 (0.44)	17.05 (0.34)	
Winter 10/11	0-10	31.80 (1.16)	36.79 (3.20)	29.86 (0.16)	32.09 (1.23)	
	40-50	28.12 (0.84)	29.37 (0.39)	27.47 (1.07)	26.35 (0.65)	C*
	60-70	25.80 (0.21)	26.78 (0.28)	27.02 (0.52)	26.80 (0.68)	
EOC [µg C g^{-1} soil]						
Summer 09	0-10 ¤	20.77 (2.44)	21.23 (3.43)	29.09 (2.12)	23.80 (0.82)	C*
	40-50	n.d.	n.d.	n.d.	n.d.	
	60-70	5.58 (0.13)	5.15 (0.67)	8.02 (1.52)	7.27 (1.37)	
Autumn 09	0-10 ¤	22.33 (2.30)	22.48 (0.77)	19.94 (0.84)	21.36 (1.24)	
	40-50	9.53 (1.41)	9.92 (1.30)	14.37 (2.53)	9.26 (2.40)	
	60-70	8.62 (1.39)	11.40 (1.84)	6.70 (0.22)	7.30 (1.37)	C*
Winter 09/10	0-10 ¤	20.95 (0.90)	30.58 (2.30)	16.75 (1.44)	23.48 (2.42)	C*,L**
	40-50	13.64 (1.73)	13.65 (0.86)	12.99 (1.99)	12.39 (1.23)	
	60-70	n.d.	n.d.	n.d.	n.d.	
Summer 10	0-10 ¤	33.69 (2.62)	34.53 (2.73)	25.00 (2.26)	27.49 (1.37)	C**
	40-50	9.18 (1.16)	8.86 (1.16)	7.17 (0.60)	8.08 (0.83)	
	60-70	9.73 (4.30)	9.84 (4.30)	10.61 (3.32)	8.93 (2.07)	
Autumn 10	0-10 ¤	22.84 (2.24)	26.03 (1.39)	13.81 (0.88)	16.99 (0.47)	C**,L*
	40-50	20.57 (2.73)	20.17 (2.60)	16.55 (1.96)	16.28 (0.76)	
	60-70	11.10 (1.69)	11.17 (1.90)	12.15 (0.78)	13.53 (1.45)	
Winter 10/11	0-10 ¤	20.43 (2.09)	23.27 (1.58)	10.99 (1.01)	19.96 (2.93)	C**,L*
	40-50	14.80 (1.42)	14.12 (1.23)	14.93 (0.76)	15.61 (2.49)	
	60-70	8.75 (1.35)	8.08 (1.19)	8.64 (0.63)	8.67 (1.45)	

PLFA content

The PLFAs of all microbial groups (total bacteria, gram positive and gram negative bacteria, and fungi) decreased significantly with depth (Figs. 5.1, S5.1) with the exception of fungal PLFA, which showed comparable values at 40-50 cm and 60-70 cm depths in summer 2010 and winter 2010/2011 (Fig. 5.1b).

PLFAs of the different microbial groups showed a strong seasonal effect at all soil depths (Tab. S5.1). In the topsoil, highest total bacterial PLFAs were measured in winter 2009/2010 (Fig. 5.1a) averaging 12 % higher than at the other dates. Gram positive and gram negative PLFAs, on the other hand, averaged 10 % higher in summer 2010 than at the other dates (Fig. S5.1). In the 40-50 cm layer, total bacterial PLFAs and gram positive and gram negative PLFAs were 20 to 30 % higher in both the wheat and maize plots in winter 2009/2010 in comparison to the other dates. In autumn and winter 2010/2011, increases in these PLFAs of around 15 % in comparison to the other dates were due mainly to increases in the wheat plots only. At 60-70 cm depth bacterial PLFAs were highest in winter 2009/2010, averaging 30 % higher than at the other dates. Highest fungal PLFA values were measured in topsoil in winter 2010/2011, at 40-50 cm depth in winter 2009/2010, and at 60-70 cm depth in summer 2010, averaging 50, 40 and 30 % higher, respectively, than the same depths at the other dates.

The type of crop (wheat vs. maize) influenced the amount of total bacterial, gram positive and gram negative bacterial and fungal PLFAs to a soil depth of 50 cm at most sampling dates, with higher PLFA amounts in wheat than in maize plots. Litter application significantly increased microbial PLFAs in the topsoil, but not in deeper layers (Figs. 5.1, S5.1, Tab. S5.1).

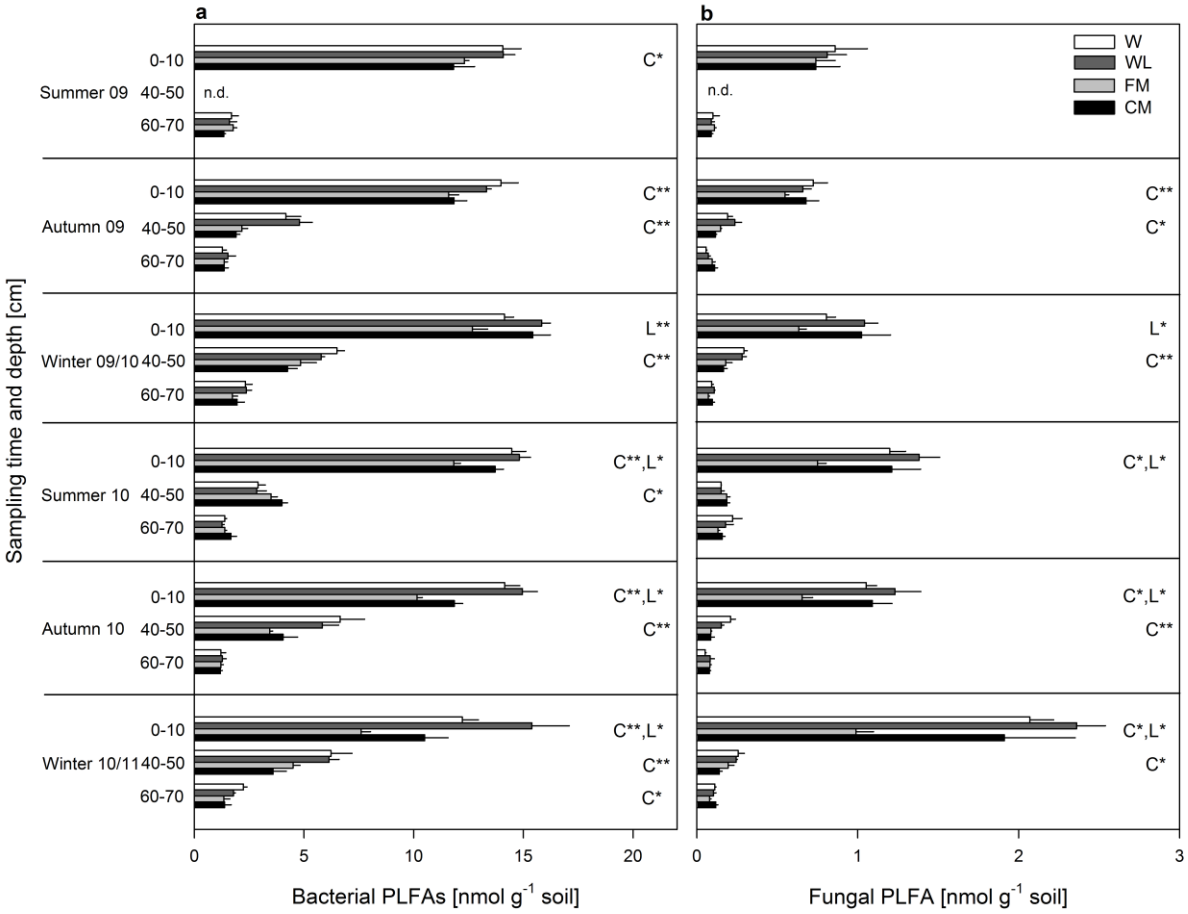

Figure 5.1 Total bacterial (a) and fungal (b) PLFAs (+SE) in the four treatments, in the three depths and at the different sampling dates. Letters indicate significant effects of treatments within depth and date (C = crop; L = litter). Depth as repeated measure was significant at every date. W = Wheat, WL = Wheat + maize Litter, FM = Fodder Maize, CM = Corn Maize. n.d. = not determined; * = $P < 0.05$; ** = $P < 0.01$.

Hydrolytic and oxidative enzyme activities

Activities of the hydrolytic enzymes β-glucosidase, N-acetyl-β-D-glucosaminidase, and xylosidase decreased significantly with soil depth (Figs. 5.2, 5.3a). Depth profiles of phenol- and peroxidase activities were less clear than for hydrolytic enzymes (Figs. 5.3b, S2). Phenoloxidase activity was highest in the 60-70 cm layer at most sampling dates (Fig. 5.3b), while peroxidase activity showed no consistent depth effect, with highest activities varying between top- and subsoil (Fig. S5.2). Seasonal variation in hydrolytic and oxidative enzyme activities was detected both in the topsoil and in deeper soil layers (Tab. S5.1). Single hydrolytic enzyme activities peaked within the soil profile either in winter 2009/2010 or 2010/2011 with the exception of β-glucosidase activity in the topsoil, which had highest values in autumn 2010. In both autumn samplings high phenoloxidase activities were

observed in the 60-70 cm depth (Fig. 5.3b). Seasonal variation in peroxidase activity could not be related to a specific season.

In the topsoil, litter application had a greater influence on hydrolytic enzyme activities over time than cultivation of the different crops (wheat vs. maize) (Tab. S5.1). This effect was reversed in deeper soil layers: N-acetyl-β-D-glucosaminidase and xylosidase activities at 40-50 cm were affected only by crop and not by litter application (Figs. 5.2b, 5.3a).

No general effect of crop or litter could be detected for phenoloxidase activity in the topsoil (Tab. S5.1). Two exceptions where crop type did show an effect were: phenoloxidase activity, in the wheat cultivated plots which increased in summer 2009 and increased in the maize cultivated plots in winter 2010/2011 (Fig. 5.3b). Wheat enhanced peroxidase activity to a depth of 40-50 cm (Fig. S5.2, Tab. S5.1).

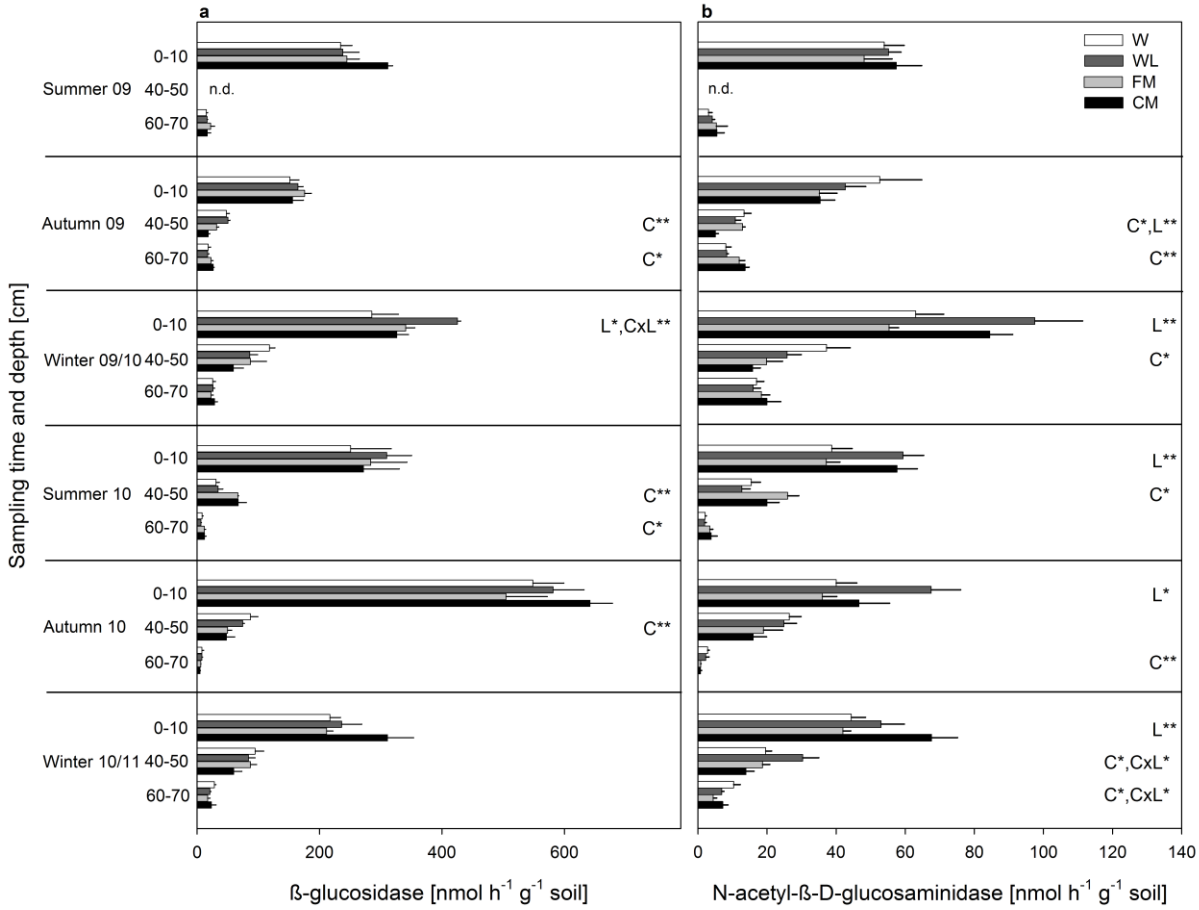

Figure 5.2 β-glucosidase (a) and N-acetyl-β-D-glucosaminidase (b) activities (+SE) in the four treatments, in the three depths and at the different sampling dates. Letters indicate significant effects of treatments within depth and date (C = crop; L = litter). Depth as repeated measure was significant at every date. W = Wheat, WL = Wheat + maize Litter, FM = Fodder Maize, CM = Corn Maize. n.d. = not determined; * = $P < 0.05$; ** = $P < 0.01$.

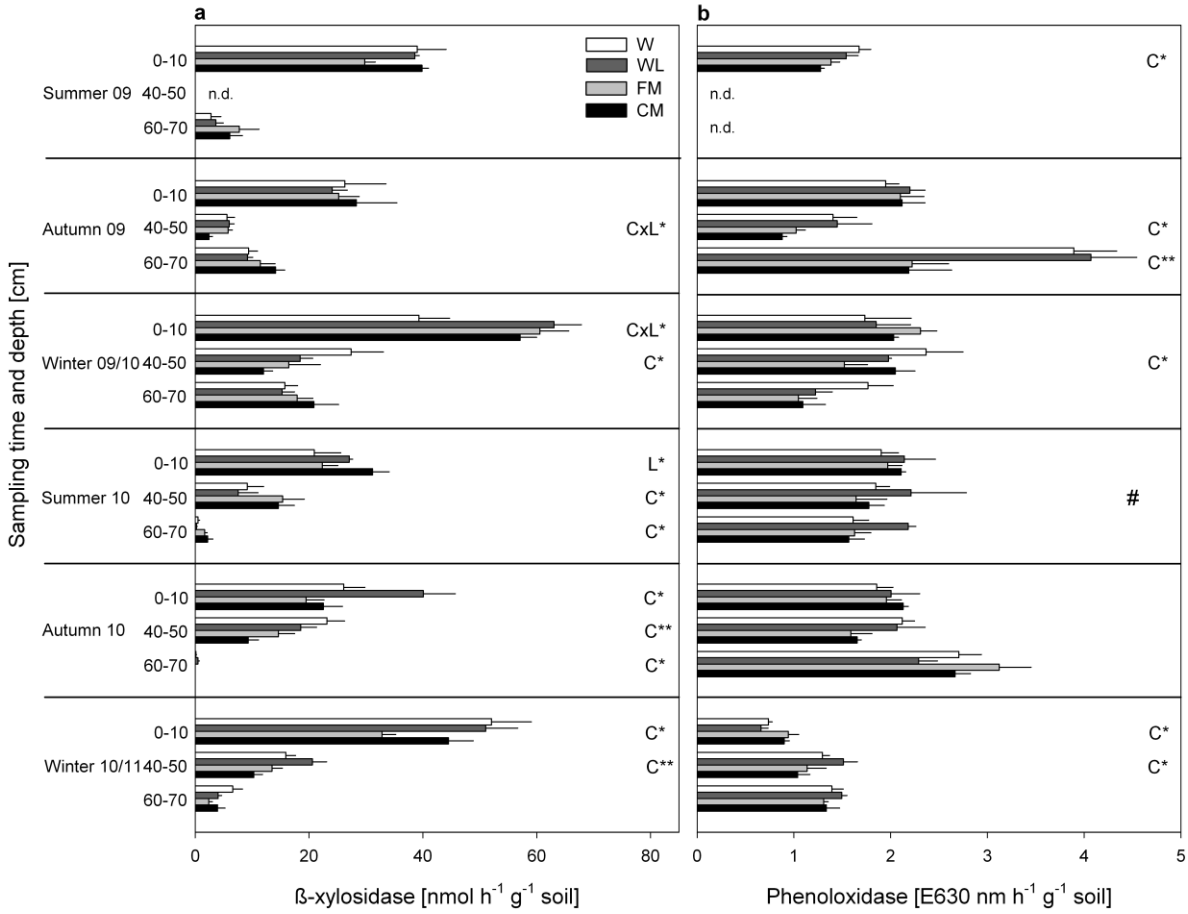

Figure 5.3 Xylosidase (a) and phenoloxidase (b) activities (+SE) in the four treatments, in the three depths and at the different sampling dates. Letters indicate significant effects of treatments within depth and date (C = crop; L = litter). Depth as repeated measure was significant at every date when not denoted differently. W = Wheat, WL = Wheat + maize Litter, FM = Fodder Maize, CM = Corn Maize. n.d. = not determined; * = $P < 0.05$; ** = $P < 0.01$; # = no significant effect of depth.

Comparison of PLFAs, enzyme activities and specific enzyme activities in top- and subsoil

Subsoils (60-70 cm) contained about 10 % of the PLFA values measured in topsoil over the study period and this percentage was similar for all microbial groups (data not shown).

Hydrolytic enzyme activities always showed lower activities in the subsoil relative to topsoil, resulting in sub- to topsoil ratios of between 0.1 and 0.5 in 2009 and between 0.01 and 0.15 in 2010/2011 (data not shown). In contrast, phenol- and peroxidase activities showed enhanced activities mostly in subsoil, leading to higher ratios for phenol- and peroxidase than for hydrolytic enzyme activities.

In general, sub- to topsoil ratios of specific enzyme activities often showed values above 1 with lower ratios for hydrolytic enzymes than for phenol- and peroxidase (Fig. 5.4). Comparison of the two years showed that ratios of N-acetyl-β-D-glucosaminidase and xylosidase were lower in the second than in the first year of the study. When topsoil enzyme activities and specific enzyme activities were related to mid-depths (40-50 cm), ratios showed an opposite trend with higher sub- to topsoil ratios for the samplings in 2010/2011 than in 2009 (Fig. 5.4).

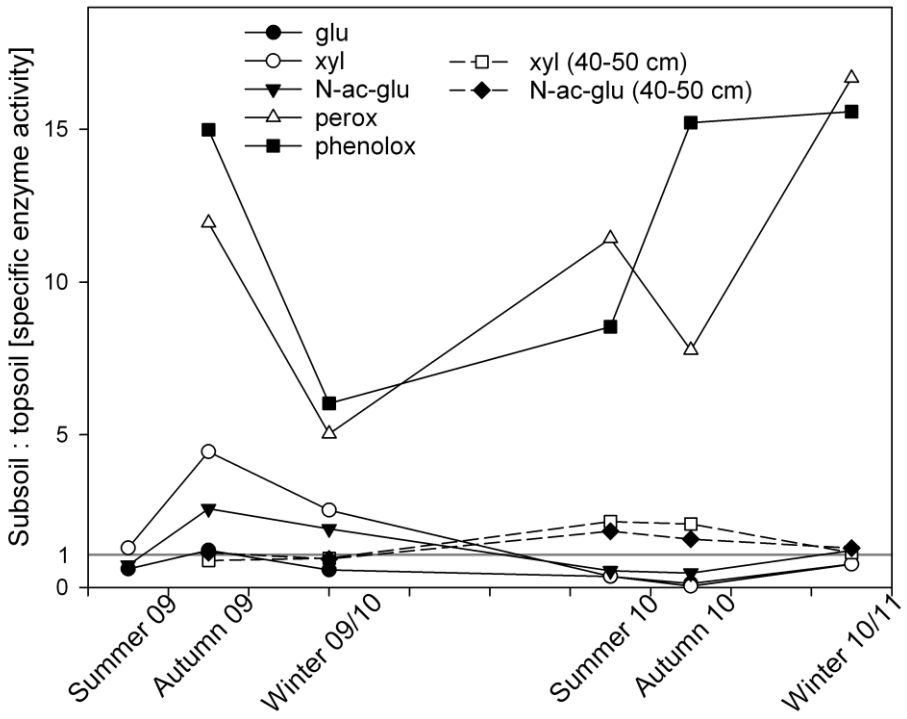

Figure 5.4 Ratios of specific enzyme activities in subsoil to topsoil. Specific enzyme activities in 60-70 cm are related to respective specific enzyme activity in 0-10 cm (solid lines). In addition specific enzyme activities of xylosidase and N-acetyl-β-D-glucosaminidase in 40-50 cm are related to respective value in 0-10 cm (dotted lines). Pictured are: β-glucosidase (β-glu), xylosidase (xyl), N-acetyl-β-D-glucosaminidase (N-ac-glu), peroxidase (perox) and phenoloxidase (phenolox). Ratio of 1 is marked by the grey line.

Discussion

Crop and litter effects at different depths

Higher PLFA amounts were more noticeable in the wheat than in the maize cultivated plots (Fig. 5.1), indicating higher substrate availability under wheat than under maize. Higher EOC content also in the wheat than in the maize cultivated plots, indicates the presence of easily

available C sources, and suggests higher exudation by wheat roots than by maize, at least in topsoil. This corresponds to the higher root biomass of wheat in comparison to maize in the topsoil in July 2009 (0.47 vs. 0.15 mg C g^{-1} soil, respectively) (Kramer et al., 2012). It is therefore likely that the topsoil in wheat cultivated plots was directly influenced by roots to a greater degree than in maize cultivated plots, leading to higher substrate availability (e.g. EOC), which could be used by microorganisms.

At depth, crop type affected PLFA abundance down to at least 50 cm soil depth, but this was not reflected by the EOC content in these soil layer (Tab. 5.1). Highest root biomass of maize (Pausch et al., 2013) and wheat (J. Pausch, pers. comm.) in the upper layers in comparison to the deeper layers (Tab. S5.2) indicates highest input of plant derived substrate into the topsoil, the most easily available of which (mainly rhizodeposits) were quickly used by microorganisms. Substrates translocated into deeper layers could be either immediately assimilated by microorganisms or be more strongly adsorbed onto mineral particles than in topsoil (Kalbitz et al., 2000).

Increased biomarker PLFAs in the topsoil in winter 2009/2010 and during the following year after litter application in November 2009 suggest enhanced substrate availability for more than nine months. Use of maize derived litter C by both bacteria and fungi as described by McMahon et al. (2005) and Williams et al. (2006) was demonstrated at this field site by following the ^{13}C flow from maize into the total microbial biomass and ergosterol (Kramer et al., 2012). In deeper soil layers, litter application did not change the amount of specific PLFAs. Litter was only dispersed on the soil surface; maize derived substrates could have been immobilised by topsoil communities, and not transported in sufficient amounts into deeper soil layers. This corresponds to the structure of the fungal community, which, as the major decomposer of recalcitrant plant substrate, was responsive to litter availability predominantly in the topsoil (Scharroba et al., 2012).

With respect to hydrolytic enzymes, effects on their activities were observable by the presence of litter derived carbon in the topsoil. In deeper soil layers, both hydrolytic and oxidative enzymes were influenced by root derived carbon from different crops (Figs. 5.2, 5.3; Tab. S5.1). Enzyme activities were often higher under wheat than under maize, suggesting that both quantity and quality of crop derived substrates were important regulators of enzyme production and expression in the different soil layers. Fresh, energy rich substrates (e.g. rhizodeposits or soluble components of litter) can result in production and activation of enzymes that decompose more recalcitrant substrates (Fontaine et al., 2003). Thus, higher root

density and root biomass combined with the derived labile carbon compounds may have stimulated enzyme activities under wheat down to 50 cm depth. The increase in hydrolytic enzyme activities by litter application in the topsoil corresponded with observed litter effects on the biomarker PLFAs in the soil profile, which suggest that litter derived compounds in deeper soil layers were of minor importance.

Seasonal effects in different depths

Season distinctly affected resource availability (EOC) and microbial community structure (PLFAs). In topsoil, high EOC content and high bacterial PLFAs in winter 2009/2010 and summer 2010 suggest advantageous nutrient conditions for bacteria, possibly due to substrate mobilization in winter and exudation in summer. High amounts of total bacterial PLFAs as well as of gram positive and gram negative PLFAs in the wheat plots corresponded to increased EOC content at 40-50 cm depth in autumn 2010.

During autumn and winter, water flux and carbon export from the topsoil may have positively influenced bacterial PLFA amounts in both deeper soil layers, predominantly under wheat (Fig. 5.1a). This may have been due to translocation of substrates after rain events, which created transient flow conditions (Totsche et al., 2007). Mobilization of organic carbon can have many causes, such as drying/rewetting (autumn) or freezing/thawing (winter) cycles, which result in microbial cell death and lysis (Majdalani et al., 2008). Moreover, mechanical stress at harvest can result in an increase in fractured plant residues, which trigger release of bacterial substrates. Our measured PLFA data imply that transport occurred in autumn (plant senescence) and winter (fallow) suggesting that water demand by plants during the growing season hampers translocation processes; this is supported by the significantly higher soil water content measured during winter samplings.

Highest fungal PLFA amount in the topsoil was measured in winter 2010/2011 (Fig. 5.1b), indicating high substrate availability after snow melt, possibly due to decomposition of remaining litter, increased availability of dead microbial biomass (Schmidt and Lipson, 2004), and destabilization of substrates due to disruption of soil structure after freeze / thaw cycles (Kalbitz et al., 2000). At 40-50 cm depth the highest fungal biomass was measured in winter 2009/2010, but not in autumn 2010 in contrast to bacteria. Hence, labile and recalcitrant resources showed differences in availability at depth. The observed fungal development indicates enhanced transport processes of plant derived recalcitrant substrates into deeper soil

layers and/or root decomposition after harvest of aboveground crops. In particular the frequent occurrence of macropores at the field site (K. Totsche, pers. comm.) may play a considerable role in rapid transport of organic resources into subsoils, thereby fueling belowground microbial communities.

Season also strongly affected hydrolytic and oxidative enzyme activities at all soil depths. Seasonal and interannual variation in enzyme activities of topsoils have been found to be highly variable due to changing patterns of temperature, precipitation and vegetation cover (Debosz et al., 1999; Kandeler et al., 1999; Bell et al., 2010; Gutknecht et al., 2010; Kaiser et al., 2010b). Studies which investigated seasonal effects on enzyme activities in depth are scarce. However, in our study, hydrolytic enzymes showed highest activities in deeper soil layers in winter. This corresponds to the biomass measurements by PLFAs and underlines the impact of abiotic factors such as higher moisture content and higher substrate availability after transport of mobile organic matter from topsoil to subsoil.

Depth distribution of microbial properties

The amounts of the biomarker PLFAs as well as EOC decreased with depth (Tab. 5.1, Figs. 5.1, S5.1). Hence, reduced substrate availability in deeper soil was the most likely reason for the decline in microbial biomass. Decreasing microbial biomass with depth has been observed by others (Blume et al., 2002; Fierer et al., 2003; Fang and Moncrieff, 2005; Bausenwein et al., 2008).

A clear difference in depth distribution between hydrolytic and oxidative enzyme activities was observed in this study. The activities of these two classes of enzymes are often not correlated to each other (Sinsabough, 2010). Hydrolytic enzyme activities generally decreased with depth while oxidative enzymes showed a different depth distribution, sometimes with even higher activities in the deeper layers (Figs. 5.2, 5.3, S5.2). Our findings are in accordance with Brockett et al. (2012) and Jackson et al. (2009) who found similar results in a forest site in Canada and a peat swamp forest in Malaysia.

The results of hydrolytic and oxidative enzyme measurements can be interpreted both on the basis of substrate distribution within the soil profile and on the basis of enzyme stability. Whereas the depth profile of ß-glucosidase, N-acetyl-β-D-glucosaminidase and xylosidase mirrored the expected availability of their respective substrates (Leinweber et al., 2008; Geisseler and Horwath, 2009), phenol- and peroxidase patterns within the profile can be

explained by the increasing adherence of these oxidative enzymes to mineral surfaces within the soil profile. Allison and Jastrow (2006) demonstrated that two cellulose-degrading enzymes and a chitin-degrading enzyme were bound mainly to the particulate organic matter (POM) fractions and that highest polyphenol oxidase activity was found in the clay-size fraction, despite a higher mean residence time for carbon in this fraction. Soluble substrates are frequently used for potential activity measurements of oxidative enzymes, even though under environmental conditions substrates are commonly insoluble and/or bound to minerals. Therefore, the actual activity and function of both oxidative enzymes might be low under field conditions as compared to laboratory measurements due to less efficient enzyme-substrate interactions in the field (Allison, 2006). A second reason for higher activities in deeper soil layers might be the pH optimum of these enzymes. Optimal conditions for phenol- and peroxidase enzyme activities were found to be at a pH of 8 ± 1 (Sinsabough et al., 2008, 2010). Soil pH_{CaCl_2} of our field site increased from pH 6.0 in the topsoil to 7.0 in deeper soil layers (>65 cm depth), leading to conditions in deeper soil layers that were closer to the optimum of oxidative enzymes.

Comparison of sub- and topsoil - effects on specific enzyme activities

The specific enzyme activities of hydrolytic and oxidative enzymes often had sub- to topsoil ratios above 1 (Fig. 5.4), meaning higher enzyme activity per microbial biomass in subsoil as compared to topsoil. This can be driven by several factors. (1) The production of enzymes by single microorganisms may be higher in subsoil in contrast to topsoil due to higher spatial separation of microbes from substrates (Salomé et al., 2010). (2) Spatial separation between microbes may lead to lower numbers of cheaters (microbes which do not produce enzymes but use enzyme products) (Allison, 2005) resulting in more microorganisms producing enzymes in subsoil than in topsoil. (3) A microbial community capable of producing higher quantities of enzymes or more efficient ones were present in the subsoil in comparison to topsoil, indicated by T-RFLP analyses (terminal restriction fragment length polymorphism) at our site (Scharroba et al., 2012). (4) Enzymes in the subsoil may also be protected to a greater extent against degradation and non-biological denaturation than in topsoil due to higher interaction with mineral components at depth, leading to stabilization of enzymes (Burns, 1982, Taylor et al., 2002). Stabilized enzymes that maintain their activity at depth may be advantageous for microorganisms because mineral-bound enzymes degrade available

substrates, resulting in “trigger” molecules (Burns, 1982) inducing production of respective enzymes.

Conclusions

We studied patterns of microbial community composition and function in an arable soil to understand whether management practice such as crop type and litter amendment affect not only topsoil but also subsoil processes. We detected a distinct crop effect on microbial communities in topsoil and the rooted zone beneath mainly due to crop specific root distribution and hence release of exudates and increased quantities of other root derived compounds. Translocation processes of microbial substrates occurred predominantly in the autumn and winter seasons due to higher water availability in the soil in comparison to summer. In contrast, litter application did not show an influence on growth of microorganisms and enzyme activities in the subsoil. This indicates that litter derived substrates were not transported in significant enough amounts into the subsoil to change the abundances of microorganisms and activity of enzymes but were instead assimilated by the communities in the topsoil. One of the most interesting results of this study was that hydrolytic and oxidative enzyme activities showed different depth gradients that could be related to microbial abundance and expected substrate availability for hydrolytic enzymes only. It is possible that hydrolytic and oxidative enzymes are bound to different particle size fractions, i.e. hydrolytic enzymes to the POM fraction and phenol- and peroxidase to minerals. In addition, specific enzyme activity increased in the subsoil, possibly due to higher production of enzymes as a result of greater spatial separation between microbes and between microbes and substrates, a community shift in the subsoil, and a higher stabilization of enzymes at depth. Future approaches to quantify turnover times of enzymes and to estimate both the activity status of enzymes and distances between microorganisms and their respective substrates in subsoil are needed to more fully understand the importance of subsoil microbial ecology for C dynamics in arable soil profiles.

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Supplementary material

Fig. S5.1 Gram positive (a) and gram negative (b) PLFAs (+SE) in the four treatments, in the three depths and at the different sampling dates. Letters indicate significant effects of treatments within depth and date (C = crop; L = litter). Depth as repeated measure was significant at every date. W = Wheat, WL = Wheat + maize Litter, FM = Fodder Maize, CM = Corn Maize. n.d. = not determined; * = P < 0.05; ** = P < 0.01.

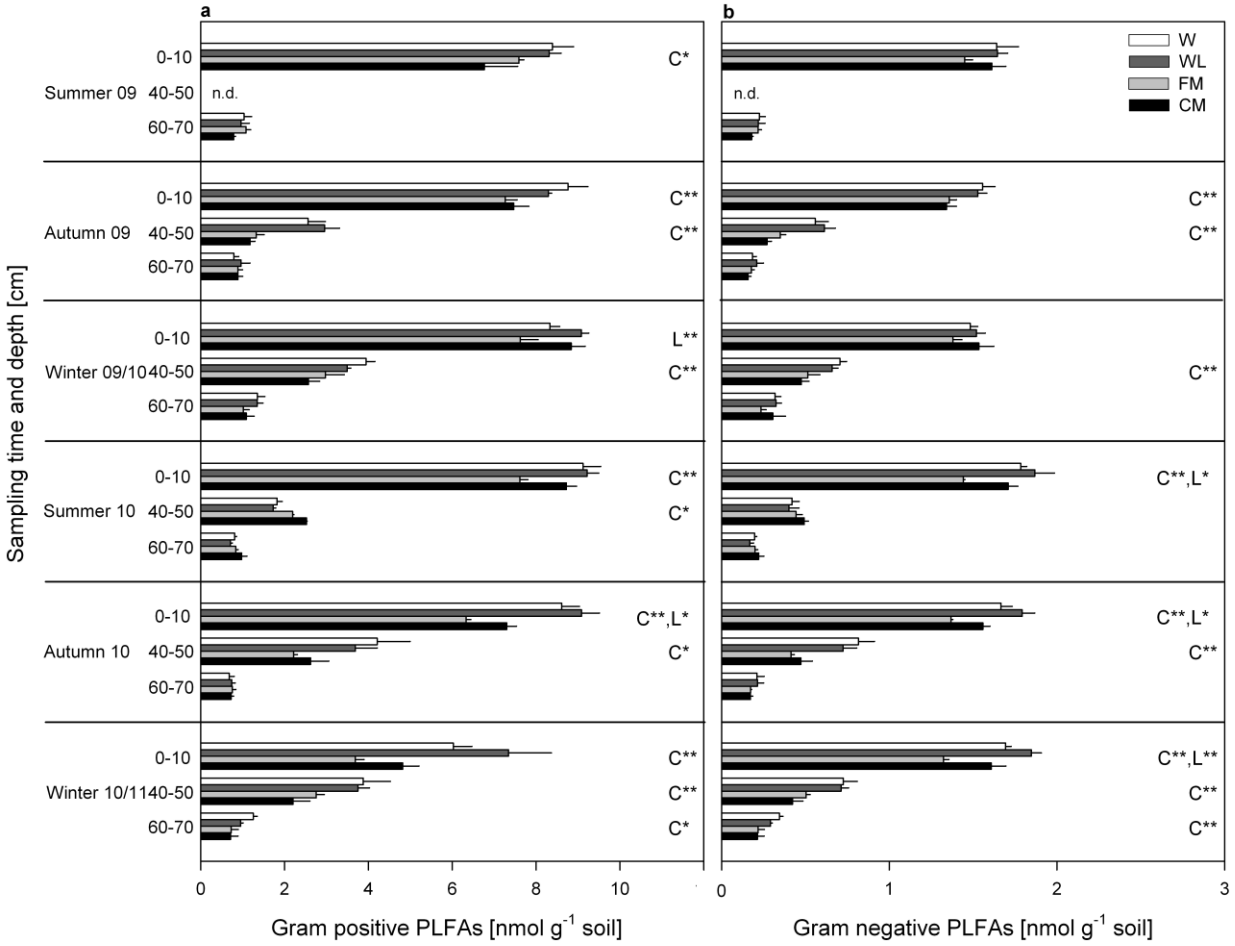

Fig. S5.2 Peroxidase activity (+SE) in the four treatments, in the three depths and at the different sampling dates. Letters indicate significant effects of treatments within depth and date (C = crop; L = litter). Depth as repeated measure was significant at every date. W = Wheat, WL = Wheat + maize Litter, FM = Fodder Maize, CM = Corn Maize. n.d. = not determined; * = P < 0.05; ** = P < 0.01. # = no significant effect of depth.

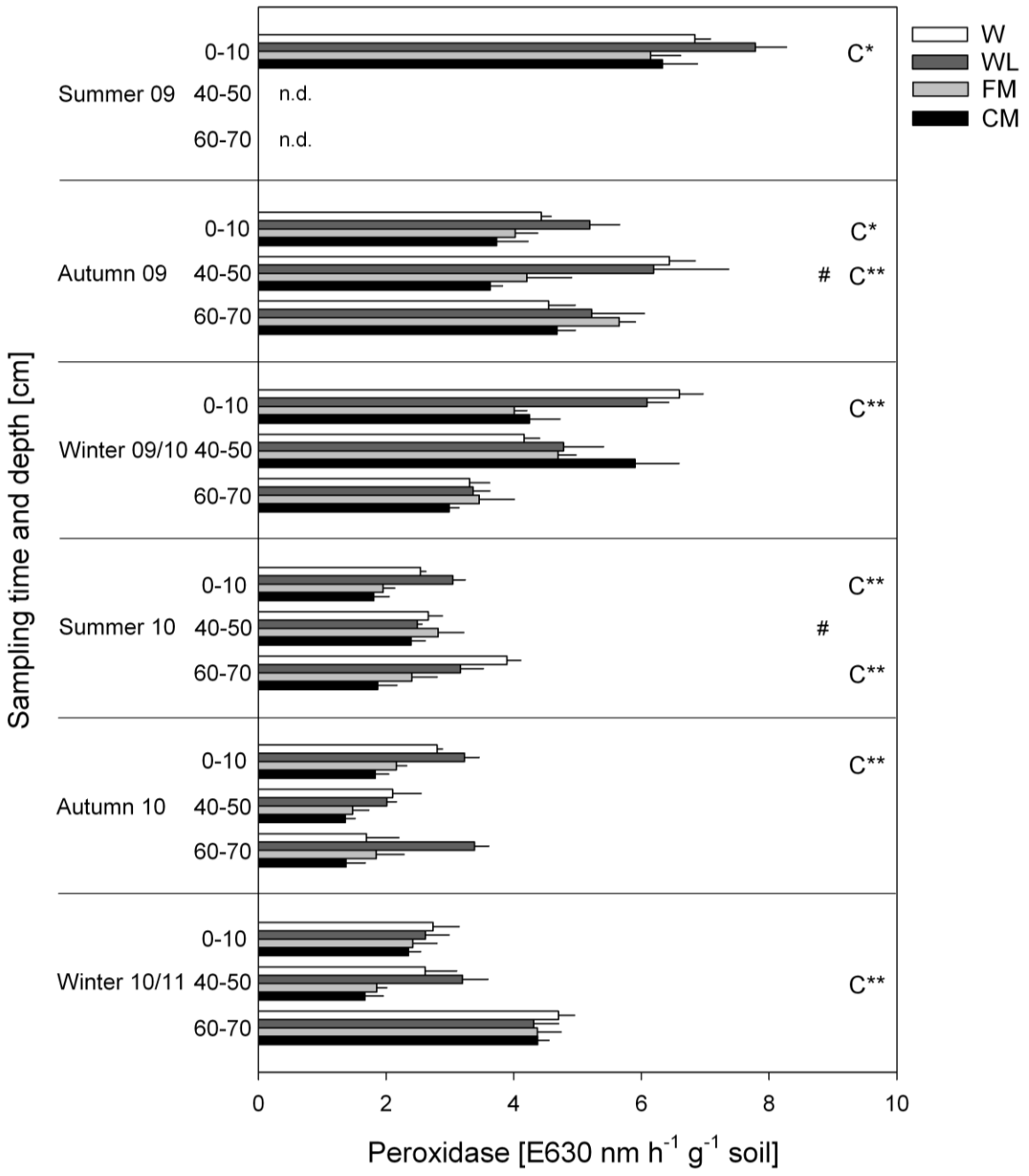

Table S5.1 Statistical results of two-way ANOVA in different depths with date of sampling as repeated measure. Significant effects are highlighted in bold. C = Crop; L = litter; T = time of sampling. β-glu = β-glucosidase; N-ac-glu = N-acetyl-β-D-glucosaminidase; Xyl = xylosidase; Perox = peroxidase; Phenolox = phenoloxidase.

Parameter	Factor	Depth [cm]					
		0-10		40-50		60-70	
		$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,12}$	P
Soil water content	C	26.433	< **0.001**	24.805	< **0.001**	5.990	**0.031**
	L	5.169	**0.042**	1.203	0.294	3.309	0.094
	C x L	0.485	0.499	3.384	0.0910	2.561	0.136
		$F_{5,60}$	P	$F_{4,48}$	P	$F_{5,60}$	P
	T	211.142	< **0.001**	227.138	< **0.001**	144.460	< **0.001**
	T x C	3.662	**0.005**	23.450	< **0.001**	29.942	< **0.001**
	T x L	1.791	0.128	0.717	0.584	0.718	0.612
		$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,12}$	P
EOC	C	25.762	< **0.001**	0.782	0.394	0.364	0.558
	L	12.131	**0.005**	0.446	0.517	0.001	0.981
	C x L	0.002	0.969	0.320	0.582	0.011	0.920
		$F_{5,60}$	P	$F_{4,48}$	P	$F_{4,48}$	P
	T	17.722	< **0.001**	21.361	< **0.001**	4.249	**0.005**
	T x C	7.680	< **0.001**	1.005	0.414	1.345	0.267
	T x L	3.834	**0.004**	0.579	0.679	0.208	0.933
		$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,12}$	P
Total Bacteria	C	63.206	< **0.001**	21.156	< **0.001**	1.442	0.253
	L	14.381	< **0.001**	0.178	0.681	0.018	0.895
	C x L	0.908	0.359	0.039	0.847	0.045	0.836
		$F_{5,60}$	P	$F_{4,48}$	P	$F_{5,60}$	P
	T	9.607	< **0.001**	30.079	< **0.001**	12.765	< **0.001**
	T x C	4.238	**0.002**	14.464	< **0.001**	3.543	**0.007**
	T x L	3.752	**0.005**	0.617	0.652	0.996	0.428
		$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,12}$	P
Gram+ Bacteria	C	45.422	< **0.001**	17.938	**0.001**	1.024	0.332
	L	5.754	**0.034**	0.179	0.680	0.121	0.735
	C x L	0.494	0.496	0.002	0.963	0.012	0.914
		$F_{5,60}$	P	$F_{4,48}$	P	$F_{5,60}$	P
	T	34.760	< **0.001**	24.829	< **0.001**	9.837	< **0.001**
	T x C	3.409	**0.009**	11.491	< **0.001**	4.008	**0.003**
	T x L	2.796	**0.025**	0.691	0.602	1.166	0.337
		$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,12}$	P
Gram- Bacteria	C	26.420	< **0.001**	22.746	< **0.001**	2.599	0.133
	L	9.730	**0.009**	0.261	0.619	0.009	0.927
	C x L	2.150	0.168	0.004	0.950	0.105	0.752
		$F_{5,60}$	P	$F_{4,48}$	P	$F_{5,60}$	P
	T	10.157	< **0.001**	18.546	< **0.001**	14.420	< **0.001**
	T x C	2.892	**0.021**	11.990	< **0.001**	3.018	**0.017**
	T x L	2.113	0.076	0.354	0.840	0.954	0.453
		$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,12}$	P
Fungi	C	55.325	< **0.001**	26.501	< **0.001**	0.008	0.931
	L	51.307	**0.021**	1.053	0.325	0.735	0.409
	C x L	13.013	**0.004**	0.224	0.644	0.377	0.551
		$F_{5,60}$	P	$F_{4,48}$	P	$F_{5,60}$	P
	T	28.411	< **0.001**	14.039	< **0.001**	13.247	< **0.001**
	T x C	2.774	**0.026**	6.739	< **0.001**	2.710	**0.028**
	T x L	2.115	0.076	0.589	0.672	0.708	0.619
		$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,12}$	P
β-glu	C	0.401	0.540	3.602	0.082	0.640	0.439
	L	5.930	**0.031**	2.926	0.113	0.000	0.989
	C x L	0.003	0.961	0.086	0.772	0.654	0.434

		$F_{5,60}$	P	$F_{4,48}$	P	$F_{5,60}$	P
	T	64.357	< **0.001**	18.662	< **0.001**	21.308	< **0.001**
	T x C	0.457	0.806	7.761	< **0.001**	1.790	0.129
	T x L	0.817	0.543	1.655	0.176	0.319	0.899
		$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,12}$	P
N-ac-glu	C	4.207	0.063	4.944	**0.046**	0.116	0.740
	L	31.312	< **0.001**	2.196	0.164	0.133	0.722
	C x L	0.161	0.696	0.664	0.431	0.828	0.381
		$F_{5,60}$	P	$F_{4,48}$	P	$F_{5,60}$	P
	T	10.983	< **0.001**	12.934	< **0.001**	41.166	< **0.001**
	T x C	1.151	0.344	8.437	< **0.001**	2.998	**0.018**
	T x L	3.347	**0.010**	1.846	0.136	0.494	0.780
		$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,12}$	P
Xyl	C	4.631	0.053	6.400	**0.026**	3.690	0.079
	L	21.610	< **0.001**	3.304	0.094	0.1222	0.733
	C x L	0.190	0.670	0.217	0.650	0.630	0.443
		$F_{5,60}$	P	$F_{4,48}$	P	$F_{5,60}$	P
	T	28.737	< **0.001**	15.578	< **0.001**	57.971	< **0.001**
	T x C	3.340	**0.009**	5.852	< **0.001**	2.070	0.082
	T x L	0.566	0.726	3.304	0.094	0.201	0.961
		$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,12}$	P
Phenolox	C	0.472	0.505	8.191	**0.014**	8.660	**0.012**
	L	0.101	0.756	0.004	0.949	0.153	0.703
	C x L	0.204	0.660	0.869	0.370	0.058	0.814
		$F_{5,60}$	P	$F_{4,48}$	P	$F_{4,48}$	P
	T	35.031	< **0.001**	7.819	< **0.001**	49.639	< **0.001**
	T x C	1.700	0.148	0.731	0.576	12.291	< **0.001**
	T x L	0.660	0.655	0.161	0.957	1.453	0.231
		$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,12}$	P
Perox	C	46.331	< **0.001**	9.990	**0.008**	3.122	0.103
	L	0.716	0.414	0.138	0.717	0.381	0.549
	C x L	1.652	0.223	0.331	0.576	1.079	0.320
		$F_{5,60}$	P	$F_{4,48}$	P	$F_{4,48}$	P
	T	124.567	< **0.001**	51.615	< **0.001**	42.600	< **0.001**
	T x C	3.690	**0.006**	8.387	< **0.001**	2.746	**0.039**
	T x L	0.623	0.681	2.167	0.087	0.930	0.455

Table S5.2 Depth distribution of root biomass of wheat and maize in July 2009 at the field site.

Depth [cm]	Root biomass [mg C g^{-1} soil]	S.E.
Wheat[+]		
0-10	0.47	0.17
10-20	0.66	0.30
20-30	0.36	0.13
30-40	0.11	0.06
40-50	0.02	0.01
Maize[++]		
0-10	0.15	0.04
10-20	0.06	0.01
20-30	0.03	0.01
30-40	0.01	0
40-50	0.01	0

[+] J. Pausch, personal communication
[++] data derived from Pausch et al., 2013

6 Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems

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Abstract

The origin and quantity of plant inputs to soil are primary factors controlling the size and structure of the soil microbial community. The present study aimed to elucidate and quantify the carbon (C) flow from both root and shoot litter residues into soil organic, extractable, microbial and fungal C pools. Using the shift in C stable isotope values associated with replacing C3 by C4 plants we followed root- vs. shoot litter-derived C resources into different soil C pools. We established the following treatments: Corn Maize (CM), Fodder Maize (FM), Wheat + maize Litter (WL) and Wheat (W) as reference. The Corn Maize treatment provided root- as well as shoot litter-derived C (without corn cobs) whereas Fodder Maize (FM) provided only root-derived C (aboveground shoot material was removed). Maize shoot litter was applied on the Wheat + maize Litter (WL) plots to trace the incorporation of C4 litter C into soil microorganisms. Soil samples were taken three times per year (summer, autumn, winter) over two growing seasons. Maize-derived C signal was detectable after three to six months in the following pools: soil organic C (C_{org}), extractable organic C (EOC), microbial biomass (C_{mic}) and fungal biomass (ergosterol). In spite of the lower amounts of root- than of shoot litter-derived C inputs, similar amounts were incorporated into each of the C pools in the FM and WL treatments, indicating greater importance of the root- than shoot litter-derived resources for the soil microorganisms as a basis for the belowground food web. In the CM plots twice as much maize-derived C was incorporated into the pools. After two years, maize-derived C in the CM treatment contributed 14.1, 24.7, 46.6 and 76.2 % to C_{org}, EOC, C_{mic} and ergosterol pools, respectively. Fungi incorporated maize-derived C to a greater extent than did total soil microbial biomass.

Introduction

Detailed knowledge on the carbon (C) flow in terrestrial ecosystems is a prerequisite for understanding ecosystem services and for managing agricultural systems in a sustainable way. Considerable information is available on total amounts, individual fractions and residence time of C in soil (Amundson 2001; Bol et al. 2009; Janzen 2004; Marschner et al. 2008; Verchot et al. 2011). In contrast, the fate of belowground C, and especially the flux of C through the soil food web, is poorly understood. The transfer of C from plant roots into soil via rhizodeposition annually recycles around 10 % of atmospheric CO_2, which is an order of magnitude greater than current rates of fossil fuel C combustion (Raich et al. 2002). A second pathway of C flow is via litter decomposition which is mediated by the microbial community, predominantly bacteria and fungi (Rosenbrock et al. 1995; Frankland 1998; Dilly et al. 2001). As primary decomposers of rhizodeposits and litter residues, soil microorganisms form the basis of the soil food web.

Plant inputs are key determinants of microbial activity and community composition in soil. Rhizodeposits lead to a proliferation of microorganisms, altering community structure, and consequently changing the C transfer through the decomposer system (Kuzyakov 2002; Butler et al. 2003; Wasaki et al. 2005). As a result, the quality and quantity of plant C resources and therefore the composition of plant communities create feedbacks to soil microorganisms affecting microbial activity and C allocation in the rhizosphere (Eisenhauer et al. 2010, Ladygina and Hedlund 2010). Generally, labile and recalcitrant plant fractions are utilised by distinct microbial communities, affecting soil C transformation (Paterson et al. 2008; Kramer and Gleixner 2008). Further, root- and litter-derived substrates are processed by different soil organisms (Elfstrand et al. 2008) and root-derived C presumably is immobilized in soil to a greater extent than shoot litter-derived C (Puget and Drinkwater 2001; Rasse et al. 2005). Such effects of resource quality are important bottom-up drivers of soil food webs (Salamon et al. 2006).

Recently, the application of stable isotope tracer methods to determine fluxes within and between C pools in soil has been increased considerably (Bowling et al. 2008). Transformations of C have been followed by switching C3 to C4 plants or resources derived from C3 or C4 plants (Steinbeiss et al. 2008; Esperschütz et al. 2009; Nottigham et al. 2009), as well as by ^{13}C or ^{14}C pulse labelling or continuous labelling to introduce a distinct C signal into the soil system (Kuzyakov and Cheng 2001, 2004; Leake et al. 2004, 2006; Williams et al. 2006a; Werth and Kuzyakov 2008).

Only few studies have separated root- and shoot litter-derived C flow and followed C incorporation into different soil and microbial C pools. Soil microorganisms are of particular interest, as they form the basis of the soil food web. For this reason the present study aims to quantify the transfer of C from both root- and shoot litter-derived C as well as of each C source separately into soil C pools [soil organic C (C_{org}) and extractable organic carbon (EOC)], total microbial biomass (C_{mic}) and ergosterol as a proxy for fungal biomass (Djajakirana et al., 1996) using ^{13}C natural abundance techniques. Typically a mixture of sterols in fungi is present which one of these is dominant and contributed over 50 % of the total sterol composition (Weete et al. 2010). Ergosterol is the predominant sterol in fungal cell membranes and only present in higher fungal phyla, i.e. Basidiomycota, Ascomycota and the majority of Zygomycota and does not occur in plants (Weete and Ghandi 1997, 1999; Klamer and Bååth 2004). Conflicting data regarding the presence of ergosterol in membranes of arbuscular mycorrhizal fungi (AM fungi) have been reported in the literature. Hart and Reader (2002) used it to quantify the biomass of inoculated AM fungi in roots and soil, but in mycelium of AM fungi and colonised roots no ergosterol could be detected by Olsson et al. (2003). The major sterol in spores of AM fungi is 24-ethyl cholesterol and no ergosterol has been detected (Grandmougin-Ferjani et al. 1999).

A field experiment was established at an agricultural site with known long-term C3 cropping history (at least 25 years). In 2009 the following treatments were set up to investigate and quantify the flow of C into the belowground soil food web: Corn Maize (CM), where the new ^{13}C (or C4) signal entered the soil system via the shoot litter and root pathway, and Fodder Maize (FM), where the aboveground parts of plants were removed at harvest and C supplies were derived mainly from roots and rhizodeposits. To gain a C4 ^{13}C signal solely through the aboveground channel, maize litter was added to plots planted with wheat [Wheat + maize Litter (WL)]. Plots with Wheat (W) only served as C3 reference for the incorporation of the maize ^{13}C signal into the different C pools. In the present study soil microorganisms as the basis of the complex soil food webs were investigated and we hypothesized that the incorporation of C into soil pools (C_{org}, EOC, C_{mic} and fungal biomass) depends on the origin of C resources (root vs shoot.) entering the soil.

Materials and methods

Study site

The experimental agricultural field is located on a terrace plain of the river Leine north-north-west of the city of Göttingen (Niedersachsen, Germany). The local climate, with a mean annual temperature of 8.7 °C and mean annual precipitation of 645 mm, represents a temperate climate zone, affected by the transgression from the maritime Atlantic climate to the west to the continental climate to the east. The elevation of the plane is 155 to 160 m, a.s.l., striking towards north-west with a mean base slope of approximately 2 %.

Geologically the area belongs to the Leinegraben, a rift formation embedded within the Harz Mountains to the east and the Weserbergland Mountains to the west. Up to 15 m of quaternary materials deposited mainly during the Weichsel glacial period, form the stratum on top of Mesozoic rocks, predominantly limestones and mudstones of the Mittlerer Keuper. The quaternary deposits are composed of clayey and fine sandy materials interbedded with silty loess materials, which are now decalcified in the upper part of the profile. These deposits are parent material for the actual soil formation.

According to IUSS (2007), the dominant soil types are Luvisols (Parabraunerden, KA5 2005) and stagnic Luvisols (Pseudogley, KA5 2005). However, long agricultural use has severely affected the build up of the soil profiles. The albic horizon typically found for these soils can no longer be detected in the field due to centuries of intensive tillage. In general, two plough layers (0.2 m and 0.3 m below surface) can be detected, with strong compaction below the second plough layer in particular. This is especially evident in the relatively high bulk density (1.6 g cm^{-3}) in and below the second plough layer (Table 6.1).

Table 6.1: Selected soil properties of the study site.

Horizon*	Depth	Texture[1] clay/silt/ sand	pH (H_2O)	pH ($CaCl_2$)	Ks	bd	Porosity
	[m]	% (w/w)			[cm d^{-1}]	[g cm^{-3}]	[cm^3 cm^{-3}]
Ap1	0-0.25	7.0/87.2/ 5.8	6.6	6.0	3.0	1.38	0.44
A(l)p2	0.25-0.37	7.1/87.8/ 5.0	6.9	6.2	1.8	1.61	0.38
Btv1	0.37-0.65	7.1/87.7/ 5.1	7.3	6.6	4.0	1.55	0.40
Btv2	>0.65	6.8/88.4/ 4.8	7.7	7.0	n.d.	1.68	0.38

*Classification according to KA5. 1: Texture according to the German classification system. Ks: Saturated hydraulic conductivity, bd: Bulk density.

Establishment of the experiment

Before the start of the experiment the isotopic signature of C and N in soil were screened in 10 cm layers to 90 cm depth in April 2009. The $\delta^{13}C$ values of the Ah horizon were typical for C3 vegetation, with a mean value of -27.3 ± 0.04 ‰ (Fig. S6.1 top). The $\delta^{13}C$ values increased with depth. The mean C and N contents of the Ah horizon were 11.6 ± 0.2 (Fig. S6.1 bottom) and 1.2 ± 0.02 mg g^{-1} dry weight, respectively, and the average C/N ratio was 9.7 ± 0.1. Background screening revealed comparable isotopic compositions and total soil C and N contents across the experimental field site.

The experimental plots were arranged in a factorial design in April 2009 (Fig. S6.1). The plots were aligned in two west-east striking rows to facilitate tillage during the experiment, 10 experimental plots (24 × 24 m) with maize (*Zea mays* L.) in one row and 10 plots with wheat (*Triticum aestivum* L.) in the other. Wheat seedlings on the maize plots were removed using a non-selective herbicide ("Round-up", Monsanto Agrar, Düsseldorf, Germany). Maize plots were then tilled with a chisel plough to a depth of 12 cm and hybrid maize ("Ronaldinio", KWS Saat Ag, Einbeck, Germany) was sown in April 2009 at a density of 11.5 grains m^{-2} (34 kg ha^{-1}). N fertilizer (ammonium nitrate urea solution: 122.4 kg N ha^{-1}) and NP fertilizer (diammonium phosphate: 32.4 kg N ha^{-1}, 82.8 kg P ha^{-1}) were added shortly before and after seeding to improve growth of maize plants. Winter wheat ("Julius", KWS Saat AG, Einbeck, Germany) was sown in October 2008 at a density of 380 grains m^{-2} (224.0 kg ha^{-1}). Fertilizers to wheat were applied as follows: NS fertilizer (granular SSA: 21.0 kg N ha^{-1}, 24.0 kg S ha^{-1}) in March 2009 and ammonium nitrate urea solution two times in April (50.4 kg N ha^{-1} each) and once in May and once in June 2009 (39.5 kg N ha^{-1} each). During the growing season different herbicide combinations were applied twice on the maize plots (Mesurol liquid 0.2 l ha^{-1}, TMTD 98 % Satec 0.1 kg ha^{-1}), whereas the wheat plots received different herbicide combinations six times (22.10.2008: Arena C 0.41 l ha^{-1}; 03.04.2009: ARTUS 25.0 g ha^{-1}, Attribut 70.0 g ha^{-1}, CCC 720 1.0 l ha^{-1}, PRIMUS 50.0 ml 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 0.1 l ha^{-1}; 19.05.2009: Matador 300 0.6 l ha^{-1}, U 46 M-Fluid 1.3 l ha^{-1}; 05.06.2009: Bulldock 0.3 l ha^{-1}, Matador 300 0.5 l ha^{-1}, Taspa 0.3 l ha^{-1}, Primor Granulate 0.2 kg ha^{-1}).

In August 2009 wheat plants were harvested and the straw was removed from the wheat plots. After harvest of maize plants which were separated from corncobs and then chopped, four treatments were established in early November differing in the source of C4 C input: Corn Maize (CM), Fodder Maize (FM), Wheat + maize Litter (WL), and Wheat as a reference (W).

In the CM plots the C4-derived ^{13}C signal was introduced belowground through rhizodeposition during the growing period and the decomposition of dead roots after harvest, and aboveground through maize shoot litter addition after harvest. On the FM plots no maize shoot litter was applied, therefore, the C4 signal derived from roots and rhizodeposits. In the WL plots the C4 signal derived only from added maize shoot litter. The W plots did not receive any C4 plant input and served as reference plots. The cultivation of wheat on the W and WL plots was necessary to maintain habitat functions of the soil without changing its isotopic C signature. To establish the CM and WL treatment the maize shoot litter (0.8 kg dry weight m^{-2}, equivalent to 0.35 kg C m^{-2}) was applied to half of both the maize and wheat plots. Each treatment was replicated five times. Because one plot of every treatment was saved for ^{13}C and ^{15}N labeling experiments, 16 plots (4 replicates of each treatment) were sampled for this study.

In April 2010 all experimental plots were tilled with a chisel plough to a depth of 12 cm, and hybrid maize ("Fernandez", KWS Saat Ag, Einbeck, Germany) was sown at a density of 12.1 grains m^{-2} (25.6 kg ha^{-1}) with additional N (ammonium nitrate urea solution: 79.2 kg N ha^{-1}) and NP fertilizer (diammonium phosphate: 32.4 kg N ha^{-1}, 82.8 kg P ha^{-1}). To improve comparability of maize and wheat plots during the growing season of 2010, summer wheat ("Melon", Saaten-Union GmbH, Isernhagen, Germany) instead of winter wheat (2009) was sown at a density of 440 grains m^{-2} (224 kg ha^{-1}), and N fertilizer (ammonium nitrate urea solution) was added in April and June (61.3 kg N ha^{-1} and 39.5 kg N ha^{-1}, respectively). During the 2010 growing season two herbicide combinations were applied on the maize plots (14.04.2010: Mesurol liquid 0.2 l ha^{-1}, TMTD 98 % Satec 0.1 kg ha^{-1}; 05.06.2010: Click 1.0 l ha^{-1}, Milagro 0.5 l ha^{-1}, Peak 14.0 g ha^{-1}), and one herbicide combination on the wheat plots (29.04.2010: Biathlon 70.0 g ha^{-1}, MCPA Berghoff 1.3 l ha^{-1}). In early November 2010 maize (without corncobs) and wheat plants were harvested after which chaffed maize straw was applied to the respective plots (0.8 kg dry weight m^{-2}, equivalent to 0.35 kg C m^{-2}).

Determination of root biomass and rhizodeposition

Root biomass of maize and wheat was determined three times in 2009. At the maize plots samples were taken directly at the position of the maize plants, 12.5 and 25 cm away from the plants within rows, 20 and 40 cm away from the plants in inter-row locations, and 23.5 and 47 cm away from the plant at the diagonal between row and inter-row. At the wheat plots frames

(25 × 25 cm) were placed randomly within four of the wheat plots and soil samples were taken at 5 positions within each frame. A soil corer (Riverside auger, inner diameter 5 cm; Eijkelkamp, Giesbeek, The Netherlands) was used and samples were taken to a depth of 50 cm in 10 cm layers. All roots were washed free of soil, dried and weighed.

In addition to root biomass, the input of C through rhizodeposition was estimated for maize based on the rhizodeposition-to-root ratio determined under controlled conditions (J. Pausch, unpubl. data).

Plant carbon and $\delta^{13}C$

Wheat shoot material (summer 2010), maize leaves (senescent; litter) and maize roots (from respective soil sampling dates) were ground and about 3 mg was analysed by an elemental analyser (Euro EA 3000, EuroVector, Milan, Italy) coupled with an isotope ratio mass spectrometer (IRMS, Delta Plus XP, Thermo Finnigan MAT, Bremen, Germany) for estimation of C and N content and $\delta^{13}C$. Glutamic acid USGS-40 (IAEA, Vienna; $\delta^{13}C$ -26.39 ± 0.04 ‰) was used as reference material for calibration of CO_2 reference gas. Acetanilide (C_8H_9NO, Merck, Darmstadt) was used as a secondary laboratory reference material for internal calibration and determination of C and N content. $\delta^{13}C$ values are expressed relative to Vienna Pee Dee belemnite (V-PDB).

Soil sampling

Soil was sampled in summer shortly before plant flowering (highest exudation), autumn (shortly before maize harvest) and winter (highest translocation of mobile organic particles). Summer sampling was conducted in July 2009 and 2010, autumn sampling in September 2009 and 2010, and winter sampling in December 2009 and in January 2011 because of heavy snow in December 2010 (Fig. S6.2).

In each plot ten soil samples were taken to 70 cm depth with a soil corer in 10 cm layers randomly between two plants within a row. The samples from each layer were thoroughly mixed and homogenized by hand. Samples were transported in a cooling box to the laboratory and stored at 4°C until sieving (no longer than one week). After sieving (< 2 mm) and careful removal of plant particles, the soil was stored at -28 °C until analysis. Water content of

samples was determined gravimetrically after drying at 105 °C for 24 h. Here we focus on the top soil layer at 0-10 cm. All data presented are related to dry weight of soil.

Soil organic carbon and $\delta^{13}C$

For estimation of C_{org} and $\delta^{13}C$ about 3 g of soil was dried at 105 °C for 24 h and subsequently ground. Soil subsamples of 15 – 30 mg were measured with a coupled system consisting of an elemental analyser (NA 1500, Carlo Erba, Milan, Italy) and an isotope ratio mass spectrometer (MAT 251, Thermo Finnigan, Bremen, Germany).

Microbial biomass

C_{mic} was estimated by chloroform-fumigation-extraction (Vance et al. 1987). In brief, 10 g soil (fresh weight) of a homogeneous subsample of each plot was fumigated under vacuum with ethanol-free chloroform in a desiccator for 24 h. After removing the chloroform, samples were extracted by adding 40 ml of a 0.025 M K_2SO_4-solution (1:4 w/v soil / extractant ratio), shaken for 30 min at 250 rev min^{-1} on a horizontal shaker and centrifuged for 30 min at 4422 g. A second subsample of 10 g was treated similarly but without fumigation for the estimation of 0.025 M extractable organic C (EOC). Organic C in the supernatants was measured with a DOC / TN-analyser (Dimatoc 100, Dimatec, Essen, Germany). EOC content of the fumigated samples was subtracted from C content of the non-fumigated samples and resulted in extractable C_{mic}. For estimation of total C_{mic} a k_{ec} factor of 0.45 was used (Joergensen 1996).

$\delta^{13}C$ of microbial biomass

For analysis of $\delta^{13}C$ values of EOC and C_{mic}, 10 ml aliquots of the supernatants of both non-fumigated and fumigated samples were dried in a vacuum rotary evaporator (RVC 2-25, Martin Christ, Osterode am Harz, Germany) at 60 °C. The remnant was ground, weighed into tin capsules within a range of 7 – 30 mg (minimum of 10 µg C) per capsule (Marhan et al. 2010), and analyzed as described for the plant material.

To calculate the $\delta^{13}C$ of C_{mic} following equation was used:

$$\delta^{13}C_{mic} = (c_{nf} \times \delta_{nf} - c_f \times \delta_f) / (c_{nf} - c_f),$$

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

Ergosterol

Ergosterol was extracted using a modified method of Djajakirana et al. (1996). To 2 g soil (fresh weight) 25 ml ethanol was added and shaken at 250 rev min^{-1} on a horizontal shaker for 30 min. Extracts were then centrifuged for 30 min at 4422 g and 10 ml of supernatant per sample was dried at 50 °C in a vacuum rotary evaporator (Martin Christ, RVC 2-25, Osterode am Harz, Germany). To dissolve the extracts 1 ml methanol was added and samples were transferred into 2 ml brown glass HPLC vials with cellulose-acetate filters (0.45 µm; Sartorius Stedim Biotech GmbH, Göttingen, Germany). Ergosterol in samples was quantitatively determined by HPLC analysis (Beckmann Coulter, System Gold 125, Fullerton, USA) using a 250 × 4.6 mm Spherisorb ODS II 5 µm column 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 Gold 166 UV-detector, Fullerton, USA). For calibration pure ergosterol (Sigma-Aldrich, St. Louis, USA) was dissolved in methanol and diluted to give final concentrations of 0.1, 0.2, 0.5, 1 and 2 µg ergosterol ml^{-1}.

^{13}C ergosterol

For extraction of ergosterol for $\delta^{13}C$ determination, 11 - 15 g soil (fresh weight) was mixed with 170 ml ethanol and 5 g NaOH, homogenised in an ultrasonic bath and saponified (30 min at 80 °C). After cooling, samples were filtered through folded filters (Ø 15 cm; Macherey-Nagel, Oensingen, Switzerland). Filtered extracts were mixed with 100 ml of deionised H_2O and 80 ml of petroleum ether and shaken for 1 min, separating into two phases. The upper phase was saved and the lower phase was mixed again with 50 ml petroleum ether and shaken for another 1 min. Both resulting upper phases were pooled and 20 µl of ethylene glycol was added. Samples were evaporated to near dryness in a rotary evaporator at 300 – 500 mbar at 40°C. Residues were re-dissolved in 2 ml of methanol / water (95/5; v/v), transferred to Eppendorf vials and centrifuged for 2 min at 12,000 g. Supernatants were transferred into brown glass HPLC-vials.

Concentration and cleaning of the extracts was performed with a Varian preparative HPLC with a Varian Pro-Star 210 pump and 701 Fraction Collector equipped with a Grohm

Nucleosil 120 C4 (250 × 16 mm) column. The eluent was methanol / water (95/5; v/v) with a flow rate of 6 ml min^{-1}. One ml of each sample was injected and the ergosterol fraction was collected. Retention time was monitored using the Galaxy Chromatography Data System (Version 1.7.4.5) software. The fraction was than evaporated under nitrogen at 60 °C, dissolved in 70 µl isooctane and transferred to a brown glass vial. A GC-C-IRMS system was used to determine the isotopic C composition of ergosterol. The system consists of a gas chromatograph (6890 series, Agilent Technologies, USA) coupled via a gas chromatography-combustion III Interface (Thermo Finnigan, Waltham, USA) to a Delta Plus XP mass spectrometer (Thermo Finnigan MAT, Bremen, Germany). An Rtx-5 (30 m × 0.25 mm, film thickness of 0.25 µm) column with helium as the carrier gas (flow rate of 1.5 ml min^{-1}) was used. The combustion reactor had a temperature of 940 °C and the reduction reactor 640 °C. The GC program was set as follows: initial temperature was 160 °C and held for 1 min, temperature was increased to 270 °C at a starting 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 and the samples were measured in a splitless mode.

Each sample was measured at least two times. For internal calibration and to check fractionation during separation of ergosterol with preparative HPLC, $\delta^{13}C$ of ergosterol standards (50 µg ml^{-1}) (ACROS Organics, Geel, Belgium, purity: 98 %) was also determined with every series of measurement. No ^{13}C isotopic fractionation could be detected as a result of cutting the ergosterol with the preparative HPLC (-10.11 ± 0.08 ‰ vs. -10.05 ± 0.14 ‰, ergosterol standards unprocessed and processed with preparative HPLC, respectively).

Calculation of maize-derived C

For calculation of the relative amounts of maize C in C_{org}, EOC, C_{mic}, and ergosterol the following mixing model was used:

$$\%C\text{-maize} = (\delta_{sample} - \delta_{reference}) / (\delta_{maize} - \delta_{wheat}),$$

where δ_{sample} is the $\delta^{13}C$ value of the respective sample, and $\delta_{reference}$ is the $\delta^{13}C$ mean value of a sample from the reference plots (with wheat crop alone). 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 because there were only small differences in $\delta^{13}C$ values between maize litter (2009: -13.04 ± 0.04 ‰; 2010: -13.34 ± 0.13 ‰) and maize roots (-12.82 ± 0.09 ‰), as well as over time. To assess the relative amount of maize C in EOC, C_{mic} and ergosterol, the average

$\delta^{13}C_{org}$ value (δ_{wheat}) over two years of the reference plots was used (-26.99 ± 0.03 ‰). For calculation of relative amounts of maize C in the C_{org} for δ_{wheat} the $\delta^{13}C$ value of the wheat plants was used (-28.31 ± 0.16 ‰). $\delta^{13}C$ mean values and standard errors of all samples in the different pools are given in Table S6.1.

Absolute amounts of maize-derived C were calculated by multiplying relative amounts of maize-derived C by C content of the respective samples.

Statistical analyses

Treatment and date effects on C_{org}, EOC, C_{mic} and ergosterol as well as on the amounts of maize-derived C in these pools were analysed by ANOVA with sampling dates as repeated factors. In addition separate ANOVAs with post hoc tests (Fischer LSD) for comparison 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.

Results

Root biomass and C_{org}

In July 2009 wheat root biomass was 0.47 ± 0.17 mg C g^{-1} soil, while maize root biomass (without crown roots) was only 0.15 ± 0.04 mg C g^{-1} soil. Before this sampling calculated rhizodeposit-derived C had been 0.37 ± 0.10 mg C g^{-1} soil.

During the sampling period C_{org} content of soils ranged from 10.59 to 13.74 mg g^{-1} soil (Fig. 6.1a). C_{org} was significantly affected by treatment only at the last sampling date in winter 2010/2011 (date × treatment effect: $F_{15,60}$ = 2.34, P = 0.011). For this date C_{org} was significantly different between treatments ($F_{3,12}$ = 9.82, P = 0.001), with 23 % lower values in the FM in comparison to the WL treatment. Maize-derived C was detected in the C_{org} of the FM and CM plots starting with the first sampling in summer 2009 and the maize signal in samples of the WL plots were detected one month after litter amendment in winter 2009/2010 (Fig. 6.1a, b). During the vegetation period 2010, the highest amount of maize-derived C was present in the CM plots, unlike both the FM and the WL plots, which showed similar amounts of maize-derived C. Maize-derived C_{org} increased continuously in each of the treatments

during the two years (Fig. 6.1a, b). After two years the amount of maize-derived C in C_{org} was 7.28, 7.50 and 14.12 % in the FM, WL and CM plots, respectively (Fig. 6.1b).

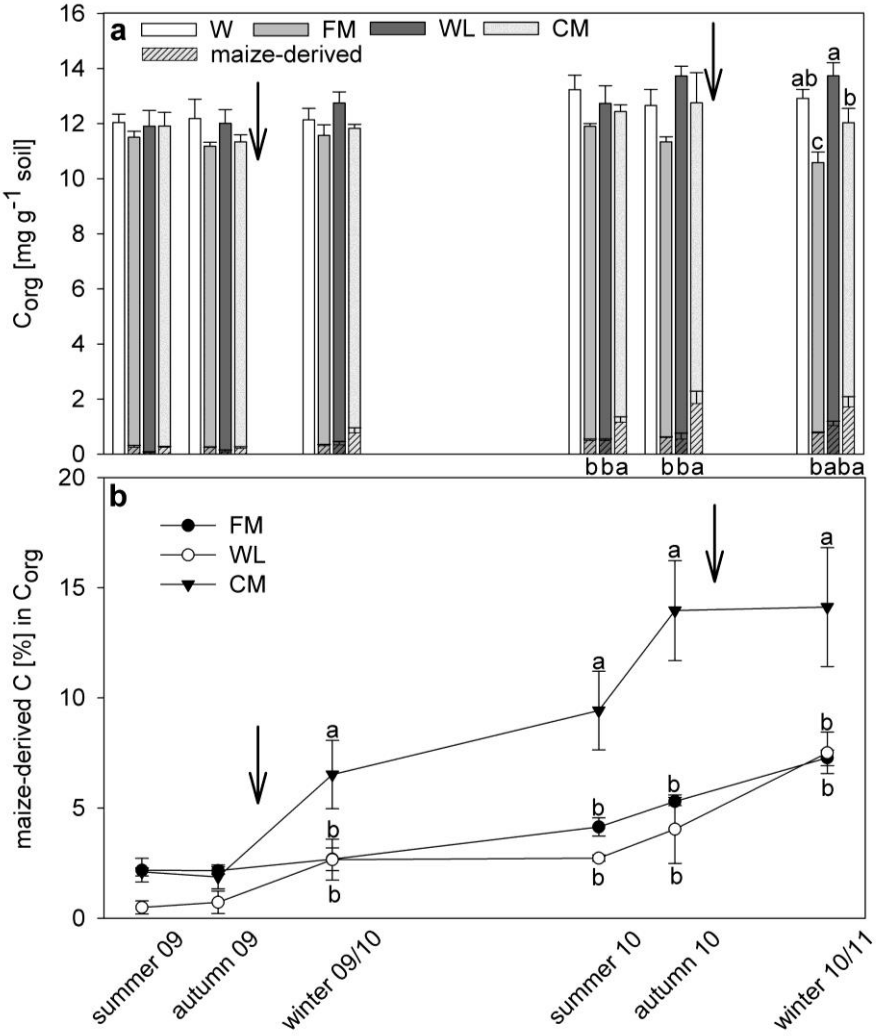

Figure 6.1 Means and standard errors of C_{org} and absolute amounts of maize-derived C (a) and relative amounts of maize-derived C (b) in the different treatments Wheat (W), Fodder Maize (FM), Wheat + maize Litter (WL) and Corn Maize (CM) at respective soil sampling dates . Different letters indicate statistically significant differences between treatments at this date (Fisher LSD: $P < 0.05$). In (a) letters above bars specify significance for total C_{org} and letters below bars for absolute amounts of maize-derived C in this pool. Arrows indicate litter application.

Extractable organic carbon

During the sampling period EOC content varied between 10.99 and 34.52 µg C g^{-1} soil (Fig. 6.2a). Effects of treatment on EOC differed over the sampling period (date × treatment effect: $F_{15,60} = 4.21$; $P < 0.001$). Although similar amounts of EOC were detected in all of the treatments in summer and autumn 2009, EOC content in soils was significantly higher in the WL than in the FM plots during the following soil sampling dates. In 2010, EOC content

tended to be higher in the wheat plots (WL and W), but the difference was only significant in autumn 2010 ($F_{3,12}$ = 15.41; P < 0.001). In winter 2010/2011 EOC content in the FM plots was significantly different ($F_{3,12}$ = 6.86; P = 0.006) between treatments, with about 50 % lower contents in the FM than in the other treatments.

Maize-derived C in EOC was first detected in the FM and CM plots in autumn 2009 (Fig. 6.2a, b). In winter 2009/2010, one month after addition of maize litter, maize-derived C was also detected in the WL plots. After summer 2010 the relative amount of maize-derived C was higher in the CM plots but no significant differences could be detected between the FM and WL plots. This was reflected at the end of this study by 12.88, 13.94 and 24.67 % maize C incorporation into EOC in the FM, WL and CM plots, respectively.

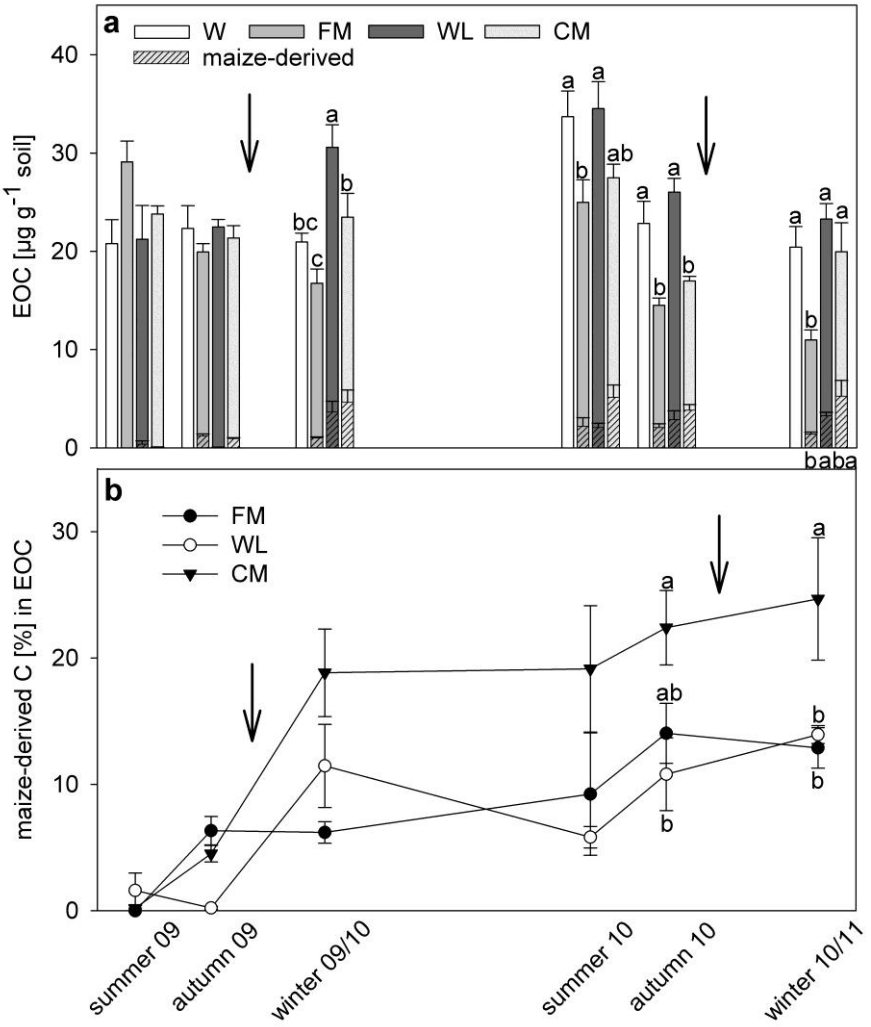

Figure 6.2 Means and standard errors of total extractable organic C (EOC) and absolute amounts of maize-derived C (a) and relative amounts of maize-derived C (b) in the different treatments at respective soil sampling dates. Different letters indicate statistically significant differences between treatments at this date (Fisher LSD: P < 0.05). Arrows indicate litter application; for legend see Fig. 6.1.

Microbial biomass C

Microbial biomass C ranged from 120.7 to 222.8 µg g^{-1} soil (Fig. 6.3a). The date of sampling significantly affected C_{mic} ($F_{5,60}$ = 7.41, P < 0.001) but the variations did not show a clear seasonal trend. In autumn 2010 C_{mic} in the FM plots was significantly lower than in WL and W plots ($F_{3,12}$ = 3.59, P = 0.046). In winter 2010/2011 C_{mic} significantly differed between treatments ($F_{3,12}$ = 4.30, P = 0.028) and was 34 % lower in the FM than in the WL plots (Fig. 6.3a).

Maize C derived from rhizodeposition was detected in C_{mic} in the FM and CM plots at the first sampling in summer 2009 (Fig. 6.3a, b). In winter 2009/2010, maize litter-derived C was also detected in C_{mic} of the WL plots. Subsequently, the amounts of maize-derived C in FM and WL plots were similar, while in the CM plots they were about twice as high (Fig. 6.3a, b). The maize-derived signal in the C_{mic} increased continuously during the two years resulting in 25.1, 25.6 and 46.6 % maize C in C_{mic} in the FM, WL and CM plots at the end of the two year period, respectively.

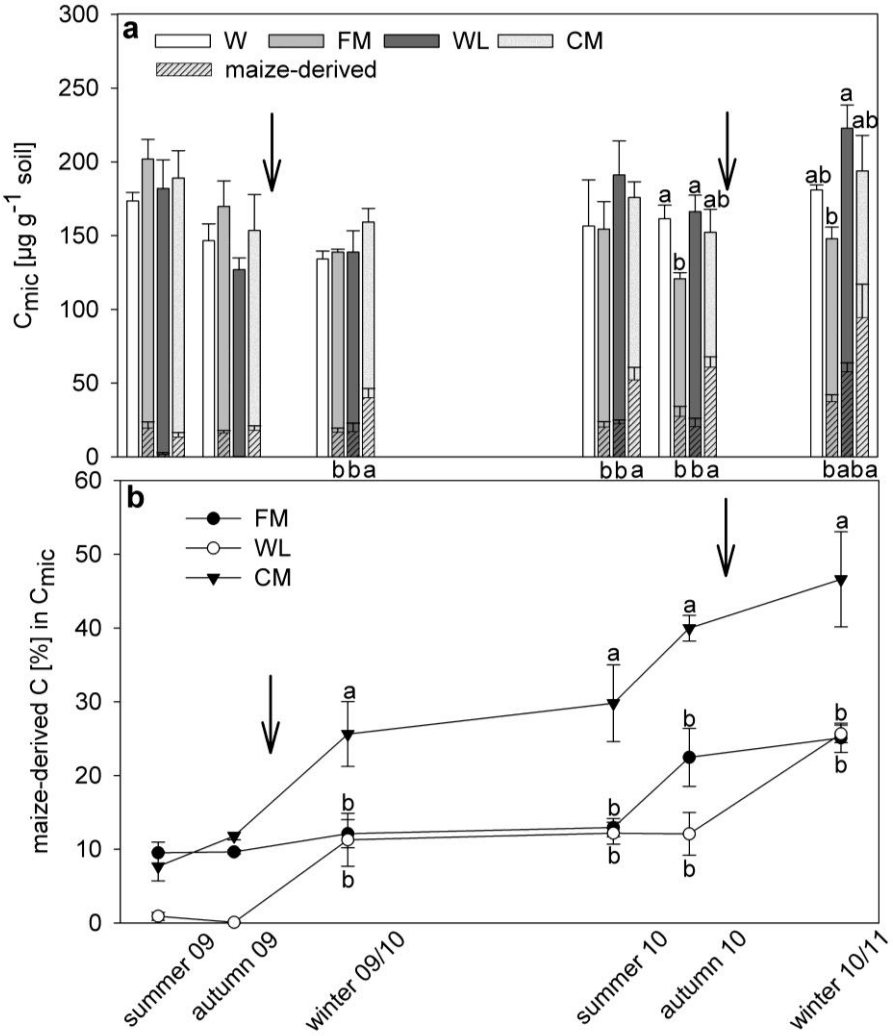

Figure 6.3 Means and standard errors of total microbial biomass (C_{mic}) and absolute amounts of maize-derived C (a) and relative amounts of maize-derived C (b) in the different treatments at respective soil sampling dates. Different letters indicate statistically significant differences between treatments at this date (Fisher LSD: $P < 0.05$). In (a) letters above bars specify significance for total C_{mic} and letters below bars for absolute amounts of maize-derived C in this pool. Arrows indicate litter application; for legend see Fig. 6.1.

Ergosterol C

Amounts of ergosterol C ranged from 0.53 to 2.23 µg C g^{-1} soil. In both winter 2009/2010 and 2010/2011 amounts of ergosterol C tended to be higher in the WL and CM plots than in the W and FM plots (Fig. 6.4a). During the vegetation period 2010, ergosterol C tended to be higher in the wheat (WL and W) plots compared to the maize plots but significant differences between treatments were only found in autumn 2010 ($F_{3,10} = 7.87$, $P = 0.005$).

In summer 2009, maize C in ergosterol was detected in both FM and CM plots, and in winter 2009, one month after litter addition, it also appeared in the WL plots (Fig. 6.4a, b). During

the growing seasons in both WL and CM plots, the amount of maize C tended to be highest at the winter samplings. Over the whole sampling period no significant differences in the relative amounts of maize-derived C could be detected between the FM and WL plots. After two years the amount of maize C in ergosterol accounted for 35.6, 39.5 and 76.2 % in the FM, WL and CM plots, respectively.

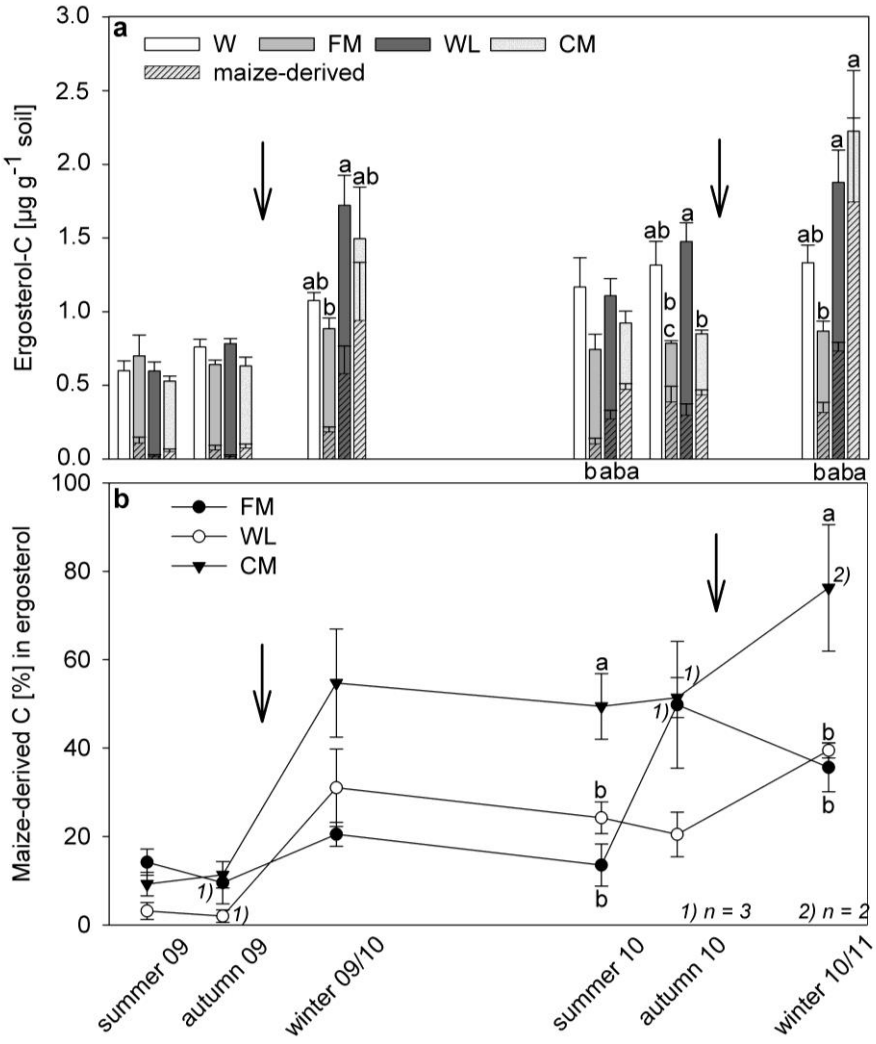

Figure 6.4 Means and standard errors of total ergosterol C and absolute amounts of maize-derived C (a) and relative amounts of maize-derived C (b) in the different treatments at respective soil sampling dates. Different letters indicate statistically significant differences between treatments at this date (Fisher LSD: $P < 0.05$). In (a) letters above bars specify significance for total ergosterol C and letters below bars for absolute amounts of maize-derived C in this pool. Arrows indicate litter application. Note numbers on some data points in (b) which also apply to (a); for legend see Fig. 6.1.

Discussion

The origin and quantity of plant inputs to soil are primary factors controlling the size and structure of the soil microbial community. The present study aimed to elucidate and quantify the C flow from both root and shoot litter residues into soil organic, extractable, microbial and fungal C pools. Our experimental approach allowed separating the flux of root-derived from shoot litter-derived C by switching from C3 (wheat) to C4 (maize) crops and by the addition of C4 shoot litter to plots with C3 plants.

Plant effects

Wheat was grown on the W and WL plots to maintain habitat functions of the soil without changing its isotopic C signature. However, the type of crop influenced the total amounts of C_{org}, EOC, C_{mic} and ergosterol, with typically greater amounts in treatments with wheat plants (W vs. FM and WL vs. CM treatment). The effect of wheat was generally more pronounced in 2010 than in 2009. In July 2009 wheat root biomass was 0.47 mg C g^{-1} soil, while maize root biomass (excluding crown roots) was only 0.15 mg C g^{-1} soil. Therefore, we suggest that higher substrate (rhizodeposit) availability in treatments with wheat plants was responsible for greater amounts of C in the organic C, microbial, and fungal pools. Furthermore, we cannot exclude the possibility that microbial diversity differences in the wheat and maize plots (WL and CM) result in assimilation of substrates by varying species. However, nearly additive amounts of maize-derived C in FM and WL to CM plots indicate that assimilated C quantities were independent of maize or wheat growing plots and the respective microorganisms present.

The wheat-derived C input, with its depleted $\delta^{13}C$ signature compared to maize, probably led to an underestimation of maize C incorporation into the different pools of the WL treatment. The increase in the EOC pool in the WL (and W) treatment during summer 2010, when the plots were planted with wheat, indicates that a certain amount of EOC was derived from the rhizodeposition of wheat. In contrast, the amount of maize C in the EOC pool of the WL treatment decreased. The wheat-derived C presumably was mineralized quickly as the total amount of EOC decreased but the maize-derived C in the EOC pool increased until the following soil sampling in autumn 2010. Similar increases in maize-derived C in almost all pools in the WL and CM treatments after litter addition between the autumn and winter

samplings further indicate that the contribution of wheat root material to the $\delta^{13}C$ signal of the different pools was negligible in the WL treatment.

Total C input

In the first year of the experiment (2009), total root-derived C input of maize was 0.52 mg C g^{-1} soil (0.37 mg C g^{-1} of total rhizodeposits until July 2009 plus 0.15 mg C g^{-1} root biomass in July 2009). In spring 2010 the maize crown roots (aboveground root material) were incorporated into the soil by chisel plough tillage, contributing to an additional input of 8.0 mg C g^{-1} into soil. Taking the incorporation of the crown root material into account, over the study period the total C input by roots was almost two times higher (9.04 mg C g^{-1} soil) than the C input derived from litter (5.08 mg C g^{-1} soil). A decomposition experiment with litter-bags in the field showed that after one year only 42 % of maize root material (without crown roots), but up to 88 % of maize shoot litter material was decomposed (N. Scheunemann, unpubl. data) indicating faster decomposition of shoot than root litter. Presumably, after incorporation into soil, the decomposition of the crown root material was further slowed down in comparison to the belowground root biomass due to its more compact and solid structure. We assume therefore that access and utilization of crown root material by decomposers was strongly limited and that the contribution of this material to the investigated C pools as a source for C was negligible. With the assumption that the root biomass and rhizodeposition were equal in both years (although maize varieties changed) root C input in the maize plots (without the crown root material) was only 1.04 mg C g^{-1} soil during the study period, which is only one fifth of the maize shoot litter input and similar to the root biomass C (without rhizodeposition) input in the wheat plots (see above). Indeed, total quantities of C in all pools of the FM treatment were slightly lower as compared to the other treatments in the second year of the study.

Absolute incorporation of maize C into different soil C pools

The pool of C_{org} is assumed to be stable with long turnover times (Flessa et al. 2008, von Lützow et al. 2008). However, maize-derived C in C_{org} was detected almost immediately, beginning with the first growing period. Balesdent and Balabane (1992) also found the $\delta^{13}C$ values of different soil particle size fractions to differ significantly in the first year after replacing C3 by C4 plants.

The total amount of C_{org} varied little over time; it decreased only slightly in the FM treatment and increased slightly in the WL treatment with significantly lower amounts in the FM treatment only in winter 2010/2011. However, the concentration of maize-derived C increased with time in each of the treatments indicating that the decrease in C_{org} in the FM treatment was due to the mineralization of C3-derived C. Clapp et al. (2000) investigated the SOC of maize cultivated fields over 13 years and showed that the SOC amount declined in treatments where aboveground plant parts were removed from the soil, which is comparable to the FM treatment in the present study.

The EOC pool represents a more mobile fraction of soil organic C than C_{org} and is assumed to be an important C source for the soil microbial community (Marschner et al. 2002). In the present study no maize-derived C from rhizodeposition was detected in the EOC pool at the first sampling date in summer 2009, three months after seeding of maize, but it was detected in C_{mic} and C_{org}. The minor contribution of maize exudates to the EOC pool in the bulk soil presumably was due to the fact that bulk soil was sampled 25 cm away from the maize plants and not directly in the rhizosphere. Marx et al. (2007) found only low amounts of C derived from rhizodeposits in the rhizosphere of maize and wheat plants, while in bulk soil rhizodeposit C was present in C_{mic} and CO_2. Relative amounts of water soluble C were shown to decrease with increasing distance to wheat roots (Merbach et al. 1999). Hütsch et al. (2002) concluded that extractable organic compounds derived from rhizodeposits were assimilated immediately by microorganisms and/or stabilized in C_{org}.

Root-derived C also was already detected in fungal biomass (ergosterol) at the first sampling in summer 2009. Since only limited amounts of ergosterol were found in Glomeromycota (see review of Weete et al. 2010), we assume minor contribution of AM fungi-derived C in the ergosterol fraction in our experiment. Only low AM colonisation of maize roots in the same field experiment during two sampling times in 2009 were detected (J. Moll, pers. communication). Therefore, we conclude that mainly saprotrophic fungi might have incorporated maize-derived C from rhizodeposits (and shoot litter) into the ergosterol fraction.

Generally, fungi can play an important role in C cycling in bulk soil because their hyphae can grow in the direction of the substrate source or from substrate into the bulk soil (Frey et al. 2003; Butenschoen et al. 2007). Esperschütz et al. (2009) found that fungi are involved in transporting ^{13}C compounds from labelled rhizodeposits into bulk soil. Soon after labelling the fungal PLFA 18:2ω6,9 was highly enriched in the rhizosphere, later it was also enriched in bulk soil. The translocation of the assimilated substrates within the hyphal network is an

advantage fungi have over bacteria which depend more on external transport processes, such as the flux of water through soil (Poll et al. 2006).

In contrast to root-derived C, litter-derived C was detected in the EOC pool at the first sampling one month after litter addition (winter 2009/2010). Even distribution of litter on the plots presumably fostered direct leaching of water soluble C from the litter into the underlying bulk soil. Diffusion of soluble C and advective transport from labelled rye litter into the soil were also found by Poll et al. (2008). A similar quick uptake of maize litter C was also detected in C_{mic} and ergosterol in winter 2009/2010. While total C_{mic} generally did not respond to litter addition, fungal biomass increased in winter in the litter addition treatments. Increased fungal biomass by the addition of maize litter has been shown previously (Helfrich et al. 2008; Potthoff et al. 2008; Rottmann et al. 2010), suggesting that fungi in bulk soil benefit from aboveground litter resources in arable systems.

Relative contribution of root- and litter-derived C to the different C pools

After two vegetation periods the proportion of maize-derived C increased in the order C_{org} < EOC < C_{mic} < ergosterol. With the exception of ergosterol, which has not been investigated before, this is in accordance with previous studies (Gregorich et al. 2000; Liang et al. 2002). For example, Gregorich et al. (2000) simulated changes in $\delta^{13}C$ values in different soil pools which had been under maize monoculture from 4 to 37 years. They calculated exponential enrichment in the first two years with higher enrichments in C_{mic} than in water soluble C, and very slow enrichment in humus C. Liang et al. (2002) found in a 110 day greenhouse study with maize 11.5, 23.3 and 48.0 % maize-derived C in SOC, water soluble organic C (WSOC) and C_{mic}, respectively. Due to higher proportions of maize-derived C in WSOC and C_{mic} they concluded that recent plant C was more bioavailable than the older soil-derived soluble C pool.

After two vegetation periods, the relative incorporation of maize-derived C was at a maximum in fungal biomass (ergosterol) with up to 76.2 % maize C in ergosterol in the CM treatment. Similarly, the fungal biomarker PLFA, 18:2ω6,9, was highly enriched in comparison to bacterial PLFAs due to the addition of litter or by rhizodeposition (Butler et al. 2003; Jin and Evans 2010; Paterson et al. 2008; Rubino et al. 2009). Flessa et al. (2008) calculated from the data of Kramer and Gleixner (2006) that after 23 years of maize cultivation 93.8 % of the C in the fungal biomarker PLFA 18:2ω6,9 derived from the C4

plant. In current concepts of food webs, the fungal energy channel, which is favoured by recalcitrant organic materials and high C/N ratio in soils, is considered a slow cycle (Scheu et al. 2005; Joergensen and Wichern 2008). Fungi are thought to use organic substrates more efficiently than bacteria (Sakamoto and Oba 1994) and to be more resistant to mortality factors as compared to bacteria (Guggenberger et al. 1999). In contrast, readily decomposable substrates are favoured by the bacterial energy channel which is characterized by rapid growth, turnover of C, and fast cycling of nutrients (Holtkamp et al. 2008; Ingwersen et al. 2008)

In spite of different C input quantities of the resources (root vs. litter) similar relative amounts of maize-derived C had been incorporated in each of the four soil C pools by the end of both vegetation periods (winter) with the effects of root and litter input being additive. The similar relative incorporation or assimilation of maize C in the treatments with only maize root or shoot litter is noteworthy because the total size of the different pools varied between treatments (with mostly lower total amounts in the FM plots; see above).

Generally, a large fraction of root-derived C is assumed to be stable in soil and hence has longer turnover time in comparison to shoot-derived C (Rasse et al. 2005). Balesdent and Balabane (1996) found more root-derived than shoot-derived maize C to be stable in different soil fractions four years after changing from C3 to C4 plants. They assumed high production but slow decomposition of belowground C compounds. Higher stability of root-derived C was also found by Puget and Drinkwater (2001). After one growing season of labelled hairy vetch (*Vicia villosa* Roth subsp. *villosa*) 48.8 % of root-derived C and 13.2 % of litter-derived C in C_{org} were retrieved, while almost equal relative amounts of root C (4.1 %) and shoot C (6.5 %) were recovered in C_{mic}. They concluded that even though the amount of shoot C input into soil was threefold higher than that of root C, relatively more root-derived C was assimilated by C_{mic}. Williams et al. (2006a) detected similar proportions of ryegrass root- and straw-derived C in the fungal biomarker PLFA 18:2w6,9 two months after mixing ryegrass and crimson clover litter into the soil, although input of straw litter was 4.5 fold greater than the root biomass.

In our experiment root-derived input of C was lower than the shoot-derived input but similar relative amounts of root- and shoot-derived C were recovered in the C_{org}, EOC, C_{mic} and fungal (ergosterol) pools. Data from the present study underline the results of the above mentioned studies in which typically more root- than shoot-litter derived C is stabilized in the soil organic C pools and assimilated in the total soil microbial and the fungal biomass.

Our results indicate that not only for soil food webs in forests (Pollierer et al. 2007) but also for the basis of food webs in agricultural ecosystems belowground C input via roots is of great importance.

Conclusions

We presented data on the incorporation of root and shoot litter C into organic and microbial C pools under field conditions over a period of two years, providing a basis for future modelling of C transfer through the belowground food web. Notably, similar amounts of C derived from the two resources differing in substrate quality and amount were incorporated into the C_{org}, EOC, C_{mic} and ergosterol pools over time, indicating the importance of root-derived C for the soil food web. High incorporation of maize C (up to 76.2 %) into ergosterol suggests fast and high assimilation of maize C into fungal biomass, with major implications for the flux of C through the bacterial and fungal energy channels of arable systems.

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Supplementary material

Fig. S6.1 $\delta^{13}C$ signature of C_{org} (top) and C_{org} amount (bottom) of the Ah horizon at the field site. Solid lines mark the experimental plots with treatment alignment, Wheat (W), Fodder Maize (FM), Wheat + maize Litter (WL) and Corn Maize (CM).

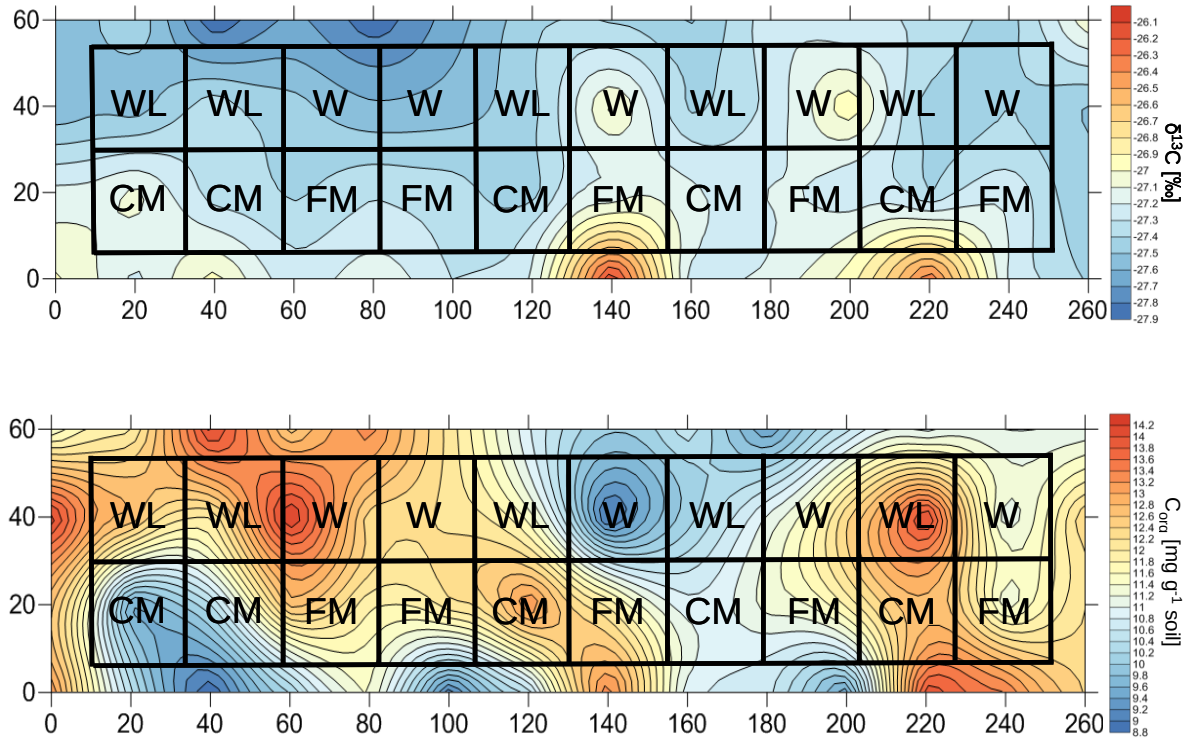

Fig. S6.2 Daily means of air temperature (°C) and precipitation (mm) over the study period in Göttingen. Arrows indicate date of soil samplings. Data were provided from the Wetterstation Göttingen in Hardegsen (Lower-Saxony) 8 km from the field site.

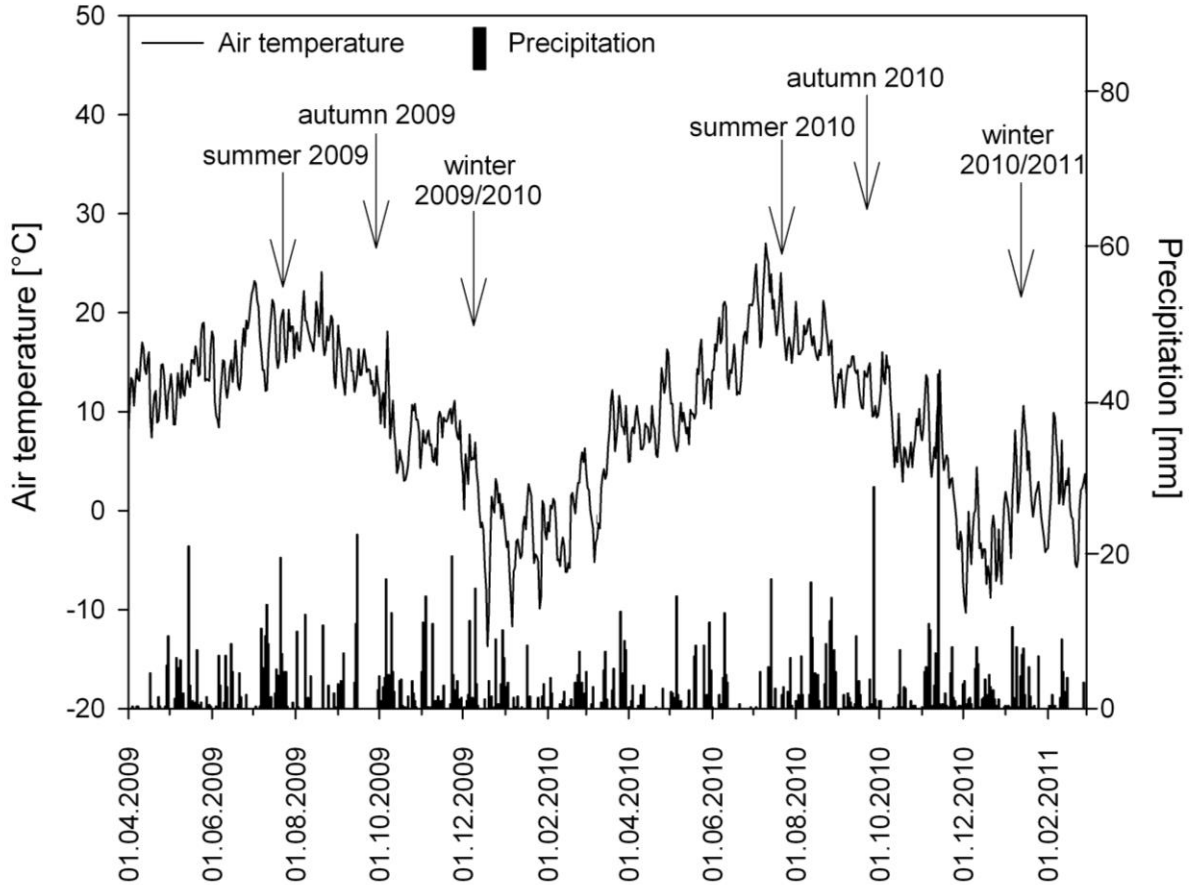

Table S6.1. Means and standard errors (in parentheses) of $\delta^{13}C$ values of different C pools in the four treatments Wheat (W), Fodder Maize (FM), Wheat + maize Litter (WL) and Corn Maize (CM) at six consecutive sampling dates.

Sampling	Treatment	C_{org}	EOC	C_{mic}	Ergosterol C
Summer 2009	W	-27.04 (0.03)	-26.69 (0.09)	-25.86 (0.27)	-27.44 (0.51)
	FM	-26.71 (0.08)	-27.20 (0.12)	-24.53 (0.20)	-25.45 (0.41)
	WL	-27.06 (0.10)	-26.90 (0.41)	-26.21 (0.36)	-27.09 (0.32)
	CM	-26.72 (0.03)	-27.19 (0.20)	-24.79 (0.27)	-26.15 (0.37)
Autumn 2009	W	-26.99 (0.11)	-27.57 (0.07)	-26.10 (0.06)	-27.51 (0.10)
	FM	-26.66 (0.03)	-26.69 (0.16)	-24.75 (0.04)	-26.17 (0.67)
	WL	-26.90 (0.09)	-27.55 (0.02)	-26.25 (0.12)	-27.50 (0.43)
	CM	-26.71 (0.08)	-26.94 (0.09)	-24.45 (0.07)	-25.92 (0.42)
Winter 2009	W	-26.98 (0.03)	-27.27 (0.04)	-25.77 (0.12)	-28.51 (0.19)
	FM	-26.57 (0.08)	-26.41 (0.12)	-24.00 (0.27)	-25.64 (0.38)
	WL	-26.59 (0.16)	-25.67 (0.46)	-24.20 (0.50)	-24.17 (1.23)
	CM	-25.98 (0.24)	-24.64 (0.48)	-22.19 (0.61)	-20.86 (1.71)
Summer 2010	W	-27.10 (0.10)	-27.50 (0.07)	-25.28 (0.29)	-28.24 (0.20)
	FM	-26.46 (0.06)	-26.26 (0.48)	-23.47 (0.17)	-26.67 (0.98)
	WL	-26.68 (0.02)	-26.68 (0.12)	-23.57 (0.21)	-24.85 (0.50)
	CM	-25.65 (0.27)	-24.82 (0.70)	-21.11 (0.73)	-21.33 (1.04)
Autumn 2010	W	-26.98 (0.02)	-27.27 (0.05)	-26.14 (0.12)	-27.71 (0.42)
	FM	-26.17 (0.03)	-25.30 (0.33)	-23.00 (0.64)	-20.73 (2.01)
	WL	-26.36 (0.24)	-25.76 (0.40)	-24.45 (0.40)	-24.84 (0.71)
	CM	-24.85 (0.35)	-24.14 (0.41)	-20.55 (0.24)	-20.51 (0.63)
Winter 2010	W	-26.86 (0.04)	-27.30 (0.14)	-25.97 (0.11)	-27.16 (0.25)
	FM	-25.75 (0.05)	-25.50 (0.22)	-22.46 (0.28)	-22.17 (0.67)
	WL	-25.72 (0.14)	-25.35 (0.10)	-22.38 (0.17)	-21.64 (0.23)
	CM	-24.70 (0.41)	-23.85 (0.68)	-19.45 (0.90)	-16.49 (1.61)

7 Eat all you can – Resource partitioning between bacteria, fungi and protists in the detritusphere of an arable soil

Submitted to the ISME journal

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Abstract

The mineralization and flow of plant-derived carbon in soil is relevant to global carbon cycling. Current models of organismic carbon fluxes in soil assume separate bacterial and fungal energy channels in the detritusphere, depending on substrate complexity and recalcitrance. Still, precise details on the most relevant microbiota involved, as well as on resource partitioning, interactions and competition between them are largely lacking. Here, a microcosm experiment was performed to trace the mineralization and assimilation of four ^{13}C-labeled detritusphere substrates (glucose, cellulose, maize leaves and roots) in an agricultural soil. Key label-assimilating bacteria, fungi and protists were identified by rRNA stable isotope probing and pyrotag sequencing. The different substrates were consumed by only a few key players within the three investigated kingdoms. Distinct lineages within the *Actinobacteria*, *Bacteroidetes* and *Gammaproteobacteria* were the main bacterial decomposers. For fungi, basidiomycetous yeasts degraded labile and ascomycetes the more recalcitrant substrates. Specific protists (*Pythium* spp.) were also highly active already in early stages of substrate decomposition. Thus, bacteria, fungi and protists were identified as primary consumers of all substrates, irrespective of complexity or recalcitrance. Secondary trophic activity was more apparent for amoeboid protozoa than for flagellates, and was observed also for bacterivorous as well as fungivorous protozoa and bacterial micropredators. Only for detritusphere bacteria, consumer diversity increased with substrate complexity. Members of all investigated kingdoms simultaneously consumed available detritusphere substrates, irrespective of resource quality. Thus, separate energy channels were not apparent in this agricultural soil, which advances current modeling concepts for litter decomposition in soil.

Introduction

Microbes fulfill crucial functions as primary decomposers of organic material such as plant litter in soil (Berg and McClaugherty 2008, Schmidt et al 2011). Bacteria have been classically understood to be mainly involved in the degradation of labile organic matter, being more active in early phases of decomposition (‘bacterial energy channel’). In contrast, fungi were assumed to be more involved in the degradation of recalcitrant compounds and to dominate in later stages of decomposition (‘fungal energy channel’, de Boer et al 2005, Moore and William Hunt 1988, Paterson et al 2008). However, more recent work has suggested a significant role of bacteria also in the mineralization of recalcitrant substrates, and of fungi in early stages of labile plant litter decomposition (Bastian et al 2009, España et al 2011, Poll et al 2010). This has fuelled a still ongoing debate on the specific functions of distinct soil microbiota in different phases of litter degradation (Strickland and Rousk 2010).

Soil microbes are known to undergo a succession during the degradation of plant residues (Bastian et al 2009, Poll et al 2010, Voriskova and Baldrian 2013). Furthermore, the diversity of soil microbiota is positively influenced by substrate complexity and has been shown to increase during decomposition (Mula-Michel and Williams 2012). Thus, resource partitioning between microbes consuming more complex substrates may be an important driver of the diversity of soil microbial communities (Zhou et al 2002). Yet to date, the role of these important mechanisms in controlling key microbial populations in the detritusphere across microbial kingdoms has not been addressed.

Higher trophic levels also significantly influence the primary degraders of organic matter. The bacterial energy channel is understood to be subject to top-down control by protozoan grazers, the next relevant trophic level of soil food-webs (De Ruiter et al 1996, Ekelund and Ronn 1994). Fungal hyphae and yeast cells as well as complex organic matter can also be consumed by protozoa (Adl and Gupta 2006, Hess et al 2012). Substrate-dependent successions of prey organisms can also be expected to affect the succession of protozoan grazers. However, the larger group of protists is also known to harbor primary detritus decomposers (Termorshuizen and Jeger 2008). Thus, although a general understanding of the ecology of protists exists for the detritusphere, a comprehensive grasp of their trophic strategies, interactions and competition for resources with other microbes remains to be elaborated (Tixier et al 2013).

Modern isotopic labeling strategies such as nucleic acid-based stable isotope probing (SIP) can be seminal for the tracing of substrate-derived carbon flows and the identification of key

microorganisms in the soil detritusphere (Stursova et al 2012). While most respective studies have been focused on bacterial consumers of defined detritusphere substrates (e.g. (Haichar et al 2007, Padmanabhan et al 2003, Schellenberger et al 2010), a number of SIP studies has also addressed the use of complex substrates over different kingdoms and/or trophic levels simultaneously (Bernard et al 2007, Drigo et al 2010, Eichorst and Kuske 2012, Lueders et al 2006, Stursova et al 2012, Vandenkoornhuyse et al 2007). Still, a comprehensive tracing of the turnover of a range of ^{13}C-labeled plant-derived substrates of distinct quality over all most relevant microbial groups in a given soil has not been reported to date.

Here, a SIP microcosms experiment was conducted with a well-investigated arable soil from an experimental maize field (Dibbern et al 2014, Kramer et al 2012, Pausch et al 2013, Scharroba et al 2013). Treatments included the amendment of ^{13}C-labeled glucose and cellulose as representative components of plant biomass, as well as maize leaves and roots as composite substrates. We traced key label-assimilating bacteria, fungi and protists by rRNA-SIP at an early and a later stage of decomposition. rRNA-SIP was combined with pyrotag sequencing, a strategy yielding superior insights into the diversity of labeled taxa (Pilloni et al 2012, Stursova et al 2012). We hypothesized that i) the complexity and recalcitrance of substrates defines primary consumers across kingdoms; ii) distinct bacterial and fungal substrate utilization channels may actually not exist; and that iii) the diversity of primary consumers as well as secondary trophic links should increase with substrate complexity. This comprehensive approach can significantly advance the current understanding of resource partitioning and trophic interactions between detritusphere microbes in arable soils.

Materials and methods

Soil

The soil originated from a recently installed agricultural field experiment located near Göttingen (Germany), designed to trace the flow of plant-derived carbon into soil food webs (Kramer et al 2012). Topsoil (0-10 cm) was taken from plots under wheat in October 2010. The dominant soil types at the sampling site are Cambisols and Luvisols. The C and N content of the soil were 1.37 and 0.14 %, respectively; soil pH_{CaCl2} was 6.0. Topsoil texture comprised 7 % clay, 87 % silt and 6 % sand. Further soil parameters can be found in (Kramer et al 2012). Rapid and pronounced incorporation of litter derived C into fungal biomass has

been shown, suggesting a high activity and assimilation potential of fungi in the detritusphere of this soil (Kramer et al 2012).

Microcosm setup for SIP

Soil corresponding to 50 g dry weight was filled into small steel cylinders (diameter = 5.5 cm, height = 4 cm). Four different substrates (glucose, cellulose, senescent maize leaves and roots) were mixed into the soil, all were ^{13}C-labeled (> 98 atom %, determined by the supplier). Soil microcosms without substrate amendment as well as with unlabeled substrates (natural abundance of $\delta^{13}C$; '^{12}C controls') were set up as controls. Substrates were purchased from IsoLife (Wageningen, Netherlands). Materials were added to the soil to a final amount of 12 mg C microcosm^{-1} (240 µg C g^{-1} soil). Soil cylinders were placed into air-tight glasses containing a small vessel attached to the lid to hold 1 M NaOH for absorbing evolving CO_2. The microcosms were incubated in a climate chamber at 12°C, representing the long term mean temperature of autumn months at the field site. ^{12}C treatments including controls were replicated three times while ^{13}C treatments were not replicated. For further details see SI.

CO_2 production, microbial biomass carbon (C_{mic}), as well as the $\delta^{13}C$ in CO_2 and C_{mic} was determined during incubation as described (SI). The relative amounts of substrate derived carbon in CO_2 and C_{mic} were inferred. Microcosms were destructively sampled after 2, 8, 16 and 32 days.

RNA extraction and rRNA stable isotope probing (rRNA-SIP)

RNA was extracted from soil as described by (Lueders et al 2004a) with minor modifications (see SI). RNA extracts from the most representative time points were selected for SIP gradient centrifugation based on substrate mineralization data, substrate derived CO_2 and assimilation. These were day 8 (high substrate use) and day 32 (later stage of decomposition) for all treatments. Soil from ^{12}C-control incubations was pooled and extracted as one sample. Isopycnic centrifugation and gradient fractionation were done as previously described (Glaubitz et al 2009, Kleindienst et al 2014) with 750 ng of total RNA loaded into each gradient resulting in 12-13 fractions per sample.

Fingerprinting and pyrotag sequencing of density resolved rRNA

Bacterial, fungal and protistan rRNA in resolved SIP fractions (fractions 2 to 10 of all gradients) were analyzed by T-RFLP fingerprinting (Euringer and Lueders 2008, Glaubitz et al 2009, Lueders et al 2004a). Only the glucose and leaf treatments could be analyzed for protists, for reasons of capacity and resources. See SI for full methodological detail. Based on these rRNA fingerprints (Figs. S7.1, S7.2, S7.3), fractions 3 ('heavy') and 8 ('light') of the ^{12}C and ^{13}C SIP gradients from day 8 and 32 were selected and subjected to 454 amplicon pyrosequencing (Kleindienst et al 2014, Pilloni et al 2012). Further details are given in the SI. All pyrotag sequencing raw data have been deposited with the NCBI sequence read archive under SRA accession numbers [*to be added in revision*] (bacterial 16S) [*t.b.a. in revision*] (protistan 18S) and SRP033337 (fungal 18S).

Calculation of taxon-specific enrichment factors in heavy fractions

To directly identify taxa involved in the assimilation of ^{13}C from amended substrates within the different groups (bacteria, fungi, protists), pyrotag 'enrichment factors' (EF) in 'heavy' rRNA fractions were deduced modified after Zumsteg et al. (2013). We calculated enrichment factors if the relative abundance of a given taxon in the 'heavy' rRNA of the ^{13}C treatment was > 2 % for at least one treatment and one time point. Only for protozoa, all taxa were included in the calculation irrespective of their relative abundance in the heavy fractions. The enrichment factors were calculated as follows:

$$\text{Enrichment} = {}^{13C}\text{heavy} / {}^{13C}\text{light} - {}^{12C}\text{heavy} / {}^{12C}\text{light},$$

where ^{13C}heavy and ^{13C}light is the relative abundance of reads of a given taxon in sequenced heavy and light rRNA fractions from ^{13}C treatments, and ^{12C}heavy and ^{12C}light is the same for the respective ^{12}C-control treatments. All taxa which showed an enrichment factor > 0.5 where considered as ^{13}C-labeled. In the interpretation of our labeling results, not only these enrichment factors, but also total relative rRNA read abundance of given taxa in 'heavy' rRNA in ^{13}C treatments, as well as labeling patterns evident from T-RF abundances linked to certain taxa across entire SIP gradients were considered (Figs. S7.1, S7.2, S7.3).

Consumer diversity in heavy fractions

The 'functional organization' (*Fo*) index was calculated for the T-RFs of 'heavy' ^{13}C-gradient fractions as a further measure of the diversity and evenness of labeled bacterial and fungal populations. *Fo* is based on Pareto-Lorenz evenness curves (Marzorati et al 2008), and as in Shannon-Wiener diversity *H'*, community richness and relative abundances of individual taxa are considered in *Fo*. However, rare taxa are less important, as the cumulative relative abundance of 20 % of all taxa is derived. This would be 0.2 at perfect evenness. The higher the *Fo* index, the more important the dominating taxa and the less diverse the respective community becomes. *Fo* was calculated and averaged over three 'heavy' rRNA fractions per ^{13}C-gradient.

Results

Mineralization of amended substrates and ^{13}C-assimilation

Mineralization of ^{13}C-labeled substrates as well as carbon flow into C_{mic} depended on the quality of the added substrate. Approximately two-thirds of added glucose-C and cellulose-C were mineralized after 32 days of incubation, but only ~45 and ~12 % of leaf- and root-C were mineralized over the same time, respectively (Fig. 7.1). Mineralization of ^{12}C and ^{13}C substrate amendments was not significantly different ($F_{1,14}$ = 0.004; P = 0.95). At day 2, almost 70 % of the CO_2 produced originated from glucose in the respective treatments, but was diluted down to around 7 % by the end of the experiment (Fig. 7.2A). In contrast, mineralization of cellulose and maize leaves peaked at day 8, with 64 and 48 % of substrate-derived CO_2, respectively. In the root treatment, the proportion of substrate-derived CO_2 remained at a constantly low level, between 12 and 17 % throughout the experiment (Fig. 7.2A). Consistently, resource-derived C incorporated into C_{mic} was highest for glucose (~40 %) after only 2 days of incubation (Fig. 7.2B). Assimilation efficiency appeared much lower for the other substrates, and substrate-derived C was at a maximum of ~15 % for cellulose on day 8, and of ~11 % and ~5 % for leaf and root, respectively, towards the end of the experiment (Fig. 7.2B).

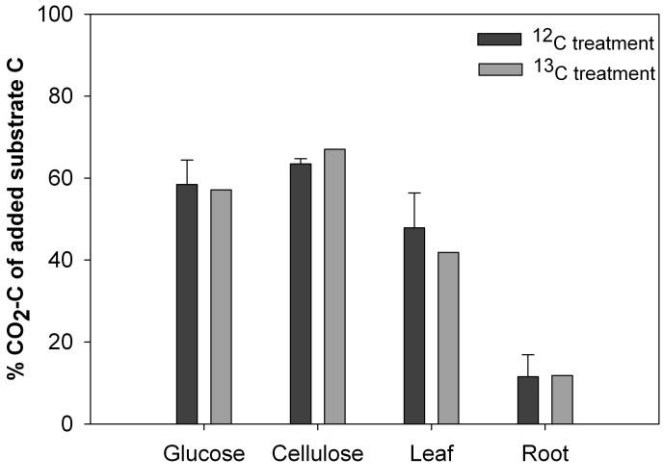

Figure 7.1 Substrate-C mineralized to CO_2 after 32 days of soil incubation.

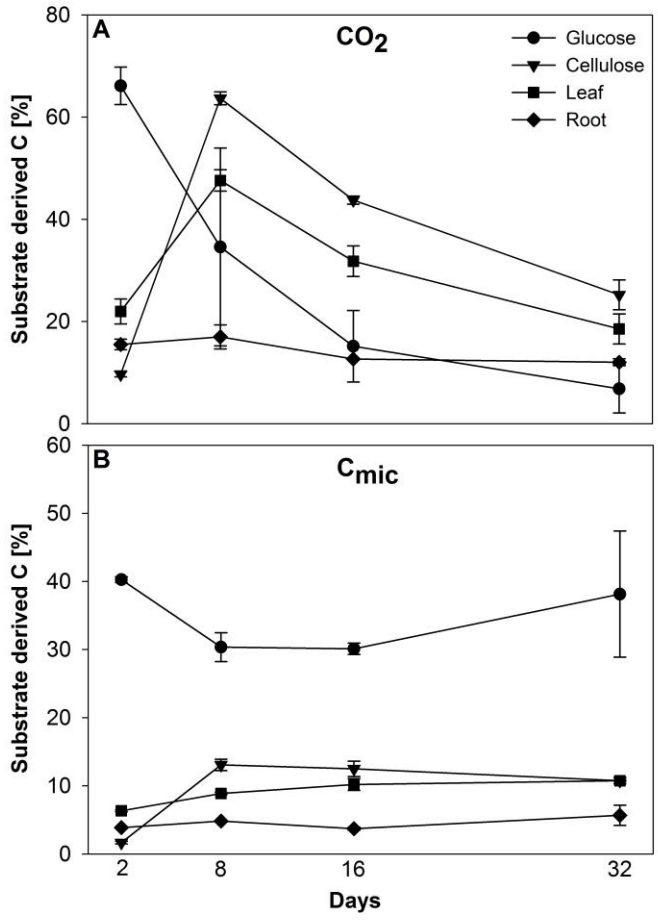

Figure 7.2 Time course of substrate derived C in CO_2 and C_{mic} during SIP incubation.

Table 7.1 Summary of most important ^{13}C-labeled taxa in the detritusphere SIP experiment.

Treatment	**Glucose**			**Cellulose**			**Leaf**			**Root**		
Time point	**8d**		**32d**	**8d**		**32d**	**8d**		**32d**	**8d**		**32d**
Bacteria	++	*Arthrobacter*	++	++	*Cellvibrio*	►	++	*Cellvibrio*	◄	++	*Cellvibrio*	►
	++	*Micrococcaceae*	◄	++	*Flavobacterium*	►	++	*Flavobacterium*	++	++	*Flavobacterium*	►
	+	*Flavobacterium*	—	—	*Streptomycetaceae*	◄	++	*Mucilaginibacter*	—	++	*Mucilaginibacter*	—
	+	*Pseudomonas*	◄	—	*Kitasatospora*	◄	+	*Cytophaga*	►	+	*Cytophaga*	—
	+	*Oxalobacteraceae*	+	+	*Cytophaga*	►	+	*Ohtaekwangia*	◄	+	*Ohtaekwangia*	◄
	+	*Humicoccus*	◄	+	*Mucilaginibacter*	—	+	*Streptomycetaceae*	+	—	*Streptomycetaceae*	+
				+	*Rugamonas*	◄	+	*Kitasatospora*	+	+	*Kitasatospora*	+
				+	*Myxobacteria*	◄	—	*Myxobacteria*	◄	—	*Myxobacteria*	◄
Fungi	++	*Cryptococcus*	++	++	*Chaetomium **1***	—	++	*Chaetomium **2***	++	++	*Chaetomium **2***	++
				—	*Geomyces*	◄	+	*Fusarium*	◄	—	*Chaetomium **1***	◄
										—	*Fusarium*	◄
Protists	++	*Pythium*	++	n.a.			++	*Pythium*	++	n.a.		
							++	*Vannellidae*	—			
							+	*Acanthamoebidae*	◄			
							+	*Nucleariidae*	—			
							+	*Vampyrellidae*	—			
							|	*Leptomyxida*	◄			
							+	*Chrysophyceae*	—			
							—	*Chlamydophryidae*	◄			
							—	*Rhynchomonas*	◄			

* ++ strongly labeled; + labeled; – not labeled or detected; ◄ increasing labeling; ► decreasing labeling; n.a. not analysed

rRNA stable isotope probing

The incorporation of label into substrate-specific subsets of bacteria, fungi and protists was evident already via the comparison of T-RFLP fingerprints from ^{13}C and ^{12}C-control gradients (Figs. S7.1, S7.2, S7.3). However, our interpretation of labeling results relies chiefly on taxon-specific pyrotag 'enrichment factors' (EF). To support this novel approach, almost all important labeled T-RFs (Figs. S7.1, S7.2, S7.3) could in fact be linked to pyrotag-defined microbial taxa (see SI for details and methodological discussion). A summary of the most relevant labeled prokaryotes and microeukaryotes detected in our experiment is given in Table 7.1.

Labeled bacterial rRNA

The different bacterial taxa incorporating ^{13}C-label belonged mainly to three bacterial phyla: *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* (Fig. 7.3). Amongst the latter, mostly *Gammaproteobacteria*, but also *Beta-* and *Deltaproteobacteria* were labeled. At day 8 of incubation, reads affiliated with *Arthrobacter* spp. (T-RFs 61, 71, 159, Fig. S7.1) were strongly enriched (EF 31, Fig. 7.3) and most abundant (~40 %, Fig. S7.4) in 'heavy' rRNA of the glucose treatment. However, also unclassified *Micrococcaceae* (T-RFs 61, 71), *Flavobacterium* spp. (T-RF 80), unclassified *Oxalobacteraceae* and *Pseudomonas* spp. (T-RF 490) were enriched, albeit at lower read abundances. Interestingly, although most glucose mineralization activity was complete after 8 days, a dynamic labeling pattern was still observed after 32 days. Here, reads related to *Flavobacterium* spp. had lost the label; those related to unclassified *Micrococcaceae*, *Humicoccus* (T-RFs 137, 145) and *Pseudomonas* spp. became more enriched. But *Arthrobacter* spp. was still the dominant taxon in 'heavy' rRNA.

In the cellulose treatment *Cellvibrio*- (T-RFs 137, 486, 487, 490) and *Flavobacterium*-(T-RFs 79, 80) related reads were most highly enriched (EF 297 & 99) and abundant (40 & 27 %) in 'heavy' rRNA after 8 days. While both were strongly reduced after 32 days, sequences of unclassified *Streptomycetaceae* and *Kitasatospora* spp. (T-RF 157) became very important in labeled rRNA at this later time point. Less enriched and/or abundant taxa were *Cytophaga* spp. after 8 days and *Rugamonas* spp. after 32 days.

The highest enrichment in leaf and root treatments was observed for the abundant taxa *Flavobacterium* (T-RFs 79, 80, 84) and *Cellvibrio* spp. (T-RFs 486, 487, 490), but also in the less frequent *Mucilaginibacter* (T-RF 524) and *Cytophaga* spp. after 8 days. After 32 days,

high enrichment was detected for *Cellvibrio*, *Flavobacterium* and *Ohtaekwangia* spp. (T-RF 205) in both leaf and root treatments. In contrast to the leaf treatment, *Cellvibrio* rRNA showed a strongly decreased ^{13}C-enrichment in the root treatment. Similarly, some of the *Actinobacteria* became more enriched in 'heavy' rRNA upon biomass degradation after 32 days. Interestingly, the unclassified *Polyangiaceae* (T-RFs 69, 500) as well as other *Myxobacteria* became noticeably more abundant (3 – 17 %) and also enriched (EF 4 – 15) with cellulose, leaf and root amendments after 32 days. Only one genus (*Ohtaekwangia* spp.; T-RF 205) appeared exclusively enriched in leaf and root treatments after 32 days, while virtually no labeling was observed under glucose or cellulose amendments.

In essence, the diversity of key taxa labeled during leaf and root decomposition was not noticeably larger than with glucose and cellulose (Table 7.1). However, the functional organization (*Fo*) of bacterial rRNA fingerprints in 'heavy' fractions showed a clear decrease towards the more complex substrates (Table 7.2) suggesting a higher diversity and fewer dominant bacterial taxa in rRNA of labeled bacterial consumers of leaves and roots.

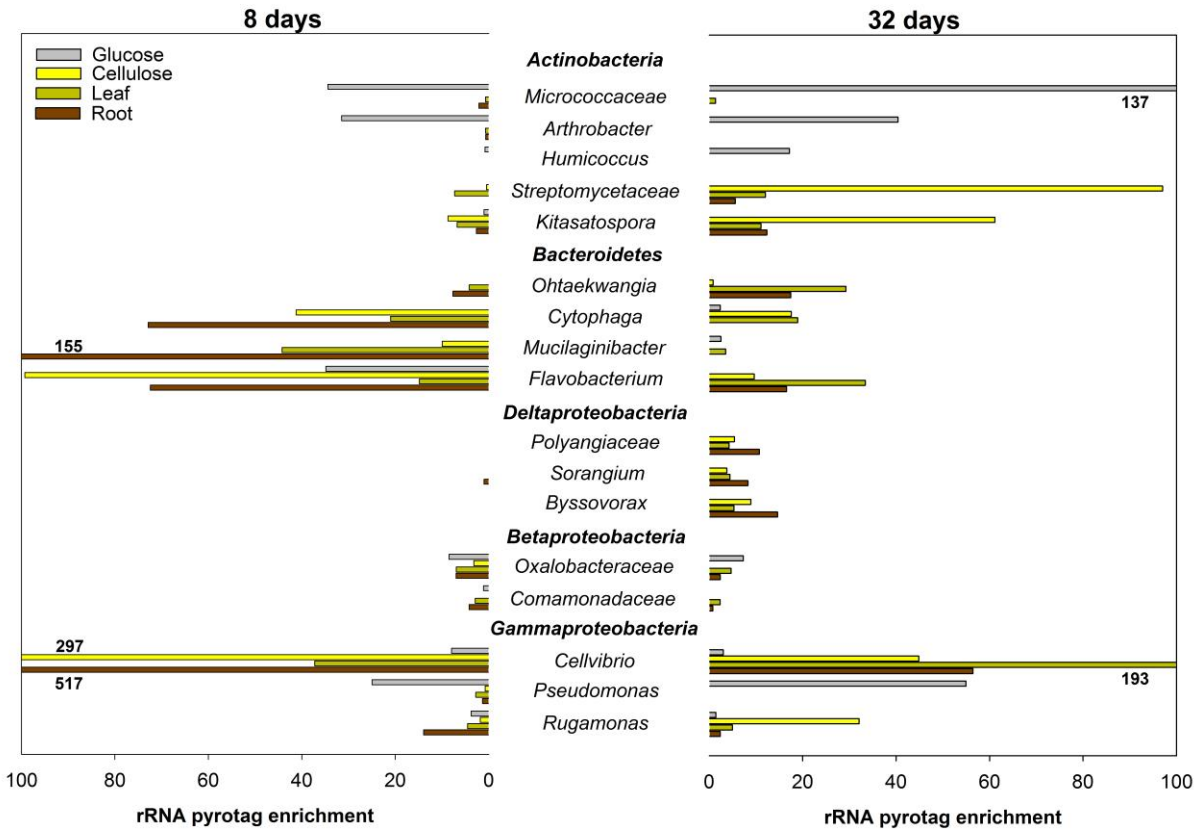

Figure 7.3 ^{13}C-labeled bacterial taxa after 8 and 32 days of incubation. Labeling was inferred via comparative pyrotag enrichment in 'heavy' vs. 'light' rRNA gradient fractions of ^{13}C and ^{12}C treatments. Only labeled taxa are shown.

Table 7.2 Functional organization (*Fo*) of heavy rRNA fingerprints as a measure of the structure of labeled microbial populations.

Time	**8 days**				**32 days**			
Treatment	Glucose	Cellulose	Leaf	Root	Glucose	Cellulose	Leaf	Root
Bacteria	0.77 ± 0.01 a	0.75 ± 0.04 a	0.68 ± 0.01 b	0.57 ± 0.03 c	0.77 ± 0.03 ab	0.83 ± 0.02 a	0.61 ± 0.05 c	0.71 ± 0.06 bc
Fungi	0.85 ± 0.03 ab	0.84 ± 0.09 ab	0.88 ± 0.02 a	0.70 ± 0.09 b	0.64 ± 0.15 b	0.80 ± 0.10 ab	0.89 ± 0.03 a	0.83 ± 0.01 ab

* *Fo* was calculated for fingerprints as introduced by (Marzorati et al 2008). Calculations were averaged over the 'heavy' rRNA fractions 2, 3 and 4 of ^{13}C-gradients and are shown ± SD. Letters indicate significant differences between the treatments at the respective date (Tukey HSD; $P < 0.05$).

Labeled fungal rRNA

Labeled fungi were less diverse and showed less pronounced enrichment in 'heavy' rRNA than bacteria. However, they showed more pronounced preferences for specific substrates. All fungal degraders of the added detritusphere substrates belonged to *Basidiomycota* and *Ascomycota* (Fig. 7.4). A consistent trend of the *Fo* of 'heavy' fungal rRNA fingerprints with substrate complexity was not observed (Table 7.2).

Glucose carbon was mainly assimilated by *Cryptococcus* spp. (T-RF 564, Fig. S7.2), as shown by highly abundant (>50 %) and enriched (EF 7) reads in 'heavy' rRNA (Figs. 7.4, S7.5). Surprisingly, no other fungi were labeled in the glucose treatment. In turn, *Cryptococcus* yeasts were not labeled in any of the other treatments. Under cellulose amendment, enrichment was only observed for *Chaetomium*-related phylotype 1 (T-RF 708) after 8 days (~23 %, EF 9), while *Geomyces* spp. belonging to the *Ascomycota* were the only labeled fungi after 32 days. In the leaf and root treatments, a second *Chaetomium*-related phylotype (T-RF 708) was enriched and highly abundant after 8 days (39 – 61 %, EF 4). After 32 days, labeling was additionally observed for *Fusarium* spp. in both plant biomass treatments, as well as for the first *Chaetomium* phylotype in the root amendment.

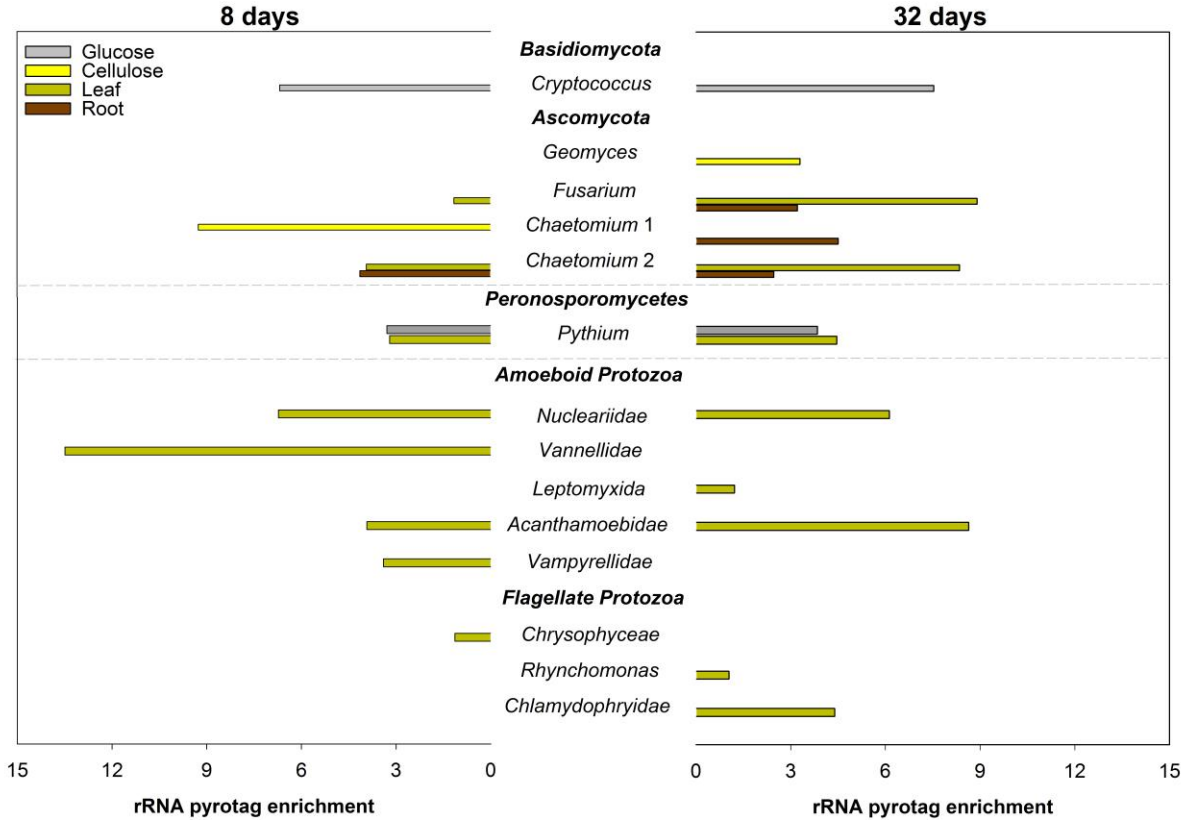

Figure 7.4 ^{13}C-labeled fungal and protist taxa after 8 and 32 days of incubation. Labeling was inferred via comparative pyrotag enrichment in 'heavy' vs. 'light' rRNA gradient fractions of ^{13}C and ^{12}C treatments. Only labeled taxa are shown.

Labeled protist rRNA

Protists within the *Peronosporomycetes* (formerly: *Oomycetes*) related to *Pythium* spp. (T-RFs 418, 421, Fig. S7.3) were clearly enriched (EF ~8) and abundant (12 – 18 %) in 'heavy' rRNA fractions of both treatments investigated for protists (glucose and leaf, Figs. 7.4, S7.5). Protozoa rRNA read abundances in the 'heavy' rRNA fractions were very low in the glucose treatment (in comparison to fungal and *Oomycete* rRNA which were also detected with the used eukaryote primer). Overall, five amoeboid and three flagellate taxa appeared enriched in the leaf amendment. After 8 days, enrichment was found in four amoeboid taxa with strongest enrichment in the highly abundant *Vannellidae* (*Amoebozoa*, T-RF 429, 4.7 %, EF 14) and in the less abundant *Nucleariidae* (*Opisthoconta*, 0.8 %, EF 7) (Figs. 7.4, S7.5). Labeling of *Vannellidae* and *Vampyrellidae* disappeared after 32 days, while still detectable in *Acanthamoebidae* (T-RF 484) and *Nucleariidae*. *Leptomyxida* (*Amoebozoa*) were also enriched at this later time. Amongst the flagellates, the *Chrysophyceae* appeared labeled after

8 days. This label had disappeared again after 32 days, but increased in *Chlamydophryidae* (T-RF 412), as well as in *Rhynchomonas* spp..

Discussion

The diversity and succession of specific pro- and microeukaryotes actively involved in the degradation of detritusphere substrates in an agricultural soil was investigated here. Our approach was based on the interpretation of rRNA labeling relying on taxon-specific pyrotag abundances in density-resolved gradient fractions, which is an advance of classical gradient interpretation based on fingerprinting (Lueders et al 2004b, Lueders et al 2006). A careful discussion of this approach can be found in the SI.

Mineralization and assimilation of detritusphere substrates

Mineralization was not influenced by the isotopic composition of amendments (Fig. 7.1). There was no difference in cumulative mineralization between the glucose and cellulose treatments, but initial mineralization rates were clearly highest for glucose (Fig. 7.2A). The much less efficient mineralization and assimilation of plant biomass amendments (Figs. 7.1, 7.2) relates directly to the higher complexity of these materials (Bertrand et al 2006, de Boer et al 2005). Additionally, roots appeared more resistant to decomposition than leaves, possibly due to their lower content of water soluble compounds and their more rigid secondary cell wall structures (Bertrand et al 2006). Overall, the observed substrate mineralization and assimilation rates indicated an adequate range of substrates chosen to address our initial hypotheses.

Bacterial key-players

The identified actinobacterial glucose consumers (unclassified members of the *Micrococcaceae* and *Arthrobacter* spp., Fig. 7.3, Table 7.1) have been previously described as glucose utilizers in soil (Padmanabhan et al 2003, Schellenberger et al 2010). The high abundance of labeled *Arthrobacter* rRNA in our study indicated a high specific activity. Still, respective rRNA enrichment was not as high as for some of the labeled taxa in the other treatments, suggesting the simultaneous use of other intrinsic and probably more recalcitrant substrates. Therefore, involvement of *Arthrobacter* spp. in priming effects is likely, as

previously proposed for *Actinobacteria* (Bastian et al 2009, Bernard et al 2007). *Pseudomonas* spp. are well known as opportunistic soil and rhizosphere bacteria (Hartmann et al 2009). Surprisingly, this taxon was labeled only under glucose amendment in our study (Fig. 7.3, Table 7.1). This is partly in contrast to previous studies, where *Pseudomonas* spp. have been shown to utilize glucose as well as more recalcitrant compounds including lignin (Goldfarb et al 2011, Padmanabhan et al 2003).

The early cellulose degrading community, dominated by *Cellvibrio, Flavobacterium* and *Cytophaga* spp., shifted to unclassified members of *Streptomycetaceae* and *Kitasatospora* spp. after 32 days. Members of *Bacteroidetes, Cellvibrio* and *Flavobacterium* spp. specifically, are known to grow on different sugars and on cellulose (Haichar et al 2007, Padmanabhan et al 2003, Schellenberger et al 2010). Although also many *Streptomycetes* can decompose polysaccharides and possess both exo- and endocellulases (Kämpfer 2006), their successional involvement as observed here has never been reported. Their capacity to form hyphae could potentially be relevant during later stages of decomposition.

The consistent labeling of most bacterial taxa identified as cellulose decomposers also in the plant residue amendments illustrates the importance of cellulose as a substrate for plant litter degraders. *Ohtaekwangia* spp., the only taxon solely labeled in the plant litter treatments, seemed to thrive specifically on biomass constituents other than glucose and cellulose. Similarly, the higher abundance and stronger label of *Mucilaginibacter* spp. in the plant biomass amendments after 8 days indicated a preferred utilization of other substrates. A clear labeling of unclassified members of *Polyangiaceae*, *Sorangium* and *Byssovorax* spp. (*Deltaproteobacteria*) emerged in cellulose, leaf and root treatments after 32 days. These *Myxobacteria* are known as micropredators (Lueders et al 2006, Reichenbach 1999), and could have been labeled via feeding on microbial biomass of primary substrate consumers.

Fungal key-players

Detritusphere fungi showed not only a clear distinction between defined substrate-utilizing taxa (glucose and cellulose), but in contrast to bacteria, also between utilizers of defined (cellulose) and more complex substrates (leaf and root, Fig. 7.4). Although *Zygomycetes*, so called ‘sugar fungi’ such as *Mortierella* and *Mucor* spp., are often considered the most important users of low molecular weight carbon sources (de Boer et al 2005), *Cryptococcus* spp. dominated glucose utilization throughout our experiment. This highlights the role of

these fast-growing yeasts as important competitors for labile resources in soil. *Cryptococcus* species were also identified as cellulose utilizers in another recent study (Stursova et al 2012), but in our experiment, they were labeled only with glucose.

The dominating early stage cellulose utilizer, *Chaetomium*-phylotype 1, belongs to a genus known to include fast growing fungi (Straatsma et al 1994). However, these were completely replaced by *Geomyces* spp. at the later stage of decomposition. *Geomyces* spp. can thrive under nutrient limitation (Hayes 2012) which could well explain their delayed involvement. Both genera have been previously identified as cellulose utilizers in SIP experiments (Eichorst and Kuske 2012, Stursova et al 2012). The distinct substrate utilization pattern of both labeled *Chaetomium* phylotypes suggests that these may have different exoenzymatic capabilities. Critical enzymes in the degradation of plant biomass are known to be generally much less prevalent in bacterial than fungal populations (Romani et al 2006). The generally high abundance of *Chaetomium*-phylotype 2, especially in 'heavy' rRNA fractions (Fig. S7.5) could indicate that these fungi were of key importance in the release of cellulose from plant biomass, thus potentially even making it available for other detritusphere microbes.

Although *Fusarium* species are opportunistic plant pathogens, known to be amongst the first colonizers of both living and dead plant biomass (Leplat et al 2013), their involvement in the degradation of leaf and root amendments became apparent only after 32 days. In our experiment, these fungi seemed only of minor importance during the initial attack on maize biomass, especially when compared to the aforementioned *Chaetomium* spp..

Protist key-players

The flow of carbon into protists was investigated for the glucose and leaf litter treatments only. Because of the large overlaps in labeled bacteria detected in the non-glucose treatments (Table 7.1), we are confident that this subset allowed inferring also the most relevant distinctions in protist labeling. Although the *Peronosporomycetes* (formerly *Oomycetes*) morphologically and physiologically resemble fungi, they are classified as heterotrophic protists in the taxon *Stramenopiles* (Adl et al 2005). *Peronosporomycetes*, such as *Pythium* are important plant pathogens (Hendrix and Campbell 1973), but can also act as pioneer saprotrophs on fresh plant residues in soil (Deacon 1997). The high abundance and ^{13}C-enrichment of protists rRNA related to *Pythium* spp. for both treatments and time points

indicated an important role of these protists during decomposition of these substrates. Remarkably, no other protists were identified as labeled in the glucose treatment.

In contrast, clear labeling of heterotrophic protozoan taxa over time indicated a substantial flow of carbon from labeled prey into protozoan grazers in the leaf treatment. ^{13}C enrichment was mainly found in amoeboid protozoa (Fig. 7.4). *Acanthamoeba* are among the most dominant protozoa in soil (Page 1976). Not surprisingly, *Acanthamoebidae* were one of the dominant labeled taxa, especially at the later stage of leaf decomposition, while *Vannellidae* dominated early in the succession (Fig. S7.5). A succession of labeled taxa during leaf decomposition was also observed for bacterivorous flagellates, although their rRNA was much less abundant. Likely the amoeboid life style was more competitive under the conditions in our soil microcosms.

Remarkably, facultative fungivorous taxa, such as *Vampyrellidae* (Hess et al 2012) and *Leptomyxida* were also labeled in the leaf treatment. Traditionally, mostly bacterivorous protists are considered as relevant in soil food webs (Moore et al 2003, Moore et al 2005, Mulder et al 2011), despite fungivorous protozoa are also ubiquitous (Ekelund 1998, Petz et al 1986). Our study clearly demonstrates a significant carbon flux from litter material to fungivorous amoebae.

Substrate complexity and consumer diversity

We show that substrate complexity and recalcitrance indeed defined the primary consumers. However, unexpectedly, we did not observe marked distinctions in key taxa which assimilated carbon from leaf and root detritus, in spite of the lower mineralization and assimilation of the roots. Although effects of substrate quality (e.g. recalcitrance) on overall microbial community structure and diversity in soils have been reported (Nicolardot et al 2007), this seems not always the case (Mula-Michel and Williams 2012). In our study, the most noticeable distinction between leaf and root treatments was that some of the labeled bacterial populations showed a much higher enrichment with root amendment after 8 days, but were sometimes less abundant in ‘heavy’ rRNA compared to the leaf treatment (i.e. *Cytophaga*, *Mucilaginibacter*, *Flavobacterium* and *Cellvibrio* spp.). This indicates that these taxa may have developed strategies to specifically access recalcitrant substrates, which may be a key determinant of bacterial niche partitioning in the detritusphere (Baldrian et al 2013, Goldfarb et al 2011). Such patterns were not observed for fungal decomposers.

Surprisingly, in view of the considerable microbial diversity present in the investigated soil (Dibbern et al 2014), all substrates appeared to be consumed by only a few key players over all investigated kingdoms. The early dominance of yeasts and *Actinobacteria* indicate an inter-kingdom competition between fast growing r-strategists for labile substrates. This is in line with the hypothesis that despite the high diversity of microbes in soil, only a minority dominates decomposition processes (Vandermeer et al 1998). However, such mechanisms seem to be less pronounced for more complex substrates, as supported by the lower *Fo* (higher evenness) of labeled bacterial rRNA in leaf and root treatments compared to defined substrates (Table 7.2).

Trophic interactions

The most marked succession of bacterial and fungal key players was observed during cellulose decomposition, a substrate of intermediate complexity and recalcitrance. Potentially, top-down rather than bottom-up controls of bacteria could have been involved here. This was especially apparent in the shifting dominance of *Cellvibrio* and *Flavobacterium* to *Actinobacteria* populations in the cellulose, but also leaf and root treatments over time (Table 7.1, Fig. 7.3). It is well known that the Gram-positive *Actinobacteria* (dominating glucose consumers after 8 days), are far less attractive prey for protozoa than Gram-negatives due to their more rigid cell walls and hyphal growth (Jezbera et al 2005). It is conceivable that the initial bursts of *Cellvibrio* and *Flavobacterium* populations in the cellulose treatment were controlled top-down by the diverse amoeboid protozoa labeled in the leaf treatment, providing niches for the development of more grazing-resistant actinobacterial cellulose utilizers over time. Similarly, the absence of labeled protozoan rRNA in the glucose treatment could well be related to *Actinobacteria* as main utilizers.

Our labeling results also provide tentative evidence for intra-bacterial predation in the detritusphere. As mentioned above, myxobacteria are known for their specialization in decomposition of biomacromolecules and complex organic matter (Eichorst and Kuske 2012, Reichenbach 1999), but they are also potential micropredators of bacterial populations (Lueders et al 2006). Their secondary rRNA labeling in the cellulose and plant treatments suggests that they consumed biomass of primary detritusphere bacteria in parallel to protozoan predators.

Conclusions and outlook

In this detritusphere SIP experiment of an agricultural soil, bacteria, fungi, and also protists were identified as primary consumers of all amendments, irrespective of substrate complexity or recalcitrance. Therefore, the notion of distinct detritusphere energy channels in soil (de Boer et al 2005, Moore and William Hunt 1988, Paterson et al 2008) appears to be an oversimplification not supported by our data. In contrast, our results support an 'eat all you can' perspective of the simultaneous activity and overlapping substrate usage patterns of bacteria, fungi and protists in the detritusphere, irrespective of resource quality. Further, this study provides an unprecedented level of detail on the microorganisms involved in detritusphere carbon flow in an agricultural soil. Distinct bacterivorous and fungivorous protozoan key players were identified as labeled. Although taxonomic detail on fungal feeding protozoa in soil exists, this is the first direct demonstration of their importance in a plant litter based microbial food web.

It may not be possible to generalize the findings of this SIP study conducted for just one specific agricultural soil. Moreover, our methodological approach does not allow for clear quantitative statements on the involvement of the identified taxa in detritusphere carbon flow. Nevertheless, we believe that this work may well be of use to improve current modelling concepts for litter decomposition in soil. More specific, functionally and trophically defined microbial components may indeed be vital to improve current ecosystem models to more accurately predict feedbacks of e.g. changing temperatures or hydrological regime on carbon cycling (Bradford 2013, McGuire and Treseder 2010, Moore et al 2005, van der Wal et al 2013). Here, the direct linking of key microbial populations to globally relevant decomposition processes is still a major challenge (Trivedi et al 2013).

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Supporting Information (SI)

Supplementary Materials and Methods

Setup of microcosm experiment

The soil was sieved (< 2 mm), stored at 4 °C for a few days and water holding capacity was determined. Two weeks before the start of the SIP experiment, soil was pre-incubated at the experimental temperature of 12 °C. Soil water content was gravimetrically determined two days before the start of the experiment. Soil moisture content was adjusted to 60 % of the water holding capacity. Soil bulk density was set at 1.4 g cm^{-3}. During incubation, microcosms were weighed repeatedly to control water content. No significant decrease in soil water content was observed, adjustment was not necessary. The amended maize leaf (C/N: ^{13}C of 82, ^{12}C of 54) and root (C/N: ^{13}C of 50, ^{12}C of 37) material was milled (< 1 mm) by the supplier. Microcosms were destructively sampled, the soil was homogenized and subsamples were stored at -80 °C for RNA extraction, and at -24 °C for all other analyses.

CO_2 production and microbial biomass C

CO_2 production was determined by titration over the entire incubation time in increasing time distances between measurements (Marhan et al 2008). CO_2 was trapped in 1 M NaOH and precipitated with 0.5 M $BaCl_2$. The remaining NaOH was titrated with 0.1 M HCl with phenolphthalein indicator (Marhan et al 2008). After sampling for titration, lids of the microcosms were left open to allow gas exchange. Another part of the precipitated $BaCO_3$ was used for $\delta^{13}C$ determination of the evolved CO_2 (only in the ^{12}C treatments).

Microbial biomass C (C_{mic}) was determined by chloroform-fumigation extraction (Vance et al 1987). 3 g (fresh weight) of homogenized soil was extracted with 0.025 M K_2SO_4 [1:4 soil solution ratio (w/v)], shaken for 30 min at 250 rev min^{-1} on a horizontal shaker and centrifuged for 30 min at 4,422 x g. Parallel subsamples were fumigated with ethanol-free chloroform in a desiccator for 24 h before extraction. Organic C in the supernatants was measured with a DOC / TN-analyser (Dimatoc 100, Dimatec, Essen, Germany).

$\delta^{13}C$ determination of CO_2 and the microbial biomass

For $\delta^{13}C$ determination in evolved CO_2, precipitated $BaCO_3$ was washed with 10 ml deionised H_2O and centrifuged at 250 x g, after which the supernatant was discarded. This was repeated three times until all residual NaOH was removed. Pellets of $BaCO_3$ were then dried at 60 °C for two days and 0.3 – 0.6 mg was weighed into tin capsules. For analysis of $\delta^{13}C$ in C_{mic} (Marhan et al 2010, only in ^{12}C treatments), 10 ml aliquots of the supernatants of both non-fumigated and fumigated samples were dried in a rotary vacuum evaporator (RVC 2-25, Martin Christ, Osterode am Harz, Germany) at 60 °C. The remnant was ground and weighed into tin capsules within a range of 7 – 30 mg (minimum of 10 µg C per capsule). For calculation of the $\delta^{13}C$ of C_{mic}, the following equation was used:

$$\delta^{13}C_{mic} = (c_{nf} \times \delta_{nf} - c_f \times \delta_f) / (c_{nf} - c_f),$$

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

$\delta^{13}C$ measurements were done with an elemental analyzer (Euro EA 3000, EuroVector, Milan, Italy) coupled with an isotope ratio mass spectrometer (IRMS, Delta Plus XP, Thermo Finnigan MAT, Bremen, Germany). Glutamic acid USGS-40 (IAEA, Vienna; $\delta^{13}C$ -26.39 ± 0.04 ‰) was used as reference material for calibration of CO_2 reference gas. Acetanilide (C_8H_9NO, Merck, Darmstadt) was used as a secondary laboratory reference material for internal calibration. $\delta^{13}C$ values are expressed relative to Vienna Pee Dee belemnite (V-PDB).

For calculation of the relative amounts of substrate derived C in CO_2 and C_{mic} the following mixing model was used:

$$\% \text{ C-substrate} = (\delta_{sample} - \delta_{reference}) / (\delta_{substrate} - \delta_{soil}),$$

where δ_{sample} is the $\delta^{13}C$ value of the respective sample, and $\delta_{reference}$ is the $\delta^{13}C$ mean value of control samples (soil without substrate amendment). $\delta_{substrate}$ is the $\delta^{13}C$ value of the respective amended material, and δ_{soil} the $\delta^{13}C$ value of the C_{org} at the beginning of the experiment.

RNA extraction

Total nucleic acids were extracted from the soil following a previously described procedure (Lueders et al 2004a) with minor modifications: 0.4 g (fresh weight) of soil were used and bead beating was done in the presence of sodium phosphate, sodium dodecyl sulphate and phenol-chloroform-isoamyl alcohol (25:24:1, pH 8). All centrifugation steps were conducted

at 20,000 x g and 4 °C for 5 min. Extracted total NAs were dissolved in 80 µl EB buffer (Qiagen GmbH, Hilden, Germany). Silica gel columns (DyeEx 2.0 Spin Kit; Qiagen) were used for further purification and elimination of humics. DNA was removed by digestion with DNAse I (Promega, Madison, WI, USA) following manufacturer protocols. Afterwards, RNA was precipitated with 2 vol. PEG solution (30 % (w/v) polyethylene glycol 6000, 1.6 mM NaCl) and centrifugation for 30 min at 4 °C and 20,000 x g. RNA pellets were washed once with ice cold 70 % (v/v) ethanol, air-dried and dissolved in 50 µl EB Buffer. The resulting RNA was quantified using the RiboGreen quantification kit (Life Technologies, Carlsbad, CA).

Quantitative gradient analyses

After fractionation and precipitation of density-resolved rRNA, bacterial, fungal and protist rRNA (the latter only for selected samples) was quantified in gradient fractions via RT-qPCR as described in Glaubitz et al. (2009). The initial screening revealed a ^{13}C-dependent increase of bacterial and microeukaryotic rRNA in 'heavy' fractions (data not shown). The buoyant density of the bulk rRNA peak remained unchanged in 'light' fractions (~1.78 – 1.79 g/ml CsTFA), indicating that only specific subsets of soil microbiota were actively involved in the utilization of the labeled detritusphere substrates.

Terminal restriction fragment length polymorphism (T-RFLP) fingerprinting

Bacterial, fungal and protistan rRNA populations in resolved SIP fractions were analyzed by T-RFLP fingerprinting. See Table S1 for a summary of all utilized PCR assays. Bacterial communities were analyzed with primers Ba27f-FAM / 907r and subsequent *Msp*I digestion as previously described (Pilloni et al 2011). Protistan communities were characterized with primers Euk20f-FAM / Euk519r and *Bsh*1236I digestion, in a minor modification of the assay originally published by Euringer and Lueders (2008). Reverse transcription of eukaryotic rRNA and PCR amplification was done with the Brilliant III Ultra-Fast SYBR Green one-step RT-qPCR Master Mix (Agilent Technologies Inc., Santa Clara, California) as specified by the manufacturer with 0.3 µl of each primer and 2 µl of RNA template. For both bacterial and protistan amplicons, digests were purified and separated by capillary electrophoresis (Pilloni et al 2011).

Fungal communities were characterized with primers nu-SSU-0817-5'- FAM / nu-SSU-1536-3' (Borneman and Hartin 2000) and *Msp*I digestion after (Edel-Hermann et al 2008). First-strand cDNA was prepared using the RevertAid Premium First Strand cDNA Synthesis kit and provided random hexamer primers (Thermo Scientific, St. Leon-Rot, Germany). PCR was performed in 40 µl reaction mixtures containing 20 µl 2x GoTaq Green Master mix (Promega, Madison, WI, USA), 20 µM of each primer and 3 µl template cDNA. PCR products were purified using 5Prime PCRExtract Mini Kit (5PRIME, Inc., Bucksfield Road, Gaithersburg, USA) followed by *Msp*I (Thermo Scientific, St. Leon-Rot, Germany) digestion of 80 ng PCR product for two hours according to the manufacturer's protocol. After purification of the reaction mixture by ethanol precipitation, fungal communities were analyzed on a ABI 3730xl capillary electrophoresis sequencer (Applied Biosystems, Foster City, CA, USA) as described previously (Scharroba et al).

Bacterial and protistan raw T-RFLP data were further processed with the T-REX online software (Culman et al 2009). Background noise filtering (Abdo et al 2006) was on default factor 1 for peak heights and the clustering threshold for aligning peaks across the samples was set to 1 using the default alignment method of T-Align (Smith et al 2005). Relative T-RF abundance was inferred from peak heights. For reduction of data complexity, T-RFs that occurred in less than 5 % of the samples were excluded from further analysis. Fungal T-RFLP data with all peaks above a threshold of 100 fluorescence units were binned and normalized with an automatic binning script (Ramette 2009) using R version 2.12.2 (R Development CoreTeam 2012). The binning frame with highest correlation values between samples and a window size of two was chosen. Peaks with a relative abundance below 0.1 % were discarded as background noise.

Amplicon sequencing

Bacterial pyrotags were generated as reported previously (Pilloni et al 2012), adapting the workflow to rRNA templates instead of DNA. Shortly, RT-PCR was done under identical conditions as for fingerprinting, applying amplicon fusion primers with respective primer A or B adapters, key sequence and multiplex identifiers (MID) as reported (Pilloni et al 2012). Amplicons were purified and pooled in equimolar 10^9 μl^{-1} concentration, and emulsion PCR, emulsion breaking and sequencing were performed as previously described in detail (Pilloni et al 2012) following manufacturer protocols using a 454 GS FLX pyrosequencer using

Titanium chemistry (Roche, Penzberg, Germany). Bidirectional reads were quality-trimmed and filtered as previously described (Pilloni et al 2012), and reads shorter than 250 bp after trimming were excluded from further analysis. Classification of bacterial taxa was done with the RDP classifier (Wang et al 2007).

Protistan pyrotags were only generated for the glucose and leaf treatments, same as for fingerprints. Amplicon preparation for protists was done as for bacteria but with modified PCR conditions (Table S7.1) and with the same Brilliant III Ultra-Fast RT-qPCR Master Mix (Agilent Technologies, Santa Clara, USA) as used for respective T-RFLP fingerprinting. Quality-trimming and filtering was the same as for bacterial pyrotags. 18S rRNA amplicon sequences were taxonomically analyzed with the CREST toolbox (Lanzén et al 2012). In brief, the amplicons were taxonomically assigned by MEGAN analysis of BLASTN files against the SilvaMod SSU rRNA reference database (LCA parameters: min. bit score 330, min. support 1, top percent 2; 50 best blast hits).

For the linking of T-RF and pyrotag data, matching sequences from bidirectional amplicon pools were assembled into contigs with the SEQMAN II software (DNAStar) using assembly thresholds of at least 97 % sequence similarity over a 50 bp match window for T-RF prediction (Pilloni et al 2012). Only contigs containing at least one forward and one reverse read were used to predict *in-silico*-T-RFs for dominating consensus phylotypes using TRiFLe (Junier et al 2008).

For sequencing of fungal rRNA, pyrotags were amplified as described for the fungal T-RFLP analyses, except of using the unlabeled forward primer nu-SSU-0817-5' combined with the fusion primer B (modified after Becklin et al. (2012). MID barcodes were inserted between the A primer and primer nu-SSU-1536-3' to allow post-sequencing sample identification. PCR products were purified from agarose gels using the QIAquick Gel Extraction kit (Qiagen, Valencia, CA, USA). The clean amplicons were quantified using the Quant-IT PicoGreen dsDNA Reagent kit (Life Technologies GmbH, Darmstadt, Germany), diluted to 10^9 molecules /µl and equimolarly pooled into an amplicon library following manufacturer protocols (Roche, Penzberg, Germany). The fungal pyrotags were sequenced in one $1/4^{th}$ plate using GS-FLX+ sequencer (Roche, Penzberg, Germany). The pyrosequences were processed and quality filtered using mothur (Schloss et al 2009). Barcodes and primers were trimmed and sequences were extracted based on 100% barcode similarity, an average quality score of 20, read length of 300 bp after trimming of the last 30 bp and homo-polymers of 8 bases. The chimera check command 'uchime' with 'template self' was used to detect potentially chimeric

sequences and remove them from the dataset. Sequences were then clustered to operational taxonomic units (OTUs) using 'cd-hit-est' with a threshold of 97 % pairwise identity. Representative sequences in the respective clusters, were extracted and taxonomically assigned according to the arb silva eukaryotic taxonomy using the 'classify seqs' command of mothur with 80 % cutoff. The taxonomic position of the dominant fungal OTUs was manually verified using the NCBI blast database. Fungal in-vitro T-RFs were generated based on full length clone library sequences done for 'heavy' fraction 3 with the software TRiFLe (Junier et al 2008).

Statistical Analysis

Cumulative CO_2 in ^{12}C and ^{13}C treatments and functional organization of bacteria and fungi after 8 and 32 days was analyzed by One-way ANOVA. Best fitted transformation (cumulative CO_2: root transformation) was used to improve homogeneity of variance (determined by Levene's test). Post hoc test (Tukey HSD) was used for comparison of means of the functional organization between treatments. Statistical analyses were done with the software STATISTICA 6.0 (Tulsa, OK, USA).

Table S7.1 Primer pairs and PCR conditions used in this study.

Group	Primers	PCR conditions
Bacteria	Ba27f (5'-3') AGA GTT TGA TCM TGG CTC AG Ba907r (5'-3') CCG TCA ATT CCT TTG AGT TT	Reverse transcription: 30 min 45°C PCR: 5 min 95 °C; 13-25 cycles [30 sec 95°C / 30 sec 52°C / 1 min 68°C]; 5 min 68°C
Protists	Euk20f (5'-3') TGC CAG TAG TCA TAT GCT TGT Euk519r (5'-3') ACC AGA CTT GYC CTC CAA T	Reverse transcription: 20 min 45°C PCR: 5 min 94°C; 25 cycles [30 sec 94°C / 30 sec 52°C / 1 min 70°C]; 5 min 70°C
Fungi	Random hexamers nu-SSU-0817-5'-FAM TTAGCATGGAATAATRRAATAGGA nu-SSU-1536-3' ATTGCAATGCYCTATCCCCA	Reverse transcription: 10 min 25°C, 30 min 60°C, 5 min 80°C PCR: 2 min 94°C, 35 cycles [45 sec 94°C / 45 sec 51°C / 1 min 72°C]; 10 min 72°C

Supplementary Methodological Discussion

Our approach involves the interpretation of rRNA labeling via taxon-specific pyrotag abundances in density-resolved gradient fractions. This is an advance of classical gradient interpretation based on fingerprinting (Lueders et al 2004b, Lueders et al 2006) in line T-RF 'subtraction values' in 'heavy' vs. 'light' rRNA fractions recently introduced by Zumsteg et al. (2013). A cautionary discussion of this methodological approach is to be found here.

Most of the pitfalls questioning the reproducibility and semi-quantitative rigor of pyrosequencing libraries reported to date concern less abundant taxa and rare OTUs, and the reliability at which they can be recovered (Gihring et al 2012, Lee et al 2012, Pinto and Raskin 2012). We have recently reported a strong reproducibility of OTU abundances across biologically replicated pyrotag libraries for the employed bacterial pyrotag sequencing approach, and shown that relative read abundances can be semi-quantitatively meaningful for templates with abundances between 0.2% and 20% (Pilloni et al 2012). Our identification of labeled detritusphere microbes in this study relied on taxa with read abundances well within that range, thus we are confident that our approach for inferring taxon-specific rRNA enrichment factors provides robust information. Since fingerprinting of gradient fractions is a well-established tool to infer labeling in SIP, we chose to combine both, T-RFLP fingerprinting and pyrotag sequencing of fractions. The fact that we could actually link most of the important labeled taxa to T-RFs consistently enriched in heavy rRNA (Figs. S7.1 to S7.3) increases the confidence in our conclusions. Nevertheless, we want to caution that replicate SIP gradients and also pyrotag libraries were not analyzed in this study. SIP is, after all, not a quantitative but a qualitative method to identify microbes involved in a given carbon flow, at best providing some cautious information on comparative labeling intensity.

In contrast to bacterial pyrotag sequencing, the sequencing and interpretation of fungal and especially protistan pyrotag libraries is still not routine. In that respect our study represents an advance. Because of the rRNA-SIP approach, we used 18S rRNA markers for fungal community analysis, instead of the much more frequently used ITS sequencing. The primers used here have been successfully applied to characterize fungal communities in soils using T-RFLP fingerprints (Edel-Hermann et al 2009, Zumsteg et al 2012), Sanger sequencing (Chen et al 2012, Jumpponen 2003, Jumpponen 2011) as well as pyrosequencing (Arfi et al 2012, Becklin et al 2012). For protists, we are in fact introducing a new bidirectional pyrotag sequencing approach here, based on primers previously optimized for protistan T-RFLP fingerprinting in subsurface environments (Euringer and Lueders 2008). These were utilized

and analyzed analogously to our bacterial pyrotag pipeline. Therefore, in summary, we are confident that our general approach of inferring taxon-specific ^{13}C-labeling via comparative pyrotag enrichment in SIP gradients is sufficiently robust to support our conclusions and, backed up by 'classical' T-RF-based gradient analyses (Figs. S7.1 – S7.3), a consistent further development of well-established SIP gradient evaluation criteria (Whiteley et al 2006).

Supplementary References

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Supplementary Figures

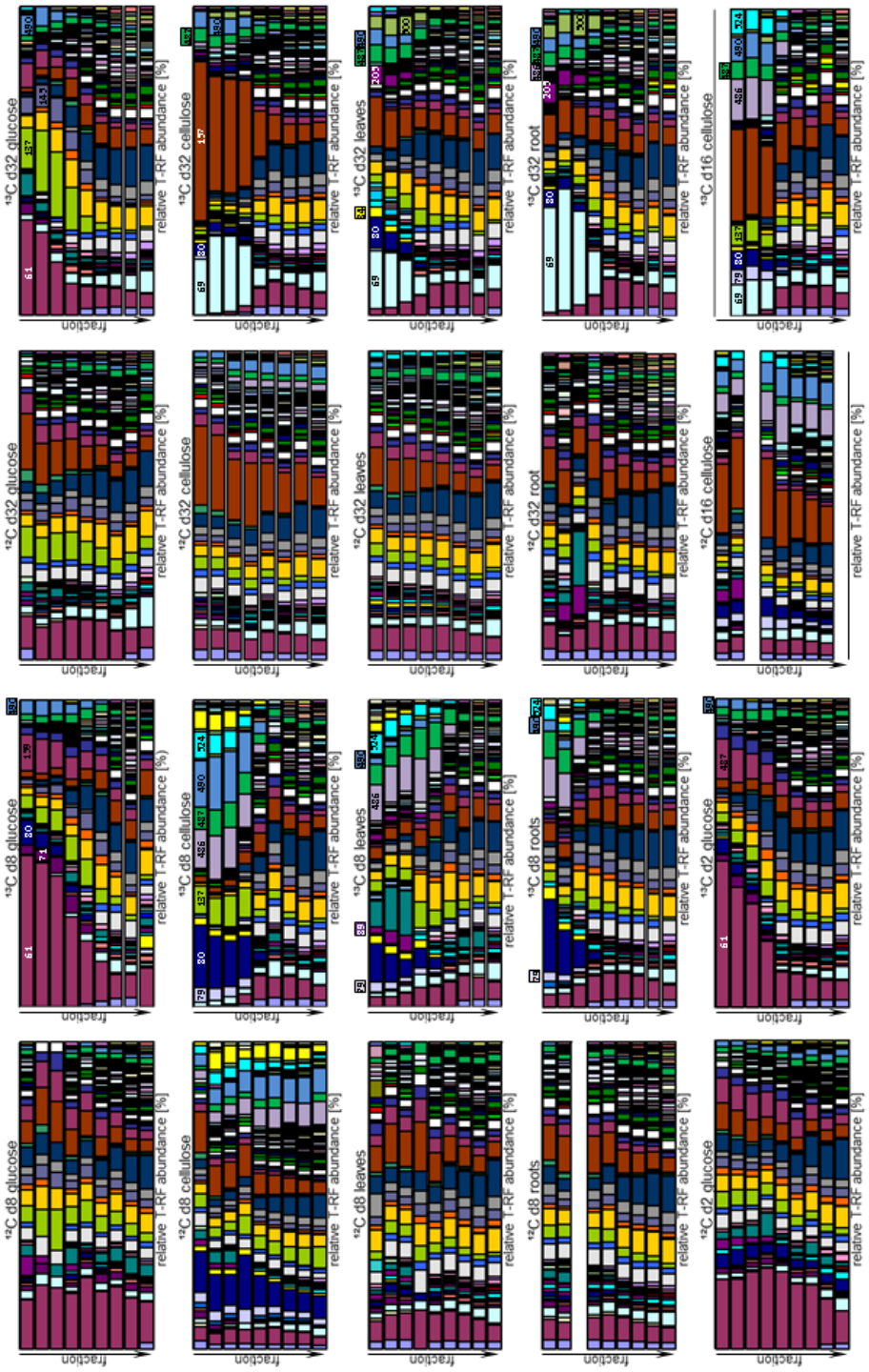

Figure S7.1 Bar plots of bacterial 16S rRNA T-RFLP fingerprints from SIP gradients. Arrows indicate 'heavy' to 'light' fractions 2 to 10 of rRNA gradients from the ^{12}C and ^{13}C treatments. Selected relevant T-RFs identified to represent labeled taxa and mentioned in the text are highlighted by numbers [fragment length in bp]. Relative abundance of all T-RFs is 100 %.

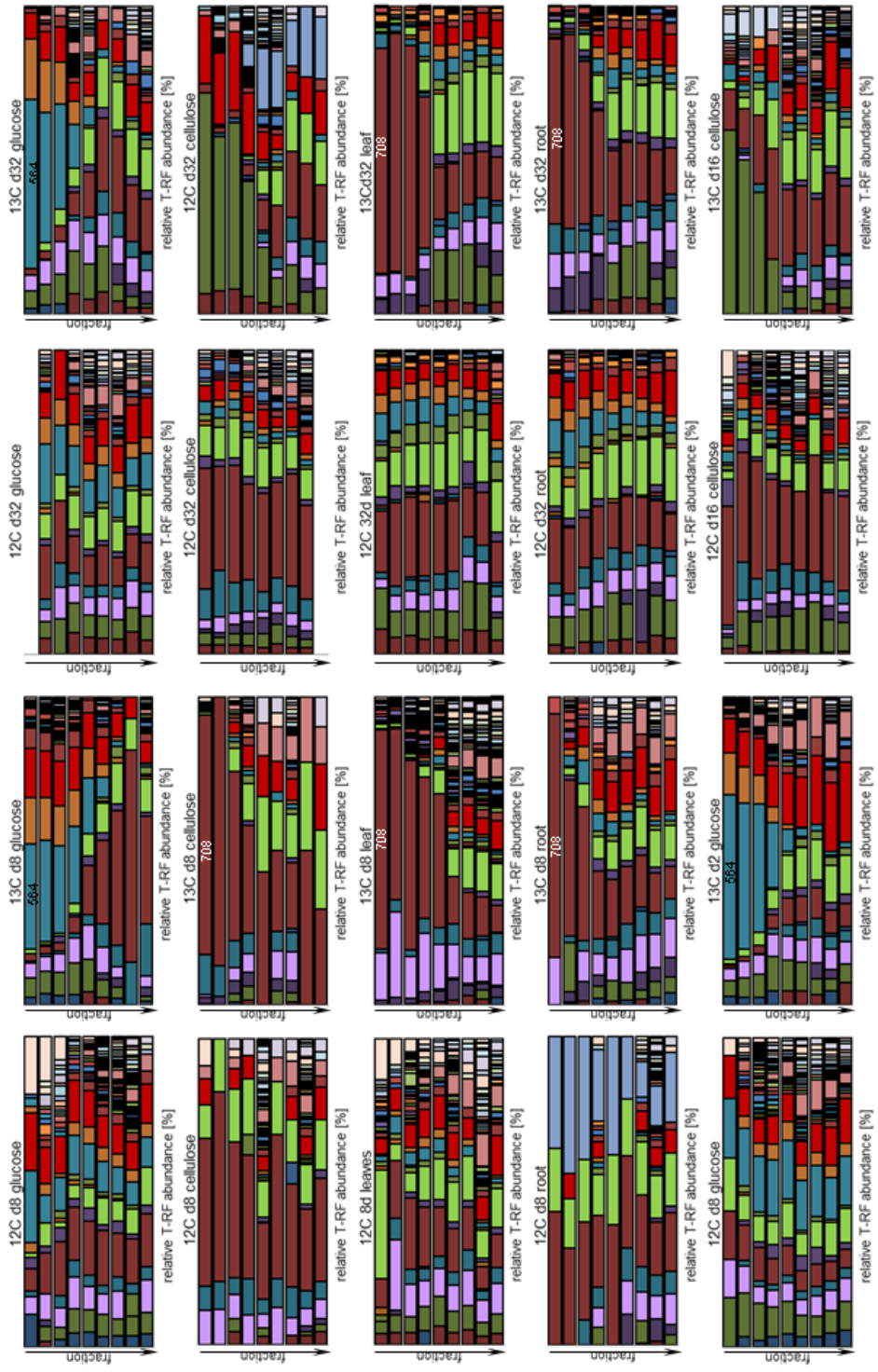

Figure S7.2 Bar plots of fungal 18S rRNA T-RFLP fingerprints from SIP gradients. Arrows indicate 'heavy' to 'light' fractions 2 to 10 of rRNA gradients from the ^{12}C and ^{13}C treatments. Selected relevant T-RFs identified to represent labeled taxa and mentioned in the text are highlighted by numbers [fragment length in bp]. Relative abundance of all T-RFs is 100 %.

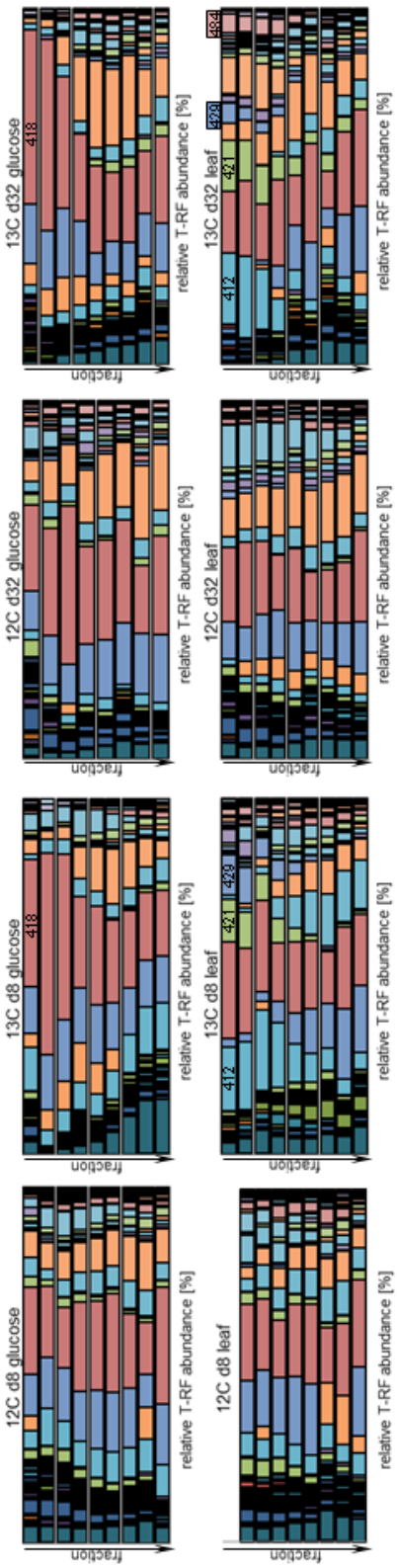

Figure S7.3 Bar plots of protist 18S rRNA T-RFLP fingerprints from SIP gradients. Arrows indicate 'heavy' to 'light' fractions 2 to 10 of rRNA gradients from the ^{12}C and ^{13}C treatments. Selected relevant T-RFs identified to represent labeled taxa and mentioned in the text are highlighted by numbers [fragment length in bp]. Relative abundance of all T-RFs is 100 %.

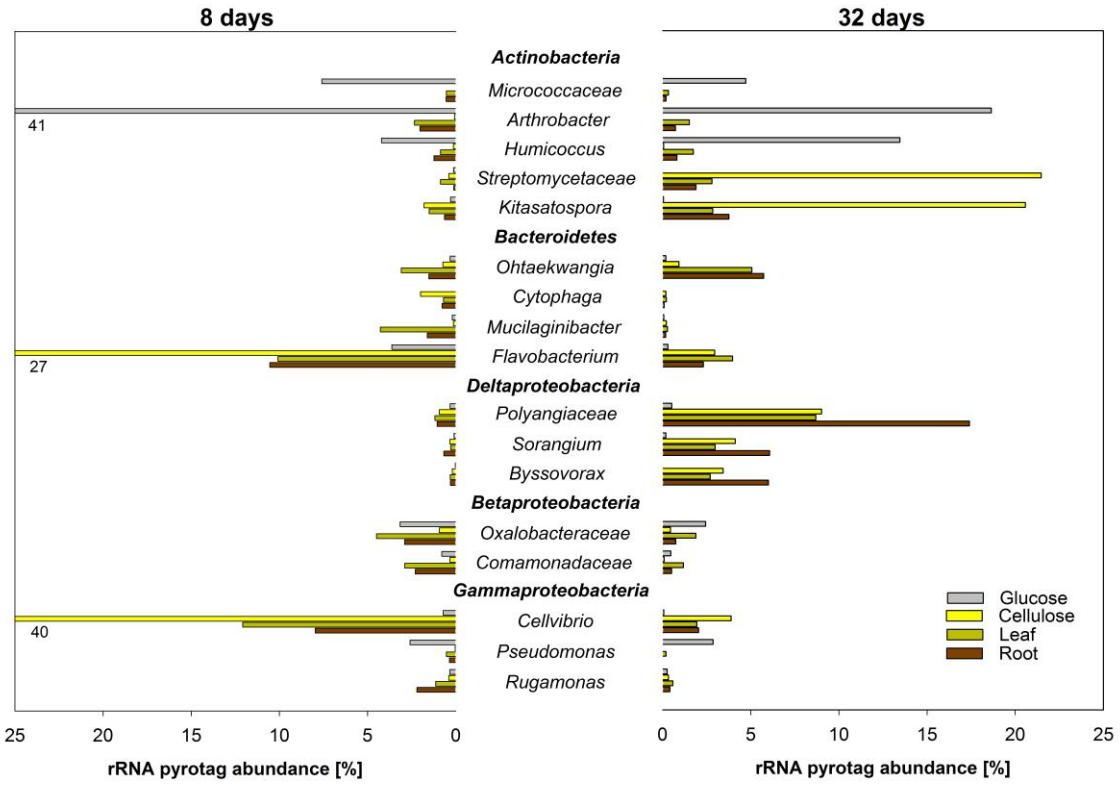

Figure S7.4 Relative abundance of ^{13}C-labeled bacterial taxa in 'heavy' rRNA after 8 and 32 days of incubation. Only labeled taxa as identified in Fig. 7.3 are shown.

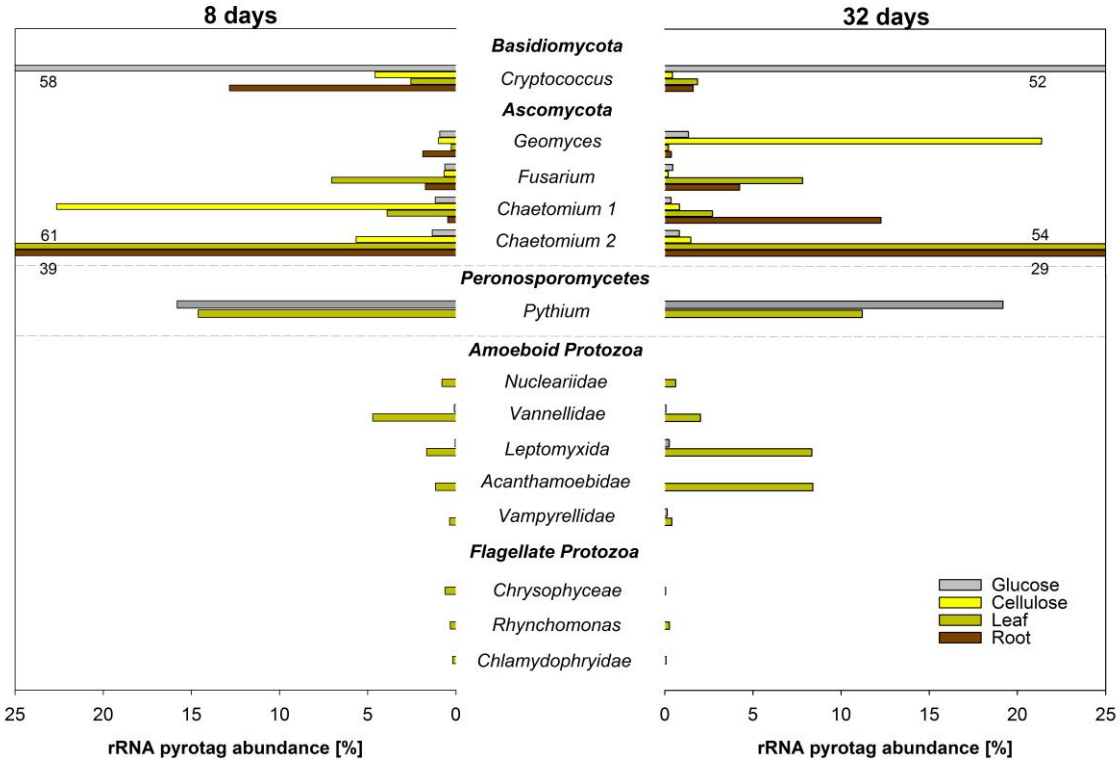

Figure S7.5 Relative abundance of ^{13}C-labeled fungal and protistan taxa in 'heavy' rRNA after 8 and 32 days of incubation. Only labeled taxa as identified in Fig. 7.4 are shown.

8 General Conclusions and final remarks

8.1 Substrate availability and its influence on abundance and function of microorganisms

Substrate availability changed with season, crop type, litter amendment and depth. Seasonal variability had a strong influence on microbial PLFA abundances and their enzyme activities in top- as well as in subsoil. This was likely due to climatic factors (temperature, moisture) and management (crop growth, litter amendment) and the corresponding changes in substrate availability. In winter especially, the relatively high abundances of bacterial and fungal PLFAs and enzyme activities in each soil layer irrespective of treatment indicated enhanced substrate availability. A release of resources due to climatic conditions (e.g. dead microbial cells, breakup of aggregates by freezing and thawing) in topsoil and transport of resources into deeper soil layers due to enhanced flow conditions after rain or thawing could have been the reason for this. The higher root biomass of wheat and therefore likely also higher rhizodeposition in comparison to maize enhanced the availability of resources in the wheat plots, which was also indicated by increased EOC. This resulted in increased bacterial and fungal PLFA abundances in wheat in comparison to maize cultivated plots to the bottom of the rooted layer (40-50 cm). However, higher enzyme activities in wheat than in maize cultivated plots were measured mainly in the 40-50 cm depth. The increased substrate availability due to litter amendment was observed only in the topsoil, indicating that litter-induced substrate availability played no role in deeper soil layers. Abundances of both bacterial and fungal PLFAs as well as enzyme activities remained elevated for nearly one year until the next litter application. At depth, bacterial and fungal PLFAs and hydrolytic enzyme activities decreased due to declining substrate availability in deeper soil layers. The depth distribution of oxidative enzyme activities, discussed below, showed a different pattern and could not be linked to substrate availability alone.

Although both bacteria and fungi profited from enhanced substrate availability in the topsoil (higher abundances of both in wheat and litter amended plots), fungi generally incorporated higher relative amounts of substrate C than did the total microbial biomass. This indicates that an increase in the abundance of microorganisms is not necessarily linked to their C assimilation efficiency of the respective resource.

8.2 Substrate quality and C assimilation by microorganisms

Substrate quality of rhizodeposits, aboveground litter and root litter differ. It was expected that high quality and more labile substrates (rhizodeposits) are used mostly by bacteria and that substrates of lower quality and higher recalcitrance (aboveground litter, root litter) are preferentially used by fungi.

We found that both bacteria and fungi assimilated C from substrates of different qualities. Surprisingly, irrespective of resource quality, fungi incorporated higher relative C amounts than the total microbial biomass at the field site. And although we did not measure the incorporation of C into bacteria directly, this indicates that bacteria, similar to fungi, assimilated root derived C as well as aboveground litter derived C, but to a lesser extent than fungi. This shows the high efficiency of fungi in assimilating resource C and supports the cited literature claims that fungal biomass contains more C and that fungi therefore have higher C needs in comparison to bacteria. But longer turnover times of fungi than of bacteria and / or a higher grazing pressure on bacteria could have contributed as well to our findings. Further studies that include higher trophic levels would help to quantify the C flow from bacteria and fungi and could help clarify the high accumulation of C in fungal biomass. Another surprise was that although root derived C was calculated as five times less than shoot litter derived C, both relative and absolute incorporated C amounts in the different soil pools were the same. This shows that root derived compounds were more strongly sequestered than aboveground litter derived C not only in SOM (Rasse et al. 2005) but also in microorganisms, supporting the findings of Puget & Drinkwater (2001).

In the NA-SIP microcosm experiment, bacteria, fungi and additionally *Peronosporomycetes* were involved in the degradation of all amended substrates irrespective of substrate recalcitrance and complexity. Although microbial diversity in the soil was high, we found that only a few key taxa contributed significantly to the degradation of the respective substrates. Among both bacteria and fungi, taxa that assimilated glucose differed from taxa which used the more complex and recalcitrant substrates. Surprisingly, although mineralization and assimilation of roots was lower than of leaves, indicating the different quality of the plant materials, we did not observe different key users. This suggests that the quality of complex substrates affects the decomposition rate but not the main degrading taxa, at least during early stages of decomposition.

Both, bacterivore and fungivore protozoa assimilated substrate derived C but the change in the user community of the bacterial community was more evident than of the fungal user community; it seems therefore that grazing affected the competitive ability of bacteria more than of fungi. The delayed enrichment in myxobacteria, which are known for feeding on bacterial biomass, indicated additional intra-bacterial grazing.

Since NA-SIP is a qualitative rather than a quantitative method, it is not possible to conclude which group, bacteria or fungi, incorporated higher amounts of the respective plant litter materials or if bacterivore or fungivore protozoa assimilated higher amounts of bacterial or fungal derived C.

8.3 Depth related effects on microorganisms and their function

Lower microbial abundances and hydrolytic enzyme activities at depth were explained by lower substrate availability. Interestingly, this did not hold true for oxidative enzymes, which showed either no depth gradient or even increased activity at depth. It is possible that because oxidative enzymes are mostly located on mineral surfaces, they were more strongly stabilized in deeper soil in comparison to hydrolytic enzymes which are mainly bound on particulate organic matter (POM). But if these stabilized enzymes were active *in situ* is uncertain.

Although different distribution patterns of hydrolytic and oxidative enzyme activities were observed with depth, the specific enzyme activities (enzyme activity per microbial biomass) of hydrolytic as well as oxidative enzymes increased in deeper soil layers. This indicated that in subsoil, either stabilization and / or production or efficiency of enzymes differs. Conditions such as greater interactions of enzymes and substrates with minerals or lower substrate availability, which could lead to greater spatial separation between microbes and substrates, would necessitate higher production or efficiency of enzymes. A microbial community shift at depth [shown by T-RFLP analyses in another study of this field experiment (Scharroba et al. 2013)] could indicate that taxa able to produce higher amounts or more efficient enzymes were present in deeper soil layers.

8.4 Final remarks

This thesis illustrates the value of stable isotope probing and demonstrates that abundance or diversity data alone do not suffice to explain decomposition processes or to identify microorganisms involved in a particular process. Although we detected high microbial

diversity in the soil, we identified only a few key players in every group (bacteria, fungi, *Peronosporomycetes*) involved in the degradation of the substrates.

We showed that both bacteria and fungi assimilate labile (such as exudates, glucose) as well as more recalcitrant (aboveground litter, roots) resources. Further, fungi are very important in decomposition of resources, as indicated by the high enrichment in the fungal biomass. The importance of fungi is generally assumed in forest and no-tillage agricultural sites. We demonstrated that also in agricultural sites with reduced tillage they are very efficient in assimilating resources of different qualities. Furthermore, due to their high enrichment, fungi could have a higher C sequestration potential based on their biomass alone than bacteria. However, since the methods we used are only adequate for relative rather than absolute comparisons of bacterial and fungal biomass, it was not possible to relate the absolute amounts of accumulated C by bacteria and fungi. In addition to availability and quality of resources, factors which are highly important in the topsoil, stabilization and spatial separation must be taken into account when considering decomposition processes in the subsoil.

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Studies	PhD thesis with the title: "Effects of resource availability and quality on soil microorganisms and their carbon assimilation" at the Institute of Soil Science and Land Evaluation, Soil Biology Section, University of Hohenheim	03/2009 – 03/2014
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Employment history	Scientific assistant at the Institute of Soil Science and Land Evaluation, Soil Biology Section, University of Hohenheim	Since 01/2013
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	Scientific assistant EU-Project: Development and evaluation of innovative tools to estimate the ecotoxicological impact of low dose pesticide application in agriculture on soil functional microbial biodiversity	05/2012 – 08/2012
	Internship Forschungsanstalt Agroscope Reckenholz-Tänikon ART, Zürich	09/2007 – 12/2007
Further Activities	Internship in England 2-weeks language course, 10-weeks voluntary work The Wildlife Trust for Birmingham and the Black Country (Leonardo da Vinci Programm)	09/2008 – 12/2008
	Internship Untere Naturschutzbehörde der Stadt Braunschweig	03/2008 – 08/2008

Stuttgart, 01.07.2014 Susanne Kramer

Publications and Presentations

This PhD thesis has been prepared cumulative. Parts of this PhD thesis are published, in preparation to publication, or otherwise presented as follows.

Publications and Manuscripts

Susanne Kramer, Dörte Dibbern, Julia Moll, Maike Huenninghaus, Robert Koller, Dirk Krueger, Sven Marhan, Tim Urich, Tesfaye Wubet, Michael Bonkowski, François Buscot, Tillmann Lueders, Ellen Kandeler. Eat all you can – Resource partitioning between bacteria, fungi and protists in detritusphere in an arable soil. Submitted to the International Society of Microbial Ecology.

Julia Moll, Kezia Goldmann, Susanne Kramer, Stefan Hempel, Ellen Kandeler, Dirk Krüger, Sven Marhan, Liliane Ruess and François Buscot. Resource quality and availability regulate fungal communities in arable soils across depth. Submitted to Microbial Ecology.

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Anika Scharroba, Dörte Dibbern, Maike Hünninghaus, Susanne Kramer, Julia Moll, Olaf Butenschoen, Michael Bonkowski, Francois Buscot, Ellen Kandeler, Robert Koller, Dirk Krüger, Tillmann Lueders, Stefan Scheu, Liliane Ruess, 2012. Effects of resource availability and quality on the structure of the micro-food web of an arable soil across depth. Soil Biology & Biochemistry 50, 1 – 11.

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Johanna Pausch, Susanne Kramer, Anika Scharroba, Nicole Scheunemann, Liliane Ruess, Olaf Butenschoen, Ellen Kandeler, Sven Marhan, Michael Riederer, Stefan Scheu, Yakov Kuzyakov. Plant carbon flow into the food web of an arable system as indicated by field ^{13}C pulse labeling. Manuscript in preparation.

Presentations

Susanne Kramer, Heike Haslwimmer, Nicole Scheunemann, Olaf Butenschoen, Liliane Ruess, Sven Marhan, Ellen Kandeler, 2013. Effects of resource availability and quality on soil microorganisms and their Carbon assimilation. Oral presentation, annual meeting of the Ecological Society of Germany, Potsdam, Germany.

Susanne Kramer, Julia Moll, Dörte Dibbern, Heike Haslwimmer, Francois Buscot, Dirk Krueger, Tesfaye Wubet, Tillmann Lueders, Sven Marhan, Ellen Kandeler. Identifizierung von Glukose und Cellulose nutzenden Bakterien und Pilzen während des Streuabbaus von Maisblättern und Maiswurzeln im Boden -ein Mikrokosmenexperiment, 2013. Oral presentation, annual meeting of the German Soil Science Society, Rostock, Germany.

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