Protists in the soil food web of an arable field:

Their density, taxonomic composition and functional role in carbon flux

I n a u g u r a l - D i s s e r t a t i o n

zur

Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Universität zu Köln

vorgelegt von

Maike Christina Hünninghaus

aus Engelskirchen

Köln 2015

Berichterstatter: Prof. Dr. Michael Bonkowski

(Gutachter) Prof. Dr. Hartmut Arndt

Tag der letzten mündlichen Prüfung: 26.11.2014

Für Hella, Gerlinde und
Runa Hünninghaus

Table of Contents

Abstract

Soil ecosystems are of fundamental importance for global carbon (C) cycling because they are the largest sinks and sources of terrestrial C. Therefore a detailed understanding of C fluxes through soil food webs is essential to predict ecosystem responses to anthropogenic CO_2 enrichment of the atmosphere. Because organic C is also fundamental to the fertility of arable soils understanding of C cycling is inevitable to develop sustainable soil management strategies.

Organic C enters the soil system via two different main routs: it is constantly released by plant roots in form of rhizodeposits and it enters the soil as dead organic matter (detritus). In both pathways C is directly acquired by diverse and highly active microorganisms. However, the C flux through microbial food webs is largely unknown predominantly due to the complex nature of trophic interactions.

This PhD thesis is focusing on the functional role of protozoa in the rhizosphere and detritusphere of agricultural systems. Protozoa being at the base of soil food webs are assumed to be key-players in controlling the C flux from bacteria to higher trophic levels. Nevertheless the community composition in soil is largely unknown and the ecological importance of different protozoan taxa is even less understood.

One aim of this thesis was to get deeper insights to the taxonomic composition and density of protozoan communities in arable soils. First the microbial food web was characterized at different soil depth and land management regimes. Further, the succession and functional roles of protozoan communities in controlling the flux of C in the rhizosphere and detritusphere was described in great detail and at high resolution.

This study revealed highly dynamic and complex microbial food web interactions in soil and confirms the key-role of protozoa for the flow of C in agricultural soils.

Kurzzusammenfassung

Bodenökosysteme sind von fundamentaler Bedeutung für den weltweiten Kohlenstoffkreislauf. Böden gelten als größte Speicher für terrestrischen Kohlenstoff, setzen gleichzeitig aber auch immense Mengen an CO_2 frei. Daher ist ein detailliertes Verständnis der Kohlenstoffflüsse durch die Bodennahrungsnetze unumgänglich um Ökosystemreaktionen auf anthropogene Anreicherungen der Atmosphäre mit Treibhausgasen vorauszusagen. Weil organischer Kohlenstoff auch für die Fruchtbarkeit landwirtschaftlich genutzter Böden eine wichtige Rolle spielt, wird dieses Wissen außerdem gebraucht, um nachhaltigere Management-Strategien für Agrarökosysteme zu entwickeln.

Organische Kohlenstoffverbindungen gelangen über zwei Hauptwege in die Böden: Sie werden kontinuierlich von Pflanzen in Form von Rhizodepositen über die Wurzeln freigesetzt und abgestorbene Reste von Lebewesen werden als Detritus zersetzt. Beiden Wegen ist gemein, dass der Kohlenstoff direkt von diversen und hochgradig aktiven Mikroorganismen aufgenommen wird. Der weitere Fluss dieses Kohlenstoffs durch die multitrophischen Nahrungsnetze im Boden ist bis heute weitgehend ungeklärt. Bedingt wird dies hauptsächlich durch die komplexe Natur trophischer Interaktionen.

Der Fokus dieser Dissertation liegt auf der funktionellen Rolle der Protozoen in der Rhizosphäre und Detritussphäre von Agrarökosystemen. Protozoen stellen die Basis der Boden-Nahrungsnetze dar und es wird angenommen, dass sie das Hauptbindeglied des Kohlenstoffflusses zwischen der Bakteriengemeinschaft und höheren trophischen Ebenen sind. Nichtsdestotrotz ist die genaue Zusammensetzung der Gemeinschaften dieser diversen Organismengruppe im Boden weitgehend unbekannt und die ökologische Bedeutung einzelner Protozoentaxa ist sogar noch weniger verstanden.

Ein Ziel der vorliegenden Arbeit war es neue Einblicke in die taxonomische Zusammensetzung und Populationsdichten von Protozoengemeinschaften in Agrarökosystemen zu gewinnen. Als erstes wurde das mikrobielle Nahrungsnetz in verschiedenen Bodentiefen und unter unterschiedlichen Managementbedingungen charakterisiert. Anschließend wurden die Sukzession und die funktionellen Rollen der Protozoengemeinschaften in der Kontrolle von pflanzenbürtigem bzw. von detritusbürtigem Kohlenstoff detailliert und in hoher Auflösung betrachtet.

Diese Dissertation deckt die hochgradig dynamischen und komplexen mikrobiellen Nahrungsnetz-Interaktionen im Boden auf und unterstreicht die Schlüsselrolle der Protozoen für den Kohlenstoffffluss in landwirtschaftlich genutzten Böden.

General Introduction

Carbon flow and microbial food webs in the soil

The flow of carbon (C) within ecosystems is a major area of interest in ecology (Cheng and Gershenson 2007). Recently C cycling belowground is increasingly recognized as one of the most significant components of the global C cycle (Cheng and Gershenson 2007, Zak and Pregitzer 1998). Soils host the largest terrestrial C pool throughout the entire soil profile (Lorenz and Lal 2005), second only to that of the ocean (Stockmann et al. 2013). Agricultural systems represent the major form of land management, covering about 5 billion ha of the global terrestrial land area (Drinkwater and Snapp 2011). Conversions of natural soils into arable fields are often followed by soil erosion and degradation of organic C pools (Lal 2004) which fundamentally reduces the fertility of arable soils. An understanding of C cycling in these particular systems is inevitable to develop sustainable soil management strategies. Yet the amount and composition of global soil C stock remains very uncertain (Scholes et al. 2009) and large parts of the distribution and sequestration of C within the soil is still a black-box for scientists.

When organic C enters the soil system it is directly acquired by microorganisms. The abundance and diversity of soil microbiota represents the greatest reservoir of biological diversity world-wide (Berendsen et al. 2012, Lopez-Guerrero et al. 2013, Curtis et al. 2002, Buee et al. 2009, Gams et al. 2007). Soil microbiota form multitrophic food webs which are central to different biogeochemical cycles (Gessner et al. 2010, Nielsen et al. 2011), especially to the C cycle as 85-90% of CO_2 leaving the soil is produced by microorganisms (Blagodatskaya 2014)

Nevertheless, the flux of C through these microbial food webs and the role of different biota are still largely unidentified and the general relationship between soil biodiversity and ecosystem functioning remains largely unknown (Bradford et al. 2014). Especially for deeper soil layers the contribution of microorganisms to C dynamics has yet received very little attention (Kramer et al. 2013, Rumpel and Kögel-Knaber 2011), even though it is estimated that over 50% of the global organic C pools in soil are found below 30cm depth (Jobbagy and Jackson 2000).

Most organic matter enters the soil in the decomposer pathway (Cebrian 2004). During decomposition plant and animal residues are initially broken down into small particles of

largely intact material which is then repeatedly recycled by the soil microbial community (Stockmann et al. 2013). This decomposing organic material is referred to as detritus. It is estimated that 80-90% of plant primary production enters the soil system as detritus (Bardgett 2005).

For a long time it was assumed that detritus was the sole source of C driving the decomposer pathway but there is also a second C route into belowground food webs: Rhizodeposition. Rhizodeposition accounts for 5–33% of daily photoassimilates (Jones et al. 2009) and was first defined by Whipps and Lynch (1985) as all material lost from plant roots. Rhizodesposits fall into two categories: those that are passively released by diffusion and those that have functional significance and at least some degree of regulation of their exudation by plant roots (Bonkowski and Clarholm 2012, Jones et al. 2004; Paterson et al. 2007). The rhizosphere, the thin soil layer directly attached to the root, is characterized by large fluxes of nutrients (rhizodeposition) and by high niche diversity (Bardgett 2005). Rhizosphere hosts a rich microbial community with significantly higher rates of metabolism and microbial biomass relative to bulk soil (Griffith 1990, Alphei et al. 1996, Bonkowski and Clarholm 2012). Several studies (e.g. Polierer et al. 2007, Albers 2006) indicate a significant role of root derived C in driving the soil food webs. Pollierer et al. (2007) for example found that most soil animal taxa predominantly took up root derived C instead of leaf litter C. This is in contrast to the, until then, common assumption that leaf litter serves as the major source of C for the soil community.

Parallel to the discrimination between rhizosphere and detritusphere the nutrient flow into soil food webs is classically divided into two separate energy channels: the bacterial energy channel where about 30% of plant derived C is metabolized in form of easily degradable substrates (e.g. Holtkamp et al. 2011, Moore et al. 2005, Jones 1998) and the fungal energy channel in charge of the degradation of recalcitrant organic materials favored by a wide C to N ratio (e.g. De Boer et al. 2005, Frankland 1998, Dilly et al. 2001). It is assumed that the fast bacterial energy channel dominates the utilization of rhizodeposits and the early stages of detritus decomposition while the much slower fungal energy channel is most important in the second half of detritus decomposition. Despite the role of soil organisms in decomposing organic matter their community structure and diversity is rarely included explicitly in models of C cycling (Nielsen et al. 2010).

Protozoa in soil

A significant part of the soil microbial community is top-down controlled by protozoan grazers. Protozoa are heterotrophic, unicellular eukaryotes with rapid turnover and high abundances in soil. Finlay et al. (2000) for example, recorded 365 protozoan species with average potential numbers of 46,400 flagellates, 17,700 naked amoebae, 11,000 testate amoebae and 4300 ciliates in 1g of arable field soil. Protozoa are globally distributed and inhabit all kinds of aquatic and terrestrial habitats (e.g. Ekelund and Rønn 1994, Finlay et al. 2000). Especially in terrestrial systems most protozoa have the ability to form drought resistant resting cysts to survive harsh environmental conditions (e.g. Rivera et al. 1992).
However, we have only scarce knowledge of the diversity of soil protozoa, their specific functional roles and there distribution along the soil profile.
Despite their small size protozoa are the major consumers of bacteria in soil. They are at the base of the heterotrophic soil food webs, supposed to control the C flux up to higher trophic levels, predominantly in the bacterial energy channel (Ekelund and Rønn 1994, Bonkowski 2004). Especially in the rhizosphere, where protozoan densities are up to 35 times higher than in bulk soil, bacteria are strongly top-down controlled by protozoa (Clarholm 1994, Bonkowski 2004). Protozoan grazing is known to keep bacteria in a more active state by promoting nutrient recycling and microbial turnover, thereby stimulating C cycling through the soil food webs (Alphei et al. 1996, Bonkowski et al. 2000, Bonkowski 2004). Grazing induced changes in microbial community composition affect fundamental ecosystem properties because soil bacteria occupy some of the most important control points for nutrient cycling and plant growth (Rosenberg et al. 2009, Krome et al. 2009, Bonkowski and Brandt 2002). Nevertheless there is only scarce information about the exact structure of microbial soil food webs in the rhizosphere and detritusphere in general and the position and role of protozoa in particular.
Although protozoa are taxonomically very diverse and originate from many different phyla (Adl et al. 2005) they are traditionally parted into three principal “functional groups”: Ciliates, flagellates and amoebae (amoebae are sometimes further distinguished into testate and naked amoebae; Darbyshire 1994) but only ciliates form a monophyletic group (Adl et al. 2012). In terms of feeding behaviour protozoa are also very diverse. Even though they are almost exclusively described as bacterial feeders in soil food web models (e.g. Hunt et al. 1987, De Ruiter et al. 1995) they in fact utilize different food sources. Some are omnivorous (Geisen 2014, Hess et al. 2012), others are strictly mycophagous (Geisen et al. in prep.) or saprophagous (Couteaux and Darbyshire 1998). The tremendous species

richness of soil protozoa has led to the assumption that functional redundancy is great among them and they are often even treated as a homogenous functional guild. On the other hand it is indicated, that protozoa feed selectively but there is a lack of clear evidence if functional differences exist between species with regard to C cycling (Nielsen et al. 2010). However, there is an urgent need for more detailed studies on the mechanisms of protozoan predation (Bonkowski and Clarholm 2012). Because of the lack of reliable data with high taxonomic resolution, protozoa are underrepresented in comparative food web studies (Brose and Scheu 2014, Kefi et al. 2012, Thomson et al. 2012).

Methods

The complex nature of trophic interactions, especially at the small scale, and the tremendous species richness of soil microbes are a challenge when investigating microbial soil food web interactions. For a long time protozoan communities were only addressed with direct counting methods or cultivation approaches. Both are very laborious and require a high taxonomic expertise. However, many protozoa cannot be directly extracted from soil and each protozoan group needs specific observation conditions (Finlay et al. 2000, Ekelund and Rønn 1994). Additionally most soil protozoa form resting cysts, so abundances determined by cultivation techniques are not really able to separate active and inactive protozoan communities. In order to standardize the variety of methods Finlay and co-workers developed a protocol combining and expanding earlier methods to quantify and identify protozoan morphotypes in soil (Finlay et al. 2000) which is applied in this thesis.

Molecular tools like high throughput sequencing deliver a high taxonomical resolution. Nevertheless these techniques need reference databases and information about morphology from direct observation, making cultivation based approaches indispensable. An additional drawback of molecular techniques is the lack of specific molecular genetic markers for species of several protozoan taxa.

For the analysis of microbial soil food webs and major energy pathways the application of ^{13}C Stable Isotope Probing (SIP) has opened a window to understanding. The analysis of incorporation of diverse energy rich compounds into different trophic levels in the microbial soil food web gives new information about the general utilization of these substrates. Combined with high throughput sequencing it enables the identification of trophic food web structure of the active microbial community in high taxonomic resolution and allows us to identify important protozoan taxa in the rhizosphere and detritusphere.

Aims and structure of the thesis

The overall goal of this PhD thesis is to understand the flux of C through the highly dynamic and diverse microbial food webs in arable soil. Special emphasis is placed on the functional role of protozoan communities as critical links for the C transfer from bacteria to higher trophic levels. In order to shed a light on abundances, diversity, feeding behavior and ecological function of protozoa in high resolution a variety of different methods was applied and field studies as well as different laboratory experiments were conducted.

Chapter I: The first chapter investigates the effects of resource availability and quality on the structure of the soil microbial food web. Abundances and community composition of bacteria, fungi, protozoa and nematodes were investigated across a depth gradient of an arable field soil (plough layer, rooted soil below plough horizon and deeper root free soil) planted with maize and wheat and displaying different management strategies. A cultivation approach after Finlay et al. (2000) was used to determine and quantify the diversity of soil protozoan morphotypes.

Chapter II: In the second chapter links in the microbial food webs of maize (*Zea mays*) rhizosphere and bulk soil were addressed by rRNA-SIP in tandem with next generation sequencing. The succession of root derived C through bacterial, fungal and protist consumer communities and their protozoan grazers on the next trophic level were assessed in high resolution. In this laboratory experiment temporal dynamics were analyzed, as well as C transfer from the plant into rhizosphere and bulk soil.

Chapter III: After addressing the microbial food webs in the rhizosphere in chapter II, chapter III describes the resource partitioning between bacteria, fungi and protists in the detritusphere. Again rRNA-SIP and next generation sequencing were used, this time to identify key-players in the decomposition of four different ^{13}C labeld C sources.

Chapter IV: The last chapter aimed at the effects of protozoan identity and species richness on the community composition and mineralization capacity of a microbial decomposer community. In two laboratory experiments the microbial decomposition of maize litter in presence of different protozoan grazer communities was followed over time. Additionally we tested whether the most abundant morphotypes of soil protozoa form distinguished functional groups.

References

Adl, S. M., Simpson, A. G., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R., Bowser, S. S., Brugerolle, G., Fensome, R. A., Fredericq, S., James, T. Y., Karpov, S., Kugrens, P., Krug, J., Lane, C. E., Lewis, L. A., Lodge, J., Lynn, D. H., Mann, D. G., McCourt, R. M., Mendoza, L., Moestrup, O., Mozley-Standridge, S. E., Nerad, T. A., Shearer, C. A., Smirnov, A. V., Spiegel, F. W., and Taylor, M. F., (2005) The new higher level classification of Eukaryotes with emphasis on the taxonomy of Protists. *Journal of Eukaryotic Microbiology*: 52-399

Adl, S. M., Simpson, A. G., Lane, C. E., Lukeš, J., Bass, D., Bowser, S. S., Brown, M.W., Burki, F., Dunthorn, M., Hampl, V., Heiss, A., Hoppenrath, M., Lara, E., le Gall, L., Lynn, D. H., McManus, H., Mitchell, E. A. D., Mozley-Stanridge, S. E., Parfrey, L. W., Pawlowski, J., Rueckert, S., Shadwick, L., Schoch, C. L., Smirnov, A., and Spiegel, F. W., (2012) The revised classification of eukaryotes. *Journal of Eukaryotic Microbiology 59*(5): 429-514

Albers, D., Schaefer, M., and Scheu, S., (2006) Incorporation of plant carbon into the soil animal food web of an arable system. *Ecology 87*(1): 235-245

Alphei, J., Bonkowski, M., and Scheu, S., (1996) Protozoa, Nematoda and Lumbricidae in the rhizosphere of Hordelymus europeaus (Poaceae): faunal interactions, response of microorganisms and effects on plant growth. *Oecologia 106*(1): 111-126

Bardgett, R., (2005) The biology of soil – A community and ecosystem approach. *Oxford University Press*

Berendsen, R. L., Pieterse, C. M. J., and Bakker, P. A. H. M., (2012) The rhizosphere microbiome and plant health. *Trends in Plant Science* 17(8):476-486

Blagodatskaya, E., Microbial interactions effect sources of priming induced by cellulose. Vortrag im Rahmen des *Ökologischen Kolloquiums des Zoologischen Instituts* am 09.07.2014, Universität zu Köln

Bonkowski, M., Griffiths, B., and Scrimgeour, C., (2000) Substrate heterogeneity and microfauna in soil organic 'hotspots' as determinants of nitrogen capture and growth of ryegrass. *Applied Soil Ecology 14*(1): 37-53

Bonkowski, M., and Brandt, F., (2002) Do soil protozoa enhance plant growth by hormonal effects?. *Soil Biology and Biochemistry 34*(11): 1709-1715

Bonkowski, M., (2004) Protozoa and plant growth: the microbial loop in soil revisited. *New Phytologist* 162: 617-631

Bonkowski, M., and Clarholm, M., (2012) Stimulation of plant growth through interactions of bacteria and protozoa: testing the auxiliary microbial loop hypothesis. *Acta Protozoologia 51*: 237-247

Bradford, M. A., Wood, S. A., Bardgett, R. D., Black, H. I., Bonkowski, M., Eggers, T., Graystone S. J., Kandeler, E., Manning, P., Setälä, H., and Jones, T. H., (2014) Discontinuity in the responses of ecosystem processes and multifunctionality to altered soil community composition. *Proceedings of the National Academy of Sciences*: 201413707

Brose, U., and Scheu, S., (2014) Into darkness: unravelling the structure of soil food webs. *Oikos 123*(10): 1153-1156

Buee, M., Reich, M., Murat, C., Morin, E., Nielsson, R. H., Uroz, S., and Martin, F., (2009) 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist* 184: 449–456

Cebrian, J., (2004) Role of first-order consumers in ecosystem carbon flow. *Ecology Letters 7*(3): 232-240

Cheng, W., and Gershenson, A., (2007) Carbon fluxes in the rhizosphere. In: *The rhizosphere: An ecological perspective*: 31-56

Clarholm, M., (1994) The microbial loop in soil. In: Ritz, K., Dighton, J., and Giller, K. E., (eds.) Beyond the biomass. *J. Wiley, New York*: 221-230

Coûteaux, M. M., and F Darbyshire, J., (1998) Functional diversity amongst soil protozoa. *Applied Soil Ecology 10*(3): 229-237

Curtis, T. P., Sloan, W. T., and Scannell, J. W., (2002) Estimating prokaryotic diversity and its limits. *Proc. Natl. Acad. Sci. USA* 99, 10494–10499

Darbyshire, J. F., (1994) Soil protozoa. *Cab International*

De Boer, W. D., Folman, L. B., Summerbell, R. C., and Boddy, L., (2005) Living in a fungal world: impact of fungi on soil bacterial niche development⋆. *FEMS microbiology reviews 29*(4): 795-811

De Ruiter, P. C., Neutel, A.-M., and Moore, J. C., (1995) Energetics, pattern of interaction strength and stability in real ecosystems. *Science* 269: 1257-1260

Dilly, O., Bartsch, S., Rosenbrock, P., Buscot, F., and Munch, J.C., (2001) Shifts in physiological capabilities of the microbiota during the decomposition of leaf litter in a black alder (*Alnus glutinosa* (Gaertn.) L.) forest. *Soil Biology and Biochemistry* 33: 921-930

Drinkwater, L., and Snapp, S., (2011) Understanding and managing the rhizosphere in agroecosystems. in *The rhizosphere: an ecological perspective*. Academic press. Cardon, Z. G., and Whitbeck, J. L., (Eds.).

Ekelund, F., and Rønn, R., (1994) Notes on protozoa in agricultural soil with emphasis on heterotrophic flagellates and naked amoebae and their ecology. *FEMS Microbiology Reviews* 15: 321-353

Finlay, B., Black, H., Brown,S., Clarke, K., Esteban, G., Hindle, R., Olmo, J., Rollett, A., and Vickerman, K., (2000) Estimating the growth potential of the soil protozoan community. *Protist* 151: 69-80

Frankland, J. C., (1998) Fungal succession—unravelling the unpredictable. *Mycological Research* 102: 1-15

Gams, W., (2007) Biodiversity of soil-inhabiting fungi. *Biodivers. Conserv*. 16: 69–72

Geisen, S., (2014) Soil Protists - Diversity, Distribution and Ecological Functioning, PhD thesis, University of Cologne: 1-273

Geisen, S., Hünninghaus, M., Dumack, K., Koller, R., Urich, T., and Bonkowski, M., (in prep.) The neclected link in soil food webs: mycophagous protists

Gessner, M. O., Swan, C. M., Dang, C. K., McKie, B. G., Bardgett, R. D., Wall, D. H., and Hättenschwiler, S., (2010) Diversity meets decomposition. *Trends in Ecology & Evolution 25*(6): 372-380

Griffiths, B. S., (1990) A comparison of microbial-feeding nematodes and protozoa in the rhizosphere of different plants. *Biology and Fertility of Soils 9*(1): 83-88

Hess, S., Sausen, N., and Melkonian, M., (2012) Shedding light on vampires: The phylogeny of vampyrellid amoebae revisited. *PLoS ONE* **7:** e31165

Holtkamp, R., van der Wal, A., Kardol, P., van der Putten, W.H., de Ruiter, P.C., and Dekker, S.C., (2011) Modelling C and N mineralisation in soil food webs during secondary succession on ex-arable land. *Soil Biology and Biochemistry* 43: 251-260

Hunt, H. W., Coleman, D. C., Ingham, E. R., Ingham, R. E., Elliott, E. T., Moore, J. C., Rose, S. L., Reid, C. P. P., and Morley, C. R. (1987) The detrital food web in a shortgrass prairie.*Biology and Fertility of Soils 3*(1-2): 57-68

Jobbágy, E. G., and Jackson, R. B., (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological applications 10*(2): 423-436

Jones, D. L., (1998) Organic acids in the rhizosphere–a critical review. *Plant and soil 205*(1): 25-44

Jones, D. L., Hodge, A., and Kuzyakov, Y., (2004) Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist 163*(3): 459-480

Jones, D. L., Nguyen, C., and Finlay, R. D., (2009) Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant and Soil 321*(1-2): 5-33

Kéfi, S., Berlow, E. L., Wieters, E. A., Navarrete, S. A., Petchey, O. L., Wood, S. A., Boit, I., Joppa, L. N., Lafferty, K. D., Williams, R. J., Martinez, N. D., Menge, B. A., Blanchette, C. A., Iles, A. C., and Brose, U., (2012) More than a meal… integrating non-feeding interactions into food webs. *Ecology letters 15*(4): 291-300

Kramer, S., Marhan, S., Haslwimmer, H., Ruess, L., and Kandeler, E., (2013) Temporal variation in surface and subsoil abundance and function of the soil microbial community in an arable soil. *Soil Biology and Biochemistry 61*: 76-85

Krome, K., Rosenberg, K., Bonkowski, M., and Scheu, S., (2009) Grazing of protozoa on rhizosphere bacteria alters growth and reproduction of *Arabidopsis thaliana*. *Soil Biology and Biochemistry 41*(9): 1866-1873

Lal, R., (2004) Soil carbon sequestration impacts on global climate change and food security. *Science 304*(5677): 1623-1627

Lopez-Guerrero, M. G., Ormeno-Orrillo, E., Rosenblueth, M., Martinez-Romero, J., and Martinez-Romero, E., (2013) Buffet hypothesis for microbial nutrition at the rhizosphere. *Frontiers in Plant Science* 4(188):1-4

Lorenz, K., and Lal, R., (2005) The depth distribution of soil organic carbon in relation to land use and management and the potential of carbon sequestration in subsoil horizons. *Advances in agronomy 88*: 35-66

Moore, J. C., McCann, K., and de Ruiter, P. C., (2005) Modeling trophic pathways, nutrient cycling, and dynamic stability in soils. *Pedobiologia 49*(6): 499-510

Nielsen, U. N., Ayres, E., Wall, D. H., and Bardgett, R. D., (2011) Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity–function relationships. *European Journal of Soil Science 62*(1): 105-116

Paterson, E., Gebbing, T., Abel, C., Sim, A., and Telfer, G., (2007) Rhizodeposition shapes rhizosphere microbial community structure in organic soil. *New Phytologist 173*(3): 600-610

Pollierer, M. M., Langel, R., Körner, C., Maraun, M., and Scheu, S., (2007) The underestimated importance of belowground carbon input for forest soil animal food webs. *Ecology Letters 10*(8): 729-736

Rivera, F., Lugo, A., Ramirez, E., Bonilla, P., Calderon, A., Rodriguez, S., Ortiz, R., Gallegos, E., Labastida, A., and Chavez, M.P., (1992) Seasonal distribution of air-borne Protozoa in Mexico-City and its suburbs. *Water Air and Soil Pollution* 61: 17-36

Rosenberg, K., Bertaux, J., Krome, K., Hartmann, A., Scheu, S., and Bonkowski, M., (2009) Soil amoebae rapidly change bacterial community composition in the rhizosphere of *Arabidopsis thaliana*. *The ISME journal 3*(6): 675-684

Rumpel, C., and Kögel-Knabner, I., (2011) Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. *Plant and Soil 338*(1-2): 143-158

Scholes, R. J., Monteiro, P. M. S., Sabine, C. L., and Canadell, J. G., (2009) Systematic long-term observations of the global carbon cycle. *Trends in ecology & evolution 24*(8): 427-430

Stockmann, U., Adams, M. A., Crawford, J. W., Field, D. J., Henakaarchchi, N., Jenkins, M., Minasny, B., McBratney, A. B., de Remy de Courcelles, V., Singh, K., Wheeler, I., Abbott, L., Angers, D. A., Baldock, J., Bird, M., Brookes, P. C., Chenu, C., Jastow, J. D., Lal, R., Lehmann, J., O'Donnell, A. G., Parton, W. J., Whitehaed, D., and Zimmermann, M., (2013) The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agriculture, Ecosystems & Environment 164*: 80-99

Thompson, R. M., Brose, U., Dunne, J. A., Hall Jr, R. O., Hladyz, S., Kitching, R. L., Martinez, N. D., Rantala, H., Romanuk, T. N., Stouffer, D. B., and Tylianakis, J. M., (2012) Food webs: reconciling the structure and function of biodiversity. *Trends in ecology & evolution 27*(12): 689-697

Whipps, J. M., and Lynch, J. M., (1985) Energy losses by the plant in rhizodeposition. Plant products and the new technology *Ann. Proc. Phytochem. Soc. Eur*. 26: 59-71

Zak, D. R., and Pregitzer, K. S., (1998) Integration of ecophysiological and biogeochemical approaches to ecosystem dynamics. In Successes, Limitations, and Frontiers in Ecosystem Science. *Springer New York*: 372-403

Chapter I: Effects of resource availability and quality on the structure of the micro-food web of an arable soil across depth*

Abstract

Soil food webs are important determinants for the C flow through terrestrial systems, with the trophic networks between microbes and microfaunal grazers forming the basis for processing plant resources. At an agricultural field site cropped with maize or wheat, plant C input to soil was experimentally manipulated by amendment with maize litter as detritus. The community structure of dominant micro-food web components, the bacteria, fungi, protozoa and nematodes, was investigated across a depth gradient comprising plough layer, rooted soil below plough horizon, and deeper root free soil. The community composition and diversity within micro-food webs, and the response to resource supply, were assessed in summer, the vegetation period with highest root exudation. In the plough layer amendment with plant residues increased microbial biomass as well as density of fan shaped amoeba morphotypes and of bacterial- and fungal-feeding nematodes. Diversity of food web assemblages was assessed by operational taxonomic units (OTU) for bacteria and fungi, protozoan morphotypes and nematode families. Changes in diversity were either not apparent (fungi, protozoa), negatively related to detritus enrichment (bacteria) or positively linked to crop plant (bacteria, nematodes). Based on the nematode faunal analysis concept general food web conditions were assigned as nutrient enriched, with a high degree of disturbance, and a dominance of the bacterial energy channel. In sum, detritus amendment fostered the abundance but not the diversity of organisms as food webs remained bottom heavy with only small amounts of C conserved at higher trophic levels. Food web structure was more affected by the abiotic (soil profile) and biotic (crop plant) environment than by the supply with detritus resources.

* for information about Co-authorships see page 154

Introduction

Soils harbour an enormous diversity of organisms in multitrophic food webs that are central to biogeochemical cycles (Gessner et al. 2010, Nielsen et al. 2011). Soil invertebrates almost uniformly are omnivorous and the number of feeding links between species is extraordinarily high, particularly in the rhizosphere (Scheu 2002, Scheu and Setälä 2002). These trophic networks are based on the availability and quality of organic C compounds that enter the soil. Two main plant dependant bottom-up factors control this input, detritus e.g. slow decomposable plant material and rhizodeposits as readily available C resource. It is generally assumed that easy degradable substrates are predominantly metabolised in the bacterial energy channel and about 30% of plant derived C enters the food web this way (Moore et al. 2005, Holtkamp et al. 2011). In contrast, the fungal energy channel is favoured by a wide C/N ratio where recalcitrant materials predominate (Frankland 1998, Dilly et al. 2001). Therewith plants provide energy resources of different complexity and accessibility to the soil food web (Ruess and Ferris 2004, Scheu et al. 2005).

The microbial environment in the rhizosphere as well as the activity and composition of the microflora in the surrounding soil are distinctly structured by a diverse soil fauna (Scheu and Setälä 2002, Ruess and Lussenhop 2006, Bonkowski et al. 2009). Grazing by the microfauna enhances mineralization processes in soils and liberates nutrients bound in microbial biomass for plant uptake (Chen and Ferris 1999, Bonkowski et al. 2000). In particular in the rhizosphere bacteria are strongly top-down regulated via grazing by protozoa (Clarholm 1994, Bonkowski 2004). Also, nematodes as most abundant and diverse soil metazoa play a significant role in regulating the composition of the microflora, detritus decomposition rates and element cycles (Bardgett et al. 1999, Ferris 2010, Neher 2010).

The majority of soil organisms are concentrated in the uppermost soil horizons, suggesting that the complexity of decomposer food webs is reduced at deeper layers. For bacteria and fungi strong decline in biomass and changes in community structure were reported across depth transects (Fierer et al. 2003, Schütz et al. 2009). This vertical distribution of the soil microflora as basis of the faunal food web is reflected by a decline in biomass and diversity of soil animals with depth, most pronounced among the meso- and macrofauna (Sadaka and Ponge 2003). This suggests protozoa and nematodes to build the dominant food web constituents in deeper soil. These modifications in horizontal (i.e. within a trophic level)

and vertical (i.e. across trophic levels) diversity along soil depth likely have major implications for C mineralization and sequestration (Gessner et al. 2010).

The present study, for the first time, systematically elaborates the effects of resource inputs on communities of all relevant constituents of the soil micro-food web across a depth transect in an arable soil. Quality and availability of plant resources were manipulated by the following treatments: fodder maize, corn maize, wheat and wheat plus maize litter. Fodder maize, where aboveground plant parts are removed at harvest, mainly supplies belowground C sources, whereas corn maize in addition adds aboveground litter to the soil. Plant detritus predominantly consist of complex, recalcitrant C compounds, such as cellulose and lignin, whereas rhizodeposits comprise labile C substrates, such as sugars, carboxylic and amino acids. Therefore, compared to fodder maize, corn maize is expected to strengthen the fungal energy channel.

The communities of bacteria, fungi, protozoa and nematodes were investigated at 0-10 cm (plough layer), 40-50 cm (rooted zone below plough layer) and 60-70 cm depth (root free soil) in summer (July), the season with the highest root exudation. Amendment with plant residues is expected to trigger bottom-up effects on bacteria and fungi, which in turn propagates to higher trophic levels of the food web. We hypothesise that organism assemblages associated with the organic layer of the soil surface exploit amended resources most, whereas those deeper in the soil are more separated from such processes. The overall effect of resource supply will be a function of the changes in horizontal and vertical diversity within food web guilds along the depth transect.

Materials and Methods

Field site and agricultural management

The experimental site was established in 2009 at an arable field located at Holtensen (51°33´N, 9°53´O; 158m NN) near Göttingen in Lower Saxony, Germany. The area has a temperate climate, with mean annual precipitation and air temperature of 720 mm and 7.9°C, respectively. The dominant soil type is Luvisol, partly with stagnic properties (IUSS, 2007). Due to the long-term agricultural use two plough layers at 0.2 and 0.3 m depth, and a strong soil compaction, particularly below the second plough layer, occur at

the field. The average depth of the A horizon is 34 ± 6.4 cm (for more details see Kramer et al. 2012).

Since 2009 the field is cropped with maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) in a stripe design of two rows. In the first vegetation period the crop was winter wheat ("Julius", sown at 224 kg ha^{-1}) and maize ("Ronaldinio" sown at 34 kg ha^{-1}). In the second year corn was a hybrid maize ("Fernandez" sown at 26 kg ha^{-1}) and cereal was changed to summer wheat ("Melon" sown at 224 kg ha^{-1}) to align vegetation periods between maize and wheat during growing season. Fertilisation practice at maize plots was ammonium nitrate as urea solution (2009: 122 kg N ha^{-1}; 2010: 79 kg N ha^{-1}) and diammonium phosphate (2009/2010: 32 kg N ha^{-1}, 83 kg P ha^{-1}) added shortly before and after seeding. Wheat received granular SN fertilizer (21 kg N ha^{-1}, 24 kg S ha^{-1}) in March and urea solution was added in April, Mai and June with rates of 40 to 61 kg N ha^{-1} per application (for details see Kramer et al. 2012).

Experimental design and soil sampling

The study was set up to manipulate resource availability and quality for the micro-food web under two different crop plants. The factorial design included four treatments: fodder maize (FM), corn maize (CM), wheat (W) and wheat + litter (WL), each with five replicates. To allow feasible agricultural management wheat and maize were arranged in a stripe design of two rows comprising 10 experimental plots of 24 x 24 m each. With fodder maize aboveground plant parts are entirely moved, whereas with corn maize, only corn cobs are harvested and plant detritus remains on the field. To establish CM and WL maize shoots (without cobs) were hackled and applied (0.8 kg m^{-2} dry weight equivalent to 0.35 kg C m^{-2}; C:N ratio 16.8) on half of the harvested maize and wheat plots each autumn. The FM and W plots did not receive additional detritus.

In July 2010 at each plot 10 replicate samples were taken randomly with a soil corer (diam. 2.5 cm) to a depth of 70 cm. Soil cores were split into 10 cm layers, equal soil layers were thoroughly mixed and subsamples were taken for analyses of bacteria, fungi, protozoa and nematodes. Four replicate plots of each treatment were sampled, except for investigation of the bacterial community with three replicates. Protozoa were assessed for maize plots only. Three soil horizons were investigated: *i*) plough layer, 0-10 cm, *ii*) rooted zone below plough horizon, 40-50 cm, and *iii*) deep root free soil, 60-70 cm. Soil samples for investigation of bacterial and fungal communities were frozen and stored at -20°C,

whereas samples for analyses of the microfauna were stored at 4°C (protozoa) and 8°C (nematodes) until animal extraction at the next day.

Microbial biomass

Microbial biomass was estimated by the amount of soil phospholipid fatty acids (PLFAs). For this 2 x 4 g soil fresh weight of each plot were extracted following the procedure described by Frostegård et al. (1993). Briefly, lipids were extracted with Bligh and Dyer (chloroform, methanol, citrate buffer (pH 4); 1:2:0.8) as solvent, fractionated via silica acid columns and the PLFA fraction subjected to alkalic methanolysis, with methylnondecanoate (19:0) as internal standard. The fatty acid methyl esters (FAMEs) were identified by retention time comparison using a gas chromatography (GC) Auto System XL (Perkin Elmer Corporation, St. Louis, Norwalk, USA) equipped with a HP-5 capillary column (50 m x 0.2 mm i.d., film thickness 0.33 μm). To verify correct identification of FAMEs a range of soil samples were analysed by GC-MS with a HP 5890 series II coupled with a 5972 mass selective detector and equipped with a DB-5MS capillary column (30 m x 0.25 mm i.d., film thickness 0.2 μm). For more details see Poll et al. (2007).

The following PLFAs were summed up to estimate bacterial biomass: i15:0, a15:0, i16:0, 16:1ω7, i17:0, cy17:0, cy19:0 (Frostegård et al., 1993; Zelles, 1999). The PLFAs i15:0, a15:0, i16:0 and i17:0 are indicative for Gram-positive, and cy17:0 and cy19:0 for Gram-negative bacteria. The PLFA 18:2ω 6,9 was used as marker for fungal biomass.

Bacterial community

Total DNA was extracted from triplicate frozen soil samples following a previously described procedure (Lueders et al. 2004) with minor modifications: 0.4 g fresh weight of soil was subjected to bead beating in extraction buffer, subsequent organic extraction and DNA precipitation. All centrifugation steps occurred at 15,000 g at 4 °C for 5 min. The extract was dissolved in 80 μl EB buffer (Qiagen GmbH, Hilden, Germany). To reduce humic acid contamination, DNA extracts were purified by silica matrix gel filtration in DyeEx 2.0 spin columns (Qiagen GmbH, Hilden, Germany).

Bacterial communities in soil samples were analysed by 16S rRNA gene-targeted terminal restriction fragment length polymorphism (T-RFLP) fingerprinting with primers Ba27f-

FAM/907r and subsequent *Msp*I digestion as described previously (Pilloni et al. 2011). Prior to the PCR the DNA from the samples of 0-10 cm depth were one hundred-fold diluted, the others ten-fold. All T-RFLP fingerprints were performed for triplicate DNA extracts for each treatment and depth. Bacterial T-RFLP data was analysed with the T-REX online T-RF analysis software (Culman et al. 2009). Background noise filtering 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. The general reproducibility of fingerprints was high. For the entire triplicate fingerprinting dataset, the mean T-RF abundance was 1.2% with a SD of ± 0.4%. Maximum SD was ± 4.8% for a T-RF of 9.9% relative abundance.

Fungal community

The total community DNA from 0.5 g bulk soil of each sample was isolated using the PowerSoil® DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol with the following modifications: all incubation steps were done at -20°C and DNA was eluted in 80µl elution buffer. Fungal ARISA PCR (Ranjard et al. 2001) of each DNA extract was done in two replicates using a FAM (6-carboxy-fluorescine) -labelled variant of primer ITS1F (Gardes and Bruns 1993) and unlabelled ITS4 (White et al. 1990) in 30 µl reaction mixtures containing 6 µl FIREPol 5x Master Mix (Solis BioDyne, Tartu, Estonia), 15 µM of each primer and 1 µl template DNA. PCR was performed with an initial denaturation step at 95°C for 5 min followed by 35 cycles at 95°C for 40s, 54°C for 30s and 72°C for 75 s. Elongation was completed with a final step of 72°C for 10 min. PCR products were purified using the E.Z.N.A.® Cycle-Pure Kit (Omega Bio-Tek, Inc., Norcross, GA, USA). Ten ng of each purified PCR product were dissolved in 14 µl of deionized Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) with 0.1 µl of internal size standard MapMarker 1000 ROX™ (BioVentures, Inc., Murfreesboro, TN, USA). After denaturation for 5 min at 95°C samples were chilled on ice for at least 10 min. Length heterogeneity of fungal ITS fragments was detected by capillary electrophoresis (ABI 3730xl, Applied Biosystems) with the following conditions: injection at 1.6 kV and separation at 15 kV for 1 h. Row profiles were analysed using Gene Mapper software 4.0 (Applied Biosystems). All peaks above a threshold of 100 fluorescence units

present in both technical replicates were kept for further analyses. OTU binning was performed with a binning script (Ramette 2009) using R resulting in a window size of 2 bp.

Protozoa

The abundance and morphotype diversity of major protozoan groups (flagellates, naked amoebae, ciliates and testate amoebae) was determined according to the protocol of Finlay et al. (2000). This method sets the focus on active protozoa at water-saturated soil conditions. From each sample three subsamples of 5 g air dried soil were re-wetted with 3 ml filtered rain water (pore size 0.22 µm), incubated at 15°C in the dark and naked amoebae and flagellates were assessed after 29 days. For the classification of flagellates the key of Jeuck and Arndt (2013) was used.

Subsamples of 1 g soil were diluted in 10 ml Neff's Modified Amoebae Saline (NMAS; Page 1976) each and gently mixed on a shaker for 20 min. After sedimentation 1 ml of supernatant again was diluted with 9 ml NMAS and 10 µl transferred into wells of two 24-well multiwell plates containing 2 ml NMAS and one sterilized amaranth seed as nutrient source. The plates were incubated at 15°C in the dark. After 14 and 35 days the wells were examined for presence of protozoa using an inverted light microscope (Nikon Eclipse TS100). Ciliates were investigated after 4 days of soil incubation with filtered rain water. To subsamples of 1 g of soil 1 ml of filtered rain water was added and two hours later 50 µl of the soil suspension were examined in a Sedgewick-Rafter counting chamber. A second investigation was performed after 10 days. After 6 weeks of soil incubation, the soil samples were analysed for the presence of testate amoebae.

Nematodes

From each replicate plot soil subsamples (50-80 g fresh weight) were extracted with a modified Baermann method according to Ruess (1995). Extraction started at room temperature (18°C) for 24 h. Then, temperature was hourly increased by 5°C starting with 20°C and ending at 45°C. Nematodes were fixed in cold 4% formaldehyde solution and total numbers of were estimated by light microscopy. Of the counted individuals 10% were determined mainly to genus level and assigned to feeding groups according to Yeates et al. (1993). Nematodes life strategy was ascribed to colonizers (*c*) and persisters (*p*) that are extremes on a scale from 1 to 5, respectively (Bongers 1990).

Community and food web indices

The Shannon-Wiener diversity index *H´* (Pielou 1971) was calculated for operational taxonomic units (OTUs) derived from bacterial and fungal fingerprints as well as for protozoa morphotypes and nematode families using the following equation:

$H' = -\Sigma (p_i \ln p_i)$

with p_i the proportion of *i*-th OTU or taxon/morphotype in a sample.
Nematode faunal analysis was performed without the dauerlarvae (non-active stage) of Rhabditidae. The Enrichment (*EI*), Structure (*SI*) and Channel Index (*CI)* were calculated according to Ferris at al. (2001). Distinctive feature is the enrichment weighting of nematode functional guilds. The following equations were used:

$EI = 100 * e / (e + b)$; $SI = 100 * s / (s + b)$ and $CI = 100 * (0.8\ Fu_2 / (3.2Ba_1 + 0.8Fu_2)$

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

Statistical analysis

The effects of resource amendment, crop plant and soil depth on density and community structure (i.e. taxa, indices) of bacteria, fungi and microfauna were analysed by ANOVA. As soil depth was significant for many protozoa and almost all nematode taxa the factors detritus and crop were set as fixed for these data sets. If significant differences were assigned, Tukey's Honestly Significant Difference (HSD) test was used for comparisons of means. Analysis was performed using STATISTICA 9.0 for Windows (StatSoft, Hamburg).
Multivariate regression trees (MRT) were applied to describe relationships between OTUs of bacterial and fungal fingerprint data with the variables detritus, crop and depth. MRT forms clusters of plots by repeated splitting of the data (de`Ath 2002). Data (relative proportions) were arcsin square root transformed before analysis and Euclidean distances used for splitting. The structure of the data was visualized by principal components analysis (PCA) biplots of the group means from the tree analysis. Finally, the intersect correlation was calculated for each axis using the scores of the group means and the scores of the observed values. Axes with a high intersect correlation (typically > 0.8) account

substantially for between group variance. MRT and PCA analysis was performed using the mvpart package (version 1.4-0) in R (version 2.11.1, R development Core Team, 2006).

Results

Microbial biomass

Microbial biomass estimated as total amount of PLFAs decreased strongly with depth (Table 1). This decline was more pronounced for bacteria than for fungi. Generally, gram-positive bacteria were the dominant group across treatments and depths. In the plough layer the total microbial biomass as well as that of bacteria and fungi was affected by both crop plant and detritus treatment. Values were low under fodder maize (FM), intermediate under corn maize (CM) and high under wheat with (WL) or without detritus (W) (total PLFAs: Crop: $F_{1,12} = 14.13$, $p = 0.003$, Detritus: $F_{1,12} = 5.92$, $p = 0.03$; gram positive PLFAs: Crop: $F_{1,12} = 11.26$, $p = 0.05$; gram negative PLFAs: Crop: $F_{1,12} = 13.40$, $p = 0.003$, Detritus: $F_{1,12} = 6.59$, $p = 0.02$; fungal PLFA: Crop: $F_{1,12} = 6.28$, $p = 0.03$, Detritus: $F_{1,12} = 6.78$, $p = 0.02$). Addition of detritus did not affect microbial biomass in the soil layers below the plough sole. Compared to wheat, maize resulted in a higher microbial biomass, predominantly in gram positive bacteria ($F_{1,12} = 8.05$, $p = 0.015$) in 40-50cm depth, but the crop effect diminished in the root free zone in 60-70 cm.

Microbial community structure

Based on OTUs multivariate regression distinctly separated bacterial communities in the plough layer from those in deeper soil (Fig. 1a). Only on the tertiary grouping level, bacterial communities at wheat plots were discriminated between 50 and 70 cm depth. Within soil layers crop plant was the dominant factor for separation, whereas detritus amendment was of minor importance. The first dimension of the PCA explained almost 64% of the total community variation, mainly separating the plough layer from deeper layers (Fig. 1b). The second dimension (21% of variation) was defined mostly by crop

plant. The relative abundance of the OTUs with 490, 146 and 135 bp increased with depth, whereas the 117, 288 and 133 bp fragments were more typical for the soil in the plough layer. For crop plants, the 119 and 142 bp peaks were more abundant under wheat, and the OTUs of 397, 533 and 447 bp under maize, respectively.

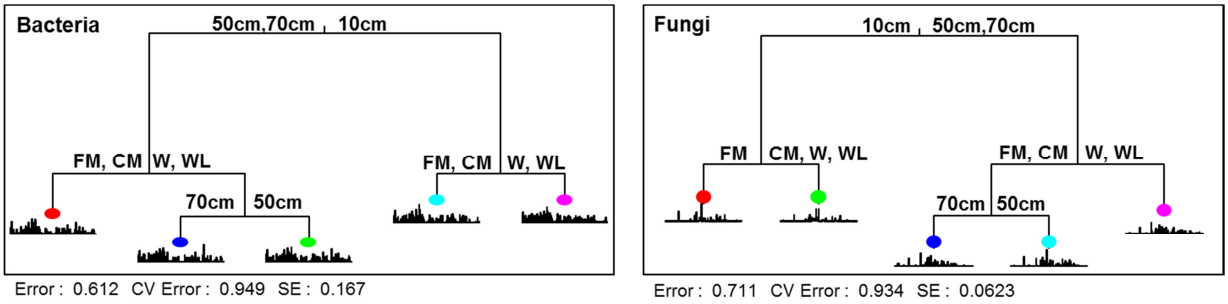

Fig. 1a: Multivariate regression tree on bacterial and fungal community composition estimated by fingerprints.

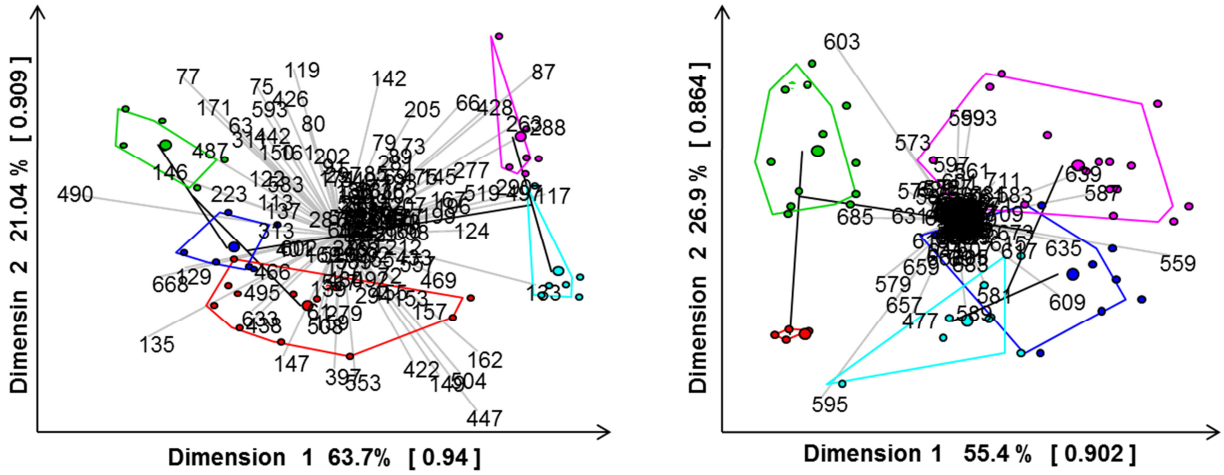

Fig. 1b: Principal component analysis for the group means of the multivariate regression tree in Fig. 1a. The large dots represent the multivariate group means, the individual plots are denoted by small dots, with matching colors to Fig. 1a. Each OTU label is located at its weighted mean from the group means. Intersect correlation is given in brackets.

Investigated are plots cropped with either fodder (FM) or corn (CM) maize and wheat without (W) or with maize detritus (WL) in 0–10, 40-50, and 60-70cm depth. Eucledian distance was used for splitting; barplots show the multivariate OTU means.

Table 1: Biomass of microorganisms and of dominant groups (gram positive/negative bacteria, fungi) determined by phospholipid fatty acids (PLFA in nmol g^{-1} DW soil ± SD). Investigated are plots cropped with either fodder (FM) or corn (CM) maize and wheat without (W) or with maize litter (WL) in 0–10, 40-50, and 60-70cm depth. ANOVA with *, **, *** at $p < 0.05$, 0.01, 0.001. Values within a soil depth with the same or no letters are not significantly different according to Tukey at $p < 0.05$. C – crop, L – litter (detritus).

	0-10cm					**40-50cm**					**60-70cm**				
	W	**WL**	**FM**	**CM**	**ANOVA**	**W**	**WL**	**FM**	**CM**	**ANOVA**	**W**	**WL**	**FM**	**CM**	**ANOVA**
PLFA total	34.2±2.5**a**	35.1±3**a**	27.7±1.7**b**	32.6±2.1**ab**	C**,L*	6.3±1.5	6.2±1.9	7.8±1.2	10±1.1	C*	3.6±0.5	3.1±0.6	3.5±0.4	4.3±1.5	
Fungi	1.2±0.2**ab**	1.4±0.3**a**	0.8±0.1**b**	1.2±0.4**ab**	C*,L*	0.2±0.01	0.2±0.04	0.2±0.04	0.2±0.03		0.2±0.1	0.2±0.1	0.13±0.02	0.2±0.03	
Gram+	9.1±0.8**a**	9.2±0.6**a**	7.6±0.4**b**	8.7±0.5**ab**	C**	1.8±0.4	1.7±0.5	2.2±0.4	2.5±0.3	C*	0.8±0.1	0.7±0.1	0.8±0.1	1±0.3	
Gram-	1.8±0.1**a**	1.9±0.2**a**	1.4±0.02**b**	1.7±0.1**ab**	C**.L*	0.4±0.1	0.4±0.1	0.4±0.1	0.5±0.1		0.2±0.02	0.2±0.1	0.2±0.03	0.2±0.1	

From the soil samples 132 ARISA-OTUs were retained, which distinctly separated plough layer communities from those in deeper soil layers by MRT (Fig. 1a). Only on the tertiary grouping level, fungal communities at maize plots differed between 50 and 70 cm depth. Additionally, in the plough layer FM was discriminated from all other treatments, whereas in deeper soil crop was the dominant factor for separation. The first two dimensions of the PCA (Fig. 1b) accounted for 85% of the variance in the dataset. The presence of the OTU with 685 bp was most apparent in the plough layer. The OTU with 603 bp was dominant in the fungal communities at CM, W, and WL but almost lacking at FM plots. Fungal communities in both deeper layers strongly correlated with the peak at 559 bp, while that at 639 bp were detected predominantly in communities under wheat and that at 589 bp under maize.

Protozoa

The protozoan community comprised naked amoeba, ciliates and flagellates but no testate amoebae (Fig. 2). Average numbers of 14,700 flagellates, 2,800 naked amoebae and 4,100 ciliates g^{-1} dry weight soil were found in the plough layer. Flagellates and ciliates reached maximum numbers in upper soil layers, whereas naked amoebae where most abundant in the root-free soil at 60-70 cm. Effects of detritus amendment on density were only apparent for amoebae with twice the numbers at CM compared to FM plots in 40-50 cm depth.

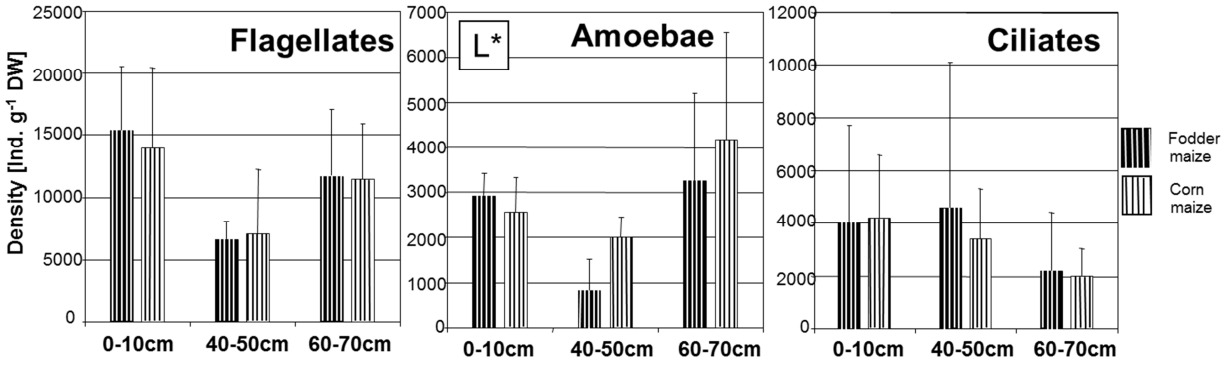

Fig. 2: Population density of protozoa (Ind. g^{-1} DW ± SD) at plots cropped with either fodder or corn maize in 0–10, 40-50, and 60-70cm depth. L – litter (detritus); ANOVA with * at $p < 0.05$.

Table 2: Community composition of amoebae, ciliates and flagellates (ind. g^{-1}DW ± SD) at plots cropped with either fodder (FM) or corn (CM) maize in 0–10, 40-50, and 60-70cm depth. ANOVA with ** at $p < 0.01$. L – litter (detritus).

	0-10 cm			40-50 cm		60-70 cm	
	FM	**CM**	**ANOVA**	**FM**	**CM**	**FM**	**CM**
Amoebae							
Acanthopodial	-	-		83±167	167±192	83±167	167±192
Branched	83±167	333±471		-	-	-	83±167
Dactylopodial	-	83±167		83±167	167±192	-	-
Eruptive	-	167±192		-	83±167	83±167	83±167
Fan-shaped	-	500±192	L**	83±167	167±192	-	83±167
Flamellian	83±167	167±333		-	-	-	83±167
Lingulate	-	83±167		-	-	-	-
Mayorellian	-	-		-	-	83±167	83±167
Monotactic	2667±272	1000±720	L**	583±569	1167±192	2917±1970	3500±1934
Orthotactic	-	83±167		-	83±167	-	83±167
Polytactic	83±167	167±192		-	167±192	83±167	-
Ciliates							
Colpoda	1800±2104	2600±1774		2400±2693	2200±1007	1800±2298	1000±766
Heterotricha	1400±766	1400±766		1400±1774	800±653	400±462	1000±400
Hypotricha	800±924	200±400		800±1131	-	-	-
Flagellates							
Apusomonads	1000±720	1583±687		250±167	500±793	83±167	250±319
Bodonids	500±430	917±419		83±167	83±167	667±816	333±471
Cercomonads	2833±1836	2417±1664		1333±770	1167±1347	1250±957	583±739
Cryptomonads	500±430	83±167		-	250±319	500±333	417±500
Dinoflagellates	250±319	500±638		-	-	167±192	417±500
Euglenids	500±192	333±471		417±631	250±319	1917±1101	1167±430
Thaumatomonadids	7750±2515	6167±3756		2500±430	2833±2589	5083±2936	6250±3108

Overall, 11 morphotypes of naked amoebae, seven groups of flagellates and three broadly defined ciliate groups were distinguished (Table 2). The flagellate community was dominated by thaumatomonads and cercomonads, the most frequent amoebae morphotype was monotactic, and the majority of the ciliates belonged to the genus *Colpoda*. Generally, numbers of morphotypes decreased with depth, particularly in fan shaped amoebae and in cercomonads, bodonids, and apusomonads among flagellates, whereas monotactic amoebae ($F_{3,16} = 4.7$, $p < 0.05$) and euglenids ($F_{2,21} = 9.93$, $p = 0.009$) increased in 60-70 cm depth. Additional detritus input affected the community composition in the plough layer (Table 2). Increased numbers of fan-shaped amoebae occurred at CM plots, while they were almost absent at FM plots ($F_{1,18} = 13.71$, $p = 0.002$). The density of monotactic amoebae in at CM plots were 30% lower compared to FM plots ($F_{2,18} = 7.7$, $p = 0.004$).

Nematodes

Across treatments nematode population density ranged from 15.1 Ind. g^{-1} dry weight in the plough layer to 0.2 Ind. g^{-1} dry weight in the root free zone, and decreased strongly with depth (Fig. 3). Crop plant did not significantly affect the density of nematodes, whereas detritus amendment resulted in highest nematode densities at CM, intermediate at W and WL, and lowest at FM plots across depths (plough layer: $F_{1,12} = 3.72$, $p = 0.02$).

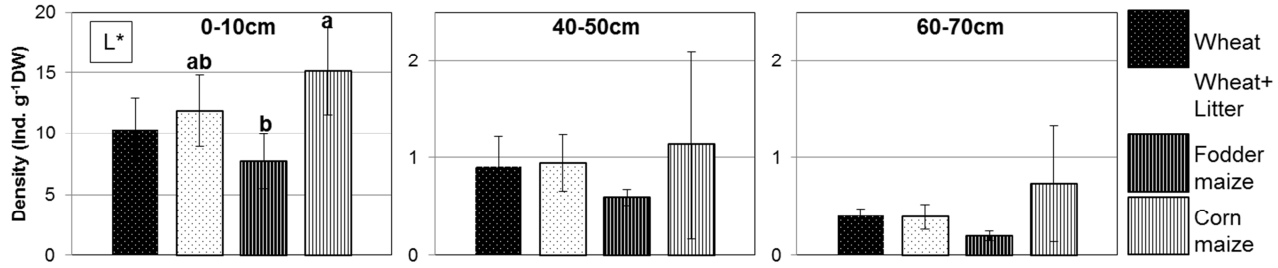

Fig. 3: Population density of nematodes (ind. g^{-1} DW ± SD) at plots cropped with either fodder or corn maize and wheat with and without maize detritus in 0–10, 40-50, and 60-70cm depth. L – litter (detritus); bars within a soil depth with the same or no letters are not significantly different according to Tukey at $p < 0.05$.

Table 3: Predominant nematode families (ind. 100 g^{-1}DW ± SD) at plots cropped with either fodder (FM) or corn (CM) maize and wheat without (W) or with maize litter (WL) in 0–10, 40-50, and 60-70cm depth. DL – dauerlarvae. ANOVA with *, **, *** at $p < 0.05$, 0.01, 0.001. Values within a soil depth with the same or no letters are not significantly different according to Tukey at $p < 0.05$. C –crop, L – litter (detritus). Families with scattered occurrence are not listed, but considered in the total sum.

	c-p value	0-10cm					40-50cm					60-70cm				
		W	WL	FM	CM	ANOVA	W	WL	FM	CM	ANOVA	W	WL	FM	CM	ANOVA
Bacterial feeders																
Cephalobidae	2	177±96	201±95	156±66	251±70		17±13	29±33	14±4	21±5		7±1	6±4	3±1	10±8	
Monhysteridae	1	2±4	20±19	-	2±4		1±2	1±0.3	0.4±1	1±1		0.2±0.4	0.3±0.4	0.3±0.4	1±1	
Panagrolaimidae	1	7±8	21±17	5±9	-	C*	-	-	-	-		-	-	-	-	
Plectidae	2	8±12	26±21	2±5	2±4	C*	0.4±1	0.2±0.5	1±1	0.3±0.4		0.3±0.5	1±1			
Rhabditidae(without DL)	1	206±40	139±46	165±100	257±107	L*	1±1**b**	4±3**b**	5±2**ab**	44±7**a**	C**	5±5	4±2	1±2	10±9	
Rhabditid DL	-	161±133	273±214	229±150	559±275		3±2	4±3	16±13	21±11		3±1	9±9	3±3	25±42	CxL*
Fungal feeders																
Anguinidae	2	13±19	23±18	2±5	10±13		0.3±1	1±1	0.3±0.4	-		0.3±0.4	0.3±1	-	0.3±1	
Aphelenchidae	2	24±12**b**	31±7**b**	39±14**b**	109±61**a**	C*,L*	13±5	12±6	6±2	11±5		6±4	4±2	3±2	2±1	
Aphelenchoididae	2	227±43**a**	229±96**a**	65±60**b**	190±60**ab**	C*	3±1	4±4	6±5	3±2		6±3	7±5	1±1	13±13	
Plant feeders																
Paratylenchidae	2	66±69	80±61	2±5	8±9	C*	1.8±3	0.2±0.5	1±1	0.4±1		-	0.2±0.3	-	-	
Pratylenchidae	3	10±9	6±12	5±1	6±7		40.1±19**a**	26.8±1.2**ab**	4±3**b**	7±9**b**	C***	10±4**a**	4±4**ab**	4±4**ab**	0.3±1**b**	C*, L**
Tylenchidae	2	110±61	111±58	85±45	101±47		11±6	11±4	4±4	4±3	C*	2±1	3±2	2±2	12±17	
Omnivores																
Qudsianematidae	4	2±4	3±6	4±5	2±4		0.1±0.3	0.3±1	-	0.2±0.4		0.4±1	0.3±0.4	-	1±1	
Thornenematidae	5	2±3	5±6	-	-		-	-	-	-		-	-	-	-	
Predators																
Anatonchidae	4	15±6	7±8	5±7	6±8		-	-	-	-		-	-	-	-	
Total number of families		14	17	14	14		11	11	10	10		10	12	7	9	

The nematode community of the arable soil comprised in total 21 families, of which the 15 predominant are presented in Table 3. Generally more families were detected under wheat compared to maize. Except for Monhysteridae all families significantly declined in density with depth (ANOVA, $p < 0.05$ for each family). Both, crop plant and detritus amendment distinctly altered nematode community composition across depth (Table 3). Briefly, as major effect wheat crop facilitated plant-feeding Pratylenchidae (40-50 cm: $F_{1,12} = 21.7$, $p = 0.0006$, 60-70cm: $F_{1,12} = 8.79$, $p = 0.01$) and Paratylenchidae (0-10 cm: $F_{1,12} = 8.2$, $p = 0.01$). Detritus amendment under maize favored the bacterial-feeding Rhabditiade (0-10 cm: $F_{1,12} = 4.85$, $p = 0.05$, 40-50 cm: $F_{1,12} = 15.34$, $p = 0.02$). The response of fungal feeders was inconsistent and limited to the plough layer; Aphelenchoididae reached highest densities at W and WL ($F_{1,12} = 8.8$, $p = 0.01$), but Aphelenchidae at CM ($F_{1,12} = 8.4$, $p = 0.01$).

The major trophic groups of nematodes across treatments were bacterial, fungal and plant feeders (Fig. 4). The density of omnivores and predators was low and mainly restricted to the top soil. The plough layer was dominated by bacterial feeders (55-72%), followed by fungal (14-26%) and plant feeders (8-18%). The different treatments most strongly affected fungal feeders with lowest proportions at FM, intermediate at W and WL, and highest at CM plots (Crop: $F_{1,12} = 5.93$, $p = 0.03$; Detritus: $F_{1,12} = 12.09$, $p = 0.005$; CxL: $F_{1,12} = 10.31$, $p = 0.007$). Wheat as crop favored plant feeders ($F_{1,12} = 10.03$, $p = 0.008$) and detritus amendment bacterial feeders ($F_{1,12} = 5.14$, $p = 0.04$). In the rooted zone below the plough layer the proportion of bacterial feeders increased (from 25 to 77%) whereas that of plant feeders deceased (from 58 to 10%; $F_{1,12} = 23.53$, $p = 0.0005$). At the root-free soil in 60-70 cm depth bacterial feeders dominated the nematode community at detritus amended plots with proportions of 52 and 62% at WL and CM, respectively ($F_{1,12} = 5.07$, $p = 0.04$).

Diversity and food web indices

General micro-food web conditions were assessed using the nematode faunal analysis concept. The Enrichment Index, with values mostly above 50, points to a good nutrient availability in the soil (Table 4). In the rooted zone below the plough layer the nutrient input to the food web was higher under maize, in particular at CM plots ($F_{1,12} = 8.8$, $p = 0.01$). The Structure Index was very low across treatments and depths, assigning an overall basal food web of low complexity. The Channel Index below 50 in the plough layer indicates C flow mainly through the bacterial channel. In the rooted zone fungal

decomposition was high at W (CI=78), whereas the bacterial pathway dominated at FM (CI=35) and CM (CI=27) plots (Crop: $F_{1,12} = 12.2$, $p = 0.04$; Detritus: $F_{1,12} = 6.5$, $p = 0.02$). The Shannon diversity index *H´* was calculated for the investigated community components of the micro-food web (Table 4). *H´* ranged from 2.2 to 2.7 for fungal communities, with no significant differences between depths or treatments. Bacterial diversity with *H´* between 3.5 and 3.9 and was lowest at CM in the plough layer (Detritus: $F_{1,8} = 23.34$, $p = 0.001$; Crop x Detritus: $F_{1,8} = 9.75$, $p = 0.01$) and the rooted zone below (Crop: $F_{1,8} = 10.73$, $p = 0.01$). The Shannon diversity of protozoa (maize plots only) ranged from 1.7 to 2.1, with a tendency to decrease with depth. Nematode H´ values were between 1.5 and 2.0, with high diversity under wheat particularly in the plough layer (Crop: $F_{1,12} = 22.42$, $p < 0.001$) and the root-free deep soil (Crop: $F_{1,12} = 7.06$, $p = 0.02$). Wheat in combination with detritus revealed the highest nematode diversity (0-10 cm: Detritus: $F_{1,12} = 5.85$, $p = 0.03$).

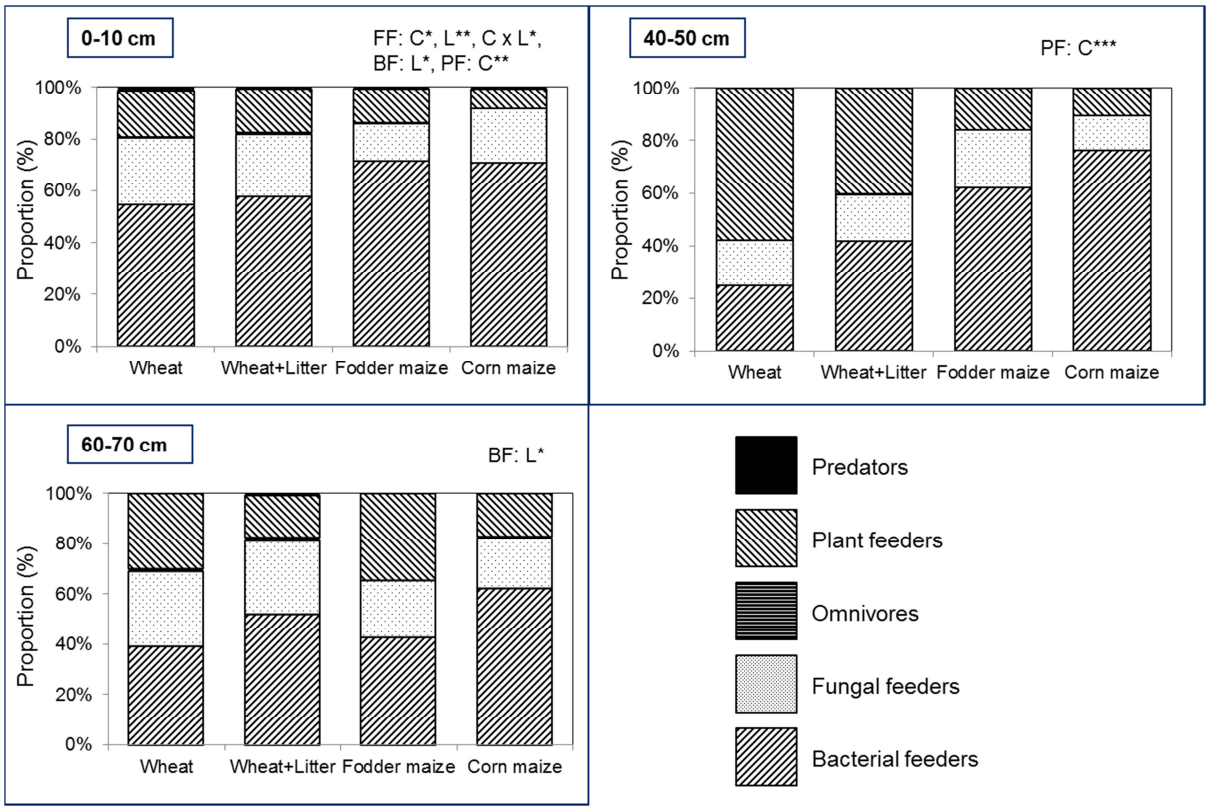

Fig. 4: Nematode trophic structure (Proportion in % ± SD) at plots cropped with either fodder or corn maize and wheat with and without maize detritus in 0–10, 40-50, and 60-70cm depth. PF – plant feeders, BF – bacterial feeders, FF – Fungal feeders. C – crop, L – litter (detritus); ANOVA with *, **, *** at $p < 0.05, 0.01, 0.001$.

Table 4: Micro-food web conditions (community indices ± SD) at plots cropped with either fodder (FM) or corn (CM) maize and wheat without (W) or with maize litter (WL) in 0–10, 40-50, and 60-70cm depth. Presented are the Shannon diversity (H`) for dominant food web groups and the EI – enrichment, SI – structure, and CI – channel index for nematode communities. ANOVA with *, **, *** at $p < 0.05$, 0.01, 0.001; values within a soil depth with the same or no letters are not significantly different according to Tukey at $p < 0.05$. C – crop, L – litter (detritus).

Food web	**0-10cm**					**40-50cm**					**60-70cm**				
	W	**WL**	**FM**	**CM**	**ANOVA**	**W**	**WL**	**FM**	**CM**	**ANOVA**	**W**	**WL**	**FM**	**CM**	**ANOVA**
Condition															
EI	72±3	66±3	72±12	70±7		32±15**b**	47±20**ab**	56±9**ab**	69±15**a**	C*	59±17	63±7	53±19	69±7	
SI	15±8	14±8	11±12	8±6		3±6	2±3	0	2±5		6±11	8±10	26±6	4±8	
CI	21±5	28±3	22±22	23±16		78±23**a**	43±10**ab**	35±13**b**	27±18**b**	C**,L*	52±37	38±18	57±37	26±6	
Diversity (H´)															
Fungi	2.5±0.2	2.5±0.3	2.2±0.2	2.4±0.3		2.7±0.2	2.7±0.2	2.4±0.5	2.6±0.2		2.4±0.6	2.5±0.6	2.4±0.3	2.5±0.5	
Bacteria	3.8±0.1**a**	3.8±0.0**ab**	3.9±0.0**a**	3.6±0.1**b**	L*,CxL*	3.9±0.1**a**	3.8±0.0**ab**	3.7±0.2**ab**	3.5±0.1**b**	C*	3.7±0.0	3.6±0.1	3.7±0.2	3.7±0.2	
Protozoa	-	-	1.7±0.1	2.1±0.1		-	-	1.7±0.1	1.9±0.4		-	-	1.8±0.3	1.7±0.1	
Nematodes	1.8±0.1**ab**	2.0±0.1**a**	1.6±0.1**b**	1.7±0.1**b**	C***,L*	1.5±0.3	1.6±0.4	1.7±0.1	1.5±0.3		1.7±0.1	1.8±0.2	1.5±0.2	1.5±0.1	C*

Discussion

Micro-food webs across depth

a) Microbial communities as base of the food web

Soil microbial communities are structured by abiotic and biotic factors, predominantly the microclimate of the soil and the presence of resources (Fierer et al. 2003, O'Brien et al. 2005, Goberna et al. 2006). Bacterial and fungal biomass was at maximum in the top soil, reflecting the distribution pattern of crop roots mainly within the plough layer (0.01-0.22 mg root C g^{-1} dry weight soil, Y. Kuzyakov pers. comm.). Microbial biomass decreased strongly with depth, most pronounced in bacteria, which is in line with studies from Schütz et al. (2009) using PLFA analyses in a transect from top soil to ground water table. Total biomass in the root free layer was approximately 10% of that in the plough layer, and thus can function as a food web basis at depth.

Depth was also the most relevant discrimination factor for bacterial and fungal community composition. Top soil samples (Ap horizon) were always separated from subsoil samples (B horizon) in MRT ordination, irrespective of crop plant. Hence, in the plough layer agricultural practice, such as fertilization or tillage, affects microbial community composition stronger than crop, which was frequently reported for arable land (Beauregard et al. 2009, Helgason et al. 2010). Additionally, water and C flux through the soil profile was strongly affected by soil management, as assigned by higher bulk density in the B horizon (db=1.60 g cm^{-3}) compared to the Ap1 horizon (db=1.38 g cm^{-3}). This resulted in a strong decrease in the amount of seepage water below the plough layer (Kramer et al. 2012).

Below the plough layer the crop plant modulated bacterial and fungal community composition. Such plant effects can be attributed to specific root exudate profiles (Bais et al. 2006, Garbeva et al. 2008) or rhizosphere priming effects in bulk soil (Dijkstra et al. 2006). Moreover, as bacteria maintain distinct substrate preferences during C transformation across soil depth (Kramer and Gleixner 2008), wheat and maize with their specific amounts of plant debris and detritus compounds (e.g. lignin) likely affected microbial community structure in resource limited deeper soil. Overall, the microbial part of the food web was predominantly shaped by farming practice in the plough layer, whereas with depth crop plant became more important.

b) Protozoa as major bacterial grazers

Protozoan communities were investigated at maize plots solely, and the recorded numbers (Ind g^{-1} dry weight soil) in the plough layer, with 15,000 flagellates, 3000 naked amoebae and 4000 ciliates, were generally lower as communities in the 0-5 cm layer of a Scottish grassland soil in with 46,400 flagellates, 17,700 naked amoebae, 11,000 testate amoebae, and 4,300 ciliates (Finlay et al., 2000). Protozoan morphotypes showed a distinct vertical distribution, with flagellates and ciliates being generally most abundant in top soil, and naked amoebae at depth. Generally, high population densities in the upper soil layers are attributed to the positive impact of increased substrate levels in the rhizosphere (Ekelund et al. 2001). However, monotactic amoebae and euglenids had their maximum abundance at the deep root-free soil. This is likely not caused by translocation of protozoan cells by water flow, as only 9% (maize) and 3% (wheat) of the total precipitation is leaving the main rooting zone (K. Totsche, pers. comm.). This indicates sufficient availability of bacterial resources to allow amoebae and specific flagellates to reside at depth.

The two most common flagellate groups, thaumatomonads and cercomonads, comprised 61% of total flagellate cells detected, which is in line with several other studies (Foissner 1991, Ekelund et al. 2001). Naked amoebae were dominated by monotactic morphotypes such as the monophyletic clade Tubulinea, which contains well known fungivore and bacterivore soil taxa, such as Deuteramoeba, Hartmannella, Sacchamoeba, among others (Smirnov and Brown 2004). Among ciliates, members of the genus *Colpoda* dominated the community with 58%, typical *r*-strategists indicating disturbed soil conditions (Foissner 1997, Verhoeven 2001). As the applied Finlay protocol (Finlay et al. 2000) focuses on active protozoa, the data give evidence of distinct niche differentiation of major protozoan taxa along the soil profile, suggesting high competition for micro-food.

c) Nematodes as consumers at different trophic levels

Nematode numbers with 8 to 15 Ind. g^{-1} dry weight in investigated arable soil were similar to those reported from other farmlands (barley, wheat, soybean) with 8 to 10 Ind. g^{-1} dry weight (Sohlenius and Sandor 1987, Neher et al. 2005). In line with other studies nematode density decreased strongly with depth, which is generally attributed to changes in soil properties and the decline in major resources (Yeates and Bongers 1999, Liang et al. 2005). In sum 21 nematode families were detected, which is half the number reported from other arable fields (Neher and Olson 1999, Neher et al. 2005).

The most dominant nematodes were bacterial-feeding *r*-strategists, common in highly managed arable soils (Ferris 2010). The density of omnivores and predators was low and mainly restricted to the top soil, indicating that the micro-food web is depleted in trophic levels with depth. As in any agro-ecosystem plant feeders made up a considerable proportion of the community, predominantly in the rooted zone (Sohlenius and Sandor 1987, Neher, 2010). Pratylenchidae and Paratylenchidae were favored by wheat as crop, which may be related to the greater root biomass of wheat than maize in the plough layer (Kramer et al. 2012). In deep root free soil plant feeders belonged almost exclusively to the Tylenchidae, facultative root-/fungal feeders (Yeates et al. 1993), suggesting a diet switch from roots to fungi as an adaptation in foraging strategy to the changes in major resources.

Impact of resource quality and availability

a) Primary decomposers

In the plough layer total microbial biomass as well as biomass of bacteria and fungi was lowest at FM, intermediate at CM and highest at W and WL plots. As FM received no above-ground detritus input, the soil harboured fewer resources for primary decomposers. Priming effects by amendment with recalcitrant plant residues have been predominantly reported in fungi but also in gram positive bacteria (Williams et al. 2006, Kramer and Gleixner 2008, Paterson et al. 2008). Irrespective of detritus application wheat as crop had an overall positive impact on the biomass of all groups, supporting the idea of plant-soil feedbacks on microorganisms as described recently (Berg and Smalla 2009, Ladygina and Hedlund 2010).

In contrast to the observed biomass changes in bacteria, detritus amendment did not affect community structure assessed by MRT. Pronounced effects usually occur within the first weeks after application, when easy degradable substrates like carbohydrates, proteins and nucleic acids are available (Baumann et al. 2009, Pascault et al. 2010). Hence, in our study eight months after detritus application, the diminishing of appropriate substrates likely accounts for the lack of a distinct response in bacterial community composition. On the other hand, the structure of the fungal community was responsive to detritus availability, most evidently in the plough layer, where FM was separated from all other treatments by MRT. Specific OTUs common across W, WL, and CM, were rare under FM, the soil with the lowest input of detritus resources. This mirrors negative effects on fungal biomass,

hence unfavourable soil conditions for decomposer fungi, which largely depend on structural plant material (Moore-Kucera 2008, Baumann et al. 2009). In sum, supply rate and quality of resources modulated the soil C channel predominantly allocating plant derived substrate, which distinctly affected the primary decomposers at the base of the food web.

b) Microbial grazers

Ciliates are excellent indicators at higher taxonomic resolution (Foissner 1997, 1999), and the dominance of colpodid ciliates, in agreement with the nematode Structure Index (see below), confirms disturbed soil conditions in the intensively managed arable soil. The density of protozoa, as dominant bacterial feeders in the micro-food web, is suggested to depend strongly on the occurrence of their bacterial prey (Ekelund and Rønn 1994, Ekelund et al. 2001). However, the response to resource enhancement under maize was minor and only naked amoebae showed detritus induced effects in one morphotype group. Therefore, the relatively strong positive response of bacterivore nematodes compared to protozoa may indicate either competitive exclusion or intraguild predation of 'bacterivorous' nematodes on protozoa (Anderson et al. 1978, Bonkowski et al. 2000).

Nematode density was highest at CM, pointing to enhanced resource availability due to detritus input. The positive effects of detritus application were apparent across all depths, which corresponds to Leroy et al. (2009) reporting increased nematode numbers after organic amendments down to 70 cm depth. Predominantly fungal feeders (e.g. Aphelenchidae, Aphelenchoididae) were fostered in the plough layer, frequently reported after enrichment with high C to N organic material (Griffiths et al. 1993, Ferris and Bongers 2006). Generally, saprotrophic fungi are stimulated by application of organic resources to soil (Broder and Wagner 1988) but fungal biomass only slightly increased at CM plots. Considering the higher density of fungal feeders, this suggests enhanced grazing pressure by nematodes controlling fungal biomass. Among bacterial feeders Rhabditidae increased most strongly at CM, indicating that detritus input enhanced the supply of resources. They are considered as strong *r*-strategists with a short life cycle and high reproduction rate, closely mirroring bacterial blooms (Ferris and Bongers 2006). In sum, nutrient amendment via detritus sustained higher biomass in the next trophic level of the food web, the fan shaped amoebae and the bacterial- and fungal-feeding nematodes. Thus resource supply rate represented a bottom-up constrain on the size and activity of the micro-food web.

Food web characteristics

The diverse biological interactions and the central position of nematodes in soil food webs are used for analysis of general food web conditions (Ferris 2010, Yeates 2010). Soil organic amendment results in enriched food webs, where bacteria and fungi increase and resources become available to the nematode community, which was reflected in the high Enrichment Index (EI). The Structure Index (SI) was very low, pointing to a disturbed basal food web, as generally reported from intensively managed agriculture sites (Neher et al. 2005, Thoden et al. 2011). The overall low Channel Index (CI) assigns C transfer predominantly in the bacterial channel of the food web, common in arable fields (Joergensen and Wichern 2008). However, with depth the fungal pathway became more important. In sum, at the investigated soil no "long" micro-food webs with substantial biomass at higher trophic levels could be sustained and the food web can be regarded as bottom heavy with only slow growth of upper predator populations across treatments.

There is evidence that the energy and nutrient flux in decomposer food webs is affected by the diversity of their components (Gessner et al. 2010, Nielsen et al. 2011). As a general pattern diversity is expected to decrease with soil depth as well as with perturbation such as enrichment pulses (Giller 1996, Hartmann et al. 2009). However, the response of the micro-food web was not entirely consistent. This may be attributed to the relatively coarse taxonomic resolution in organism determination. Nevertheless, the calculated Shannon index is based on 132 OTUs for bacteria and fungi each, and 21 protozoa morphotypes and nematode families each. Based on this the diversity of fungal communities remained stable across depth, crop plant or detritus amendment. Some OTUs were almost exclusively present in the B horizon, suggesting disturbance in the intensively managed top soil. The apparently stable diversity therefore is rather a consequence of the overall low diversity, particularly in plough layer. In contrast, wheat as crop had an overall positive impact on bacterial and nematode communities, and detritus amendment a negative impact on bacteria assemblages under maize. Overall, the variable diversity pattern indicates that dominant groups of the micro-food web, even with apparently similar function, exploit different microsites and niches. Organisms of the same functional guild, e.g. bacterial-feeding protozoa and nematodes, differ in their adaptation to prevailing soil conditions, providing complementary rather than redundant functions in soil services such as N mineralisation.

Conclusions

The input of resources is generally expected to impose strong bottom-up forces on food web lower trophic levels, the bacteria and fungi, and that this increase propagates to higher trophic levels. However, our results are not consistent with this hypothesis, at least not for the investigated summer vegetation period after detritus amendment in the previous autumn. Among the primary consumers of the incoming substrate bacteria and fungi only moderately increased in abundance by detritus amendment, while within the next trophic level, guilds of nematodes that feed on bacteria and fungi were strongly responsive to resource enhancement. On the other hand, a considerable part of protozoa (flagellates, ciliates) remained unaffected. The complexity of the micro-food web expressed by the Shannon diversity index revealed distinct responses only within bacteria and nematodes. These different pattern within microbial and microfaunal groups underlines their various roles in soil C flux, suggesting complementary resource use as well as facilitative interactions among decomposer organisms. Overall, the micro-food web in the arable soil was of low complexity with a high degree of disturbance, and rather recalcitrant to enhanced resource supply as no large biomass at higher trophic levels was sustained.

References

Anderson, R., Elliott, E., McClellan, J., Coleman, D., Cole, C., and Hunt, H., (1978) Trophic interactions in soils as they affect energy and nutrient dynamics. III. Biotic interactions of bacteria, amoebae, and nematodes. *Microbial Ecology* 4: 361-371

Ath, de G., (2002) Multivariate regression trees: a new technique for modeling species-environment relationships. *Ecology* 83: 1105-1117

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., and Vivanco, J. M., (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology* 57: 233-266

Bardgett, R. D., Cook, R., Yeates, G. W., and Denton, C. S., (1999) The influence of nematodes on below-ground processes in grassland ecosystems. *Plant and Soil* 212: 23-33

Baumann, K., Marschner, P., Smernik, R. J., and Baldock, J. A., (2009) Residue chemistry and microbial community structure during decomposition of eucalypt, wheat and vetch residues. *Soil Biology and Biochemistry* 41: 1966-1975

Beauregard, M. S., Hamel, C., Atul, N., and St-Arnaud, M., (2009) Longterm phosphorus fertilization impacts soil fungal and bacterial diversity but not AM fungal community in alfalfa. *Microbiology Ecology* 59: 379-389

Berg, G., and Smalla, K., (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology and Ecology* 68: 1-13

Bongers, T., (1990) The maturity index, an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* 83: 14-19

Bonkowski, M., (2004) Protozoa and plant growth: the microbial loop in soil revisited. *New Phytologist* 162: 617-631

Bonkowski, M., Griffiths, B., and Scrimgeour, C., (2000) Substrate heterogeneity and microfauna in soil organic `hotspots' as determinants of nitrogen capture and growth of ryegrass. *Applied Soil Ecology* 14: 37-53

Bonkowski, M., Villenave, C., and Griffiths, B., (2009) Rhizosphere fauna: the functional and structural diversity of intimate interactions of soil fauna with plant roots. *Plant and Soil* 321: 213-233

Broder, M. W., and Wagner, G. H., (1988) Microbial colonization and decomposition of corn, wheat, and soybean residue. *Soil Science Society of America Journal* 52: 112-117

Chen, J., and Ferris, H., (1999) The effects of nematode grazing on nitrogen mineralization during fungal decomposition of organic matter. *Soil Biology and Biochemistry* 31: 1265-1279

Clarholm, M., (1994) The microbial loop. In: Ritz, K., Dighton, J., Giller, K. E. (Eds.), Beyond the biomass. *Wiley, New York*: 221-230

Culman, S. W., Bukowski, R., Gauch, H. G., Cadillo-Quiroz, H., and Buckley, D. H., (2009) T-REX: software for the processing and analysis of T-RFLP data. *BMC Bioinformatics* 10: 171

Dijkstra, F. A., Cheng, W., and Johnson, D. W., (2006) Plant biomass influences rhizosphere priming effects on soil organic matter decomposition in two differently managed soils. *Soil Biology and Biochemistry* 38: 2519-2526

Dilly, O., Bartsch, S., Rosenbrock, P., Buscot, F., and Munch, J. C., (2001) Shifts in physiological capabilities of the microbiota during the decomposition of leaf detritus in a black alder (*Alnus glutinosa* (Gaertn.) L.) forest. *Soil Biology and Biochemistry* 33: 921-930

Ekelund, F., and Rønn, R., (1994) Notes on protozoa in agricultural soil with emphasis on heterotrophic flagellates and naked amoebae and their ecology. *FEMS Microbiology Reviews* 15: 321-353

Ekelund, F., Rønn, R., and Griffiths, B. S., (2001) Quantitative estimation of flagellate community structure and diversity in soil samples. *Protist* 152: 301-314

Ferris, H., (2010) Contribution of nematodes to the structure and function of the soil food web. *Journal of Nematology* 42: 63-67

Ferris, H., and Bongers, T., (2006) Nematode indicators of organic enrichment. *Journal of Nematology* 38: 3-12

Ferris, H., Bongers, T., and de Goede, R. G. M., (2001) A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Applied Soil Ecology* 18: 13-29

Fierer, N., Schimel, J. P., and Holden, P. A., (2003) Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry* 35. 167-176

Finlay, B., Black, H., Brown, S., Clarke, K., Esteban, G., Hindle, R., Olmo, J., Rollett, A., and Vickerman, K., (2000) Estimating the growth potential of the soil protozoan community. *Protist* 151: 69-80

Foissner, W., (1991) Diversity and ecology of soil flagellates. In: Patterson, D.J., Larsen, J. (Eds.), The biology of free-living heterotrophic flagellates. Systems Associates Spezification. 45, *ClarPress Oxford*: 93-112.

Foissner, W., (1997) Protozoa as bioindicators in agroecosystems, with emphasis on farming practices, biocides, and biodiversity. *Agriculture, Ecosystems and Environment* 63: 93-103

Foissner, W., (1999) Soil protozoa as bioindicators: pros and cons, methods, diversity, representative examples. *Agriculture, Ecosystems and Environment* 74: 95-112

Frankland, J. C., (1998) Fungal succession - unravelling the unpredictable. *Mycological Research* 102: 1-15

Frostegård, Å., Tunlid, A., and Bååth, E., (1993) Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipids fatty acid analysis. *Soil Biology and Biochemistry* 25: 723-730

Garbeva, P., van Elsas, J. D., and van Veen, J. A., (2008) Rhizosphere microbial community and its response to plant species and soil history. *Plant and Soil* 302: 19-32

Gardes, M., and Bruns, T. D., (1993) ITS primers with enhanced specificity for basidiomycetes — application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118

Gessner, M. O., Swan, C. M., Dang, C. K., McKie, B. G., Bardgett, R. D., Wall, D. H., and Hättenschwiler, S., (2010) Diversity meets decomposition. *Trends in Ecology and Evolution* 25: 372-380

Giller, P. S., (1996) The diversity of soil communities, the "poor man´s tropical rainforest". *Biodiversity and Conservation* 5: 135-168

Goberna, M., Sánchez, J., Pascual, J. A., and García, C., (2006) Surface and subsurface organic C, microbial biomass and activity in a forest soil sequence. *Soil Biology and Biochemistry* 38: 2233-2243

Griffiths, B. S., Ekelund, F., Rønn, R., and Christensen, S., (1993) Protozoa and nematodes on decomposing barley roots. *Soil Biology and Biochemistry* 25: 1293-1295

Hartmann, M., Lee, S., Hallam, S. J., and Mohn, W. W., (2009) Bacterial, archaeal and eukaryal community structures throughout soil horizons of harvested and naturally disturbed forest stands. *Environmental Microbiology* 11: 3045–3062

Helgason, B. L., and Walley, F. L., (2010) No-till soil management increases microbial biomass and alters community profiles in soil aggregates. *Applied Soil Ecology* 46: 390-397

Holtkamp, R., van der Wal, A., Kardol, P., van der Putten, W. H., de Ruiter, P. C., and Dekker, S. C., (2011) Modelling C and N mineralisation in soil food webs during secondary succession on ex-arable land. *Soil Biology and Biochemistry* 43: 251-260

Jeuck, A., and Arndt, H., (2013) A Short Guide to Common Heterotrophic Flagellates of Freshwater Habitats Based on the Morphology of Living Organisms. *Protist 164*(6): 842

Joergensen, R. J., and Wichern, F., (2008) Quantitative assessment of the fungal contribution to microbial tissue in soil. *Soil Biology and Biochemistry* 40: 2977 - 2991

Kramer, C., and Gleixner, G., (2008) Soil organic matter in soil depth profiles: Distinct C preferences of microbial groups during C transformation. *Soil Biology and Biochemistry* 40: 425-433

Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Schoene, J., Scheunemann, N., Schmalwasser, A., Totsche, K. U., Walker, F., Scheu, S., and Kandeler, E., (2012) C flow into microbial and fungal biomass as basis for the belowground food web in an agroecosystem. *Pedobiologia* 55(2): 111-119

Ladygina, N., and Hedlund, K., (2010) Plant species influence microbial diversity and C allocation in the rhizosphere. *Soil Biology and Biochemistry* 42: 162-168

Leroy, B. L. M., de Sutter, N., Ferris, H., Moens, M. and Reheul, D., (2009) Short term nematode population dynamics as influenced by the quality of exogenous organic matter. *Nematology* 11: 23-38

Liang, W., Zhang, X., Li, Q., Jiang, Y., Ou, W., and Neher, D. A., (2005) Vertical distribution of bacterivorous nematodes under different land uses. *Journal of Nematology* 37: 254-258

Lueders, T., Manefield, M., and Friedrich, M. W., (2004) Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. *Environmental Microbiology* 6: 73-78

Moore, J. C., McCann, K., and de Ruiter, P. C., (2005) Modeling trophic pathways, nutrient cycling, and dynamic stability in soils. *Pedobiologia* 49: 499-510

Moore-Kucera, J., and Dick, R. P., (2008) Application of ^{13}C-labeled detritus and root materials for in situ decomposition studies using phospholipid fatty acids. *Soil Biology and Biochemistry* 40: 2485-2493

Neher, D. A., (2010) Ecology of plant and free-living nematodes in natural and agricultural soil. *Annual Review of Phytopathology* 48: 371-394

Neher, D. A., and Olson, R. K., (1999) Nematode communities in soils of four farm cropping management systems. *Pedobiologia* 5: 430-438

Neher, D. A., Wu, J., Barbercheck, M. E., and Anas, O., (2005) Ecosystem type affects interpretation of soil nematode community measures. *Applied Soil Ecology* 30: 47-64

Nielsen, U. N., Ayres, E., Wall, D. W., and Bradgett, R. D., (2011) Soil biodiversity and C cycling: a review and synthesis of studies examining diversity-function relationships. *European Journal of Soil Science* 62: 105-116

O'Brien, H. E., Parrent, J. L., Jackson, J. A., Moncalvo, J-M., and Vilgalys, R., (2005) Fungal community analysis by largescale sequencing of environmental samples. *Applied and Environmental Microbiology* 71: 5544–5550

Page, F. C., (1976) Taxonomic criteria for Limax amoebae with descriptions of three new species of *Hartmanella* and three of *Vahlkampfia*. *Journal of Protozoology* 14: 499-521

Pascault, N., Cecillon, L., and Mathieu, O., (2010) In situ dynamics of microbial communities during decomposition of wheat, rape, and alfalfa residues. *Microbiology Ecology* 60: 816-828

Paterson, E., Osler, G., Dawson, L. A., Gebbing, T., Sim, A., and Ord, B., (2008) Labile and recalcitrant plant fractions are utilized by distinct microbial communities in soil: Independent of the presence of roots and mycorrhizal fungi. *Soil Biology and Biochemistry* 40: 1103-1113

Pielou, E. C., (1971) An introduction to mathematical ecology. *Wiley Interscience,* New York: 286

Pilloni, G., von Netzer, F., Engel, M., and Lueders, T., (2011) Electron acceptor-dependent identification of key anaerobic toluene degraders at a tar-oil-contaminated aquifer by Pyro-SIP. *FEMS Microbiology Ecology.* DOI: 10.1111/j.1574-6941.2011.01083

Poll, J., Marhan, S., Haase, S., Hallmann, J., Kandeler, E., and Ruess, L., (2007) Low amounts of herbivory by root-knot nematodes affect microbial community dynamics and C allocation in the rhizosphere. *FEMS Microbiology Ecology* 62: 268-279

Ramette, A., (2009) Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities. *Applied and Environmental Microbiology* 75: 2495–2505

Ranjard, L., Poly, F., Lata, J. C., Mougel, C., Thioulouse, J., and Nazaret, S., (2001) Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. *Applied and Environmental Microbiology* 67: 4479-4487

Ruess, L., (1995) Nematode fauna in spruce forest soils: a qualitative/ quantitative comparison. *Nematologica* 41: 106-124

Ruess, L., and Ferris, H., (2004) Decomposition pathways and successional changes. *Nematology Monographs Perspectives* 2: 547-556

Ruess, L., and Lussenhop, J., (2006) Trophic interactions of fungi and animals. In: Dighton, J., Oudemans, P., White, J. (Eds.), The fungal community: Its organization and role in the ecosystem. *CRC, Boca Raton*: 581-598

Sadaka, N., and Ponge, J-F., (2003) Soil animal communities in holm oak forests: influence of horizon, altitude and year. *European Journal of Soil Biology* 39: 197-207

Scheu, S., (2002) The soil food web: structure and perspectives. *European Journal Soil Biology* 38: 11-20

Scheu, S., Ruess, L., and Bonkowski, M., (2005) Interactions between micro-organisms and soil micro- and mesofauna. In: Buscot, F., Varma, A. (Eds.), Microorganisms in soils: roles in genesis and functions. *Springer Verlag*: 253-275

Scheu, S., and Setälä, H., (2002) Multitrophic interactions in decomposer food-webs. In: Tscharntke, T., Hawkins, B. A. (Eds.), Multitrophic level interactions. *Cambridge University Press*, Cambridge: 223-264

Schütz, K., Nagel, P., Vetter, W., Kandeler, E. and Ruess, L., (2009) Flooding forested groundwater areas modifies microbial communities from top soil to groundwater table. *FEMS Microbiology Ecology* 67: 171-182

Smith, C. J., Danilowicz, B. S., Clear, A.K., Costello, F. J., Wilson, B., and Meijer, W. G., (2005) T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles. *FEMS Microbiology Ecology* 54: 375-380

Smirnov, A.V., and Brown, S., (2004) Guide to the methods of study and identification of soil gymnamoebae. *Protistology* 3: 148-190

Sohlenius, B., and Sandor, A., (1987) Vertical distribution of nematodes in arable soil under grass (*Festuca pratensis*) and barley (*Hordeum distichum*) *Biology and Fertility of Soils* 3: 19-25

Thoden, T. C., Korthals, G. W., and Termorshuizen, A. J., (2011) Organic amendments and their influences on plant- parasitic and free-living nematodes: a promising method for nematode management? *Nematology* 13: 133-153

Verhoeven, R., (2001) Response of soil microfauna to organic fertilization in sandy virgin soils of coastal dunes. *Biology and Fertility of Soils* 34: 390-396

White, T. J., Bruns, T., Lee, S., and Taylor, J., (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: a Guide to Methods and Applications, *Academic Press*, San Diego, California: 315–322

Williams, M. A., Myrold, D. D., and Bottomely, P. J., (2006) C flow from ^{13}C-labeled straw and root residues into the phospholipid fatty acids of a soil microbial community under field conditions. *Soil Biology and Biochemistry* 38: 759-768

Yeates, G. W., Bongers, T., Goede, de R. G. M, Freckman, D. W. and Georgieva S. S., (1993) Feeding habits in soil nematode families and genera - an outline for soil ecologists. *Journal of Nematology* 25: 315-331

Yeates, G. W., (2010) Nematodes in ecological webs. In: Encyclopedia of Life Sciences. Wileyand Sons, Chichester: 1-11

Yeates, G. W., and Bongers, T., (1999) Nematode diversity in agroecosystems. *Agriculture, Ecosystems and Environment* 74: 113-135

Zelles, L., (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. *Biology Fertility Soils* 29: 111-129

Rhizosphere food web links uncovered – Carbon fluxes from maize (*Zea mays* L.) roots to microbial key-consumers and their predators*

Abstract

The flow of C via plant roots into root associated microorganisms and subsequently into soil food webs, and thus the coupling of belowground and aboveground systems, is one of the key processes determining ecosystem functioning. With the advent of high throughput sequencing methods it became clear that the rhizosphere of a particular plant species and even plant genotype selects for specific subsets of the soil microbial community, termed the 'rhizosphere microbiome'. Given the highly dynamic nature of growing roots, and the ease by which root derived C spreads through root associated microorganisms, the impression of a plant's rhizosphere microbiome as a static community might be strongly misleading. In particular the roles of root infecting microorganisms, such as arbuscular mycorrhizal fungi (AMF), as C sinks need further investigation. A comparison to free-living assimilate utilizing microorganisms and revealing the species composition of rhizosphere associated microbes will greatly advance our functional understanding of rhizosphere processes. Furthermore, the identity of active microbial predators as well as the organization of rhizosphere food webs is virtually unknown, despite the ability of microbial grazers to significantly influence the composition and function of root associated microorganisms. Only few studies to date revealed that the microbial food web actively depends on C input from root exudates, even though great efforts have been spent to describe microbial communities in the plant rhizosphere.

In a plant labeling experiment using a combined rRNA-SIP and pyrotag sequencing approach with ^{13}C-labeled rhizodeposits we traced the C flow from roots of maize (*Zea mays* L.) through the communities of bacteria, fungi and heterotrophic protists. Surprisingly we found that only few taxa of the diverse microbial community in rhizosphere as well as in bulk soil actively rely on root exudates. AMF were the determining factor for the allocation of plant derived C into bulk soil microbial

* for information about Co-authorships see page 154

communities, thereby providing food webs in the rhizosphere and in bulk soil with energy from plant photosynthates.

We revealed the organization of the plant C associated microbial soil food web, identified specific microbial key players and outlined a succession of plant derived C through bacterial, fungal and protistan communities. These findings provide crucial insights into the temporal dynamics and functioning of the root associated microbiome and the identity of their microbial key predators in soil food webs.

Introduction

According to current knowledge soil microbiota represent the greatest reservoir of biological diversity world-wide (Berendsen et al. 2012, Lopez-Guerrero et al. 2013, Curtis et al. 2002, Buee et al. 2009, Gams et al. 2007). This tremendous soil microbial diversity is mainly fuelled by plant derived C that enters the soil via rhizodeposition or as detritus. In particular the energy flux from plants to the rhizosphere has recently been assumed to drive the entire soil food web (Albers et al. 2006, Bonkowski et al. 2009, Frank and Groffman 2009). However, little is known about the paths of low-molecular weight C into soil-borne communities and resulting feedbacks on ecological functions (Drigo et al. 2010) especially for higher trophic levels in the soil food web like protozoan grazers.

Further, the understanding of the complex interactions of plants and microorganisms is also very limited (Bisseling et al. 2009) but recent findings lead to the assumption that plants are able to shape their rhizosphere communities and that these microbiomes are crucial for plant health (Berendsen et al. 2012). Similar to other eukaryotes, plants and their microbiomes can be described as a "super-organism" since the plant relies on the soil microbiota for specific functions and traits (Mendes et al. 2011). The plant in turn cultivates its microbiome by adjusting pH, reducing competition for beneficial microbes and providing C rich rhizodeposits (Peiffer et al. 2013, Bais et al. 2006). Despite this tremendous importance of soil microbial communities the structure of belowground food webs and its key-players are still unknown to a large extend. Even though strong influence of selectively grazing protozoa on bacterial community structure was found in several studies (Bonkowski and Brandt 2002, Kreuzer et al. 2006, Rosenberg et al. 2009) trophic links between both, bacteria or fungi and protozoa, in soil are insufficiently characterized. Protozoa are known as one of the most abundant groups of bacterivores, but they are also

consumers of a number of other food sources (Crotty et al. 2012). Microcosm studies provide strong evidence of the important role of protozoa in the rhizosphere, but these experiments are often restricted to only a few model organisms disregarding the high diversity of soil protozoa and the fundamental difference the presents or absence of AMF mean to food web organisation. The identity of protozoan taxa relevant for C fluxes in the rhizosphere is unknown. In sum soil protozoa form the critical link for the C transfer to upper trophic levels, still they have been largely ignored in relation to the "whole" soil food web, biogeochemical cycling or microbial ecology in general (Crotty et al. 2012, Wilkinson and Mitchell 2010). Understanding the C flow in food webs involving bacteria and protozoa can provide insides into how protozoan grazing affects bacterial function in a wide range of systems (Moreno et al. 2010).

The application of ^{13}C Stable Isotope Probing (SIP) to trace plant derived C fluxes into microbial nucleic acids (Whiteley et al. 2007, Vandenkoornhuyse et al. 2007) or other biomarkers (Boschker et al. 1998) has opened up a window to understand the fluxes of C through plant associated microbial communities (Drigo et al. 2010). RNA-SIP is directly linked to microbial activity (Molin and Givskov 1999) and enables us to characterize structure and *in situ* functions of specifically active microbial populations (Lueders et al. 2004). RNA becomes labeled more rapidly than DNA because ribosomes are more abundant and have a faster turnover and transcription is not semiconservative. This makes RNA-SIP very fast and allows short term responses to be investigated (Vandenkoornhuyse et al. 2007). Especially for higher trophic levels like protozoan grazers, this method is suitable because the amount of ^{13}C incorporated is proportional to the activity of the organism during the incubation period with the tracer. As a result we have a direct integrating measure on the identity of the taxonomic groups actively preying primary consumers of plant derived C. The use of SIP to analyse C sequestration in a microbial soil food web provides strong evidence for the utility of this technique in unravelling the turnover and flow of complex C pools in natural systems (Lueders et al. 2006). This has already been demonstrated for exudate based (Prosser et al. 2006, Neufeld et al. 2007, Mao et al. 2014, Lu and Conrad 2005, Rangel-Castro et al. 2005) and detritus based (see chapter III) food webs.

Here we follow the largely unknown avenues of C fluxes from the rhizosphere into the bulk soil to elucidate the microbial key-players and food web components directly relying on rhizodeposits and to differentiate them from those that rely on detritus inputs (chapter

III). A ^{13}C-labeling experiment was conducted using maize plants in agricultural soil and rRNA-SIP combined with subsequent pyrotag sequencing to identify pro- and eukaryotic soil microbes. We characterized the active bacterial, fungal and protistan, especially protozoan, communities in the rhizosphere and bulk soil. We expected to find different rhizodeposit dependent communities in rhizosphere and bulk soil, with more enriched taxa in the rhizosphere. Furthermore we predicted food web relevant bacterial/fungal/protozoan populations to be temporally and spatially dynamic because of the constantly changing conditions in the rhizosphere.

Materials and Methods

Microcosm experiment for SIP

The experiment was conducted in two acrylic glass chamber (l: 95 cm, w: 42 cm, h: 70 cm) for the ^{13}C-labeling and a ^{12}C-control, respectively. To mimic summer conditions in temperate agricultural fields, maize plants were exposed to light for 12 hours each day with 600 µmol PAR. Temperature was 28°C by day and 18°C at night. Plants were germinated on wet cellulose tissue and after five days, seeds and root tips were truncated to ensure highly branched roots and CO_2 as sole C source for the plants. The next day, maize seedlings were planted into rhizoboxes with 135 g fresh soil from an agricultural field site near Göttingen, Germany (see Kramer et al. 2012). Soil was sieved (< 5mm) and well homogenized. The plants were watered with 15 ml water daily. One week after planting 0.5 g l^{-1} KNO_3-fertilizer was added to the water for five days. The labeling started 24 days after germination and three control plants were sampled directly before labeling. In both chambers 24 plants were inserted and $^{13}CO_2$ (^{13}C-source: sodium bicarbonate-^{13}C, 98 atom %, Campro scientific, Berlin, Germany) or unlabeled CO_2 ($^{12}CO_2$, source: anhydrous sodium carbonate, Sigma-Aldrich, St. Louis, USA), were pumped into the respective chambers. A constant CO_2 concentration of 418 ± 27 ppm was established to ensure optimal C fixation rates. CO_2 concentration was controlled via IRGA (Carbocap GM70, Vaisala, Vantaa, Finland). The CO_2 concentration applied was only little higher than the natural CO_2 concentration in air (about 390 ppm; Andrews et al. 2013). Soil was covered with parafilm to reduce CO_2 efflux from soil respiration. Labeling lasted for 6 days but the

experimental conditions were maintained for 10 more days. Plants were watered during the experiment with 25 – 30 ml each day and 7.5 mg KNO_3 per plant was added at day 4, 6, 9, and 12 as plants showed signs of nitrogen deficiency. Plants were harvested at 0.5, 1, 2, 3, 5, 8, 11 and 16 days, respectively. For sampling the fronts of the rhizoboxes were opened and bulk soil was carefully removed with a spatula. Roots were collected and shaken. Remaining adherent soil to roots was defined as rhizosphere soil (Buddrus-Schiemann et al. 2010). At each sampling triplicate plants were harvested, but for RNA-SIP the soil samples of all three plants were pooled and homogenously mixed before RNA extraction for rhizosphere and bulk soil samples, respectively. Dry weight of plant shoots and roots were determined and ^{13}C excess mass was estimated for bulk soil, roots and shoots (Fig. 1). The $\delta^{13}C$ values of shoot, root and soil samples were measured using an elemental analyzer NA 2500 (Carlo Erba Instruments, Milano, Italy) interfaced to a Delta XP isotope ratio mass spectrometer (Thermo Electron Cooperation, Bremen, Germany).

RNA extraction and rRNA-SIP

RNA was extracted from soil as described by Lueders et al. (2004) with minor modifications: 0.4 g (fresh weight) of homogenously mixed 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 nucleic acids (NA) 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 humic substances. 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).

RNA extracts from the sampling time points 1, 3, 5, 8, 11 and 16 days after the experiment started were selected for gradient centrifugation. Replicate rRNA extracts from ^{12}C-control incubations were pooled and centrifuged in one composite sample. Isopycnic centrifugation and gradient fractionation were done as described in Glaubitz et al. (2009) 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

Prokaryotic rRNA populations in resolved SIP fractions (fractions 2 to 10 of all gradients) were analyzed by T-RFLP fingerprinting (Lueders et al. 2004, Glaubitz et al. 2009). Labeled T-RFs were identified by comparison of T-RF abundances of "heavy" and "light" rRNA fractions from the ^{13}C treatments, as well as with "heavy" fractions of ^{12}C-controls. Only if a T-RF was noticeably more abundant in "heavy" ^{13}C fractions than in all other ^{12}C and ^{13}C fractions, it was considered as labeled. As the bacterial labeling was strongest on day 5 and 8 these time points were chosen to analyze for C transfer into eukaryotic RNA. Based on these rRNA fingerprints (Fig. S 1, page 81), fractions 3 ('heavy') and 8 ('light') of all ^{12}C and ^{13}C SIP gradients from day 5 and 8 were subjected to 454 amplicon pyrosequencing.

Bacterial pyrotags were generated as reported by 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). 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 by Pilloni et al. (2012) following manufacturer protocols using a 454 GS FLX pyrosequencer with Titanium chemisty (Roche Applied Biosystems, Penzberg, Germany). Bidirectional reads were quality-trimmed and filtered as described by 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).

Eukaryotic pyrotags were generated in parallel for day 5 and 8. Amplicon preparation for eukaryotes was done as for bacteria but with modified PCR conditions and using Brilliant III Ultra-Fats RT-qPCR Master Mix (Agilent Technologies, Santa Clara, USA). Quality trimming, filtering and exclusion of short reads were done following the same protocol as for prokaryotes. SSU rRNA amplicon sequences were taxonomically analyzed with the CREST toolbox (Lanzen 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).

Prokaryotic and eukaryotic in-vitro T-RFs were generated for the contigs with the software TRiFLe (Junier et al. 2008) to compare T-RF and sequencing data sets and to designate potential phylogenetic groups detected in-vivo TRFs.

Data handling

The excess (above background) of the ^{13}C tracer in a certain C pool (χ^E (^{13}C)) was determined by subtracting the relative ^{13}C abundance in the unlabeled pool ($\chi(^{13}C)_{Std}$, atom %) from the relative ^{13}C abundance in this pool (*P*) after labeling ($\chi(^{13}C)_p$, atom %) and was expressed as $g^{13}C$ g C^{-1} (Coplen 2011):

$$\chi^E(^{13}C) = \chi(^{13}C)_p - \chi(^{13}C)_{Std}$$

To identify taxa directly involved in the assimilation of ^{13}C from rhizodeposits within the different groups, pyrotag 'enrichment factors' (EF) in 'heavy' rRNA fractions were deduced. All taxa with EF > 0.5 and a relative read abundance of at least 0.25% in the corresponding heavy ^{13}C fraction were considered as being ^{13}C-labeled.
The enrichment factors were calculated as:

Enrichment factor = ^{13C} heavy / ^{13C} light – ^{12C} heavy / ^{12C} light

with ^{13C} heavy and ^{13C} light being the relative abundance of pyrotag reads affiliated to a given taxon in sequenced heavy and light rRNA SIP fractions from the ^{13}C treatment, and ^{12}C heavy and ^{12}C light being the relative abundance of pyrotag reads affiliated to a given taxon for the respective ^{12}C-control gradient.

Results

Plant and soil

Plants approximately doubled their shoot and root biomass in the 16 days of the experiment. Between the samplings on day 5 and day 8 the biggest average increase in plant growth was observed (Fig 1 A). On day 5 the plant biomass was 0.69 g DW on average (shoot: 0.34 g ± 0.07 g DW, root: 0.35g ± 0.09 g DW) and increased on day 8 to 0.98 g DW (shoot: 0.48g ± 0.05 g DW, root: 0.50g ± 0.08 g DW). Each plant assimilated 2.84 ± 0.75 mmol C d^{-1} on average during the labeling period of six days, resulting in an ^{13}C excess mass of about 0.26, 0.23, and 0.007 $g^{13}C$ g C^{-1} for maize shoots, roots and bulk soil after 6 days of labeling, respectively (Fig. 1 B).

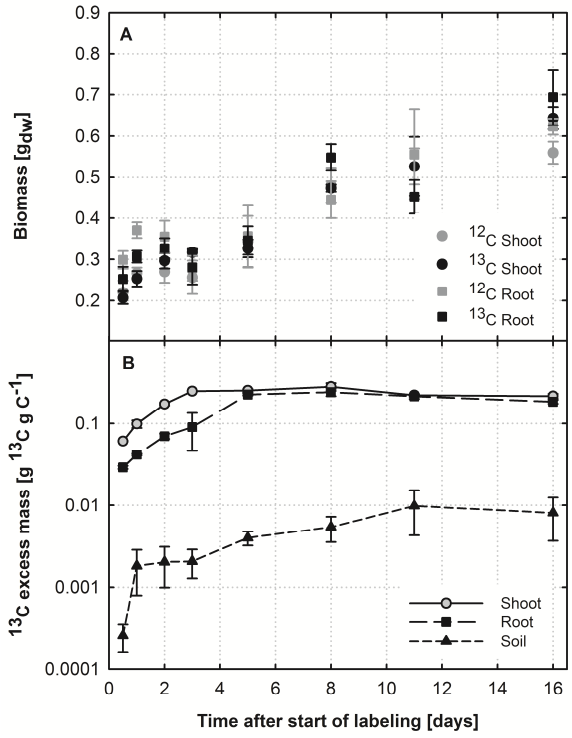

Fig. 1: mean values of plant biomass in ^{12}C and ^{13}C treatment (A) and ^{13}C enrichment in soil, roots and shoots (B) of the three samples harvested each time. Error bars indicate standard deviation.

Fungi

Fungal reads accounted for between 40 % (day 5, bulk soil) and 48 % (day 8, bulk soil) of all eukaryotic reads. Most sequences were assigned to the supergroup Ascomycota, the Mortierellales, belonging to the Mucoromycotina (including the so called "sugar fungi") were also very abundant (Fig. 2). Nevertheless, neither ascomycotan taxa (except some yeast genera), nor sugar fungi or taxa belonging to the supergroup Basidiomycota showed ^{13}C enrichment in any of the investigated samples. Only taxa in the fungal supergroups Glomeromycota and Chytridomycota were enriched in ^{13}C (Fig. 3).

Arbuscular mycorrhizal Glomeromycota were more abundant in bulk soil, were they represent 7 % (d5 bulk) and 11 % (d8 bulk) of all eukaryotic reads, respectively. In the rhizosphere only 2 % (day 5) and 3 % (day 8) of eukaryotic reads belonged to Glomeromycota. Glomeromycota were the most heavily labeled fungal group on both days in rhizosphere and bulk soil (especially on day 8). Within the Glomeromycota the Paraglomerales were by far the most abundant and enriched taxon, but also the taxa Glomerales, Diversisporales and Archaeosporales were enriched (Fig. 3).

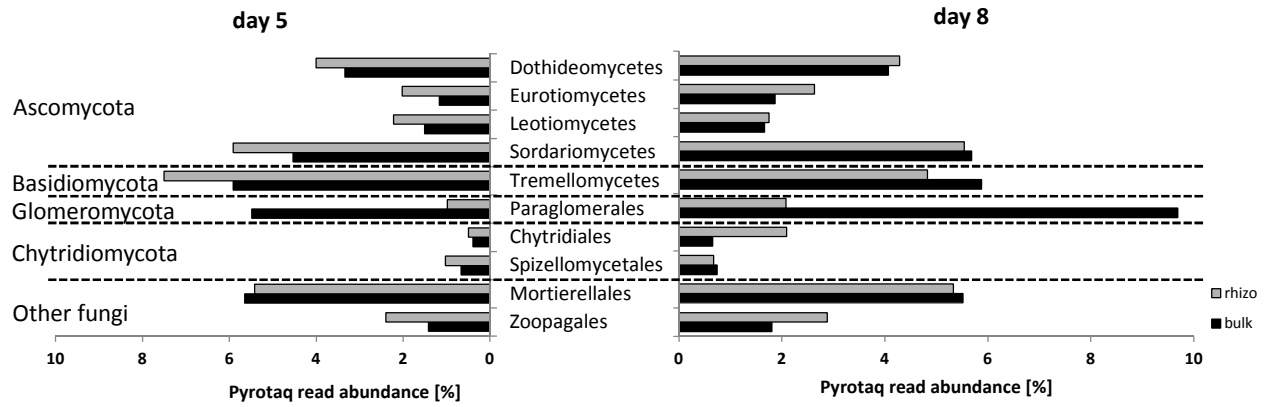

Fig. 2: Relative pyrotaq read abundance of the most common fungal taxa in rhizosphere (grey) and bulk soil (black) on day 5 and 8 after start of labeling

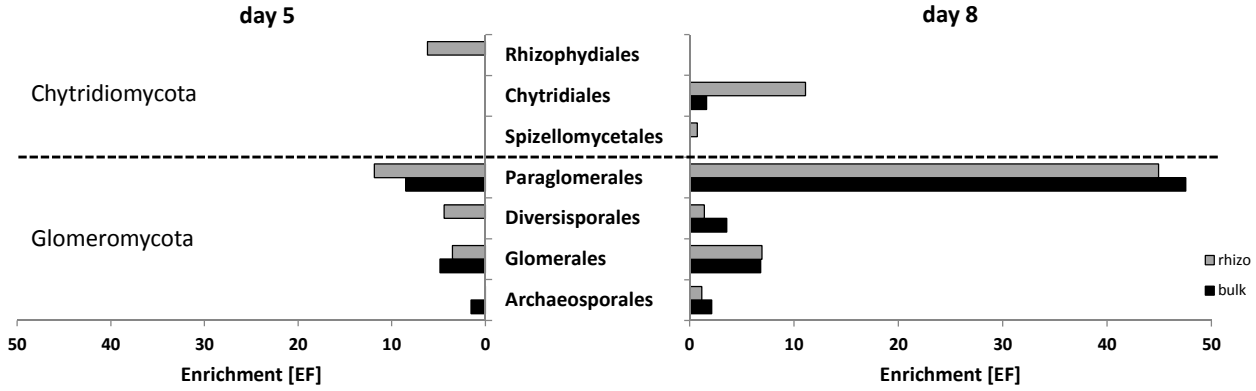

Fig. 3: Enrichment factors of labeled fungal taxa in rhizosphere (grey) and bulk soil (black) on day 5 and 8 after start of labeling

The chytridiomycotan taxa Chytridiales, Rhizophydiales and Spizellomycetales were enriched in the rhizosphere; Rhizophydiales on day 5 and Chytridiales and Spizellomycetales on day 8. Chytridiales were also labeled in bulk soil on day 8. Yeasts were almost exclusively labeled in the rhizosphere and only 8 days post labelling (Fig. 5), even though the percentage of yeast reads in the rhizosphere did not exceed the percentage in bulk soil (Fig. 4). Concerning pyrotag reads *Cryptococcus* was by far the most abundant yeast genus (Fig.4), however it was not enriched. The rhizodeposit-utilizing yeasts were represented by the genera *Saitoella* in bulk soil, and *Candida* and *Torulaspora* in the rhizosphere of maize.

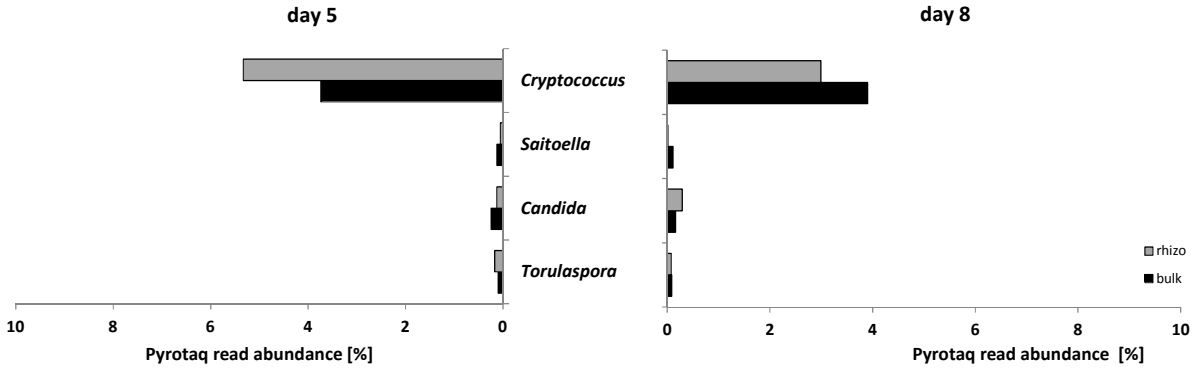

Fig. 4: Relative read-abundance of the most common yeast genera in rhizosphere (grey) and bulk soil (black) on day 5 and 8 after start of labeling

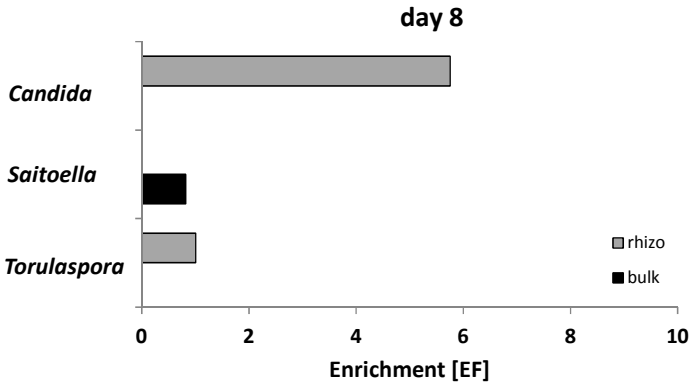

Fig. 5: Enrichment factors of labeled yeast genera in rhizosphere (grey) and bulk soil (black) on day 8 after start of labeling (5 days after labelling start: no enriched yeast genera detected)

Bacteria

From day 5 onwards, selected bacterial taxa became enriched in ^{13}C. Generally, bacterial taxa that were highly labeled were not the most abundant taxa according to relative pyrotag read abundances (Fig. 6). The exception was *Opitutus* which was both, abundant and strongly enriched. Enriched bacterial communities in both, rhizosphere and bulk soil resembled each other. After 5 days of labeling *Azospirillum* (Alphaproteobacteria) and *Arthrobacter* (Actinobacteria) were enriched in the maize rhizosphere, *Mucilaginibacter* (Bacteroidetes), *Ohtaekwangia* (Bacteroidetes) and *Massilia* were enriched in bulk soil. *Sphingobium* (Alphaproteobacteria), *Opitutus* (Verrucomicrobia) and unclassified Oxalobacteriacea were enriched in both, rhizosphere and bulk soil. On day 8, two days after labeling stopped, *Arthrobacter* was the only bacterial species exclusively labeled in the rhizosphere whereas *Kitasatospora* (Actinobacteria), *Ohtaekwangia* and *Pseudonocardia* were exclusively labeled in bulk soil. *Opitutus, Mucilaginibacter,*

Massilia, Azospirillum, and *Sphingobium* were enriched in both the rhizosphere and bulk soil, respectively.

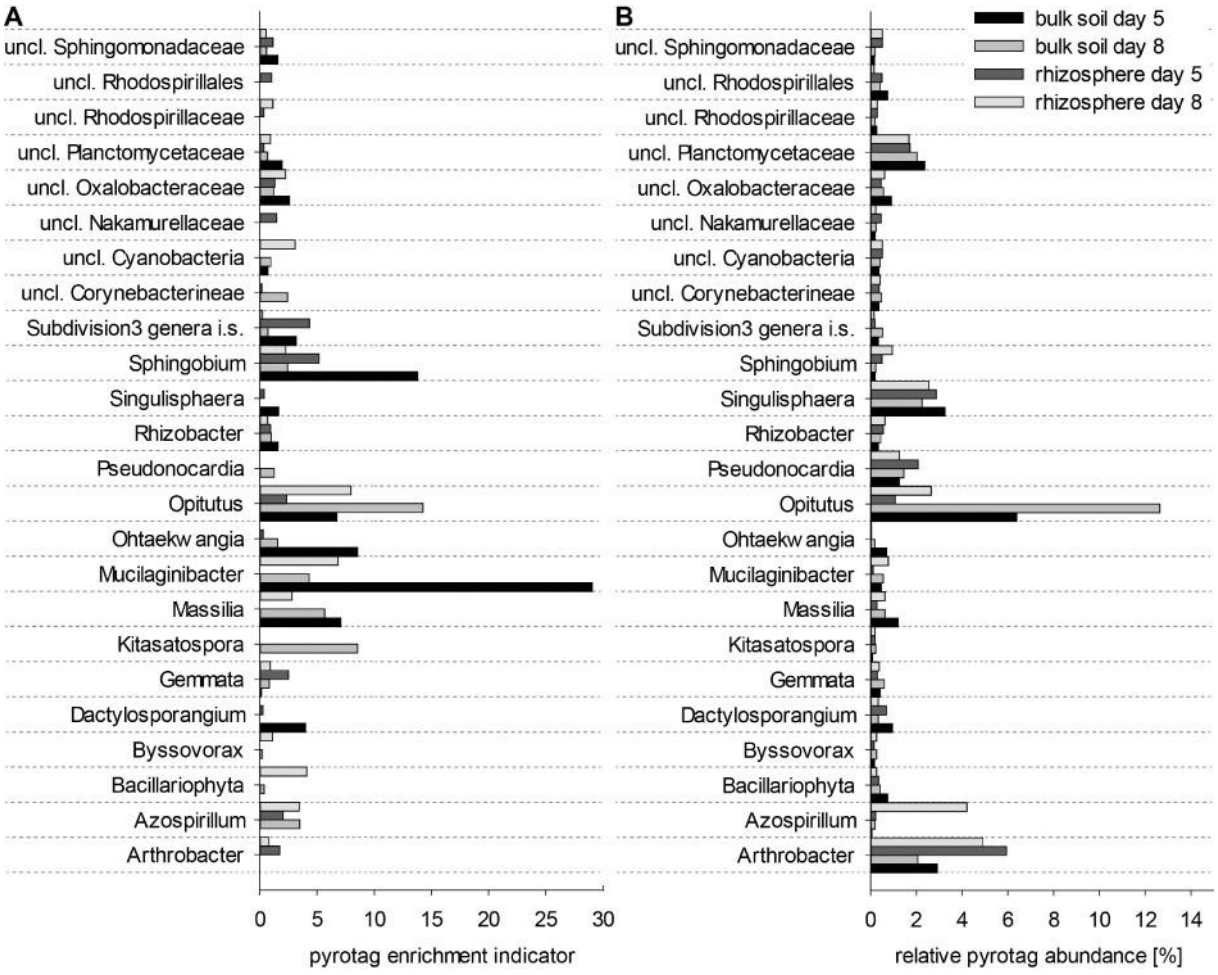

Fig. 6: Enrichment factors (A) and relative sequence abundance (B) in "heavy" ^{13}C-rRNA fractions for bacterial taxa labeled by ^{13}C-rhizodeposits in bulk soil and in the rhizosphere on day 5 and 8 of the experiment.

Heterotrophic protists

Although the Peronosporomycetes (formerly Oomycetes) morphologically and physiologically resemble the fungi (Dick, 2001), they have been classified as heterotrophic protists in the taxon Stramenopiles (Adl et al., 2005, Krings et al. 2011). Peronosporomycetes accounted for between 1 % (day 8, rhizosphere and bulk soil) and 4% (day 5, bulk soil) of all eukaryotic reads. The most abundant Peronosporomycete taxon was the Pythiales with 0.46 % (day 5, rhizosphere), 0.49 % (day 5, bulk soil), 0.36 % (day 8, rhizosphere) and 0.60 % (day 8, bulk soil) of all eukaryotic reads, respectively. The second common Peronosporomycete taxon was Peronosporales with 0.26 % (day 5,

rhizosphere), 0.74 % (day 5, bulk soil), 0.07 % (day 8, rhizosphere) and 0.02 % (day 8, bulk soil) of all eukaryotic reads. The peronosporomycete taxa were only enriched on day 5, especially in bulk soil. Labeled peronosporomycetes almost exclusively belonged to the taxon Peronosporales (bulk soil, day 5, EF = 30.89).

Protozoan sequences accounted for about 20 – 30 % of all eukaryotic reads, being slightly higher in rhizosphere (day 5: 25.6 %, day 8: 28.3 %) than in bulk soil (day 5: 22.4 %, day 8: 23.2 %). The protozoan communities turned out to be phylogenetically very diverse in rhizosphere and in bulk soil, in total we found representatives of 58 different orders in our samples.

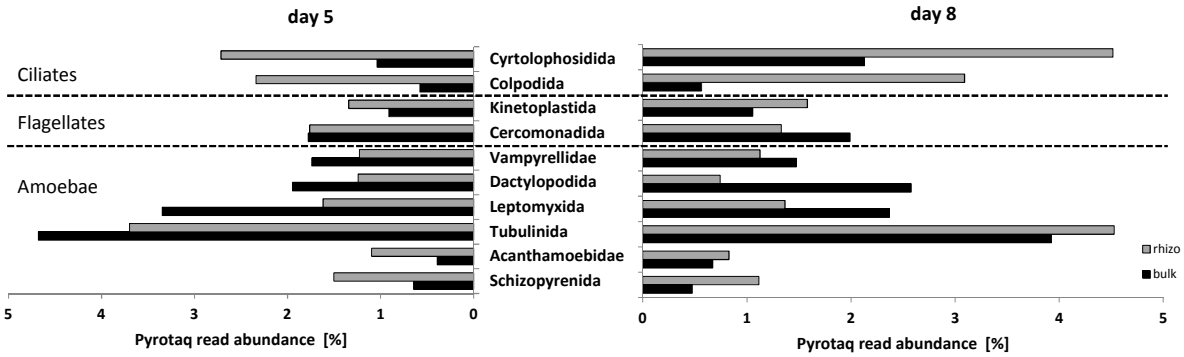

Fig. 7: Relative read-abundance of the most common protozoan taxa in the rhizosphere (grey) and bulk soil (black) on day 5 and 8 after start of labeling

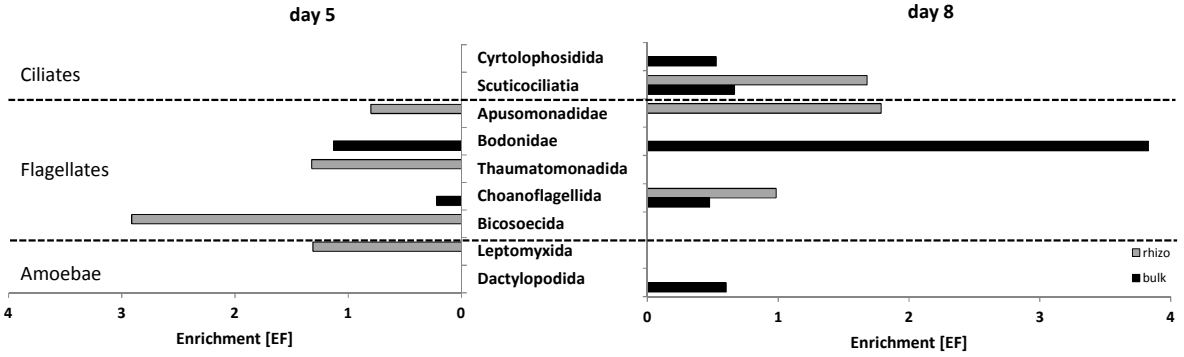

Fig. 8: Enrichment factors of labeled protozoan taxa in the rhizosphere (grey) and bulk soil (black) on day 5 and 8 after start of labeling

Kinetoplastida and Cercomonadida, both being common and abundant soil flagellates, represented more than 1 % of all eukaryotic reads (Fig. 7), but only a small subgroup of the Kinetoplastida, the Bodonidae, were enriched with ^{13}C (Fig. 8). The flagellate taxa Bicosoecida (stramenopiles), Thaumatomonadida and, at a higher taxonomic resolution, Apusomonadidae were enriched in the rhizosphere on day 5. Bodonidae were the only protozoa enriched in bulk soil on day 5 (EF = 1.1). On day 8 Choanoflagellida were

labelled in both, rhizosphere (EF = 1) and bulk soil (EF = 0.5) while Apusomonadidae were again enriched in the rhizosphere (EF = 1.8) and Bodonidae in bulk soil (EF = 3.8), respectively.

The ciliate taxa Colpodida and Cyrtolophosidida were very abundant, especially in the rhizosphere (Fig. 7), but only on day 8 ciliate taxa showed signs of ^{13}C enrichment (Fig. 8). These taxa were Scuticociliatia (EF = 1.68 in the rhizosphere, EF = 0.66 in bulk soil) and Cyrtolophosidida (EF = 0.5 in bulk soil).

Highest read abundances were found in naked amoebae, since six out of the ten most abundant protozoan taxa displayed an amoeba morphotype (Fig. 7). Nevertheless, only two of these taxa were labeled. These taxa were the Leptomyxida on day 5 and 8 in the rhizosphere and the Dactylopodida on day 8 in bulk soil (Fig. 8).

The plant associated microbial food web

A very diverse microbial community was detected in the rhizosphere and in bulk soil, but only specific subsets of the fungal and bacterial community did directly benefit from plant-derived C. AMF were highly enriched and apparently transferred a large proportion of this C into bulk soil where Peronosporales became labeled. Single celled fungi like ascomycotan yeasts and chytridiomycotan taxa were enriched in both, rhizosphere and bulk soil whereas filamentous saprophytic fungi did not benefit from root derived C. Only after a labeling period of 5 days specific groups of bacteria were enriched in plant derived ^{13}C. These labeled subsets of the bacterial community turned out to be surprisingly similar in the rhizosphere and in bulk soil. On the next trophic level protozoa participated from root C in rhizosphere and bulk soil, respectively.

The pulse of C spread through the microbial community with time of labeling. The explicit enrichment of mycorrhiza as well as the diversity of labeled taxa was higher on day 8. While the enrichment on day 5 was mainly found in bulk soil organisms the proportion of ^{13}C bound in microbial biomass on day 8 was far more balanced between rhizosphere and bulk soil.

Discussion

A significant part of the variation in the composition of maize rhizosphere bacteria could be attributed to an interaction of plant host genetics and soil factors (Peiffer and Ley 2013). Using a combined rRNA-SIP and pyrotag sequencing approach we found only a small subset of the present microbial community to be part of the microbial food web associated with the rhizosphere of maize plants in natural agricultural soil. Since rRNA in contrast to DNA is only transcribed by living and active organisms (Urich et al. 2008, Tveit et al. 2012) we were able to portray the flow of rhizosphere C through the active root associated microorganisms into the bulk soil, and further into the rhizosphere and bulk soil microbial food webs. Because methodological differences hamper the comparability and interpretation of results from different SIP-studies, it must be mentioned beforehand that we used a very conservative approach to assign taxa as being labeled. As unlabeled rRNA tends to occur also in 'heavy' fractions and over entire caesium trifluoroacetate gradients, only T-RFs or pyrotag reads distinctly more abundant in the 'heavy' ^{13}C rRNA fractions than in all other ^{13}C and ^{12}C fractions were considered as labeled in our study (Lueders et al. 2004). This approach will prevent false positive assignments.

In contrast, one could define all sequences in the heavy fractions as being labeled (Vandenkoornhuyse et al. 2007), although identical sequences are frequently found also in the light fractions. Vandenkoornhuyse et al. (2007) argued that those bacteria used both fresh ^{13}C labeled and also old unlabeled substrates as C source. Therefore, if only a small subgroup of a given population actually used ^{13}C-labeled rhizodeposits, e.g. due to favorable localization near the root tips, or when the ^{13}C signal was diluted in higher trophic levels, these taxa likely were not identified as being labeled with our conservative approach. For example, rhizodeposits can be taken up and incorporated into rRNA from rhizosphere microorganisms within hours (Vandenkoornhuyse et al. 2007, Henkes et al. 2011), but based on T-RFLP fingerprinting we could not detect any isotope enrichment in bacteria during the first three days after labeling in our experiment. For analysis of eukaryotic communities primers optimized for protistan T-RFLP fingerprinting in subsurface environments (Euringer and Lueders 2008) were used. As in all primer-based sequencing studies targeting the entire eukaryotic community, general primers are never universal so that some groups are likely to be underrepresented (Berney et al. 2004, Epstein and Lopez-Garcia 2008, Stephenson et al. 2011, Bohmann et al. 2014). A more detailed discussion of the applied approach is found in the SI of chapter III (page 113).

Root exudate consuming microbial key-organisms

It is often assumed that bacteria monopolize most of the easily available rhizosphere C (Kent and Triplett 2002), but more and more evidence accumulates that simultaneously saprophytic fungi, in particular root pathogens like *Fusarium* (Lueders et al. 2004), and so-called 'sugar fungi', can be important competitors for C rich root exudates (De Boer et al. 2005, De Boer et al. 2006, Mougel et al. 2006). Therefore we were particularly interested to identify enriched fungi in the maize rhizosphere. Although typical sugar fungi like *Mortierella* and other hyphal Basidiomycota and Ascomycota were common and diverse in our soils (Fig. 2), they were clearly not enriched. In chapter III we studied plant decomposition in soil from the same location as in this study and found the basidiomycote yeast *Cryptococcus* to be the strongest competitor and the major utilizer of glucose C next to bacteria. Accordingly, we expected *Cryptococcus* to be also a major competitor for root derived C. Interestingly, despite *Cryptococcus* reads were highly abundant in both, the maize rhizosphere and bulk soil, this yeast apparently assimilated no rhizodeposit derived label. Instead, yeasts of the genera *Candida* and *Torulaspora* were enriched in the maize rhizosphere 8 days post labeling. Also Chytridiomycota, evolutionary an early diverging fungal lineage consisting of mainly single celled 'zoosporic fungi' (Barr 2001) were clearly enriched in the maize rhizosphere. Interestingly, these were predominantly the taxa Rhizophydiales and Spizellomycetales which both contain AMF parasites (Ross and Ruttencutter 1977, Wakefield et al. 2010). Progressive labeling of Rhizophydiales (day 5) and of Spizellomycetales (day 8) in the rhizosphere, and of Chytridiales (day 8) in both rhizosphere and bulk soil indicates a succession of root derived C through Chytridiomycota taxa between the sampling events. These results unambiguously demonstrate that single-celled fungi, such as most Chytridiomycota and yeasts can play a major role as competitors for readily available exudates in the plant rhizosphere, but more research on yeasts is needed to explain the apparent differences in C uptake between different yeast taxa.

Bacteria, despite being highly abundant and diverse in our soil, received surprisingly little C rich root metabolites compared to fungi, and only a small subset of the bacterial community was significantly enriched in the maize rhizosphere. The bacterial taxa most clearly depending on root exudates consisted of *Opitutus* (Verrucomicrobia), *Sphingobium* and *Mucilaginibacter* (EF > 5), further enriched species were e.g. *Massilia, Azospirillum* and *Arthrobacter.*

The four taxa, *Massilia* (Ofek et al. 2012), *Mucilaginibacter* (Madhaiyan et al. 2010, Lee et al. 2013), *Azospirillum* and *Arthrobacter* (Babalola 2010, Gaiero et al. 2013) are all

known to contain plant growth promoting rhizosphere bacteria (PGPR). However, *Opitutus* and *Arthrobacter* appeared to be the bacterial key-players in the maize rhizosphere of our agricultural field soil as these taxa were not only heavily labeled but also highly abundant on the root surface. Verrucomicrobia have been shown to be abundant and possibly functional important bacteria in the rhizosphere of plants, but they have been generally little studied (Kielak et al. 2010). The finding that *Opitutus* had by far the highest read numbers and high enrichment indicates that this taxon was highly successful foraging for plant C in the maize rhizosphere and might play a critical role in the microbial food web. Also bacterial taxa affiliated to *Sphingobium* and *Massilia* can be very abundant in the rhizosphere of maize (Balkwill et al. 2006, Dohrmann et al. 2013, Peiffer et al. 2013), and both taxa well participated on root C. Bacterial isolates affiliated to the genus *Mucilaginibacter* are generally non-motile and supposed to produce high amounts of extracellular polymeric substances (Pankratov et al. 2007). *Mucilaginibacter* is often found in the rhizosphere of plants (Lee et al. 2013) but our results show that they play as well an important role in bulk soil food webs.

The outstanding role of AM fungi as strong sinks for plant C is well known (Farrar et al. 2003, Olsson and Johnson 2005, Vandenkoornhuyse et al. 2007, Johnson et al. 2002). Depending on the nutrient demand of the plant AMF can consume more than 20 % of plant assimilated C (Bago et al. 2000). Our results exactly match the findings of Olsson and Johnson (2005) and Drigo et al. (2010) where almost all root derived C was first captured by AMF, and gradually released by mycorrhizal plants between 5 - 8 days post labeling.
Plant roots are simultaneously colonized by different AMF taxa (Kiers et al. 2012); in the maize rhizosphere the orders Paraglomerales, Glomerales, Diversisporales and Archaeosporales were important sinks of root C but the Paraglomerales were the key mycorrhizal taxon that captured by far most plant assimilates. In our experiment, the hyphal network of AMF was the only conceivable “C bridge” for the translocation of significant amounts of recently fixed plant C from rhizosphere into the bulk soil and further into bulk soil bacteria. This is in line with several other studies (Koller et al. 2013, Mao et al. 2014, Drigo et al. 2010). The labeled subset of bulk soil bacteria resembled the labeled rhizosphere community with *Ohtaekwangia*, *Dactylospoarangium*, *Singulisphaera*, Corynebacterineae and *Pseudonocaria* as enriched groups. *Sphingobium, Arthrobacter,* Nakamurellaceae, Sphingomonadacea, Rhodospirillacea, *Azospirillum* and *Byssovorax* were only enriched in bulk soil. Highest ^{13}C enrichment was found for *Mucilaginibacter*, *Opitutus* (Verrucomicrobia), *Ohtaekwangia* and *Massilia.* Except *Opitutus,* none of these

taxa had high relative sequence abundances despite their high ^{13}C incorporation. Other bacteria, like *Arthrobacter*, *Singulisphaera*, *Azospirillum* and unclassified Planctomycetaceae were quite abundant in the 'heavy' ^{13}C fraction but had only little ^{13}C enrichment as they were also abundant in all other SIP gradient fractions. This second group of bacteria could be important in using rhizodeposits as 'activation energy' to oxidize other pools of SOM, the so-called 'priming effect' (Kuzyakov 2010).

High throughput sequencing methods targeting rhizosphere microbial DNA leave the impression of a plant's microbiome as a static community. SIP experiments have the advantage that the dynamics of the C flow through the prominent rhizodeposit utilizers can be identified. Exudates are mainly released at the growing root tip (Jones et al. 2009) and is used by a succession of microbes as root growth proceeds. Semenov et al. (1999) described that fast-growing (mostly copiotrophic) bacteria that utilize easily available substrates at the root tip and slow-growing (possibly oligotrophic) bacteria with limited nutrient use capacity will oscillate wavelike along roots due to recurrent growth and death cycles (Liljeroth et al. 1991, Maloney et al. 1997). In addition to root exudates, also more complex C sources like root border cells will become labeled during the 5 - 8 days of incubation (Jones et al. 2009) but it is doubtful that users of more recalcitrant C might have taken up enough label to be detected with our conservative approach. Although we probably underestimated the rhizodeposit utilizing bacteria, our conservative approach enabled us to clearly identify the bacterial key taxa connected to the belowground C flux from maize plants. Some of them are known as plant growth promoting rhizosphere bacteria (*Azospirillum*, *Arthrobacter*, *Mucilaginibacter*, and *Massilia*); others are well-known rhizosphere inhabitants (i.e. *Sphingobium* and *Rhizobacter*). In addition, we could identify bacterial key-players whose roles in rhizosphere processes were so far unknown, such as *Ohtaekwangia*, and in particular *Opitutus*. The findings of this experiment demonstrate that rhizodeposits are mainly converted by specific subpopulations of rhizosphere bacteria at a given time and it might be not adequate to consider overall bacterial biomass as a foundation for the modeling of C fluxes in soil food webs. Rather, the specific sub-populations utilizing defined substrates should be discerned.

Besides bacteria and fungi the protistan Peronosporomycetes were important users of plant derived C 5 days post labeling. Among Peronosporomycetes are well known plant pathogens (Hendrix and Campell 1973, Nowiki et al. 2012) but others can also act as pioneer saprotrophs on fresh organic matter (Deacon 1997). Especially some *Pythium* species are known as highly competitive utilizers of easily available C compounds in soil

(Deacon 1997). In a decomposition study using a variety of C sources and maize litter, *Pythium* turned out to be the most important protist at all stages of decomposition, outcompeting all other protists in particular at glucose utilization (chapter III). Also Mao et al. (2014) found *Pythium* in the rhizosphere of switchgrass to be heavily labeled, thus actively utilizing root exudates. In fact, most of the Peronosporomycete pyrotag reads in bulk soil and rhizosphere belonged to *Pythium*. However, despite abundant in our experiment, *Pythium* was only slightly labeled in the rhizosphere, while Peronosporales were heavily labeled in bulk soil on day 5 of our experiment. Well known members of the taxon Peronosporales are the mainly plant pathogenic genera *Phytophthora*, *Peronospora* and *Plasmopara*. Together with the bacterial taxa *Mucilaginibacter, Ohtaekwangis, Massilia* and *Opitutus* and the glomeromycotan taxa Paraglomerales, the Peronosporales were identified as the main utilizers of root C on day 5 in bulk soil, possibly feeding as parasites on AMF as described for an unclassified "*Pythium*-like" organism by Ross and Ruttencutter (1977).

Except for Peronosporomycetes all the other soil protist sequences considered in this study belonged to microbial grazers. These soil protozoa are important consumers of bacteria linking primary producers to higher trophic levels (e.g. Hunt et al. 1987, Clarholm 1985, Bonkowski 2004). A very diverse and temporarily highly dynamic protozoan community was detected in both, rhizosphere and bulk soil. Soil ciliates were more abundant in the rhizosphere while most taxa of naked amoebae, except Acanthamoeba and Schizopyrenida, were more abundant in bulk soil. Naked amoebae were the most abundant protozoan supergroup in pyrotag reads with six out of the ten most abundant orders belonging to this taxon (Fig. 7). Contrastingly, the active protozoan community directly incorporating root C was very different from those that were most abundant since we found the highest enrichment of ^{13}C in flagellates with Bicosoecida, Apusomonadidae, Thaumatomonadida and Scuticociliatia as heavily labeled groups (EF > 1) in the rhizosphere and Bodonidae in the bulk soil, respectively (Fig. 8). Based on cultivation methods, Thaumatomonadida were already identified as a dominant protozoan group in soil originating from the same agricultural field site in a former study (chapter I). Another heavily enriched protozoan group in the rhizosphere were the facultative mycophagous Leptomyxida, a taxon with amoeboid morphology (Chakraborty 1982, Geisen et al. in prep.), indicating possible feeding on labeled fungi like yeasts or mycorrhiza. Except for slightly enriched Dactylopodida in bulk soil on day 8, surprisingly no other labeled naked amoebae taxon was found, even though naked amoebae are supposed to be one of the most competitive

bacterial feeders at high bacterial densities (e.g. Clarholm et al. 2006) and were found to be the most important grazers in other plant labeling experiments (Murase and Frenzel 2007).

Microbial food web links in the maize rhizosphere

In this study for the first time we were able to describe a complex microbial food web actively relying on rhizodeposits during the succession of plant derived C through microbial key-organisms in the maize rhizosphere.

Plant derived C was initially kept in arbuscular mycorrhizal fungi and only from 5 days onwards spread through other soil microorganisms and the soil food web. The Glomeromycota served as the major plant-soil interface, allocating a significant amount of recently fixed C to bulk soil microbial communities. Bacteria became enriched only 5 days post labeling. At this time the microbial food web in the rhizosphere using plant derived C was much more diverse than the bulk soil microbial community even though at that time most of the label was found in a few heavily enriched bulk soil taxa like *Mucilaginibacter* or Peronosporales (Fig. 9 A). In this food web AMFs were already the major C sink, but they were far from being as dominant as they were 8 days post labeling. Flagellates were the main grazers in the rhizosphere and the only grazers in bulk soil that incorporated ^{13}C label. Since the ^{13}C signal is diluted in higher trophic levels due to increased respiration losses and ineffectiveness of trophic energy transfer, all enriched protozoan grazers, according to our stringent definition of enrichment, must have been primary consumers of other highly enriched microorganisms. The assimilation efficiency of microbial consumers is generally low(< 37 % in protists, Crotty et al. 2012). Especially at higher trophic levels like bacterivorous protozoa, that feed primarily on fast growing bacteria with a high turnover, ^{13}C losses due to respiration must be tremendous (Crotty et al. 2013) resulting in apparently reduced assimilation ratios (Lueders et al. 2004). Eight days post labeling we found a higher number of ^{13}C enriched taxa in the rhizosphere compared to 5 days post labeling. Labeled ciliates appeared for the first time in the protozoan community. A broader spectrum of labeled bacteria and fungi appeared to create more nutritional niches thereby supporting an increased number of taxa of protozoan grazes.

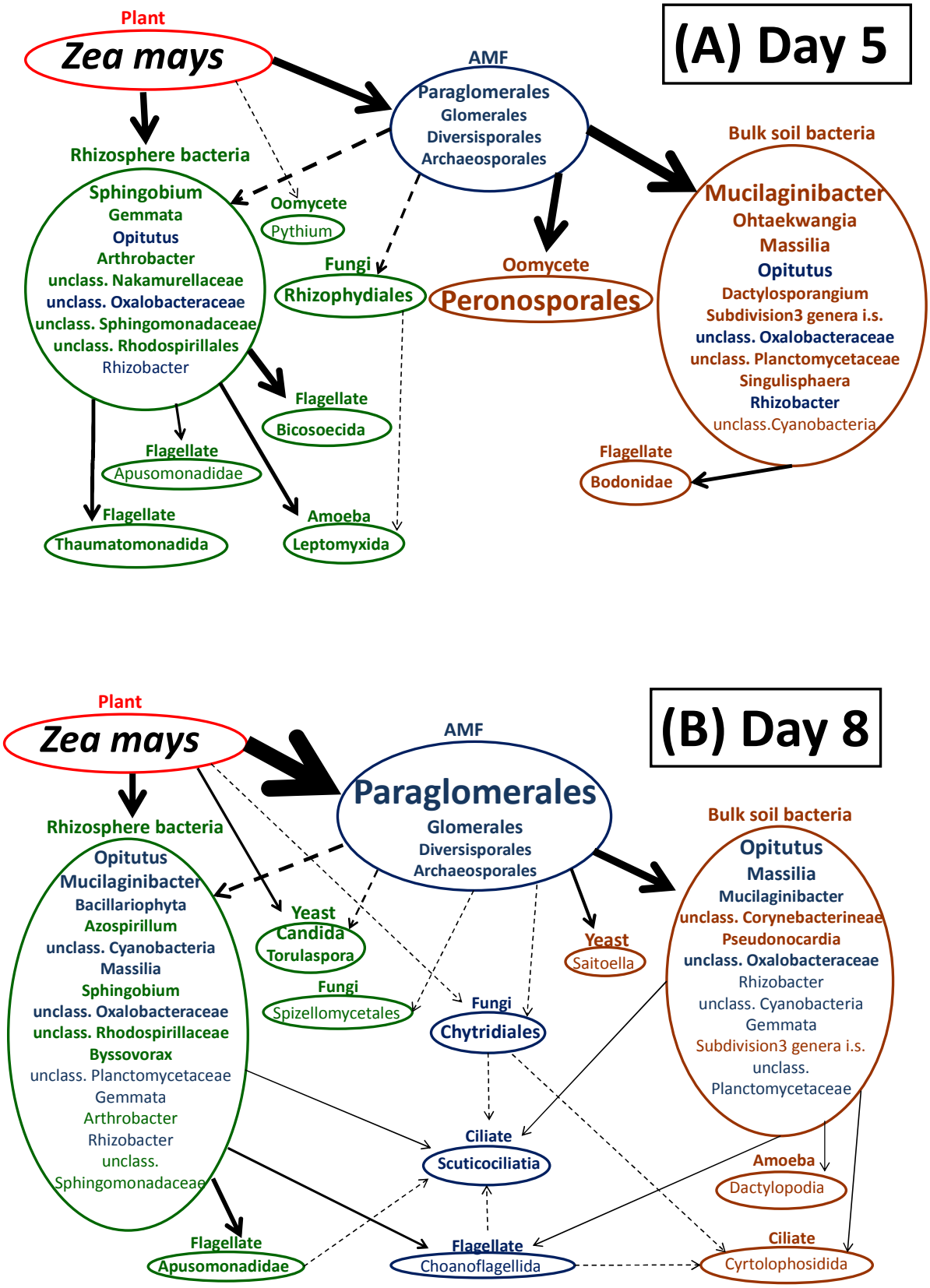

Fig. 9: Plant derived microbial soil food webs in rhizosphere and bulk soil based on plant derived carbon (^{13}C) incorporation into RNA 5 (A) and 8 (B) days post labeling. Rhizosphere taxa are shown in green, bulk soil taxa in brown, taxa labelled in both, rhizosphere and bulk soil, in blue. Arrows indicate C-flow, size of arrows and taxa names indicate strength of ^{13}C enrichment, dotted arrows indicate assumed C flow

Although our very conservative definition of ^{13}C enrichment may have excluded potential users of plant derived C our results confirm the existence of a distinct, diverse and temporarily dynamic microbial food web strongly associated with the belowground C flux of maize roots in an agricultural soil (Berendsen et al. 2012).

Conclusion

Understanding the C flow into and through belowground food webs is a crucial task in soil ecology. This study gives detailed novel insights concerning the composition of these food webs in agricultural soil and describes the spread of C from maize roots through different microbial taxa and trophic levels.

Even though we worked with a rather simple system, a complex, species rich and dynamic food web fuelled by maize exudates is portrayed. We found striking differences between the subset of microbial taxa with particularly high read abundances and the part of the community incorporating plant derived C. Our results indicate that in the rhizosphere most root exudates are consumed by AMF rather than by the bacterial community. As a result the proportion of C allocated into bacterial feeding protozoa was low. Further AMF are forming a highly effective C bridge from root into bulk soil, where a fraction of the plant derived C is liberated with a delay of several days. As a consequence the area of influence of the plant is much bigger than anticipated and the C supply within this zone turns out to be much more homogeneous than generally expected. Except for AMF, the labeled fungal community predominantly consisted of single celled organisms like yeasts and possibly AMFs parasitizing Chytridiomycota. Fast growing saprophytic sugar fungi could surprisingly not be identified as root exudate consumers, they seem to be less relevant in food webs that are fuelled by plant derived C.

Generally, the portrayed plant C associated microbial soil food web has a high turnover and we observed a rapid succession of enriched organisms. 5 days post labeling mainly rhizosphere protozoa, especially flagellates, thrive on labeled organisms like the rhizosphere bacteria *Sphingobium*, 3 days later a complex protozoan community of flagellates, amoebae and ciliates in the rhizosphere and bulk soil is (directly or) indirectly consuming root-derived C.

Taken together, we reveal that food webs of root associated soil microbes are fundamentally different to those in bulk soil and that AMFs, yeasts and Peronosporales are primary consumers of plant derived nutrients, contrasting the classic notion of bacteria as the major recipients of nutrients.

References

Adl S. M., Simpson A. G., Farmer M. A., Andersen R. A., Anderson O. R., Barta J. R., Bowser S. S., Brugerolle G., Fensome R. A., Fredericq S., James T. Y., Karpov S., Kugrens P., Krug J., Lane C. E., Lewis L. A., Lodge J., Lynn D. H., Mann D. G., McCourt R. M., Mendoza L., Moestrup O., Mozley-Standridge S. E., Nerad T. A., Shearer C. A., Smirnov A. V., Spiegel F. W., and Taylor M. F., (2005) The new higher level classification of Eukaryotes with emphasis on the taxonomy of Protists. *Journal of Eukaryotic Microbiology* 52: 399-451

Albers, D., Schaefer, M., and Scheu, S., (2006) Incorporation of plant carbon into the soil animal food web of an arable system. *Ecology* 87: 235-245

Andrews, A. E., Kofler, J. D., Trudeau, M. E., Williams, J. C., Neff, D. H., Masarie, K. A., Chao, D. Y., Kitzis, D. R., Novelli, P. C., Zhao, C. L., Dlugokencky, E. J., Lang, P. M., Crotwell, M. J., Fischer, M. L., Parker, M. J., Lee, J. T., Baumann, D. D., Desai, A. R., Stanier, C. O., de Wekker, S. F. J., Wolfe, D. E., Munger, J. W., and Tans, P. P., (2013) CO_2, CO and CH_4 measurements from the NOAA Earth System Research Laboratory's Tall Tower Greenhouse Gas Observing Network: instrumentation, uncertainty analysis and recommendations for future high-accuracy greenhouse gas monitoring efforts. *Atmospheric Measurement Techniques Discussions 6*(1): 1461-1553.

Babalola, O. O., (2010) Beneficial bacteria of agricultural importance. *Biotechnology letters 32*(11): 1559-1570

Badri, D. V., Chaparro, J. M., Zhang, R., Chen, Q., and Vivanco, J. M., (2013) Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. *J. Biol. Chem.* 288: 4502-4512

Badri, D. V., and Vivanco, J. M., (2009) Regulation and function of root exudates. *Plant Cell Environ.* 32:666-681

Bago, B., Pfeffer, P. E., and Shachar-Hill, Y., (2000) Carbon metabolism and transport in arbuscular mycorrhizas. *Plant Physiology 124*(3): 949-958

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., and Vivanco, J. M., (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu Rev Plant Biol* 57(1): 233–266

Balkwill, D. L., Fredrickson, J. K., and Romine, M. F., (2006) Sphingomonas and related genera. In The prokaryotes. *Springer New York*: 605-629

Barr, D. J. S., (2001) Chytridiomycota. In Systematics and Evolution, *Springer Berlin* Heidelberg: 93-112

Berendsen, R. L., Pieterse, C. M. J., and Bakker, P. A. H. M., (2012) The rhizosphere microbiome and plant health. *Trends in Plant Science* 17(8):476-486

Berney, C., Fahrni, J., and Pawlowski, J., (2004) How many novel eukaryotic 'kingdoms'? Pitfalls and limitations of environmental DNA surveys. *BMC Biol* 2: 13

Bisseling, T., Dangl, J. L., and Schulze-Lefert, P., (2009) Next-generation communication. *Science 324*(5928): 691-691

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W., and de Bruyn, M., (2014) Environmental DNA for wildlife biology and biodiversity monitoring. *Trends Ecol Evol* 29(6): 358-367

Bonkowski, M., and Brandt, F., (2002) Do soil protozoa enhance plant growth by hormonal effects? *Soil Biology and Biochemistry* 34: 1709-1715

Bonkowski, M., (2004) Protozoa and plant growth: the microbial loop in soil revisited. *New Phytologist*, *162*(3): 617-631

Bonkowski, M., Villenave, C., and Griffiths, B., (2009) Rhizosphere fauna: the functional and structural diversity of intimate interactions of soil fauna with plant roots. *Plant and Soil* 321: 213-233

Boschker, H. T. S., Nold, S. C., Wellsbury, P., Bos, D., De Graaf, W., Pel, R., Parkes, R. J., and Cappenberg, T. E., (1998) Direct linking of microbial populations to specific biogeochemical processes by ^{13}C-labelling of biomarkers. *Nature 392*(6678): 801-805

Bouffaud, M.-L., Poirier, M.-A., Muller, D., and Moënne-Loccoz, Y., (2014) Root microbiome relates to plant host evolution in maize and other Poaceae. *Environmental Microbiology* 16(9): 2804-2814

Brown, S. A., Palmer, K. L. and Whiteley, M., (2008) Revisiting the host as growth medium. *Nat. Rev. Microbiol.* 6: 657-666

Buddrus-Schiemann, K., Schmid, M., Schreiner, K., Welzl, G., and Hartmann, A. (2010) Root colonization by Pseudomonas sp. DSMZ 13134 and impact on the indigenous rhizosphere bacterial community of barley. *Microbial Ecology 60*(2): 381-393

Buee, M., Reich, M., Murat, C., Morin, E., Nielsson, R. H., Uroz, S., and Martin, F., (2009) 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist* 184: 449–456

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F. O., Amann, R., Eickhorst, T., and Schulze-Lefert, P., (2012) Revealing structure and assembly cues for arabidopsis root-inhabiting bacterial microbiota. *Nature* 488: 91-95

Chakraborty, S., and Old, K., (1982) Mycophagous soil amoeba: Interactions with three plant pathogenic fungi. *Soil Biology and Biochemistry* 14: 247-255

Clarholm, M., (1985) Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. *Soil Biology and Biochemistry 17*(2): 181-187

Clarholm, M., Bonkowski, M., and Griffiths, B., (2007) Protozoa and other protista in soil. *Modern soil microbiology, CRC, Boca Raton London New York:* 148-175

Coplen, T. B., (2011) Guidelines and recommended terms for expression of stable isotope-ratio and gas-ratio measurement results. *Rapid Commun. Mass Spectrom*. 25: 2538–2560

Curtis, T. P., Sloan, W. T., and Scannell, J. W., (2002) Estimating prokaryotic diversity and its limits. *Proc. Natl. Acad. Sci. USA* 99: 10494–10499

Crotty, F. V., Adl, S. M., Blackshaw, R. P., and Murray, P. J., (2012) Using stable isotopes to differntiate trophic feeding channels within soil food webs. *The Journal of Eukaryotic Microbiology* 59(6): 520-526

Crotty, F. V., Adl, S. M., Blackshaw, R. P., and Murray, P. J., (2013) Measuring soil protist respiration and ingestion rates using stable isotopes. *Soil Biology and Biochemistry* 57: 919-921

Deacon, J.W., (1997) Modern mycology. *Blackwell, London*. 188-191

De Boer, W., Folman, L., Summerbell, R., and Boddy, L., (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* 29: 795-811

De Boer, W., Kowalchuk, G. A., and van Veen, J. A., (2006) 'Root-food' and the rhizosphere microbial community composition. *New Phytologist* 170: 3-6

Dennis, P. G., Miller, A. J., and Hirsch, P. R., (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Mircobiol. Ecology* 72: 313-327

Dick, M. W., (2001) Straminipilous fungi. Systematics of the Peronosporomycetes including accounts of the marine straminipilous protists, the plasmodiophorids and similar organisms. Dordrecht, the Netherlands: *Kluwer Academic Publishers*: 670

Dohrmann, A. B., Küting, M., Jünemann, S., Jaenicke, S., Schlüter, A., and Tebbe, C. C., (2012) Importance of rare taxa for bacterial diversity in the rhizosphere of Bt-and conventional maize varieties. *The ISME journal 7*(1): 37-49

Drigo, B., Pijl, A. S., Duyts, H., Kielak, A. M., Gamper, H. A., Houtekamer, M. J., Boschker, H. T. S., Bodelier, P. L. E., Whiteley, A. S., van Veen, J. A., and Kowalchuk, G. A., (2010) Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2. *PNAS* 107(24):10938-10942

Epstein, S., and López-García, P., (2008) "Missing" protists: a molecular prospective. *Biodivers Conserv* 17: 261-276

Euringer, K., and Lueders, T., (2008) An optimised PCR/T-RFLP fingerprinting approach for the investigation of protistan communities in groundwater environments. *J Microbiol Methods* 75: 262-268

Farrar, J., Hawes, M., Jones, D., and Lindow, S., (2003) How roots control the flux of carbon to the rhizosphere. *Ecology* 84: 827-837

Frank, D. A., and Groffman, P. M., (2009) Plant rhizospheric N processes: what we don't know and why we should care. *Ecology* 90: 1512-1519

Gaiero, J. R., McCall, C. A., Thompson, K. A., Day, N. J., Best, A. S., and Dunfield, K. E., (2013) Inside the root microbiome: Bacterial root endophytes and plant growth promotion. *American journal of botany 100*(9): 1738-1750

Gams, W., (2007) Biodiversity of soil-inhabiting fungi. *Biodivers. Conserv*. 16: 69–72

Geisen, S., Hünninghaus, M., Dumack, K., Koller, R., Urich, T., and Bonkowski, M., (in prep.) The neclected link in soil food webs: mycophagous protists.

Glaubitz, S., Lueders, T., Abraham, W.-R., Jost, G., Jurgens, K., and Labrenz, M., (2009) ^{13}C-isotope analyses reveal that chemolithoautotrophic *Gamma*- and *Epsilonproteobacteria* feed a microbial food web in a pelagic redoxcline of the central Baltic Sea. *Environ Microbiol* 11: 326-337

Haichar, F. Z., Marol, C., Berge, O., Rangel-Castro, J. I., Prosser, J. I., Balesdent, J., Heulin, T., and Achouak, W., (2008) Plant host habitat and root exudates shape bacterial community structer. *ISME* 2:1221-1230

Hendrix, F. F., and Campbell, W. A., (1973) Pythiums as plant pathogens. *Annual review of Phytopathology 11*(1): 77-98

Henkes, G. J., Jousset, A., Bonkowski, M., Thorpe, M. R., Scheu, S., Lanouel, A., Schurr, U., and Rösel, U. S. R., (2011) *Pseudomonas fluorescens* CHA0 maintains carbon delivery to *Fusarium graminearum*-infected roots and prevents reduction in biomass of barley shoots through systemic interactions. *Journal of Experimental Botany* 62: 4337-4344

Hunt, H. W., Coleman, D. C., Ingham, E. R., Ingham, R. E., Elliott, E. T., Moore, J. C., Rose, S. L., Reid, C. P. P., and Morley, C. R. (1987) The detrital food web in a shortgrass prairie.*Biology and Fertility of Soils 3*(1-2): 57-68

Johnson, D., Leake, J. R., Ostle, N., Ineson, P., and Read, D. J., (2002) In situ $^{13}CO_2$ pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytologist 153*(2): 327-334

Jones, D. L., Nguyen, C., and Finlay, R. D., (2009) Carbon flow in the rhizosphere: carbon trading at the soil-root interface. *Plant and Soil* 321: 5-33

Junier, P., Junier, T., and Witzel, K. P., (2008) TRiFLe, a program for in silico terminal restriction fragment length polymorphism analysis with user-defined sequence sets. *Applied and Environmental Microbiology* 74(20): 6452-6456

Kent, A. D., and Triplett, E. W., (2002) Microbial communities and their interactions in soil and rhizosphere ecosystems. *Annual Review of Microbiology* 56: 211-236

Kielak, A., Rodrigues, J. L., Kuramae, E. E., Chain, P. S., Van Veen, J. A., and Kowalchuk, G. A., (2010) Phylogenetic and metagenomic analysis of Verrucomicrobia in former agricultural grassland soil. *FEMS microbiology ecology 71*(1): 23-33

Kiers, E. T., Duhamel, M., Beesetty, Y., Mensah, J. A., Franken, O., Verbruggen, E., Fellbaum, C. R., Kowalchuk, G. A., Hart, M. M., Bago, A., Palmer, T. M., West, S. A., Vandenkoornhuyse, P., Jansa, J., and Bücking, H., (2012) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333: 880-882

Koller, R., Robin, C., Bonkowski, M., Ruess, L., and Scheu, S., (2013) Litter quality as driving factor for plant nutrition via grazing of protozoa on soil microorganisms. *FEMS Microbiology Ecology* 85: 241-250

Koller, R., Scheu, S., Bonkowski, M., and Robin, C., (2013) Protozoa stimulate N uptake and growth of arbuscular mycorrhizal plants. *Soil Biology and Biochemistry 65*: 204-210

Kramer, S., Dibbern, D., Moll, J., Hünninghaus, M., Koller, R., Krueger, D., Marhan, S., Urich, T., Wubet, T., Bonkowski, M., Buscot, F., Lueders, T., and Kandeler, E., (in prep.) Eat all you can – Resource partioning between bacteria, fungi and protists in the detritusphere

Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Scheunemann, N., Schoene, J., Schmalwasser, A., Totsche, K. U., Walker, F., Scheu, S., and Kandeler, E., (2012) Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems. *Pedobiologia* 55: 111-119

Kreuzer, K., Adamczyk, J., Iijima, M., Wagner, M., Scheu, S., and Bonkowski, M., (2006) Grazing of a common species of soil protozoa (*Acanthamoeba castellanii*) affects rhizosphere bacterial community composition and root architecture of rice (*Oryza sativa* L.). *Soil Biology & Biochemistry* 38: 1665-1672

Krings, M., Taylor, T. N., and Dotzler, N., (2011) The fossil record of the Peronosporomycetes (Oomycota). *Mycologia* 103(3): 445–457

Kuzyakov, Y., (2010) Priming effects: interactions between living and dead organic matter. *Soil Biology and Biochemistry*, *42*(9): 1363-1371

Lanzén, A., Jørgensen, S. L., Huson, D. H., Gorfer, M., Grindhaug, S. H., Jonassen, I., Øvreås, L., and Urich, T., (2012) CREST – Classification Resources for Environmental Sequence Tags. *PLoS ONE* 7(11): e49334. doi:10.1371/journal.pone.0049334

Lee, H. R., Han, S. I., Rhee, K. H., and Whang, K. S., (2013) *Mucilaginibacter herbaticus sp*. nov., isolated from the rhizosphere of the medicinal plant *Angelica sinensis*. *International journal of systematic and evolutionary microbiology 63*(Pt 8): 2787-2793

Liljeroth, E., Burgers, S. L. G. E., and Van Veen, J. A., (1991) Changes in bacterial populations along roots of wheat (*Triticum aestivum* L.) seedlings. *Biology and fertility of soils 10*(4): 276-280

Lopez-Guerrero, M. G., Ormeno-Orrillo, E., Rosenblueth, M., Martinez-Romero, J., and Martinez-Romero, E., (2013) Buffet hypothesis for microbial nutrition at the rhizosphere. *Frontiers in Plant Science* 4(188): 1-4

Lu, Y., and Conrad, R., (2005) In situ stable isotope probing of methanogenic archaea in the rice rhizosphere. *Science 309*(5737): 1088-1090

Lueders, T., Kindler, R., Miltner, A., Friedrich, M. W., and Kaestner, M., (2006) Identification of bacterial micropredators distinctivly active in a soil microbial food web. *Applied and Environmental Microbiology* 72(8): 5342-5348

Lueders, T., Manefield, M., and Friedrich, M. W., (2004) Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. *Environmental Microbiology* 6(1): 73

Lueders, T., Wagner, B., Claus, P., and Friedrich, M. W., (2004) Stable isotope probing of rRNA and DNA reveals a dynamic methylotroph community and tropic interactions with fungi and protozoa in oxic rice field soil. *Environmental Microbiology* 6(1): 60-72

Maloney, P. E., Van Bruggen, A. H. C., and Hu, S., (1997) Bacterial community structure in relation to the carbon environments in lettuce and tomato rhizospheres and in bulk soil. *Microbial ecology 34*(2): 109-117

Mao, Y., Li, X., Smyth, E. M., Yannarell, A. C., and Mackie, R. I., (2014) Enrichment of specific bacterial and eukaryotic microbes in the rhizosphere of switchgrass (*Panicum virgatum* L.) through root exudates. *Environmental Microbiology Reports* doi: 10.1111/1758-2229.12152

Marzorati, M., Wittebolle, L., Boon, N., Daffonchio, D., and Verstraete, W., (2008) How to get more out of molecular fingerprints: Practical tools for microbial ecology. *Environmental Microbiology* 10(6): 1571

Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J. H. M., Piceno, Y. M., DeSantis, T. Z., Andersen, G. L., Bakker, P. A. H. M., and Raaijmakers, J. M., (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332: 1097-1100

Molin, S., and Givskov, M., (1999) Application of molecular tools for in situ monitoring of bacterial growth activity. *Environmental microbiology 1*(5): 383-391

Moreno, A. M., Matz, C., Kjelleberg, S., and Manefield, M., (2010) Identification of ciliate grazers of autotrophic bacteria in ammonia-oxidizing activated sludge by RNA stable isotope probing. *Applied and environmental microbiology 76*(7): 2203-2211

Mougel, C., Offre, P., Ranjard, L., Corberand, T., Gamalero, E., Robin, C., and Lemanceau, P., (2006) Dynamic of the genetic structure of bacterial and fungal communities at different development stages of *Medicago truncatula* Gaertn. cv. Jemalong line J5. *New Phytologist* 170: 165-175

Murase, J., and Frenzel, P., (2007) A methane-driven microbial food web in wetland rice soil. *Environmental Microbiology* 9(12): 3025-3034

Neufeld, J. D., Vohra, J., Dumont, M. G., Lueders, T., Manefield, M., Friedrich, M. W., and Murrell, J. C., (2007) DNA stable-isotope probing. *Nature protocols 2*(4): 860-866

Nowicki, M., Foolad, M. R., Nowakowska, M., and Kozik, E. U., (2012) Potato and tomato late blight caused by Phytophthora infestans: An overview of pathology and resistance breeding. *Plant Disease 96*(1): 4-17

Ofek, M., Hadar, Y., and Minz, D., (2012) Ecology of root colonizing Massilia (Oxalobacteraceae). *PloS one 7*(7). e40117

Olsson, P. A., and Johnson, N. C., (2005) Tracking carbon from the atmosphere to the rhizosphere. *Ecology Letters* 8: 1264-1270.

Pankratov, T. A., Tindall, B. J., Liesack, W., and Dedysh, S. N., (2007) *Mucilaginibacter paludis* gen. nov., sp. nov. and *Mucilaginibacter gracilis* sp. nov., pectin-, xylan- and laminarin-degrading members of the family Sphingobacteriaceae from acidic Sphagnum peat bog. *International Journal of Systematic and Evolutionary Microbiology 57*(10): 2349-2354

Pilloni, G., Granitsiotis, M. S., Engel, M., and Lueders, T., (2012) Testing the Limits of 454 Pyrotag Sequencing: Reproducibility, Quantitative Assessment and Comparison to T-RFLP Fingerprinting of Aquifer Microbes. *PLoS One* 7(7):e40467

Peiffer, J. A., and Ley, R. E., (2013) Exploring the maize rhizosphere microbiome in the field - A glimpse into a highly complex system. *Communicative and Integrative Biology* 6:5, e25177

Peiffer, J. A., Spor, A., Koren, O., Jin, Z., Green Tringe, S., Dangl, J. L., Buckler, E. S., and Ley, R. E., (2013) Diversity and heritability of the maize rhizosphere microbiome under field conditions. *PNAS* 110(16):6548-6553

Prosser, J. I., Rangel-Castro, J. I., and Killham, K., (2006) Studying plant–microbe interactions using stable isotope technologies. *Current opinion in biotechnology*, *17*(1), 98-102.

Rangel-Castro, J. I., Killham, K., Ostle, N., Nicol, G. W., Anderson, I. C., Scrimgeour, C. M., Ineson, P., Mehark, A., and Prosser, J. I., (2005) Stable isotope probing analysis of the influence of liming on root exudate utilization by soil microorganisms.*Environmental Microbiology 7*(6): 828-838

Rosenberg, K., Bertaux, J., Krome, K., Hartmann, A., Scheu, S., and Bonkowski, M., (2009) Soil amoebae rapidly change bacterial community composition in the rhizosphere of *Arabidopsis thaliana*. *ISME Journal* 3: 675-684

Ross, J. P., and Ruttencutter, R., (1977) Population dynamics of two vesicular-arbuscular endomycorrhizal fungi and the role of hyperparasitic fungi. *Phytopathology 67*: 490-496

Scharroba, A., Dibbern, D., Hünninghaus, M., Kramer, S., Moll, J., Butenschoen, O., Bonkowski, M., Buscot, F., Kandeler, E., Koller, R., Krüger, D., Lueders, T., Scheu, S., and Ruess, L., (2012) Effects of resource availability and quality on the structure of the micro-food web of an arable soil across depth. *Soil Biology and Biochemistry* 50: 1-11

Schüßler, A., (2009) Struktur, Funktion und Ökologie der abuskulären Mykorrhiza. *Rundgespräch der Kommision für Ökologie* bd. 37: 97-108

Semenov, A. M., van Bruggen, A. H. C., and Zelenev, V. V., (1999) Moving waves of bacterial populations and total organic Carbon along roots of wheat. *Microbial ecology* 37: 116-128

Shaw, I. J., Morris, P., and Hooker, J. E., (2006) Perception and modification of plant flavonoid signals by rhizosphere microorganisms. *Environmental Microbiology* 8: 1867-1880

Stephenson, S. L., Fiore-Donno, A. M., and Schnittler, M., (2011) Myxomycetes in soil. *Soil Biol Biochem* 43: 2237-2242

Torsvik, V., Ovreas, L., and Thingstad, T. F., (2002) Prokaryotic diversity – magnitude, dynamics, and controlling factors. *Science* 296: 1064–1066

Tveit, A., Schwacke, R., Svenning, M. M., and Urich, T., (2012) Organic carbon transformations in high-Arctic peat soils: key functions and microorganisms. *The ISME journal 7*(2): 299-311

Urich, T., Lanzén, A., Qi, J., Huson, D. H., Schleper, C., and Schuster, S. C., (2008) Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. *PloS one 3*(6), e2527

Vandenkoornhuyse, P., Mahe, S., Ineson, P., Staddon, P., Ostle, N., Cliquet, J. B., Francez, A. J., Fitter, A., and Young, J. P. W., (2007) Active root-inhabitating microbes identified by rapid incorporation of plant-derived carbon into RNA. *PNAS* 104(43): 16970-16975

Wakefield, W. S., Powell, M. J., Letcher, P. M., Barr, D. J., Churchill, P. F., Longcore, J. E., and Chen, S. F., (2010) A molecular phylogenetic evaluation of the Spizellomycetales. *Mycologia 102*(3): 596-604

Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R., (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* 73(16): 5261-5267

Whiteley, A. S., Thomson, B., Lueders, T., and Manefield, M., (2007) RNA stable-isotope probing. *Nature protocols 2*(4): 838-844

Wilkinson, D. M. and Mitchell, E. A. D., (2010) Testate amoebae and nutrient cycling with particular reference to soils. *Geomicrobiol Journal (*27): 520–533

Supplementary Information (SI)

No labeled bacterial T-RFs were observed before day 5. On the other days, only few labeled T-RFs were identified (Table 1) in the bulk soil. At day 5, T-RFs of, 428 bp, 447 bp and 487 bp were labeled, the T-RFs of 132 bp, 447 bp and 487 bp on day 8, and the T-RFs of 447 bp and 487 bp on day 11. In the rhizosphere, only few bacterial T-RFs were labeled on day 5 and 8, similar as in bulk soil samples. On day 5, one T-RF with 72 bp appeared weakly labeled. On day 8, T-RFs with 147 bp, 435 bp and 447 bp were ^{13}C enriched in the rhizosphere (Table 1).

Table 1: Labeled T-RFs with assigned taxa. exp. T-RF: T-RF recognized as labeled by comparing T-RFLP fingerprint of SIP gradient fractions from ^{13}C treatments and ^{12}C-controls; *in-silico* T-RFs: best matching T-RF found in according contiq sequences. ???: no distinct taxa were found for this T-RF.

	Day 5			**Day 8**		
	exp. T-RF	taxa	*in-silico* T-RFs	exp. T-RF	taxa	*in-silico* T-RFs
bulk soil	428	???		132	???	
	447	Opitutus	451	447	Opitutus	450
	487	Opitutus	487	487	Opitutus	487
rhizosphere	72	Arthrobacter	67	147	Azospirillum	150
				435	Mucilaginibacter	435
				447	Opitutus	450

Only a small subset of the soil bacterial community was found to be enriched in ^{13}C by T-RFLP fingerprinting, additional taxa with low abundances were labeled as indicated by pyrotag sequencing. This discrepancy results from the generally low abundance of ^{13}C enriched taxa in our experiment. Hence, T-RFs of most labeled bacteria were probably masked by unlabeled rRNA with the same T-RFs, and therefore not detected in the fingerprint approach.

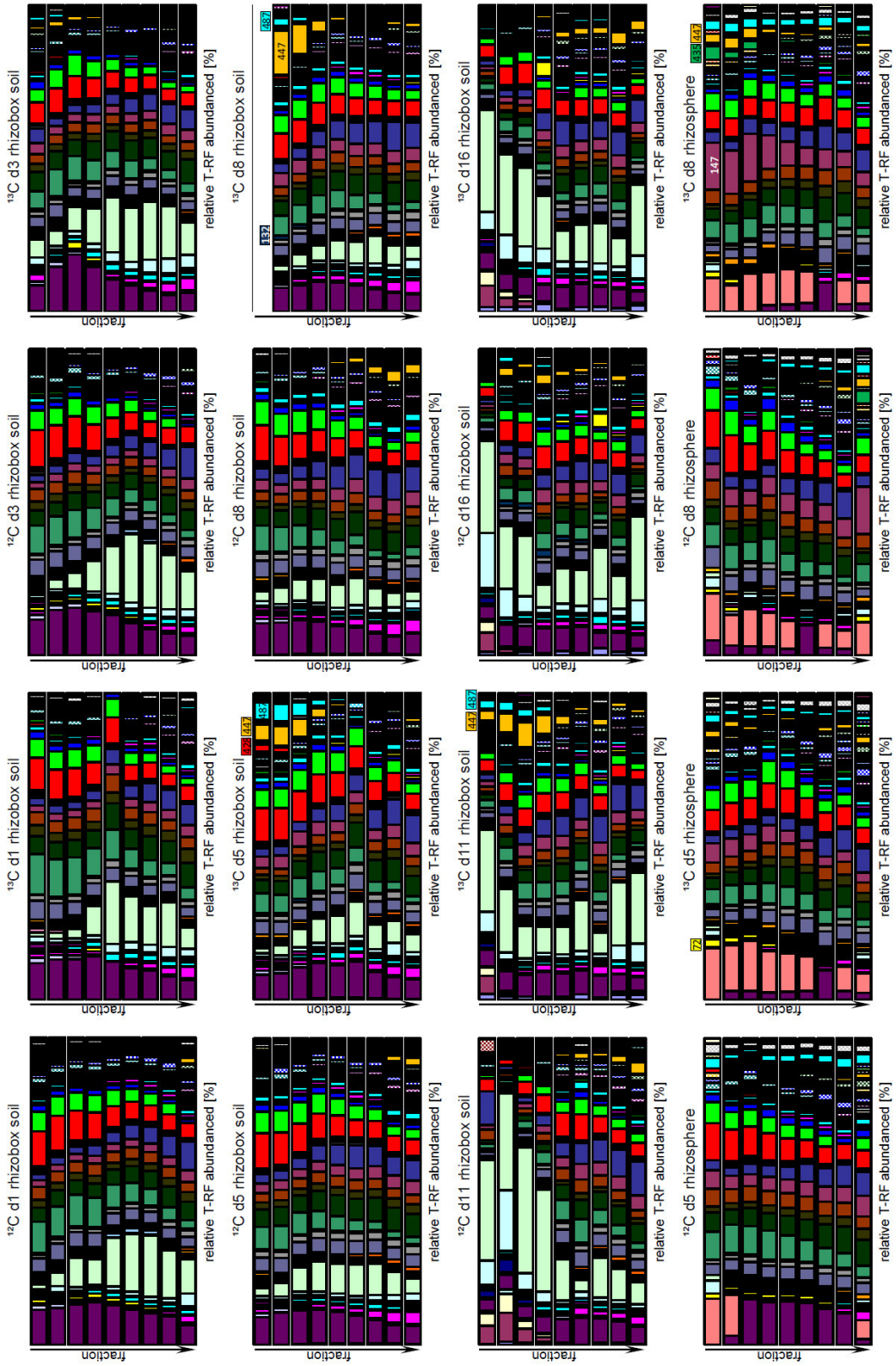

Fig. S 1: Bar plots of the 16S rRNA T-RFLP fingerprints. Arrows indicate fractions from 'heavy' (base) to 'light' (point) of the ^{12}C and ^{13}C gradients. d1, d3, d5, d8, d11 and d16 are the sampling time points 1, 3, 5, 8, 11 and 16 days after the experiment started.

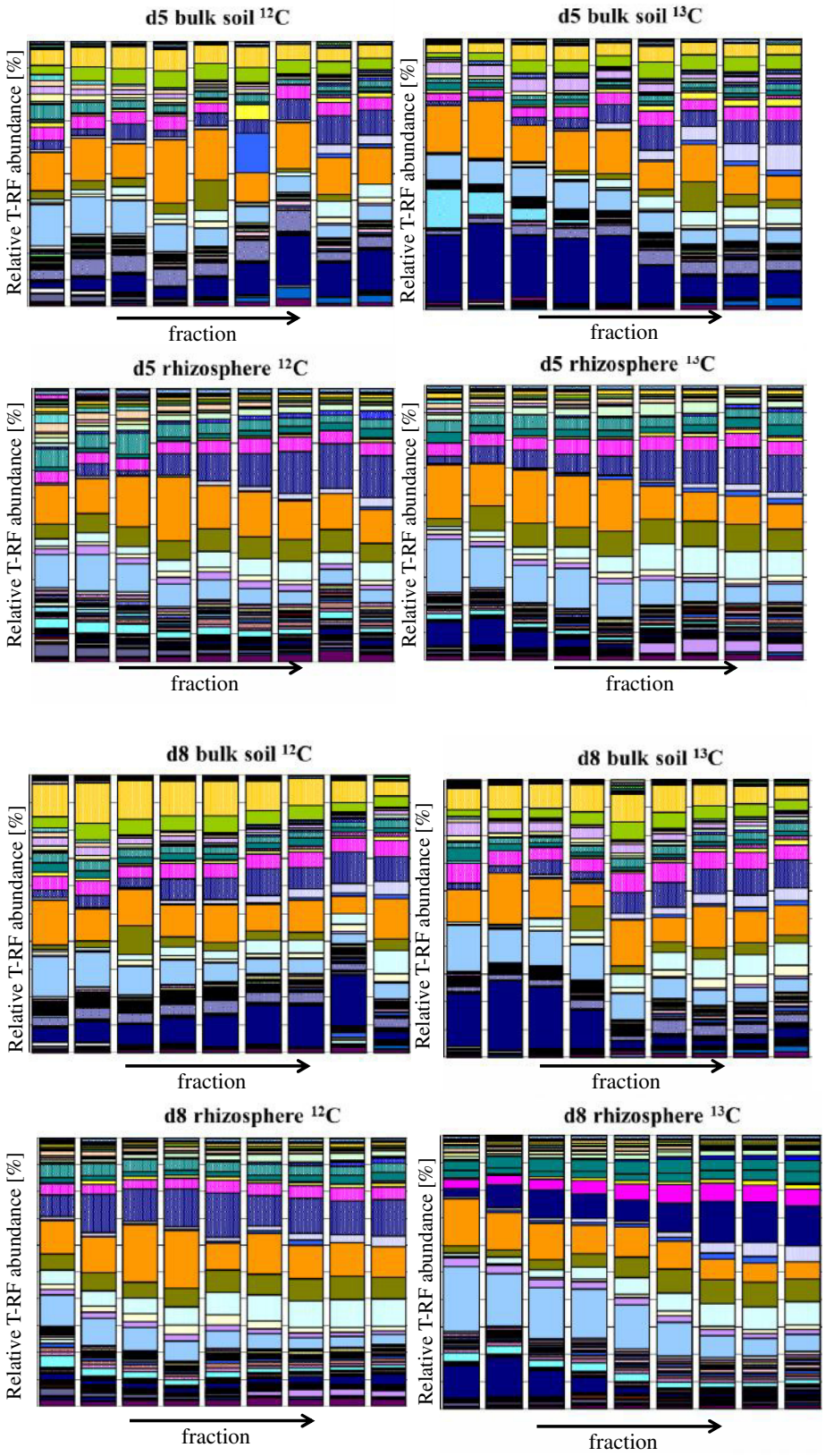

Fig. S 2: Bar plots of the eukaryotic rRNA T-RFLP fingerprints. d5 and d8 are the sampling time points 5 and 8days after the experiment started.

Chapter III: Eat all you can - Resource partitioning between bacteria, fungi and protists in the detritusphere*

Abstract

The mineralization and flow of plant derived C in soil is relevant to global C cycling. Current models of organismic C 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-SIP 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.

* for information about Co-authorships see page 155

Introduction

Microbes fulfil 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 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 detritus degradation (Strickland and Rousk 2010).

Soil microbes are known to undergo a succession during the degradation of detritus (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 harbour 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 SIP can be seminal for the tracing of substrate derived C 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 C 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 ^{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 C (C_{mic}), as well as the ^{13}C in CO_2 and C_{mic} was determined during incubation as described (SI). The relative amounts of substrate derived C 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 analysed by T-RFLP fingerprinting (Euringer and Lueders 2008, Glaubitz et al. 2009, Lueders et al. 2004a). Only the glucose and leaf treatments were analysed for protists. See SI for full methodological detail. Based on these rRNA fingerprints (Figs. S 1, S 2, S 3, see page 115 - 117), 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.

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. S 1, S 2, S 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 C 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. 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. 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. 2A). Consistently, resource-derived C incorporated into C_{mic} was highest

for glucose (~40 %) after only 2 days of incubation (Fig. 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. 2B).

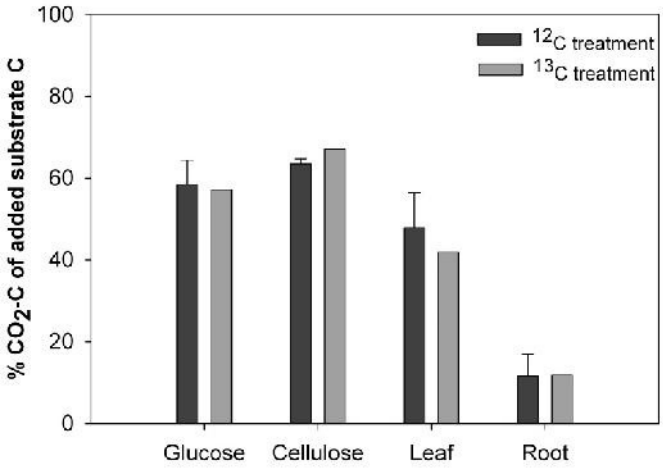

Fig. 1: Substrate-C mineralized to CO_2 after 32 days of soil incubation.

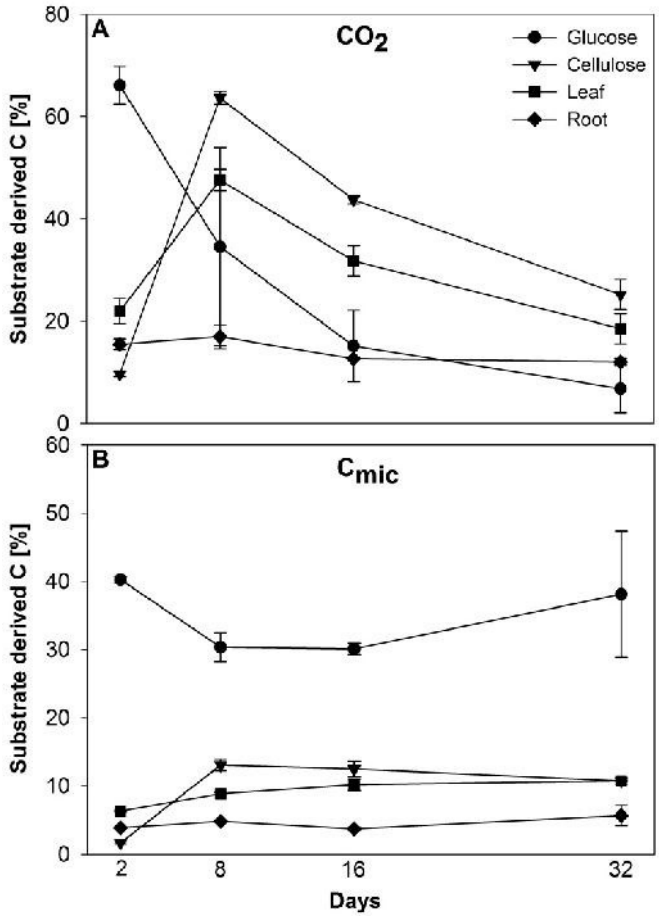

Fig. 2: Time course of substrate derived C in CO_2 (A) and C_{mic} (B) during SIP incubation

rRNA stable isotope probing

The incorporation of ^{13}C into substrate-specific subsets of bacterial, fungal and protistan communities was evident already via the comparison of T-RFLP fingerprints from ^{13}C and ^{12}C-control gradients (Figs. S1, S2, S3). 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. S1, S2, S3) could in fact be linked to pyrotag-defined microbial taxa (see SI for details and methodological discussion). An overview of the most relevant labeled prokaryotes and microeukaryotes detected in our experiment is given in Table 1.

Labeled bacterial rRNA

The different bacterial taxa incorporating ^{13}C-label belonged mainly to three bacterial phyla: *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* (Fig. 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. S 1) were strongly enriched (EF 31, Fig. 3) and most abundant (~40 %, Fig. S 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 and 99) and abundant (40 and 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.

Table 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

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.

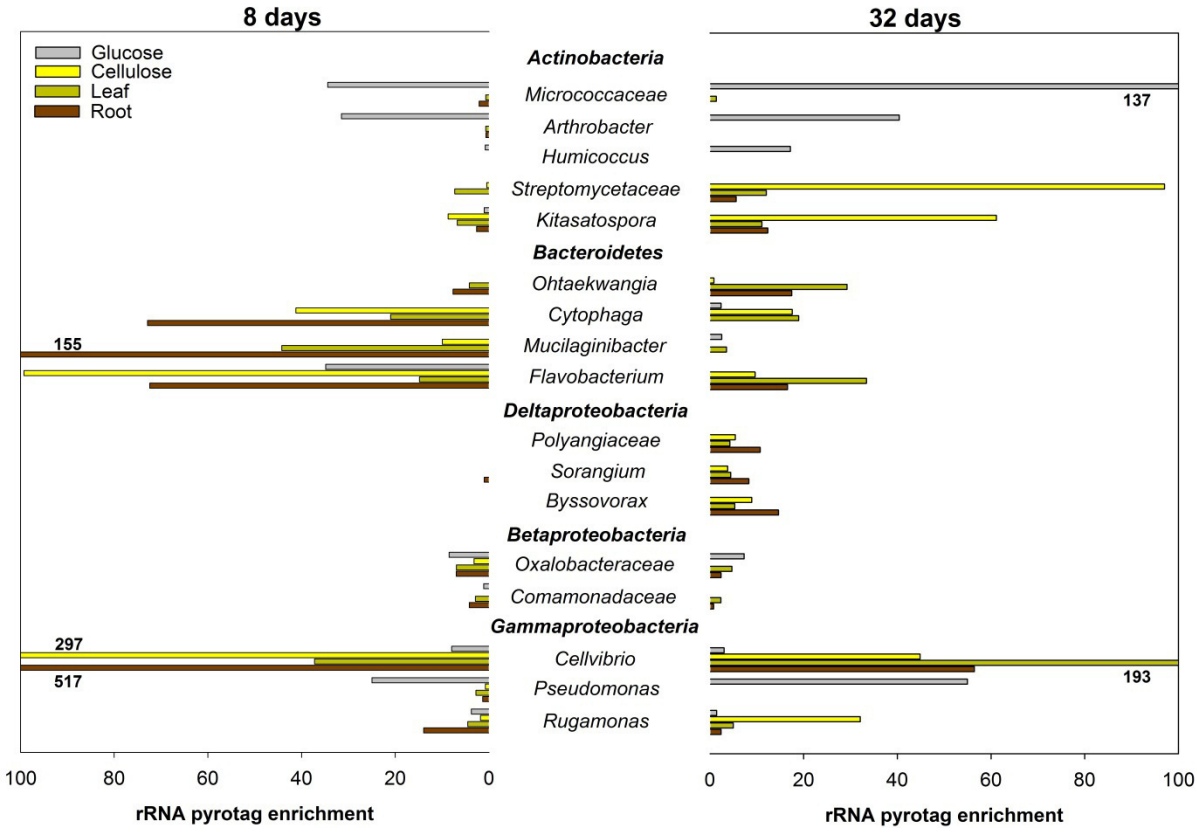

Fig. 3: ^{13}C-labeled bacterial taxa after 8 and 32 days of incubation. Labeling was inferred via comparative pyrotag enrichment in 'heavy' vs. 'light' rRNA gradient fractions of ^{13}C and ^{12}C treatments. Only labeled taxa are shown.

In essence, the diversity of key taxa labeled during leaf and root decomposition was not noticeably larger than with glucose and cellulose (Table 1). However, the functional organization (*Fo*) of bacterial rRNA fingerprints in 'heavy' fractions showed a clear decrease towards the more complex substrates (Table 2) suggesting a higher diversity and fewer dominant bacterial taxa in rRNA of labeled bacterial consumers of leaves and roots.

Table 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. 4). A consistent trend of the *Fo* of 'heavy' fungal rRNA fingerprints with substrate complexity was not observed (Table 2).

Glucose C was mainly assimilated by *Cryptococcus spp*. (T-RF 564, Fig. S 2), as shown by highly abundant (>50 %) and enriched (EF 7) reads in 'heavy' rRNA (Figs. 4, S 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.

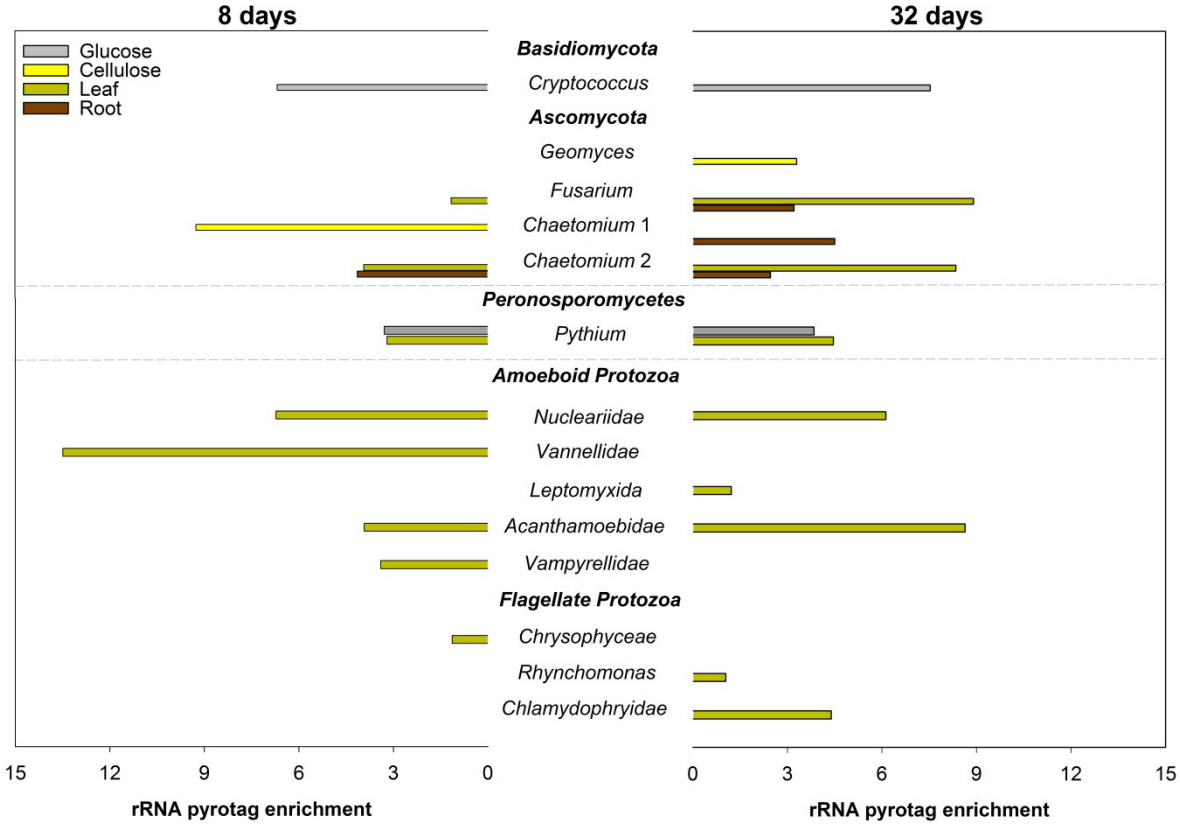

Fig. 4: ^{13}C-labeled fungal and protist taxa after 8 and 32 days of incubation. Labeling was inferred via comparative pyrotag enrichment in 'heavy' vs. 'light' rRNA gradient fractions of ^{13}C and ^{12}C treatments. Only labeled taxa are shown.

Labeled protist rRNA

Protists within the Peronosporomycetes (formerly: Oomycetes) related to *Pythium spp*. (T-RFs 418, 421, Fig. S 3) were clearly enriched (EF ~8) and abundant (12 – 18 %) in 'heavy' rRNA fractions of both treatments investigated for protists (glucose and leaf, Figs. 4, S 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. 4, S 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. 1). There was no difference in cumulative mineralization between the glucose and cellulose treatments, but initial mineralization rates were clearly highest for glucose (Fig. 2A). The much less efficient mineralization and assimilation of plant biomass amendments (Figs. 1, 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. 3, Table 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. 3, Table 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). Also many Streptomycetes can decompose polysaccharides and possess both exo- and endocellulases (Kämpfer 2006), but 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. 4). Although Zygomycetes, so called 'sugar fungi' such as *Mortierella* and *Mucor spp.,* are often considered the most important users of low molecular weight C 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. S 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 C 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 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 protozoan taxa over time indicated a substantial flow of C from labeled prey into protozoan grazers in the leaf treatment. ^{13}C enrichment was mainly found in amoeboid protozoa (Fig. 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. S 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 C flux from litter material to facultative 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 C 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 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 1, Fig. 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

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 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 C flow in an agricultural soil. Distinct bacterivorous and presumably even fungivorous protozoan key-players were identified as labeled. Although taxonomic detail on fungal feeding protozoa in soil exists, this might be 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 C 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 C 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).

References

Adl, S. M., and Gupta, V. S., (2006) Protists in soil ecology and forest nutrient cycling. *Can J Forest Res* 36: 1805-1817

Adl, S. M., Simpson, A. G. B., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R., Bowser, S. S., Brugerolle, G., Fensome, R., Fredericq, S., James, T. Y., Karpov, S., Kugrens, P., Krug, J., Lane, C., Lewis, L. A., Lodge, G., Lynn, D. H., Mann, D. G., McCourt, R. M. Mendoza, L., Moestrup, Ø., Mozley-Standridge, S., Nerad, T. A., Shearer, C., Smirnov, A. V., and Spiegelz, F. W., (2005) The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J Eukaryot Microbiol* 52: 399-451

Baldrian, P., Kolarik, M., Stursova, M., Kopecky, J., Valaskova, V., Vetrovsky, T., Žifčákova, L., Šnajdr, J., Rídl, J., Vlček, C., and Voříšková, J., (2013) Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME Journal* 6: 248-258

Bastian, F., Bouziri, L., Nicolardot, B., and Ranjard, L. (2009) Impact of wheat straw decomposition on successional patterns of soil microbial community structure. *Soil Biol Biochem* 41: 262-275

Berg, B., and McClaugherty, C. (2008) Plant litter, 2nd edn. *Springer: Berlin*

Bernard, L., Mougel, C., Maron, P. A., Nowak, V., Lévêque, J., Henault, C., Zahar Haichar, F., Berge, O., Marol, C., Balesdent, J., Gibiat, F., Lemanceau, P., and Ranjard, L., (2007) Dynamics and identification of soil microbial populations actively assimilating C from ^{13}C-labelled wheat residue as estimated by DNA- and RNA-SIP techniques. *Environ Microbiol* 9: 752

Bertrand, I., Chabbert, B., Kurek, B., and Recous, S. (2006) Can the biochemical features and histology of wheat residues explain their decomposition in soil? *Plant Soil* 281: 291-307

Bradford, M. A., (2013) Thermal adaptation of decomposer communities in warming soils. *Front Microbiol* 4

De Boer, W., Folman, L. B., Summerbell, R. C., and Boddy, L. (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiol Rev* 29: 795-811

De Ruiter, P., Neutel, A.-M., and Moore, J., (1996) Energetics and stability in belowground food webs. In: Polis G, Winemiller K (eds) *Food Webs*. Springer US: 201-210

Deacon, J. W. (1997) *Modern Mycology*, 3rd edn. Blackwell: London

Dibbern, D., Schmalwasser, A., Lueders, T., and Totsche, K. U. (2014) Selective transport of plant root-associated bacterial populations in agricultural soils upon snowmelt. *Soil Biol Biochem* 69: 187-196

Drigo, B., Pijl, A. S., Duyts, H., Kielak, A. M., Gamper, H. A., Houtekamer, M. J., Boschker, H. T. S., Bodelier, P. L. E., Whiteley, A. S., van Veen, J. A., and Kowalchuk, G. A., (2010) Shifting C flow from roots into associated microbial communities in response to elevated atmospheric CO_2. *Proc Natl Acad Sci USA* 107: 10938-10942

Eichorst, S. A., and Kuske, C. R., (2012) Identification of cellulose-responsive bacterial and fungal communities in geographically and edaphically different soils by using stable isotope probing. *Appl Environ Microbiol* 78: 2316-2327

Ekelund, F., and Ronn, R. (1994) Notes on protozoa in agricultural soil with emphasis on heterotrophic flagellates and naked amoebae and their ecology. *FEMS Microbiol Rev* 15: 321-353

Ekelund, F., (1998) Enumeration and abundance of mycophagous protozoa in soil, with special emphasis on heterotrophic flagellates. *Soil Biol Biochem* 30: 1343-1347

España, M., Rasche, F., Kandeler, E., Brune, T., Rodriguez, B., Bending, G. D., and Cadish, G., (2011) Assessing the effect of organic residue quality on active decomposing fungi in a tropical Vertisol using ^{15}N-DNA stable isotope probing. *Fung Ecol* 4: 115-119

Euringer, K., and Lueders, T., (2008) An optimised PCR/T-RFLP fingerprinting approach for the investigation of protistan communities in groundwater environments. *J Microbiol Methods* 75: 262-268

Glaubitz, S., Lueders, T., Abraham, W.-R., Jost, G., Jurgens, K., and Labrenz, M., (2009) ^{13}C-isotope analyses reveal that chemolithoautotrophic *Gamma-* and *Epsilonproteobacteria* feed a microbial food web in a pelagic redoxcline of the central Baltic Sea. *Environ Microbiol* 11: 326-337

Goldfarb, K. C., Karaoz, U., Hanson, C. A., Santee, C. A., Bradford, M. A., Treseder, K. K., Wallenstein, M. D., and Brodie, E. L., (2011) Differential growth responses of soil bacterial taxa to C substrates of varying chemical recalcitrance. *Front Microbiol* 2.

Haichar, F. E. Z., Achouak, W., Christen, R., Heulin, T., Marol, C., Marais, M. F., Mougel, C., Ranjard, L., Balesdent, J., and Berge, O., (2007) Identification of cellulolytic bacteria in soil by stable isotope probing. *Environ Microbiol* 9: 625

Hartmann, A., Schmid, M., van Tuinen, D., and Berg, G., (2009) Plant-driven selection of microbes. *Plant Soil* 321: 235-257

Hayes, M. A., (2012) The *Geomyces* fungi: Ecology and distribution. *Bioscience* 62: 819-823

Hendrix, F. F. J., and Campbell, W. A. (1973) Pythiums as plant pathogens. Baker, Kenneth F. (Ed.) *Annual Review Of Phytopathology, Vol. 11*. Illus. Annual Reviews Inc.: Palo Alto, Ca.: 77-98

Hess, S., Sausen, N., and Melkonian, M., (2012) Shedding light on vampires: The phylogeny of vampyrellid amoebae revisited. *PLoS ONE* 7: e31165

Jezbera, J., Horňák, K., and Šimek, K., (2005) Food selection by bacterivorous protists: insight from the analysis of the food vacuole content by means of fluorescence in situ hybridization. *FEMS Microbiol Ecol* 52: 351-363

Kämpfer, P. (2006) The Family Streptomycetaceae, Part I: Taxonomy. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E., (eds) *The Prokaryotes*. Springer New York: 538-604

Kleindienst, S., Herbst, F.-A., Stagars, M., von Netzer, F., von Bergen, M., Seifert, J., Peplies, J., Amann, R., Musat, F., Lueders, T., and Knittel, K., (2014) Diverse sulfate-reducing bacteria of the *Desulfosarcina/Desulfococcus* clade are the key alkane degraders at marine seeps. *ISME J*

Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Scheunemann, N., Schoene, J., Schmallwasser, A., Totsche, K. U., Walker, F., Scheu, S., & Kandeler, E. (2012) C flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems.*Pedobiologia*, *55*(2), 111-119

Leplat, J., Friberg, H., Abid, M., and Steinberg, C., (2013) Survival of *Fusarium graminearum*, the causal agent of Fusarium head blight. A review. *Agron Sustain Dev* 33: 97-111

Lueders, T., Manefield, M., and Friedrich, M. W., (2004a) Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. *Environ Microbiol* 6: 73

Lueders, T., Wagner, B., Claus, P., and Friedrich, M. W. (2004b) Stable isotope probing of rRNA and DNA reveals a dynamic methylotroph community and trophic interactions with fungi and protozoa in oxic rice field soil. *Environ Microbiol* 6: 60

Lueders, T., Kindler, R., Miltner, A., Friedrich, M. W., and Kaestner, M., (2006) Identification of bacterial micropredators distinctively active in a soil microbial food web. *Appl Environ Microbiol* 72: 5342-5348

Marzorati, M., Wittebolle, L., Boon, N., Daffonchio, D., and Verstraete, W. (2008) How to get more out of molecular fingerprints: Practical tools for microbial ecology. *Environ Microbiol* 10: 1571

McGuire, K. L., and Treseder, K. K., (2010) Microbial communities and their relevance for ecosystem models: Decomposition as a case study. *Soil Biol Biochem* 42: 529-535

Moore, J. C., and Hunt, H. W., (1988) Resource compartmentation and the stability of real ecosystems. *Nature* 333: 261-263

Moore, J. C., McCann, K., Setälä, H., and de Ruiter, P. C., (2003) Top-down is bottom-up: does predation in the rhizosphere regulate aboveground dynamics? *Ecology* 84: 846-857

Moore, J. C., McCann, K., and de Ruiter, P. C., (2005) Modeling trophic pathways, nutrient cycling, and dynamic stability in soils. *Pedobiologia* 49: 499-510

Mula-Michel, H. P., and Williams, M. A., (2012) Soil type modestly impacts bacterial community succession associated with decomposing grass detrituspheres. *Soil Sci Soc Am J* 77: 133

Mulder, C., Boit, A., Bonkowski, M., De Ruiter, P. C., Mancinelli, G., Van der Heijden, M. G. A., van Wijnen, H. J., Vonk, J. A., and Rutgers, M., (2011) A belowground perspective on dutch agroecosystems: How soil organisms interact to support ecosystem services. In: Guy W (ed) *Advances in Ecological Research*. Academic Press.: 277-357

Nicolardot, B., Bouziri, L., Bastian, F., and Ranjard, L., (2007) A microcosm experiment to evaluate the influence of location and quality of plant residues on residue decomposition and genetic structure of soil microbial communities. *Soil Biol Biochem* 39: 1631

Padmanabhan, P., Padmanabhan, S., DeRito, C., Gray, A., Gannon, D., Snape, J. R., Tsai, C. S., Park, W., Jeon, C., and Madsen, E. L., (2003) Respiration of ^{13}C-labeled substrates added to soil in the field and subsequent 16S rRNA gene analysis of ^{13}C-labeled soil DNA. *Appl Environ Microbiol* 69: 1614-1622

Page, F. C., (1976) An illustrated key to freshwater and soil amoebae with notes on cultivation and ecology. Freshwater Biological Association: The Ferry House, Far Sawrey, Ambleside, Cumbria.

Paterson, E., Osler, G., Dawson, L. A., Gebbing, T., Sim, A., and Ord, B., (2008) Labile and recalcitrant plant fractions are utilised by distinct microbial communities in soil: Independent of the presence of roots and mycorrhizal fungi. *Soil Biol Biochem* 40: 1103

Pausch, J., Tian, J., Riederer, M., and Kuzyakov, Y., (2013) Estimation of rhizodeposition at field scale: upscaling of a ^{14}C labeling study. *Plant Soil*: 1

Petz, W., Foissner, W., Wirnsberger, E., Krautgartner, W. D., and Adam, H., (1986) Mycophagy, a new feeding strategy in autochthonous soil ciliates. *Naturwissenschaften* 73: 560-562

Pilloni, G., Granitsiotis, M. S., Engel, M., and Lueders, T., (2012) Testing the limits of 454 pyrotag sequencing: reproducibility, quantitative assessment and comparison to T-RFLP fingerprinting of aquifer microbes. *PLoS One* 7: e40467

Poll, C., Brune, T., Begerow, D., and Kandeler, E., (2010) Small-scale diversity and succession of fungi in the detritusphere of rye residues. *Microb Ecol* 59: 130-140

Reichenbach, H., (1999) The ecology of the myxobacteria. *Environ Microbiol* 1: 15

Romani, A. M., Fischer, H., Mille-Lindblom, C., and Tranvik, L. J., (2006) Interactions of bacteria and fungi on decomposing litter: Differential extracellular enzyme activities. *Ecology* 87: 2559-2569

Scharroba, A., Dibbern, D., Hünninghaus, M., Kramer, S., Moll, J., Butenschoen, O., Bonkowski, M., Buscot, F., Kandeler, E., Koller, R., Krüger, D., Lueders, T., Scheu, S., and Ruess, L., (2013) Effects of resource availability and quality on the structure of the micro-food web of an arable soil across depth. *Soil Biol Biochem* 50: 1

Schellenberger, S., Kolb, S., and Drake, H. L., (2010) Metabolic responses of novel cellulolytic and saccharolytic agricultural soil Bacteria to oxygen. *Environ Microbiol* 12: 845.

Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kögel-Knaber, I., Lehmann, J., Manning, D. A. C., Nannipieri, P., Rasse, D. P., Weiner, S., and Trumbore, S. E., (2011) Persistence of soil organic matter as an ecosystem property. *Nature* 478: 49-56

Straatsma, G., Samson, R. A., Olijnsma, T. W., Op Den Camp, H. J. M., Gerrits, J. P. G., and Van Griensven, L. J. L. D., (1994) Ecology of thermophilic fungi in mushroom compost, with emphasis of *Scytalidium thermophilum* and growth stimulation of *Agaricus bisporus* mycelium. *Appl Environ Microbiol* 60: 454-458

Strickland, M. S., and Rousk, J., (2010) Considering fungal:bacterial dominance in soils – Methods, controls, and ecosystem implications. *Soil Biol Biochem* 42: 1385-1395

Stursova, M., Zifcakova, L., Leigh, M. B., Burgess, R., and Baldrian, P., (2012) Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. *FEMS Microbiol Ecol* 80: 735-746

Termorshuizen, A. J., and Jeger, M. J., (2008) Strategies of soilborne plant pathogenic fungi in relation to disease suppression. *Fung Ecol* 1: 108-114

Tixier, P., Duyck, P.-F., Côte, F.-X., Caron-Lormier, G., and Malézieux, E., (2013) Food web-based simulation for agroecology. *Agron Sustain Dev* 33: 663-670

Trivedi, P., Anderson, I. C., and Singh, B. K., (2013) Microbial modulators of soil C storage: integrating genomic and metabolic knowledge for global prediction. *Trends Microbiol* 21: 641-651

van der Wal, A., Geydan, T. D., Kuyper, T. W., and de Boer, W., (2013) A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. *FEMS Microbiol Rev* 37: 477-494

Vandenkoornhuyse, P., Mahe, S., Ineson, P., Staddon, P., Ostle, N., Cliquet, J.-B., Francez, A.-J., Fitter, A. H., and Young, J. P. W., (2007) Active root-inhabiting microbes identified by rapid incorporation of plant-derived C into RNA. *Proc Natl Acad Sci USA* 104: 16970-16975

Vandermeer, J., Van Noordwijk, M., Anderson, J., Ong, C., and Perfecto, I., (1998) Global change and multi-species agroecosystems: Concepts and issues. *Agric Ecosyst Environ* 67: 1-22

Voriskova, J., and Baldrian, P., (2013) Fungal community on decomposing leaf litter undergoes rapid successional changes. *ISME Journal* 7: 477-486

Zhou, J., Xia, B., Treves, D. S., Wu, L. Y., Marsh, T. L., O'Neill, R. V., Palumbo, A. V., and Tiedje, J. M., (2002) Spatial and resource factors influencing high microbial diversity in soil. *Appl Environ Microbiol* 68: 326-334

Zumsteg, A., Schmutz, S., and Frey, B., (2013) Identification of biomass utilizing bacteria in a C-depleted glacier forefield soil by the use of 13C DNA stable isotope probing. *Environ Microbiol Rep* 5: 424

Supplementary 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:

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

with c_{nf} and c_f 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 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:

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

With δ_{sample} the $\delta^{13}C$ value of the respective sample, and $\delta_{reference}$ the $\delta^{13}C$ mean value of control samples (soil without substrate amendment). $\delta_{substrate}$ 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 analysed by T-RFLP fingerprinting. See Table S 1 for a summary of all utilized PCR assays. Bacterial communities were analysed 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. 2012).

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 S1) 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 were 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 S 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. S 1 to S 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 C 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. S 1 – S 3), a consistent further development of well-established SIP gradient evaluation criteria (Whiteley et al. 2006).

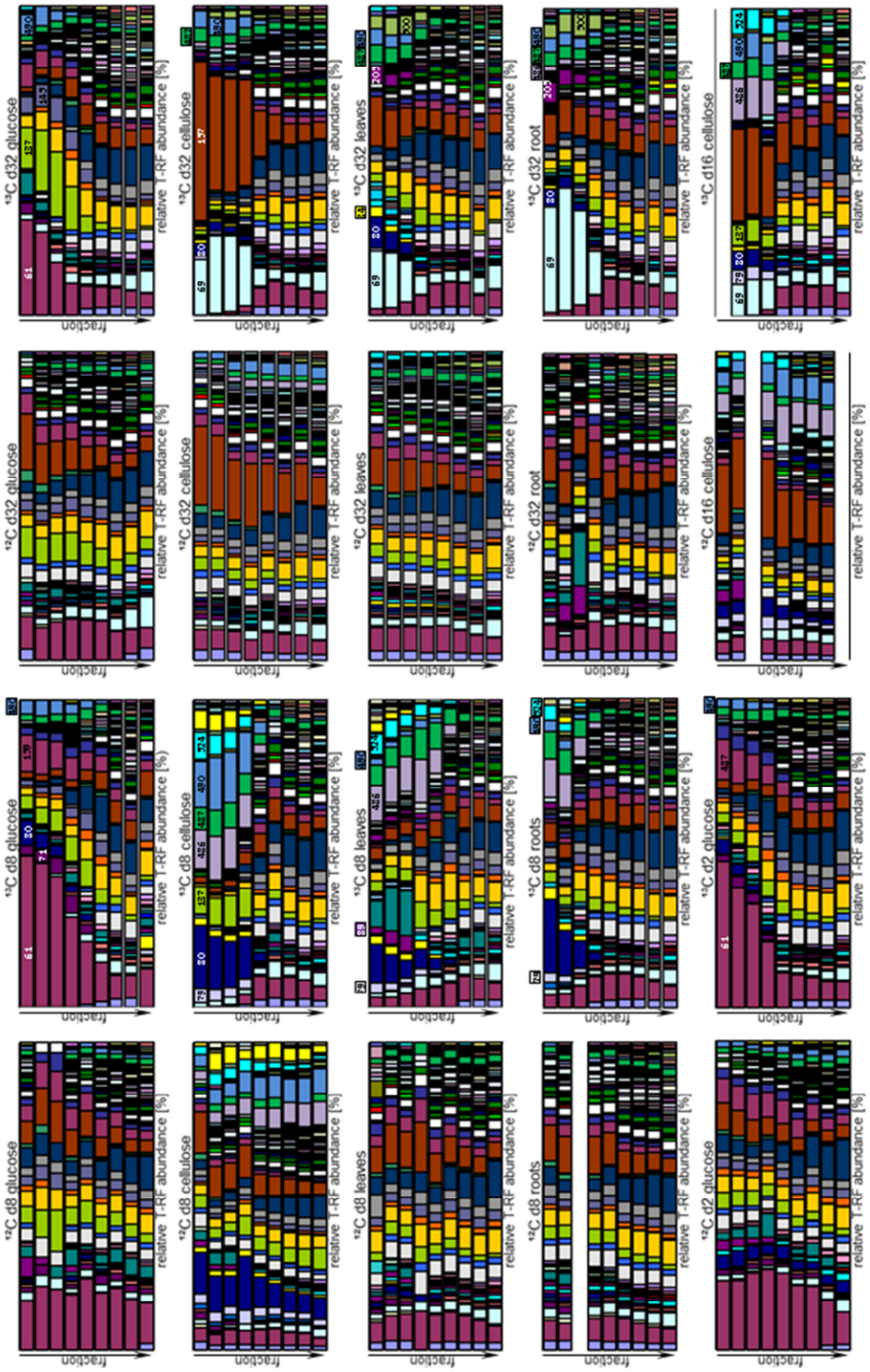

Fig. S 1: Fractions 2 to 10 of rRNA gradients from the ^{12}C and ^{13}C treatments. Selected relevant T-RFs identified to represent labeled taxa and mentioned in the text are highlighted by numbers [fragment length in bp]. Relative abundance of all T-RFs is 100 %.

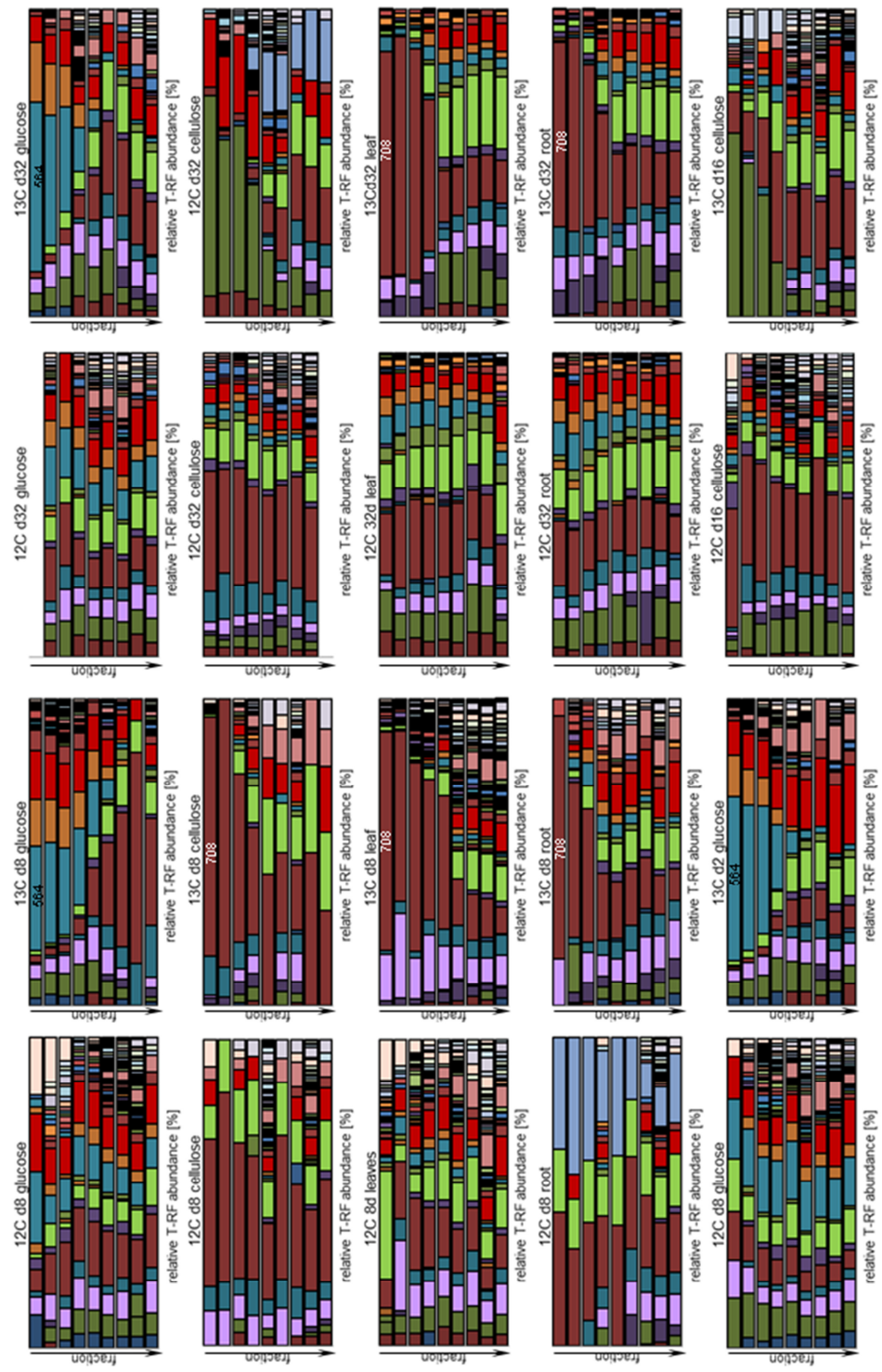

Fig. S 2: Bar plots of fungal 18S rRNA T-RFLP fingerprints from SIP gradients. Arrows indicate 'heavy' to 'light' fractions 2 to 10 of rRNA gradients from the ^{12}C and ^{13}C treatments. Selected relevant T-RFs identified to represent labeled taxa and mentioned in the text are highlighted by numbers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

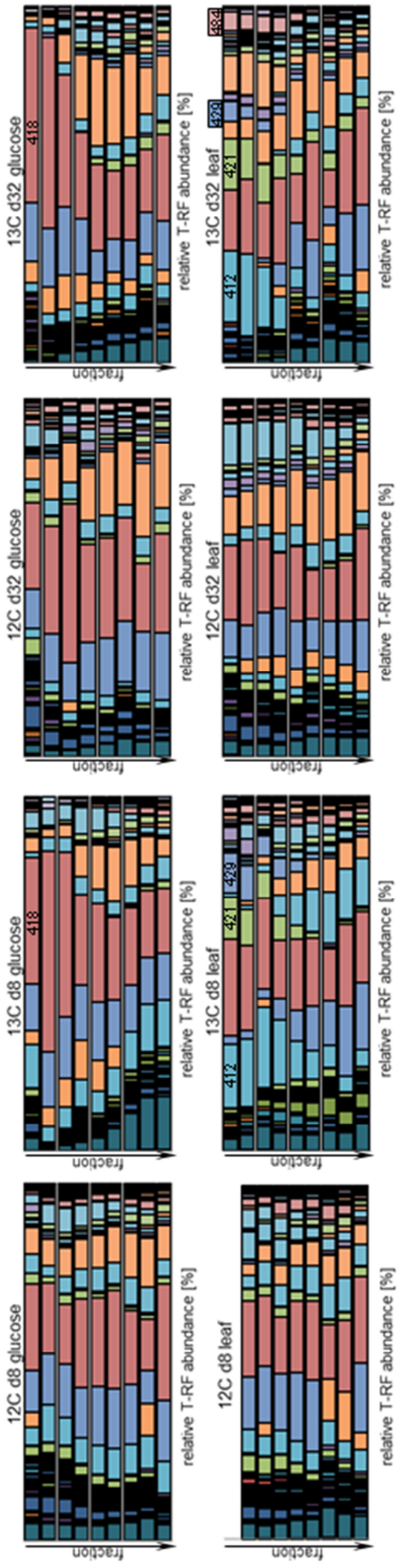

Fig. S 3: Bar plots of protist 18S rRNA T-RFLP fingerprints from SIP gradients. Arrows indicate 'heavy' to 'light' fractions 2 to 10 of rRNA gradients from the ^{12}C and ^{13}C treatments. Selected relevant T-RFs identified to represent labeled taxa and mentioned in the text are highlighted by numbers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

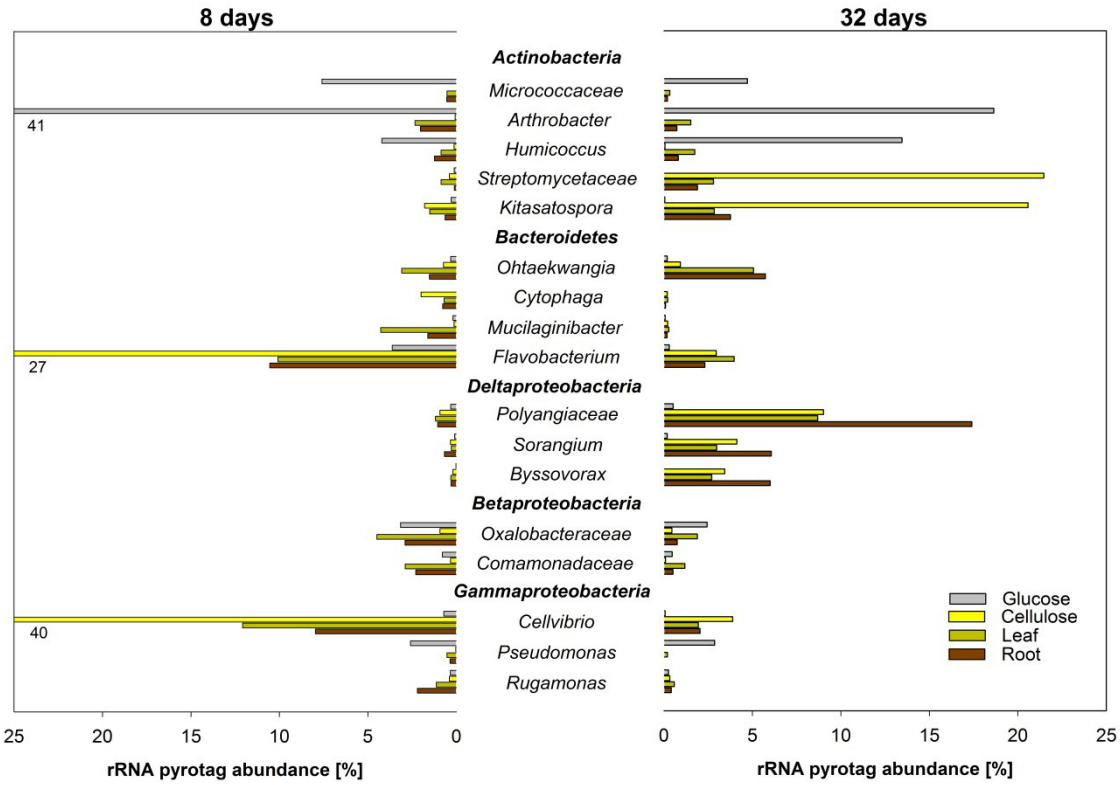

Fig. S 4: Relative abundance of ^{13}C-labeled bacterial taxa in 'heavy' rRNA after 8 and 32 days of incubation. Only labeled taxa as identified in Fig. 3 are shown.

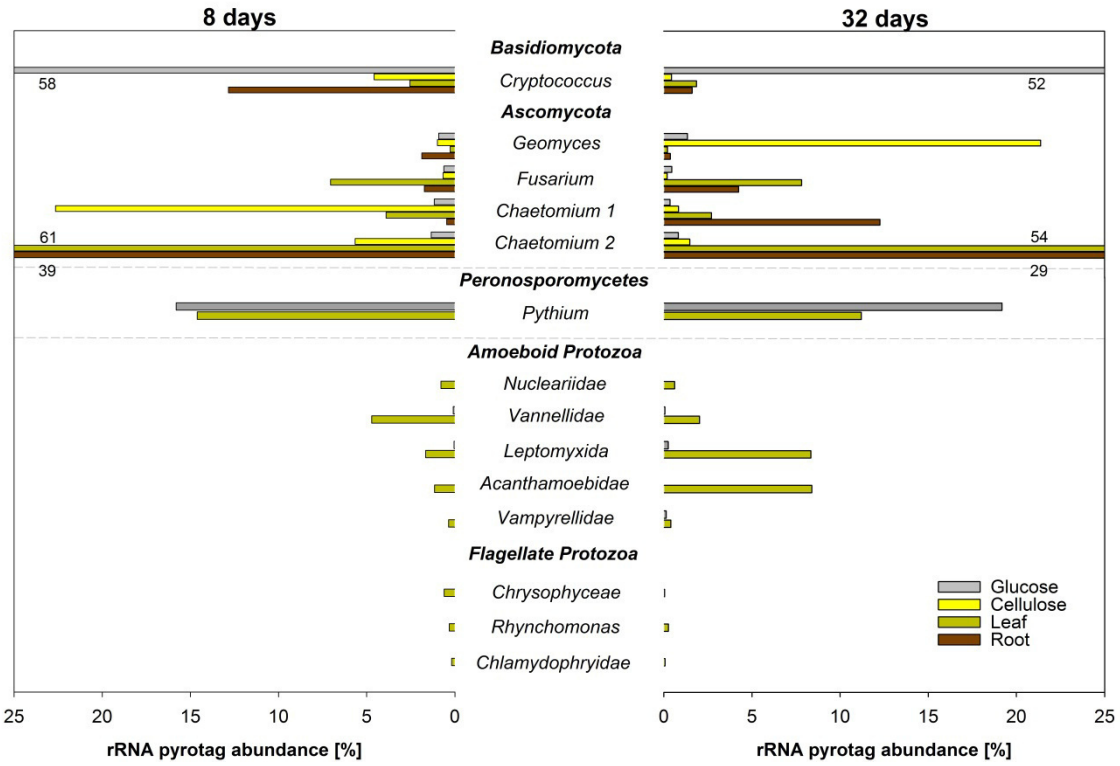

Fig. S 5: Relative abundance of ^{13}C-labeled fungal and protistan taxa in 'heavy' rRNA after 8 and 32 days of incubation. Only labeled taxa as identified in Fig. 4 are shown.

Supplementary References

Abdo, Z., Schuette, U. M. E., Bent, S. J., Williams, C. J., Forney, L. J., and Joyce, P., (2006) Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. *Environ Microbiol* 8: 929-938

Arfi, Y., Buée, M., Marchand, C., Levasseur, A., and Record, E., (2012) Multiple markers pyrosequencing reveals highly diverse and host-specific fungal communities on the mangrove trees *Avicennia marina* and *Rhizophora stylosa*. *FEMS Microbiol Ecol* 79: 433-444

Becklin, K., Hertweck, K., and Jumpponen, A., (2012) Host identity impacts rhizosphere fungal communities associated with three alpine plant species. *Microb Ecol* 63: 682-693

Borneman, J., and Hartin, R. J., (2000) PCR primers that amplify fungal rRNA genes from environmental samples. *Appl Environ Microbiol* 66: 4356-4360

Chen, M., Li, X., Yang, Q., Chi, X., Pan, L., Chen, N., Yang, Z., Wang, T., Wang, M., and Yu, S., (2012) Soil eukaryotic microorganism succession as affected by continuous cropping of peanut - pathogenic and beneficial fungi were selected. *PLoS ONE* 7: e40659

Culman, S. W., Bukowski, R., Gauch, H. G., Cadillo-Quiroz, H., and Buckley, D. H., (2009) T-REX: software for the processing and analysis of T-RFLP data. *BMC Bioinformatics* 10: 171-171

Edel-Hermann, V., Gautheron, N., Alabouvette, C., and Steinberg, C., (2008) Fingerprinting methods to approach multitrophic interactions among microflora and microfauna communities in soil. *Biol Fertility Soils* 44: 975-984

Edel-Hermann, V., Brenot, S., Gautheron, N., Aimé, S., Alabouvette, C., and Steinberg, C., (2009) Ecological fitness of the biocontrol agent Fusarium oxysporum Fo47 in soil and its impact on the soil microbial communities. *FEMS Microbiol Ecol* 68: 37-45

Euringer, K., and Lueders, T., (2008) An optimised PCR/T-RFLP fingerprinting approach for the investigation of protistan communities in groundwater environments. *J Microbiol Methods* 75: 262-268

Gihring, T. M., Green, S. J., and Schadt, C. W., (2012) Massively parallel rRNA gene sequencing exacerbates the potential for biased community diversity comparisons due to variable library sizes. *Environ Microbiol* 14: 285-290

Glaubitz, S., Lueders, T., Abraham, W.-R., Jost, G., Jurgens, K., and Labrenz, M., (2009) ^{13}C-isotope analyses reveal that chemolithoautotrophic *Gamma*- and *Epsilonproteobacteria* feed a microbial food web in a pelagic redoxcline of the central Baltic Sea. *Environ Microbiol* 11: 326-337

Jumpponen, A., (2003) Soil fungal community assembly in a primary successional glacier forefront ecosystem as inferred from rDNA sequence analyses. *New Phytol* 158: 569-578

Jumpponen, A., (2011) Analysis of ribosomal RNA indicates seasonal fungal community dynamics in *Andropogon gerardii* roots. *Mycorrhiza* 21: 453-464

Junier, P., Junier, T., and Witzel, K.-P., (2008) TRiFLe, a program for in silico terminal restriction fragment length polymorphism analysis with user-defined sequence sets. *Appl Environ Microbiol* 74: 6452-6456

Lanzén, A., Jørgensen, S. L., Huson, D. H., Gorfer, M., Grindhaug, S. H., Jonassen, I., Øvreås, L., and Urich, T., (2012) CREST – Classification Resources for Environmental Sequence Tags. *PLoS ONE* 7(11): e49334. doi:10.1371/journal.pone.0049334

Lee, C. K., Herbold, C. W., Polson, S. W., Wommack, K. E., Williamson, S. J., McDonald, I. R., and Craigh Cary, S., (2012) Groundtruthing next-gen sequencing for microbial ecology – biases and errors in community structure estimates from PCR amplicon pyrosequencing. *PLoS ONE* 7: e44224

Lueders, T., Manefield, M., and Friedrich, M. W., (2004a) Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. *Environ Microbiol* 6: 7

Lueders, T., Wagner, B., Claus, P., and Friedrich, M. W., (2004b) Stable isotope probing of rRNA and DNA reveals a dynamic methylotroph community and trophic interactions with fungi and protozoa in oxic rice field soil. *Environ Microbiol* 6: 60

Lueders, T., Kindler, R., Miltner, A., Friedrich, M. W., and Kaestner, M., (2006) Identification of bacterial micropredators distinctively active in a soil microbial food web. *Appl Environ Microbiol* 72: 5342-5348

Marhan, S., Derain, D., Erbs, M., Kuzyakov, Y., Fangmeier, A., and Kandeler, E., (2008) Soil organic matter mineralization and residue decomposition of spring wheat grown under elevated CO_2 atmosphere. *Agric Ecosyst Environ* 123: 63-68

Marhan, S., Kandeler, E., Rein, S., Fangmeier, A., and Niklaus, P. A., (2010) Indirect effects of soil moisture reverse soil C sequestration responses of a spring wheat agroecosystem to elevated CO_2. *Global Change Biol* 16: 469-483

Pilloni, G., von Netzer, F., Engel, M., and Lueders, T., (2011) Electron acceptor-dependent identification of key anaerobic toluene degraders at a tar-oil-contaminated aquifer by Pyro-SIP. *FEMS Microbiol Ecol* 78: 165-175

Pilloni, G., Granitsiotis, M. S., Engel, M., and Lueders, T., (2012) Testing the limits of 454 pyrotag sequencing: reproducibility, quantitative assessment and comparison to T-RFLP fingerprinting of aquifer microbes. *PLoS One* 7: e40467

Pinto, A. J., and Raskin, L., (2012) PCR biases distort bacterial and archaeal community structure in pyrosequencing datasets. *PLoS ONE* 7: e43093

R Development CoreTeam (2012) R: A language and environment for statistical computing. Vienna: R foundation for statistical computing

Ramette, A., (2009) Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities. *Appl Environ Microbiol* 75: 2495-2505

Scharroba, A., Dibbern, D., Hünninghaus, M., Kramer, S., Moll, J., Butenschoen, O., Bonkowski, M., Buscot, F., Kandeler, E., Koller, R., Krüger, D., Lueders, T., Scheu, S., and Ruess, L., (2012) Effects of resource availability and quality on the structure of the micro-food web of an arable soil across depth. *Soil Biology and Biochemistry* 50: 1-11

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J., and Weber, C. F., (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 75: 7537-7541

Smith, C. J., Danilowicz, B. S., Clear, A. K., Costello, F. J., Wilson, B., and Meijer, W. G., (2005) T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles. *FEMS Microbiol Ecol* 54: 375-380

Vance, E. D., Brookes, P. C., and Jenkinson, D. S., (1987) An Extraction Method For Measuring Soil Microbial Biomass C. *Soil Biol Biochem* 19: 703-708

Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R., (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73: 5261-5267

Whiteley, A. S., Manefield, M., and Lueders, T., (2006) Unlocking the 'microbial black box' using RNA-based stable isotope probing technologies. *Curr Opin Biotechnol* 17: 67-71

Zumsteg, A., Luster, J., Göransson, H., Smittenberg, R., Brunner, I., Bernasconi, S., Zeyer, J., and Frey, B., (2012) Bacterial, archaeal and fungal succession in the forefield of a receding glacier. *Microb Ecol* 63: 552-564

Zumsteg, A., Schmutz, S., and Frey, B., (2013) Identification of biomass utilizing bacteria in a C-depleted glacier forefield soil by the use of 13C DNA stable isotope probing. *Environ Microbiol Rep* 5: 424

Chapter IV: Effects of protozoa – microbe interactions on carbon fluxes in the detritusphere*

Abstract

Decomposition of organic matter is crucial for ecosystem functioning. Microorganisms, which are responsible for the mineralization and recycling of nutrients required by plants, are usually treated as a homogeneous functional guild. However, there is strong evidence that microbes differ in mineralization capacity. In addition, a significant part of the microbial community is top-down controlled by microbial grazers, such as protozoa. Since protozoan grazing is highly selective, and selectivity differs among species, we hypothesised that protozoan taxa specifically affect bacterial community structure and function (mineralization), and as a result, that protozoan taxa complement each other in grazing intensity. Further we expected that influence of protozoan species on soil bacteria changes during detritus decomposition. We tested the effects of different bacterivorous protozoan taxa on the mineralization rate of maize litter in a short-term (88h) and a long-term (3 weeks) experiment. Arable field soil was inoculated with different species combinations and species richness levels using two species from each of following "functional groups": flagellates, ciliates and amoeba. The effects of protozoan species richness, composition of functional groups within the community and the effects of specific species are discussed with respect to CO_2-production and corresponding microbial C mineralization over time, taking C mineralization as a measure of overall microbial function. Further we tested whether changes in microbial function could be related to changes in microbial community composition (measured by phospholipid fatty acids analyses). During microbial decomposition, different protozoan grazers gained influence for different time intervals leading to a succession of protozoan impacts on bacterial mineralization. Effects of protozoan grazers on microbial respiration turned out to be species specific. The "functional group" turned out to be no reliable indicator of the way a species affects prey organisms and communities.

* for information about Co-authorships see page 155

Introduction

The fluxes of C as an energy source into belowground food webs are a key processes of terrestrial ecosystems as it determines the mineralization and transfer of belowground C back to the atmosphere and has been estimated to be an order of magnitude larger than anthropogenic CO_2 emissions (Nielsen et al. 2011). About 90 % of plant biomass enters the soil system as detritus and fuels food webs (Gessner et al. 2010). In the food web microbial decomposers catalyse the mineralization of C from detritus and recycle nutrients for primary production (Verhoef and Morin 2010). Thereby microorganisms determine a critical balance between C mineralization and sequestration (Gessner et al. 2010, Bardgett 2005). Protozoa, due to their significance as the major consumers of microbial biomass in soils (Krivtsov et al. 2003, Hunt et al. 1987, Brussaard et al. 1990), hold a key role in the balance between C loss from the ecosystem by respiratory CO_2 release and conservation through sequestration in living biotic particulate fractions (Anderson 2011). Protozoa strongly control bacterial populations (Glücksman et al. 2010, Koller et al. 2013, Fuhrman et al. 1992, Rønn et al. 2002) and are able to reduce bacterial biomass by as much as 60 % (Clarholm 1981). Selective grazing influences the growth condition of individual bacteria species (Hahn and Höfle 1999, Saleem et al. 2012), can substantially enhance bacterial production (Bonkowski et al. 2000, Sundin et al. 1990) and alter microbial community dynamics (Marschner et al. 2011, Griffiths et al. 1999, Kreuzer et al. 2006, Rosenberg et al. 2009, Koller et al. 2013).

However, the microbial decomposer community has long been treated as a single functional guild but there is ample evidence that that microbial community composition plays a critical role in determining ecosystem process rates (Strickland et al. 2009, Hendrix et al. 1986, Schimel and Gulledge 1998, Reed and Martiny 2007). The large species richness of soil communities has led to the hypothesis that functional redundancy is great among soil organisms (Bengtsson 1998, Andren et al. 1999, Setälä et al. 2005) but there are clear examples that functional diversity between species affects specific ecosystem processes like nitrogen fixation and denitrification (Coleman and Whitman 2005) or specific aspects of C cycling (Nielsen et al. 2011). Although the diversity of protists in morphology and phylogeny is huge (Cavalier-Smith 1993, Cavalier-Smith 1998, Adl et al. 2012) it is still an open debate whether protozoan function differs across morphotypes (Geisen 2014) and even more whether the identity and diversity within a functional group matters to ecosystem functioning (Glücksman et al. 2010, Reed and Martiny 2007, Saleem et al. 2012). Identifying key players in detritus decomposition is of pivotal significance as

it is essential to assess the consequences of biodiversity loss and subsequent effects on C and nutrient cycles (Gessner et al. 2010). Further, given that natural communities typically have multiple predators feeding on most prey, understanding emergent multiple predator effects on detritus decomposition is another crucial issue for community ecology (Sih et al. 1998).

We hypothesized that I) protozoan taxa specifically affect bacterial community structure and function (mineralization), II) complement each other in grazing intensity on the bacterial community and III) that the influence of protozoan species on soil bacteria changes during detritus decomposition. We expected no strong influence of protozoan identity on bacterial community structure and mineralization in the beginning of detritus decomposition with ample easily available C sources present (short term), but with reducing detritus quality (long term) we expected protozoan identity to strongly feedback on bacterial mineralization capacity and bacterial community.
To test these hypotheses we manipulated species richness and functional group composition of protozoan communities in two related lab experiments using up to six different protozoan species out of the three "functional groups" ciliates, flagellates and naked amoebae. We measured resulting changes in bacterial respiration and protozoan impacts on bacterial community composition. The experiments were performed in microcosms with autoclaved soil and sterilized maize shoot litter as detritus which was re-inoculated with a diverse bacterial community. Short term effects (first 3 days) of functional diversity and community composition of protozoa on C mineralization, as a measure of overall mineralization capacity, were approached using a microrespirometer (Griffiths et al. 2001). Long term (3 weeks) effects were investigated by using KOH titration. At the end of both experiments bacterial community composition was determined by Phospholipid fatty acid (PLFA) analyses to link group specific effects of bacterivorous protozoa to microbial community composition and C mineralization. This experimental set up allows the differentiation between species specific and functional group specific effects of protozoa on microbial C mineralization, allowing a high resolution of the C flow through microbial communities.

Material and Methods

Experimental set-up

A protozoa-free bacteria inoculum was produced according to the protocol of Kreutzer et al. (2006). Briefly, 50 g of air-dried grassland soil (0 - 10 cm, Cologne, Germany) were mixed on a shaker (Köttermann Typ 4020, Uetze, Germany) at 90 rpm for 20 min in 100 ml Neff's modified amoebae saline (NMAS, Page 1976). After 2.5 h of sedimentation the supernatant was passed twice through a filter (Rundfilter 595 ½, Schleicher and Schuell, Dassel, Germany) and the flowthrough was subsequently filtered through a 3 µm sterile syringe filter (Whatman, Buckinghamshire, England) and through a 1.2 µm sterile syringe filter (Sactorius Minisart, Göttingen, Germany), respectively.

The protozoa-free filtrate was then added to a sterilized soil-sand mixture (1:1) and incubated in darkness at 20°C. Two subsamples were checked for protozoan contamination at 100 x magnification (Nikon Eclipse, Japan) on every second day during this incubation time.

After two weeks 40 g of the bacteria/sand/soil-mixture were diluted in 60 ml NMAS and shaken for 30 min at 90 rpm. Finally, autoclaved soil (originating from an agricultural field site near Göttingen, Germany, see Kramer et al. 2012 for further information) was inoculated with 25 µl bacteria solution per g soil.

Two sets of 124 microcosms were prepared: 50 ml centrifugation tubes (Falcon, Oneonta, NY, USA) were filled with 20 g ± 0.4 g of this soil for the long-term experiment and 2 g ± 0.04 g soil were filled into plastic 6 ml screw cap vessels for a parallel short term experiment. All soil treatments were incubated at 14.6 % water-content for nine days to ensure establishment of the bacterial community in the soil.

Protozoan cultures were transferred to 15 ml plastic tubes and washed three times to remove nutrient broth by centrifugation at 700 rpm for 3 min and resuspending the pellet in NMAS. Protozoan densities were microscopically estimated using a Neubauer counting chamber.

About 500 protozoa/g dw soil were added as single species or in combination (Table 1) and incubated for 9 days to establish stable protozoa and bacteria communities.

For the short term experiment 0.02 g ± 0.0005 g sterilized powdered maize litter (43.45 % C, 1.11 % N) and 200 µl sterile distilled water were mixed into the soil immediately before the start of the respiration measurements. Respiration was measured over the first 88 h

after detritus inoculation (ADI) using a microrespirometer (Griffiths 2001). After termination the soil was frozen for subsequent PLFA-extraction.

In the long term experiment 0.2 g ± 0.005 g sterilized powdered maize litter and 2 ml distilled water were added into each tube, relatively the same amount (5 mg C per g soil DW) as in the short term experiment. Open tubes and a test tube containing 1 ml KOH (1N), to absorb produced CO_2, were placed in 1l amber bottles for three weeks. Titrimetric determination of the CO_2 production with BaCl and HCl was performed after 2, 4, 7, 10, 14 and 21 days. CO_2-content was calculated using the following formula:

$$CO2 = \frac{0.1 * (nv - tv) \frac{KOHtot}{KOHtit} * 22.4}{d}$$

with nv = neutral value, tv = titrated value; KOHtot = total KOH, KOHtit = titrated KOH, d = days

After three weeks these samples were frozen for subsequent PLFA-extraction, too.

C to N content of the used maize litter was estimated using Flash 2000 NC soil analyser (Thermo fisher scientific, Waltham, USA) and 5 x 5 g of litter.

Table 1: Experimental set-up to determine effects of species (SP) and functional group (FG) of specific protozoan grazers (two species each of amoebae (*Acanthamoeba castellanii* and *Hartmannella vermiformis*), flagellates (*Cercomonas longicauda* and *Spumella sp.*) and ciliates (*Tetrahymena pyriformis* and *Colpoda steinii*)) on microbial carbon fluxes in the detritusphere

	Treatment number																						
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
Acanthamoeba		x						x			x	x					x	x		x	x		x
Hartmannella			x					x					x	x			x	x		x		x	x
Cercomonas				x					x		x				x		x		x	x	x		x
Spumella					x				x				x			x	x		x		x	x	x
Tetrahymena						x				x		x				x		x	x	x		x	x
Colpoda							x			x				x	x			x	x		x	x	x
SP	0	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	4	4	4	4	4	4	6
FG	0	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3

Organisms

The grazer model community consisted of two different species of each of the major protozoan morphotoypes representing different "functional groups" (flagellates, ciliates, amoebae). The feeding behaviour, morphology and cell size of these groups is supposed to differ tremendously. While soil flagellates are usually small, free swimming and selectively feeding, ciliates are rather big and consume high numbers of bacteria. The amoebae chosen for this experiment are in terms of body size in between the flagellates and ciliates. They preferentially stay attached to soil particles and graze the bacteria growing there. Protozoa were incubated as single species or species combination treatments. The species richness of the microcosms was 0, 1, 2, 4 or 6 protozoan species out of 0, 1, 2 or 3 functional groups, respectively.

As ciliate species *Tetrahymena pyriformis* and *Colpoda steinii* were chosen. *Tetrahymena pyriformis* was taken because it is a model organism often used in literature. *Colpoda steinii* was taken because ciliates of the genus Colpoda usually dominate ciliate communities in soil.

As flagellate species the cercomonad *Cercomonas longicauda* and the crysomonad *Spumella sp.* were used. Cercomonads represent one of the main flagellate groups found in agricultural soil. Because of their ability to show amoeboid as well as "classical" flagellate movement they are very effective grazers. Even though many Crysomonads are mixotrophic organisms, *Spumella sp.* is an obligate phagotroph raptorial feeder (Jones 2000) which obtains its food by creating a water current with its longest flagellum (Zwart and Darbyshire 1992), whereas *Cercomonas* forms ventral pseudopodia during a gliding movement over soil particles (Bouwman and Zwart 1994).

As amoeba species *Acanthamoeba castellanii*, a commonly used model organism that has shown massive influence on the performance of plants and bacterial community in previous studies (e.g. Rosenberg et al. 2009, Bonkowski and Brandt 2002) was chosen. The second amoeba species used was *Hartmannella vermiformis*. This species was taken because it is a relatively fast and active amoeba.

The cultures of *Colpoda*, *Cercomonas* and *Hartmannella* were obtained from specimen isolated from the same soil used in the experiments.

PLFA-Analysis

PLFAs were extracted from 2 g of soil for the short term experiment and 4 g of soil for the long term experiment following the procedure described by Frostegård et al. (1993).

Briefly, Bligh & Dyer (chloroform, methanol, citrate buffer (pH 4); 1 : 2 : 0.8) was used as extraction solvent. Lipid fractionation was performed with silica columns (Bond-Elute-SI, 500 mg, 3 ml; Agilent Technologies, Lake Forest, USA) and phospholipids were eluted with methanol. Methanolysis of PLFAs was conducted with 0.2 M methanolic KOH. As internal standard methylnondecanoate (19:0) was used to perform quantitative analyses. The fatty acid methyl esters (FAMEs) were kept at –20°C until analysis with isooctane as solvent.

FAMEs were identified by chromatographic retention time comparison with a standard mixture composed of 37 different FAMEs that ranged from C11 to C24 (Sigma-Aldrich, St Louis, USA). Analysis was performed by gas chromatography (GC) using an Auto System XL (Perkin Elmer Corporation, Norwalk, USA) equipped with a HP-5 capillary column (50 m x 0.2 mm i.d., film thickness 0.33 μm). The injector temperature was 260°C and that of detector 280°C. The temperature program started with 70°C (hold time 2 min) and increased with 30°C/min to 160°C, and then with 3°C/min to 280°C and held for 15 min. The injection temperature was 260°C and helium was used as carrier gas. Evaluation was performed with the software Turbochrom Workstation Version 6.1.0.0 (Perkin Elmer Corporation, Norwalk, USA).

To verify correct identification of FAMEs (chain length and saturation) a range of soil samples were analysed by GC-MS with a HP 5890 series II coupled with a 5972 mass selective detector and equipped with a DB-5MS capillary column (30 m x 0.25 mm i.d., film thickness 0.2 μm). Helium 5.0 was used as carrier gas at a flow of 1 ml/min. The GC oven temperature program started at 50°C (hold time 1 min), increased with 9°C/min to 180°C, then 5°C/min to 260°C, and 20°C/min to 300°C (hold time 11 min). The transfer line temperature was 300°C. A mass range of 50 to 500 m/z was monitored in Scan mode.

The following PLFAs were summed up to estimate bacterial biomass: i15:0, a15:0, i16:0, 16:1ώ7, i17:0, cy17:0, 18:1ώ7/18:1ώ9t and cy19:0 (Frostegård et al. 1993, Zelles 1999). The PLFAs i15:0, a15:0, i16:0 and i17:0 are indicative for Gram-positive, and 16:1ώ7, cy17:0, 18:1ώ7/18:1ώ9t and cy19:0 for Gram-negative bacteria. The ratio of the PLFAs cy17:0 to 16:1ώ7 was used to estimate growth phase of gram negative bacteria (Fouchard et al. 2005, Knivett and Cullen 1965).

Statistics

Unknown until today were the dynamics of the protist populations. The specific experimental design enabled us to calculate the explained variance of CO_2 production by

each protist species as a measure of its influence on mineralization rate. Hereby we were able to follow the succession of the functional influence of the different protist species on microbial mineralization through time; a measure of interaction strength (Berlow et al. 1999).

Statistical analyses were performed in R 2.10.1.

Results

I. Short term experiment

Mineralization

In the short term experiment, microbial respiration was neither affected by species richness, functional group richness or functional group identity. Except for *Spumella* and *Hartmannella,* all protozoan taxa enhanced microbial respiration in single species treatments compared to the protozoa-free controls.

In contrast microbial respiration was strongly influenced by specific protozoan taxa at different time intervals indicating a rapid succession of protozoan species affecting decomposition of detritus (Fig. 1). Generally flagellate and ciliate species were the main drivers of this succession at the short time scale (Fig. 1). The succession of the influence of different protozoan species on CO_2 production can clearly be seen by the change in F-values of the statistical model. Peaks of explained variation of microbial respiration after 10-20 h, 10-60 h, 30-70 h and 50-88 h were successively determined by *Colpoda, Spumella*, *Tetrahymena* and *Cercomonas* (Fig. 1). Interestingly, specific species had either negative (*Spumella*) or positive (*Tetrahymena, Cercomonas, Colpoda*) effects on microbial respiration, that partly cancelled out each other.

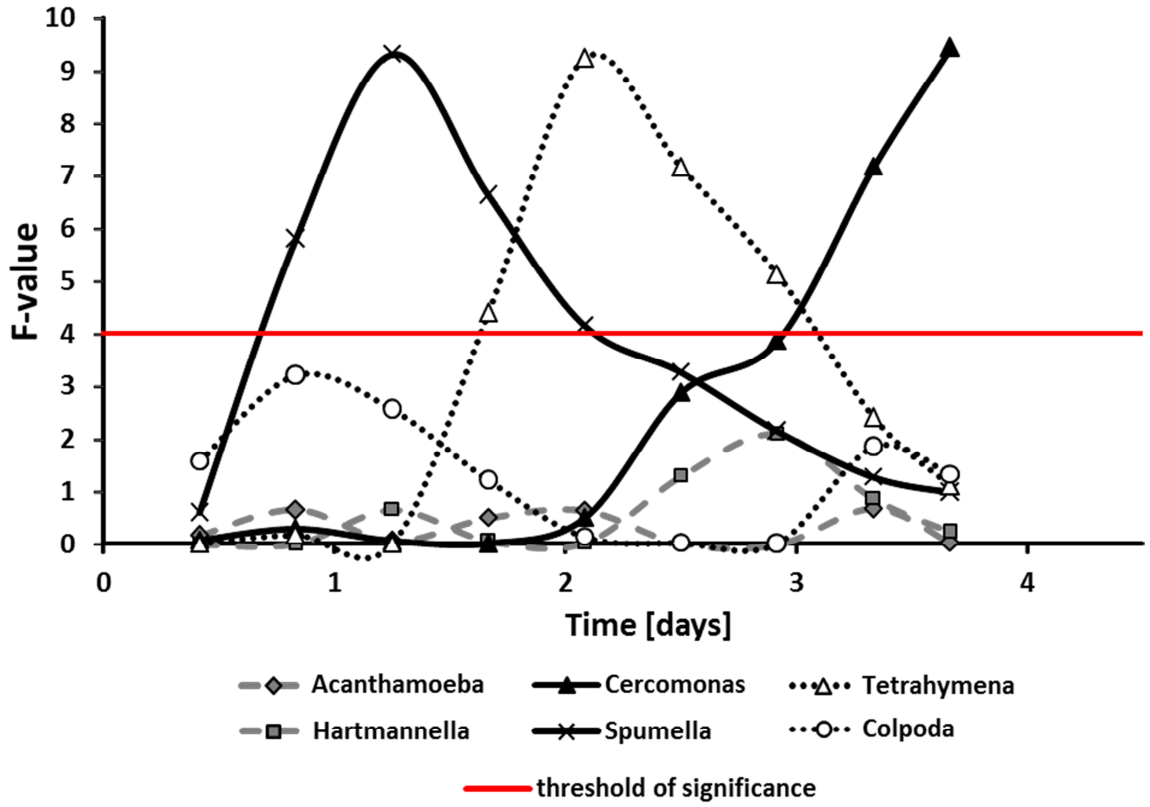

Fig. 1: Effects of specific protozoan species on microbial respiration during the first 4 days ADI, red line indicates threshold of significance ($p < 0.05$)

Community composition

In line with overall microbial respiration, PLFA composition was determined by specific protozoan taxa rather than by species richness or functional group identity and richness (Fig. 2). Especially *Tetrahymena* appeared to have a striking influence on microbial PLFA composition.

A more detailed examination of bacteria-specific PLFAs emphasised the different effectiveness of the different grazers (Table 2) and revealed a negative linear effect of the number of functional groups on the ratio of cy17:0 to 16:1ώ7 ($F_{1,74} = 18.81$, $p < 0.0001$). *Cercomonas* was the only protozoan species with a significant effect on overall bacterial PLFAs ($F_{1,78} = 14.80$, $p < 0.0001$) by reducing gram positive ($F_{1,76} = 14{,}24$, $p = 0.0441$) as well as gram negative bacteria ($F_{1,77} = 14{,}21$, $p = 0.0003$) and the ratio of cy17:0 to 16:1ώ7 ($F_{1,74} = 4.95$, $p = 0.0291$). The other flagellate species, *Spumella*, only led to a decrease of the ratio of cy17:0 to 16:1ώ7 ($F_{1,74} = 4.05$, $p = 0.0478$). Both ciliate species had generally few effects on bacterial PLFAs, but the presence of *Colpoda* increased the relative biomass of gram negative bacteria ($F_{1,77} = 4.40$, $p = 0.0393$). Even though they had no overall effect

on microbial respiration, both amoeba species reduced the ratio of cy17:0 to 16:1ώ7 (*Acanthamoeba*: $F_{1,74} = 5.31$, $p = 0.0240$ and *Hartmannella*: $F_{1,74} = 6.828$, $p = 0.0109$) and affected gram positive bacteria in opposite directions, *Acanthamoeba* increased the relative biomass of gram positive bacteria ($F_{1,76} = 18.85$, $p < 0.0001$)., while *Hartmannella* reduced it ($F_{1,76} = 4.79$, $p = 0.0318$).

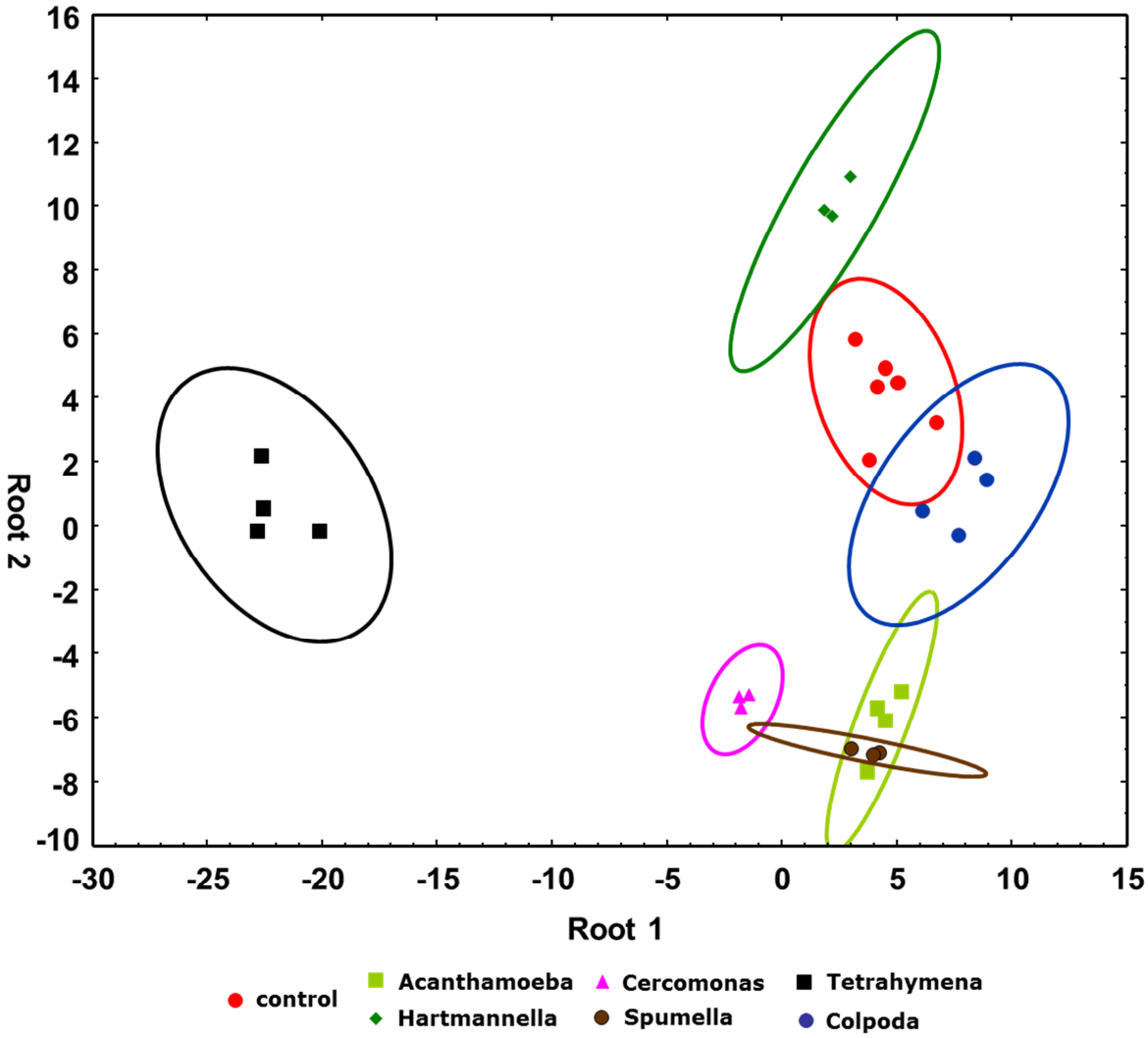

Fig. 2: Discriminant function analysis of the effects of grazer species in single species treatments on overall PLFA composition in soil 88h after inoculation of detritus (Wilks`Lamda: 0.000001, $F_{96,35} = 4.39$, $p < 0.001$), ellipses represents 80% confidence interval

Table 2: Effect ($p < 0.05$) of protozoan grazers on bacterial PLFA factors 88 h ADI (grNgro is the ratio of cy17:0 to 16:1ώ7 (growth factor of gram negative bacteria)), empty circles indicate increase, black circles indicate decrease, black line indicate no effects

	Bacteria	gram+	gram-	grNgro
Colpoda	—	—	○	—
Tetrahymena	—	—	—	—
Spumella	—	—	—	●
Cercomonas	●	●	●	●
Hartmannella	—	●	—	●
Acanthamoeba	—	○	—	●

II. Long term experiment

Mineralization

As in the short term experiment mineralization (CO_2 evolution) of maize litter was always lowest in the control treatment and increased in presence of protozoan grazers. In the long term experiment mineralization was highest in treatments with 2 and 3 functional groups (230 h ADI, $F_{3,120} = 2.79$, $p = 0.0433$). At the first time point (41 h ADI) mineralization increased linear with increasing number of protozoa functional groups ($F_{1,121} = 5.68$, $p = 0.0187$). In contrast to the short term experiment, protozoan species effects cumulated in a late peak of grazer influence rather than a rapid succession (Fig. 3).

However, at the beginning of the long term experiment *Colpoda* had a major positive effect on microbial respiration, followed by a peak of *Tetrahymena* influence although the latter was only marginally significant (Fig. 3). After 9.5 days, when overall CO_2-release had strongly declined, a second peak of protozoan activity and influence on the microbial community followed. This second phase of maize mineralization was dominated by positive effects of *Acanthamoeba* and *Spumella* and negative effects of *Cercomonas* and *Colpoda* on microbial respiration. *Acanthamoeba* constantly increased C mineralization until the end of the experiment (Fig. 3). Interestingly, *Colpoda* enhanced microbial respiration in the early phase of detritus decomposition and reduced it at a later phase.

These observations show that protozoan effects on overall microbial functioning are complex and far from simply complementary.

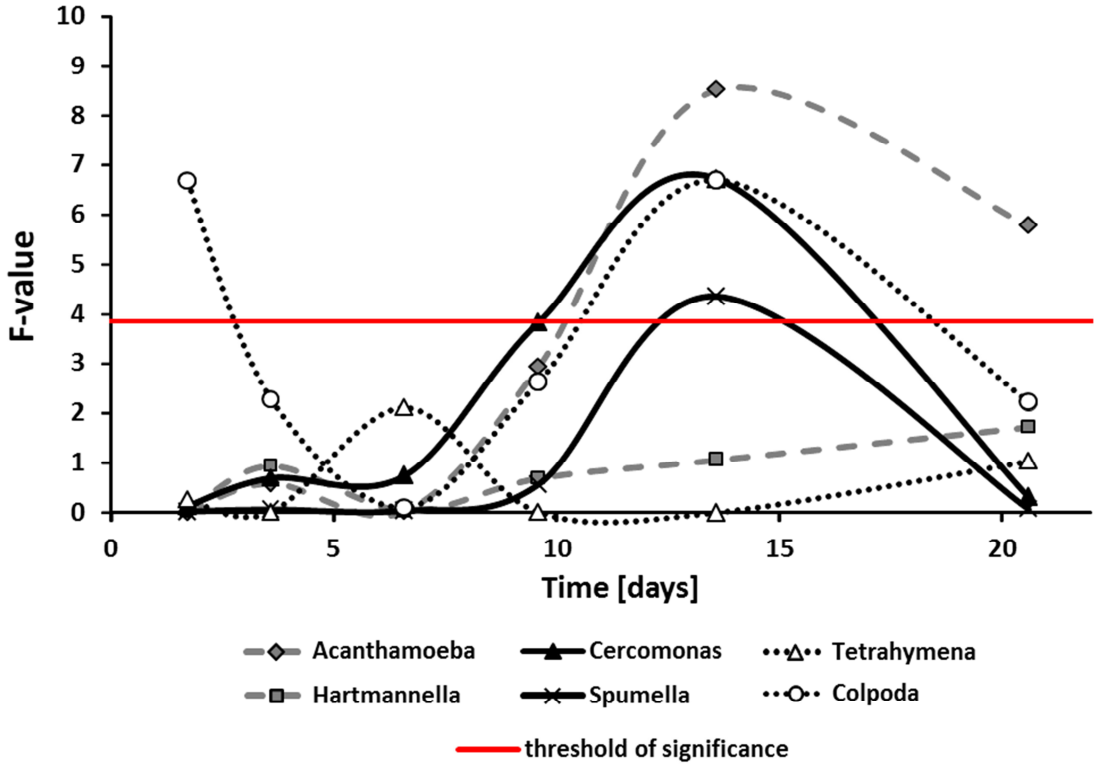

Fig. 3: Effects of specific protozoan species on microbial respiration during the first 21 days ADI, red line indicates threshold of significance ($p < 0.05$)

Community composition

3 weeks after start of detritus decomposition by the microbial community clear effects of grazer species richness on PLFA composition became visible (Fig. 4). The number of functional groups of protozoa had gradually shifted the bacterial community at this time, indicating niche complementarity between functional groups at the level of microbial community composition. No specific effects of single grazer species could be detected at the end of the experiment.

A linear reduction of relative bacterial biomass, with increasing grazer diversity was observed ($F_{1,94} = 14.04$, $p = 0.0003$), which can be partly explained by a strong linear reduction of gram positive bacteria ($F_{1,93} = 5.47$, $p = 0.0215$). In contrast, the ratio of cy17:0 to 16:1ώ7 linearly increased with more protozoan functional groups present ($F_{1,93} = 6.03$, $p = 0.0160$). A negative correlation between gram positive and gram negative

bacteria was found at the end of the long term experiment (y = -0.5207x + 49.496, R^2 = 0.2023, p < 0.001).

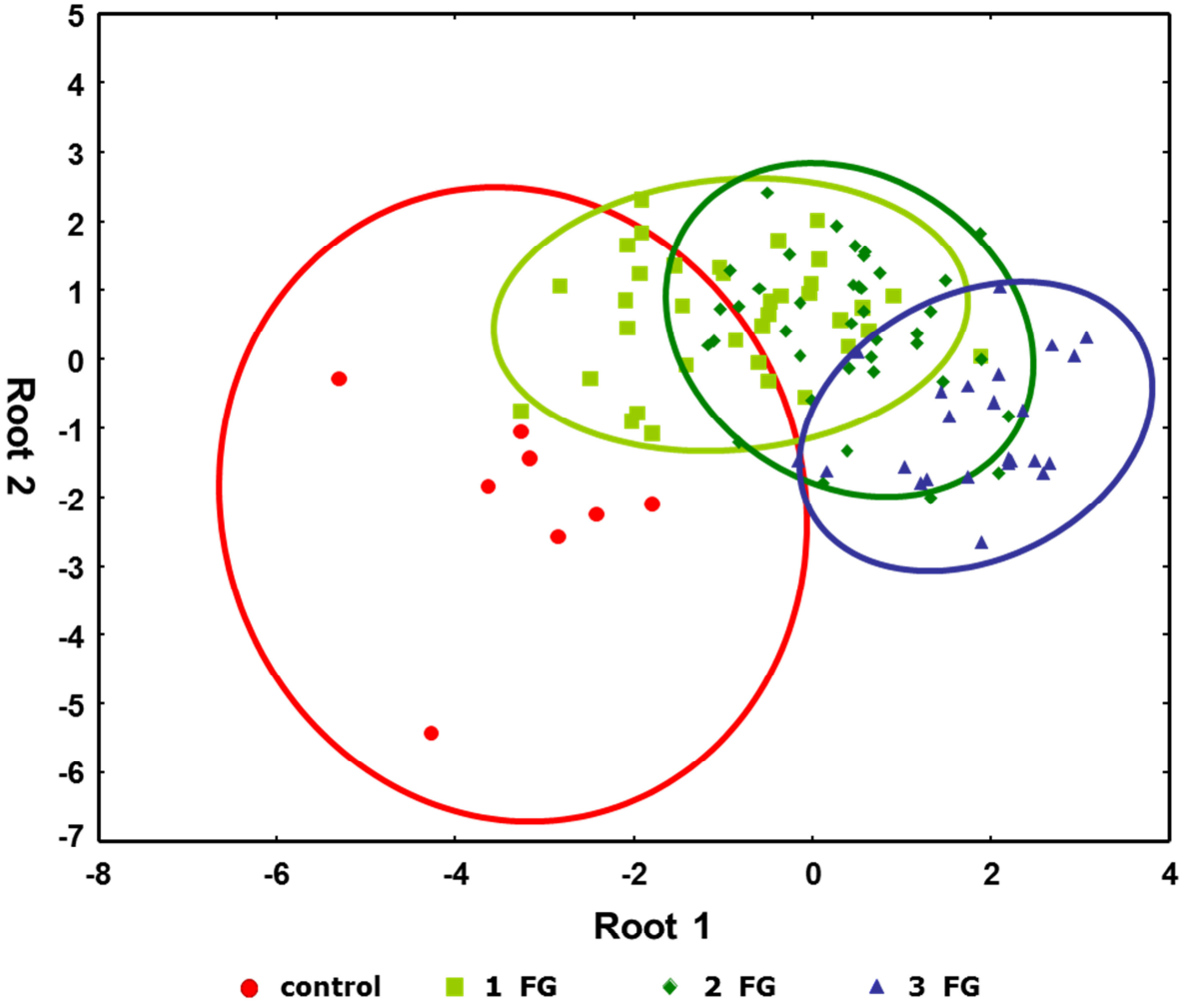

Fig. 4: Discriminant function analysis of the effects of grazer functional group diversity on PLFA composition in soil three weeks ADI (Wilks`Lamda: 0.1442, $F_{51,259}$= 4.67, p < 0.001), ellipses represents 80% confidence interval

Even though we did not find clear species specific effects of protozoan grazers on total PLFA composition an examination of bacteria specific PLFAs proved, that grazer identity was still affecting the composition of bacterial community composition (Table 3). Each species influenced at least one bacteria-correlated PLFA factor. While the flagellates displayed a negative impact on total bacterial biomass, *Cercomonas* reduced gram positive bacteria ($F_{1,93}$ = 6.56, p = 0.0120) and reduced the ratio of cy17:0 to 16:1ώ7 ($F_{1,93}$ = 4.24, p = 0.04234), while both amoebae reduced the ratio of cy17:0 to 16:1ώ7 (*Acanthamoeba*

($F_{1,93}$ = 4.94, p = 0.0287) and *Hartmannella* ($F_{1,93}$ = 30.66, p < 0.0001)). Ciliate species had opposite effects on total bacterial biomass. *Tetrahymena* reduced total bacterial PLFAs ($F_{1,94}$ = 5.67, p = 0.0193), even though it increased relative biomass of gram positive bacteria ($F_{1,93}$ = 6.22, p = 0.0144). *Colpoda,* in turn, enhanced total relative bacterial biomass ($F_{1,94}$ = 4.69, p = 0.0328), especially of gram positive bacteria ($F_{1,93}$ = 5.15, p = 0.0255) and enhanced the ratio of cy17:0 to 16:1ώ7 ($F_{1,93}$ = 8.43, p = 0.0046).

Table 3: Effect (p < 0.05) of protozoan grazers on bacterial PLFA factors 3 weeks ADI (empty circles indicate enhancement, black circles indicate decrease, circle in brackets indicate p < 0.1)

	Bacteria	**gram+**	**gram-**	**grNgro**
Colpoda	○	○	—	○
Tetrahymena	●	○	(●)	—
Spumella	●	—	—	—
Cercomonas	●	●	—	●
Hartmannella	—	—	—	●
Acanthamoeba	—	●	—	●

Discussion

Crop residues decompose in two distinct phases, an initial rapid phase where labile compounds are broken down and a slower phase during which the more recalcitrant fraction is decomposed (Marschner et al. 2011, Moore et al. 2004, Swan and Kominoski 2012). In line with this, our measurements show that most of the easily available C sources have been metabolized by microorganisms 4 days ADI (CO_2 evolution decreased by ~80 % compared to experiment start), confirming a rapid exhaustion of easily available C pools that was followed by a gradual decrease in CO_2 production until three weeks ADI at the end of the long-term experiment (decrease of ~97 % compared to experiment start). Accordingly, both phases of detritus decomposition were well reflected by our respective short-term and long-term experiments. Overall, microbial mineralization gradually

increased in presence of protozoa. This is in line with the observation that grazing in general (Coleman et al. 1978, Griffiths 1994a) and a diverse protozoan community in particular promotes C turnover and decomposition (Coleman et al. 1978, Cutler and Crump 1929, De Telegdy-Kovats 1932, Holubar et al. 2000, Ratsak et al. 1996, Stout 1973). Nevertheless our results indicate that specific protozoan species had positive, while others had negative impacts on microbial C mineralization at a specific time point, and some species even exerted both, positive and negative effects over the course of the experiments. Because each species might have a different environmental optimum, as well as its own functional response and growth efficiency, detritivores and decomposers specialize on particular successional stages of their resource (Anderson 1975, Bastow 2012) while strong competition is likely to cause rapid changes in community composition (Marschner et al. 2011). Therefore, using vastly different protist taxa and morphotypes we hypothesized overall detritus C mineralization rate to increase with increasing species richness of protozoan grazers due to mechanisms of niche complementarity (e.g. shown by Saleem et al. 2012). This hypothesis was based on the fact that different protozoan species exhibit distinct grazing preferences, and different taxonomic groups fundamentally differ in their grazing strategies (Boenigk and Arndt 2002; Parry 2004, Saleem et al. 2013).

The microbial PLFA pattern at the end of the short term experiment (~ 3.5 d ADI) revealed that grazer identity indeed had caused specific changes in the microbial community patterns. This confirms our hypothesis that the species specific grazing activity of protozoa has the potential to rapidly modify the composition and physiological status (i.e. ratio of cy17:0 to 16:1ω7 for gram negative bacteria) of soil bacterial communities through differential grazing pressures (Glücksmann et al. 2010, Corno and Jürgens 2008, Rønn et al. 2002). At the end of the short term experiment we could not detect any complementarity effects of species richness or functional groups. This changed after 3 weeks: species specific effects on total PLFA composition had disappeared, but a gradual shift in microbial community composition with increasing FG richness of protozoa indicates overall functional complementarity between amoebae, flagellates and ciliates. Likewise a linear increase in the ratio of cy17:0 to 16:1ω7 with increasing FG richness of protozoa indicates a shift in physiology towards the stationary growth phase in gram negative bacteria (Knivett and Cullen 1965). The linear reduction of bacterial biomass with increasing grazer species richness further demonstrates that complementarity effects gained influence on microbial community composition also at the species level. Nevertheless grazer identity had still significant and partly opposite effects on specific

marker PLFAs, sometimes even two species within the same functional group displayed these differences. Thus our hypothesis that protozoan taxa can specifically affect bacterial community structure and function was confirmed to a large extend. If protozoa of the same morphotype differ in their feeding preferences, the large number of protozoan genotypes being discovered can have significant consequences for both, understanding bacterial community dynamics and explaining variations in ecosystem C processes (Glücksman et al. 2010).

Microbial C mineralization of detritus was controlled by a rapid succession of protozoan grazers with ciliates and flagellates dominating early successional stages. Effects of amoebae on microbial mineralization became visible only at late successional stages when bacterial production doubtlessly had ceased. At this stage, ~ 14 d ADI, the ability of amoebae to invade bacterial biofilms likely provided a functional advantage. This supports our second hypothesis that influence of protozoan species on soil bacteria changes during decomposition of detritus.

Protozoan grazing can influence C mineralization in two ways. On the one hand strong reductions of bacterial numbers will result in decreased mineralization (Crump 1923, Ekelund and Rønn 1994). On the other hand excretion of nutrients by protozoan grazers often alleviates resource limitation of microbes to such an extent that reproduction rates of bacteria keep up with protozoan grazing. This can result in an almost unchanged pool of microbial biomass with greatly enhanced turnover and a stimulation of mineralization rates (Alphei et al. 1996, Bonkowski et al. 2000, Griffiths 1994b). Indeed, overall mineralization rate turned out to be the net result of a complex succession of grazers with partly opposite effects on microbial mineralization.

A common pattern observed in bacteria and fungi is a succession of fast-growing microbial r-strategists (copiotrophs) that are replaced by slow-growing K-strategists (oligotrophs) during the distinct phases of litter decomposition (Bastian et al. 2009, Frankland 1998, Rui et al. 2009). High consumption rates and fast reproduction are likely the functional attributes that give certain protozoan taxa a head-start over others when bacterial food is in ample supply. These functional attributes were reflected in highly mobile ciliate filter feeders, being able to engulf large amounts of bacteria, and in tiny flagellate taxa with their fast reproduction rates, both clearly dominating during the early stages of litter decomposition. Such priority effects are assumed to lead to monopolization of food resources by distinct taxa (Urban and De Meester 2009). Succession is a dominant feature of detrital food webs and critical in understanding soil communities (Frankland 1998;

Freckman 1988; Ponge 1991; Rønn et al. 1996; Swift et al. 1979). Our results show that this common pattern is also reflected in behavior and influence of protozoan grazers and that the rapidity of these successions might be higher than generally assumed.

Protozoa have been shown to alter the taxonomic composition and activity of bacterial communities in soil within three days (Rosenberg et al. 2009). This can easily lead to system instability (Moore and Hunt 1988) but the rapidly changing strength of species specific effects of protozoa on bacterial mineralization and community structure shows that the effects of grazer identity on total system mineralization were strong but only transient. This suggests weak interaction strength between generalist microbial predators. At small time scales these weak interaction strength stabilized overall grazer impacts and in the sum led to enhanced microbial mineralization thus supporting food webs dynamics (Closs et al. 1999, De Ruiter et al. 1995, Neutel et al. 2007). Enhanced grazer diversity may also lead to a stabilization of ecosystem performance by weak interaction strength on major ecosystem process such as microbial mineralization of detritus.

This study provides a clear example that the overall outcome of ecosystem processes, such as mineralization rate are regulated by the sum of positive and negative effects of complex species interactions operating at a very fine scale. This is in line with Glücksman et al. (2010) who found that grazing preferences are both more specific and less predictable than assumptions based on simple morphological similarities suggest.

Because the microbial community has specific effects on decomposition, the modifications of bacterial community structure and activity shown in this study have important consequences for the entire C flow through the microbial compartment of the soil food web. Predation modifies bacterial turnover, thereby also affecting the mobilization of different C sources. Interestingly, the assumption that shared characteristics of species reflect similar functions is seldom tested (Verhoef and Morin 2010). Further, this “one-taxonomic group-one-diet” hypothesis that is generally applied to generalize soil ecology (Eggers and Jones 2000) has very limited scope for furthering the understanding of food-web interactions, or the impact of linkages between organisms and nutrient cycling within the soil (Crotty et al. 2012). For Cercomonads Glücksman et al. (2010) already found that even closely related and morphologically almost undistinguishable protozoan species can differently impact bacterial community composition and abundances. Using a much broader spectrum of protist morphotypes, we confirm that microbial functioning (e.g. mineralization capacity) is affected by protist community composition.

Conclusions

Shedding light on the influence of the diverse soil protozoan community on bacterial community structure and detritus mineralization is of fundamental importance for understanding C fluxes through the soil system. Protozoa affect C fluxes in the detritusphere via diversity dependent top-down control of composition and function (mineralization) of the microbial community. Mineralization rate, being a key ecosystem process, overall increased in the presence of protozoa. The six different protozoan taxa in this study had species specific impacts on composition and mineralization capacity of the microbial community. The influence of different protozoan species on the bacterial community was constantly changing and seems to depend extremely on the stage of detritus decomposition. As a result a succession of protozoan effects was described.

In general, protozoan identity seems to be more relevant for the composition of the microbial community at the beginning of decomposition while the species richness of the protozoan community turns out to be the critical determinant at a later stage.

As a consequence of these results it has to be emphasized that the general "functional group concept", classifying protozoa into the categories ciliate, amoeba and flagellate has to be applied with care since it is not a reliable indicator of the way a species effect prey organisms and communities.

References

Adl, S. M., Simpson, A. G., Lane, C. E., Lukeš, J., Bass, D., Bowser, S. S., Brown, M. W., Burki, F., Dunthorn, M., Hampl, V., Heiss, A., Hoppenrath, M., Lara, E., le Gall, L., Lynn, D. H., McManus, H., Mitchell, E. A. D., Mozley-Stanridge, S. E., Parfrey, L. W., Pawlowski, J., Rueckert, S., Shadwick, L., Schoch, C. L., Smirnov, A., and Spiegel, F. W., (2012) The revised classification of eukaryotes. *Journal of Eukaryotic Microbiology 59*(5): 429-514

Alphei, J., Bonkowski, M., and Scheu, S., (1996) Protozoa, Nematoda and Lumbricidae in the rhizosphere of *Hordelymus europaeus* (Poaceae): Faunal interactions, response of microorganisms and effects on plant growth. *Oecologia* 106: 111-126

Anderson, J. M, (1975) Succession, diversity and trophic relationships of some soil animals in decomposing leaf litter. *Journal of Animal Ecology* 44: 475-495

Anderson, O. R., (2011) Soil respiration, climate change and the role of microbial communities. *Protist 162*(5): 679-690

Andrén, O., Brussaard, L., and Clarholm, M., (1999) Soil organism influence on ecosystem-level processes–bypassing the ecological hierarchy?. *Applied Soil Ecology 11*(2): 177-188

Bardgett, R. D., (2005) The biology of soil. *A community and ecosystem approach*.

Bastian, F., Bouziri, L., Nicolardot, B., and Ranjard, L., (2009) Impact of wheat straw decomposition on successional patterns of soil microbial community structure. *Soil Biology and Biochemistry* 41: 262-275. doi: 10.1016/j.soilbio.2008.10.024

Bastow, J., (2012) Succession, Resource Processing, and Diversity in Detrital Food Webs. *Soil Ecology and Ecosystem Services*

Bengtsson, J., (1998) Which species? What kind of diversity? Which ecosystem function? Some problems in studies of relations between biodiversity and ecosystem function. *Applied Soil Ecology 10*(3): 191-199

Berlow, E., Navarette, S., Briggs, C., Power, M., and Menge, B., (1999) Quantifying variation in the strength of species interactions. *Ecology* 80: 2206-2224

Boenigk, J., and Arndt, H., (2002) Bacterivory by heterotrophic flagellates: community structure and feeding strategies. Antonie van Leeuwenhoek 81: 465-480

Bonkowski, M., and Brandt, F., (2002) Do soil protozoa enhance plant growth by hormonal effects?. *Soil Biology and Biochemistry 34*(11): 1709-1715

Bonkowski, M., Griffiths, B., and Scrimgeour, C., (2000) Substrate heterogeneity and microfauna in soil organic 'hotspots' as determinants of nitrogen capture and growth of ryegrass. *Applied Soil Ecology 14*(1): 37-53

Bouwman, L. A., and Zwart, K. B., (1994) The ecology of bacterivorous protozoans and nematodes in arable soil. *Agriculture, ecosystems & environment 51*(1): 145-160

Bruno, J. F., and Cardinale, B. J., (2008) Cascading effects of predator richness. *Frontiers in Ecology and the Environment* 6: 539-546. doi: 10.1890/070136

Brussaard, L., Bouwman, L. A., Geurs, M., Hassink, J., and Zwart, K. B., (1990) Biomass, composition and temporal dynamics of soil organisms of a silt loam soil under conventional and integrated management. *Netherlands Journal of Agricultural Science 38*(3A): 283-302

Cavalier-Smith, T., (1993) Kingdom protozoa and its 18 phyla. *Microbiological reviews 57*(4): 953

Cavalier-Smith, T., (1998) A revised six-kingdom system of life. *Biological Reviews 73*(3): 203-266

Clarholm, M., (1981) Protozoan grazing of bacteria in soil—impact and importance. *Microbial Ecology 7*(4): 343-350

Closs, G. P., Balcombe, S. R., Shirley, M. J., Fitter, A. H., and Raffaelli, D., (1999) Generalist Predators, Interaction Strength and Food-web Stability. *Advances in Ecological Research*. Academic Press

Coleman, D. C., Anderson, R. V., Cole, C. V., Elliott, E. T., Woods, L., and Campion, M. K., (1978) Trophic interactions in soils as they affect energy and nutrient dynamics. IV. Flows of metabolic and biomass carbon. *Microbial Ecology* 4: 373-380

Coleman, D. C., and Whitman, W. B., (2005) Linking species richness, biodiversity and ecosystem function in soil systems. *Pedobiologia 49*(6): 479-497

Corno, G., and Jürgens, K., (2008) Structural and functional patterns of bacterial communities in response to protist predation along an experimental productivity gradient. *Environmental microbiology 10*(10): 2857-2871

Coûteaux, M. M., and F Darbyshire, J., (1998) Functional diversity amongst soil protozoa. *Applied Soil Ecology 10*(3): 229-237

Crotty, F. V., Adl, S. M., Blackshaw, R. P., and Murray, P. J., (2012) Using stable isotopes to differentiate trophic feeding channels within soil food webs. *Journal of Eukaryotic Microbiology 59*(6): 520-526

Crump, D. W., (1923) The action of protozoa on bacteria when inoculated into sterile soil. *Ann Appl Biol* 10: 137-141

Cutler, D. W., and Crump, L. M., (1929) Carbon dioxide production in sands and soils in the presence and absence of amoebae. *Ann Appl Biol* 16: 472-482

De Ruiter, P. C., Neutel, A.-M., and Moore, J. C., (1995) Energetics, pattern of interaction strength and stability in real ecosystems. *Science* 269: 1257-1260

De Telegdy-Kovats, L., (1932) The growth and respiration of bacteria in sand cultures in the presence and absence of protozoa. *Ann Apl Biol* 19: 65-86

Eggers, T., and Jones, T. H., (2000) You are what you eat…or are you? *Trends in Ecology and Evolution* 15: 265-266

Ekelund, F., and Rønn, R., (1994) Notes on protozoa in agricultural soil with emphasis on heterotrophic flagellates and naked amoebae and their ecology. *FEMS Microbiology Reviews* 15:321-353

Fouchard, S., Abdellaoui-Maâne, Z., Boulanger, A., Llopiz, P., and Neunlist, S., (2005) Influence of growth conditions on Pseudomonas fluorescens strains: a link between metabolite production and the PLFA profile. *FEMS microbiology letters 251*(2): 211-218

Frankland, J. C., (1998) Fungal succession—unravelling the unpredictable. *Mycological Research* 102: 1-15

Freckman, D. W., (1988) Bacterivorous nematodes and organic-matter decomposition. Agriculture, *Ecosystems and Environment* 24: 195-217

Frostegård, Å., Tunlid, A., and Bååth, E., (1993) Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Applied and Environmental Microbiology 59*(11): 3605-3617

Fuhrman, J. A., (1992) Novel major archaebacterial group from marine plankton. *Nature 356*(6365): 148-149

Geisen, S., (2014) Soil Protists - Diversity, Distribution and Ecological Functioning, PhD thesis, University of Cologne: 1-273

Gessner, M. O., Swan, C. M., Dang, C. K., McKie, B. G., Bardgett, R. D., Wall, D. H., and Hättenschwiler, S., (2010) Diversity meets decomposition. *Trends in Ecology & Evolution 25*(6): 372-380

Glücksman, E., Bell, T., Griffiths, R. I., and Bass, D., (2010) Closely related protist strains have different grazing impacts on natural bacterial communities.*Environmental microbiology 12*(12): 3105-3113

Griffiths, B. S., and Caul, S., (1993) Migration of bacterial-feeding nematodes, but not protozoa, to decomposing grass residues. *Biology and Fertility of Soils15*(3): 201-207

Griffiths, B. S., (1994a) Microbial-feeding nematodes and protozoa in soil: Their effectson microbial activity and nitrogen mineralization in decomposition hotspots and the rhizosphere. *Plant and Soil* 164: 25-33

Griffiths, B. S., (1994b) Soil nutrient flow. In: J Darbyshire (ed) *Soil Protozoa*. CAB International, Wallingford.

Griffiths, B. S., Bonkowski, M., Dobson, G., and Caul, S., (1999) Changes in soil microbial community structure in the presence of microbial-feeding nematodes and protozoa. *Pedobiologia 43*(4): 297-304

Griffiths, B. S., Bonkowski, M., Roy, J., and Ritz, K., (2001) Functional stability, substrate utilisation and biological indicators of soils following environmental impacts. *Applied Soil Ecology 16*(1): 49-61

Hahn, M. W., and Höfle, M. G., (1999) Flagellate predation on a bacterial model community: interplay of size-selective grazing, specific bacterial cell size, and bacterial community composition. *Applied and environmental microbiology 65*(11): 4863-4872

Hendrix, P. F., Parmelee, R. W., Crossley, D. A., Coleman, D. C., Odum, E. P., and Groffman, P. M., (1986) Detritus food webs in conventional and no-tillage agroecosystems. *Bioscience*: 374-380

Holubar, P., Grudke, T., Moser, A., Strenn, B., and Braun, R., (2000) Effects of bacterivorous ciliated protozoans and degradation efficiency of a petrochemical activated sludge process. *Water Research* 34: 2051-2060

Hunt, H. W., Coleman, D. C., Ingham, E. R., Ingham, R. E., Elliott, E. T., Moore, J. C., Rose, C. L., Reid, C. P. P., and Morley, C. R., (1987) The detrital food web in a shortgrass prairie.*Biology and Fertility of Soils 3*(1-2): 57-68

Jones, R. I., (2000) Mixotrophy in planktonic protists: an overview. *Freshwater Biology*, *45*(2), 219-226.

Knivett, V.A., and Cullen, J., (1965) Some factors affecting cyclopropane acid formation in *Escherichia coli. Biochemical Journal* 96: 771-776

Koller, R., Robin, C., Bonkowski, M., Ruess, L., and Scheu, S., (2013) Litter quality as driving factor for plant nutrition via grazing of protozoa on soil microorganisms. *FEMS microbiology ecology 85*(2). 241-250

Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Scheunemann, N., Schoene, J., Schmallwasser, A., Totsche, K. U., Walker, F., Scheu, S., and Kandeler, E., (2012) Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems.*Pedobiologia 55*(2). 111-119

Kreuzer, K., Adamczyk, J., Iijima, M., Wagner, M., Scheu, S., and Bonkowski, M., (2006) Grazing of a common species of soil protozoa (*Acanthamoeba castellanii*) affects rhizosphere bacterial community composition and root architecture of rice (*Oryza sativa* L.). *Soil Biology and Biochemistry 38*(7): 1665-1672

Krivtsov, V., Liddell, K., Bezginova, T., Salmond, R., Garside, A., Thompson, J., Palfreyman, J. W., Staines, H. J., Watling, R., Brendler, A., and Griffiths, B., (2003) Ecological interactions of heterotrophic flagellates, ciliates and naked amoebae in forest litter of the Dawyck Cryptogamic Sanctuary (Scotland, UK). *European journal of protistology 39*(2): 183-198

Marschner, P., Umar, S., and Baumann, K., (2011) The microbial community composition changes rapidly in the early stages of decomposition of wheat residue. *Soil Biology and Biochemistry 43*(2): 445-451

Moore, J. C., and Hunt, W. H., (1988) Resource compartmentation and the stability of real ecosystems. *Nature* 333: 261-263

Moore, J. C., Berlow, E. L., Coleman, D. C., Ruiter, P. C., Dong, Q., Hastings, A., Johnson, N. C., McCann, K. S., Melville, K., Morin, P. J., Nadelhoffer, K., Rosemond, A. D., Post, D. M., Sabo, J. L., Scow, K. M., Vanni, M. J., and Wall, D. H., (2004) Detritus, trophic dynamics and biodiversity. *Ecology letters 7*(7): 584-600

Müller, M. S., Scheu, S., and Jousset, A., (2013) Protozoa Drive the Dynamics of Culturable Biocontrol Bacterial Communities. *PloS one 8*(6): e66200

Nielsen, U. N., Ayres, E., Wall, D. H., and Bardgett, R. D., (2011) Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity–function relationships. *European Journal of Soil Science 62*(1): 105-116

Neutel, A. M., Heesterbeek, J. A. P., van de Koppel, J., Hoenderboom, G., Vos, A., Kaldeway, C., Berendse, F., and de Ruiter, P. C., (2007) Reconciling complexity with stability in naturally assembling food webs. *Nature* 449: 599-U511. doi: 10.1038/nature06154

Page, F. C., (1976). Illustrated key to freshwater and soil amoebae. *Freshwater Biological Association*, The Ferry House, Ableside, Cumbria.

Parry, J., (2004) Protozoan grazing of freshwater biofilms. *Advances in Applied Microbiology* 54: 167-196

Ponge, J. F., (1991) Succession of fungi and fauna during decomposition of needles in a small area of Scots pine litter. *Plant and Soil* 138: 99-113

Ratsak, C. H., Maarsen, K. A., and Kooijman, S. A. L. M., (1996) Effects of protozoa on carbon mineralization in activated sludge. Water Research 30: 1-12

Reed, H. E., and Martiny, J. B., (2007) Testing the functional significance of microbial composition in natural communities. *FEMS microbiology ecology 62*(2): 161-170

Rønn, R., Griffiths, B. S., Ekelund, F., and Christensen, S., (1996) Spatial distribution and successional pattern of microbial activity and micro-faunal populations on decomposing barley roots. *Journal of Applied Ecology* 33: 662-672

Rønn, R., McCaig, A. E., Griffiths, B. S., and Prosser, J. I., (2002) Impact of protozoan grazing on bacterial community structure in soil microcosms. *Applied and Environmental Microbiology 68*(12): 6094-6105

Rosenberg, K., Bertaux, J., Krome, K., Hartmann, A., Scheu, S., and Bonkowski, M., (2009) Soil amoebae rapidly change bacterial community composition in the rhizosphere of Arabidopsis thaliana. *The ISME journal 3*(6): 675-684

Rui, J., Peng, J., and Lu, Y., (2009) Succession of bacterial populations during plant residue decomposition in rice field soil. *Applied and environmental microbiology* 75: 4879-4886

Saleem, M., Fetzer, I., Dormann, C. F., Harms, H., and Chatzinotas, A., (2012) Predator richness increases the effect of prey diversity on prey yield. *Nature communications 3*: 1305

Saleem, M., Fetzer, I., Harms, H., and Chatzinotas, A., (2013) Diversity of protists and bacteria determines predation performance and stability. *The ISME journal*: 1-10

Schimel, J. P., and Gulledge, J. A. Y., (1998) Microbial community structure and global trace gases. *Global change biology 4*(7): 745-758

Setälä, H., Berg, M. P., and Jones, T. H., (2005) Trophic structure and functional redundancy in soil communities. *Biological diversity and function in soils*: 236-249

Sih, A., Englund, G., and Wooster, D., (1998) Emergent impacts of multiple predators on prey. *Trends in Ecology & Evolution 13*(9): 350-355

Stout, J.D., (1973) The relationship between protozoan populations and biological activity in soils. *Amer Zool* 13: 193-201

Strickland, M. S., Lauber, C., Fierer, N., and Bradford, M. A., (2009) Testing the functional significance of microbial community composition. *Ecology 90*(2): 441-451

Sundin, P., Valeur, A., Olsson, S., and Odham, G., (1990) Interactions between bacteria-feeding nematodes and bacteria in the rape rhizosphere: effects on root exudation and distribution of bacteria. *FEMS microbiology letters 73*(1): 13-22

Swan, C. M., and Kominoski, J. S., (2012) Biodiversity and ecosystem function of decomposition. *eLS*.

Swift, M. J., Heal, O. W., and Anderson, J. M., (1979) Decomposition in terrestrial ecosystems. Univ of California Press.

Urban, M. C., and De Meester, L., (2009) Community monopolization: local adaptation enhances priority effects in an evolving metacommunity. *Proceedings of the Royal Society B: Biological Sciences* 276: 4129-4138. doi: 10.1098/rspb.2009.1382.

Verhoef, H. A., and Morin, P. J., (Eds.). (2010) *Community ecology: processes, models, and applications*. New York: Oxford University Press.

Zelles, L., (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biology and Fertility of Soils 29*(2): 111-129

Zwart, K. B., and Darbyshire, J. F., (1992) Growth and nitrogenous excretion of a common soil flagellate *Spumella sp.*—a laboratory experiment. *Journal of Soil Science 43*(1): 145-157

General Discussion

Despite the fundamental importance of the flux of C through microbial soil food webs for ecosystem functioning, global C balance and fertility of arable soils little is known about their structure and the key-taxa actually utilizing the supplied C sources. Further, differences between the microbial food webs in rhizosphere and detritusphere with different origins of C are scarcely investigated. This thesis reveals a new detailed view on soil microbial community compositions and C flow through different compartments of the soil system and illuminates the functional role of protozoa in soil food webs of an arable field in high taxonomic resolution. The results presented here allow a comparison of the microbial communities in diverse microhabitats like different soil layers, bulk soil, rhizosphere and detritusphere. A closer look on detritus driven systems portrayed successions of the microbial community in presence of different detrital energy sources and while decomposing the same detrital C source but in presence of different protozoan grazers.

In **chapter I** the microbial community structure of an arable field where rhizodeposits and detritus serve as energy sources is portrayed in detail. Maize litter as detritus enhanced abundances of primary consumers but not their diversity. In general protozoan abundance and community composition barely responded to detritus amendment. Flagellate and ciliates taxa dominated top-soil layers while naked amoebae were common inhabitants of deep soil layers. Abundance of monotactic amoebae and euglenids even peaked in deep root free soil. An unexpected high diversity and abundance of protozoa beneath the ploughshare suggests strong food web interactions in deeper soil layers. These results indicate distinct niche differentiation of major protozoan taxa along the soil profile, suggesting high competition for microbial food.

Chapter I delivered a detailed overview of the soil microbial communities in arable soil. However, the experimental set-up in the field could only scarcely distinguish between rhizodeposit and detritus driven microbial communities. Further the applied cultivation approach after Finlay et al. (2000) for determination and quantification of protozoa allowed a morphotype based grouping but failed in taxonomic classification. The field study focused on a spatial resolution and did not include temporal changes. For complementing the field experiment the latter aspects were addressed in subsequent lab experiments. **Chapter II** and **chapter III** focus on C fluxes in rhizosphere and detritusphere microbial communities, respectively and were able to describe the microbial

key-players directing these fluxes. By using high throughput sequencing in tandem with stable isotope probing for both experiments a high eukaryotic diversity in bulk soil, rhizosphere and detritusphere was found, but only a small subset of the microbial community actively participated in the food webs utilizing the corresponding C source. In **chapter II** complex microbial food webs relying on rhizodeposits are described during the succession of plant derived C through microbial key-organisms in the maize rhizosphere. The portrayed food webs had a high turnover and we observed a rapid succession of enriched organisms proving that a plant's rhizosphere microbiome is not only characterized by high microbial diversity (Berendsen et al. 2012) but also extremely dynamic. In line with other studies (Koller et al. 2013, Mao et al. 2014, Drigo et al. 2010) mycorrhizal fungi turned out to be the major route for the flux of plant derived C into the bulk soil communities. The area of influence of mycorrhizal plants turned out to be much bigger than previously assumed.

In the detritusphere (**chapter III**) bacteria, fungi and protists were identified as primary consumers of all substrates offered, irrespectively of their recalcitrance. We found that different amoeba and flagellate taxa played an active role in the detrital microbial food webs mineralizing leaf litter. Surprisingly protozoa were not significantly involved in the food webs utilizing glucose, even though other protists like the peronosporomycete *Pythium* played a major role in the glucose driven food webs. In **chapter II** several ciliate, flagellate and amoebae taxa turned out to be important key-players in rhizodeposit driven food webs. Since a large fraction of rhizodeposits consist of low-molecular weight and easily available C compounds like sugars (Farrar et al. 2003, Bardgett 2005) we expected at least some of these protozoa would also participate in detrital food webs fuelled by sugars. This was not the case. The results presented here underline that scaling up experimental results for describing ecological processes has to be done with caution, predominantly due to specificity and complexity of multitrophic interactions and dependency on environmental conditions which may easily lead to false assumptions. The two experiments illustrate the value of Stable Isotope Probing (SIP) and subsequent high throughput sequencing for the investigation of active microorganisms and interactions in food webs. Neither diversity nor abundance data alone would have been able to reveal the patterns of trophic level interactions in major energy pathways and to identify key organisms of C transfer.

We know that protozoa in soil systems are both, highly abundant and morphologically as well as phylogenetically very diverse (Cavalier-Smith 1998, Adl et al. 2012). Nevertheless

our results show that only a small fraction of the protozoan community in arable soil is active at a specific time point in rhizosphere as well as in detritusphere. This lead to the question whether specific protozoan species differ in their ecological function and if the commonly applied division of soil protozoa into the so called "functional groups" ciliates, flagellates and amoeba mirror real functionally different impacts on prey communities or strongly oversimplify complexity. These questions were addressed in **chapter IV** of this thesis.

During microbial decomposition of maize litter microbial C mineralization was controlled by a rapid succession of protozoan grazers with ciliates and flagellates dominating early and amoebae late successional stages. Effects of protozoan grazers on microbial respiration turned out to be species specific. The results showed that the general "functional group concept" is not a reliable indicator of the way protozoan species affect prey organisms and communities. Until present, most food web models only consider "functional groups" of protozoa (Holtkamp et al. 2011, Hunt et al. 1987, De Ruiter et al. 1995), this needs to be reconsidered in the light of our results. The overall outcomes of ecosystem processes like C mineralization are regulated by the sum of positive and negative effects of complex species interactions operating at a very fine scale.

The fast successions described in all chapters capturing temporal changes (**chapter II-IV**) suggest that soil microbial communities, especially protozoan communities, are characterized by a high degree of competition in all investigated systems since strong competition is generally thought to cause rapid changes in community composition (Marschner et al. 2011). It is well-known that succession is a dominant feature, especially of detrital food webs, and critical for understanding soil communities (Frankland 1998; Freckman 1988; Ponge 1991; Rønn et al. 1996; Swift et al. 1979). Nevertheless this thesis revealed an unexpected strong and rapid succession of microbial food web composition and influence on C flux in terrestrial ecosystems.

The highly dynamic nature of microbial soil food webs, expressed in high turnover rates and rapid succession of different taxa, together with the extreme heterogeneity of soil systems make it extremely difficult to predict reactions to environmental changes like climate change and management conditions (Eisenhauer et al. 2012). Soil communities form strongly interwoven food webs (Brose and Scheu 2014) even in presumably simple structured habitats like arable fields and and the same organisms may have different food preferences and trophic positions depending on food web composition. In summary, the presented data provides a new level of resolution and detail about microbial food webs and

the ecology of soil protozoa. This thesis shows that data on microbial food web dynamics and detailed knowledge of protozoan species and communities are a prerequisite to understand food web functioning and to develop accurate food web models for C fluxes through the 'black box' of microbial communities in arable soil. Further, these findings are crucial for development of sustainable management strategies for agricultural systems. The "one-taxonomic group - one-diet" hypothesis that is generally applied to generalize soil ecology (Eggers and Jones 2000) has very limited scope for improving the understanding of food web interactions, or the impact of microorganisms on nutrient cycling within the soil (Crotty et al. 2012). More detailed studies considering small scale variations in the structure of soil food webs are needed, especially at the basis of the food webs where different energy channels are supposed to originate (Brose and Scheu 2014).

We found rhizosphere and detritusphere to host functional and phylogenetically different microbial communities that support different food web structures but a strict differentiation between a bacterial and a fungal energy channel (e.g. De Boer et al. 2005, Moore and Hunt 1988, Paterson et al. 2008) is not supported by our results. This thesis shows simultaneous activity and overlapping patterns of the usage of different quality substrates by bacteria, fungi and protists (*Pythium*) in the detritusphere. In the rhizosphere, where bacteria were supposed to be the main utilizers of rhizodeposits, single celled fungi, mycorrhiza and protists (*Peronosporales*) also played an important role as primary consumers. This is in line with other recent findings (Crotty et al. 2012, Brose and Scheu 2014, Nieminen and Setälä 2001).

Overall this thesis contributes to a great extend to disentangling complex and highly dynamic microbial food web interactions with protozoa in soil. As the presented results further underline the role of protozoa as critical links in the C transfer in root and detritus derived food webs as well as in deeper soil layers, the quantification and identification of key-taxa in the field and equally detailed analysis of other soil systems are major tasks for future research.

References

Adl, S. M., Simpson, A. G., Lane, C. E., Lukeš, J., Bass, D., Bowser, S. S., Brown, M. W., Burki, F., Dunthorn, M., Hampl, V., Heiss, A., Hoppenrath, M., Lara, E., le Gall, L., Lynn, D. H., McManus, H., Mitchell, E. A. D., Mozley-Stanridge, S. E., Parfrey, L. W., Pawlowski, J., Rueckert, S., Shadwick, L., Schoch, C. L., Smirnov, A., and Spiegel, F. W., (2012) The revised classification of eukaryotes. *Journal of Eukaryotic Microbiology 59*(5): 429-514

Bardgett, R., (2005) The biology of soil – A community and ecosystem approach. *Oxford University Press*

Berendsen, R. L., Pieterse, C. M. J., and Bakker, P. A. H. M., (2012) The rhizosphere microbiome and plant health. *Trends in Plant Science* 17(8):476-486

Brose, U., and Scheu, S., (2014) Into darkness: unravelling the structure of soil food webs. *Oikos 123*(10): 1153-1156

Cavalier-Smith, T., (1998) A revised six-kingdom system of life. *Biological Reviews 73*(3): 203-266

Crotty, F. V., Adl, S. M., Blackshaw, R. P., and Murray, P. J., (2012) Using stable isotopes to differntiate trophic feeding channels within soil food webs. *The Journal of Eukaryotic Microbiology* 59(6): 520-526

De Boer, W., Folman, L., Summerbell, R., and Boddy, L., (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* 29: 795-811

De Ruiter, P. C., Neutel, A.-M., and Moore, J. C., (1995) Energetics, pattern of interaction strength and stability in real ecosystems. *Science* 269: 1257-1260

Drigo, B., Pijl, A. S., Duyts, H., Kielak, A. M., Gamper, H. A., Houtekamer, M. J., Boschker, H. T. S., Bodelier, P. L. E., Whiteley, A. S., van Veen, J. A., and Kowalchuk, G. A., (2010) Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2. *PNAS* 107(24):10938-10942

Eggers, T., and Jones, T. H., (2000) You are what you eat…or are you? *Trends in Ecology and Evolution* 15: 265-266

Eisenhauer, N., Cesarz, S., Koller, R., Worm, K., Reich, P. B., (2012) Global change belowground: Impacts of elevated CO_2, nitrogen, and summer drought on soil food webs and biodiversity. *Global change biology* 18: 435-447

Farrar, J., Hawes, M., Jones, D., and Lindow, S., (2003) How roots control the flux of carbon to the rhizosphere. *Ecology* 84: 827-837

Finlay, B., Black, H., Brown, S., Clarke, K., Esteban, G., Hindle, R., Olmo, J., Rollett, A., and Vickerman, K., (2000) Estimating the growth potential of the soil protozoan community. *Protist* 151: 69-80

Frankland, J. C., (1998) Fungal succession - unravelling the unpredictable. *Mycological Research* 102: 1-15

Freckman, D. W., (1988) Bacterivorous nematodes and organic-matter decomposition. Agriculture, *Ecosystems and Environment* 24: 195-217

Holtkamp, R., Van der Wal, A., Kardol, P., Van der Putten, W. H., De Ruiter, P. C., and Dekker, S. C., (2011) Modelling C and N mineralisation in soil food webs during secondary succession on ex-arable land. *Soil Biology and Biochemistry 43*(2): 251-260

Hunt, H. W., Coleman, D. C., Ingham, E. R., Ingham, R. E., Elliott, E. T., Moore, J. C., Rose, S. L., Reid, C. P. P., and Morley, C. R. (1987) The detrital food web in a shortgrass prairie.*Biology and Fertility of Soils 3*(1-2): 57-68

Koller, R., Scheu, S., Bonkowski, M., and Robin, C., (2013) Protozoa stimulate N uptake and growth of arbuscular mycorrhizal plants. *Soil Biology and Biochemistry 65*: 204-210

Mao, Y., Li, X., Smyth, E. M., Yannarell, A. C., and Mackie, R. I., (2014) Enrichment of specific bacterial and eukaryotic microbes in the rhizosphere of switchgrass (*Panicum virgatum* L.) through root exudates. *Environmental Microbiology Reports* doi: 10.1111/1758-2229.12152

Marschner, P., Umar, S., and Baumann, K., (2011) The microbial community composition changes rapidly in the early stages of decomposition of wheat residue. *Soil Biology and Biochemistry 43*(2): 445-451

Moore, J. C., and Hunt, W. H., (1988) Resource compartmentation and the stability of real ecosystems. *Nature* 333: 261-263

Nieminen, J. K., and Setälä, H., (2001) Bacteria and microbial-feeders modify the performance of a decomposer fungus. *Soil Biology and Biochemistry 33*(12): 1703-1712

Paterson, E., Osler, G., Dawson, L. A., Gebbing, T., Sim, A., and Ord, B., (2008) Labile and recalcitrant plant fractions are utilized by distinct microbial communities in soil: Independent of the presence of roots and mycorrhizal fungi. *Soil Biology and Biochemistry* 40: 1103-1113

Ponge, J. F., (1991) Succession of fungi and fauna during decomposition of needles in a small area of Scots pine litter. *Plant and Soil* 138: 99-113

Rønn, R., Griffiths, B. S., Ekelund, F., and Christensen, S., (1996) Spatial distribution and successional pattern of microbial activity and micro-faunal populations on decomposing barley roots. *Journal of Applied Ecology* 33: 662-672

Swift, M. J., Heal, O. W., and Anderson, J. M., (1979) Decomposition in terrestrial ecosystems. Univ of California Press

Danksagung

Als erstes möchte ich mich bei meinem Doktorvater Prof. Michael Bonkowski bedanken, der mir die Möglichkeit gab diese Arbeit zu schreiben. Danke Michael für die Einführung in ein spannendes Thema, die vielseitigen Erfahrungen die ich sammeln durfte und die gute Betreuung.

Auch dem Rest der AG Bonkowski gebührt großer Dank. Ich hatte das Glück Teil eines tollen Teams mit netten Kollegen sein zu dürfen. Vielen Dank für all die Unterstützung!

Ganz besonders Robert Koller und Stefan Geisen haben viel zum Gelingen dieser Arbeit beigetragen, mich stets motiviert und für viel Spaß an der Arbeit gesorgt. Ihr ward die besten Büro-Mitbewohner, die man sich nur wünschen kann und es wird mir sehr fehlen mit euch zusammenzuarbeiten! Robert danke ich zusätzlich noch für all die Zeit, die er in mich und diese Arbeit investiert hat, für all die langen Gespräche und die guten Ratschläge. Ohne dich hätte ich mir diese Arbeit nicht vorstellen können!

Bei meinen Kollegen aus der Forschergruppe möchte ich mich auch bedanken, besonders bei den Münchnern, Dörte Dibbern und Tillmann Lüders, für die gute Zusammenarbeit und die spannenden Aufenthalte bei euch im Helmholtz Zentrum. Ich habe viel von euch gelernt.

Prof. Hartmut Arndt danke ich für die Zweitkorrektur dieser Arbeit. Bei Dr. Anja Scherwaß und bei Prof. Tim Mansfeldt bedanke ich mich für die Übernahme des Beisitzerpostens bzw. des Prüfungsvorsitzes bei meiner Verteidigung.

Nicht zuletzt möchte ich mich bei meiner Familie bedanken. Meiner Mutter danke ich für all ihre Unterstützung, das Rücken-freihalten und Ermutigen. Danke, dass du an mich glaubst und immer für mich da bist! Außerdem danke ich Mama und Bernd für mein “Arbeitszimmer“ in ihrem Haus und alles was sie für mich getan haben, besonders in der letzten, stressigen Zeit.

Kai und Runa: Ihr seid mein Leben, meine Motivation und mein Glück. Danke für alles, ich liebe euch!

Declaration of Co-Authorships

Authors contributed to the work according to the following categories:

A – more than 75% B – 51 to 75% C – 20 to 50% D – up to 19% 0 – no contribution

Chapter I – Effects of resource availability and quality on the structure of the micro-food web of an arable soil across depth

Name	Literature review	Development of theoretical model/ framework	Concept and design of the study/ analysis	Execution, choice and development of methods	Evaluation and interpretation of results	Concept and writing of the article
A. Scharroba	D	0	D	C	D	D
D. Dibbern	D	0	D	C	D	D
M. Hünninghaus	D	0	D	C	D	D
S. Kramer	D	0	D	C	D	D
J. Moll	D	0	D	C	D	D
O. Butenschoen	0	0	D	0	0	0
M. Bonkowski	0	D	D	0	D	0
F. Buscot	0	D	D	0	0	0
E. Kandeler	0	D	D	0	D	0
R. Koller	0	D	D	0	0	0
D. Krüger	0	D	D	0	D	0
T. Lueders	0	D	D	0	D	0
S. Scheu	0	C	D	0	0	0
L. Ruess	D	C	D	0	D	D

Chapter II - Rhizosphere food web links uncovered – Carbon fluxes from maize (*Zea mays* L.) roots to microbial key-consumers and their predators

Name	Literature review	Development of theoretical model/ framework	Concept and design of the study/ analysis	Execution, choice and development of methods	Evaluation and interpretation of results	Concept and writing of the article
M. Hünninghaus	C	D	C	C	C	B
D. Dibbern	C	0	0	D	D	D
R. Koller	D	C	C	C	C	D
T. Urich	0	0	0	D	0	0
T. Lueders	D	C	C	C	0	0
M. Bonkowski	C	C	C	D	C	C

Chapter III – Eat all you can - Resource partitioning between bacteria, fungi and protists in the detritusphere

Name	Literature review	Development of theoretical model/ framework	Concept and design of the study/ analysis	Execution, choice and development of methods	Evaluation and interpretation of results	Concept and writing of the article
S. Kramer	C	D	D	B	B	B
D. Dibbern	D	D	D	D	C	C
M. Hünninghaus	D	D	D	D	C	C
J. Moll	D	D	D	D	C	C
R. Koller	0	0	0	D	D	D
D. Krüger	0	0	0	0	D	0
S. Marhan	D	C	C	D	0	D
T. Urich	0	0	0	D	0	0
T. Wubet	0	0	0	D	D	0
M. Bonkowski	D	D	0	0	D	D
F. Buscot	0	D	0	0	0	D
T. Lueders	D	D	D	D	D	C
E. Kandeler	D	C	C	D	D	D

S. Kramer, D. Dibbern, M. Hünninghaus and J. Moll contributed equally to this work

Chapter IV – Effects of Protozoa - microbe interactions on carbon fluxes in the detritusphere

Name	Literature review	Development of theoretical model/ framework	Concept and design of the study/ analysis	Execution, choice and development of methods	Evaluation and interpretation of results	Concept and writing of the article
M. Hünninghaus	B	D	C	B	B	B
R. Koller	D	C	D	D	C	D
S. Kramer	0	0	0	D	0	0
E. Kandeler	0	0	0	D	0	0
M. Bonkowski	C	C	C	D	C	C

Liste der Teilpublikationen

Scharroba, A., Dibbern, D., **Hünninghaus, M.**, Kramer, S., Moll, J., Butenschoen, O., Bonkowski, M., Buscot, F., Kandeler, E., Koller, R., Krüger, D., Lueders, T., Scheu, S., and Ruess, L. (2012) Effects of resource availability and quality on the structure of the micro-food web of an arable soil across depth. *Soil Biology and Biochemistry* 50: 1-11

Kramer, S., Dibbern, D., Moll, J., **Hünninghaus, M.**, Koller, R., Krueger, D., Marhan, S., Urich, T., Wubet, T., Bonkowski, M., Buscot, F., Lüders, T., and Kandeler, E., (submitted to the ISME Journal) Eat all you can – Resource partioning between bacteria, fungi and protists in the detritusphere

Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit − einschließlich Tabellen, Karten und Abbildungen −, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie − abgesehen von unten angegebenen Teilpublikationen − noch nicht veröffentlicht worden ist, sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen der Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Michael Bonkowski betreut worden.

Wiehl, den 03.10.2014

(Maike Hünninghaus)