The role of root-derived carbon in trophic interactions within soil food webs as indicated by analyses of stable isotope, lipids and amino acids in microarthropods (Collembola)

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Summary

A considerable amount of photosynthates is released into soil via living roots as rhizodeposits. This root-derived carbon (C) is an important source of energy and nutrients for microorganisms and higher trophic levels in soil food webs. However, as soil food webs are highly complex with considerable proportions of generalist feeders, it is not well studied how root-derived C affects trophic interactions between microorganisms and microbivores, such as Collembola. Therefore, in this thesis I traced the root-derived C flux into Collembola species belonging to three functional groups, i.e. epedaphic, hemiedaphic and euedaphic, in three cropping systems (oil-seed rape, grass and willow) using $^{13}CO_2$ pulse labeling and bulk stable isotope ^{13}C analysis. The contribution of bacterial and fungal channels to the incorporation of root-derived C into Collembola was investigated in more detail using compound specific ^{13}C lipid analysis. To study the influences of vertical heterogeneity and root energy supply on the trophic structure of soil food webs in forest systems, I identified the trophic niches of Collembola functional groups in a root-trenching experiment using compound-specific isotope analysis of carbon and nitrogen (N) in amino acids of Collembola.

In the first study (chapter 2), using a pulse labeling experiment with $^{13}CO_2$, I identified the incorporation of root-derived C into nine abundant species of Collembola belonging to three functional groups (epedaphic, hemiedaphic and euedaphic) over a period of 28 days in agricultural fields with different plant types, i.e. rape, grass and willow. The incorporation of ^{13}C into Collembola was slower in rape than in grass and willow, suggesting a lower incorporation efficiency of root-derived C into soil food webs in rape as compared to grass and willow. In addition, the utilization of root-derived C and litter/old soil organic matter differed between functional groups/species of Collembola, with the differentiation being dependent on cropping system. These findings highlight that cropping system impacts the flux of root C into soil food webs, presumably due to differences in plant species, application of fertilizer and rhizosphere microbial communities. In addition, partitioning in the utilization of root-derived C and soil organic matter presumably is an important mechanism for local coexistence of Collembola species in soil.

A shortcoming of the first study (chapter 2) was the inability to disentangle the contribution of bacterial and fungal channels to the incorporation of root-derived C into Collembola. Therefore, I further

identified the incorporation of root-derived ^{13}C into bacterial and fungal biomarkers in microbial phospholipid fatty acids (PLFAs) in bulk soil and neutral lipid fatty acids (NLFAs) in five Collembola species belonging to different functional groups (chapter 3). Generally, Collembola incorporated more root-derived C from the bacterial channel in rape than in grass and willow, where fungi were the dominant C source. The proportional abundance of bacterial and fungal biomarkers in Collembola NLFAs corresponded with the ^{13}C incorporation into microbial PLFAs, while it did not correlate with the proportion of microbial PFLAs in different cropping systems. This suggests that the differences in dietary composition of Collembola among cropping systems are mainly driven by changes in microbial community in the rhizosphere but not in bulk soil. Finally, hemiedaphic Collembola incorporated more root-derived C from bacterial resources than eu-/epedaphic Collembola. Hence, changes in root C flux into bacterial and fungal channels among cropping systems resulted in differential utilization of these resources by soil microbivores, suggesting that in particular microorganisms fueled by rhizodeposits are vital resources for the nutrition of higher trophic levels in soil food webs.

In contrast to arable soils, the soil in forest systems receives a considerable amount of aboveground inputs via dead organic matter, resulting in a strong vertical heterogeneity of available resources and environmental conditions. To investigate the vertical heterogeneity of trophic interactions in forest soil food webs and its response to deprivation of root energy supply, a state-of-the-art method, i.e. compound specific isotope analysis (CSIA) of C and N in amino acids, was used to identify the trophic niches of different functional groups of Collembola and their responses to the deprivation of root-derived resources in a root-trenching experiment (chapter 4). The ^{13}C fingerprinting suggested saprotrophic microorganisms rather than mycorrhizal fungi to be the dominant resource for Collembola. The $\delta^{15}N$ values of phenylalanine as indicator of isotopic baseline were higher in euedaphic Collembola than in ep-/hemiedaphic Collembola, suggesting that euedaphic Collembola mainly utilized N from processed organic substrate in deeper soil, while leaf litter was the dominant N source for ep-/hemiedaphic Collembola. In addition, the trophic position calculated from CSIA (TP_{CSIA}) of euedaphic Collembola was constantly higher than that of epedaphic Collembola, reflecting a higher number of trophic transfers in the soil than in the litter layer. Further, the deprivation of root energy supply reduced the TP_{CSIA} of Collembola, suggesting that root derived C increases the number of trophic transfers and food chain length in soil food webs. However, this varied among functional groups of Collembola and forest stands,

suggesting that the association between root energy supply and trophic interactions is mediated by vertical resource heterogeneity, regional conditions and feeding strategies of consumers.

Overall, results of this thesis advance our understanding of the important role of root-derived resources for soil food webs; root-derived C as major resource fueling soil food webs profoundly influences the niche partitioning in soil consumers, trophic interactions between microbes and microbivores and trophic structure of soil food webs.

Chapter 1

General introduction

1. Belowground C input and its importance for soil food webs

About 20% of belowground allocated carbon (C) is released by living roots into the soil as rhizodeposits (Kuzyakov and Domanski, 2000; Nguyen, 2003). This root-derived C, mainly comprising low molecular substances, such as sugars, amino acids, and organic acids, thus are preferentially utilized by soil microorganisms (Anderson et al., 1993; Buée et al., 2009). Root-derived C fluxes thereby regulate a wide range of soil ecological processes and accelerate the cycling of virtually all elements (Kuzyakov, 2002). In the last two decades, the importance of root-derived C for the nutrition of consumers at higher levels in soil food webs has been widely recognized (Pollierer et al., 2007; Högberg et al., 2010; Goncharov et al., 2016; Scheunemann et al., 2016). However, the flux of root-derived C varies between vegetation systems, due to differences in plant species, microbial community composition, and soil chemical and physical conditions (Sechi et al., 2014; Zieger et al., 2017). How this affects the incorporation of root-derived C into soil food webs, and the trophic interactions between microorganisms and microbivores, such as Collembola and nematodes, is not well understood.

2. The incorporation of root-derived C into soil food webs in different cropping systems

Incorporation of root C into the soil food web depends on the allocation of recently fixed C by plants to roots and rhizodeposits (Kuzyakov and Domanski, 2000; Pausch and Kuzyakov, 2018), and on the partitioning into energy channels based on different microbial groups (Ngosong et al., 2014). Crops allocate large amounts of fixed C to aboveground biomass since they typically are selected to maximize the growth of fruits and grains, leading to low flux of fixed C to belowground plant compartments (Pausch and Kuzyakov, 2018). In addition, the application of fertilizers results in low allocation of assimilates to belowground plant compartments due to decreasing plant investment in nutrient absorption (Phillips et al., 2011). By contrast, grass and trees are likely to allocate more fixed C below the ground compared to annual crops, as they invest more into the exploitation of nutrients from soil (Grayston et al., 1997; Pausch and Kuzyakov, 2018). The incorporation of root-derived C into microbial groups varies between cropping systems (Sechi et al., 2014; Zieger et al., 2017). For instance, trees and grasses allocate high amounts of C to roots and rhizodeposits, which are used by mycorrhizal and saprotrophic fungi (Denef et al., 2007; Högberg et al., 2010; De Deyn et al., 2011; Zieger et al., 2017). By contrast, bacteria are likely to be important in channeling root-derived C to higher trophic levels in systems with more intensive management, with their relative importance affected by crop species, soil fertility and management practices (Wardle et al., 2004; Elfstrand et al., 2008; Pausch et al., 2016a, b)

3. Collembola

Collembola are among the most widespread and abundant microarthropods in soils, contributing to C transport at the litter-soil interface (Chamberlain et al., 2006), and channeling C and nutrients from multiple resources to predators at higher trophic levels (Oelbermann et al., 2008; Liu et al., 2016). Collembola species with contrasting life forms are assigned to different functional groups: epedaphic (surface and upper litter layer dwelling), euedaphic (soil dwelling) and hemiedaphic (intermediate) Collembola (Faber, 1991; Potapov et al., 2016b), reflecting structural heterogeneity of soil food webs. Stable isotope studies suggest that Collembola are generally highly dependent on root-derived C (Garrett et al., 2001; Pollierer et al., 2012; Ferlian et al., 2015). However, Collembola species with contrasting life forms may differentially utilize root-derived resources and other C resources such as leaf litter and soil organic matter (Potapov et al., 2016a). As euedaphic Collembola inhabit mineral soil, they are assumed to be more closely associated with roots and to incorporate more root-derived C compared to epedaphic and hemiedaphic Collembola (Scheunemann et al., 2010; Potapov et al., 2016a). However, incorporation of root-derived C by different functional groups may differ between cropping systems due to trophic plasticity of Collembola species (Ruess et al., 2005; Endlweber et al., 2009; Scheunemann et al., 2015; Eerpina et al., 2017).

The incorporation of root-derived C into microbial groups varies between cropping systems, due to differences in plant species, and soil chemical and physical conditions (Sechi et al., 2014; Zieger et al., 2017). Hence, the channeling of root-derived C into Collembola and higher trophic levels of soil food webs likely also varies between cropping systems. As generalist feeders, Collembola incorporate root-derived C from different channels based on bacteria, fungi and plants (Crotty et al., 2011; Pollierer et al., 2012), and change diet according to resource availability (Endlweber et al., 2009; Scheunemann et al., 2015; Eerpina et al., 2017). However, factors determining the variation in dietary composition of Collembola between cropping systems and its association with differences in microbial resources, especially those actively utilizing root-derived C, are not well studied.

4. The influence of root energy supply and vertical heterogeneity on soil food webs in forest systems

In contrast to the arable soil, the soil in forests receives a considerable amount of aboveground inputs, promoting a strong vertical heterogeneity of available resources and environmental conditions, leading to pronounced compartmentalization of soil food webs (Okuzaki et al., 2009; Stouffer and Bascompte, 2011). Investigating the trophic niches of different functional groups of Collembola may provide new insights into the structural heterogeneity of soil food webs. Although forest soil food webs are fueled by multiple energy resources, root-derived C, serving as a high quality energy resource, is preferentially utilized by microorganisms and microbivores in soil food webs (Anderson et al., 1993; Pollierer et al., 2007; Buée et al., 2009), potentially exerting strong impacts on the trophic structure of soil food webs.

Root energy supply may affect the trophic niches of Collembola in two ways, i.e. by changing the trophic position and the utilization of basal resources. The ‘productivity hypothesis’ suggests that food chains should increase in length as productivity and the availability of resources increases (Pimm, 1982; Thompson and Townsend, 2005). This is due to the high inefficiency of energy transfer through food webs, leading to a substantial loss of energy during each trophic transfer, until a further trophic level cannot be supported. As root-derived C is an important energy supply, the shortage of root input may result in shorter food chain length, visible in a decrease of trophic position in omnivores by shifting diet towards feeding more on basal resources (Stenroth et al., 2008). However, the strong compartmentalization of food webs may mitigate the impacts of deprivation of root energy supply. Ectomycorrhizal fungi are the major sink for photosynthates in forest soils (Högberg and Högberg, 2002; Courty et al., 2010). Consumption of ectomycorrhizal fungi may contribute to the transfer of root-derived resources to the soil animal food web in forests. Hence, as root input declines, the substantial reduction in biomass of ectomycorrhizal fungi (Siira-Pietikäinen et al., 2001; Brant et al., 2006) may cause

Collembola to rely more on other resources such as saprotrophic fungi. However, recent studies suggest that saprotrophic microorganisms rather than mycorrhizal fungi are the dominant resources for soil microbivores (Malmström and Persson, 2011; Potapov and Tiunov, 2016; Bluhm et al., 2019).

5. Methods for tracing the incorporation of root-derived carbon into soil animal consumers

Stable isotopes refer to atoms with the same number of protons and different number of neutrons. There are two types of stable C isotopes ^{12}C and ^{13}C. The recently fixed C can be pulse labeled by exposing plant shoots to a $^{13}CO_2$ enriched atmosphere for a short period of time, e.g. six hours. As shown in previous studies (Goncharov et al., 2016; Scheunemann et al., 2016), microorganisms and microbivores such as Collembola incorporate this ^{13}C-enriched C shortly after labeling, presumably via feeding on roots or microorganisms actively utilizing rhizodeposits released by living roots. Hence, by measuring the ^{13}C signature of bulk tissue of Collembola, we can identify the magnitude and dynamics of root C flux to soil food webs and how it is affected by Collembola functional groups/species and cropping systems (chapter 2).

A limitation of bulk stable isotope analysis is that it does not allow to identify the C fluxes to Collembola from different basal resources including plants, bacteria and fungi. Hence, lipid analysis was employed in the second experiment. The principle of lipid analysis is based on a phenomenon termed ‘dietary routing’: to save energy, consumers incorporate fatty acids from the diet, such as microbial phospholipid fatty acids, into storage lipids, i.e. neutral lipid fatty acids, without major modification (Blem, 1976). As the ^{13}C signature of individual biomarkers in neutral lipid fatty acids of consumers is similar to that in lipids of food resources, the root-derived C fluxes from basal resources to consumers can be traced by measuring the ^{13}C signature of specific biomarkers in neutral lipid fatty acids of consumers. For instance, the incorporation of root C from the fungal channel can be traced by the ^{13}C values of the fungal biomarker 18:2ω6,9, while that from the bacterial channel is indicated by the ^{13}C values of bacterial biomarkers such as 16:1ω7, 18:1ω7 and a15:0 in neutral lipid fatty acids of consumers.

In the third experiment, we used compound-specific isotope analysis of C and N in amino acids to investigate the trophic niches of different functional groups of Collembola and its response to the deprivation of root energy supply. The ^{15}N values of source amino acids, e.g. phenylalanine, change only slightly (~0.4 ‰) with trophic transfer, while the ^{15}N values of trophic amino acids, e.g. glutamine, increase significantly (~8.0 ‰), resulting in a relatively constant trophic discrimination factor of 7.6 ± 1.2 ‰ during each trophic transfer (Chikaraishi et al., 2014). Therefore, the trophic position of a consumer can be calculated based on the difference in its ^{15}N values between phenylalanine and glutamine. Unique patterns of ^{13}C values of essential amino acids are generated by different major linkages of prokaryotes and eukaryotes, due to the differences in the isotopic fractionations caused by the individual biosynthetic pathways and associated branch points for each amino acid (Hayes, 2001). As animals generally cannot synthesize essential amino acids, these '^{13}C fingerprints' of essential amino acids are passed on to consumers and allow to trace their trophic links to different basal resources (Dadd, 1973; O'Brien et al., 2002; Nation, Sr., 2015). The ^{13}C fingerprinting method can provide additional information to lipid analysis. For instance, ectomycorrhizal and saprotrophic fungi share the same biomarkers in lipids, whereas they are distinct to each other in respect to amino acids ^{13}C fingerprinting (Pollierer et al. 2020 accepted).

6. Study sites

The first and second studies were conducted at sites located in Reiffenhausen, south of Göttingen in central Germany (51° 39′ 83″ N/9° 98′ 75″ E; 325 m a.s.l.). Average annual temperature is 9.1 °C, and the mean annual precipitation is 635 mm (Richter et al., 2015). The soil at the study sites is sedimentary deposits of Middle and Upper Triassic Sandstone material, partly mixed with claystone material and covered by loess sediments. The texture of soil varies from loamy sand in the eastern part to silty loam in the western part (Hartmann and Lamersdorf, 2015; Tariq et al., 2018). The experiment was established on former cropland in March 2011. The preceding crop grown on the experimental sites was winter barley (*Hordeum vulgare* L.).

Fields planted with three cropping systems including rape (oilseed rape, *Brassica napus* L.), grass (dominated by *Lolium perenne* L., mixed with clover *Trifolium repens* L.), and tree (willow, *Salix schwerinii* E.L. Wolf and *Salix viminalis* L.) were established (Ehret et al., 2015; Tariq et al., 2018). The willow and grass sites were established in March 2011. In September 2015, part of the grass sites were transformed into rape fields. The rape site represents cropland of high land-use intensity including fertilizer application, annual harvest, and crop rotation. The grass site represents low input grassland without fertilizer application but with three cuts per year (Ehret et al., 2015). The willow site was planted as low input short-term forest rotation system with a rotation cycle of three years without application of fertilizer. The willow trees were about 3 years old and 4 m high in our experiment. The willow site and grass site were arranged as an agroforest with rows of willow stripes and grassland strips in between (three willow stripes, each 7.5 m wide and 75 m long, and three grassland stripes, each 9 m wide and 75 m long). The rape site was next to one of the outer willow stripes (18 m wide and 75 m long).

The third experiment was established in two regions across Germany, i.e. the Hainich-Dün (Hainich) and the Schorfheide-Chorin (Schorfheide), and formed part of the 'Biodiversity Exploratories', a large project serving as open platform for biodiversity and ecosystem research (www.biodiversity-exploratories.de; Fischer et al., 2010). The Schorfheide is a young glacial landscape with an altitude of 3–140 m a.s.l., a mean annual temperature of 8.0–8.5 °C and a mean annual precipitation of 500–600 mm. Soils in the Schorfheide are mainly cambisols. The Hainich is dominated by calcareous bedrock and varies in altitude from 285 to 550 m a.s.l. Soils in the Hainich are mainly luvisols with few stagnosols. The mean annual temperature is 6.5–8.0 °C and the mean annual precipitation is 600–800 mm. For more details on the study sites see Fischer et al. (2010). In each region two forest types were selected. The forest types included managed coniferous forests and 70 years old managed beech forests. Coniferous forests consisted of spruce (*Picea abies*) in the Hainich, and pine (*Pinus sylvestris*) in the Schorfheide. Beech forests are dominated by *Fagus sylvatica*, mixed with ash (*Fraxinus excelsior*) and sycamore (*Acer pseudoplatanus*). Coniferous forests are more intensively managed than beech forests since they had been planted to replace naturally occurring beech forests. Each forest type was replicated four times per region, resulting in 16 forest plots. All plots were randomly arranged, the minimum distance between plots was 500 m.

In each of the 16 forest plots, one 'root-trenching' and one 'control' subplot of an area of 120 × 120 cm were established between September and October 2011. In the root-trenching treatment, roots were excluded by inserting polyethylene barriers (120 × 60 × 0.5 cm) into 40–50 cm deep trenches along the four sides of the subplots. In addition, aluminum linings were inserted at the edges to close the gap between adjacent barriers. Polyethylene barriers extended ca. 10 cm above the ground to prevent animal migration between the trenched plots and the surrounding. Aboveground barriers were also established in control subplots to control for potential side effects. At regular intervals during the growth period above-ground parts of herbaceous plants and grasses in trenched and control plots were removed to minimize input of root-derived resources. Soil moisture was measured gravimetrically from soil cores and water was added to control plots equalizing the amount of water in the upper 10 cm of the soil in control and trenched plots.

7. Objectives and chapter outline

The present studies address the question of the role of root-derived C for trophic interactions in soil food webs in arable and forest systems. The chapters 2 and 3 are studies conducted within the experimental framework of a pulse labeling experiment with $^{13}CO_2$ in three cropping systems. Chapter 2 investigated the dynamics and magnitude of the flux of root-derived C into Collembola species/functional groups in different cropping systems. Chapter 3 investigated the differentiation in root-derived C fluxes into bacterial and fungal channels among cropping systems and how it impacts the trophic associations between microorganisms and Collembola. Chapter 4 represents a study using a root-trenching experiment to shed light on the role of vertical heterogeneity and root energy supply for the structural heterogeneity of soil food webs in temperate forest systems.

We examined the following hypotheses:

(i) The incorporation of root C from grass and willow into Collembola is faster than that from rape, due to higher belowground C allocation in grasses and trees than in annual crops, and/or to application of fertilizer and lack of mycorrhizal symbiosis in rape (chapter 2).

(ii) Euedaphic Collembola incorporate more root-derived C than epedaphic and hemiedaphic Collembola due to closer association of euedaphic Collembola with plant roots (chapter 2).

(iii) The relative importance of energy channels for the incorporation of root-derived C into Collembola differs among cropping systems, with higher incorporation of root-derived C from the bacterial channel in rape than in grass and willow, where fungi are the primary root-derived C source (chapter 3).

(iv) The trophic niches differ between functional groups of Collembola in forest systems (chapter 4):

  a) Euedaphic Collembola rely more on ^{15}N-enriched organic substrate in deeper soil, while litter depleted in ^{15}N is the predominant N source for ep-/hemiedaphic Collembola

b) The trophic position of euedaphic Collembola is higher than that of ep-/hemiedaphic Collembola, reflecting a higher number of trophic transfers in soil than in the litter layer.

c) Saprotrophic microbes are the predominant basal resources for Collembola, whereas the contribution of mycorrhizal fungi and plants is subordinate.

(v) Deprivation of root-derived resources reduces the trophic position of Collembola, especially at higher trophic levels, as suggested by the ‘productivity hypothesis’ (chapter 4).

In the following, the content of the chapters is summarized

Chapter 2:

In a pulse labeling experiment with $^{13}CO_2$, we measured the incorporation of root-derived C into nine abundant species of Collembola over a period of 28 days in the field of rape, grass and willow. The dynamics of incorporation into Collembola markedly varied among cropping systems, with slower fluxes in rape than in grass and willow. This suggests that the efficiency of incorporation of root-derived C into soil food webs was lower in rape than in grass and willow, presumably due to differences in plant species, application of fertilizer and rhizosphere microbial community. In addition, the utilization of root-derived C and litter/old soil organic matter differed between functional groups/species of Collembola and this differentiation was dependent on cropping system. In contrast to previous assumptions, euedaphic Collembola incorporated less root-derived C but more soil organic C than ep-/hemiedaphic Collembola in rape and willow, whereas in grass the incorporation was similar among functional groups. Furthermore, differential incorporation of ^{13}C in euedaphic species points to strong niche differentiation within the same functional group. Our findings highlight that cropping system impacts the flux of root C into soil microarthropods. In addition, partitioning in the utilization of root-derived C and soil organic matter is an important mechanism promoting coexistence of Collembola species in soil at local scales.

Chapter 3:

A shortcoming of the study presented in chapter 2 is the inability to investigate the contribution of bacterial and fungal channels to the incorporation of root-derived C into Collembola. Therefore, we measured the incorporation of root-derived ^{13}C into bacterial and fungal biomarkers in microbial phospholipid fatty acids in bulk soil and in neutral lipid fatty acids in five Collembola species belonging to three functional groups: epedaphic, hemiedaphic and euedaphic. Collembola incorporated more root-derived C from the bacterial channel in rape than in grass and willow, where fungi were the dominant C source. This corresponded to the higher ^{13}C incorporation in bacterial biomarkers in microbial phospholipid fatty acids in rape as compared to grass and willow. The proportional abundance of bacterial and fungal biomarkers in neutral lipid fatty acids of Collembola also corresponded with the ^{13}C incorporation into microbial phospholipid fatty acids, while it did not correlate with the proportion of microbial phospholipid fatty acids in different cropping systems. This suggests that the differences in dietary composition of Collembola among cropping systems are mainly dependent on the changes in microbial community in the rhizosphere but not in bulk soil. Finally, hemiedaphic Collembola incorporated more root-derived C from bacterial resources than eu-/epedaphic Collembola. Hence, changes in root C flux into bacterial and fungal resources among cropping systems resulted in differential utilization of these resources by soil microbivores, suggesting that in particular microorganisms fueled by rhizodeposits are vital resources for the nutrition of higher trophic levels in soil food webs.

Chapter 4:

In contrast to the arable soil, the soil in forest systems receives a considerable amount of aboveground inputs, comprising mainly leaf litter. This results in a strong vertical heterogeneity of available resources and environmental conditions in forest soils. However, it remains unclear how vertical heterogeneity interacts with root-derived resources to influence the trophic structures of soil food webs. We used compound specific isotope analysis of carbon and nitrogen in amino acids to identify the trophic niches of different functional groups of Collembola in a root-trenching experiment. In line with a recent study, the ^{13}C fingerprinting suggests that saprotrophic microorganisms, especially saprotrophic fungi, are the predominant basal resources for all functional groups of Collembola, whereas the contribution of mycorrhizal fungi and plants is subordinate. The $\delta^{15}N$ values of phenylalanine were higher in euedaphic Collembola than in ep-/hemiedaphic Collembola, indicating that euedaphic Collembola mainly utilized N from processed organic substrate in deeper soil, while leaf litter was the dominant N source for ep-/hemiedaphic Collembola. In addition, the trophic position calculated from compound-specific isotope analysis of euedaphic Collembola was constantly higher than that of epedaphic Collembola, reflecting a higher number of trophic transfers in the soil than in the litter layer. Finally, the deprivation of root energy supply decreased the trophic position of soil omnivores, suggesting that, in line with the ‘productivity hypothesis’, root derived C increases the number of trophic transfers and food chain length in soil food webs. However, this varied among functional groups of Collembola and forest stands, suggesting that compartmentalization of food webs and other energy sources, i.e. litter and old soil organic matter, reduce the impact of shortage in root energy supply.

Reference

Anderson, T.A., Guthrie, E.A., Walton, B.T., 1993. Bioremediation in the rhizosphere. Environmental Science & Technology 27, 2630–2636. doi:10.1021/es00049a001

Blem, C.R., 1976. Patterns of lipid storage and utilization in birds. Integrative and Comparative Biology 16, 671–684. doi:10.1093/icb/16.4.671

Bluhm, S.L., Potapov, A.M., Shrubovych, J., Ammerschubert, S., Polle, A., Scheu, S., 2019. Protura are unique: First evidence of specialized feeding on ectomycorrhizal fungi in soil invertebrates. BMC Ecology 19, 1–7. doi:10.1186/s12898-019-0227-y

Brant, J.B., Myrold, D.D., Sulzman, E.W., 2006. Root controls on soil microbial community structure in forest soils. Oecologia 148, 650–659. doi:10.1007/s00442-006-0402-7

Buée, M., De Boer, W., Martin, F., van Overbeek, L., Jurkevitch, E., 2009. The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Plant and Soil 321, 189–212. doi:10.1007/s11104-009-9991-3

Chamberlain, P., Mcnamara, N., Chaplow, J., Stott, A., Black, H., 2006. Translocation of surface litter carbon into soil by Collembola. Soil Biology and Biochemistry 38, 2655–2664. doi:10.1016/j.soilbio.2006.03.021

Chikaraishi, Y., Steffan, S.A., Ogawa, N.O., Ishikawa, N.F., Sasaki, Y., Tsuchiya, M., Ohkouchi, N., 2014. High-resolution food webs based on nitrogen isotopic composition of amino acids. Ecology and Evolution 4, 2423–2449. doi:10.1002/ece3.1103

Courty, P.E., Buée, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.P., Uroz, S., Garbaye, J., 2010. The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. Soil Biology and Biochemistry 42, 679–698 . doi:10.1016/j.soilbio.2009.12.006

Crotty, F.V., Blackshaw, R.P., Murray, P.J., 2011. Tracking the flow of bacterially derived ^{13}C and ^{15}N through soil faunal feeding channels. Rapid Communications in Mass Spectrometry 25, 1503–1513. doi:10.1002/rcm.4945

Dadd, R.H., 1973. Insect nutrition: current developments and metabolic implications. Annual Review of Entomology 18, 381–420 . doi:10.1146/annurev.en.18.010173.002121

De Deyn, G.B., Quirk, H., Oakley, S., Ostle, N., Bardgett, R.D., 2011. Rapid transfer of photosynthetic carbon through the plant-soil system in differently managed species-rich grasslands. Biogeosciences 8, 1131–1139. doi:10.5194/bg-8-1131-2011

Denef, K., Bubenheim, H., Lenhart, K., Vermeulen, J., Van Cleemput, O., Boeckx, P., Müller, C., 2007. Community shifts and carbon translocation within metabolically-active rhizosphere microorganisms in grasslands under elevated CO_2. Biogeosciences 4, 769–779. doi:10.5194/bg-4-769-2007

Eerpina, R., Boiteau, G., Lynch, D.H., 2017. Collembola diet switching in the presence of maize roots varies with species. Canadian Journal of Soil Science 97, 171–177. doi:10.1139/cjss-2016-0057

Ehret, M., Bühle, L., Graß, R., Lamersdorf, N., Wachendorf, M., 2015. Bioenergy provision by an alley cropping system of grassland and shrub willow hybrids: biomass, fuel characteristics and net energy yields. Agroforestry Systems 89, 365–381. doi:10.1007/s10457-014-9773-7

Elfstrand, S., Lagerlöf, J., Hedlund, K., Mårtensson, A., 2008. Carbon routes from decomposing plant residues and living roots into soil food webs assessed with ^{13}C labelling. Soil Biology and Biochemistry 40, 2530–2539. doi:10.1016/j.soilbio.2008.06.013

Endlweber, K., Ruess, L., Scheu, S., 2009. Collembola switch diet in presence of plant roots thereby functioning as herbivores. Soil Biology and Biochemistry 41, 1151–1154. doi:10.1016/j.soilbio.2009.02.022

Faber, J.H., 1991. Functional classification of soil fauna: A new approach. Oikos 62, 110. doi:10.2307/3545458

Ferlian, O., Klarner, B., Langeneckert, A.E., Scheu, S., 2015. Trophic niche differentiation and utilisation of food resources in collembolans based on complementary analyses of fatty acids and stable isotopes. Soil Biology and Biochemistry 82, 28–35. doi:10.1016/J.SOILBIO.2014.12.012

Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., Korte, G., Nieschulze, J., Pfeiffer, S., Prati, D., Renner, S., Schöning, I., Schumacher, U., Wells, K., Buscot, F., Kalko, E.K.V., Linsenmair, K.E., Schulze, E.D., Weisser, W.W., 2010. Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic and Applied Ecology 11, 473–485. doi:10.1016/j.baae.2010.07.009

Garrett, C.J., Crossley, D.A., Coleman, D.C., Hendrix, P.F., Kisselle, K.W., Potter, R.L., 2001. Impact of the rhizosphere on soil microarthropods in agroecosystems on the Georgia piedmont. Applied Soil Ecology 16, 141–148. doi:10.1016/S0929-1393(00)00114-1

Goncharov, A.A., Tsurikov, S.M., Potapov, A.M., Tiunov, A.V., 2016. Short-term incorporation of freshly fixed plant carbon into the soil animal food web: field study in a spruce forest. Ecological Research 31, 923–933. doi:10.1007/s11284-016-1402-7

Grayston, S.J., Vaughan, D., Jones, D., 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5, 29–56. doi:10.1016/S0929-1393(96)00126-6

Hartmann, L., Lamersdorf, N., 2015. Site Conditions, Initial Growth and Nutrient and Litter Cycling of Newly Installed Short Rotation Coppice and Agroforestry Systems, in: Bioenergy from Dendromass for the Sustainable Development of Rural Areas. pp. 121–138. doi:10.1002/9783527682973.ch10

Hayes, J.M., 2001. Fractionation of carbon and hydrogen isotopes in biosynthetic processes. Reviews in Mineralogy and Geochemistry 43, 191–277. doi:10.2138/gsrmg.43.1.225

Högberg, M.N., Briones, M.J.I., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton, B., Hurry, V., Linder, S., Näsholm, T., Högberg, P., 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. New Phytologist 187, 485–493. doi:10.1111/j.1469-8137.2010.03274.x

Högberg, M.N., Högberg, P., 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. New Phytologist 154, 791–795. doi:10.1046/j.1469-8137.2002.00417.x

Kuzyakov, Y., 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science 165, 382–396. doi:10.1002/1522-2624(200208)165:4<382::AID-JPLN382>3.0.CO;2-#

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163, 421–431. doi:10.1002/1522-2624(200008)163:4<421::AID-JPLN421>3.0.CO;2-R

Liu, S., Behm, J.E., Chen, J., Fu, S., He, X., Hu, J., Schaefer, D., Gan, J., Yang, X., 2016. Functional redundancy dampens the trophic cascade effect of a web-building spider in a tropical forest floor. Soil Biology and Biochemistry 98, 22–29. doi:10.1016/J.SOILBIO.2016.03.020

Malmström, A., Persson, T., 2011. Responses of Collembola and Protura to tree girdling–some support for ectomycorrhizal feeding. Soil Org. 83, 279–285.

Nation, Sr., J.L., 2015. Insect Physiology and Biochemistry. CRC press. doi:10.1201/b18758

Ngosong, C., Gabriel, E., Ruess, L., 2014. Collembola grazing on arbuscular mycorrhiza fungi modulates nutrient allocation in plants. Pedobiologia 57, 171–179. doi:10.1016/j.pedobi.2014.03.002

Nguyen, C., 2003. Rhizodeposition of organic C by plants: Mechanisms and controls, in: Agronomie. pp. 375–396. doi:10.1051/agro:2003011

O’Brien, D.M., Fogel, M.L., Boggs, C.L., 2002. Renewable and nonrenewable resources: Amino acid turnover and allocation to reproduction in Lepidoptera. Proceedings of the National Academy of Sciences of the United States of America 99, 4413–4418. doi:10.1073/pnas.072346699

Oelbermann, K., Langel, R., Scheu, S., 2008. Utilization of prey from the decomposer system by generalist predators of grassland. Oecologia 155, 605–617. doi:10.1007/s00442-007-0927-4

Okuzaki, Y., Tayasu, I., Okuda, N., Sota, T., 2009. Vertical heterogeneity of a forest floor invertebrate food web as indicated by stable-isotope analysis. Ecological Research 24, 1351–1359. doi:10.1007/s11284-009-0619-0

Pausch, J., Hofmann, S., Scharroba, A., Kuzyakov, Y., Ruess, L., 2016a. Fluxes of root-derived carbon into the nematode micro-food web of an arable soil. Food Webs 9, 32–38.

doi:10.1016/J.FOOWEB.2016.05.001

Pausch, J., Kramer, S., Scharroba, A., Scheunemann, N., Butenschoen, O., Kandeler, E., Marhan, S., Riederer, M., Scheu, S., Kuzyakov, Y., Ruess, L., 2016b. Small but active - pool size does not matter for carbon incorporation in below-ground food webs. Functional Ecology 30, 479–489. doi:10.1111/1365-2435.12512

Pausch, J., Kuzyakov, Y., 2018. Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale. Global Change Biology 24, 1–12. doi:10.1111/gcb.13850

Phillips, R.P., Finzi, A.C., Bernhardt, E.S., 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecology Letters 14, 187–194. doi:10.1111/j.1461-0248.2010.01570.x

Pimm, S.L., 1982. Food webs. SPRINGER.

Pollierer, M.M., Dyckmans, J., Scheu, S., Haubert, D., 2012. Carbon flux through fungi and bacteria into the forest soil animal food web as indicated by compound-specific ^{13}C fatty acid analysis. Functional Ecology 26, 978–990. doi:10.1111/j.1365-2435.2012.02005.x

Pollierer, M.M., Langel, R., Körner, C., Maraun, M., Scheu, S., 2007. The underestimated importance of belowground carbon input for forest soil animal food webs. Ecology Letters 10, 729–736. doi:10.1111/j.1461-0248.2007.01064.x

Potapov, A.M., Goncharov, A.A., Tsurikov, S.M., Tully, T., Tiunov, A.V., 2016a. Assimilation of plant-derived freshly fixed carbon by soil collembolans: Not only via roots? Pedobiologia 59, 189–193. doi:10.1016/j.pedobi.2016.07.002

Potapov, A.M., Semenina, E.E., Korotkevich, A.Y., Kuznetsova, N.A., Tiunov, A.V., 2016b. Connecting taxonomy and ecology: Trophic niches of collembolans as related to taxonomic identity and life forms. Soil Biology and Biochemistry 101, 20–31. doi:10.1016/j.soilbio.2016.07.002

Potapov, A.M., Tiunov, A.V, 2016. Stable isotope composition of mycophagous collembolans versus mycotrophic plants: Do soil invertebrates feed on mycorrhizal fungi? Soil Biology and Biochemistry 93, 115–118. doi:10.1016/j.soilbio.2015.11.001

Richter, F., Döring, C., Jansen, M., Panferov, O., Spank, U., Bernhofer, C., 2015. How to predict hydrological effects of local land use change: How the vegetation parameterisation for short rotation coppices influences model results. Hydrology and Earth System Sciences 19, 3457–3474. doi:10.5194/hess-19-3457-2015

Ruess, L., Schütz, K., Haubert, D., Häggblom, M.M., Kandeler, E., Scheu, S., 2005. Application of lipid analysis to understand trophic interactions in soil. Ecology 86, 2075–2082. doi:10.1890/04-1399

Scheunemann, N., Digel, C., Scheu, S., Butenschoen, O., 2015. Roots rather than shoot residues drive soil arthropod communities of arable fields. Oecologia 179, 1135–1145. doi:10.1007/s00442-015-3415-2

Scheunemann, N., Pausch, J., Digel, C., Kramer, S., Scharroba, A., Kuzyakov, Y., Kandeler, E., Ruess, L., Butenschoen, O., Scheu, S., 2016. Incorporation of root C and fertilizer N into the food web of an arable field: Variations with functional group and energy channel. Food Webs 9, 39–45. doi:10.1016/J.FOOWEB.2016.02.006

Scheunemann, N., Scheu, S., Butenschoen, O., 2010. Incorporation of decade old soil carbon into the soil animal food web of an arable system. Applied Soil Ecology 46, 59–63. doi:10.1016/j.apsoil.2010.06.014

Sechi, V., D’Annibale, A., Ambus, P., Sárossy, Z., Krogh, P.H., Eriksen, J., Holmstrup, M., 2014. Collembola feeding habits and niche specialization in agricultural grasslands of different composition. Soil Biology and Biochemistry 74, 31–38. doi:10.1016/j.soilbio.2014.02.019

Siira-Pietikäinen, A., Pietikäinen, J., Fritze, H., Haimi, J., 2001. Short-term responses of soil decomposer communities to forest management: Clear felling versus alternative forest harvesting methods. Canadian Journal of Forest Research 31, 88–99. doi:10.1139/x00-148

Stenroth, P., Holmqvist, N., Nyström, P., Berglund, O., Larsson, P., Granéli, W., 2008. The influence of productivity and width of littoral zone on the trophic position of a large-bodied omnivore. Oecologia 156, 681–690. doi:10.1007/s00442-008-1019-9

Stouffer, D.B., Bascompte, J., 2011. Compartmentalization increases food-web persistence. Proceedings of the National Academy of Sciences of the United States of America 108, 3648–3652. doi:10.1073/pnas.1014353108

Tariq, A., Gunina, A., Lamersdorf, N., 2018. Initial changes in soil properties and carbon sequestration potential under monocultures and short-rotation alley coppices with poplar and willow after three years of plantation. Science of The Total Environment 634, 963–973. doi:10.1016/J.SCITOTENV.2018.03.391

Thompson, R.M., Townsend, C.R., 2005. Energy availability, spatial heterogeneity and ecosystem size predict food-web structure in streams. Oikos 108, 137–148. doi:10.1111/j.0030-1299.2005.11600.x

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van Der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633. doi:10.1126/science.1094875

Zieger, S.L., Ammerschubert, S., Polle, A., Scheu, S., 2017. Root-derived carbon and nitrogen from beech and ash trees differentially fuel soil animal food webs of deciduous forests. PLoS ONE 12. doi:10.1371/journal.pone.0189502

Chapter 2

Incorporation of root-derived carbon into soil microarthropods varies between cropping systems

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Abstract

As the dynamics and magnitude of rhizodeposition vary considerably among cropping systems, we investigated effects of cropping system on the incorporation of root-derived carbon (C) into Collembola, a dominant taxon of soil microarthropods. In the field, we used $^{13}CO_2$ to pulse label a crop monoculture (oilseed rape, *Brassica napus* L.), a mixed grass community (dominated by *Lolium perenne* L. mixed with clover *Trifolium repens* L.), and a tree plantation (willow, *Salix schwerinii* E.L. Wolf and *Salix viminalis* L.). During 28 days, the incorporation of ^{13}C was traced in nine species of Collembola including epedaphic (surface-dwelling), hemiedaphic (litter-dwelling), and euedaphic (soil-dwelling) functional groups. Incorporation of ^{13}C into Collembola reached a plateau before day 3 after the labeling in grass and willow, but increased up to day 14 in rape. While euedaphic Collembola incorporated less root-derived C than epedaphic and hemiedaphic Collembola in rape and willow, the incorporation of ^{13}C was similar among functional groups in grass. Differential incorporation of ^{13}C in euedaphic species points to niche differentiation within the same functional group. Our findings highlight that cropping system not only affects the flux of root C into soil mesofauna, being slower in rape than in grass and willow, but also the utilization of root-derived resources by functional groups and species of Collembola. The results indicate that pronounced differences in belowground C inputs between cropping systems affect microbivores as basal species and thereby soil food webs and their functioning and services.

Key-words: ^{13}C labeling, Collembola, functional group, grass, rape, willow

1. Introduction

Considerable amounts (10-20%) of C are released by plants into soil as rhizodeposition shortly after fixation (Kuzyakov and Domanski 2000; Dennis et al. 2010). This root-derived C comprises mainly low-molecular-weight compounds, such as glucose, organic acids, and amino acids, which are preferentially taken up by soil organisms (Dennis et al. 2010). Collembola play a major role in soil food webs, contributing to C transport at the litter-soil interface (Chamberlain et al. 2006), and channeling C and nutrients from multiple resources to predators at higher trophic levels (Oelbermann et al. 2008; Liu et al. 2016). Stable isotope studies showed that Collembola quickly incorporate recently fixed C via multiple pathways, including plants, bacteria, saprotrophic/mycorrhizal fungi, and other soil animals (Pollierer et al. 2012; Ferlian et al. 2015; Scheunemann et al. 2016). However, how cropping systems affect the incorporation of root-derived C into Collembola is little understood.

Incorporation of root C into the soil food web depends on the allocation of recently fixed C by plants to roots and rhizodeposits, and this varies with the strategies of plants to exploit nutrients from soil (Kuzyakov and Domanski 2000; Pausch and Kuzyakov 2018). Crops allocate large amounts of fixed C to aboveground biomass since they typically are selected to maximize the growth of fruits and grains, leading to low flux of fixed C to belowground plant compartments (Pausch and Kuzyakov 2018). The application of fertilizers also results in low allocation of assimilates to belowground plant compartments due to decreasing plant investment in nutrient absorption (Phillips et al. 2011). By contrast, grass and trees are likely to allocate more fixed C below the ground as compared to annual crops, as they invest more into the exploitation of nutrients from soil (Grayston et al. 1997; Pausch and Kuzyakov 2018). In addition, rhizosphere microorganisms differ between cropping systems; e.g. mycorrhizal fungi effectively distribute recently fixed plant C to the soil microbial community and may facilitate the uptake of root-derived C by microbivores.

Different functional groups of Collembola living in different soil depths potentially differ in the utilization of root-derived C (Potapov et al. 2016a). Due to closer association with roots, euedaphic Collembola inhabiting the mineral soil presumably incorporate more root-derived C than hemiedaphic and epedaphic Collembola, which preferentially colonize organic layers or the soil surface (Scheunemann et al. 2010; Potapov et al. 2016a). However, incorporation of root-derived C by different functional groups may differ between cropping systems due to trophic plasticity of Collembola species (Ruess et al. 2005; Endlweber et al. 2009; Scheunemann et al. 2015; Eerpina et al. 2017).

To explore variations in the incorporation of plant-derived C into Collembola we pulse labeled three cropping systems with $^{13}CO_2$ and traced the incorporation of root C into nine Collembola species of different functional groups. The cropping systems included a herbaceous crop monoculture (oilseed rape, *Brassica napus* L.), a mixed-grass community (grassland dominated by *Lolium perenne* L. mixed with clover *Trifolium repens* L.), and a tree plantation (willow, *Salix schwerinii* E.L. Wolf and *Salix viminalis* L.). We tested the following hypotheses: (i) The incorporation of root C from grass and willow into Collembola is faster than that from rape, due to higher belowground C allocation in grasses and trees than in annual crops, and/or to application of fertilizer and lack of mycorrhizal symbiosis in rape. (ii) Euedaphic Collembola incorporate more root-derived C than epedaphic and hemiedaphic Collembola due to closer association of euedaphic Collembola with plant roots. (iii) Incorporation of root-derived C differs between species within functional groups and the differences will vary among cropping systems.

2. Materials and methods

2.1 Site description

The study sites were located in Reiffenhausen, south of Göttingen in central Germany (51°39′83”N/9°98′75″E; 325m a.s.l.). Average annual temperature is 9.1 °C and the mean annual precipitation is 635 mm (Richter et al. 2015). The soil at the study sites is sedimentary deposits of Middle and Upper Triassic Sandstone material, partly mixed with claystone material and covered by loess sediments. The texture of soil varies from loamy sand in the eastern part to silty loam in the western part (Hartmann and Lamersdorf 2015; Tariq et al. 2018). The experiment was established on former cropland in March 2011. The preceding crop grown on the experimental sites was winter barley (*Hordeum vulgare* L.).

2.2 Experimental design

Fields planted with three cropping systems comprising rape (oilseed rape, *Brassica napus* L.), grass (dominated by *Lolium perenne* L., mixed with clover *Trifolium repens* L.), and tree (willow, *Salix schwerinii* E.L. Wolf and *Salix viminalis* L.) were established (Ehret et al. 2015; Tariq et al. 2018). The willow and grass sites were established in March 2011. In September 2015, part of the grass sites were transformed into rape fields. The rape site represents cropland of high land-use intensity including fertilizer application, annual harvest, and crop rotation. The grass site represents low input grassland without fertilizer application but with three cuts per year (Ehret et al. 2015). The willow site was planted as low input short-term forest rotation system with a rotation cycle of three years without application of fertilizer. The willow trees were about 3 years old and 4 m heigh in our experiment. The willow site and grass site were arranged as an agroforest with rows of willow stripes and grassland strips in between (three willow stripes, each 7.5 m wide and 75 m long, and three grassland stripes, each 9 m wide and 75 m long). The rape site was next to one of the outer willow stripes (18 m wide and 75 m long).

In each of cropping systems, five labeling chambers were installed. In the rape field, the chambers were randomly positioned at a distance of 5 to 10 m from each other in May 2017. For labeling willow, one stripe adjacent to the rape field was selected and the chambers were spaced at least by 10 m and installed in July 2017. In the grass stripes, grass plots were established as blocks differing in e.g., application of fertilizer (Ehret et al. 2015). Since the size of individual blocks (9.0 × 6.5 m) was too small to install five chambers, we installed the chambers in two blocks with the same treatment (no fertilizer addition) in August 2017; two chambers in block one which was next to the selected willow stripe, and three chambers in block two spaced by 50 m to block one. The chambers consisted of stainless steel frames of 1 × 1 m which were inserted into the soil to a depth of 10 cm, and of a plastic frame on top (height 1 m for rape and grass, 2 m for willow) that was covered with translucent LDPE (low-density polyethylene) foil. Plants were labeled by the addition of HCl to 20 g $Na_2{}^{13}CO_3$ in a plastic beaker. In willow, upper branches were bent to fit the height of the chamber. In addition, understory plants were removed and the soil surface was covered with black plastic foil before labeling to exclude ^{13}C incorporation by herbaceous plants and algae. The produced $^{13}CO_2$ was circulated in the chambers by a fan for 6 h. After labeling, the foil and plastic frame were removed, while the steel frame remained in the soil to prevent migration of animals between labeled and unlabeled areas (Scheunemann et al. 2016). The fact that ^{13}C enrichment did not decrease in most Collembola species throughout the experiment indicated that this procedure was effective.

Sites of different cropping systems were close to each other and were established on one former field. Therefore, our study sites may not be perfectly independent of each other. However, establishing the study sites on similar background provided a number of advantages for testing our hypothesis. First, small spatial distances between sites allowed us to sample the same Collembola species across all study sites, a crucial precondition to study variations in nutrition of Collembola species between cropping systems. In addition, it restricted effects of confounding variables, such as differences in soil type, on the flux of root C into soil food webs. Finally, the distance between labeling chambers was at least 5 m to minimize spatial dependence.

2.3 Sampling

Soil samples were taken 3, 7, 14, and 28 days after labeling to analyze the dynamics of ^{13}C incorporation into Collembola. Additionally, unlabeled samples were taken at adjacent plots and served as control for analyzing ^{13}C and ^{15}N natural abundance in each cropping system. In each chamber, one soil sample was taken at each sampling date using a stainless steel soil corer (diameter 20 cm, depth 10 cm). Soil arthropods were extracted by heat (Kempson et al. 1963) and stored in 70% ethanol at -20 °C. Collembola were identified to species level using a Zeiss microscope (Hopkin 2007). The nine abundant Collembola species across all sites were selected for stable isotope analysis: epedaphic species including *Isotoma viridis* (Bourlet), *Lepidocyrtus cyaneus* (Tullberg), *Lepidocyrtus paradoxus* (Uzel), *Orchesella villosa* (Geoffroy); hemiedaphic species including *Folsomia quadrioculata* (Tullberg), *Parisotoma notabilis* (Schäffer), *Pseudosinella alba* (Packard); euedaphic species including *Protaphorura armata* (Tullberg), *Stenaphorura denisi* (Bagnall). The classification of functional groups was based on Potapov et al. (2016b).

2.4 Stable isotope analysis

Approximately 50 µg of dry weight of each Collembola species (from 1 to 30 individuals) were weighed into tin capsules, which were analyzed for $^{13}C/^{12}C$ with a coupled system of an elemental analyzer (NA1110, CE -Instruments, Rodano, Milano, Italy) and an isotope ratio mass spectrometer (Delta Plus, Finnigan MAT, Bremen, Germany) located at the Centre for Stable Isotope Research and Analysis, Göttingen, Germany. V-PDB was used as a standard for ^{13}C. Acetanilide was used for internal calibration. Isotope natural abundance was expressed using the delta notation with $\delta^{13}C = [(R_{sample} - R_{standard}) / R_{standard}] \times 1000$. R_{sample} and $R_{standard}$ refer to the $^{13}C/^{12}C$ in samples and standard, respectively.

Incorporation of ^{13}C into Collembola was calculated for each species as shift in the isotope signature between labeled and control sample as ^{13}C enrichment $\Delta^{13}C$ [‰] = $\delta^{13}C_{\text{labeled sample}} - \delta^{13}C_{\text{unlabeled sample}}$. Data on Δ^{13}C of shoot/leave and root (visible fine roots shorted by hand), soil dissolved organic C, and soil PLFAs were provided by Lingling Shi (unpubl. data).

2.5 Statistical analysis

Δ^{13}C values of Collembola were compared between sampling days, Collembola species, and functional groups using linear mixed effects models for each cropping system. In the first model, species and sampling days were set as fixed effects. As different spatial scales investigated were nested in a hierarchical structure (‘soil core’ nested within ‘chamber’, ‘chamber’ nested within ‘cropping system’), stable isotope data of Collembola from the same soil core and from the same chamber were not independent. We accounted for this by setting ‘chamber’ and ‘soil core’ nested within ‘chamber’ as random effects. In the second model, Δ^{13}C values of Collembola were compared between functional groups of Collembola and days with ‘species’, ‘chamber’, and ‘soil core’ nested within ‘chamber’ as random effects. In the third model, we averaged Δ^{13}C values from different sampling days for each chamber and species, and used the natural abundance δ^{13}C values as fixed effects; random effects were ‘chamber’ and ‘species’. Since chambers in grass were distributed in two blocks, we also used ‘block’ as random effect in the model of grass. Because the epedaphic species *L. paradoxus* had considerably lower Δ^{13}C values in rape and distinct dynamics compared to the other three epedaphic species, it was excluded in the second and third models. Similar to the third model, we also analyzed the relationship between average Δ^{13}C values of Collembola species and their body length and abundance in rape, grass, and willow.

For each model, heteroscedasticity was inspected by plotting the residuals against the predicted values and normality was checked by Quantile-Quantile plots. Δ^{13}C values were transformed (logarithmic or Box-cox transformations) if homogeneity of variance and normality were not satisfied. All statistical analyses were conducted using R 3.4.2 (R Core team, 2016), packages *lme4* (Bates et al. 2015), and *lmerTest* (Kuznetsova et al. 2017). Plots were drawn in R using the package *ggplot2* (Wickham 2016).

3. Results

3.1 *Incorporation of ^{13}C into Collembola in different cropping systems*

Three days after labeling, the ^{13}C enrichment of shoots of rape, grass, and willow was 399.6 ± 91.7, 826.6 ± 209.6, and 355.1 ± 176.5 ‰, respectively (Table S1). High ^{13}C concentration in plant tissue early after labeling allowed us to reliably trace the incorporation of recently fixed C into Collembola.

The incorporation of ^{13}C into Collembola differed markedly between sampling days in rape, but stayed relatively constant in grass and willow (Table 1). Except for *L. paradoxus* and *S. denisi*, ^{13}C incorporation into Collembola in rape increased gradually after labeling, reached a plateau at day 7 or 14 and then stayed constant until day 28 (Fig. 1). In grass, ^{13}C incorporation was already high at day three and stayed relatively constant in most species except for *L. paradoxus* and *O. villosa*, which reached their peaks at day 7 and 14, respectively (Fig. 2). In willow, ^{13}C incorporation (overall mean enrichment 13.6 ± 19.9 ‰) was generally lower than in grass (187.3 ± 216 ‰) and rape (80.99 ± 74.8 ‰), and stayed more constant than in rape after labeling. Incorporation of ^{13}C in willow was lowest in *S. denisi* and *P. armata* (Fig. 3).

Table 1. Linear mixed effects model of type III error for the effect of species, sampling day, and their interaction on the incorporation of ^{13}C into Collembola in different cropping systems (rape, grass and willow).

Cropping system	Factor	Sum of Square	Mean of Square	df1	df2	F-value	P-value
rape	species	22.09	2.76	8	97.22	**66.68**	**<0.001**
	day	9.83	3.28	3	13.07	**79.15**	**<0.001**
	species × day	4.54	0.19	24	96.97	**4.57**	**<0.001**
grass	species	9.47	1.18	8	79.81	**23.70**	**<0.001**
	day	0.05	0.05	3	14.75	0.33	0.804
	species × day	3.83	0.16	24	80.25	**3.19**	**<0.001**
willow	species	7.02	0.88	8	73.85	**18.33**	**<0.001**
	day	0.47	0.16	3	15.13	3.25	0.052
	species × day	1.19	0.05	24	74.93	1.03	0.439

Significant effects are in bold; df1, numerator degree of freedom, df2, denominator degree of freedom.

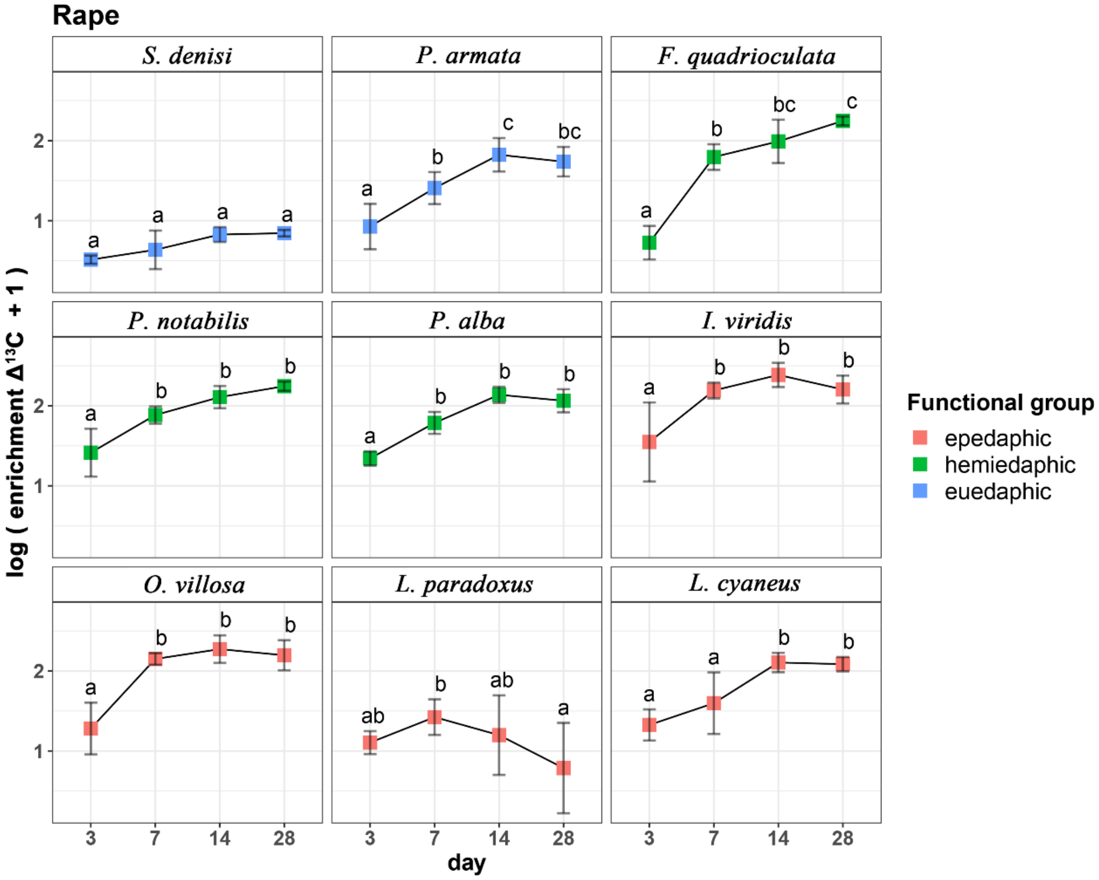

Fig. 1 Incorporation of ^{13}C into Collembola species (*Stenaphorura denisi*, *Protaphorura armata*, *Folsomia quadrioculata*, *Parisotoma notabilis*, *Pseudosinella alba*, *Isotoma viridis*, *Orchesella villosa*, *Lepidocyrtus paradoxus*, *Lepidocyrtus cyaneus*) 3, 7, 14 and 28 days after labeling in rape; means ± SD. Values of different sampling dates within a species with the same letters are not significantly different ($p < 0.05$; Tukey's HSD test).

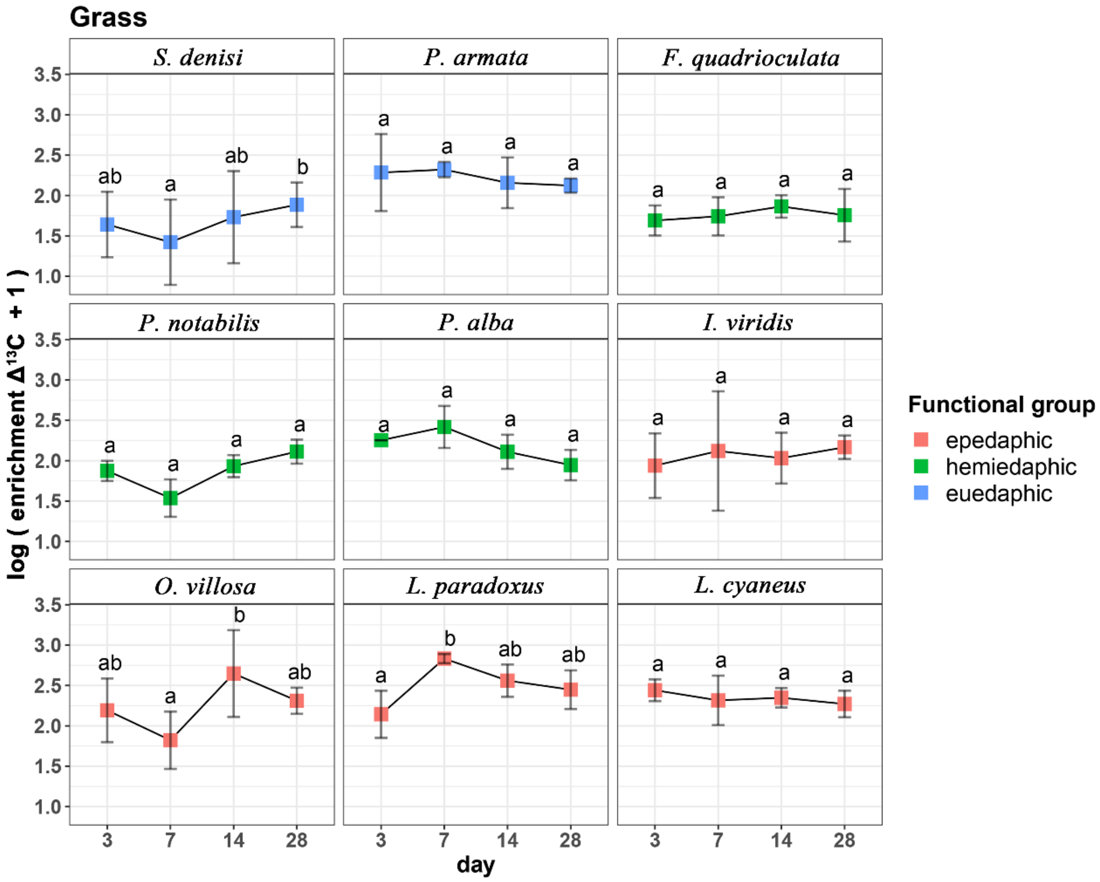

Fig. 2 Incorporation of ^{13}C into Collembola species 3, 7, 14 and 28 days after labeling in grass; means ± SD. Values of different sampling dates within a species with the same letters are not significantly different ($p < 0.05$; Tukey's HSD test). For abbreviations see Fig.1.

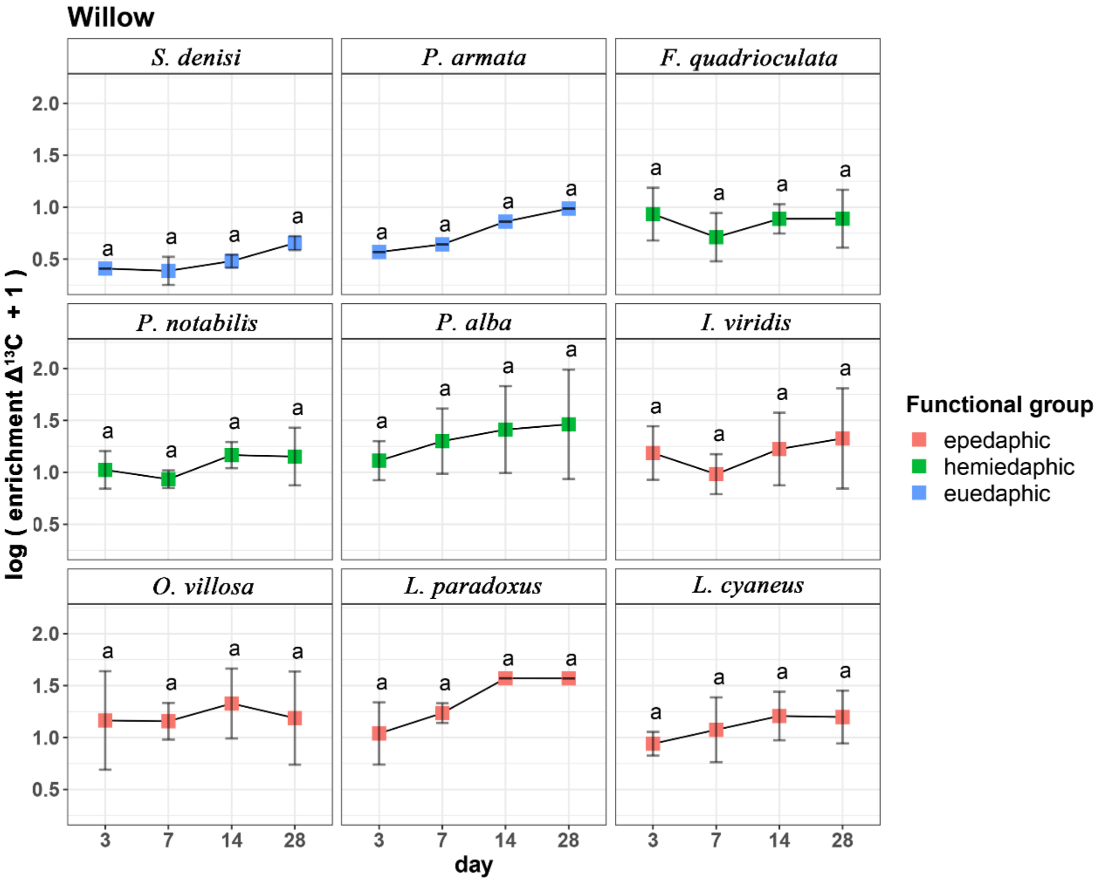

Fig. 3 Incorporation of ^{13}C into Collembola species 3, 7, 14 and 28 days after labeling in willow; means ± SD. Values of different sampling dates within a species with the same letters are not significantly different ($p < 0.05$; Tukey's HSD test). For abbreviations see Fig.1.

3.2 Incorporation of ^{13}C into Collembola functional groups

Incorporation of ^{13}C differed significantly between Collembola functional groups in willow and rape, with epedaphic and hemiedaphic Collembola being more enriched than euedaphic Collembola (Table 2, Fig. 4). In rape, differences between functional groups depended on sampling day, being more pronounced after day 7. By contrast, incorporation of ^{13}C did not differ significantly between functional groups in grass (Table 2, Fig. 4).

Table 2. Linear mixed effects model table of type III error for the effect of functional group, sampling day, and their interaction on the incorporation of ^{13}C into Collembola in different cropping systems (rape, grass and willow).

Cropping system	**Factor**	**Sum of Square**	**Mean of**	**df1**	**df2**	**F-value**	**P-value**

			Square				
rape	fg	0.64	0.32	2	5.01	**6.60**	**0.039**
	day	13.58	4.53	3	14.05	**94.06**	**<0.001**
	fg × day	0.68	0.11	6	105.42	**2.34**	**0.036**
grass	fg	0.28	0.14	2	5.85	1.87	0.235
	day	0.13	0.04	3	15.79	0.59	0.634
	fg × day	0.42	0.07	6	98.76	0.93	0.481
willow	fg	1.38	0.66	2	5.90	**6.62**	**0.031**
	day	1.21	0.40	3	19.58	**4.01**	**0.022**
	fg × day	0.77	0.13	6	96.91	1.27	0.276

Significant effects are in bold; df1, numerator degree of freedom, df2, denominator degree of freedom; fg, functional group.

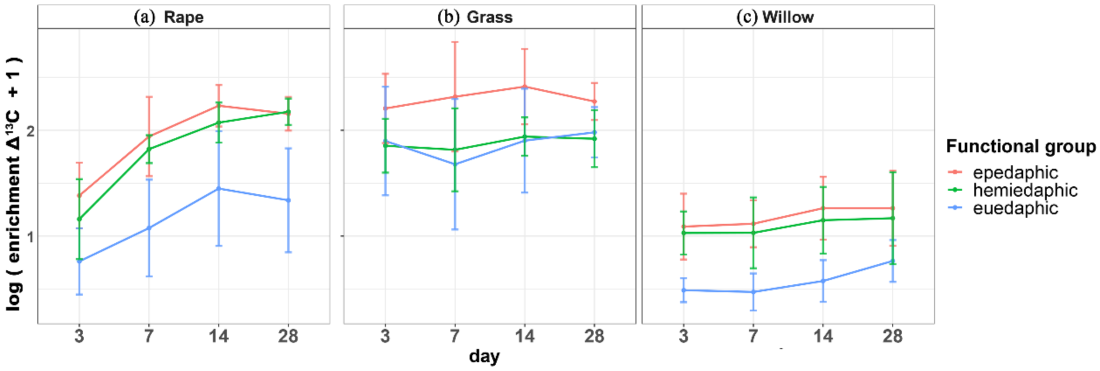

Fig. 4 Incorporation of ^{13}C into Collembola functional groups 3, 7, 14 and 28 days after labeling in fields planted with (a) rape, (b) grass and (c) willow; means ± SD.

3.3 Species-specific differences in the incorporation of ^{13}C

The random effects of species in the model of functional groups were significant in all cropping systems ($p < 0.001$); i.e. the incorporation of ^{13}C into Collembola significantly differed between species within the same functional group (Table 1). In the euedaphic group, Δ^{13}C values in *P. armata* were significantly higher than in *S. denisi* in each of the cropping systems (Fig. 5). Species-specific differences in the other functional groups depended on cropping system. For instance, *P. alba* was more enriched than the other hemiedaphic species in grass and willow, but not in rape. Δ^{13}C values in *L. paradoxus* were lower than in the other epedaphic species in rape, but similar in grass and willow.

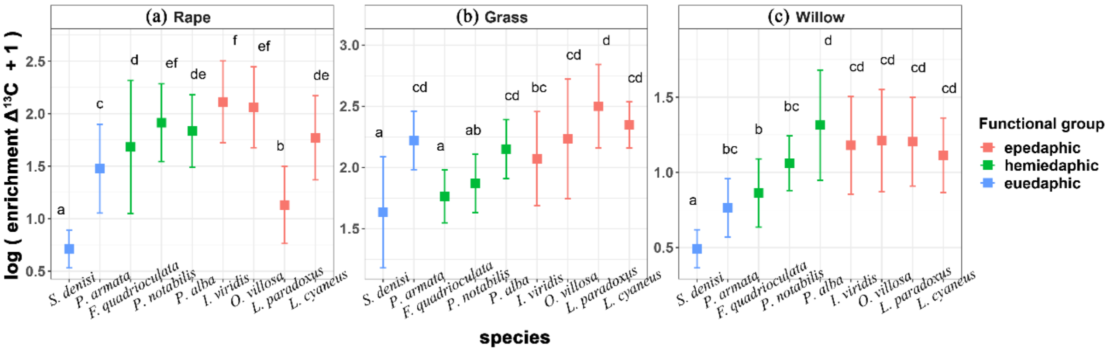

Fig. 5 Incorporation of ^{13}C into Collembola species in fields planted with (a) rape, (b) grass and (c) willow; means ± SD. All sampling dates are bulked together. Values of different species within a cropping system with the same letters are not significantly different ($p < 0.05$; Tukey's HSD test). For abbreviations see Fig.1.

Δ^{13}C values of Collembola species negatively correlated with their natural abundance values of ^{13}C (δ^{13}C) in rape, grass, and willow, however, the correlation was not significant in the latter (Table S2, Fig. 6). In none of the cropping systems, Δ^{13}C values of Collembola species were significantly correlated with their abundance nor their body length (data not shown).

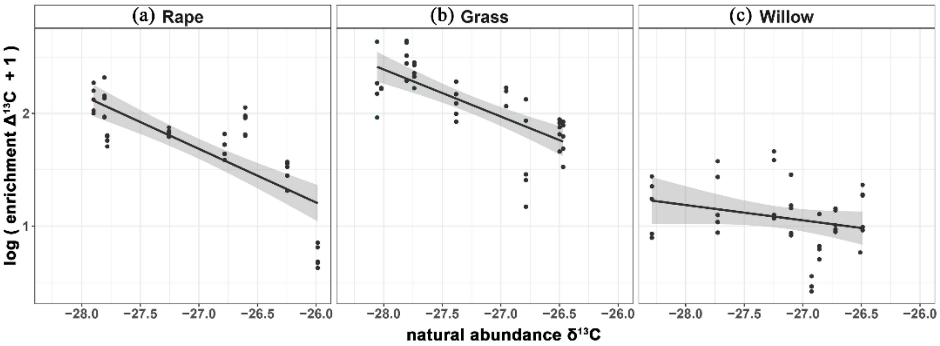

Fig. 6 Correlation between the incorporation of ^{13}C (Δ^{13}C) and the natural abundance of ^{13}C (δ^{13}C) of the same Collembola species in fields planted with (a) rape, (b) grass and (c) willow; data are means per species and chamber.

4. Discussion

4.1 *Influence of cropping system on the utilization of root-derived carbon*

Collembola rapidly incorporated the labeled C in each of the cropping systems (rape, grass, and willow), indicating that soil food webs under these cropping systems are fueled by recently fixed C. This is in line with previous studies showing that soil invertebrates incorporated recently fixed C in arable systems, grasslands, temperate, and boreal forests, most likely via rhizodeposits and associated microorganisms (Pollierer et al. 2007; Högberg et al. 2010; Seeber et al. 2012; Eissfeller et al. 2013; Goncharov et al. 2016; Scheunemann et al. 2016). Our study for the first time showed that incorporation dynamics of recently fixed C and its distribution among different functional groups and species of Collembola are markedly different among cropping systems.

Supporting our first hypothesis, the incorporation of ^{13}C into Collembola reached a peak on days 0-3 in grass and willow but at day 7 or 14 in rape (Fig. 1-3), indicating slower root C flux into Collembola in rape than in grass and willow. In other labeling experiments, incorporation of ^{13}C into Collembola also increased with time in cropland (Pausch et al. 2016; Scheunemann et al. 2016), while staying relatively constant or without clear trend in grassland (Ostle et al. 2007) and forest (Högberg et al. 2010; Fujii et al. 2016). This may reflect the lower C allocation to roots in rape compared to grass and willow (Kuzyakov and Domanski 2000; Pausch and Kuzyakov 2018), presumably because crop species are selected to maximize the growth of harvested plant compartments (Pausch and Kuzyakov 2018). By contrast, perennial grasses translocate more C belowground (30-50%) than crops (20-30%) since they rely on belowground C reserves for regrowth in spring and after grazing or mowing (Pausch and Kuzyakov 2018). Application of fertilizer likely further decreased belowground C allocation in rape. Typically, fertilization with N reduces the allocation of C to roots and the root-to-shoot biomass ratio (Hermans et al. 2006), which directly or indirectly results in lower C supply to soil microorganisms and animals (Ai et al. 2015, Denef et al. 2009, Högberg et al. 2010). Supporting this assumption, the ^{13}C enrichment of dissolved organic C in soil increased gradually and peaked at day 14 in rape, while it peaked at day 3 in grass and willow (Table S3), reflecting lower exudation and/or lower microbial assimilation efficiency of root exudates in rape than in grass and willow. Thus, the transfer efficiency of root-derived C to microorganisms and to microbivorous arthropods presumably was lower in rape than in grass and willow.

Association between plants and soil microorganisms differs between cropping systems and this is likely to be another important factor affecting the flux of root C into rhizosphere animal consumers such as Collembola. Grasses and willows form symbiotic associations with mycorrhizal fungi (Sumorok and Kiedrzynska 2007; Baum et al. 2009; Nicolson and Johnston 2009) and this might be associated with higher root exudation compared to non-mycorrhizal plants such as oilseed rape (Okubo et al. 2016). High allocation of plant C to mycorrhizal fungi and root exudates assimilated by saprotrophic rhizosphere microorganisms in grass and willow (Denef et al. 2007; Kušliene et al. 2014) is likely to result in fast incorporation of root C into rhizosphere consumers. In addition, mycorrhizal and saprotrophic fungal mycelia efficiently transfer root C from the rhizosphere to root-free bulk soil (Butler et al. 2003; Kušliene et al. 2014) facilitating the capture of root C by Collembola. By contrast, incorporation of root C into animal consumers may be hampered by the absence of mycorrhizal associations in rape (Kühn et al. 2019). This likely results in a more uneven distribution of root C in bulk soil, thus reducing the incorporation of root-derived C into Collembola.

The ^{13}C incorporation into Collembola species was remarkably lower in willow than in grass and rape. Potentially, low enrichment in willow was due to high amounts of unlabeled C in the phloem that diluted the signal of ^{13}C, leading to lower Δ^{13}C values in Collembola even when incorporating similar amounts of root C as in grass and rape. Supporting this assumption, the ^{13}C enrichment of roots (Table S1) and of the biomarker PLFA 18:2ω6,9 in soil were also lower in willow than in grass and rape (Lingling Shi, unpubl. data). Although the PLFA 18:2ω6,9 originates from both roots and fungi, its close correlation with other fungal biomarkers (Högberg 2006, Pausch et al. 2016) and the fact that only sieved soil was analyzed in the present study suggest that it predominantly reflects fungal tissue. Besides dilution effects, ^{13}C loss via respiration of aboveground parts or low belowground allocation of recent photosynthate C may also have contributed to the low ^{13}C incorporation into Collembola in willow.

Unlike the sites of grass and willow, which were established directly from crop fields, the rape site was established from part of the grass site. Differences in preceding vegetation may have influenced the incorporation of root-derived C into Collembola due to differentially affecting soil properties and microbial communities. However, legacy effects of the preceding vegetation presumably were minor compared to effects of the present plant types/species and soil management. For instance, Detheridge et al. (2016) showed that the fungal community of cereal fields with different preceding crop species converged already one year after establishment. Similarly, Crotty et al. (2016) found that the legacy effect of preceding crop species on the diversity and abundance of soil fauna diminished in the second cropping season during crop rotation, and the abundance of Collembola did not vary any more with the previous crop species.

In the present study, we could not capture variation in belowground C allocation as influenced by growth period. As our study was conducted during the late growth period of oilseed rape, the rapid growth of flowers and seeds likely diminished the flux of C to roots and into the soil food web, whereas belowground C allocation and incorporation presumably is higher in rape at early growth stages (Pausch and Kuzyakov 2018). In grasses, the variation in C allocation with growth stage is relatively minor as compared to annual crops (Pausch and Kuzyakov 2018), whereas the belowground C input from trees potentially is higher in the late than in the early growth period (Mordacq et al. 1986, Kagawa et al. 2006, Högberg et al. 2010). As patterns of ^{13}C incorporation into Collembola potentially differ between growth periods, in particular in rape and willow, further research on seasonal variation in belowground C inputs in different cropping systems is needed.

4.2 Incorporation of root-derived carbon into Collembola functional groups

Functional groups of Collembola reflect the habitat they live in, such as litter, humus, and mineral soil (Faber 1991; Ponge 2000; Rusek 2007), which allows to infer distribution of root-derived C in soil food webs at different soil depths (Potapov et al. 2016b). In contrast to our second hypothesis, hemiedaphic and epedaphic Collembola incorporated more ^{13}C than euedaphic Collembola in rape and willow (Fig. 4), indicating that soil-dwelling species have limited access to recently fixed C in these cropping systems. This contradicts previous findings in forests where hemiedaphic Collembola were not strongly labeled (Potapov et al. 2016a; Fujii et al. 2016), possibly because they relied on litter as major C source in these systems. By contrast, our studies were conducted in arable soil with relatively few litter resources; therefore, hemiedaphic Collembola may shift from litter-derived to root-derived resources. Similar to the results of Scheunemann et al. (2015), the hemiedaphic species *P. alba* incorporated more root-derived C than the euedaphic species *P. armata*.

The relatively low incorporation of root-derived resources into euedaphic species in rape and willow suggests that they more heavily rely on old C sources, i.e. soil organic matter and litter, than hemi- and epedaphic species in these cropping systems. Supporting this conclusion, Ponge (2000) found certain euedaphic species to preferentially consume humus rather than root tissue or fungal hyphae. Further, old soil C accounted for more than 50% of the body C of Collembola in maize fields even after prolonged growth of maize (Albers et al. 2006; Scheunemann et al. 2010). This is supported by the relatively high natural $\delta^{15}N$ and $\delta^{13}C$ values of euedaphic Collembola in rape and willow (Table S4), which correspond to the enriched $\delta^{15}N$ and $\delta^{13}C$ values of soil organic matter relative to plant material (Hyodo et al. 2010; Potapov et al. 2019). However, high $\delta^{15}N$ values in Collembola may also reflect trophic fractionation due to occupying higher trophic positions within the food web (Chahartaghi et al. 2005).

While the incorporation of root-derived C differed between functional groups in rape and willow, there was no significant difference between functional groups in grass (Fig, 4). This was mainly due to higher incorporation of root-derived C into euedaphic species in grassland as compared to rape and willow. Roots of grass may be more palatable than those of rape and willow. This may especially be true for *P. armata*, since its natural abundance $\delta^{13}C$ values decreased from comparatively high values in rape and willow to low values in grass (Table S4), indicating a more direct trophic relationship to grass roots, presumably via feeding on roots, arbuscular mycorrhizal fungi or root-feeding nematodes. Previous studies also found euedaphic Collembola species to shift their food preference from root C to soil organic C depending on resource availability (Gillet and Ponge 2003; Endlweber et al. 2009; Eerpina et al. 2017). In addition, high belowground C allocation in grass may promote its utilization by different functional groups of Collembola as well as their microbial prey.

Previous studies suggest that epedaphic species potentially have a high preference for aboveground resources such as microalgae, lichens, and organic matter deposited by plants, including pollen and honeydew from aphids (Scheunemann et al. 2010; Potapov et al. 2016a). However, the temporal variation in $\Delta^{13}C$ values of epedaphic and hemiedaphic species in individual cropping systems was synchronous with that of euedaphic species; since euedaphic species are unlikely to be linked to aboveground resources, the main source of their recently fixed C is likely to be root-derived. Although photoautotrophic microorganisms might be an important resource for Collembola in upper litter layers or extreme habitats such as rock surface (Potapov et al. 2018), their importance in presence of plant roots remains unknown. Further research separating different channels of recently fixed C to epedaphic Collembola is required.

4.3 Species-specific differences in the incorporation of root-derived carbon

Functional group could only partially explain the variation in the incorporation of ^{13}C into Collembola species; species within the same functional group also differed in the incorporation of ^{13}C

supporting our third hypothesis. Notably, the variation was most pronounced in euedaphic species, with *P. armata* incorporating considerably more root-derived C than *S. denisi* (Fig. 5). In contrast to variations within epedaphic and hemiedaphic groups, this difference persisted in each of the studied cropping systems, indicating remarkable niche differentiation between euedaphic species.

Strong niche differentiation in euedaphic Collembola has also been observed by Potapov et al. (2016a), who found higher dependence on root-derived resources in *P. armata* than in *Isotomiella minor*, and by Fujii et al. (2016), who found incorporation of root-derived C into *Onychiurus flavescens*, but not into *I. minor*. While *Protaphorura* and *Onychiurus* belong to the family Onychiuridae, *Isotomiella* and *Stenaphorura* belong to families Isotomidae and Tullbergiidae, respectively, characterized by more slender bodies allowing to access smaller soil pores. Thus, the niche-differentiation in euedaphic Collembola may be explained partly by morphological differences within functional groups (Potapov et al. 2016b).

More pronounced niche-differentiation in euedaphic Collembola potentially points to higher demand for resource partitioning in the mineral soil. The relatively stable environment in mineral soil may facilitate resource exploitation and thereby competition, promoting the differential utilization of root-associated and litter/humus-associated resources among euedaphic species. By contrast, resources in surface soil presumably varied considerably between cropping systems and this likely contributed to the variable uptake of ^{13}C of species of the same functional group in epedaphic and hemiedaphic Collembola. For instance, ^{13}C enrichment in *L. paradoxus* was lower than in the other epedaphic species in rape, but similar in grass and willow.

Collembola species with low incorporation of ^{13}C presumably rely less on root-derived C, but more on other unlabeled resources such as soil organic matter. Supporting this assumption, we found the incorporation of ^{13}C into Collembola species ($\Delta^{13}C$ values) to be negatively correlated with bulk tissue ^{13}C natural abundance of Collembola ($\delta^{13}C$ values) in rape and grass (Fig. 6). This is in line with previous studies indicating that low $\delta^{13}C$ values reflect preferential incorporation of recently fixed plant C; by contrast, high $\delta^{13}C$ values indicate high incorporation of C originating from soil organic matter and associated microorganisms (Potapov et al. 2016b, 2019). Interestingly, *L. paradoxus* in rape had low natural $\delta^{13}C$ values, but also low incorporation of ^{13}C, contrasting other epedaphic species (Fig, 5, Table S4). In addition, ^{13}C enrichment of *L. paradoxus* declined after it peaked at day 7. This suggests that, unlike the other epedaphic species, *L. paradoxus* incorporated recently fixed C from other photoautotrophs than plants, presumably algae or cyanobacteria.

Body size may be another factor affecting the incorporation of ^{13}C into Collembola species, since smaller species with higher mass-specific metabolic rates (Brown and Sily, 2006) may also have a higher rate of ^{13}C incorporation than larger species (Larsen et al. 2009). However, there was no significant correlation between body size and the incorporation of ^{13}C into Collembola species, suggesting that dietary differentiation may play a more important role in governing incorporation of root-derived C than body size. Although the present study cannot provide details on the exact mechanisms responsible for the differential incorporation of root-derived C into Collembola species, e.g. by feeding on different microorganisms, our results suggest that the differential use of basal resources may be an important mechanism favoring local coexistence of Collembola species.

5. Conclusions

Results of the present study demonstrate that the flux of root-derived C into soil microbivores, and thus to higher trophic levels of soil food webs, strongly varies between cropping systems. Cropping system not only affected the flux of root C into Collembola, with slower fluxes in rape than in grass and willow, but also the utilization of root-derived resources vs. litter/soil organic matter-derived resources by Collembola functional groups and species. Thereby, our findings suggest that the role of root-derived C as major resource fueling soil food webs varies markedly with cropping system, potentially due to differences in plant species/plant type and application of fertilizer. Future studies using novel techniques, such as compound-specific fatty acid, amino acid and molecular gut content analysis, will allow deeper insight into the relative contributions of fungi and bacteria in channeling recently fixed C from roots into soil food webs of arable fields with different cropping systems and fertilizer regimes.

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References

Ai, C., Liang, G., Sun, J., Wang, X., He, P., Zhou, W., He, X., 2015. Reduced dependence of rhizosphere microbiome on plant-derived carbon in 32-year long-term inorganic and organic fertilized soils. Soil Biology and Biochemistry 80, 70–78. doi:10.1016/j.soilbio.2014.09.028

Albers, D., Schaefer, M., Scheu, S., 2006. Incorporation of plant carbon into the soil animal food web of an arable system. Ecology 87, 235–245. doi:10.1890/04-1728

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48. doi:10.18637/jss.v067.i01

Baum, C., Toljander, Y.K., Eckhardt, K.U., Weih, M., 2009. The significance of host-fungus combinations in ectomycorrhizal symbioses for the chemical quality of willow foliage. Plant and Soil 323, 213–224. doi:10.1007/s11104-009-9928-x

Brown, J.H., Sibly, R.M., 2006. Life-history evolution under a production constraint. Proceedings of the National Academy of Sciences of the United States of America 103, 17595–17599. doi:10.1073/pnas.0608522103

Butler, J.L., Williams, M.A., Bottomley, P.J., Myrold, D.D., 2003. Microbial Community Dynamics Associated with Rhizosphere Carbon Flow. Applied and Environmental Microbiology 69, 6793–6800. doi:10.1128/AEM.69.11.6793-6800.2003

Chahartaghi, M., Langel, R., Scheu, S., Ruess, L., 2005. Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biology and Biochemistry 37, 1718–1725. doi:10.1016/J.SOILBIO.2005.02.006

Chamberlain, P., Mcnamara, N., Chaplow, J., Stott, A., Black, H., 2006. Translocation of surface litter carbon into soil by Collembola. Soil Biology and Biochemistry 38, 2655–2664. doi:10.1016/j.soilbio.2006.03.021

Crotty, F.V., Fychan, R., Sanderson, R., Rhymes, J.R., Bourdin, F., Scullion, J., Marley, C.L., 2016. Understanding the legacy effect of previous forage crop and tillage management on soil biology, after conversion to an arable crop rotation. Soil Biology and Biochemistry 103, 241–252.

doi:10.1016/j.soilbio.2016.08.018

Denef, K., Bubenheim, H., Lenhart, K., Vermeulen, J., Van Cleemput, O., Boeckx, P., Müller, C., 2007. Community shifts and carbon translocation within metabolically-active rhizosphere microorganisms in grasslands under elevated CO_2. Biogeosciences 4, 769–779. doi:10.5194/bg-4-769-2007

Denef, K., Roobroeck, D., Manimel Wadu, M.C.W., Lootens, P., Boeckx, P., 2009. Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. Soil Biology and Biochemistry 41, 144–153. doi:10.1016/j.soilbio.2008.10.008

Dennis, P.G., Miller, A.J., Hirsch, P.R., 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiology Ecology. doi:10.1111/j.1574-6941.2010.00860.x

Detheridge, A.P., Brand, G., Fychan, R., Crotty, F.V., Sanderson, R., Griffith, G.W., Marley, C.L., 2016. The legacy effect of cover crops on soil fungal populations in a cereal rotation. Agriculture, Ecosystems and Environment 228, 49–61. doi:10.1016/j.agee.2016.04.022

Eerpina, R., Boiteau, G., Lynch, D.H., 2017. Collembola diet switching in the presence of maize roots varies with species. Canadian Journal of Soil Science 97, 171–177. doi:10.1139/cjss-2016-0057

Ehret, M., Bühle, L., Graß, R., Lamersdorf, N., Wachendorf, M., 2015. Bioenergy provision by an alley cropping system of grassland and shrub willow hybrids: biomass, fuel characteristics and net energy yields. Agroforestry Systems 89, 365–381. doi:10.1007/s10457-014-9773-7

Eissfeller, V., Beyer, F., Valtanen, K., Hertel, D., Maraun, M., Polle, A., Scheu, S., 2013. Incorporation of plant carbon and microbial nitrogen into the rhizosphere food web of beech and ash. Soil Biology and Biochemistry 62, 76–81. doi:10.1016/J.SOILBIO.2013.03.002

Endlweber, K., Ruess, L., Scheu, S., 2009. Collembola switch diet in presence of plant roots thereby functioning as herbivores. Soil Biology and Biochemistry 41, 1151–1154. doi:10.1016/J.SOILBIO.2009.02.022

Endlweber, K., Ruess, L., Scheu, S., 2009. Collembola switch diet in presence of plant roots thereby functioning as herbivores. Soil Biology and Biochemistry 41, 1151–1154. doi:10.1016/j.soilbio.2009.02.022

Faber, J.H., 1991. Functional classification of soil fauna: A new approach. Oikos 62, 110.

doi:10.2307/3545458

Ferlian, O., Klarner, B., Langeneckert, A.E., Scheu, S., 2015. Trophic niche differentiation and utilisation of food resources in collembolans based on complementary analyses of fatty acids and stable isotopes. Soil Biology and Biochemistry 82, 28–35. doi:10.1016/J.SOILBIO.2014.12.012

Fujii, S., Mori, A.S., Kominami, Y., Tawa, Y., Inagaki, Y., Takanashi, S., Takeda, H., 2016. Differential utilization of root-derived carbon among collembolan species. Pedobiologia 59, 225–227. doi:10.1016/J.PEDOBI.2016.05.001

Gillet, S., Ponge, J.F., 2003. Changes in species assemblages and diets of Collembola along a gradient of metal pollution. Applied Soil Ecology 22, 127–138. doi:10.1016/S0929-1393(02)00134-8

Goncharov, A.A., Tsurikov, S.M., Potapov, A.M., Tiunov, A.V., 2016. Short-term incorporation of freshly fixed plant carbon into the soil animal food web: field study in a spruce forest. Ecological Research 31, 923–933. doi:10.1007/s11284-016-1402-7

Grayston, S.J., Vaughan, D., Jones, D., 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5, 29–56. doi:10.1016/S0929-1393(96)00126-6

Hartmann, L., Lamersdorf, N., 2015. Site Conditions, Initial Growth and Nutrient and Litter Cycling of Newly Installed Short Rotation Coppice and Agroforestry Systems, in: Bioenergy from Dendromass for the Sustainable Development of Rural Areas. pp. 121–138. doi:10.1002/9783527682973.ch10

Hermans, C., Hammond, J.P., White, P.J., Verbruggen, N., 2006. How do plants respond to nutrient shortage by biomass allocation? Trends in Plant Science 11, 610–617. doi:10.1016/j.tplants.2006.10.007

Högberg, M.N., 2006. Discrepancies between ergosterol and the phospholipid fatty acid 18:2ω6,9 as biomarkers for fungi in boreal forest soils. Soil Biology and Biochemistry 38, 3431–3435. doi:10.1016/j.soilbio.2006.06.002

Högberg, M.N., Briones, M.J.I., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton, B., Hurry, V., Linder, S., Näsholm, T., Högberg, P., 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. New Phytologist 187, 485–493. doi:10.1111/j.1469-

8137.2010.03274.x

Hopkin, S.P., 2007. A Key to the Collembola (Springtails) of Britain and Ireland. Field Studies Council, Shrewsbury.

Hyodo, F., Kohzu, A., Tayasu, I., 2010. Linking aboveground and belowground food webs through carbon and nitrogen stable isotope analyses. Ecological Research 25, 745–756. doi:10.1007/s11284-010-0719-x

Kagawa, A., Sugimoto, A., Maximov, T.C., 2006. Seasonal course of translocation, storage and remobilization of ^{13}C pulse-labeled photoassimilate in naturally growing Larix gmelinii saplings. New Phytologist 171, 793–804. doi:10.1111/j.1469-8137.2006.01780.x

Kempson, D., Lloyd, M., Ghelardi, R., 1963. A new extractor for woodland litter. Pedobiologia 3, 1–21.

Kühn, J., Schweitzer, K., Ruess, L., 2019. Diversity and specificity of lipid patterns in basal soil food web resources. PLoS ONE 14, e0221102. doi:10.1371/journal.pone.0221102

Kušliene, G., Rasmussen, J., Kuzyakov, Y., Eriksen, J., 2014. Medium-term response of microbial community to rhizodeposits of white clover and ryegrass and tracing of active processes induced by ^{13}C and ^{15}N labelled exudates. Soil Biology and Biochemistry 76, 22–33. doi:10.1016/j.soilbio.2014.05.003

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. LmerTest package: Tests in linear mixed effects models. Journal of Statistical Software 82, 1–26. doi:10.18637/jss.v082.i13

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163, 421–431. doi:10.1002/1522-2624(200008)163:4<421::AID-JPLN421>3.0.CO;2-R

Larsen, T., Ventura, M., Damgaard, C., Hobbie, E.A., Krogh, P.H., 2009. Nutrient allocations and metabolism in two collembolans with contrasting reproduction and growth strategies. Functional Ecology 23, 745–755. doi:10.1111/j.1365-2435.2009.01564.x

Liu, S., Behm, J.E., Chen, J., Fu, S., He, X., Hu, J., Schaefer, D., Gan, J., Yang, X., 2016. Functional redundancy dampens the trophic cascade effect of a web-building spider in a tropical forest floor. Soil Biology and Biochemistry 98, 22–29. doi:10.1016/J.SOILBIO.2016.03.020

Mordacq, L., Mousseau, M., Deleens, E., 1986. A ^{13}C method of estimation of carbon allocation to roots in a young chestnut coppice. Plant, Cell & Environment 9, 735–739. doi:10.1111/j.1365-3040.1986.tb02106.x

Nicolson, T.H., Johnston, C., 2009. Mycorrhiza in the Gramineae. Transactions of the British Mycological Society 72, 261–268. doi:10.1016/s0007-1536(79)80041-8

Oelbermann, K., Langel, R., Scheu, S., 2008. Utilization of prey from the decomposer system by generalist predators of grassland. Oecologia 155, 605–617. doi:10.1007/s00442-007-0927-4

Okubo, A., Matsusaka, M., Sugiyama, S., 2016. Impacts of root symbiotic associations on interspecific variation in sugar exudation rates and rhizosphere microbial communities: a comparison among four plant families. Plant and Soil 399, 345–356. doi:10.1007/s11104-015-2703-2

Ostle, N., Briones, M.J.I., Ineson, P., Cole, L., Staddon, P., Sleep, D., 2007. Isotopic detection of recent photosynthate carbon flow into grassland rhizosphere fauna. Soil Biology and Biochemistry 39, 768–777. doi:10.1016/j.soilbio.2006.09.025

Pausch, J., Kramer, S., Scharroba, A., Scheunemann, N., Butenschoen, O., Kandeler, E., Marhan, S., Riederer, M., Scheu, S., Kuzyakov, Y., Ruess, L., 2016. Small but active - pool size does not matter for carbon incorporation in below-ground food webs. Functional Ecology 30, 479–489. doi:10.1111/1365-2435.12512

Pausch, J., Kuzyakov, Y., 2018. Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale. Global Change Biology 24, 1–12. doi:10.1111/gcb.13850

Phillips, R.P., Finzi, A.C., Bernhardt, E.S., 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecology Letters 14, 187–194. doi:10.1111/j.1461-0248.2010.01570.x

Pollierer, M.M., Dyckmans, J., Scheu, S., Haubert, D., 2012. Carbon flux through fungi and bacteria into the forest soil animal food web as indicated by compound-specific ^{13}C fatty acid analysis. Functional Ecology 26, 978–990. doi:10.1111/j.1365-2435.2012.02005.x

Pollierer, M.M., Langel, R., Körner, C., Maraun, M., Scheu, S., 2007. The underestimated importance of belowground carbon input for forest soil animal food webs. Ecology Letters 10, 729–736. doi:10.1111/j.1461-0248.2007.01064.x

Ponge, J.F., 2000. Vertical distribution of Collembola (Hexapoda) and their food resources in organic horizons of beech forests. Biology and Fertility of Soils 32, 508–522.

doi:10.1007/s003740000285

Potapov, A.M., Goncharov, A.A., Tsurikov, S.M., Tully, T., Tiunov, A. V., 2016a. Assimilation of plant-derived freshly fixed carbon by soil collembolans: Not only via roots? Pedobiologia 59, 189–193. doi:10.1016/j.pedobi.2016.07.002

Potapov, A.M., Korotkevich, A.Y., Tiunov, A.V., 2018. Non-vascular plants as a food source for litter-dwelling Collembola: Field evidence. Pedobiologia 66, 11–17. doi:10.1016/j.pedobi.2017.12.005

Potapov, A.M., Semenina, E.E., Korotkevich, A.Y., Kuznetsova, N.A., Tiunov, A.V., 2016b. Connecting taxonomy and ecology: Trophic niches of collembolans as related to taxonomic identity and life forms. Soil Biology and Biochemistry 101, 20–31. doi:10.1016/j.soilbio.2016.07.002

Potapov, A.M., Tiunov, A.V., Scheu, S., 2019. Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. Biological Reviews 94, 37–59. doi:10.1111/brv.12434

Richter, F., Döring, C., Jansen, M., Panferov, O., Spank, U., Bernhofer, C., 2015. How to predict hydrological effects of local land use change: How the vegetation parameterisation for short rotation coppices influences model results. Hydrology and Earth System Sciences 19, 3457–3474. doi:10.5194/hess-19-3457-2015

Ruess, L., Schütz, K., Haubert, D., Häggblom, M.M., Kandeler, E., Scheu, S., 2005. Application of lipid analysis to understand trophic interactions in soil. Ecology 86, 2075–2082. doi:10.1890/04-1399

Rusek, J., 2007. A new classification of Collembola and Protura life forms. Contributions to Soil Zoology in Central Europe II 109–115.

Scheunemann, N., Digel, C., Scheu, S., Butenschoen, O., 2015. Roots rather than shoot residues drive soil arthropod communities of arable fields. Oecologia 179, 1135–1145. doi:10.1007/s00442-015-3415-2

Scheunemann, N., Pausch, J., Digel, C., Kramer, S., Scharroba, A., Kuzyakov, Y., Kandeler, E., Ruess, L., Butenschoen, O., Scheu, S., 2016. Incorporation of root C and fertilizer N into the food web of an arable field: Variations with functional group and energy channel. Food Webs 9, 39–45. doi:10.1016/J.FOOWEB.2016.02.006

Scheunemann, N., Scheu, S., Butenschoen, O., 2010. Incorporation of decade old soil carbon into the soil animal food web of an arable system. Applied Soil Ecology 46, 59–63. doi:10.1016/j.apsoil.2010.06.014

Seeber, J., Rief, A., Richter, A., Traugott, M., Bahn, M., 2012. Drought-induced reduction in uptake of recently photosynthesized carbon by springtails and mites in alpine grassland. Soil Biology and Biochemistry 55, 37–39. doi:10.1016/J.SOILBIO.2012.06.009

Sumorok, B., Kiedrzynska, E., 2007. Mycorrhizal status of native willow species in the Pilica River floodplain along the moisture gradient. Wetlands: Monitoring, Modelling and Management 281–286. Taylor & Francis, London.

Tariq, A., Gunina, A., Lamersdorf, N., 2018. Initial changes in soil properties and carbon sequestration potential under monocultures and short-rotation alley coppices with poplar and willow after three years of plantation. Science of The Total Environment 634, 963–973. doi:10.1016/J.SCITOTENV.2018.03.391

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Supporting information

Table S1 ^{13}C enrichment (Δ^{13}C) of above- and belowground parts of rape, grass and willow 3, 7, 14, 28 days after labeling.

Part	**Day**	**Grass**	**Rape**	**Willow**
Shoot/leave	3	826.59 ± 209.60c	399.61 ± 91.73c	355.14 ± 176.49c
	7	436.56 ± 339.25bc	379.27 ± 100.05bc	290.53 ± 84.04bc
	14	611.99 ± 153.23b	263.47 ± 76.82b	202.79 ± 59.21b
	28	297.32 ± 58.04a	191.24 ± 27.44a	150.37 ± 69.27a
Root	3	320.98 ± 364.95a	61.25 ± 41.71a	1.75 ± 2.47a
	7	133.83 ± 88.41a	88.69 ± 34.88a	7.22 ± 11.39a
	14	138.05 ± 106.52a	85.03 ± 60.24a	11.74 ± 7.75a
	28	281.41 ± 231.26a	76.03 ± 30.14a	12.09 ± 12.36a

Values of different sampling days within cropping systems marked with the same letter do not differ significantly ($p < 0.05$; Tukey's HSD test). Means ± SD.

Table S2 Linear mixed effect model of type III error for the effect of natural abundance of ^{13}C (δ^{13}C) on $\log_{10}$ transformed Δ^{13}C values of the same Collembola species in different cropping systems (rape, grass and willow).

Cropping system	**Slope**	**Std. error**	**t-value**	**p-value**
Rape	-0.48	0.15	**-3.2**	**0.019**
Grass	-0.41	0.10	**-4.20**	**0.004**
Willow	-0.15	0.13	-1.14	0.293

Significant effects are in bold.

Table S3 ^{13}C enrichment (Δ^{13}C) of soil dissolved organic carbon 3, 7, 14, 28 days after labeling in rape, grass, and willow

Day	**Grass**	**Rape**	**Willow**
3	1.07 ± 2.4a	3.24 ± 2.49a	9.22 ± 8.06b
7	2.6 ± 0.73a	12.51 ± 9.74b	7.41 ± 0.81b
14	0.96 ± 0.89a	15.85 ± 5.92b	9.35 ± 4.95b

28	0.81 ± 0.66a	3.02 ± 1.97a	1.5 ± 2.18a

Values of different sampling days within cropping systems marked with the same letter do not differ significantly ($p < 0.05$; Tukey's HSD test). Means ± SD.

Table S4 Natural abundance of ^{13}C ($\delta^{13}C$) and ^{15}N ($\delta^{15}N$) in Collembola species (*Stenaphorura denisi, Protaphorura armata, Folsomia quadrioculata, Parisotoma notabilis, Pseudosinella alba, Isotoma viridis, Orchesella villosa, Lepidocyrtus paradoxus, Lepidocyrtus cyaneus*) in different cropping systems (rape, grass and willow).

	$\delta^{13}C$			**$\delta^{15}N$**		
Species	Grass	Willow	Rape	Grass	Willow	Rape
O. villosa	-28.06 ± 0.85	-27.73 ± 1.48	-27.81 ± 0.67	3.56 ± 0.32	-1.39 ± 0.97	4.39 ± 0.5
I. viridis	-27.38 ± 0.19	-28.29 ± 0.46	-27.9 ± 0.7	2.55	-0.95 ± 1.57	1.74 ± 1.12
L. cyaneus	-27.74 ± 0.25	-27.1 ± 0.53	-27.78 ± 0.5	3.88 ± 2.85	0.69 ± 0.88	3.42 ± 0.91
L. paradoxus	-27.8 ± 0.49	-26.49 ± 1.02	-27.26 ± 0.41	3.21 ± 0.64	2.01 ± 3.08	1.98 ± 1.22
F. quadrioculata	-26.47 ± 0.41	-26.86 ± 0.22	-26.78 ± 0.43	4.54 ± 1.49	1.28 ± 0.37	3.89 ± 0.43
P. notabilis	-26.5 ± 0.52	-26.72 ± 0.32	-26.61 ± 0.2	5.7 ± 2.46	1.32 ± 0.15	6.48 ± 0.82
P. alba	-26.95 ± 1.37	-27.25 ± 0.55	-27.26 ± 0.44	10.81 ± 2.31	2.75 ± 3.1	6.06 ± 0.63
P. armata	-28.02 ± 1.13	-26.51 ± 0.02	-26.25 ± 0.36	7.32 ± 2.25	6.63 ± 0.62	6.24 ± 0.57
S. denisi	-26.79 ± 0.38	-26.93 ± 0.64	-25.99	6.8 ± 1.81	6.92 ± 0.65	4.55

means ± SD.

Chapter 3

The flux of root-derived carbon via fungi and bacteria into soil microarthropods (Collembola) differs markedly between cropping systems

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Abstract

Recently fixed plant carbon (C) being released as rhizodeposits is a major resource fueling soil food webs. Soil microorganisms predominate in incorporating root-derived C and subsequently transfer it to higher trophic levels. However, variation in microbial community structure between cropping systems and its consequences for the incorporation of root-derived C into soil microbivores remain unclear. In the present study, we used $^{13}CO_2$ to pulse label a crop monoculture (oilseed rape, *Brassica napus* L.), a mixed grass community (dominated by *Lolium perenne* L. mixed with clover *Trifolium repens* L.), and a young tree plantation (willow, *Salix schwerinii* E.L. Wolf and *Salix viminalis* L.). During 28 days, we traced the incorporation of root-derived ^{13}C into phospholipid fatty acids (PLFAs) of soil microorganisms and neutral lipid fatty acids (NLFAs) of five Collembola species belonging to three functional groups: epedaphic (surface-dwelling), hemiedaphic (litter-dwelling), and euedaphic (soil-dwelling). The contribution of bacterial and fungal channels to the incorporation of root-derived C into Collembola varied considerably between cropping systems. Collembola incorporated more ^{13}C from the bacterial channel in rape than in grass and willow, where fungi were the major C source. This corresponded to a similarly higher ^{13}C incorporation into bacterial marker PLFAs in rape as compared to grass and willow. In contrast, while the proportion of bacterial and fungal biomarkers in Collembola NLFAs was related to the ^{13}C incorporation into microbial PLFAs, it did not correlate with the proportion of microbial PLFAs in the different cropping systems. This suggests that Collembola rely on specific microbial pools, presumably related to recent plant inputs. Within the same cropping system, hemiedaphic Collembola incorporated more root-derived ^{13}C from the bacterial channel compared to euedaphic and epedaphic Collembola. The results demonstrate the remarkable importance of cropping system for the flux of root

C into microorganisms and microbivore soil invertebrates. Changes in root C flux into bacterial and fungal resources among cropping systems resulted in differential utilization of these resources by soil microbivores, suggesting that in particular microorganisms fueled by rhizodeposits are vital resources for the nutrition of higher trophic levels in soil food webs.

Key-words: bacterial energy channel, bioenergy crops, ^{13}C labeling, Collembola, fungal energy channel, lipids

1. Introduction

A considerable amount of plant photosynthates is transferred to roots shortly after being fixed from the atmosphere and is released into the soil as rhizodeposits (Dennis et al., 2010; Pausch and Kuzyakov, 2018). This root-derived carbon (C), mainly comprising low molecular substances such as sugars, amino acids, and organic acids, is the major C source for soil microorganisms (Anderson et al., 1993; Buée et al., 2009). Root-derived C fluxes thereby regulate a wide range of soil ecological processes and accelerate the cycling of virtually all elements (Kuzyakov, 2002). Although soil microorganisms are the primary consumers of rhizodeposits, their activity and biomass, and thereby C fluxes to higher trophic levels, are significantly influenced by microbial grazers, such as nematodes and springtails (Collembola) (Maboreke et al., 2017; Ngosong et al., 2014). However, as soil food webs are highly complex with considerable proportions of generalist consumers (Digel et al. 2014; Scheu, 2002), trophic links between microorganisms and higher trophic levels in food webs are difficult to disentangle.

Collembola are important members of soil food webs, contributing to C transport at the litter-soil interface (Ruf et al., 2006), and channeling C and nutrients from basal resources, including bacteria, fungi, and plants, to higher trophic levels (Liu et al., 2016; Oelbermann et al., 2008). Collembola heavily rely on root-derived C (Garrett et al., 2001; Larsen et al., 2007; Pollierer et al., 2007; Scheunemann et al., 2015), potentially due to their high preference for root-associated microorganisms (Ostle et al., 2007; Maboreke et al., 2017). Collembola incorporate root-derived C from different channels based on bacteria, fungi, and plants (Crotty et al., 2011; Pollierer et al., 2012), and change diet according to resource availability (Eerpina et al., 2017; Endlweber et al., 2009; Scheunemann et al., 2015). However, determinants of the variation in dietary composition of Collembola between cropping systems and its dependence on differences in microbial resources, especially those utilizing root-derived C, are not well studied.

The incorporation of root-derived C into microbial groups varies between cropping systems, due to differences in plant species, and soil chemical and physical conditions (Sechi et al., 2014; Zieger et al., 2017). Therefore, the channeling of root-derived C into Collembola and higher trophic levels of soil food webs likely also varies between cropping systems. For instance, trees and grasses allocate high amounts of C to roots and rhizodeposits, which are used by mycorrhizal and saprotrophic fungi (De Deyn et al., 2011; Denef et al., 2007; Zieger et al., 2017). By contrast, bacteria are likely to be important in channeling root-derived C to higher trophic levels in more intensively managed systems, with their relative importance affected by crop species and management practices (Elfstrand et al., 2008; Pausch et al., 2016a, b). For instance, some crop species, such as oilseed rape, do not form mycorrhizal associations and accumulate secondary compounds that are toxic to saprotrophic fungi in their rhizosphere (Kirkegaard and Sarwar, 1998; Okubo et al., 2016), which may facilitate the utilization of root-derived C by bacteria.

Collembola species with different functional traits dwell in different soil depth, likely resulting in differential incorporation of root-derived C from bacterial and fungal channels. Epedaphic Collembola are surface-dwelling species inhabiting the upper litter layer, where they may incorporate root-derived C from the fungal channel since fungal hyphae transfer root-derived C from rhizosphere to bulk soil and litter (Klironomos et al. 1995). By contrast, hemiedaphic and euedaphic Collembola inhabiting the lower litter layer and mineral soil (Potapov et al., 2016) are more likely to access microbial resources in the rhizosphere, and to incorporate root C from both bacterial and fungal channels.

Fatty acid (FA) analysis provides biomarkers for basal resources such as bacteria, fungi, and plants, allowing to detail the trophic links between Collembola and basal resources (Chamberlain et al., 2004; Ruess et al., 2007; Ruess and Chamberlain, 2010; Ruess and Müller-Navarra, 2019). The principle of FA analysis is based on “dietary routing”; to save energy, consumers incorporate FAs from the diet, such as microbial phospholipid fatty acids (PLFAs), into storage lipids, i.e. neutral lipid fatty acids (NLFAs), without major modification (Blem, 1976). Therefore, trophic links of consumers to basal resources can be traced by measuring the proportion of specific biomarkers in NLFAs of consumers. In labeling experiments, or when resources differ in isotopic composition (Ngosong et al., 2014; Pollierer et al., 2012; Scheunemann et al., 2016), stable isotope ratios of C within these biomarkers can provide information on the flux of C through specific energy channels or compartments of soil food webs. Previous studies have shown that Collembola gain plant C via consumption of bacteria, fungi, and roots (Ferlian et al., 2015; Haubert et al., 2009; Ngosong et al., 2011; Pollierer et al., 2012; Sechi et al., 2014). However, most studies analyzing natural abundance isotope ratios or long-term labeling experiments did not separate the flux of C from rhizodeposits from that of C from leaf or root litter. In pulse labeling studies, Collembola rapidly incorporated root-derived C, with peaks of label after three days (Garrett et al., 2001; Högberg et al., 2010; Zieger et al., 2017). Therefore, combining FA analysis with short-term oriented methods, such as ^{13}C pulse labeling, may allow to separate the role of different basal resources in channeling root-derived C into soil food webs (Maboreke et al., 2017).

To investigate the relative importance of bacterial and fungal channels for the incorporation of root-derived C into Collembola, we pulse labeled three cropping systems in an agroforestry field with $^{13}CO_2$ and traced the incorporation of root-derived C into biomarker NLFAs of five Collembola species belonging to epedaphic, hemiedaphic, and euedaphic groups. The three cropping systems included a herbaceous crop monoculture (oilseed rape, *Brassica napus* L.), a mixed-grass community (grassland dominated by *Lolium perenne* L. mixed with clover *Trifolium repens* L.), and a tree plantation (willow, *Salix schwerinii* E.L. Wolf and *Salix viminalis* L.). To investigate the incorporation of root-derived C from different basal resources into Collembola NLFAs, we employed ^{13}C pulse labeling and compound-specific PLFA and NLFA analysis.

We tested the following hypotheses:

i) The relative importance of energy channels for the incorporation of root-derived C into Collembola differs among cropping systems, with higher incorporation of root-derived C from the bacterial channel in rape than in grass and willow, where fungi are the primary root-derived C source.

ii) Within the same cropping system, relative fluxes of root-derived C via the bacterial and fungal energy channels differ between Collembola functional groups, with higher relevance of the fungal channel for epedaphic Collembola, whereas hemiedaphic and euedaphic Collembola are more closely associated with bacterial resources.

2. Materials and methods

2.1 Site description

The study sites were located in Reiffenhausen, south of Göttingen in central Germany (51º39’83’’ N/9º98’75’’ E a.s.l.). The mean annual precipitation is 635 mm and average annual temperature is 9.1 °C (Richter et al., 2015). The soil at the study sites is characterized by sedimentary deposits of Middle and Upper Triassic Sandstone material, partly mixed with claystone material and covered by loess sediments. The texture of the soil varies from loamy sand in the eastern part to silty loam in the western part (Hartmann and Lamersdorf, 2015; Tariq et al., 2018). The experiment was established on former cropland in March 2011. The preceding crop grown on the study sites was winter barley (*Hordeum vulgare* L.).

2.2 Experimental design

Fields comprising the three cropping systems rape (oilseed rape, *Brassica napus* L.), grass (dominated by *Lolium perenne* L., mixed with clover *Trifolium repens* L.), and tree (willow, *Salix schwerinii* E.L. Wolf and *Salix viminalis* L.) were established (Tariq et al., 2018). The willow and grass sites were established in March 2011. In September 2015, part of the grass sites were transformed into rape fields. The rape site represents cropland of high land-use intensity including fertilizer application, annual harvest, and crop rotation. The grass site represents low input grassland without fertilizer application but with three cuts per year. The willow site was planted as low input short-term forest rotation system with a rotation cycle of three years without application of fertilizer. The willow trees were about 3 years old and 4 m high when our experiment was conducted. The willow site and grass site were arranged as an agroforest with rows of willow stripes and grassland stripes in between (three willow stripes, each 7.5 m wide and 75 m long, and three grassland stripes, each 9 m wide and 75 m long). The rape site was next to one of the outer willow stripes (18 m wide and 75 m long). The biomass of leaves and stems of rape, grass, and willow was 737, 137, and 1034 g/m^2, respectively; the root biomass was 120, 101, and 387 g/m^2, respectively. The values of $\delta^{13}C$ of soil total organic C were -26.3, -27.3, and -27.8 ‰, the bulk soil density was 1.4, 1.5, and 1.5 g/cm^3, and pH was 5.4, 6.6, and 5.6 in rape, grass, and willow, respectively.

In each of the cropping systems, five labeling chambers were installed. In the rape field, the chambers were randomly positioned at a distance of 5 to 10 m from each other in May 2017. For labeling willow, one stripe adjacent to the rape field was selected and the chambers were spaced at least by 10 m and installed in July 2017. In the grass field, the chambers were installed in two blocks of 9.0 × 6.5 m in August 2017, two chambers in block one, which was next to the selected willow stripe, and three chambers in block two spaced by 50 m to block one. The chambers consisted of stainless steel frames of 1 × 1 m which were inserted into the soil to a depth of 10 cm, and of a plastic frame on top (height 1 m for rape and grass, 2 m for willow) that was covered with translucent LDPE (low-density polyethylene) foil. Plants were labeled by the addition of HCl to 20 g $Na_2{}^{13}CO_3$ in a plastic beaker. In willow, upper braches were bent down to fit the height of the chambers and held in position with a rope. Understory plants were removed and the soil surface was covered with black plastic foil before labeling to exclude ^{13}C incorporation by herbaceous plants and algae. The produced $^{13}CO_2$ was circulated in the chambers by a fan for 6 h. After labeling, the foil and plastic frame were removed, while the steel frame bases remained in the soil to prevent migration of animals between labeled and unlabeled areas.

2.3 Sampling

Soil samples were taken 3, 7, 14, and 28 days after labeling to analyze the dynamics of ^{13}C incorporation into Collembola. Additionally, unlabeled samples were taken at adjacent plots and served as control for analyzing ^{13}C and ^{15}N natural abundance in each cropping system. In each chamber, one soil sample was taken at each sampling date using a stainless steel soil corer (diameter 20 cm, depth 10 cm) for Collembola analysis. Soil arthropods were extracted by heat (Kempson et al., 1963) and stored in 70% ethanol at -20 °C. The extraction started two to three hours after soil sampling. Collembola were identified to species level under the microscope using Hopkin (2007). After identification, Collembola were transferred into methanol and stored at -20 °C. Five Collembola species from three functional groups were selected for further analysis: epedaphic species including *Lepidocyrtus cyaneus* (Tullberg) and *Orchesella villosa* (Geoffroy), hemiedaphic species including *Folsomia quadrioculata* (Tullberg) and *Parisotoma notabilis* (Schäffer), and the euedaphic species *Protaphorura armata* (Tullberg) (Potapov et al., 2016). For phospholipid fatty acid (PLFA) analysis one soil sample to a depth of 15 cm was taken randomly from three chambers in each cropping system at 1, 3, 7, and 14 days after labeling.

2.4 Analysis of fatty acids

Collembola NLFAs were extracted as described in Zelles (1999) and Haubert et al. (2004). The NLFA fractions were saponified and methylated following the procedures given for the Sherlock Microbial Identification System (MIDI Inc., Newark, NJ, USA; see Ruess et al., 2002). Soil PLFAs were extracted and purified following the method of Frostegård et al. (1993), with some modifications as described in Gunina et al. (2014). The lipid-containing phase was transferred to test tubes and stored at -20 °C until analysis.

2.5 Analysis of $^{13}C/^{12}C$ ratios of fatty acids

The isotopic composition of individual FAs in Collembola and soil was determined using a Thermo Finnigan Trace GC coupled via a GP interface to a Delta Plus mass spectrometer (Finnigan, Bremen, Germany). The GC was equipped with a fused silica capillary column (Phenomenex Zebron ZB-5, 30 m, 0.32 mm i.d., film thickness 0.25 lm, Torrance, CA, USA). The temperature program started with 60 °C (held for 1 min) and increased by 6 °C per minute to 310 °C and held for 15 min at 310 °C. The injection temperature was 250 °C and helium was used as carrier gas. The flow rate of helium was 2.2 mL min^{-1}. FAMEs were identified by chromatographic retention time based on comparison with standard mixtures composed of 37 different Fatty Acid Methyl Esters (FAMEs) ranging from C11 to C24 and 26 Bacterial Fatty Acid Methyl Esters (BAMEs; Sigma Aldrich, St Louis, MO, USA). The C isotope composition is reported in δ notation (‰) relative to Vienna Pee Dee Belemnite standard (V-PDB) as $\delta^{13}C$ [‰] = $((^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard} - 1) \times 1000$.

2.6 Calculations

Incorporation of ^{13}C

Incorporation of ^{13}C into individual FAs was expressed as the percentage of ^{13}C tracer in specific FAs in a single sample of Collembola or soil as

$$\text{Incorporation of } {}^{13}C_{FA}\,(\%) = {}^{13}C_{FA} / {}^{13}C_{FA\text{-}total} \times 100.$$

$^{13}C_{FA}$ is the amount of tracer ^{13}C in specific biomarker fatty acids and calculated as $^{13}C_{FA} = (atom\%_{FA\text{-}labeled} - atom\%_{FA\text{-}control}) \times C_{FA}$, with $atom\%_{FA\text{-}labeled}$ the ^{13}C / (^{12}C + ^{13}C) of specific FAs in labeled samples, and C_{FA} the amount of C (expressed as µV·sec) in specific biomarker fatty acids. $^{13}C_{FA\text{-}total}$ is the total amount of tracer ^{13}C across all detected fatty acids (27 biomarker PLFAs in soil and 9 biomarker NLFAs in Collembola) in a single sample (Chaudhary and Dick, 2016). Incorporation of ^{13}C is used to compare individual biomarkers in Collembola NLFAs and soil PLFAs among cropping systems and thereby to identify the relative importance of specific energy channels in the uptake of root-derived C among cropping systems.

Proportional contribution of C of individual FAs to total C was expressed as the percentage of C in specific FAs in a single sample of Collembola or soil as

$$Proportion\ of\ C_{FA}\ (\%) = C_{FA} / C_{FA\text{-}total} \times 100$$

with $C_{FA\text{-}total}$ the amount of C in total fatty acids. The amount of C was measured by GC and expressed as µV·sec. The proportion of C in individual FA markers relative to total C in Collembola NLFAs and soil PLFAs is used to identify the relative importance of basal resources in the diet of Collembola and the relative dominance of microbial groups in the soil microbial community, respectively.

Nine common biomarkers in soil PLFAs and Collembola NLFAs were detected. The ^{13}C incorporation and proportion in individual FAs were grouped (summed up) for bacterial (a15:0, 16:1ω7, 18:1ω7), fungal (18:2ω6,9), plant (18:1ω9) and general biomarkers (14:0, 15:0, 16:0, 18:0). Since the present study mainly focused on the incorporation of root-derived C from microbial channels into Collembola, only the nine common biomarkers in Collembola NLFAs and soil PLFAs were analyzed. Variations in the full spectrum of soil microbial PLFAs with cropping systems will be presented elsewhere (L. Shi, unpubl. data); the data are deposited at https://doi.org/10.5061/dryad.gqnk98skf.

Enrichment of ^{13}C

^{13}C enrichment of specific FAs was calculated as

$$\Delta^{13}C_{FA}\ [‰] = \delta^{13}C_{FA\text{-}labeled} - \delta^{13}C_{FA\text{-}control}$$

In contrast to ^{13}C incorporation, ^{13}C enrichment is independent of pool size and indicative of the replacement of C of certain biomarker FAs by newly incorporated ^{13}C; it reflects the relevance of newly deposited root-derived C for specific energy channels. The ^{13}C enrichment of biomarkers in Collembola NLFAs is likely to be closely related to the ^{13}C enrichment of the respective biomarkers in microbial PLFAs, i.e. food resources; thus, it allows to compare the dynamics and magnitude of the incorporation of root-derived C via bacterial and fungal channels into Collembola within the same cropping system. The ^{13}C enrichment of each biomarker group (bacteria, fungi, plant) was calculated as the weighted average of $\Delta^{13}C_{FA}$ by their proportional abundance in total FAs of a sample.

To investigate the influence of cropping systems on the diet of Collembola and on their incorporation of root-derived C from bacterial and fungal channels, we calculated the ratio between bacterial and fungal biomarkers (B-to-F ratio), ^{13}C incorporation (B-to-F $^{13}C_{incorporation}$ ratio), and ^{13}C enrichment (B-to-F $^{13}C_{enrichment}$ ratio) for Collembola NLFAs and soil PLFAs, respectively.

2.7 Statistical analysis

Incorporation of ^{13}C

For Collembola NLFAs, effects of 'cropping system', 'functional group' of Collembola, 'biomarker', and their interaction on the ^{13}C incorporation and proportion of biomarkers were inspected using linear mixed effects models. Since different groups of Collembola dominated in different cropping systems, we did not collect enough biomass of each functional group of Collembola for lipid analysis across all cropping systems, e.g. the epedaphic group was present in all cropping systems, while hemiedaphic and euedaphic groups were only present in rape and grass. Therefore, to investigate the effects of 'cropping system' on ^{13}C incorporation and proportion of specific biomarkers, we built models for epedaphic and hemi-/euedaphic Collembola separately. In the model of epedaphic Collembola, we included 'cropping system', 'biomarker FA', and their interaction as fixed effects and 'chamber nested within cropping system', 'Collembola species', 'ID of NLFA sample' (identity of the sample of individual Collembola species in each soil core), and 'sampling day' and its interaction with fixed effects as random effects. The model for hemi-/euedaphic Collembola was similar but included 'functional group' as fixed effect.

For soil PLFAs, the effects of 'cropping system', 'biomarker', 'sampling day', and their interaction on the concentration, proportion, and ^{13}C incorporation into biomarkers were analyzed by linear mixed effects models with 'ID of PLFA sample' (identity of the soil core PLFAs were extracted from) as random effect.

To investigate the association between microbial resources and the diet of Collembola, we compared the variations in B-to-F ratio and B-to-F $^{13}C_{incorporation}$ ratio among cropping systems between

Collembola NLFAs and soil PLFAs. For Collembola NLFAs, we used linear mixed effects models to analyze the effects of ‘cropping system’, and/or ‘functional group’, and/or their interaction on B-to-F ratio and B-to-F $^{13}C_{incorporation}$ ratio, with ‘Collembola species’, ‘chamber nested within cropping system’, ‘sampling day’ and its interaction with fixed effects as random effects. In these models, analyses were performed separately for epedaphic and hemi-/euedaphic Collembola. For soil PLFAs, effects of ‘cropping system’, ‘sampling day’, and their interaction on B-to-F ratio and B-to-F $^{13}C_{incorporation}$ ratio were tested with linear models.

To analyze the differences in the B-to-F ratio and the B-to-F $^{13}C_{incorporation}$ ratio between functional groups of Collembola, we excluded the data of willow since we lacked data on euedaphic and hemiedaphic Collembola in this cropping system. In this model, we used ‘cropping system’, ‘functional group’ and their interaction as fixed effects, and the same random effects as in the model of B-to-F $^{13}C_{incorporation}$ ratio for hemi-/euedaphic Collembola.

When analyzing ^{13}C incorporation, proportion, B-to-F ratio, and B-to-F $^{13}C_{incorporation}$ ratio of Collembola NLFAs, we used ‘functional group’ rather than ‘Collembola species’ as fixed effects since the B-to-F ratio and B-to-F $^{13}C_{incorporation}$ ratio of Collembola species was similar within functional groups (Table S1). In addition, ‘sampling day’ was not included as fixed effect but as random effect since we did not collect enough biomass of Collembola at each sampling day; therefore, analyses mainly focused on overall effects of ‘cropping system’, ‘biomarker’, ‘functional group’ and their interaction on properties of biomarker NLFAs across the whole experimental period.

Enrichment of ^{13}C

The differences in ^{13}C enrichment between NLFA biomarkers and sampling days were analyzed for each cropping system separately. In these models, we included ‘sampling day’, ‘biomarker’, and their interaction as fixed effects and ‘functional group’, ‘Collembola species’, ‘ID of NLFA sample’, and ‘chamber nested within cropping system’ as random effects. ‘Functional group’ was not included as fixed effect because within the same cropping system the effects of ‘functional group’ on ^{13}C enrichment were minor compared to the effects of ‘biomarker’ and ‘sampling day’ (see Table S2 in Supporting Information). For each cropping system, the effects of ‘biomarker’, ‘sampling day’, and their interaction on the ^{13}C enrichment of biomarker PLFAs were investigated with linear mixed effects models with ‘ID of PLFA sample' as random effect. In addition, we used linear models to analyze the responses of the B-to-F $^{13}C_{enrichment}$ ratio to 'cropping system', 'sampling day', 'lipid type' (NLFA/PLFA), and their interaction. As the B-to-F $^{13}C_{enrichment}$ ratio did not differ significantly between Collembola species ($P > 0.05$) in the same cropping systems, we did not include 'species' in this model.

Data were transformed (arcsine, square-root, or log) prior to the analyses to improve normality and homogeneity of variance. Data for general biomarkers were excluded from statistical analyses since they provided little information on the trophic association with different basal resources. All statistical analyses were conducted using R 3.4.2 (R Core team, 2016), packages 'lme4' (Bates et al., 2017), 'lmerTest' (Kuznetsova et al., 2016), and 'emmeans' (Lenth, 2019). All figures were drawn using 'ggplot2' (Wickham, 2016).

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3. Results

3.1 Incorporation of ^{13}C

The ^{13}C incorporation into NLFAs differed between biomarkers, cropping systems, and functional groups of Collembola (epedaphic: significant biomarker × cropping system interaction, $F_{4,64} = 3.28$, $P = 0.017$; hemi-/euedaphic: significant biomarker × cropping system × functional group interaction, $F_{2,94} = 3.176$, $P = 0.046$; Table S3). The incorporation of ^{13}C into the bacterial biomarker NLFAs of Collembola differed significantly between cropping systems, with similar trends among functional groups of Collembola (Fig. 1). In epedaphic Collembola, the incorporation was highest in rape, lowest in willow, and intermediate in grass. In hemiedaphic and euedaphic Collembola, the incorporation of ^{13}C in bacterial biomarkers was also higher in rape than in grass. Although the ^{13}C incorporation into bacterial biomarkers in epedaphic Collembola did not differ significantly between rape and grass, the analysis of ^{13}C incorporation into Collembola NLFAs in rape and grass indicated that the ^{13}C incorporation into bacterial biomarker NLFAs was higher in rape than in grass for all functional groups of Collembola (significant cropping system × biomarker interaction, $F_{1,18} = 9.98$, $P = 0.001$). Generally, changes in the proportion of bacterial biomarkers in Collembola NLFAs were similar to changes in the relative ^{13}C incorporation, with the exception that the proportion of bacterial biomarkers in epedaphic Collembola did not differ significantly between cropping systems (Fig. S1). In euedaphic Collembola, both the incorporation and proportion of fungal and plant biomarkers was higher in grass than in rape. By contrast, no differences were detected in epedaphic and hemiedaphic Collembola.

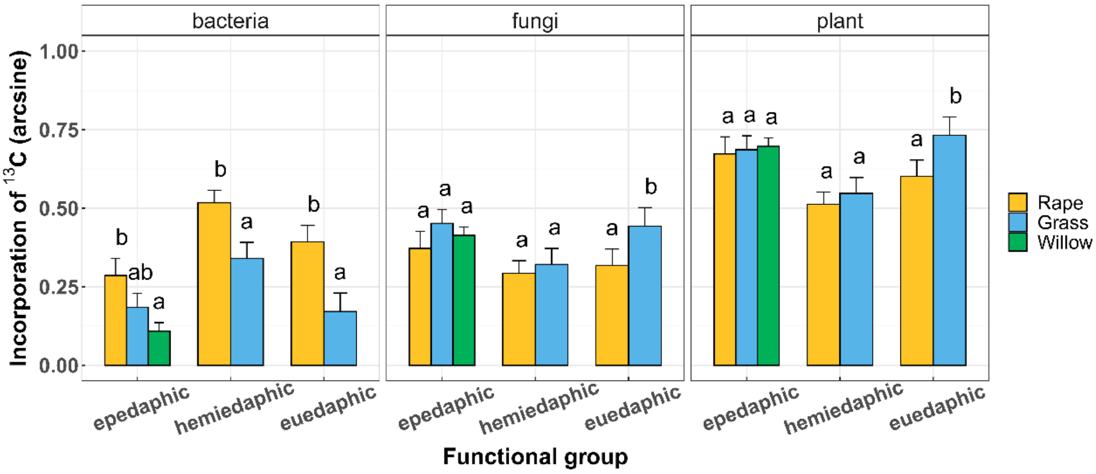

Figure 1. Incorporation of ^{13}C into bacterial, fungal, and plant biomarkers in NLFAs of Collembola functional groups (epedaphic, hemiedaphic, and euedaphic) in rape, grass, and willow; least square means ± SE; values with different letters are significantly different between cropping systems in the same functional group and biomarker NLFAs ($p < 0.05$; Tukey's HSD test); data are arcsine transformed.

The pattern of ^{13}C incorporation into soil PLFAs was similar to that of ^{13}C incorporation into biomarker NLFAs in Collembola, i.e. ^{13}C incorporation in bacterial biomarker PLFAs was higher in rape than in grass and willow (Table 1). However, the proportion of biomarker PLFAs differed from the proportion of Collembola NLFAs. For instance, the proportion of bacterial biomarker PLFAs in rape and grass was higher than in willow (Table 1), whereas the proportion of bacterial biomarker NLFAs in Collembola was highest in rape, lowest in willow, and intermediate in grass (Fig. S1). In line with this pattern, the variation in the B-to-F ratio and B-to-F $^{13}C_{incorporation}$ ratio in Collembola NLFAs was similar to the B-to-F $^{13}C_{incorporation}$ in soil PLFAs; the ratios were highest in rape, lowest in willow, and intermediate in grass. By contrast, the B-to-F ratio in soil PLFAs followed a markedly different pattern, with higher values in grass than in rape and willow (Fig. 2). Notably, the B-to-F $^{13}C_{incorporation}$ ratio in soil PLFAs differed between sampling days (Table S4). From day 1 to 7, the B-to-F $^{13}C_{incorporation}$ ratio was higher in rape than in grass and willow ($F_{2,12} = 8.65$, $P = 0.004$). At day 14, the B-to-F $^{13}C_{incorporation}$ ratio increased in grass to a higher level than that in rape and willow ($F_{2,5} = 15.21$, $P = 0.007$). In line with this pattern, in epedaphic and hemiedaphic Collembola in grass the B-to-F $^{13}C_{incorporation}$ ratio also increased in trend (Table S5). The concentration and the proportion of biomarker PLFAs in soil varied in similar ways among cropping systems, however, differences in concentrations were only significant in the fungal biomarker, but not in bacterial and plant biomarkers (Table 1).

Table 1. Incorporation of ^{13}C, proportion, and concentration of biomarker PLFAs from bulk soil in rape, grass, and willow.

	Biomarker	Rape	Grass	Willow
Incorporation of ^{13}C (%)	bacteria	31.88 ± 2.21b	23.76 ± 2.23a	24.82 ± 2.02a
	fungi	10.59 ± 2.12a	13.36 ± 2.23a	16.48 ± 2.02a
	plant	11.94 ± 2.12a	9.65 ± 2.23a	8.68 ± 2.02a
Proportion (%)	bacteria	27.80 ± 0.46b	28.37 ± 0.51b	24.49 ± 0.41a
	fungi	3.11 ± 0.15b	2.34 ± 0.15a	5.18 ± 0.19c
	plant	11.46 ± 0.29b	10.67 ± 0.31ab	10.06 ± 0.26a
Concentration (ug g^{-1} dry soil)	bacteria	13.55 ± 1.77a	12.18 ± 1.67a	10.54 ± 1.32a
	fungi	1.48 ± 0.19ab	0.98 ± 0.13a	2.18 ± 0.27b
	plant	5.53 ± 0.72a	4.72 ± 0.65a	4.29 ± 0.54a

Least square means ± SE; values with different letters are significantly different between cropping systems in incorporation of ^{13}C, proportion, or concentration in the same biomarker in soil PLFAs ($p < 0.05$; Tukey's HSD test).

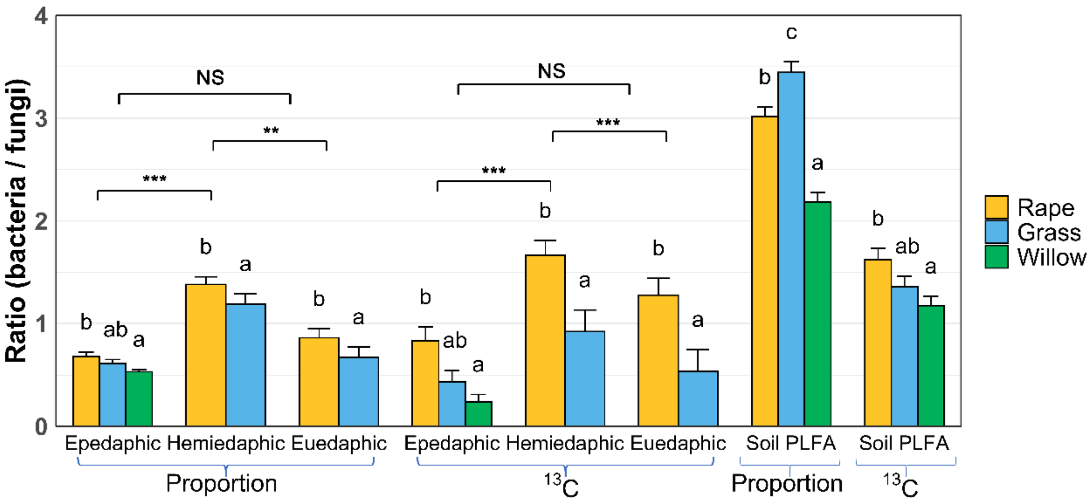

Figure 2. Ratio of proportion and ^{13}C incorporation (^{13}C) between bacterial and fungal biomarkers in Collembola NLFAs (epedaphic/hemiedaphic/euedaphic) and soil PLFAs; least square means ± SE; values with different letters are significantly different between cropping systems ($p < 0.05$; Tukey's HSD test); significant differences between Collembola functional groups are indicated by $^{**}p < 0.01$, $^{***}p < 0.001$; NS, not significant; please note that ratios are square root transformed.

The relative contribution of bacterial and fungal biomarkers to the incorporation of ^{13}C into

Collembola NLFAs differed between functional groups of Collembola. The B-to-F $^{13}C_{incorporation}$ ratio was higher in hemiedaphic than euedaphic and epedaphic Collembola ($F_{2,36} = 17.68$, $P < 0.001$, Fig. 2, Table S6). Similarly, the B-to-F ratio was also higher in hemiedaphic than euedaphic and epedaphic Collembola ($F_{2,10} = 23.2$, $P < 0.001$).

3.2 Enrichment of ^{13}C

In rape, the ^{13}C enrichment of bacterial biomarkers increased more quickly than that of fungal and plant biomarkers in Collembola NLFAs, resulting in higher enrichment of bacterial compared to fungal and plant biomarkers from day 3 to 7, while the differences diminished afterwards (Fig. 3; significant biomarker × day interaction; $F_{6,60} = 10.2$, $P < 0.001$). By contrast, the ^{13}C enrichment of the bacterial biomarkers was consistently lower than that of fungal and plant biomarkers in Collembola NLFAs in grass and willow. In soil PLFAs, the ^{13}C enrichment of bacterial biomarkers was lower than that of the fungal biomarker in rape, grass, and willow (Fig. S2). This resulted in a markedly higher B-to-F $^{13}C_{enrichment}$ ratio in Collembola NLFAs than in soil PLFAs in rape especially at day 3 and 7, but with minor or no difference in grass and willow (Fig. 4).

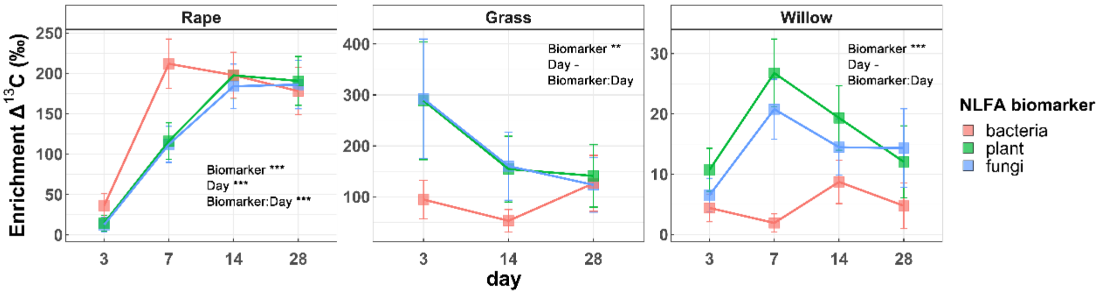

Figure 3. ^{13}C enrichment ($\Delta^{13}C$) in bacterial, fungal, and plant biomarker NLFAs of Collembola 3, 7, 14, 28 days after labeling in rape, grass, and willow; least square means ± SE; $-p < 0.10$, $^{**}p < 0.01$, $^{***}p < 0.001$.

The ^{13}C enrichment of fungal biomarkers in Collembola NLFAs declined in trend in grass, while showing a humped shape pattern in willow (Fig. 3), whereas the ^{13}C enrichment of bacterial biomarker NLFAs stayed relatively constant in grass and willow. This was similar to the dynamics of ^{13}C enrichment in soil PLFAs in grass and willow (Fig. S2). By contrast, in rape the dynamics of Collembola NLFAs only partially matched the dynamics of soil PLFAs. For instance, at days 3 – 14 the ^{13}C enrichment of the fungal biomarker increased in both Collembola NLFAs and soil PLFAs, although the differences between sampling days were not significant in soil PLFAs (Fig.3, Fig. S2). As the significance of the effects of sampling day on the ^{13}C enrichment of biomarker PLFAs was generally low, temporal changes should be interpreted with caution.

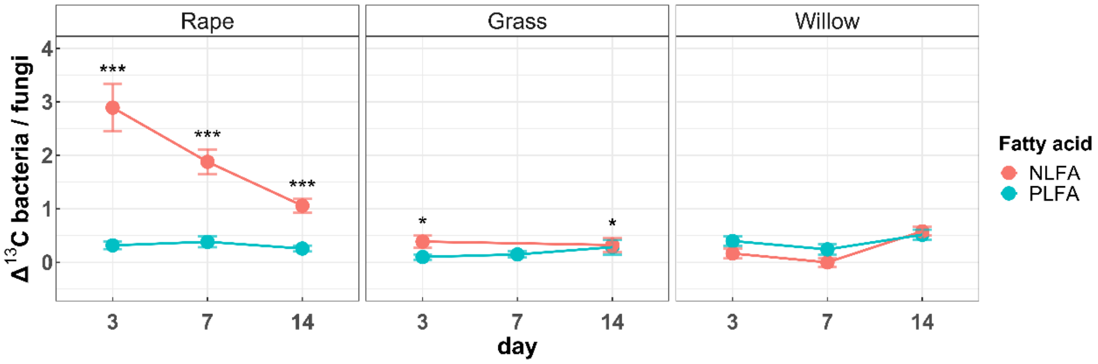

Figure 4. Ratio of ^{13}C enrichment (Δ^{13}C) between bacterial and fungal biomarkers in NLFAs and PLFAs 3, 7, 14 days after labeling in rape, grass, and willow; least square means ± SE; significant differences in values between NLFAs and PLFAs at individual sampling dates are indicated by *$p < 0.05$, ***$p < 0.001$.

4. Discussion

4.1 Incorporation of root-derived C into Collembola NLFAs

Despite Collembola are among the major microbivores in soil food webs, it is unknown to what extent trophic relationships between Collembola and microorganisms differ among cropping systems. Supporting our first hypothesis, the incorporation of root-derived C from bacterial and fungal channels into Collembola differed markedly between cropping systems, with more root-derived C channeled into the bacterial biomarker NLFAs in rape compared to grass and willow, where relatively more root-derived C was incorporated into the fungal biomarker NLFA.

Differential fluxes of root-derived C into Collembola among cropping systems can be attributed to changes in microbial community structure in the rhizosphere. The incorporation of ^{13}C into NLFAs of Collembola paralleled the incorporation into microbial PLFAs, suggesting that rhizosphere bacteria play a more important role in channeling root-derived C into Collembola in rape compared to grass and willow. Potentially, bacteria outcompete fungi by utilizing rhizodeposits of rape, as ^{13}C was incorporated more rapidly into bacterial biomarker NLFAs than into the fungal biomarker NLFA, and bacterial biomarker NLFAs were more enriched in ^{13}C at days 3-7 in rape. Previous studies in arable systems provided inconsistent results. While in a maize field substantially more root-derived C was channeled via fungi than via bacteria into invertebrates of higher trophic levels (Pausch et al., 2016b), in another arable system the bacterial channel was more pronounced than the fungal channel (Pausch et al., 2016a). In soil cultivated with leek, Gram-negative bacterial biomarker PLFAs were the most ^{13}C enriched and incorporated more root C than other PLFA biomarkers (Elfstrand et al., 2008). The conflicting results suggest that factors such as plant species, growth stage, and fertilizer application, strongly impact the incorporation of root C fluxes into bacterial and fungal channels in arable systems.

Oilseed rape is a species of the Brassicaceae that generally does not form mycorrhizal associations. Toxic compounds such as glucosinolates and their hydrolysate isothiocyanates in exudates of Brassicaceae significantly affect microbial communities, e.g. via inhibitory effects on arbuscular mycorrhizal fungi as well as on other fungi ("Biofumigation"; Kirkegaard and Sarwar, 1998; Okubo et al., 2016). Bacteria are generally more tolerant to these compounds than fungi (Smith and Kirkegaard, 2002) and this may explain why bacteria were considerably more abundant in the rhizosphere of oilseed rape compared to wheat (family Poaceae; Solaiman et al., 2007). In the present study, the concentration and proportion of bacterial biomarkers in soil PLFAs did not differ significantly between rape and grass, potentially because we sampled the bulk soil and not the rhizosphere soil. Management practices such as fertilizer application may also enhance the C flux into the bacterial energy channel and diminish the flux into the fungal channel (Denef et al., 2009; Hannula et al., 2017; Paterson et al., 2007). Interestingly, although root-derived C was incorporated at a slower rate into fungal than into bacterial biomarker NLFAs in Collembola, its incorporation into the fungal NLFA reached a similarly high level as into bacterial biomarker NLFAs at day 14 and then stayed constant, reflecting either incorporation of root exudates at different rates or transfer of C from dead bacterial cells into fungi. The abundance of hemiedaphic Collembola (*F. quadrioculata* and *P. notabilis*) in rape was at least twice as high as in grass and willow (Z. Li, unpubl. data); via grazing this high abundance may result in strong effects on rhizosphere bacteria and thereby facilitate the incorporation of bacterial C into fungi.

Fungi played a more dominant role in channeling root-derived C into Collembola in grass and willow than in rape as indicated by the generally lower B-to-F $^{13}C_{incorporation}$ ratio in Collembola in grass and willow than in rape, although the differences between rape and grass were not significant in epedaphic Collembola. In addition, the ^{13}C enrichment of the fungal biomarker was higher than that of bacterial biomarkers in Collembola NLFAs in grass and willow, but similar or lower than that of bacterial biomarkers in rape. Hence, the flux of root-derived C via the fungal channel was more pronounced in grass and willow than in rape. Previous studies in grassland and tree plantations also suggest that root-derived C is dominantly processed by the fungal community and only much later by bacteria (De Deyn et al., 2011; Denef et al., 2009; Esperschütz et al., 2009). Despite mycorrhizal fungi play a major role in the utilization of root-derived C and may be important for the channeling of root C into soil animals (Jonas et al., 2007; Ngosong et al., 2014), their importance for the diet of Collembola has been questioned (Bluhm et al., 2019; Caravaca and Ruess, 2014; Potapov and Tiunov, 2016). The NLFA 16:1ω5 is used as indicator for arbuscular mycorrhizal fungi (Olsson et al., 1999). In grass, the low proportion of the NLFA 16:1ω5 (< 1%) and the high ^{13}C incorporation into 18:2ω6,9 in Collembola NLFAs imply a dominant role of non-mycorrhizal fungi in channeling root-derived C into Collembola. This is consistent with saprotrophic fungi forming a significant fraction of microbial biomass (20–66 %) in the rhizosphere of grassland plants (Joergensen, 2000), and with saprotrophic or root pathogenic fungi being of higher food quality for Collembola compared to arbuscular mycorrhizal fungi (Larsen et al. 2008). Willows form both arbuscular mycorrhizal and ectomycorrhizal symbiosis (Püttsepp et al., 2004; van der Heijden and Vosatka, 2000). As the FA 18:2ω6,9 is present in both saprotrophic as well as ectomycorrhizal fungi, separating their contribution to the channeling of root C into Collembola using FA analysis is not possible.

Collembola switch diet according to the availability of resources (Eerpina et al., 2017; Endlweber et al., 2009; Scheunemann et al., 2015; Sechi et al., 2014). Since Collembola as generalist feeders rely on a wide range of diets (Scheu, 2002), it is reasonable to assume that they shift diet to feed on the most abundant microbial resources depending on cropping system. However, while changes in the relative importance of bacteria and fungi in the diet of Collembola (B-to-F ratio of NLFAs) paralleled those in the incorporation of root-derived C into soil PLFAs (B-to-F $^{13}C_{incorporation}$ ratio into PLFAs), the B-to-F ratio of PLFAs in soil differed markedly from the B-to-F ratio of NLFAs in Collembola. This suggests that Collembola preferentially fed on microorganisms utilizing rhizodeposits rather than microorganisms in bulk soil utilizing soil organic matter resources (Maboreke et al., 2017). Potentially, microorganisms incorporating root exudates are less substrate limited, resulting in higher activity and local biomass compared to those in bulk soil, making them a more palatable resource for Collembola (Buée et al., 2009; De Boer et al., 2005).

In rape, the relative importance of root-derived C in fungal and bacterial biomarkers, as indicated by their enrichment in ^{13}C, differed markedly between Collembola NLFAs and soil PLFAs, while the differences were minor in grass and willow. Potentially, Collembola NLFAs reflect the composition of microorganisms in the rhizosphere, while soil PLFAs mainly reflect microorganisms in bulk soil. Therefore, in the rhizosphere of rape, root C presumably is channeled mainly by bacteria, whereas in bulk soil it is predominantly channeled by fungi as fungal hyphae efficiently transfer root C from the rhizosphere to more distant soil environments (Buée et al., 2009; Kuzyakov, 2002). This may result in a more heterogeneous distribution of root C in soil, potentially reducing the incorporation of root C into the soil food web and thus explaining at least in part the slower increase in ^{13}C enrichment in Collembola NLFAs in rape than in grass and willow. Overall, the dynamics and magnitude of ^{13}C enrichment were more similar between Collembola NLFAs and soil PLFAs in grass and willow than in rape, indicating that the transfer of root-derived C from basal microbial resources to higher trophic levels in the food web is more efficient in a fungi-dominated than in a bacteria-dominated rhizosphere. Unfortunately, we did not analyze PLFAs in rhizosphere soil and thus cannot prove this assumption. In addition, other factors, such as growth stage, climatic conditions, assimilate transport in phloem, and mobilization of storage C, may also impact the flux of root C into soil food webs (Pausch and Kuzyakov, 2018, Kuzyakov and Gavrichkova 2010).

The ^{13}C enrichment of Collembola NLFAs was remarkably lower in willow than in grass and rape. This may be due to high amounts of unlabeled C in the phloem that diluted the signal of ^{13}C, leading to a lower content of ^{13}C in Collembola in willow compared to those in grass and rape. This is supported by the lower ^{13}C enrichment of biomarker PLFAs in willow than in grass and rape. Besides dilution effects, ^{13}C loss via respiration of aboveground parts or low belowground allocation of recent photosynthate C may also have contributed to the low ^{13}C enrichment of Collembola in willow. In the present study, data on hemiedaphic and euedaphic Collembola were lacking in willow, hence we cannot provide direct evidence that fungal resources also play a more important role in the incorporation of root-derived C and dietary nutrition for hemiedaphic and euedaphic Collembola in willow than in rape. However, a previous study also found fungi to be the dominant channel for root-derived C in a willow plantation (Elias et. al. 2017).

4.2 Incorporation of root-derived C into NLFAs of functional groups of Collembola

Dependence on bacterial and fungal resources markedly differed between functional groups of Collembola within the same cropping system. This is in line with previous studies pointing to trophic niche differentiation among functional groups of Collembola in forests, grasslands and arable systems (Chahartaghi et al., 2005; Ngosong et al., 2011; Ruess et al., 2007; Sechi et al., 2014). In contrast to our assumptions, the ^{13}C incorporation into biomarker NLFAs of epedaphic and euedaphic Collembola was similar, while hemiedaphic Collembola had a higher B-to-F $^{13}C_{incorporation}$ ratio in NLFAs, suggesting that they incorporated more root C from the bacterial channel compared to epedaphic and euedaphic species in the same cropping system. This was also mirrored in their NLFA composition, with a higher B-to-F ratio than in epedaphic and euedaphic species, pointing to more pronounced feeding on bacteria-associated resources. The composition of Collembola functional groups/species may influence the community composition of soil microorganisms and thus may impact soil ecological processes such as nutrient cycling and soil organic matter stabilization (Coulibaly et al., 2019; Erktan et al., 2020; Filser, 2002). Further research linking the differential food preferences of functional groups/species of Collembola and their regulatory effects on microbial community composition, especially in the presence of roots, is needed.

Interestingly, in euedaphic Collembola (*P. armata*) the ^{13}C incorporation and proportion of fungal and plant biomarker NLFAs was higher in grass than in rape, while this was not the case in epedaphic and hemiedaphic Collembola. This is in line with a study at the same sites, in which *P. armata* was more intensively labeled and more depleted in natural abundance of ^{13}C in grass compared to rape and willow, pointing to more pronounced consumption of roots or root associated resources, such as herbivorous nematodes or rhizosphere fungi in grassland (Li et al., 2020). Lemanski et al. (2014) also found predominance of root feeding in *P. armata* in grasslands. *P. armata* and other euedaphic species potentially switch their preference for root C and soil organic C according to the palatability of root resources (Crotty et al., 2011; Endlweber et al., 2009; Scheunemann et al., 2015).

5. Conclusions

Results of the present study demonstrate that the flux of root C into soil microbivores via the bacterial and fungal energy channel strongly varies between cropping systems, with higher incorporation of root-derived C from the bacterial channel in rape than in the grass and willow systems, where the fungal channel dominates. The differences in dietary composition of Collembola among cropping systems mainly reflects the change of microorganisms in the rhizosphere but not in bulk soil, suggesting that Collembola selectively feed on microorganisms in the rhizosphere rather than feeding in a generalist way on bulk soil resources. This suggests that the food quality and community composition in microbial hotspots are more important than the quantity of total microbial biomass in determining microbial food resources of soil microbivores. Within the same cropping system, functional groups of Collembola differ in the incorporation of root-derived C from different basal resources, with hemiedaphic Collembola incorporating more root C from the bacterial channel than epedaphic and euedaphic Collembola. The partitioning in utilization of microbial resources is an important mechanism allowing soil microbivore species to coexist locally.

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Reference

Anderson, T.A., Guthrie, E.A., Walton, B.T., 1993. Bioremediation in the rhizosphere. Environmental Science & Technology 27, 2630–2636. doi:10.1021/es00049a001

Bates, D., Maechler, M., Bolker, B., 2017. Lme4: Linear Mixed-effects Models Using S4 Classes (version 1.1-13). R Package. R.

Blem, C.R., 1976. Patterns of lipid storage and utilization in birds. Integrative and Comparative Biology 16, 671–684. doi:10.1093/icb/16.4.671

Bluhm, S.L., Potapov, A.M., Shrubovych, J., Ammerschubert, S., Polle, A., Scheu, S., 2019. Protura are unique: First evidence of specialized feeding on ectomycorrhizal fungi in soil invertebrates. BMC Ecology 19, 1–7. doi:10.1186/s12898-019-0227-y

Buée, M., De Boer, W., Martin, F., van Overbeek, L., Jurkevitch, E., 2009. The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Plant and Soil 321, 189–212. doi:10.1007/s11104-009-9991-3

Caravaca, F., Ruess, L., 2014. Arbuscular mycorrhizal fungi and their associated microbial community modulated by Collembola grazers in host plant free substrate. Soil Biology and Biochemistry 69, 25–33. doi:10.1016/J.SOILBIO.2013.10.032

Chahartaghi, M., Langel, R., Scheu, S., Ruess, L., 2005. Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biology and Biochemistry 37, 1718–1725. doi:10.1016/J.SOILBIO.2005.02.006

Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P., Evershed, R.P., 2004. Lipid content and carbon assimilation in Collembola: implications for the use of compound-specific carbon isotope analysis in animal dietary studies. Oecologia 139, 325–335. doi:10.1007/s00442-003-1422-1

Chaudhary, D.R., Dick, R.P., 2016. Linking microbial community dynamics associated with rhizosphere carbon flow in a biofuel crop (Jatropha curcas L.). Communications in Soil Science and Plant Analysis 47, 1193–1206. doi:10.1080/00103624.2016.1166243

Coulibaly, S.F.M., Winck, B., Akpa-Vinceslas, M., Mignot, L., Gras, M. Le, Forey, E., Chauvat, M., 2019. Functional assemblages of Collembola determine soil microbial communities and associated functions. Frontiers in Environmental Science 7, 52. doi:10.3389/fenvs.2019.00052

Crotty, F.V., Blackshaw, R.P., Murray, P.J., 2011. Tracking the flow of bacterially derived ^{13}C and ^{15}N through soil faunal feeding channels. Rapid Communications in Mass Spectrometry 25, 1503–1513. doi:10.1002/rcm.4945

De Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: Impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29, 795–811 . doi:10.1016/j.femsre.2004.11.005

De Deyn, G.B., Quirk, H., Oakley, S., Ostle, N., Bardgett, R.D., 2011. Rapid transfer of photosynthetic carbon through the plant-soil system in differently managed species-rich grasslands. Biogeosciences 8, 1131–1139. doi:10.5194/bg-8-1131-2011

Denef, K., Bubenheim, H., Lenhart, K., Vermeulen, J., Van Cleemput, O., Boeckx, P., Müller, C., 2007. Community shifts and carbon translocation within metabolically-active rhizosphere microorganisms in grasslands under elevated CO_2. Biogeosciences 4, 769–779. doi:10.5194/bg-4-769-2007

Denef, K., Roobroeck, D., Manimel Wadu, M.C.W., Lootens, P., Boeckx, P., 2009. Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. Soil Biology and Biochemistry 41, 144–153. doi:10.1016/j.soilbio.2008.10.008

Dennis, P.G., Miller, A.J., Hirsch, P.R., 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiology Ecology 72, 313–327. doi:10.1111/j.1574-6941.2010.00860.x

Digel, C., Curtsdotter, A., Riede, J., Klarner, B., Brose, U., 2014. Unravelling the complex structure of forest soil food webs: Higher omnivory and more trophic levels. Oikos 123, 1157–1172. doi:10.1111/oik.00865

Eerpina, R., Boiteau, G., Lynch, D.H., 2017. Collembola diet switching in the presence of maize roots varies with species. Canadian Journal of Soil Science 97, 171–177. doi:10.1139/cjss-2016-0057

Elfstrand, S., Lagerlöf, J., Hedlund, K., Mårtensson, A., 2008. Carbon routes from decomposing plant residues and living roots into soil food webs assessed with ^{13}C labelling. Soil Biology and Biochemistry 40, 2530–2539. doi:10.1016/j.soilbio.2008.06.013

Elias, D.M.O., Rowe, R.L., Pereira, M.G., Stott, A.W., Barnes, C.J., Bending, G.D., McNamara, N.P., 2017. Functional differences in the microbial processing of recent assimilates under two contrasting perennial bioenergy plantations. Soil Biology and Biochemistry 114, 248–262. doi:10.1016/j.soilbio.2017.07.026

Endlweber, K., Ruess, L., Scheu, S., 2009. Collembola switch diet in presence of plant roots thereby functioning as herbivores. Soil Biology and Biochemistry 41, 1151–1154. doi:10.1016/j.soilbio.2009.02.022

Erktan, A., Rillig, M., Carminati, A., Jousset, A., Scheu, S., 2020. Bacterial and fungal predator – prey interactions modulate soil aggregation. Biogeosciences Discussions 1–31. doi:10.5194/bg-2020-

48

Esperschütz, J., Gattinger, A., Buegger, F., Lang, H., Munch, J.C., Schloter, M., Winkler, J.B., 2009. A continuous labelling approach to recover photosynthetically fixed carbon in plant tissue and rhizosphere organisms of young beech trees (Fagus sylvatica L.) using ^{13}C depleted CO_2. Plant and Soil 323, 21–29. doi:10.1007/s11104-009-9998-9

Ferlian, O., Klarner, B., Langeneckert, A.E., Scheu, S., 2015. Trophic niche differentiation and utilisation of food resources in collembolans based on complementary analyses of fatty acids and stable isotopes. Soil Biology and Biochemistry 82, 28–35. doi:10.1016/J.SOILBIO.2014.12.012

Filser, J., 2002. The role of Collembola in carbon and nitrogen cycling in soil. Pedobiologia 46, 234–245. doi:10.1016/S0031-4056(04)70139-0

Frostegård, Å., Bååth, E., Tunlio, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25, 723–730. doi:10.1016/0038-0717(93)90113-P

Garrett, C.J., Crossley, D.A., Coleman, D.C., Hendrix, P.F., Kisselle, K.W., Potter, R.L., 2001. Impact of the rhizosphere on soil microarthropods in agroecosystems on the Georgia piedmont. Applied Soil Ecology 16, 141–148. doi:10.1016/S0929-1393(00)00114-1

Gunina, A., Dippold, M.A., Glaser, B., Kuzyakov, Y., 2014. Fate of low molecular weight organic substances in an arable soil: From microbial uptake to utilisation and stabilisation. Soil Biology and Biochemistry 77, 304–313. doi:10.1016/j.soilbio.2014.06.029

Hannula, S.E., Morriën, E., De Hollander, M., Van Der Putten, W.H., Van Veen, J.A., De Boer, W., 2017. Shifts in rhizosphere fungal community during secondary succession following abandonment from agriculture. ISME Journal 11, 2294–2304. doi:10.1038/ismej.2017.90

Hartmann, L., Lamersdorf, N., 2015. Site conditions, initial growth and nutrient and litter cycling of newly installed short rotation coppice and agroforestry systems, in: Bioenergy from Dendromass for the Sustainable Development of Rural Areas. pp. 121–138. doi:10.1002/9783527682973.ch10

Haubert, D., Birkhofer, K., Fliessbach, A., Gehre, M., Scheu, S., Ruess, L., 2009. Trophic structure and major trophic links in conventional versus organic farming systems as indicated by carbon stable isotope ratios of fatty acids. OIKOS 118, 1579–1589. doi:10.1111/j.1600-0706.2009.17587.x

Haubert, D., Häggblom, M.M., Scheu, S., Ruess, L., 2004. Effects of fungal food quality and starvation on the fatty acid composition of Protaphorura fimata (Collembola). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 138, 41–52. doi:10.1016/j.cbpc.2004.02.009

Högberg, M.N., Briones, M.J.I., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton, B., Hurry, V., Linder, S., Näsholm, T., Högberg, P., 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. New Phytologist 187, 485–493. doi:10.1111/j.1469-8137.2010.03274.x

Hopkin, S.P., 2007. A Key to the Collembola (Springtails) of Britain and Ireland. Field Studies Council, Shrewsbury.

Joergensen, R.G., 2000. Ergosterol and microbial biomass in the rhizosphere of grassland soils. Soil Biology and Biochemistry 32, 647–652. doi:10.1016/S0038-0717(99)00191-1

Jonas, J.L., Wilson, G.W.T., White, P.M., Joern, A., 2007. Consumption of mycorrhizal and saprophytic fungi by Collembola in grassland soils. Soil Biology and Biochemistry 39, 2594–2602. doi:10.1016/j.soilbio.2007.05.004

Kempson, D., Lloyd, M., Ghelardi, R., 1963. A new extractor for woodland litter. Pedobiologia 3, 1–21.

Kirkegaard, J.A., Sarwar, M., 1998. Biofumigation potential of brassicas. Plant and Soil 201, 71–89. doi:10.1023/A:1004364713152

Klironomos, J.N., Kendrick, B., 1995. Relationships among microarthropods, fungi, and their environment. Plant and Soil 170, 183–197. doi:10.1007/BF02183066

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2016. lmerTest: Tests for random and fixed effects for linear mixed effect models. R Package Version. doi:http://CRAN.R-project.org/package=lmerTest

Kuzyakov, Y., 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science 165, 382–396. doi:10.1002/1522-2624(200208)165:4<382::AID-JPLN382>3.0.CO;2-#

Kuzyakov, Y., Gavrichkova, O., 2010. Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. Global Change Biology 16, 3386–3406.

doi:10.1111/j.1365-2486.2010.02179.x

Larsen, J., Johansen, A., Larsen, S.E., Heckmann, L.H., Jakobsen, I., Krogh, P.H., 2008. Population performance of collembolans feeding on soil fungi from different ecological niches. Soil Biology and Biochemistry 40, 360–369. doi:10.1016/j.soilbio.2007.08.016

Larsen, T., Gorissen, A., Krogh, P.H., Ventura, M., Magid, J., 2007. Assimilation dynamics of soil carbon and nitrogen by wheat roots and Collembola. Plant and Soil 295, 253–264. doi:10.1007/s11104-007-9280-y

Lenth, R.V. (2019). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.3.5.1. https://CRAN.R-project.org/package=emmeans

Lemanski, K., Scheu, S., 2014. Fertilizer addition lessens the flux of microbial carbon to higher trophic levels in soil food webs of grassland. Oecologia 176, 487–496. doi:10.1007/s00442-014-3037-0

Li, Z., Scheunemann, N., Potapov, A.M., Shi, L., Pausch, J., Scheu, S., Pollierer, M.M., 2020. Incorporation of root-derived carbon into soil microarthropods varies between cropping systems. Biology and Fertility of Soils 56, 839–851. doi:10.1007/s00374-020-01467-8

Liu, S., Behm, J.E., Chen, J., Fu, S., He, X., Hu, J., Schaefer, D., Gan, J., Yang, X., 2016. Functional redundancy dampens the trophic cascade effect of a web-building spider in a tropical forest floor. Soil Biology and Biochemistry 98, 22–29. doi:10.1016/J.SOILBIO.2016.03.020

Maboreke, H.R., Graf, M., Grams, T.E.E., Herrmann, S., Scheu, S., Ruess, L., 2017. Multitrophic interactions in the rhizosphere of a temperate forest tree affect plant carbon flow into the belowground food web. Soil Biology and Biochemistry 115, 526–536. doi:10.1016/j.soilbio.2017.09.002

Ngosong, C., Gabriel, E., Ruess, L., 2014. Collembola grazing on arbuscular mycorrhiza fungi modulates nutrient allocation in plants. Pedobiologia 57, 171–179. doi:10.1016/j.pedobi.2014.03.002

Ngosong, C., Raupp, J., Richnow, H.H., Ruess, L., 2011. Tracking Collembola feeding strategies by the natural C-13 signal of fatty acids in an arable soil with different fertilizer regimes. Pedobiologia 54, 225–233. doi:10.1016/j.pedobi.2011.02.004

Oelbermann, K., Langel, R., Scheu, S., 2008. Utilization of prey from the decomposer system by generalist predators of grassland. Oecologia 155, 605–617. doi:10.1007/s00442-007-0927-4

Okubo, A., Matsusaka, M., Sugiyama, S., 2016. Impacts of root symbiotic associations on interspecific variation in sugar exudation rates and rhizosphere microbial communities: a comparison among four plant families. Plant and Soil 399, 345–356. doi:10.1007/s11104-015-2703-2

Olsson, P.A., Thingstrup, I., Jakobsen, I., Bååth, E., 1999. Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field. Soil Biology and Biochemistry 31, 1879–1887. doi:10.1016/S0038-0717(99)00119-4

Ostle, N., Briones, M.J.I., Ineson, P., Cole, L., Staddon, P., Sleep, D., 2007. Isotopic detection of recent photosynthate carbon flow into grassland rhizosphere fauna. Soil Biology and Biochemistry 39, 768–777. doi:10.1016/j.soilbio.2006.09.025

Paterson, E., Gebbing, T., Abel, C., Sim, A., Telfer, G., 2007. Rhizodeposition shapes rhizosphere microbial community structure in organic soil. New Phytologist 173, 600–610. doi:10.1111/j.1469-8137.2006.01931.x

Pausch, J., Hofmann, S., Scharroba, A., Kuzyakov, Y., Ruess, L., 2016a. Fluxes of root-derived carbon into the nematode micro-food web of an arable soil. Food Webs 9, 32–38. doi:10.1016/J.FOOWEB.2016.05.001

Pausch, J., Kramer, S., Scharroba, A., Scheunemann, N., Butenschoen, O., Kandeler, E., Marhan, S., Riederer, M., Scheu, S., Kuzyakov, Y., Ruess, L., 2016b. Small but active - pool size does not matter for carbon incorporation in below-ground food webs. Functional Ecology 30, 479–489. doi:10.1111/1365-2435.12512

Pausch, J., Kuzyakov, Y., 2018. Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale. Global Change Biology 24, 1–12. doi:10.1111/gcb.13850

Pollierer, M.M., Dyckmans, J., Scheu, S., Haubert, D., 2012. Carbon flux through fungi and bacteria into the forest soil animal food web as indicated by compound-specific ^{13}C fatty acid analysis. Functional Ecology 26, 978–990. doi:10.1111/j.1365-2435.2012.02005.x

Pollierer, M.M., Langel, R., Körner, C., Maraun, M., Scheu, S., 2007. The underestimated importance of belowground carbon input for forest soil animal food webs. Ecology Letters 10, 729–736. doi:10.1111/j.1461-0248.2007.01064.x

Potapov, A.M., Semenina, E.E., Korotkevich, A.Y., Kuznetsova, N.A., Tiunov, A.V., 2016. Connecting taxonomy and ecology: Trophic niches of collembolans as related to taxonomic identity and life forms. Soil Biology and Biochemistry 101, 20–31. doi:10.1016/j.soilbio.2016.07.002

Potapov, A.M., Tiunov, A.V, 2016. Stable isotope composition of mycophagous collembolans versus mycotrophic plants: Do soil invertebrates feed on mycorrhizal fungi? Soil Biology and Biochemistry 93, 115–118. doi:10.1016/j.soilbio.2015.11.001

Püttsepp, Ü., Rosling, A., Taylor, A.F.S., 2004. Ectomycorrhizal fungal communities associated with Salix viminalis L. and S. dasyclados Wimm. clones in a short-rotation forestry plantation. Forest Ecology and Management 196, 413–424. doi:10.1016/j.foreco.2004.04.003

R Core Team, 2016. R Development Core Team. R: A Language and Environment for Statistical Computing 55, 275–286. doi:http://www.R-project.org

Richter, F., Döring, C., Jansen, M., Panferov, O., Spank, U., Bernhofer, C., 2015. How to predict hydrological effects of local land use change: How the vegetation parameterisation for short rotation coppices influences model results. Hydrology and Earth System Sciences 19, 3457–3474. doi:10.5194/hess-19-3457-2015

Ruess, L., Chamberlain, P.M., 2010. The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biology and Biochemistry 42, 1898–1910. doi:10.1016/J.SOILBIO.2010.07.020

Ruess, L., Häggblom, M.M., García Zapata, E.J., Dighton, J., 2002. Fatty acids of fungi and nematodes—possible biomarkers in the soil food chain? Soil Biology and Biochemistry 34, 745–756. doi:10.1016/S0038-0717(01)00231-0

Ruess, L., Müller-Navarra, D.C., 2019. Essential biomolecules in food webs. Frontiers in Ecology and Evolution 7, 269. doi:10.3389/fevo.2019.00269

Ruess, L., Schütz, K., Migge-Kleian, S., Häggblom, M.M., Kandeler, E., Scheu, S., 2007. Lipid composition of Collembola and their food resources in deciduous forest stands—Implications for feeding strategies. Soil Biology and Biochemistry 39, 1990–2000. doi:10.1016/J.SOILBIO.2007.03.002

Ruf, A., Kuzyakov, Y., Lopatovskaya, O., 2006. Carbon fluxes in soil food webs of increasing complexity revealed by ^{14}C labelling and ^{13}C natural abundance. Soil Biology and Biochemistry 38, 2390–2400. doi:10.1016/J.SOILBIO.2006.03.008

Scheu, S., 2002. The soil food web: structure and perspectives. European Journal of Soil Biology 38, 11–20. doi:10.1016/S1164-5563(01)01117-7

Scheunemann, N., Digel, C., Scheu, S., Butenschoen, O., 2015. Roots rather than shoot residues drive soil arthropod communities of arable fields. Oecologia 179, 1135–1145. doi:10.1007/s00442-015-3415-2

Scheunemann, N., Pausch, J., Digel, C., Kramer, S., Scharroba, A., Kuzyakov, Y., Kandeler, E., Ruess, L., Butenschoen, O., Scheu, S., 2016. Incorporation of root C and fertilizer N into the food web of an arable field: Variations with functional group and energy channel. Food Webs 9, 39–45. doi:10.1016/J.FOOWEB.2016.02.006

Sechi, V., D'Annibale, A., Ambus, P., Sárossy, Z., Krogh, P.H., Eriksen, J., Holmstrup, M., 2014. Collembola feeding habits and niche specialization in agricultural grasslands of different composition. Soil Biology and Biochemistry 74, 31–38. doi:10.1016/j.soilbio.2014.02.019

Smith, B.J., Kirkegaard, J.A., 2002. In vitro inhibition of soil microorganisms by 2-phenylethyl isothiocyanate. Plant Pathology 51, 585–593. doi:10.1046/j.1365-3059.2002.00744.x

Solaiman, Z., Marschner, P., Wang, D., Rengel, Z., 2007. Growth, P uptake and rhizosphere properties of wheat and canola genotypes in an alkaline soil with low P availability. Biology and Fertility of Soils 44, 143–153. doi:10.1007/s00374-007-0188-8

Tariq, A., Gunina, A., Lamersdorf, N., 2018. Initial changes in soil properties and carbon sequestration potential under monocultures and short-rotation alley coppices with poplar and willow after three years of plantation. Science of The Total Environment 634, 963–973. doi:10.1016/J.SCITOTENV.2018.03.391

van der Heijden, E.W., Vosatka, M., 2000. Mycorrhizal associations of Salix repens L. communities in succession of dune ecosystems. Canadian Journal of Botany 77, 1833–1841. doi:10.1139/b99-178

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

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, 111–129. doi:10.1007/s003740050533

Zieger, S.L., Ammerschubert, S., Polle, A., Scheu, S., 2017. Root-derived carbon and nitrogen from beech and ash trees differentially fuel soil animal food webs of deciduous forests. PLoS ONE 12. doi:10.1371/journal.pone.0189502

Supporting information

Table S1. ^{13}C incorporation (^{13}C), proportion, ratio of proportion (B-to-F ratio) and ratio of ^{13}C incorporation (B-to-F $^{13}C_{incorporation}$ ratio) between bacterial and fungal biomarker NLFAs of Collembola species in rape, grass, and willow.

Cropping system	**Functional group**	**Species**	**Bacterial**		**Fungi**		**B-to-F ratio**	**B-to-F $^{13}C_{incorporation}$ ratio**
			^{13}C (%)	**Proportion (%)**	**^{13}C (%)**	**Proportion (%)**		
Rape	Epedaphic	*L. cyaneus*	8.50 ± 1.90a	6.49 ± 1.06a	12.77 ± 2.33b	16.54 ± 1.69c	0.39 ± 0.12a	0.63 ± 0.22a
	Euedaphic	*P. armata*	14.72 ± 2.27b	10.64 ± 1.21b	9.57 ± 1.83ab	12.97 ± 1.33bc	0.82 ± 0.15b	1.44 ± 0.44ab
	Hemiedaphic	*P. notabilis*	17.09 ± 2.73b	13.77 ± 1.57b	7.15 ± 1.76a	6.92 ± 1.11a	2.24 ± 0.29c	2.51 ± 0.89bc
	Hemiedaphic	*F. quadrioculata*	27.94 ± 2.87c	22.73 ± 1.57c	10.04 ± 1.71ab	12.31 ± 1.15b	1.82 ± 0.18c	2.65 ± 0.71c
Grass	Epedaphic	*L. cyaneus*	3.66 ± 1.01a	6.51 ± 1.17a	17.62 ± 2.21a	14.61 ± 1.75b	0.45 ± 0.06a	0.21 ± 0.15a
	Epedaphic	*O. villosa*	2.75 ± 1.24a	5.41 ± 1.20a	21.97 ± 3.49a	20.93 ± 2.36c	0.27 ± 0.08a	0.30 ± 0.16a
	Euedaphic	*P. armata*	2.86 ± 1.03a	6.09 ± 1.20a	19.50 ± 2.68a	18.74 ± 2.10bc	0.31 ± 0.06a	0.29 ± 0.15a
	Hemiedaphic	*F. quadrioculata*	13.76 ± 2.25b	14.9 ± 1.87b	12.82 ± 2.18a	10.43 ± 1.56a	1.33 ± 0.06b	1.13 ± 0.15b
Willow	Epedaphic	*L. cyaneus*	1.09 ± 0.01a	4.94 ± 0.61a	15.50 ± 2.57a	19.43 ± 1.21a	0.27 ± 0.02a	0.09 ± 0.04a
	Epedaphic	*O. villosa*	1.33 ± 1.12a	5.87 ± 0.99a	17.00 ± 0.04a	17.84 ± 1.72a	0.33 ± 0.04a	0.15 ± 0.05a

Least square means ± SE; values with different letters are significantly different between Collembola species in the same cropping system ($p < 0.05$; Tukey's HSD test).

Table S2. Linear mixed effect model table (type III error) on the effect of functional group, sampling date, biomarker, and their interaction on the ^{13}C enrichment of Collembola NLFAs.

Cropping system	Factor	Sum of square	Mean square	Df1	Df2	F-value	*p*-value
Rape	Day	69.54	23.18	3	20.80	**24.02**	< **0.001**
	Biomarker	30.26	15.13	2	46.00	**15.68**	< **0.001**
	Functional group	10.56	5.28	2	21.77	**5.47**	**0.012**
	Day : Biomarker	73.94	12.32	6	46.00	**12.77**	< **0.001**
	Day : Functional group	8.99	1.80	5	20.96	1.86	0.144
	Biomarker : Functional group	2.89	0.72	4	46.00	0.75	0.563
	Day : Biomarker : Functional group	20.07	2.01	10	46.00	**2.08**	**0.046**
Grass	Day	63.10	31.55	2	3.04	4.48	0.124
	Biomarker	86.86	43.43	2	8.00	**6.16**	**0.024**
	Functional group	17.81	8.90	2	2.00	1.26	0.442
	Day : Biomarker	74.35	18.59	4	8.00	2.64	0.113
	Day : Functional group	0.01	0.01	1	3.05	0.00	0.978
	Biomarker : Functional group	51.06	12.77	4	8.00	1.81	0.220
	Day : Biomarker : Functional group	31.33	15.665	2	8.00	2.22	0.171
Willow	Day	238.89	79.63	3	10.60	1.70	0.226
	Biomarker	1192.60	596.30	2	24.00	**12.73**	<**0.001**
	Day : Biomarker	686.73	114.46	6	24.00	2.44	0.055

Significant effects are given in bold; Df1, numerator degree of freedom, Df2, denominator degree of freedom.

Table S3. Linear mixed effect model table (type III error) on the effect of functional group, cropping system, biomarker, and their interaction on the incorporation of ^{13}C into Collembola NLFAs.

Functional group	Factor	Sum of square	Mean square	Df1	Df2	F-value	*p*-value
Hemiedaphic / euedaphic	Cropping system	0.00	0.00	1	20.60	0.19	0.668
	Biomarker	0.20	0.10	2	20.47	**44.09**	**<0.001**
	Functional group	0.00	0.00	1	1.03	0.14	0.769
	Cropping system : Biomarker	0.06	0.03	2	20.47	**13.37**	**<0.001**
	Cropping system : Functional group	0.01	0.01	1	93.78	2.88	0.093
	Biomarker : Functional group	0.18	0.09	2	93.78	**38.91**	**<0.001**
	Cropping system : Biomarker : Functional group	0.01	0.01	2	93.78	**3.18**	**0.046**
epedaphic	Cropping system	0.01	0.00	2	11.81	0.36	0.706
	Biomarker	2.35	1.18	2	63.65	**129.70**	**< 0.001**
	Cropping system : Biomarker	0.12	0.03	4	63.65	**3.28**	**0.017**

Significant effects are in bold; Df1, numerator degree of freedom, Df2, denominator degree of freedom.

Table S4. The incorporation of ^{13}C (%) into bacterial, fungal, and plant biomarkers and the ratio of ^{13}C incorporation between bacterial and fungal biomarkers in soil PLFAs 1, 3, 7, 14 days after labeling in rape, grass, and willow.

Cropping system	Day	Bacteria	Fungi	Plant	Bacteria/Fungi
Rape	1	28.30 ± 2.91a	8.24 ± 2.91a	11.82 ± 2.91ab	2.29 ± 0.71a
Rape	3	26.37 ± 2.96a	10.20 ± 2.96a	18.09 ± 2.96b	2.56 ± 0.41a
Rape	7	29.47 ± 3.15a	10.35 ± 3.15a	14.29 ± 3.15b	3.74 ± 0.50a
Rape	14	43.38 ± 2.96b	13.57 ± 2.96a	3.58 ± 2.96a	2.14 ± 0.41a
Grass	1	20.18 ± 2.94a	11.01 ± 2.94a	9.53 ± 2.94ab	1.22 ± 0.50a
Grass	3	18.25 ± 3.18a	12.97 ± 3.18a	15.79 ± 3.18b	1.66 ± 0.50a
Grass	7	21.34 ± 2.99a	13.13 ± 2.99a	11.99 ± 2.99b	1.53 ± 0.41a
Grass	14	35.25 ± 3.18b	16.34 ± 3.18a	1.28 ± 3.18a	4.12 ± 0.50b
Willow	1	21.24 ± 2.88a	14.13 ± 2.88a	8.56 ± 2.88ab	1.10 ± 0.41a
Willow	3	19.32 ± 2.94a	16.10 ± 2.94a	14.82 ± 2.94b	1.37 ± 0.50a
Willow	7	22.41 ± 2.95a	16.25 ± 2.95a	11.02 ± 2.95b	1.18 ± 0.41a
Willow	14	36.32 ± 2.94 b	19.46 ± 2.94a	0.31 ± 2.94a	2.09 ± 0.41a

Least square means ± SE; values of different sampling day with the different letters are significantly different in the same biomarker and cropping system ($p < 0.05$; Tukey's HSD test).

Table S5. The ratio of ^{13}C incorporation between bacterial and fungal biomarkers in Collembola NLFAs (epedaphic/hemiedaphic/euedaphic) 3, 7, 14, 28 days after labeling in rape, grass, and willow.

Cropping system	Day	epedaphic	hemiedaphic	euedaphic
Rape	3		2.84 ± 0.48ab	3.70 ± 0.88b
	7	1.97 ± 0.67c	3.57 ± 0.46b	3.36 ± 1.14b
	14	0.57 ± 0.14b	2.50 ± 0.32ab	0.51 ± 0.17a
	28	0.16 ± 0.06a	1.80 ± 0.27a	0.61 ± 0.12a
Grass	3	0.05 ± 0.03a	0.94 ± 0.45a	0.18 ± 0.08a
	14	0.15 ± 0.07a		0.12 ± 0.08a
	28	0.49 ± 0.23a	1.45 ± 0.97a	
Willow	3	0.10 ± 0.08a		
	7	0.02 ± 0.02a		
	14	0.15 ± 0.13a		
	28	0.06 ± 0.07a		

Least square means ± SE; values with different letters are significantly different between sampling days in the same functional group and cropping system ($p < 0.05$; Tukey's HSD test).

Table S6. Linear mixed effect model table (type III error) on the effect of functional group, cropping system, and their interaction on the ratio of ^{13}C incorporation between bacterial and fungal biomarkers (B-to-F $^{13}C_{incorporation}$ ratio), and on the ratio between bacterial and fungal biomarkers (B-to-F ratio) in Collembola NLFAs in rape and grass.

Functional group	Factor	Sum of square	Mean square	Df1	Df2	F-value	*p*-value
B-to-F $^{13}C_{incorporation}$ ratio	Functional group	2.28	1.14	2	36	**17.69**	**< 0.001**
	Cropping system	0.49	0.49	1	5	**7.60**	**0.03**
	Functional group : Cropping system	0.15	0.08	2	36	1.17	0.32
B-to-F ratio	Functional group	0.75	0.37	2	10	**23.20**	**< 0.001**
	Cropping system	0.12	0.12	1	36	**7.63**	**0.008**
	Cropping system : Functional group	0.10	0.05	2	36	3.16	0.055

Significant effects are in bold; Df1, numerator degree of freedom, Df2, denominator degree of freedom.

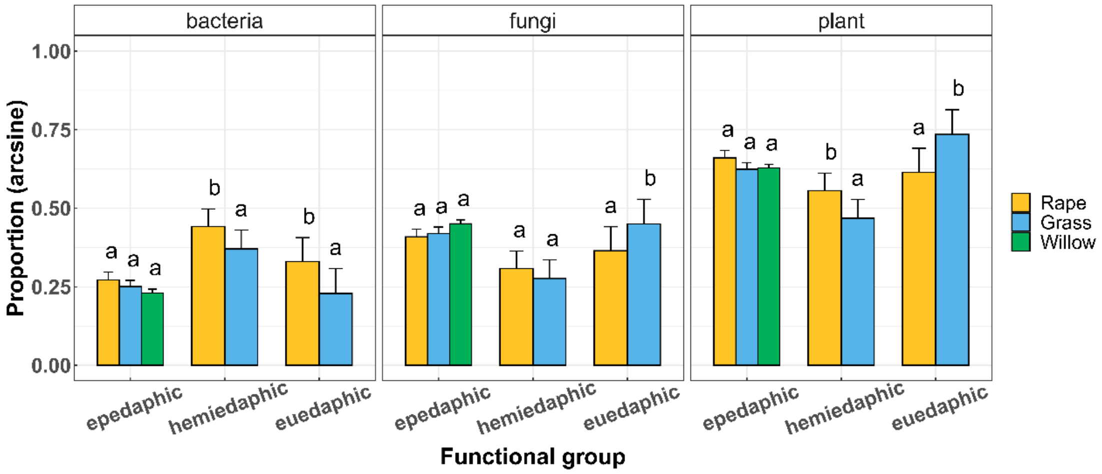

Figure S1. Proportion of bacterial, fungal, and plant biomarkers in NLFAs of Collembola functional groups (epedaphic, hemiedaphic, and euedaphic) in rape, grass, and willow; least square means ± SE; values of different cropping systems with different letters are significantly different in the same functional group and biomarker NLFAs ($p < 0.05$; Tukey's HSD test). Data are arcsine transformed.

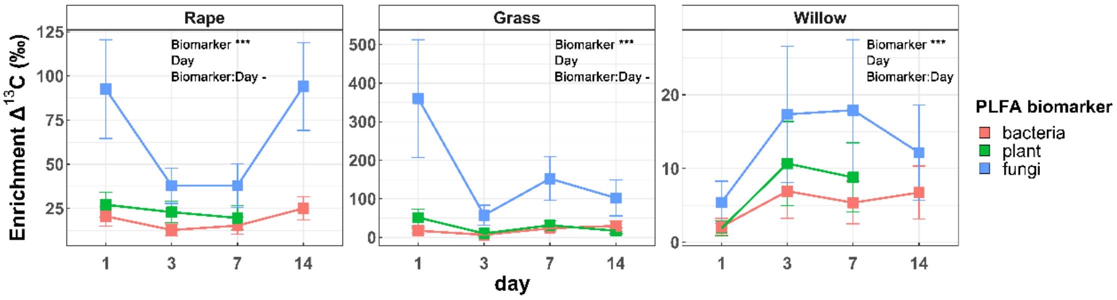

Figure S2. ^{13}C enrichment ($\Delta^{13}C$) in bacterial, fungal, and plant biomarkers in soil PLFAs 1, 3, 7, 14 days after labeling in rape, grass, and willow; least square means ± SE; -$p < 0.10$, ***$p < 0.001$.

Chapter 4

Amino acid isotopes in functional assemblages of microarthropods reveal the influence of vertical heterogeneity and root energy supply on trophic interactions in soil food webs

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Abstract

The vertical heterogeneity of trophic interactions in soil food webs in temperate forest systems and its response to deprivation of root energy supply are poorly understood. In the present study, trophic niches of Collembola functional groups (epedaphic, hemiedaphic and euedaphic) were analyzed using compound specific isotope analysis (CSIA) of carbon and nitrogen in amino acids (AAs) in a root-trenching experiment. The δ^{15}N values of phenylalanine, serving as proxy for nitrogen (N) resource, were higher in euedaphic Collembola than in ep-/hemiedaphic Collembola, suggesting that euedaphic Collembola mainly utilized N from processed organic substrate in deeper soil, while leaf litter was the dominant N source for ep-/hemiedaphic Collembola. The trophic position calculated from CSIA (TP_{CSIA}) of euedaphic Collembola was constantly higher than that of epedaphic Collembola, potentially due to (1) more intensive feeding on microbial resources from old soil organic matter that has been subject to more trophic transfers, (2) higher uptake of animal resources, such as microfauna or animal carcass. Both reflects a higher number of trophic transfers in the soil than in the litter layer. For the first time, we found evidence that the deprivation of root energy supply decreases the TP_{CSIA} of soil omnivores, suggesting that, in line with the 'productivity hypothesis', root derived C increases the number of trophic transfers and food chain length in soil food webs. However, effects varied among functional groups of Collembola in different forest stands, indicating that the association between root energy supply and trophic interactions is mediated by vertical resource heterogeneity, regional conditions and feeding strategies of consumers. Finally, our results were in line with recent studies, suggesting that saprotrophic microorganisms, especially saprotrophic fungi, are the predominant resources for Collembola, whereas the contribution of mycorrhizal fungi and plants is subordinate. Overall, by using the CSIA of AAs, our study provides novel information on trophic niches of microarthropods in forest soils, extending our

understanding of the influences of vertical heterogeneity and root energy supply on trophic interactions in soil food webs.

Key words: *amino acid CSIA;* ^{13}*C fingerprinting; Collembola; functional group; mycorrhizal fungi; root trenching; saprotrophic microorganisms; trophic position*

1. Introduction

Soil food webs are strongly compartmentalized due to the vertical heterogeneity of available resources, environmental conditions and physical soil structure (Okuzaki et al., 2009; Stouffer and Bascompte, 2011). Collembola are among the most widespread and abundant microarthropods in soils. They occupy a central position in soil food webs, channeling carbon (C) and nutrients from basal resources, including plants, bacteria and fungi, to predators at higher trophic levels (Pollierer et al., 2012). Collembola species with contrasting life forms are assigned to different functional groups: epedaphic (surface and upper litter layer dwelling), euedaphic (soil dwelling) and hemiedaphic (intermediate) Collembola (Faber, 1991; Potapov et al., 2016). Investigating the trophic niche of different functional groups of Collembola may provide new insights into the structural heterogeneity of soil food webs. However, only few studies have investigated Collembola at functional assemblage level and little is known about the factors affecting the trophic niche of different functional groups of Collembola (Coulibaly et al., 2019).

Soil food webs in forests are based on different resources, i.e. plant litter, soil organic matter and root-derived resources (mainly root exudates). Unlike plant detritus with a relatively high content of recalcitrant compounds, root-derived resources mainly comprise low-molecular-weight compounds such as glucose, organic acids, lipids and amino acids, and are thus preferentially utilized by microorganisms and rapidly transferred to higher trophic levels in soil food webs (Anderson et al., 1993; Pollierer et al., 2007; Buée et al., 2009). In forest ecosystems, resource manipulation experiments, such as root-trenching and tree girdling, have been widely used to study the impacts of root-derived energy supply on soil microbial and faunal communities in respects of biomass and community composition (Siira-Pietikäinen et al., 2001; Brant et al., 2006; Li et al., 2009). However, knowledge on how deprivation of root energy supply influences trophic niches of soil microarthropods is lacking.

Root energy supply may affect the trophic niche of Collembola in two ways, i.e. by changing the trophic position (TP) and the utilization of basal resources. The ‘productivity hypothesis’ suggests that food chains should increase in length as productivity and the availability of resources increases (Pimm, 1982; Thompson and Townsend, 2005). This is caused by the high inefficiency of energy transfer through food webs, i.e. substantial amounts of energy are lost during trophic transfer until a further trophic level cannot be supported. Given the important role of root-derived C as energy supply, the shortage of root input may result in shorter food chain length and e.g. decrease the TP of omnivores by shifting diet towards feeding more on basal resources (Stenroth et al., 2008). However, strong compartmentalization and multiple basal resources in soil food webs may mitigate the effect of deprivation of root-derived resources on soil fauna.

Ectomycorrhizal fungi are the major sink for photosynthates in forest soils, receiving ca. 30 % of the net primary production from tree hosts (Högberg and Högberg, 2002; Courty et al., 2010). Consumption of ectomycorrhizal fungi may contribute to the transfer of root-derived resources to the forest soil animal food web. Hence, as root input declines, the substantial reduction in biomass of ectomycorrhizal fungi (Siira-Pietikäinen et al., 2001; Brant et al., 2006) may cause Collembola to rely more on other resources such as saprotrophic fungi. Euedaphic Collembola might be more affected than other functional groups, as mycorrhizal fungal tissue was mainly found in the gut of Collembola living at a greater depth (Ponge, 2000). However, recent studies indicate that saprotrophic microorganisms rather than mycorrhizal fungi are the dominant resources for soil microbivores (Malmström and Persson, 2011; Potapov and Tiunov, 2016; Bluhm et al., 2019b).

Trophic niches of soil omnivores have been well characterized using bulk stable isotope and neutral lipid fatty acid (NLFA) analysis (Ruess and Chamberlain, 2010; Digel et al., 2014), however, there are major gaps remaining. First, disentangling the contribution of ectomycorrhizal and saprotrophic fungal resources to the diet of soil omnivores is tricky as both fungal groups share the same lipid biomarkers. Second, inferring TP from bulk stable isotope composition remains challenging, as the $\delta^{15}N$ signature of organisms is affected not only by trophic fractionation but also by variations in stable isotope values of basal resources (Ohkouchi et al., 2017; Potapov et al., 2019a). Compound specific isotope analysis (CSIA) of amino acids (AAs) represents a new and powerful tool that has been shown to provide accurate and precise estimates of TP and basal resources of organisms in aquatic and terrestrial ecosystems (McClelland and Montoya, 2002). The TP estimated by CSIA of AAs (TP_{CSIA}) is based on the differential isotopic fractionation between the canonical 'trophic' and 'source' AAs glutamine (Glu) and phenylalanine (Phe) during metabolic processes. Furthermore, using $\delta^{13}C$ signatures of essential AAs (eAAs), the AA fingerprinting approach allows to distinguish between saprotrophic fungi, mycorrhizal fungi, bacteria and plants as basal resources of soil organisms (Larsen et al., 2009; Pollierer et al., 2019, 2020).

In the present study, we investigated the trophic niche differentiation between functional groups of Collembola in forests differing in tree species composition and soil types in two regions of Germany. In addition, a root-trenching experiment was established to investigate the effects of deprivation of root-derived resources on the trophic niches of Collembola. We analyzed the isotopic C and N signatures of AAs (CSIA-AAs) to estimate the TP, N sources (N baseline) and basal resources of Collembola. Here, basal resources refer to fungi (ectomycorrhizal vs. saprotrophic fungi), bacteria and higher plant tissue. Specifically, we analyzed the following hypotheses:

(1) The ^{15}N signature of Phe is higher in euedaphic than ep-/hemiedaphic Collembola, as euedaphic Collembola rely more on ^{15}N-enriched organic substrate in deeper soil, while litter depleted in ^{15}N is the predominant N source for ep-/hemiedaphic Collembola.

(2) The TP_{CSIA} of euedaphic Collembola is higher than that of ep-/hemiedaphic Collembola, due to a higher number of trophic transfers in soil than in the litter layer.

(3) Deprivation of root-derived resources reduces the TP of Collembola, especially at higher trophic levels, as suggested by the 'productivity hypothesis'.

(4) The reduction of ectomycorrhizal fungi in trenched plots decreases the dependence of Collembola on ectomycorrhizal fungi and increases the utilization of saprotrophic fungi; the dietary shift is stronger in euedaphic Collembola than in ep-/hemiedaphic Collembola.

2. Materials and Methods

2.1 *Study sites*

The study sites comprised two regions across Germany, i.e. the Hainich-Dün (Hainich) and the Schorfheide-Chorin (Schorfheide), and form part of the 'Biodiversity Exploratories', a large project serving as open platform for biodiversity and ecosystem research (www.biodiversity-exploratories.de; Fischer et al., 2010). The Schorfheide is a young glacial landscape with an altitude of 3–140 m a.s.l., a mean annual temperature of 8.0–8.5 °C and a mean annual precipitation of 500–600 mm. Soils in the Schorfheide are mainly cambisols. The Hainich is dominated by calcareous bedrock and varies in altitude from 285 to 550 m a.s.l. Soils in the Hainich are mainly luvisols with few stagnosols. The mean annual temperature is 6.5–8.0 °C and the mean annual precipitation is 600–800 mm. For more details on the study sites see Fischer et al. (2010).

In each region two forest types were selected. The forest types included managed coniferous forests and 70 years old managed beech forests. Coniferous forests consisted of spruce (*Picea abies*) in the Hainich, and pine (*Pinus sylvestris*) in the Schorfheide. Beech forests are dominated by *Fagus sylvatica*, mixed with ash (*Fraxinus excelsior*) and sycamore (*Acer pseudoplatanus*). Coniferous forests are more intensively managed than beech forests since they had been planted to replace naturally occurring beech forests. Each forest type was replicated four times per region, resulting in 16 forest plots. All plots were randomly arranged, the minimum distance between plots was 500 m.

2.2 Establishment and maintenance of root-trenching plots

In each of the 16 forest plots, one ‘root-trenching’ and one ‘control’ subplot of an area of 120 × 120 cm were established between September and October 2011. In the root-trenching treatment, roots were excluded by inserting polyethylene barriers (120 × 60 × 0.5 cm) into 40–50 cm deep trenches along the four sides of the subplots. In addition, aluminum linings were inserted at the edges to close the gap between adjacent barriers. Polyethylene barriers extended ca. 10 cm above the ground to prevent animal migration between the trenched plots and the surrounding. Aboveground barriers were also established in control subplots to control for potential side effects. At regular intervals during the growth period above-ground parts of herbaceous plants and grasses in trenched and control plots were removed to minimize input of root-derived resources. Soil moisture was measured gravimetrically from soil cores and water was added to control plots equalizing the amount of water in the upper 10 cm of the soil in control and trenched plots; for more details see Bluhm et al. (2019a).

2.3 Sampling

Soil samples were taken in October 2015. In each subplot, the litter layer and upper 5 cm of the soil layer from an area 0.7 m² were sampled. Invertebrates were extracted using a heat gradient (Kempson et al., 1963) from litter and soil samples separately. The extracted animals were stored in glycerin at -20ºC. Collembola were sorted to three functional groups, i.e. epedaphic, hemiedaphic and euedaphic Collembola, under a dissecting microscope and transferred into 70 % ethanol.

2.4 Extraction and derivatization of amino acids

For CSIA, samples were transferred to Pyrex culture tubes and flushed with N_2 gas, sealed and hydrolyzed in 6 mol/L HCl at 110°C in a heating block for 20 h (Larsen et al., 2013). After hydrolysis, lipophilic compounds were removed by adding n-hexane/DCM to the Pyrex tubes that were flushed shortly with N_2 gas and sealed before vortexing for 30 s. The aqueous phase was then filtered through a Pasteur pipette lined with glass wool that had been pretreated at 450°C. All samples were transferred into 4 mL vials before evaporating the samples to dryness under a steam of N_2 gas at 110°C in a heating block for 30 min. The samples were then stored at 18°C. To volatize the AAs, we followed the derivatization procedure of Corr et al. (2007), methylating the dried samples with acidified methanol and subsequently acetylating them with a mixture of acetic anhydride, trimethylamine and acetone (N-acetyl methyl ester derivatives). As a precautionary measure to reduce oxidation of AAs during derivatization, reaction vials were flushed with N_2 and sealed prior to the methylation and acetylation reactions. To account for C added during derivatization and variability of isotope fractionation during analysis, pure AAs with known $\delta^{13}C$ and $\delta^{15}N$ values were also derivatized and analyzed. Nor-leucine was used as internal reference. AA derivatives were injected into a Thermo Finnigan Trace GC coupled via a GP interface to a Delta Plus mass spectrometer (Finnigan, Bremen, Germany), located at the Centre for Stable Isotope Research and Analysis, Göttingen, Germany. The GC was equipped with an Agilent J&W VF-35ms GC column (30 m × 0.32 mm × 1.00 µm). The temperature program started with 80°C held for 1 min, increased by 20°C/min to 135°C, then by 5°C/min to 160°C and held for 3 min, then increased again by 8°C/min to 300°C and held for 3 min. The injection temperature was 280°C and helium was used as carrier gas. The flow rate of helium was 2 mL/min. All samples were analyzed in triplicate. The N isotopic composition of AAs in samples was expressed relative to atmospheric N by normalizing measured values (vs. reference gas) using scales derived from known $\delta^{15}N$ values of the reference mixture. The C isotopic composition was corrected for carbon added during derivatization following O'Brien et al. (2002) and expressed relative to Vienna PD Belemnite.

2.5 Calculation and statistical analysis

CSIA-based trophic position (TP_{CSIA}) was calculated using the difference in $\delta^{15}N$ values between Glu and Phe according to Chikaraishi et al. (2011) as $TP_{CSIA} = 1 + (\delta^{15}N_{Glu} - \delta^{15}N_{Phe} + \beta)/\Delta_{Glu\text{-}Phe}$, with $\beta = 8.4 \pm 1.6‰$ the 'baseline' difference between $\delta^{15}N$ values in Glu and Phe in C3 plants and $\Delta_{Glu\text{-}Phe} = 7.6 \pm 1.2‰$ the trophic discrimination between Glu and Phe per trophic level (Chikaraishi et al., 2014). Primary producers were treated as first trophic level (TP = 1). When calculating TP_{CSIA}, we propagated variance by using the differential solution of equation (1) according to Blum et al. (2013) and Ohkouchi et al. (2017) (Table S1).

To inspect the effects of root-trenching, forest type and functional group of Collembola on the TP_{CSIA} of Collembola, linear mixed effects models were conducted with the random term 'forest plot identity' for Schorfheide and Hainich, respectively. Due to the lack of ^{15}N data on euedaphic Collembola in coniferous forests of the Hainich, we conducted analyses for beech and coniferous forests in the Hainich separately. To test whether Collembola from litter and soil layers differ in TP_{CSIA} irrespective of functional group, we calculated correlations between the proportional abundance of Collembola from the litter layer (abundance in litter / combined abundance in litter and soil) and residuals of the model of TP_{CSIA} for each functional group of Collembola in the Hainich and Schorfheide, respectively. The residuals of TP_{CSIA}, i.e. the observed minus the fitted values, are independent of forest type, root-trenching and functional group of Collembola. As $\delta^{15}N_{Phe}$ reflects the isotopic baseline and may provide important information on N sources for Collembola, we also analyzed the variation in $\delta^{15}N_{Phe}$ using linear mixed effects models with 'site', 'forest type', 'root trenching', 'functional group' and their interaction as fixed effects and 'forest plot identity' as random term.

For the analysis of $\delta^{13}C$ values of AAs, we used eAAs that cannot be synthesized by animal consumers including invertebrates such as Collembola (Dadd, 1973; O'Brien et al., 2002; Larsen et al., 2011; Nation, Sr., 2015). To estimate the contribution of different resources to the diet of Collembola, linear discriminant function analysis (LDA) was performed with $\delta^{13}C$ values of three eAAs, i.e. Thr, Phe and Met. To predict the biosynthetic origin of these eAAs in consumers, we used classifier variables (i.e., 'training' data) comprising the resources from previous studies (Larsen et al., 2009, 2013; Pollierer et al., 2020). Due to technical issues (potential co-elution with glycerin-remains from storage solution) the $\delta^{13}C$

values of Leu and Ile were not reliable, and were thus excluded from LDA. As Leu is an important AA in disentangling bacterial and saprotrophic fungal resources in LDA (Potapov et al., 2019b), our analysis may over-/underestimate the contribution of bacterial resources relative to saprotrophic fungal resources. All statistical analyses were conducted using R 3.6.1 (R Core Team, 2019), packages 'MASS' (Venables and Ripley, 2002), 'lme4' (Bates et al., 2015), 'lmerTest' (Kuznetsova et al., 2017) and 'emmeans' (Lenth, 2019). All figures were drawn using the package ggplot2 (Wickham, 2016).

3. Results

3.1 Trophic position as indicated by $\delta^{15}N$ values of glutamic acid and phenylalanine

In control plots, differences in TP_{CSIA} between functional groups of Collembola were more pronounced in the Schorfheide than in the Hainich (Fig. 1, Tables S1-S2). In the Schorfheide, the TP_{CSIA} of hemiedaphic and euedaphic Collembola was higher than that of epedaphic Collembola. In the Hainich, the TP_{CSIA} of hemiedaphic Collembola was similar to that of epedaphic Collembola. In Hainich beech forests, the TP_{CSIA} of euedaphic Collembola was higher than that of hemiedaphic and epedaphic Collembola, although the difference was only marginally significant ($F_{2,12} = 3.7$, $P = 0.056$; Table S2).

Root trenching affected the TP_{CSIA} of Collembola; however, the effects varied with study site, functional group and forest type. In the Schorfheide, root-trenching reduced the TP_{CSIA} of hemiedaphic Collembola in beech and coniferous forests, but did not affect the TP_{CSIA} of epedaphic and euedaphic Collembola (Collembola functional group × Trenching interaction; $F_{2,26} = 6.27$, $P = 0.006$; Fig. 1, Table S2). In the Hainich, root-trenching reduced the TP_{CSIA} of epedaphic Collembola in coniferous forests (Collembola functional group × Trenching interaction; $F_{1,6} = 8.13$, $P = 0.03$). The TP_{CSIA} of euedaphic Collembola was not affected by root-trenching in all study sites and forest types ($P > 0.05$).

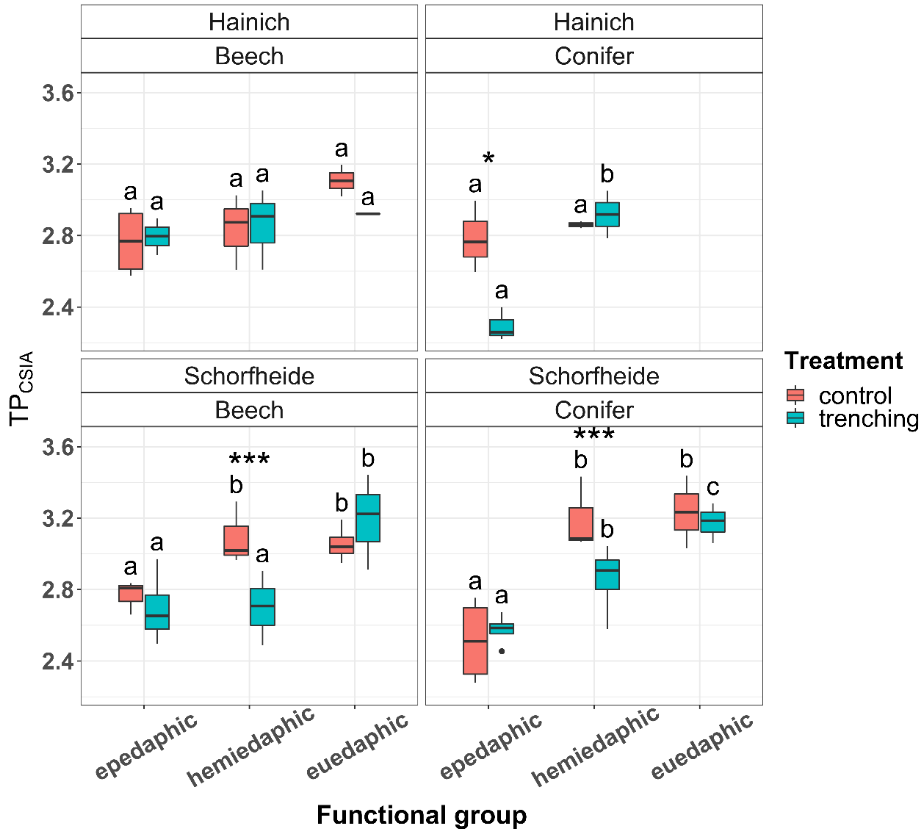

Figure 1. The trophic position (TP_{CSIA}) of Collembola as calculated from $\delta^{15}N$ values of glutamine (Glu) and phenylalanine (Phe). Different letters indicate significant differences between Collembola functional groups within one treatment, i.e. control or trenching ($p < 0.05$; Tukey's HSD test); significant effects of root trenching on respective functional group are indicated by $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

The correlations between the proportional abundance of Collembola in the litter layer (abundance in litter / combined abundance in litter and soil) and residuals of the model of TP_{CSIA} (i.e., the observed minus the fitted values) for each functional group of Collembola differed markedly between the Hainich and Schorfheide. In the Schorfheide, the residuals of TP_{CSIA} negatively correlated with the proportional abundance in the litter layer, although the correlation was not significant in epedaphic Collembola (Fig. 2). In the Hainich, the correlation was not significant in all functional groups of Collembola.

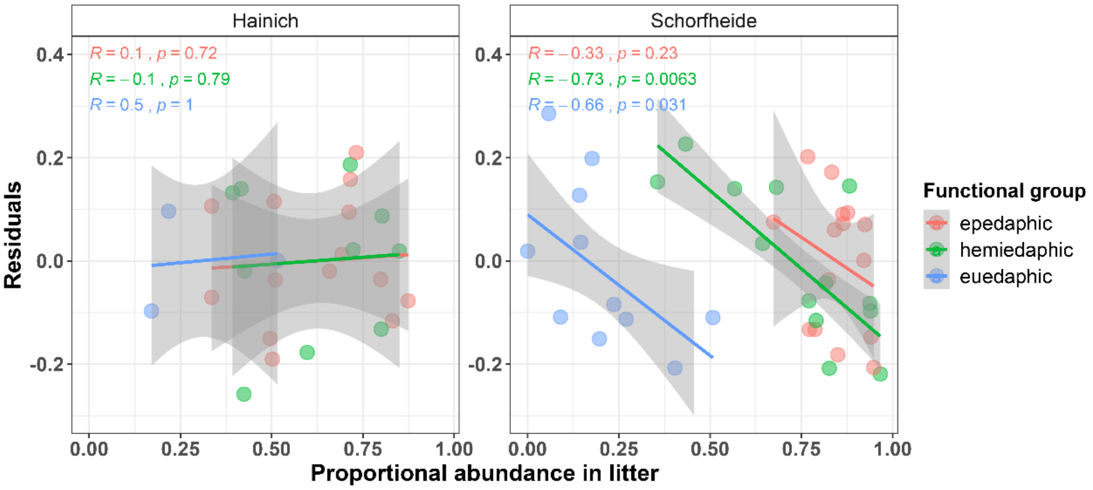

Figure 2. Correlation between proportional abundance in the litter layer (abundance in litter / combined abundance in litter and soil) and residuals of trophic position (TP_{CSIA}) of the respective functional group of Collembola in the Hainich and Schorfheide; R, spearman correlation coefficient.

The $\delta^{15}N_{Phe}$ values differed markedly between functional groups of Collembola, with higher $\delta^{15}N_{Phe}$ values in euedaphic Collembola than in epedaphic and hemiedaphic Collembola (significant effect of functional group; $F_{2,54} = 19.19$, $P < 0.001$; Fig. 3). In addition, $\delta^{15}N_{Phe}$ values of Collembola were higher in the Hainich than in the Schorfheide (significant effect of study site; $F_{1,11} = 19.16$, $P = 0.001$). The effects of forest type and root trenching on $\delta^{15}N_{Phe}$ were not significant ($P > 0.05$).

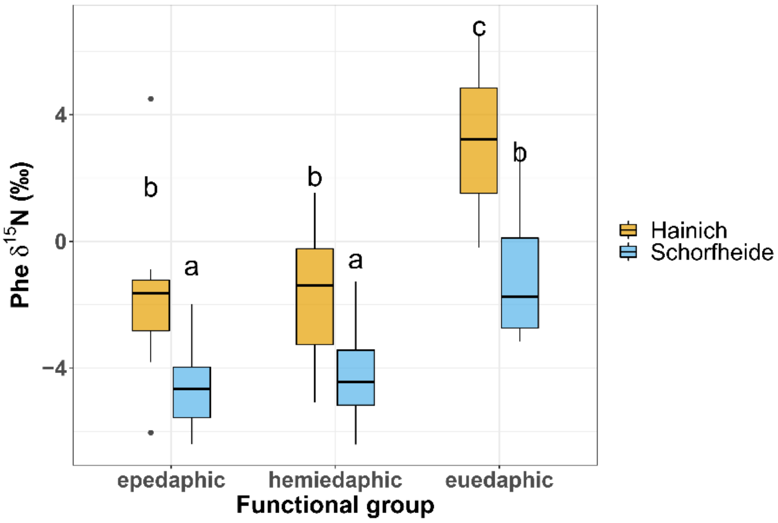

Figure 3. $\delta^{15}N$ values of phenylalanine (Phe) in epedaphic, hemiedaphic and euedaphic Collembola in the Hainich and Schorfheide. Values from control and trenched plots in different forest types were pooled. Different letters indicate significant differences ($p < 0.05$; Tukey's HSD test).

3.2 Basal resources as indicated by $\delta^{13}C$ values of essential amino acids

According to the LDA the $\delta^{13}C_{eAAs}$ patterns of Collembola were more close to saprotrophic microbes, i.e. bacteria and saprotrophic fungi, than to plants and mycorrhizal fungi (Fig. 4). Almost all samples of Collembola were classified as feeders on saprotrophic microorganisms (84/85).

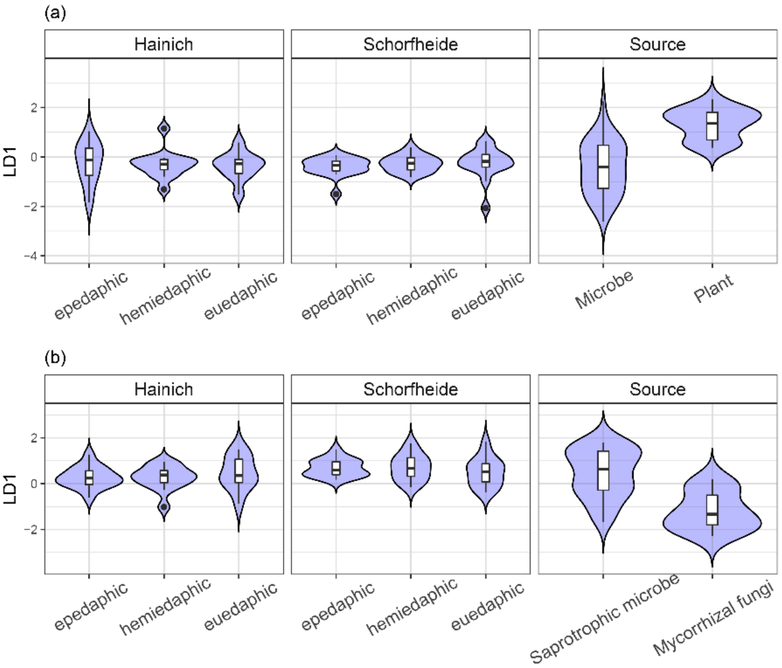

Figure 4. Linear discriminant analysis (LDA) scores based on $\delta^{13}C$ values of essential AAs of Collembola in the Hainich and Schorfheide and respective scores of training data sets of basal resources: (a) plants and microbes (bacteria, saprotrophic fungi and mycorrhizal fungi), and (b) mycorrhizal fungi and saprotrophic microorganisms including bacteria and saprotrophic fungi. LDA was based on eAAs methionine (Met), phenylalanine (Phe) and threonine (Thr).

As saprotrophic microorganisms were identified as the major food resources for Collembola, we employed a training data set consisting of eAA $\delta^{13}C$ signatures of bacteria, saprotrophic fungi and mycorrhizal fungi to explore the contribution of bacteria and saprotrophic fungi to the diet of Collembola using LDA (Fig. 5). Most samples of Collembola clustered with saprotrophic fungi, i.e. 87% of the samples were classified as feeding on saprotrophic fungi, 12 % as feeding on bacteria and 1 % as feeding on mycorrhizal fungi. However, the $\delta^{13}C_{eAA}$ pattern of Collembola differed between sites (MANOVA, site, $F_{1,83} = 3.9$, $P = 0.02$), mainly because samples were positioned slightly closer to mycorrhizal fungi in Hainich than in Schorfheide (ANOVA, the effect of site on LD1, $F_{1,83} = 6.7$, $P = 0.01$). There were no significant effects of root-trenching, forest type and functional group ($P > 0.05$).

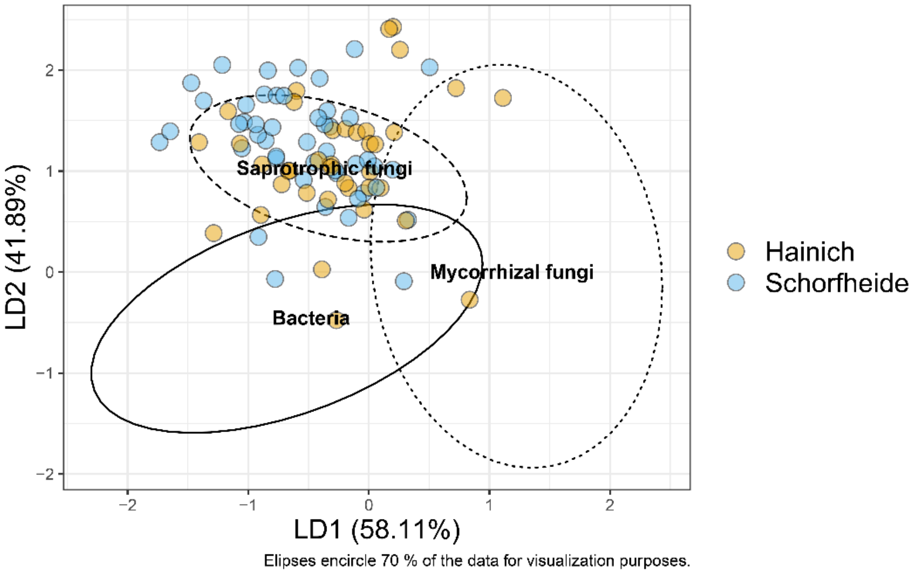

Figure 5. Linear discriminant analysis (LDA) scores based on δ^{13}C values of essential AAs of Collembola in the Hainich and Schorfheide and respective scores of training data sets of basal resources including bacteria, saprotrophic fungi and mycorrhizal fungi. LDA was based on eAAs methionine (Met), phenylalanine (Phe) and threonine (Thr).

4. Discussion

Using compound-specific stable isotope composition of C and N in amino acids, we identified the trophic niche of Collembola functional groups, providing new insights into the structural heterogeneity of soil food webs in forest systems. In addition, for the first time we provide evidence how the deprivation of root-derived resources influences the trophic structure of soil food webs in the context of the 'productivity hypothesis'.

4.1 The trophic niches of Collembola functional groups

The trophic niche differentiation between functional groups of Collembola was related to the layers they inhabited, reflecting the vertical heterogeneity of trophic interactions in soils. As indicated by δ^{15}N values of the source AA Phe, the basal N sources differed markedly between functional groups of Collembola; the δ^{15}N values of Phe were higher in euedaphic Collembola than in ep-/hemiedaphic Collembola. This is consistent with previous studies that soil invertebrates feeding on organic substrate in deeper soil are generally more enriched in ^{15}N compared to those mainly depending on litter substrate in shallower layers (Briones and Bol, 2003; Hyodo et al., 2008; Potapov et al., 2019a). δ^{15}N values of organic matter typically increase with soil depth, due to isotopic fractionation during decomposition, mineralization, leaching and plant-mycorrhizal N transfer (Högberg et al., 1996; Hobbie and Ouimette, 2009). Hence, the ^{15}N enrichment of the source AA Phe in euedaphic Collembola suggests intensive feeding on ^{15}N-enrichend soil organic matter including humiphagous microorganisms in the N mobilization stage (Osono and Takeda, 2004). By contrast, saprotrophic fungi in the immobilization stage of litter decomposition presumably are the primary resources for ep-/hemiedaphic Collembola (Fujii and Takeda, 2012). The δ^{15}N values of Phe in Collembola also differed between study sites, matching the regional variation in ^{15}N signature of available resources including tree leaves, fine roots and soil organic matter (Klarner et al., 2014). This reflects regional variations in the ecosystem N balance, related to temperature, precipitation, nitrogen deposition and soil nutrient level (Robinson, 2001; Templer et al., 2007). Thus, the δ^{15}N of Phe reflects variations in the N baseline in space, which is the prerequisite for the accurate estimation of TP of soil organisms (McClelland and Montoya, 2002).

The TP calculated from CSIA of Collembola ranged from ~ 2.3 to 3.2, suggesting that Collembola as omnivores feed on resources of different trophic levels or include varying proportions of microorganisms with different nutritional strategies in their diet (Steffan et al., 2015). In general, the TP_{CSIA} of euedaphic Collembola was higher than that of epedaphic Collembola. Consumption of microalgae, higher plant tissue and pollen may partially explain the relatively low TP_{CSIA} of epedaphic Collembola, whereas euedaphic Collembola presumably mainly rely on microbial resources (Chahartaghi et al., 2005; Potapov et al., 2018). However, the ^{13}C fingerprinting suggests that saprotrophic fungi are the dominant resource for both epedaphic and euedaphic Collembola. Previous studies found the gut content of surface dwelling Collembola to predominantly comprise fungal materials (Bodvarsson, 1970; Anderson and Healey, 1972), suggesting selective feeding on saprotrophic fungi involved in the initial stage of litter decomposition (Hishi et al., 2007; Fujii and Takeda, 2012; Anslan et al., 2018). This may result in relatively short trophic chains between plant resources and epedaphic Collembola. By contrast, euedaphic Collembola mainly feed on saprotrophic microorganisms associated with highly processed organic matter in deeper soil layers (Ponge, 2000). In soil the amount of N from microbial residues can reach 40–100 % of the total organic N pool (Liang et al., 2019). Reprocessing of the proteinaceous components in microbial necromass by microorganisms themselves may result in long microbial food chains, leading to inflation of TP_{CSIA} in euedaphic Collembola (Steffan et al., 2015). In addition, the TP of fungal species may also depend on whether they synthesize AAs using exogenous inorganic N or directly incorporate AAs released from decomposing organic matter (Geisseler et al., 2010; Pollierer et al., 2020). Finally, higher consumption of living or dead animal resources, such as rotifers, nematodes, protozoa and enchytraeids, may also contribute to the high TP_{CSIA} of euedaphic Collembola (Brown, 1954; Heidemann et al., 2014).

In contrast to euedaphic and epedaphic Collembola, the TP_{CSIA} of hemiedaphic Collembola was less stable and varied markedly between study sites. In the Schorfheide, the TP_{CSIA} of hemiedpahic Collembola was higher than that of epedaphic Collembola and similar to euedaphic Collembola. Higher dependence on old soil organic matter may explain the higher TP_{CSIA} in hemiedaphic Collembola relative to epedaphic Collembola. However, since the N source as inferred from $\delta^{15}N$ values of Phe was similar for epedaphic and hemiedaphic Collembola, the higher TP_{CSIA} of hemiedaphic Collembola may rather be due to more intensive feeding on resources at higher trophic levels, such as animal prey or carcass. By contrast, in the Hainich the TP_{CSIA} of hemiedaphic Collembola was similar to that of epedaphic Collembola. This may be due to differences in species composition between regions. In the Schorfheide, the small species *P. notabilis* was more dominant than in the Hainich where the large species *F. quadrioculata* predominated (data not shown). The small body size of *P. notabilis* may allow them to penetrate through small pores and thereby to access microfauna in refuge habitats inaccessible for large Collembola species. Further, higher consumption of microfauna in the Schorfheide may have been due to higher abundance of soil microfauna; in fact the abundance of nematodes is higher in the Schorfheide (2,181,568 ± 1,588,685 ind. m^{-2}) than in the Hainich (194,805 ± 117,890 ind. m^{-2}), likely due to higher root C input (Bluhm et al., 2019a).

The generally higher TP_{CSIA} of euedaphic Collembola as compared to epedaphic Collembola indicates that the number of trophic transfers increases with depth. This is in line with Okuzaki et al. (2009) who found that the range of $\delta^{15}N$ of invertebrates was greater in the humus than in the litter layer, suggesting a larger number of trophic transfers than in the litter layer. More trophic interactions at the basal level in food webs (longer microbial food chains) or more diverse resources, including animals directly or indirectly feeding on litter at later stages of decay, may both contribute to this pattern. Interestingly, the relationship between the vertical distribution of Collembola and TP_{CSIA} also existed within functional groups of Collembola. In the Schorfheide, after removing the variation explained by forest type, functional group and trenching, the residuals of TP_{CSIA} still correlated negatively with the proportional abundance of Collembola in the litter layer. The correlation suggests that irrespective of functional group, TP_{CSIA} generally is higher in Collembola in the soil layer compared to those in the litter layer. As the residuals of TP_{CSIA} were not correlated with $\delta^{15}N$ values of Phe ($P > 0.05$, data not shown), the increase in TP_{CSIA} with depth within functional groups was unlikely due to the increasing incorporation of saprotrophic microorganisms associated with old soil organic matter; other mechanisms such as higher incorporation of food resources of higher trophic level e.g., animal tissue, presumably play a more important role. Notably, the correlation between TP_{CSIA} and the vertical distribution within Collembola functional groups was only found in the Schorfheide but not in the Hainich. This indicates higher niche partitioning between individuals within functional groups in the Schorfheide than in the Hainich, potentially related to the more complex microhabitat structure and the resulting stronger compartmentalization of food webs in the Schorfheide. In fact, the high soil acidity in the Schorfheide is associated with low macrodecomposer biomass (Pollierer et al., submitted) and the formation of thick organic layers including L, F and H materials (i.e., moder soil), thereby providing a wider range of microhabitats than in the Hainich where organic layers predominantly comprise of L material only (i.e., mull soil) (Erdmann et al., 2012; Ferlian and Scheu, 2014).

Disentangling the contribution of ectomycorrhizal and saprotrophic fungal resources to the diet of soil animals is a challenge due to the lack of specific biomarkers for ectomycorrhizal fungi, e.g. in lipid analysis. In the present study, using ^{13}C AA fingerprinting we found that saprotrophic microorganisms, especially saprotrophic fungi, rather than mycorrhizal fungi form the dominant food resource of Collembola. This is in line with recent studies suggesting that soil decomposers generally do not use mycorrhizal fungi as main food source (Potapov and Tiunov, 2016; Bluhm et al., 2019b, Pollierer and Scheu, 2021). Results of earlier studies suggested euedaphic Collembola to also ingest mycorrhizal fungi and root tissue (Ponge 2000). However, Malmström and Persson (2011) found that the abundance of the euedaphic Collembola did not decrease in girdled plots, arguing against mycorrhizal fungi as major food resource. Notably, our finding that different functional groups of Collembola utilize similar basal resources contrasts previous studies on the lipid composition of Collembola, suggesting that the dietary composition differs between Collembola species (Ruess et al., 2007; Pollierer et al., 2012; Ferlian et al., 2015). Potentially, pooling different species into functional groups may have masked species-specific variation in the use of basal resources. In addition, the lack of data on $\delta^{13}C$ of leucine may have biased the estimation of the contribution of bacterial and saprotrophic fungi to the diet of Collembola, since normalized $\delta^{13}C$ values of leucine differ markedly between bacteria and fungi (Potapov et al., 2019b). Despite this limitation our conclusion that saprotrophic microorganisms are the predominant resources for Collembola, whereas the contribution of mycorrhizal fungi and plants is relatively minor, remains valid and this is supported by a recent study using CSIA-AAs fingerprinting approach (Pollierer and Scheu, 2021).

4.2 The effect of root energy supply on trophic niches of Collembola

In the cases where root trenching exerted significant effects on TP_{CSIA} of Collembola, it was consistently reduced when Collembola were deprived of root energy supply. This supports the 'productivity hypothesis', stating that the length of food chains increases with availability of basal resources (Thompson and Townsend, 2005). However, the deprivation of root-derived resources reduced the TP_{CSIA} of hemiedaphic Collembola only in the Schorfheide but not in the Hainich. In acidic soils such as the Schorfheide, roots are concentrated in upper soil and high numbers of root tips grow into the humus layer (Godbold et al., 2003). Hence, the input of root-derived resources, such as exudates and dead fine roots, supplying additional resources for microorganisms and microbivores, and supporting more trophic transfers and longer food chains, into the litter layer is likely to be higher in the Schorfheide than in the Hainich. Supporting this conclusion, the amount of microbial PLFAs in the litter layer was reduced markedly by root-trenching in the Schorfheide, whereas the reduction was minor in the Hainich (Bluhm et al., 2019a). The elimination of root energy supply may shorten the food chain length, affecting omnivores in two ways (Stenroth et al., 2008): (1) they may shift diet to increase feeding on lower trophic levels, and (2) decreased biodiversity leads to a decreased number of links at the basal end of food web. As discussed before, hemiedaphic Collembola in the Schorfheide are likely to consume relatively high amounts of animal tissue (as predators or scavengers). Hence, the reduction in animal prey resulting from the shortage of root-derived resources may cause hemiedaphic Collembola to feed more on resources at lower trophic levels such as saprotrophic microorganisms. We did not collect data on microfauna in the litter layer, however, the abundance of bacterivorous nematodes in soil decreased by more than 50% in trenched plots in the Schorfheide but not in the Hainich (Sarah Bluhm, pers. comm.), supporting the assumption of reduced prey availability as they are known to also feed on nematodes (Heidemann et al., 2014). In aquatic systems, TP of omnivores such as crayfish also decreased with a reduction of primary productivity, caused by reduced consumption of animal prey and in favor of feeding more on basal resources (Olsson et al., 2008; Stenroth et al., 2008).

In contrast to our hypothesis, although euedaphic Collembola occupied a relatively high TP_{CSIA}, their TP_{CSIA} was not affected by root-trenching. Potentially, unlike hemiedaphic Collembola in the Schorfheide, which likely in part feed on animal tissue, euedaphic Collembola presumably mainly feed on saprotrophic microorganisms. Therefore, a shift to feeding on more basal resources, such as plant litter and soil organic matter, is unlikely due to the low quality and low accessibility of these materials. Similarly, the TP_{CSIA} of epedaphic Collembola was generally not affected by root-trenching, presumably due to high dependence on saprotrophic fungi in decomposing leaf litter. In coniferous forests in the Schorfheide, the TP_{CSIA} of epedaphic Collembola was lower in trenched plots than in control plots. Although the mechanisms responsible for the decrease in TP_{CSIA} are not clear, potentially, it was related to changes in Collembola species composition, e.g. due to reduced numbers of epedaphic Neanuridae known to feed on animal prey including tardigrades, rotifers and Collembola eggs (Potapov et al., 2016).

Deprivation of root-derived resources did not change the relative contribution of eAAs from different basal resources to Collembola, with a consistently high contribution of saprotrophic microorganisms. In line with these results, the lipid composition of euedaphic Collembola was also not affected by root-trenching (Sarah Bluhm, pers. comm.), potentially because the majority of fungal biomarker lipids originated from saprotrophic fungi rather than from mycorrhizal fungi. The high dependence on saprotrophic fungi may also explain the relatively minor response of Collembola to the deprivation of root resources in a previous study (Malmström and Persson, 2011). Notably, our conclusions may be restricted to forest ecosystems as euedaphic Collembola in grassland depend more on roots and root-associated microorganisms than those in tree plantations, as gramineous and herbaceous roots presumably are more palatable than tree roots (Lemanski and Scheu, 2014; Li et al., 2020). Analogously, roots of herbaceous plants in the forest understory potentially serve as food resources for euedaphic Collembola. However, in the present study herbaceous plants were removed from both trenching and control plots, and thus their contribution to the diet of Collembola remains unknown.

5. Conclusions

In this study, we compared TP_{CSIA} and basal resources of Collembola of different life-forms in temperate forest ecosystems, and explored their response to the deprivation of root-derived resources. Generally, our results support results of recent studies suggesting that saprotrophic microorganisms, especially saprotrophic fungi, are the predominant resource of Collembola, whereas the contribution of mycorrhizal fungi and plants to their diet is subordinate. In more detail, however, we found that the trophic niches among functional groups of Collembola differ markedly in respect to N sources and TP_{CSIA}. Euedaphic Collembola mainly utilized N from processed organic matter in deeper soil, while leaf litter was the dominant N source for epedaphic and hemiedaphic Collembola. The TP_{CSIA} of euedaphic Collembola was consistently higher than that of epedaphic Collembola, presumably due to (1) more intensive feeding on microorganisms living on old soil organic matter or their residues, undergoing more trophic transfers, or (2) higher consumption of animal tissue, such as microfauna or animal carcasses. Both reflects the higher number of trophic transfers in soil than in the litter layer. For the first time, we provided evidence that the deprivation of root energy supply decreases the trophic position of soil omnivores, suggesting that root-derived resources increase the trophic transfer and food chain length in soil food webs. However, this varied among functional groups of Collembola and forest stands, suggesting that compartmentalization of food webs and multiple energy sources reduce the impact of shortage in root energy supply. In addition, omnivores at higher trophic levels, especially those relying on animal resources, are more likely to shift diet towards increased feeding on lower trophic levels. This is presumably due to the reduction in animal prey (or carcasses) such as nematodes when the availability of root resources declines. Hence, in complex soil food webs the associations between root energy supply and trophic interactions are affected by vertical resource heterogeneity, regional conditions and feeding strategies of consumers.

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References

Anderson, J.M., Healey, I.N., 1972. Seasonal and inter-specific variation in major components of the gut contents of some woodland Collembola. The Journal of Animal Ecology 41, 359. doi:10.2307/3473

Anderson, T.A., Guthrie, E.A., Walton, B.T., 1993. Bioremediation in the rhizosphere. Environmental Science & Technology 27, 2630–2636. doi:10.1021/es00049a001

Anslan, S., Bahram, M., Tedersoo, L., 2018. Seasonal and annual variation in fungal communities associated with epigeic springtails (Collembola spp.) in boreal forests. Soil Biology and Biochemistry 116, 245–252. doi:10.1016/j.soilbio.2017.10.021

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48. doi:10.18637/jss.v067.i01

Bluhm, S.L., Eitzinger, B., Ferlian, O., Bluhm, C., Schröter, K., Pena, R., Maraun, M., Scheu, S., 2019a. Deprivation of root-derived resources affects microbial biomass but not community structure in litter and soil. PLoS ONE 14. doi:10.1371/journal.pone.0214233

Bluhm, S.L., Potapov, A.M., Shrubovych, J., Ammerschubert, S., Polle, A., Scheu, S., 2019b. Protura are unique: First evidence of specialized feeding on ectomycorrhizal fungi in soil invertebrates. BMC Ecology 19, 1–7. doi:10.1186/s12898-019-0227-y

Blum, J.D., Popp, B.N., Drazen, J.C., Anela Choy, C., Johnson, M.W., 2013. Methylmercury production below the mixed layer in the North Pacific Ocean. Nature Geoscience 6, 879–884. doi:10.1038/ngeo1918

Bodvarsson, H., 1970. Alimentary studies of seven common soil-inhabiting Collembola of southern Sweden. Insect Systematics & Evolution 1, 74–80. doi:10.1163/187631270x00366

Brant, J.B., Myrold, D.D., Sulzman, E.W., 2006. Root controls on soil microbial community structure in forest soils. Oecologia 148, 650–659. doi:10.1007/s00442-006-0402-7

Briones, M.J.I., Bol, R., 2003. Natural abundance of ^{13}C and ^{15}N in earthworms from different cropping treatments. Pedobiologia 47, 560–567. doi:10.1078/0031-4056-00229

Brown, W.L., 1954. Collembola feeding upon nematodes. Ecology 35, 421. doi:10.2307/1930111

Buée, M., De Boer, W., Martin, F., van Overbeek, L., Jurkevitch, E., 2009. The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Plant and Soil 321, 189–212. doi:10.1007/s11104-009-9991-3

Chahartaghi, M., Langel, R., Scheu, S., Ruess, L., 2005. Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biology and Biochemistry 37, 1718–1725. doi:10.1016/J.SOILBIO.2005.02.006

Chikaraishi, Y., Ogawa, N.O., Doi, H., Ohkouchi, N., 2011. ^{15}N/^{14}N ratios of amino acids as a tool for studying terrestrial food webs: A case study of terrestrial insects (bees, wasps, and hornets). Ecological Research 26, 835–844. doi:10.1007/s11284-011-0844-1

Chikaraishi, Y., Steffan, S.A., Ogawa, N.O., Ishikawa, N.F., Sasaki, Y., Tsuchiya, M., Ohkouchi, N., 2014. High-resolution food webs based on nitrogen isotopic composition of amino acids. Ecology and Evolution 4, 2423–2449. doi:10.1002/ece3.1103

Corr, L.T., Berstan, R., Evershed, R.P., 2007. Development of N-acetyl methyl ester derivatives for the determination of δ^{13}C values of amino acids using gas chromatography-combustion-isotope ratio mass spectrometry. Analytical Chemistry 79, 9082–9090. doi:10.1021/ac071223b

Coulibaly, S.F.M., Winck, B., Akpa-Vinceslas, M., Mignot, L., Gras, M. Le, Forey, E., Chauvat, M., 2019. Functional assemblages of Collembola determine soil microbial communities and associated functions. Frontiers in Environmental Science 7, 52. doi:10.3389/fenvs.2019.00052

Courty, P.E., Buée, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.P., Uroz, S., Garbaye, J., 2010. The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. Soil Biology and Biochemistry 42, 679–698. doi:10.1016/j.soilbio.2009.12.006

Dadd, R.H., 1973. Insect nutrition: current developments and metabolic implications. Annual Review of Entomology 18, 381–420. doi:10.1146/annurev.en.18.010173.002121

Digel, C., Curtsdotter, A., Riede, J., Klarner, B., Brose, U., 2014. Unravelling the complex structure of forest soil food webs: Higher omnivory and more trophic levels. Oikos 123, 1157–1172. doi:10.1111/oik.00865

Erdmann, G., Scheu, S., Maraun, M., 2012. Regional factors rather than forest type drive the community structure of soil living oribatid mites (Acari, Oribatida). Experimental and Applied Acarology 57, 157–169. doi:10.1007/s10493-012-9546-9

Faber, J.H., 1991. Functional classification of soil fauna: A new approach. Oikos 62, 110. doi:10.2307/3545458

Ferlian, O., Klarner, B., Langeneckert, A.E., Scheu, S., 2015. Trophic niche differentiation and utilisation of food resources in collembolans based on complementary analyses of fatty acids and stable isotopes. Soil Biology and Biochemistry 82, 28–35. doi:10.1016/J.SOILBIO.2014.12.012

Ferlian, O., Scheu, S., 2014. Shifts in trophic interactions with forest type in soil generalist predators as indicated by complementary analyses of fatty acids and stable isotopes. Oikos 123, 1182–1191. doi:10.1111/j.1600-0706.2013.00848.x

Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., Korte, G., Nieschulze, J., Pfeiffer, S., Prati, D., Renner, S., Schöning, I., Schumacher, U., Wells, K., Buscot, F., Kalko, E.K.V., Linsenmair, K.E., Schulze, E.D., Weisser, W.W., 2010. Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic and Applied Ecology 11, 473–485. doi:10.1016/j.baae.2010.07.009

Fujii, S., Takeda, H., 2012. Succession of collembolan communities during decomposition of leaf and root litter: Effects of litter type and position. Soil Biology and Biochemistry 54, 77–85. doi:10.1016/j.soilbio.2012.04.021

Geisseler, D., Horwath, W.R., Joergensen, R.G., Ludwig, B., 2010. Pathways of nitrogen utilization by soil microorganisms - A review. Soil Biology and Biochemistry 42, 2058–2067.

doi:10.1016/j.soilbio.2010.08.021

Godbold, D.L., Fritz, H.W., Jentschke, G., Meesenburg, H., Rademacher, P., 2003. Root turnover and root necromass accumulation of Norway spruce (Picea abies) are affected by soil acidity. Tree Physiology 23, 915–921. doi:10.1093/treephys/23.13.915

Heidemann, K., Hennies, A., Schakowske, J., Blumenberg, L., Ruess, L., Scheu, S., Maraun, M., 2014. Free-living nematodes as prey for higher trophic levels of forest soil food webs. Oikos 123, 1199–1211. doi:10.1111/j.1600-0706.2013.00872.x

Hishi, T., Hyodo, F., Saitoh, S., Takeda, H., 2007. The feeding habits of collembola along decomposition gradients using stable carbon and nitrogen isotope analyses. Soil Biology and Biochemistry 39, 1820–1823. doi:10.1016/J.SOILBIO.2007.01.028

Hobbie, E.A., Ouimette, A.P., 2009. Controls of nitrogen isotope patterns in soil profiles. Biogeochemistry 95, 355–371 . doi:10.1007/s10533-009-9328-6

Högberg, M.N., Högberg, P., 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. New Phytologist 154, 791–795. doi:10.1046/j.1469-8137.2002.00417.x

Högberg, P., Hogbom, L., Schinkel, H., Hogberg, M., Johannisson, C., Wallmark, H., 1996. ^{15}N abundance of surface soils, roots and mycorrhizas in profiles of European forest soils. Oecologia 108, 207–214. doi:10.1007/BF00334643

Hyodo, F., Tayasu, I., Konaté, S., Tondoh, J.E., Lavelle, P., Wada, E., 2008. Gradual enrichment of ^{15}N with humification of diets in a below-ground food web: Relationship between ^{15}N and diet age determined using ^{14}C. Functional Ecology 22, 516–522. doi:10.1111/j.1365-2435.2008.01386.x

Kempson, D., Lloyd, M., Ghelardi, R., 1963. A new extractor for woodland litter. Pedobiologia 3, 1–21.

Klarner, B., Ehnes, R.B., Erdmann, G., Eitzinger, B., Pollierer, M.M., Maraun, M., Scheu, S., 2014. Trophic shift of soil animal species with forest type as indicated by stable isotope analysis. Oikos 123, 1173–1181. doi:10.1111/j.1600-0706.2013.00939.x

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. LmerTest package: Tests in linear mixed effects models. Journal of Statistical Software 82, 1–26. doi:10.18637/jss.v082.i13

Larsen, T., Taylor, D.L., Leigh, M.B., O'Brien, D.M., 2009. Stable isotope fingerprinting: a novel method for identifying plant, fungal, or bacterial origins of amino acids. Ecology 90, 3526–3535. doi:10.1890/08-1695.1

Larsen, T., Ventura, M., Andersen, N., O'Brien, D.M., Piatkowski, U., McCarthy, M.D., 2013. Tracing carbon sources through aquatic and terrestrial food webs using amino acid stable isotope fingerprinting. PLoS ONE 8. doi:10.1371/journal.pone.0073441

Larsen, T., Ventura, M., O'Brien, D.M., Magid, J., Lomstein, B.A., Larsen, J., 2011. Contrasting effects of nitrogen limitation and amino acid imbalance on carbon and nitrogen turnover in three species of Collembola. Soil Biology and Biochemistry 43, 749–759. doi:10.1016/j.soilbio.2010.12.008

Lemanski, K., Scheu, S., 2014. Incorporation of ^{13}C labelled glucose into soil microorganisms of grassland: Effects of fertilizer addition and plant functional group composition. Soil Biology and Biochemistry 69, 38–45. doi:10.1016/j.soilbio.2013.10.034

Lenth, R., 2019. emmeans: Estimated Marginal Means, aka Least-Squares Means.

Li, Y.J., Yang, X.D., Zou, X.M., Wu, J.H., 2009. Response of soil nematode communities to tree girdling in a subtropical evergreen broad-leaved forest of southwest China. Soil Biology and Biochemistry 41, 877–882. doi:10.1016/j.soilbio.2008.07.031

Li, Z., Scheunemann, N., Potapov, A.M., Shi, L., Pausch, J., Scheu, S., Pollierer, M.M., 2020. Incorporation of root-derived carbon into soil microarthropods varies between cropping systems. Biology and Fertility of Soils 56, 839–851 . doi:10.1007/s00374-020-01467-8

Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Global Change Biology 25, 3578–3590. doi:10.1111/gcb.14781

Malmström, A., Persson, T., 2011. Responses of Collembola and Protura to tree girdling–some support for ectomycorrhizal feeding. Soil Org. 83, 279–285.

McClelland, J.W., Montoya, J.P., 2002. Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. Ecology 83, 2173–2180. doi:10.1890/0012-9658(2002)083[2173:TRATNI]2.0.CO;2

Nation, Sr., J.L., 2015. Insect Physiology and Biochemistry. CRC press. doi:10.1201/b18758

O'Brien, D.M., Fogel, M.L., Boggs, C.L., 2002. Renewable and nonrenewable resources: Amino acid turnover and allocation to reproduction in Lepidoptera. Proceedings of the National Academy of Sciences of the United States of America 99, 4413–4418. doi:10.1073/pnas.072346699

Ohkouchi, N., Chikaraishi, Y., Close, H.G., Fry, B., Larsen, T., Madigan, D.J., McCarthy, M.D., McMahon, K.W., Nagata, T., Naito, Y.I., Ogawa, N.O., Popp, B.N., Steffan, S., Takano, Y., Tayasu, I., Wyatt, A.S.J., Yamaguchi, Y.T., Yokoyama, Y., 2017. Advances in the application of amino acid nitrogen isotopic analysis in ecological and biogeochemical studies. Organic Geochemistry 113, 150–174 . doi:10.1016/j.orggeochem.2017.07.009

Okuzaki, Y., Tayasu, I., Okuda, N., Sota, T., 2009. Vertical heterogeneity of a forest floor invertebrate food web as indicated by stable-isotope analysis. Ecological Research 24, 1351–1359. doi:10.1007/s11284-009-0619-0

Olsson, K., Nyström, P., Stenroth, P., Nilsson, E., Svensson, M., Granéli, W., 2008. The influence of food quality and availability on trophic position, carbon signature, and growth rate of an omnivorous crayfish. Canadian Journal of Fisheries and Aquatic Sciences 65, 2293–2304. doi:10.1139/F08-137

Osono, T., Takeda, H., 2004. Accumulation and release of nitrogen and phosphorus in relation to lignin decomposition in leaf litter of 14 tree species. Ecological Research 19, 593–602. doi:10.1111/j.1440-1703.2004.00675.x

Pimm, S.L., 1982. Food webs. SPRINGER.

Pollierer, M.M., Dyckmans, J., Scheu, S., Haubert, D., 2012. Carbon flux through fungi and bacteria into the forest soil animal food web as indicated by compound-specific ^{13}C fatty acid analysis. Functional Ecology 26, 978–990. doi:10.1111/j.1365-2435.2012.02005.x

Pollierer, M.M., Langel, R., Körner, C., Maraun, M., Scheu, S., 2007. The underestimated importance of belowground carbon input for forest soil animal food webs. Ecology Letters 10, 729–736. doi:10.1111/j.1461-0248.2007.01064.x

Pollierer, M.M., Larsen, T., Potapov, A., Brückner, A., Heethoff, M., Dyckmans, J., Scheu, S., 2019. Compound-specific isotope analysis of amino acids as a new tool to uncover trophic chains in soil food webs. Ecological Monographs 89. doi:10.1002/ecm.1384

Pollierer, M.M., Scheu, S., Tiunov, A.V., 2020. Isotope analyses of amino acids in fungi and fungal feeding Diptera larvae allow differentiating ectomycorrhizal and saprotrophic fungi-based food chains. Functional Ecology. doi:10.1111/1365-2435.13654

Ponge, J.F., 2000. Vertical distribution of Collembola (Hexapoda) and their food resources in organic horizons of beech forests. Biology and Fertility of Soils 32, 508–522. doi:10.1007/s003740000285

Potapov, A.A., Semenina, E.E., Korotkevich, A.Y., Kuznetsova, N.A., Tiunov, A.V, 2016. Connecting taxonomy and ecology: Trophic niches of collembolans as related to taxonomic identity and life forms. Soil Biology and Biochemistry 101, 20–31. doi:10.1016/j.soilbio.2016.07.002

Potapov, A.M., Korotkevich, A.Y., Tiunov, A.V., 2018. Non-vascular plants as a food source for litter-dwelling Collembola: Field evidence. Pedobiologia 66, 11–17. doi:10.1016/j.pedobi.2017.12.005

Potapov, A.M., Tiunov, A.V., Scheu, S., 2019a. Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. Biological Reviews 94, 37–59. doi:10.1111/brv.12434

Potapov, A.M., Tiunov, A.V., Scheu, S., Larsen, T., Pollierer, M.M., 2019b. Combining bulk and amino acid stable isotope analyses to quantify trophic level and basal resources of detritivores: a case study on earthworms. Oecologia 189, 447–460. doi:10.1007/s00442-018-04335-3

Potapov, A.M., Tiunov, A.V, 2016. Stable isotope composition of mycophagous collembolans versus mycotrophic plants: Do soil invertebrates feed on mycorrhizal fungi? Soil Biology and Biochemistry 93, 115–118. doi:10.1016/j.soilbio.2015.11.001

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Robinson, D., 2001. $\delta^{15}N$ as an integrator of the nitrogen cycle. Trends in Ecology & Evolution 16, 153–162. doi:10.1016/S0169-5347(00)02098-X

Ruess, L., Chamberlain, P.M., 2010. The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biology and Biochemistry 42, 1898–1910. doi:10.1016/j.soilbio.2010.07.020

Ruess, L., Schütz, K., Migge-Kleian, S., Häggblom, M.M., Kandeler, E., Scheu, S., 2007. Lipid composition of Collembola and their food resources in deciduous forest stands—Implications for feeding strategies. Soil Biology and Biochemistry 39, 1990–2000. doi:10.1016/J.SOILBIO.2007.03.002

Siira-Pietikäinen, A., Pietikäinen, J., Fritze, H., Haimi, J., 2001. Short-term responses of soil decomposer communities to forest management: Clear felling versus alternative forest harvesting methods. Canadian Journal of Forest Research 31, 88–99. doi:10.1139/x00-148

Steffan, S.A., Chikaraishi, Y., Currie, C.R., Horn, H., Gaines-Day, H.R., Pauli, J.N., Zalapa, J.E., Ohkouchi, N., 2015. Microbes are trophic analogs of animals. Proceedings of the National Academy of Sciences of the United States of America 112, 15119–15124. doi:10.1073/pnas.1508782112

Stenroth, P., Holmqvist, N., Nyström, P., Berglund, O., Larsson, P., Granéli, W., 2008. The influence of productivity and width of littoral zone on the trophic position of a large-bodied omnivore. Oecologia 156, 681–690. doi:10.1007/s00442-008-1019-9

Stouffer, D.B., Bascompte, J., 2011. Compartmentalization increases food-web persistence. Proceedings of the National Academy of Sciences of the United States of America 108, 3648–3652. doi:10.1073/pnas.1014353108

Templer, P.H., Arthur, M.A., Lovett, G.M., Weathers, K.C., 2007. Plant and soil natural abundance δ^{15}N: Indicators of relative rates of nitrogen cycling in temperate forest ecosystems. Oecologia 153, 399–406. doi:10.1007/s00442-007-0746-7

Thompson, R.M., Townsend, C.R., 2005. Energy availability, spatial heterogeneity and ecosystem size predict food-web structure in streams. Oikos 108, 137–148. doi:10.1111/j.0030-1299.2005.11600.x

Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, Fourth. ed. Springer, New York.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Supporting information

Table S1. Trophic position (TP_{CSIA}) of Collembola as calculated from compound-specific δ^{15}N values of the amino acids Phe and Glu.

Site	Forest	Treatment	Functional group	N	δ^{15}N Phe	δ^{15}N Glu	TP_{CSIA}		
							Average	1σ[1]	1σ[2]
Hainich	Beech	control	epedaphic	4	-1.16	3.79	2.77	0.19	0.09
Hainich	Beech	control	hemiedaphic	3	-1.55	3.93	2.84	0.21	0.29
Hainich	Beech	control	euedaphic	2	3.14	10.67	3.11	0.12	0.09
Hainich	Beech	trenching	epedaphic	4	-1.97	3.2	2.79	0.09	0.09
Hainich	Beech	trenching	hemiedaphic	3	-2.00	3.64	2.86	0.23	0.13
Hainich	Beech	trenching	euedaphic	1	3.22	9.35	2.92	-	0.1
Hainich	Conifer	control	epedaphic	3	-2.87	2.23	2.79	0.2	0.12
Hainich	Conifer	control	hemiedaphic	2	-1.88	3.78	2.86	0.03	0.13
Hainich	Conifer	trenching	epedaphic	3	-1.46	-0.08	2.29	0.09	0.07
Hainich	Conifer	trenching	hemiedaphic	2	-0.47	5.63	2.92	0.19	0.1
Schorfheide	Beech	control	epedaphic	3	-4.44	0.52	2.77	0.09	0.1
Schorfheide	Beech	control	hemiedaphic	3	-4.42	3.00	3.09	0.18	0.07
Schorfheide	Beech	control	euedaphic	4	-0.89	6.25	3.06	0.1	0.09
Schorfheide	Beech	trenching	epedaphic	4	-5.33	-0.92	2.69	0.2	0.14
Schorfheide	Beech	trenching	hemiedaphic	3	-3.63	0.83	2.7	0.21	0.09
Schorfheide	Beech	trenching	euedaphic	3	-1.86	6.31	3.19	0.27	0.11
Schorfheide	Conifer	control	epedaphic	4	-4.05	-1.01	2.51	0.24	0.06
Schorfheide	Conifer	control	hemiedaphic	3	-5.08	3.12	3.19	0.21	0.13
Schorfheide	Conifer	control	euedaphic	2	-2.35	6.14	3.23	0.29	0.26
Schorfheide	Conifer	trenching	epedaphic	4	-4.17	-0.66	2.57	0.09	0.07
Schorfheide	Conifer	trenching	hemiedaphic	4	-3.85	1.81	2.86	0.2	0.09
Schorfheide	Conifer	trenching	euedaphic	3	0.10	8.15	3.18	0.11	0.2

[1] Standard deviation (1σ) for the comparison of TP_{CSIA} values from multiple samples.

[2] Average of potential uncertainty in TP_{CSIA} calculated by propagating uncertainty of measurement for $\delta^{15}N_{Phe}$, $\delta^{15}N_{Glu}$, β, and TDF after Ohkouchi et al., (2017).

Table S2. Linear mixed effects model (type III error) for the effect of forest type (Forest), functional group (Fg) and root trenching (Trenching) on the trophic position (TP_{CSIA}) of Collembola in the Hainich and Schorfheide. Due to the lack of data on euedaphic Collembola in coniferous forests of the Hainich, analyses were conducted for beech and coniferous forests in the Hainich separately.

Site	Forest	Factor	Sum of Square	Mean of Square	Df1	Df2	*F* value	*P* value
Schorfheide		Forest	0.00	0.00	1	6.15	0.04	0.849
		Fg	1.85	0.92	2	26.93	33.89	<0.001
		Trenching	0.10	0.10	1	25.82	3.73	0.065
		Forest × Fg	0.19	0.09	2	27.00	3.42	0.047
		Fg × Trenching	0.34	0.17	2	26.25	6.27	0.006
		Forest × Trenching	-	-	-	-	-	-
		Forest × Fg × Trenching	-	-	-	-	-	-
Hainich	Beech	Fg	0.16	0.08	2	12.18	3.7	0.056
		Trenching	-	-	-	-	-	-
		Fg × Trenching	-	-	-	-	-	-
	Conifer	Fg	0.29	0.29	1	6	13.21	0.011
		Trenching	0.11	0.11	1	6	5.06	0.065
		Fg × Trenching	0.18	0.18	1	6	8.13	0.029

Df1, numerator degree of freedom; *Df2*, denominator degree of freedom; *Fg*, functional group of Collembola; non-significant interactions were excluded from the model.

Chapter 5

General discussion

Results of this thesis represent a major advance in the understanding of the role of root-derived carbon (C) for trophic interactions in soil food webs. By $^{13}CO_2$ pulse labeling and bulk stable isotope ^{13}C analysis, I traced the root-derived C flux into Collembola species belonging to different functional groups in three cropping systems. Further, the contribution of bacterial and fungal channels to the incorporation of root-derived C into Collembola was estimated using compound specific ^{13}C lipid analysis. To investigate the influences of vertical heterogeneity and root energy supply on trophic structure of soil food webs in forest systems, the trophic niches of Collembola functional groups in a root-trenching experiment were identified by compound-specific isotope analysis (CSIA) of carbon and nitrogen (N) in amino acids (AAs).

In the first study, I pulse labeled the plants with $^{13}CO_2$ to trace the flux of root-derived C into nine Collembola species belonging to three functional groups. Hemiedaphic and epedaphic Collembola incorporated more ^{13}C than euedaphic Collembola in rape and willow, indicating that soil-dwelling species have limited access to recently fixed C in these cropping systems. This contradicts previous findings in forests where euedaphic Collembola were strongly labeled (Potapov et al., 2016a). The contrasting results indicate that the dependence of Collembola on root-derived C is not only determined by inhabited depths but also by other factors such as palatability of root resources and availability and quality of other organic resources such as leaf litter. For instance, the incorporation of root-derived C into euedaphic Collembola was higher in grass than in rape and willow, suggesting that euedaphic Collembola species shift their food preference from root-derived C to soil organic C depending on resource availability (Gillet and Ponge, 2003; Endlweber et al., 2009; Eerpina et al., 2017). In addition, this study was conducted in arable soil with relatively few litter resources; therefore, epedaphic and hemiedaphic Collembola may shift from litter-derived to root-derived resources.

Within the same functional groups, I observed species-specific variation in the uptake of root-derived C. Notably, the variation was most pronounced in euedaphic species, with *Protaphorura armata* incorporating considerably more root-derived C than *Stenaphorura denisi* in all cropping systems. *S. denisi* are characterized by more slender bodies, potentially allowing access to smaller soil pores inaccessible for *P. armata*. Hence, the niche-differentiation in euedaphic Collembola may be explained partly by morphological differences within functional groups (Potapov et al., 2016b). In epedaphic Collembola, *Lepidocyrtus paradoxus* in rape incorporated only little labelled C, contrasting other epedaphic species. In addition, ^{13}C enrichment of *L. paradoxus* declined after it peaked at day 7 and declined afterwards. This suggests that, unlike the other epedaphic species, *L. paradoxus* incorporated recently fixed C from other photoautotrophs than plants, presumably algae or cyanobacteria.

The flux of root C into Collembola strongly differed between cropping systems. The incorporation of ^{13}C into Collembola was markedly slower in rape than in grass and willow. This may reflect lower C allocation to roots of rape, since annual crops are selected to maximize the growth of aboveground compartments. The experiment was conducted during the late growth period of oil-seed rape, the fast growth of flowers and seeds may diminish the C input to belowground (Pausch and Kuzyakov, 2018). In addition, application of fertilizer may further reduce the belowground C allocation in rape. In addition to belowground C allocation, the rhizosphere microbial community may also affect the flux of root C into soil food webs. Bacteria may be more predominant in utilizing root-derived C in rape than in grass and willow, as secondary compounds in root exudates of oil-seed rape are toxic to mycorrhizal and saprotrophic fungi (Kirkegaard and Sarwar, 1998; Okubo et al., 2016). Here, bulk stable isotope analysis reached its limitation since it is unable to disentangle the C fluxes from different microbial channels. Therefore, fatty acids analysis was employed in the second study to further resolve root-derived energy flux from bacterial and fungal channels to higher trophic levels in soil food webs.

The results of the second study demonstrate that the flux of root C into soil microbivores via the bacterial and fungal energy channel strongly varies between cropping systems, with higher incorporation of root-derived C from the bacterial channel in rape than in the grass and willow systems, where the fungi are the dominant C sources. This is consistent with the higher incorporation of root-derived C into bacterial biomarkers in soil phospholipid fatty acids (PLFAs) in rape, suggesting that rhizosphere bacteria play a more important role in channeling root-derived C into soil food webs in rape compared to grass and willow. Oilseed rape is a species of the Brassicaceae that generally does not form mycorrhizal associations. Toxic compounds such as glucosinolates and their hydrolysate isothiocyanates in exudates of Brassicaceae significantly affect microbial communities, e.g. via inhibitory effects on arbuscular mycorrhizal fungi as well as on other fungi (“Biofumigation”; Kirkegaard and Sarwar, 1998; Okubo et al., 2016). Bacteria are generally more tolerant to these compounds than fungi (Smith and Kirkegaard, 2002). In addition, management practices such as fertilizer application may enhance the C flux into the bacterial energy channel and diminish the flux into the fungal channel (Wardle et al., 2004; Denef et al., 2009; Hannula et al., 2017).

In rape, the relative importance of root-derived C in fungal and bacterial biomarkers, as indicated by their enrichment in ^{13}C, differed markedly between Collembola neutral lipid fatty acids (NLFAs) and soil PLFAs, while the differences were minor in grass and willow. Potentially, Collembola NLFAs reflect the composition of microorganisms in the rhizosphere, while soil PLFAs mainly reflect microorganisms in bulk soil. Therefore, in the rhizosphere of rape, root C presumably is channeled mainly by bacteria and immobilized in the rhizosphere, whereas in grass and willow root C is predominantly channeled by fungi; fungal hyphae efficiently transfer root C from the rhizosphere to more distant soil environments (Kuzyakov, 2002; Buée et al., 2009). The highly heterogeneous distribution of root C in soil potentially reduces the incorporation of root C into the soil food web and thus explains at least in part the slower increase in ^{13}C enrichment in Collembola in rape than in grass and willow. Hence, the transfer of root-derived C from basal microbial resources to higher trophic levels in the food web is more efficient in a fungi-dominated than in a bacteria-dominated rhizosphere.

Collembola switch diet according to the availability of resources (Endlweber et al., 2009; Eerpina et al., 2017). As Collembola are generalist feeders relying on a wide range of resources (Scheu, 2002), it is reasonable to assume that they shift diet to feed on the most abundant microbial resources depending on cropping system. However, changes in the relative importance of bacteria and fungi in the diet of Collembola did not correspond to the changes in relative abundance of bacteria and fungi in the soil. Such mismatches in lipid composition between Collembola NLFAs and microbial PLFAs in bulk soil and litter were also found in previous studies (Ngosong et al., 2009; Sechi et al., 2014; Ferlian et al., 2015). Interestingly, changes in the relative importance of bacteria and fungi in the diet of Collembola paralleled those in the incorporation of root-derived C into soil PLFAs, suggesting that the dietary composition of Collembola is determined by microbial communities in the rhizosphere actively utilizing recently fixed C, rather than by those in bulk soil. Potentially, microorganisms incorporating root exudates are less substrate limited, resulting in higher activity and local biomass compared to those in bulk soil, making them a more palatable resource for Collembola (De Boer et al., 2005; Buée et al., 2009).

Hence, the food quality and community composition in microbial hotspots are more important than the quantity of total microbial biomass in determining microbial food resources of soil microbivores.

Unlike the arable soil, the soil in forests receives a considerable amount of aboveground inputs, promoting a strong vertical heterogeneity of available resources and environmental conditions. However, the vertical heterogeneity of trophic interactions in soil food webs in temperate forest systems and its response to deprivation of root energy supply are poorly understood. To answer these questions, trophic niches of Collembola functional groups were identified using compound specific isotope analysis of carbon and nitrogen in amino acids in a root-trenching experiment.

^{13}C AA fingerprinting suggested that saprotrophic microorganisms, especially saprotrophic fungi, rather than mycorrhizal fungi form the dominant food resource for Collembola. This is in line with recent studies that soil decomposers generally do not use mycorrhizal fungi as main food source (Klironomos et al., 1999; Potapov and Tiunov, 2016; Bluhm et al., 2019; Pollierer et al. 2021 accepted). In contrast to basal resources, the N sources and trophic position calculated from CSIA (TP_{CSIA}) differed markedly among functional groups of Collembola. The $\delta^{15}N$ values of phenylalanine, serving as proxy for N resource, were higher in euedaphic Collembola than in ep-/hemiedaphic Collembola. This indicates that euedaphic Collembola have a higher dependence on ^{15}N-enrichend soil organic matter including humiphagous microorganisms in the N mobilization stage (Osono and Takeda, 2004). By contrast, saprotrophic fungi in the immobilization stage of litter decomposition presumably are the primary resources for ep-/hemiedaphic Collembola (Fujii and Takeda, 2012). The trophic position of euedaphic Collembola was generally higher than that of epedaphic Collembola. Feeding on saprotrophic fungi involved in the early stage of litter decomposition (Bodvarsson, 1970; Anderson and Healey, 1972) may result in a short trophic chain between epedaphic Collembola and plants. By contrast, euedaphic Collembola feed on saprotrophic fungi relying on highly processed soil organic matter, of which microbial necromass is the major component. In this case, reprocessing of the proteinaceous components in microbial necromass by microorganisms themselves may lead to higher trophic positions of saprotrophic microorganisms (Steffan et al., 2015), thereby resulting in trophic inflation of euedaphic Collembola. Another reason for high trophic position of euedaphic Collembola may be the higher availability of animal resources, including animal prey and carcasses, in the litter at late stages of decomposition (Heijboer et al., 2018). The TP_{CSIA} of hemiedaphic Collembola varied among regions, with higher TP_{CSIA} in Schorfheide than in Hainich as compared to epedaphic Collembola in the same region. As $\delta^{15}N$ values of Phe were similar for epedaphic and hemiedaphic Collembola in both regions, the higher TP_{CSIA} of hemiedaphic Collembola in Schorfheide likely was not due to higher dependence on old soil organic matter, but presumably rather to more intensive feeding on resources at higher trophic levels, such as animal prey or carcass.

The generally higher TP_{CSIA} of euedaphic Collembola relative to epedaphic Collembola indicates that the number of trophic transfers increases with depth of inhabited layer (Okuzaki et al., 2009). Both more trophic interactions at the basal level in food webs (longer microbial food chains) and more diverse resources, including animals feeding on litter at later stages of decay, may contribute to the longer food chain length in the soil than in the litter layer. In line with this hypothesis, in the Schorfheide the trophic positons of Collembola increased with depth irrespective of functional group, whereas the $\delta^{15}N$ values of phenylalanine did not change, reflecting higher availability of animal resources in deeper layers. However, this pattern was only observed in the Schorfheide but not in the Hainich, potentially due to regional differences in distribution of animal resources and microhabitat structure (Erdmann et al., 2012; Ferlian and Scheu, 2014).

Taking advantage of CSIA-AAs, for the first time I provide evidence that deprivation of root energy supply decreases the trophic transfer in soil food webs, which is in line with the ‘productivity hypothesis’ (Thompson and Townsend, 2005). In the cases where root trenching affected TP_{CSIA} of Collembola, it was consistently reduced when Collembola were deprived of root energy supply. This reflects that the length of food chains increases with availability of basal resources. However, the reduction in TP_{CSIA} varied between functional groups and forest stands. The deprivation of root-derived resources reduced the TP_{CSIA} of hemiedaphic Collembola only in the Schorfheide but not in the Hainich. As discussed before, hemiedaphic Collembola in the Schorfheide are likely to consume relatively high amounts of animal tissue (as predators or scavengers). Hence, the reduction in animal prey resulting from the shortage of root-derived resources may lead hemiedaphic Collembola to feed more on resources at lower trophic levels such as saprotrophic microorganisms. Supporting this hypothesis, the abundance of bacterivorous nematodes in soil decreased by more than 50% in trenched plots in the Schorfheide but not in the Hainich (Sarah Bluhm, pers. comm.). The TP_{CSIA} of euedaphic and epedaphic Collembola was generally not affected by root-trenching. Potentially, they may mainly feed on saprotrophic microorganisms. Hence, a shift to feeding on more basal resources, such as plant litter and soil organic matter, is unlikely due to the low quality and low accessibility of these materials. In addition, the strong compartmentalization of soil food webs may reduce the impact of deprivation of root energy supply. Therefore, the association between root energy supply and trophic interactions in soil are affected by vertical heterogeneity, regional factors and feeding strategies of consumers.

This thesis represents a major advance in the understanding of the role of root-derived C for trophic interactions in soil food webs in arable and forest systems. Cropping system not only affects the flux of root C into Collembola, but also the utilization of root-derived resources vs. litter/soil organic matter-derived resources by Collembola functional groups and species. In addition, the flux of root C into soil microbivores via the bacterial and fungal energy channel strongly varied between cropping systems depending on plant species/plant type and fertilizer application. This change in energy flux into different microbial groups significantly affects the transfer efficiency of root C from basal microbial resources to higher trophic levels, and represents a major driver of dietary variation in microbivorous microarthropds among cropping systems. Using compound-specific stable isotope composition of C and N in amino acids, I identified the trophic niche of Collembola functional groups, providing new insights into the structural heterogeneity of soil food webs in forest systems. Importantly, as the baseline isotopic signature is recorded in the δ^{15}N signature of phenylalanine, we were able to overcome a long-standing problem of bulk isotope analyses, i.e. to clarify whether the apparent increase in bulk δ^{15}N of consumers with soil depth is due to elevated N isotope baseline or more trophic transfers. Hence, trophic positions of soil consumers could be estimated reliably. This allows for the first time to provide direct evidence that root energy supply increases trophic transfers and food chain length in soil food webs, which is in line with the ‘productivity hypothesis’. Overall, root-derived C as major resource fueling soil food webs profoundly influences the niche partitioning in soil consumers, trophic interactions between microbes and microbivores and trophic structure of soil food webs. Plants invest a considerable part of the energy in root C input, linking the above- and belowground functioning of arable and forest ecosystems. Our study stresses the importance of root-derived C in the trophic interactions in soil food webs and it may have significant implications for the future changes in root mediated ecosystem functions when, e.g. the ratio of CO_2, water and nutrient availability and land use are changing.

Reference

Anderson, J.M., Healey, I.N., 1972. Seasonal and inter-specific variation in major components of the gut contents of some woodland Collembola. The Journal of Animal Ecology 41, 359. doi:10.2307/3473

Bluhm, S.L., Potapov, A.M., Shrubovych, J., Ammerschubert, S., Polle, A., Scheu, S., 2019. Protura are unique: First evidence of specialized feeding on ectomycorrhizal fungi in soil invertebrates. BMC Ecology 19, 1–7. doi:10.1186/s12898-019-0227-y

Bodvarsson, H., 1970. Alimentary studies of seven common soil-inhabiting Collembola of southern Sweden. Insect Systematics & Evolution 1, 74–80. doi:10.1163/187631270x00366

Buée, M., De Boer, W., Martin, F., van Overbeek, L., Jurkevitch, E., 2009. The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Plant and Soil 321, 189–212. doi:10.1007/s11104-009-9991-3

De Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: Impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29, 795-811 . doi:10.1016/j.femsre.2004.11.005

Denef, K., Roobroeck, D., Manimel Wadu, M.C.W., Lootens, P., Boeckx, P., 2009. Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. Soil Biology and Biochemistry 41, 144–153. doi:10.1016/j.soilbio.2008.10.008

Eerpina, R., Boiteau, G., Lynch, D.H., 2017. Collembola diet switching in the presence of maize roots varies with species. Canadian Journal of Soil Science 97, 171–177. doi:10.1139/cjss-2016-0057

Endlweber, K., Ruess, L., Scheu, S., 2009. Collembola switch diet in presence of plant roots thereby functioning as herbivores. Soil Biology and Biochemistry 41, 1151–1154. doi:10.1016/j.soilbio.2009.02.022

Erdmann, G., Scheu, S., Maraun, M., 2012. Regional factors rather than forest type drive the community structure of soil living oribatid mites (Acari, Oribatida). Experimental and Applied Acarology 57, 157–169. doi:10.1007/s10493-012-9546-9

Ferlian, O., Klarner, B., Langeneckert, A.E., Scheu, S., 2015. Trophic niche differentiation and utilisation of food resources in collembolans based on complementary analyses of fatty acids and stable isotopes. Soil Biology and Biochemistry 82, 28–35. doi:10.1016/J.SOILBIO.2014.12.012

Ferlian, O., Scheu, S., 2014. Shifts in trophic interactions with forest type in soil generalist predators as indicated by complementary analyses of fatty acids and stable isotopes. Oikos 123, 1182–1191. doi:10.1111/j.1600-0706.2013.00848.x

Fujii, S., Takeda, H., 2012. Succession of collembolan communities during decomposition of leaf and root litter: Effects of litter type and position. Soil Biology and Biochemistry 54, 77–85. doi:10.1016/j.soilbio.2012.04.021

Gillet, S., Ponge, J.-F., 2003. Changes in species assemblages and diets of Collembola along a gradient of metal pollution. Applied Soil Ecology 22, 127–138. doi:10.1016/S0929-1393(02)00134-8

Hannula, S.E., Morriën, E., De Hollander, M., Van Der Putten, W.H., Van Veen, J.A., De Boer, W., 2017. Shifts in rhizosphere fungal community during secondary succession following abandonment from agriculture. ISME Journal 11, 2294–2304. doi:10.1038/ismej.2017.90

Heijboer, A., de Ruiter, P.C., Bodelier, P.L.E., Kowalchuk, G.A., 2018. Modulation of Litter Decomposition by the Soil Microbial Food Web Under Influence of Land Use Change. Frontiers in Microbiology 9, 2860. doi:10.3389/fmicb.2018.02860

Kirkegaard, J.A., Sarwar, M., 1998. Biofumigation potential of brassicas. Plant and Soil 201, 71–89. doi:10.1023/A:1004364713152

Klironomos, J.N., Bednarczuk, E.M., Neville, J., 1999. Reproductive significance of feeding on saprobic and arbuscular mycorrhizal fungi by the collembolan, Folsomia candida. Functional Ecology 13, 756–761. doi:10.1046/j.1365-2435.1999.00379.x

Kuzyakov, Y., 2002. Review: Factors affecting rhizosphere priming effects, in: Journal of Plant Nutrition and Soil Science. pp. 382–396. doi:10.1002/1522-2624(200208)165:4<382::AID-JPLN382>3.0.CO;2-#

Ngosong, C., Raupp, J., Scheu, S., Ruess, L., 2009. Low importance for a fungal based food web in arable soils under mineral and organic fertilization indicated by Collembola grazers. Soil Biology and Biochemistry 41, 2308–2317. doi:10.1016/j.soilbio.2009.08.015

Okubo, A., Matsusaka, M., Sugiyama, S., 2016. Impacts of root symbiotic associations on interspecific variation in sugar exudation rates and rhizosphere microbial communities: a comparison among four plant families. Plant and Soil 399, 345–356. doi:10.1007/s11104-015-2703-2

Okuzaki, Y., Tayasu, I., Okuda, N., Sota, T., 2009. Vertical heterogeneity of a forest floor invertebrate food web as indicated by stable-isotope analysis. Ecological Research 24, 1351–1359. doi:10.1007/s11284-009-0619-0

Osono, T., Takeda, H., 2004. Accumulation and release of nitrogen and phosphorus in relation to lignin decomposition in leaf litter of 14 tree species. Ecological Research 19, 593–602. doi:10.1111/j.1440-1703.2004.00675.x

Pausch, J., Kuzyakov, Y., 2018. Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale. Global Change Biology 24, 1–12. doi:10.1111/gcb.13850

Potapov, A.M., Goncharov, A.A., Tsurikov, S.M., Tully, T., Tiunov, A.V., 2016a. Assimilation of plant-derived freshly fixed carbon by soil collembolans: Not only via roots? Pedobiologia 59, 189–193. doi:10.1016/j.pedobi.2016.07.002

Potapov, A.M., Semenina, E.E., Korotkevich, A.Y., Kuznetsova, N.A., Tiunov, A.V., 2016b. Connecting taxonomy and ecology: Trophic niches of collembolans as related to taxonomic identity and life forms. Soil Biology and Biochemistry 101, 20–31. doi:10.1016/j.soilbio.2016.07.002

Potapov, A.M., Tiunov, A.V, 2016. Stable isotope composition of mycophagous collembolans versus mycotrophic plants: Do soil invertebrates feed on mycorrhizal fungi? Soil Biology and Biochemistry 93, 115–118. doi:10.1016/j.soilbio.2015.11.001

Scheu, S., 2002. The soil food web: structure and perspectives. European Journal of Soil Biology 38, 11–20. doi:10.1016/S1164-5563(01)01117-7

Sechi, V., D'Annibale, A., Ambus, P., Sárossy, Z., Krogh, P.H., Eriksen, J., Holmstrup, M., 2014. Collembola feeding habits and niche specialization in agricultural grasslands of different composition. Soil Biology and Biochemistry 74, 31–38. doi:10.1016/j.soilbio.2014.02.019

Smith, B.J., Kirkegaard, J.A., 2002. In vitro inhibition of soil microorganisms by 2-phenylethyl isothiocyanate. Plant Pathology 51, 585–593. doi:10.1046/j.1365-3059.2002.00744.x

Steffan, S.A., Chikaraishi, Y., Currie, C.R., Horn, H., Gaines-Day, H.R., Pauli, J.N., Zalapa, J.E., Ohkouchi, N., 2015. Microbes are trophic analogs of animals. Proceedings of the National

Academy of Sciences of the United States of America 112, 15119–15124. doi:10.1073/pnas.1508782112

Thompson, R.M., Townsend, C.R., 2005. Energy availability, spatial heterogeneity and ecosystem size predict food-web structure in streams. Oikos 108, 137–148. doi:10.1111/j.0030-1299.2005.11600.x

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van Der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633. doi:10.1126/science.1094875

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List of publications

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Xiao, H., Wang, W., Xia, S., **Li, Z.**, Gan, J., Yang, X., 2020. Distributional patterns of soil nematodes in relation to environmental variables in forest ecosystems. Soil Ecology Letters, 1-10. doi: 10.1007/s42832-020-0069-8

He, X., Liu, S., Wang, J., Gan, J., **Li, Z.**, Liu, T., Zhang, W., Zou, X., Yang, X., 2020. Disturbance intensity overwhelms propagule pressure and litter resource in controlling the success of Pontoscolex corethrurus invasion in the tropics. Biological Invasions 22, 1705–1721. doi:10.1007/s10530-020-02214-8

Li, Z., Wei, Z., Yang, X., 2016. Seasonal variations of soil nematode community at different secondary succession stages of evergreen broad-leaved forests in Ailao mountain. Chinese Journal of Ecology 35, 3023–3031. doi:10.13292/j.1000-4890.201611.008 (in Chinese with English abstract)

Liu, S., **Li, Z.**, Sui, Y., Schaefer, D.A., Alele, P.O., Chen, J., Yang, X., 2015. Spider foraging strategies dominate pest suppression in organic tea plantations. BioControl 60, 839–847. doi:10.1007/s10526-015-9691-2

Submitted

Li, Z., Shi, L., Kuzyakov, Y., Pausch, J., Scheu, S., Pollierer, M.M., The flux of root-derived carbon via fungi and bacteria into soil microarthropods (Collembola) differs markedly between cropping systems. (submitted, Soil Biology and Biochemistry)

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Thesis declarations

Declaration of the author's own contribution to manuscripts with multiple authors

Chapters 2 comprises a manuscript that has been published in peer-reviewed journals; Chapter 3 and 4 comprise mansucripts that are currently submitted to a peer reviewed journal. In all manuscripts I am the first author and I have collected and analyzed the data, written the manuscripts, developed the main ideas, created tables, figures and appendices and contributed significantly to the study design. All co-authors contributed to finalising the manuscripts.

Plagiarism declaration

I declare that I have written this doctoral thesis independently. All persons contributing to the manuscripts have been named so. All sentences or passages quoted from other people's work have been specifically acknowledged by clear cross-referencing. I have not submitted this thesis in any form for another degree at any university or institution.

Zhipeng Li
Göttingen, February 2021