Soil Biology & Biochemistry 115 (2017) 526–536

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Multitrophic interactions in the rhizosphere of a temperate forest tree affect plant carbon flow into the belowground food web

H.R. Maboreke [a, *], M. Graf [b], T.E.E. Grams [c], S. Herrmann [d, e], S. Scheu [b], L. Ruess [a]

[a] Humboldt-Universität zu Berlin, Institute of Biology, Ecology Group, Philippstr. 13, 10115 Berlin, Germany
[b] J.F. Blumenbach Institute of Zoology and Anthropology, Georg August University Göttingen, Germany
[c] Ecophysiology of Plants, Technische Universität München, Hans-Carl-von-Carlowitz-Platz 2, 85354 Freising, Germany
[d] Dept. Soil Ecology, UFZ – Helmholtz Centre for Environmental Research, Theodor-Lieser-Str. 4, 06120 Halle/Saale, Germany
[e] Dept. Community Ecology, UFZ – Helmholtz Centre for Environmental Research, Theodor-Lieser-Str. 4, 06120 Halle/Saale, Germany

ARTICLE INFO

Article history:
Received 29 November 2016
Received in revised form
29 August 2017
Accepted 5 September 2017
Available online 29 September 2017

Keywords:
Oak
Plant parasitic nematodes
Collembola
PLFAs
^{13}C labelling

ABSTRACT

Tree roots and mycorrhizal fungi form an important resource for soil microinvertebrates; however, there is little knowledge on the role of root feeders and microbial grazers on belowground carbon flux of forest trees. Using oak (*Quercus robur*) microcuttings (Clone DF159) inoculated with an ectomycorrhizal symbiont partner (*Piloderma croceum*), the present study investigated the impact of multitrophic interactions in the rhizosphere on the carbon flow to the soil food web in a pulse-chase labelling experiment. Changes in plant biomass, microbial communities (soil phospholipid fatty acids - PLFAs) and Collembola lipid pattern (total lipid fatty acids - TLFAs) as well as the ^{13}C incorporation into fatty acids were assessed at 2, 5 and 20 days post labelling of oak microcuttings. Microcosms were inoculated with *Pratylenchus penetrans* (root-feeding nematode), *Protaphorura armata* (fungal-feeding Collembola) or their combination. The interaction of *P. penetrans* and *P. armata* synergistically reduced oak biomass. Both soil animals independently impacted growth of microorganisms in the rhizosphere, *P. penetrans* inhibited whilst *P. armata* promoted bacterial biomass. Irrespective of treatment and time, Gram-positive bacteria derived most of the recent photoassimilated oak carbon, with amounts ranging between 80 and 92% of the total ^{13}C allocated in microbial PLFAs. Presence of either *P. armata* or *P. penetrans* enhanced the incorporation of plant carbon in bacteria, whereas collective effects of these two functional groups was scarce. Predominantly the impact of the root-feeding nematode on belowground carbon flow diminished with time. In sum, root feeders and detritivores played independent roles in carbon allocation patterns and community structure of microorganisms in the rhizosphere of oaks.

1. Introduction

Shifts in carbon allocation between aboveground and belowground compartments play a major role in the carbon cycle of forest ecosystem (Norby et al., 2004; Cheng and Gershenson, 2007; Litton et al., 2007). The ability of these systems to sequester carbon, e.g. under different climate scenarios, is driven by diverse biotic interactions controlling plant carbon flux belowground (Farrar and Jones, 2000). In forest soils, ectomycorrhizal symbiosis acts as key-driver for these nutrient and carbon translocation processes (Smith and Read, 2008; Mueller et al., 2012; Cesarz et al., 2013a). Tree roots and mycorrhizal fungi form an important resource for soil animals, however, in contrast to annual plants, the role of root feeders and microbial grazers for belowground carbon fluxes by forest trees has been largely neglected.

The rhizosphere associated fauna in forests ecosystems is highly diverse, and includes about 60–100 species of nematodes (Boag and Yeates, 1998; Ettema, 1998) and 30–50 species of Collembola (Hopkin, 1997; Rusek, 1998), that occupy various ecological niches. The density and diversity of nematodes is especially high in deciduous forests (Hanel, 1996, 1997). Here the proportion of root-feeding taxa can reach up to 34% of the total community (Cesarz et al., 2013b), with their population development linked to tree belowground carbon allocation (Hoeksema et al., 2000). These root feeders affect plant symbiotic relationships and suppress colonization of roots by and sporulation of mycorrhizal fungi (Francl,

* Corresponding author.
E-mail addresses: maborekh@hu-berlin.de, zeemaboreke@yahoo.co.uk (H.R. Maboreke).

https://doi.org/10.1016/j.soilbio.2017.09.002
0038-0717/

1993; Kesba and Al-Sayed, 2006). Further, plant-parasitic nematodes impose diverse direct (e.g. carbon loss via feeding) and indirect (e.g. "leakage" of root cell contents) effects on their hosts (Yeates et al., 1998; Williamson and Gleason, 2003). Nematode feeding induces metabolic sinks that alter allocation pattern of photoassimilates, biomass (shoot-to-root ratio) and nutrient concentrations in plants (Van der Putten, 2003; Hofmann et al., 2010; Kaplan et al., 2011). The transfer of carbon to roots is facilitated, enhancing exudation and thereby altering microbial community structure in the rhizosphere (Bardgett et al., 1999; Haase et al., 2007; Poll et al., 2007). Thus, besides their direct uptake of plant carbon via feeding, plant-parasitic nematodes additionally contribute to the regulation of soil microbial communities and thus to soil nutrient and carbon dynamics.

While plant-parasitic nematodes are important root herbivores, Collembola are major fungal feeders in the soil, consuming mycorrhiza as well as saprotrophic fungi in forest ecosystems (Klironomos et al., 1996; Hopkin, 1997; Kaneda and Kaneko, 2004; Scheu and Folger, 2004). Therefore, in contrast to nematodes, the mechanisms by which Collembola affect plants are to a greater extent indirect. Some studies suggest that the activity of these fungivores has positive effects on plant performance, with moderate grazing stimulating mycorrhiza fungal growth as well as enhancing nutrient release from low vital or dead mycorrhizal mycelium (Kaneda and Kaneko, 2004; Steinacker and Wilson, 2008). They have also been shown to modify plant growth by altering nutrient supply or by feeding on mycorrhizal fungi (Lussenhop and Bassiri, 2005; Tordoff et al., 2008). On the other hand, depending on the resources available, Collembola can switch from fungal to root feeding as shown in e.g. herbs and corn (Endlweber et al., 2009; Ngosong et al., 2014). These omnivorous grazers therefore affect forest trees mainly indirectly via feeding on mycorrhizal fungi; however, they may also directly affect trees by acting as root herbivores.

Owning to the complexity of belowground processes and the technical difficulties to manipulate rhizosphere environments in long-lived forest trees, significant gaps remain in our current understanding how soil microorganisms, fauna and plants interact in affecting the transfer and allocation of photoassimilates. Only recently, the common view that mycorrhizal fungi form a main food source for Collembola is being debated as stable isotope data do not support this opinion (Potapov and Tiunov 2016). We investigated the impact of these multitrophic interactions in the rhizosphere of forest trees on belowground carbon flux in a pulse-chase labelling experiment with $^{13}CO_2$. Microcuttings of pedunculate oak, *Quercus robur* (L.), pre-inoculated with the ectomycorrhizal fungus *Piloderma croceum* (J. Erikss. & Hjortst), and grown in a soil based culture system, were used as the model forest tree. Oak microcuttings, from the clone DF159 (Herrmann et al., 2016), were inoculated with the nematode *Pratylenchus penetrans* (Cobb, 1917), the Collembola *Protaphorura armata* (Tullberg, 1869) or both. Migratory root-feeding nematodes such as *Pratylenchus* spp. were shown to negatively interact with the ectomycorrhiza of forest trees (Marks et al., 1987; Hanel, 1998). The euedaphic Collembola species *P. armata* is known to feed on both roots and fungi (Endlweber et al., 2009). The allocation of plant-derived carbon within rhizosphere microorganisms in response to the multitrophic interactions of the oak with herbivore and fungivore soil animals was assessed. The flux of photoassimilates into the belowground food web was traced by the ^{13}C signal in the compartments plant tissue, soil microbial biomass, soil phospholipid fatty acids and Collembola total lipid fatty acids. We expected the allocation of plant carbon in oak and the carbon flux into the belowground food web to be affected by the type of trophic interaction with: i) root-feeding nematodes enhancing carbon flux to roots and into rhizosphere microbial communities, ii) Collembola predominantly acting as fungivores, thereby assimilating root carbon directly from the ectomycorrhizal fungus, and iii) the interaction of both functional groups, i.e. the loss of carbon and nutrients by direct nematode feeding, and the impaired mycorrhizal symbiosis due to Collembola grazing, reducing the resilience of oak microcuttings.

2. Materials and methods

2.1. Microcosm model system

Soil substrate for microcosm set up and preparation of bacterial filtrate was performed as presented by Herrmann et al. (2016) and briefly summarized here. Soil was collected from the upper soil horizons A0 (humus layer, 0–10 cm depth) and A1+A2 (mineral soil, 10–30 cm depth) from an oak forest stand at the Dölauer Heide (51.51016°N, 11.91291°E) close to Halle/Saale, Germany. Subsamples of these two soils were immediately frozen at −20 °C and used as stock soil for the production of a microbial filtrate inoculum. The remaining bulk soil samples were air dried and sieved though 5 mm mesh, equal volumes of the two soil layers A0 and A1+A2 were thoroughly mixed together. Aliquots of 500 ml were gamma sterilized at 50 kGy by BGS Beta-Gamma-Service (Wiehe, Germany) and subsequently stored at 8 °C. Sterility tests were performed by plating soil aliquots on Lysogeny broth agar (LB) before use in the microcosms.

Micropropagated pedunculate oaks (*Quercus robur*) from a long-term established clone DF159 were used as models for hardwood ectomycorrhiza-associated tree species (Herrmann et al., 1998, 2016). Microcuttings were rooted as described in Herrmann et al. (2004) and transplanted into sterile 12 × 12 cm square petri dish microcosms filled with a mixture of equal volumes of γ-sterilized soil and mycorrhizal inoculum (for details see, Caravaca et al., 2015; Herrmann et al., 2016). *Piloderma croceum*, a basidiomycete ectomycorrhizal fungus of hardwood and conifer species was used as symbiotic partner. The employed strain *P. croceum* F1598 has previously been used in studies on the oak clone DF159 (Herrmann et al., 1998, 2004, 2016; Tarkka et al., 2013). The fungus was grown on a modified Melin-Norkrans (MMN) medium (Marx, 1969) as described in Herrmann et al. (1998).

To establish a natural bacterial community, a soil bacterial filtrate excluding fungi was prepared as described by Rosenberg et al. (2009). Each microcosm received 5 ml of the bacterial filtrate inoculum five weeks after establishment of the oak-fungus culture system. The oaks were grown in a climate chamber throughout the experiment at 23 °C, with a 16 h day and 8 h night regime, photosynthetic photon flux density of 180 μmol m^{-2} s^{-1}, 400 ppm CO_2 and 80% relative humidity.

2.2. Soil fauna

Pratylenchus penetrans, a cosmopolitan nematode, was used as the belowground plant herbivore model. Axenic cultures of *P. penetrans* were grown on carrot discs following a protocol by O'Bannon and Taylor (1968). Nematodes were extracted from the carrot discs and surface sterilized using 0.01% Mercury chloride before treatment inoculation. Nematode density was determined by counting individuals in a known volume of water, which was then adjusted to obtain the desired nematode inoculum density per millilitre.

The Collembola *Protaphorura armata* was used as soil fungivorous animal model. Laboratory cultures established from field populations close to Darmstadt (Germany) were reared for several generations in a glass crib on a mixture of sterilized potting soil and clay pellets (3:1). The soil substrate was amended with baker's