Disentangling carbon flow across microbial kingdoms in the rhizosphere of maize

Maike Hünninghaus[a], Dörte Dibbern[b], Susanne Kramer[c], Robert Koller[a,d], Johanna Pausch[e], Brigitte Schloter-Hai[f], Tim Urich[g], Ellen Kandeler[c], Michael Bonkowski[a], Tillmann Lueders[b,*]

Highlights:

- We use rRNA-SIP to trace the succession of the uptake of plant-derived carbon flows across all relevant microbial kingdoms (bacteria, fungi, protists) in the rhizosphere of maize.
- ^{13}C-labeling of mycorrhizal *Paraglomerales* and several bacteria including *Opitutus, Mucliaginibacter* and *Massilia spp.* was especially apparent in soil surrounding the strict rhizosphere after 5 d, highlighting the pivotal role of AMF as a rapid shunt of fresh plant assimilates to microbes outside the strict rhizosphere.
- Labeling of filamentous saprotrophic *Ascomycota* or *Basidiomycota* was not apparent, challenging proposed "sapro-rhizosphere" concepts.

Disentangling carbon flow across microbial kingdoms in the rhizosphere of maize

Maike Hünninghaus[a], Dörte Dibbern[b], Susanne Kramer[c], Robert Koller[a,d], Johanna Pausch[e], Brigitte Schloter-Hai[f], Tim Urich[g], Ellen Kandeler[c], Michael Bonkowski[a], Tillmann Lueders[b,h*]

[a] Institute of Zoology, Terrestrial Ecology, University of Cologne and Cluster of Excellence on Plant Sciences (CEPLAS), Cologne, Germany

[b] Institute of Groundwater Ecology, Helmholtz Zentrum München, Neuherberg, Germany

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

[d] Institute of Bio- and Geosciences, IBG-2 Plant Sciences, Forschungszentrum Jülich, Germany

[e] Agroecology, University of Bayreuth, Germany

[f] Research Unit for Comparative Microbiome Analysis, Helmholtz Zentrum München, Neuherberg, Germany

[g] Institute of Microbiology, University of Greifswald, Germany

[h] Department of Ecological Microbiology, University of Bayreuth, Germany

* Corresponding author. Department of Ecological Microbiology, University of Bayreuth, Dr.-Hans-Frisch-Straße 1-3, 95440 Bayreuth, Germany.

E-mail: tillmann.lueders@uni-bayreuth.de

Keywords: rhizosphere microbes, mycorrhizosphere, arbuscular mycorrhizal fungi, rhizodeposition, rRNA-stable isotope probing, amplicon sequencing

Abstract

Numerous $^{13}CO_2$ labeling studies have traced the flow of carbon from fresh plant exudates into rhizosphere bacterial communities. However, the succession of the uptake of carbon leaving the roots by distinct rhizosphere microbiota has rarely been resolved between microbial kingdoms. This can provide valuable insights on the niche partitioning of primary rhizodeposit consumption, as well as on community interactions in plant-derived carbon flows in soil. Here, we have traced the flow of fresh plant assimilates to rhizosphere microbiota of maize (*Zea mays* L.) by rRNA-stable isotope probing (SIP). Carbon flows involving bacteria, unicellular fungi, as well as protists were observed over 5 and 8 days. Surprisingly, labeling of *Paraglomerales* and several bacteria including *Opitutus, Mucliaginibacter* and *Massilia spp.* was especially apparent in soil surrounding the strict rhizosphere after 5 d. This highlights the central role of arbuscular mycorrhizal fungi (AMF) as a shunt for fresh plant assimilates to soil microbes not directly influenced by root exudation. Distinct trophic webs involving different flagellates, amoeba and ciliates were also observed in rhizosphere and surrounding soil, while labeling of filamentous saprotrophic *Ascomycota* or *Basidiomycota* was not apparent. This challenges the proposed "sapro-rhizosphere" concept and demonstrates the utility of rRNA-SIP to disentangle inter-kingdom microbial relationships in the rhizosphere.

Introduction

Soil microbiota represent one of the greatest reservoirs of biodiversity and organismic carbon on our planet (Torsvik and Øvreås, 2002; Roesch et al., 2007). This tremendous biomass is mainly fueled by plant-derived carbon inputs that enter the soil via two distinct pathways: rhizodeposition and plant detritus. Rhizodeposition is able to shape rhizosphere microbiota, which in turn are important for plant health in many ways (Berendsen et al., 2012; Philippot et al., 2013; Sasse et al., 2018). The carbon flux from plants to the rhizosphere is understood to drive a major component of the entire soil food web, which exerts a top-down selection on rhizosphere microbiomes in return (Bonkowski et al., 2009). Next to direct root exudation, an important share of rhizodeposition can also be transferred via arbuscular mycorrhizal fungi (AMF, (Jones et al., 2004; Roth and Paszkowski, 2017). In return, AMF facilitate the access of plants to nutrients and water and contribute significantly to root system functioning via their hyphal network (Jones et al., 2004; Veresoglou et al., 2012). However, the comparative importance of the mycorrhizal pathway vs. direct exudation in shaping rhizosphere communities has rarely been addressed (Kaiser et al., 2015). Research on rhizosphere microbiomes has often been limited to a kingdom-level perspective, and the complex interactions of AMF, rhizosphere bacteria, protists and other saprotrophic fungi remain poorly resolved (Henkes et al., 2018).

While bacteria were originally thought to monopolize the consumption of fresh exudates in the rhizosphere (Kent and Triplett, 2002), non-AMF fungi were conceived to be less competitive for labile organic substrates (de Boer et al., 2005). However, the incorporation of plant-derived ^{13}C in fungal phospholipid fatty acids (Butler et al., 2003; Treonis et al., 2004) has led to the hypothesis that filamentous “sugar-fungi” (e.g. *Mucor* or *Mortierella* spp. within the *Mucoromycotina*), directly compete with bacteria for the uptake of

69 labile exudates (De Boer et al., 2006). Moreover, the interactions of AMF with bacteria not
70 directly drawing on root exudation may also be more important than originally assumed
71 (Drigo et al., 2010; Scheublin et al., 2010; Qin et al., 2016). Further complexity is added to
72 inter-kingdom carbon flows in the rhizosphere by bacterivorous and fungivorous
73 micropredators, both within the protists and bacteria (Ballhausen and de Boer, 2016; Geisen et
74 al., 2016; Zhang and Lueders, 2017; Henkes et al., 2018). Accordingly, the understanding of
75 such intra-microbial trophic loops is still at an early stage.

76 Here, microcosm studies making use of $^{13}CO_2$ labeling via growing plants, in
77 combination with stable isotope probing (SIP) of nucleic acids, have opened an important
78 experimental route (Vandenkoornhuyse et al., 2007; Drigo et al., 2010; Mao et al., 2014;
79 Haichar et al., 2016; Uksa et al., 2017). However, only one previous study has realized the
80 potential of this approach to trace the flux of plant-assimilated C across all relevant microbial
81 kingdoms, *i.e.* bacteria, fungi and protists (Drigo et al., 2010). For a number of C-3 plants, the
82 authors showed that AMF acted as a rapid shunt for the transfer of C between plant and soil,
83 and indirectly released root C to secondary bacterial and fungal consumers. Here, we aimed to
84 disentangle the comparative involvement of members of the three microbial kingdoms as
85 consumers of rhizodeposits in the rhizosphere of maize, a C-4 plant. A $^{13}CO_2$-labeling
86 experiment was conducted with young maize plants growing in rhizoboxes with soil from an
87 intensively investigated agricultural field (Scharroba et al., 2012; Kramer et al., 2013; Moll et
88 al., 2015; Müller et al., 2016; Pausch et al., 2016). Key assimilators of plant-derived C were
89 identified by rRNA-SIP across all relevant microbial kingdoms (bacteria, fungi and protists).
90 We have previously investigated microbial carbon flows in the detritusphere (Kramer et al.,
91 2016) and also between micropredators in the same soil (Zhang and Lueders, 2017). Together
92 with the present study, this allows for a highly comprehensive inventory of population-
93 specific microbial activities in the same plant-soil system.

Materials and methods

Microcosm experiment for SIP

The experiment was conducted in two acrylic glass chambers (l: 95 cm, w: 42 cm, h: 70 cm) for the growth of ^{13}C-labeled and ^{12}C-control plants. To mimic summer conditions in temperate agricultural fields, maize plants (*Zea mays*) were exposed to light for 12 hours each day with 600 µmol PAR. Temperature was 28 °C by day and 18 °C at night. Plants were germinated on wet cellulose tissue and after five days, seeds and root tips were truncated to generate highly branched roots and support the use of CO_2 as sole C-source for the plants. The next day, maize seedlings were planted into ethanol sterilized (70% v/v) rhizoboxes (17.0 x 10.0 x 0.5 cm) containing 135 g of fresh, sieved (< 5mm) soil collected in November 2010 from the upper 10 cm of an experimental maize field near Göttingen, Germany (Kramer et al., 2012; Scharroba et al., 2012). Soil was stored at room temperature, sieved (< 5mm) and homogenized before being distributed into the rhizoboxes for the experiment in March 2011. The plants were watered every day with 15 ml of water. One week after germination, 0.5 g l^{-1} KNO_3 were added to the water as fertilizer over a total period of five days.

The labeling started 24 days after germination and three control plants were sampled directly before the onset of labeling. By then, soil in the rhizoboxes was densely rooted, suggesting the adequate development of a rhizosphere in the original bulk soil, as intended. 24 plants, each, were placed into both chambers and either $^{13}CO_2$ (from sodium bicarbonate-^{13}C, 98 atom %, Campro Scientific, Berlin, Germany) or unlabeled CO_2 (source: anhydrous sodium carbonate, Sigma-Aldrich, St. Louis, USA), were pumped into the respective chambers. Constant CO_2 concentrations of 418 ± 27 ppm were established to ensure optimal C fixation rates. CO_2 concentrations were controlled via an infrared gas analyzer (Carbocap GM70, Vaisala, Vantaa, Finland). Soil was covered with parafilm to reduce CO_2 efflux from

soil respiration, and also to minimize direct labeling of soil microbes via CO_2-fixation. Labeling lasted for 6 d but the experiment was continued for another 10 d after end of labeling. During the experiment, plants were irrigated with 25 – 30 ml of water each day. 7.5 mg of KNO_3 per plant were amended to the water at days 4, 6, 9, and 12 of the experiment, respectively, as plants developed signs of nitrogen deficiency.

Triplicate plants were destructively harvested per treatment after 0.5, 1, 2, 3, 5, 8, 11 and 16 days. Upon sampling, the front plates of the rhizoboxes were opened and roots were carefully removed using sterile spatula. Soil remaining adherent to the roots after manual shaking was considered as the rhizosphere *sensu stricto* (Andrade et al., 1997; Buddrus-Schiemann et al., 2010), which is largely restricted to the root hair zone for maize (Bengough, 2012). Aliquots of ~5 g of strict rhizosphere soil or ~25 g of remaining rhizobox soil (i.e. surrounding soil) were transferred into sterile plastic tubes and stored frozen (-20°C) until further analysis. Due to the limited availability of strict rhizosphere soil, biogeochemical and isotopic downstream analyses could only be done for surrounding soil. rRNA-SIP, however, was conducted for both sets of soil samples.

Isotopic analysis of plants and soil

Total dry weight of plant shoots and roots were determined after each sampling and the incorporation of isotopic label was estimated for plant biomass and soil of triplicate ^{13}C-treatments. For this, the $\delta^{13}C$ values of bulk shoot, root and surrounding soil samples were measured on an Elemental Analyzer NA 2500 (Carlo Erba Instruments, Milano, Italy) interfaced to a Delta XP isotope ratio mass spectrometer (Thermo Electron Cooperation, Bremen, Germany, (Pausch et al., 2016). Enrichment of ^{13}C label in a certain C pool was determined by subtracting the relative abundance of ^{13}C in the unlabeled pool ($\chi(^{13}C)_{Std}$, in

atom %) from the relative ^{13}C abundance in the same pool (*P*) after labeling ($\chi(^{13}C)_P$, atom %). Carbon excess was expressed in $g^{13}C\ g\ C^{-1}$:

$$\chi^E(^{13}C) = \chi(^{13}C)_P - \chi(^{13}C)_{Std} \quad \text{(Pausch et al., 2016)}$$

Biogeochemical analyses

Organic carbon was extracted from sets of triplicate surrounding soil samples with 0.025M K_2SO_4 (1:4; w/v), shaken at 250 rev min^{-1} on a horizontal shaker and centrifuged for 30 min at 4422 x g. Organic C content in supernatants was measured by a N/C analyzer (Multi N/C 2100S, Analytik Jena, Jena, Germany). Lipids were extracted from 6 g (fresh weight) of soil with a Bligh & Dyer solution [chloroform, methanol, citrate buffer (pH = 4; 1: 2: 0.8; v/v/v, (Frostegård et al., 1991) and separated into glyco-, neutral fatty acids (NLFA) and phospholipid fatty acids (PLFA) with silica acid columns (0.5 g silicic acid, 3 ml; Varian Medical Systems, Palo Alto, CA, USA). Neutral and phospholipid fatty acids were transformed into fatty acid methyl esters (FAME) by mild alkaline methanolysis. FAMEs were measured with a GC as described in Kramer et al. (2013). An internal FAME standard was added to the extracts for quantification before methanolysis. PLFAs i15:0, a15:0, i16:0, 16:1ω7, i17:0, cy17:0, cy19:0 were used as bacterial markers, 18:2ω6,9c as a marker for saprotrophic fungi, and NLFA 16:1ω5 for arbuscular mycorrhiza (Ruess and Chamberlain, 2010).

rRNA stable isotope probing (rRNA-SIP)

RNA extraction from soil followed previous protocols (Kramer et al., 2016; Zhang and Lueders, 2017) and was extracted from rhizosphere and surrounding soil samples. RNA extracts from time points after 1, 3, 5 and 8 d of labeling were selected for gradient centrifugation. To account for analytical capacities assigned to the project, rRNA extracts

164 from triplicate microcosms were pooled and centrifuged as composite samples in SIP.
165 Isopycnic centrifugation and gradient fractionation were done as described (Kramer et al.,
166 2016; Zhang and Lueders, 2017), with 750 ng of total RNA loaded into each gradient and a
167 resolution of 12 gradient fractions per sample.

168 Bacterial rRNA in density-resolved SIP fractions was first analyzed by T-RFLP
169 fingerprinting as described (Kramer et al., 2016; Zhang and Lueders, 2017). In brief, bacterial
170 communities were analyzed with primers Ba27f-FAM / 907r and subsequent *Msp*I digestion.
171 T-RFs representing labeled community members were identified by comparison of T-RF
172 abundances in heavy vs. light rRNA fractions of ^{13}C-gradients as well as of ^{12}C-controls.
173 Based on fingerprinting results (Fig. S1), fractions 3 and 8 were identified as representative
174 heavy and light rRNA fractions of all ^{12}C and ^{13}C SIP gradients and subjected to amplicon
175 sequencing (Kramer et al., 2016; Zhang and Lueders, 2017).

176 Bacterial rRNA amplicons were generated and sequenced on a 454 GS FLX sequencer
177 using Titanium chemisty (Roche Applied Biosystems, Penzberg, Germany) as previously
178 described (Kramer et al., 2016). Bidirectional reads were quality-trimmed and filtered (Pilloni
179 et al., 2012; Kramer et al., 2016), and reads <250 bp after trimming were excluded from
180 further analysis. Classification of bacterial taxa was done with the RDP classifier (Wang et al.,
181 2007). Eukaryotic rRNA amplicons were generated with the same primers as used for
182 eukaryote T-RFLP, and with modified PCR chemistry using the Brilliant III Ultra-Fast RT-
183 qPCR Master Mix (Agilent Technologies, Santa Clara, USA). Quality trimming, filtering and
184 exclusion of short reads were done following the same workflow as for prokaryotes. Then,
185 eukaryotic SSU rRNA sequences were taxonomically analyzed with the CREST toolbox
186 (Lanzén et al., 2012). In brief, the amplicons were taxonomically assigned by MEGAN
187 analysis of BLASTN files against the SilvaMod SSU rRNA reference database (LCA
188 parameters: min. bit score 330, min. support 1, top percent 2; 50 best blast hits). The

189 classification of flagellates followed the key of Jeuck and Arndt (2013), with all *Bicosoecida*
190 and *Cercomonadida* considered as flagellates.

191 The sequencing libraries generated for light, unlabeled rRNA (fractions 3 of ^{12}C-
192 gradients) were considered to closely represent total rRNA pools extracted from the different
193 time points and soil compartments and used to illustrate overall community composition
194 (Zhang and Lueders, 2017). T-RFs were predicted *in silico* for sequencing contigs generated
195 for dominating bacterial taxa of all libraries (Pilloni et al., 2012) by using TRiFLe (Junier et
196 al., 2008). This allowed to tentatively link labeling patterns observed for T-RFs to that of
197 sequencing data (Kramer et al., 2016; Zhang and Lueders, 2017). All sequencing data have
198 been deposited with the NCBI sequence read archive and are available under the BioProject
199 accession number PRJNA521477 for bacterial and microeukaryotic rRNA reads.

200 **Inference of taxon-specific isotopic enrichment factors in rRNA**

201 To identify taxa involved in the assimilation of ^{13}C from rhizodeposits within the
202 different groups (bacteria, fungi, protists), sequencing read 'enrichment factors' (EF) in heavy
203 rRNA fractions were inferred as reported (Kramer et al., 2016; Zhang and Lueders, 2017).
204 Briefly, EFs were calculated as follows:

205 $$EF = {}^{13}C_{heavy} / {}^{13}C_{light} - {}^{12}C_{heavy} / {}^{12}C_{light}$$

206 where $^{13}C_{heavy}$ and $^{13}C_{light}$ were the relative abundance of taxon-specific reads in heavy and
207 light rRNA fractions of ^{13}C treatments, and $^{12}C_{heavy}$ and $^{12}C_{light}$ were the same for the
208 respective ^{12}C-controls. EFs were calculated for all bacterial taxa with >1 % read abundance
209 in heavy rRNA of at least one ^{13}C treatment and one time point, >0.25% for eukaryotes, and >
210 0.1% for yeasts, as the latter were resolved to a finer taxonomic resolution.

Statistics

The concentrations of extractable organic carbon (EOC), PLFAs and NLFAs over time and between treatments were analyzed with factorial ANOVA using STATISTICA 12 (Tulsa, OK, USA). Post Hoc tests for comparison of means were done with Tukey HSD. When necessary, data were transformed to obtain homoscedasticity (proven by Levene`s test). All error bars are given as standard deviations of triplicate analyses.

Results

Plant growth and rhizobox labeling

During the 16 d of our labeling incubation, the maize plants approximately doubled in root and shoot biomass (Fig. 1A). The biggest increase in plant growth was observed between days 5 and 8, where the average total plant biomass increased from 0.69 to 0.98 g_{dw} per rhizobox. Significant differences in plant growth between ^{13}C and ^{12}C-treatments were not observed. During the labeling period of 6 d, each plant assimilated 2.84 ± 0.75 mmol C d^{-1}, resulting in an isotopic enrichment of ^{13}C of ~26, 23, and 0.7 atom % ^{13}C for maize shoots, roots and soil, respectively. Between the end of labeling (6 d) and the end of the experiment, enrichment in plant did not increase any further (Fig. 1B).

The content of total EOC in rhizobox soil varied over time ($F_{8,36}$ = 59.9; P < 0.01), with highest concentrations found on days 2-3 and 11, respectively (Fig. 2A). The abundance of bacterial PLFAs and of the fungal PLFA 18:2ω6,9c showed a significant short-term increase within the first day of labeling (Fig. 2B, C). Afterwards, the abundance of bacterial PLFAs remained constant over time, while that of fungi steadily increased. Mycorrhizal NLFAs (16:1ω5) also showed an initial peak and then a constant increase in abundance until

234 day 16 (Fig. 2D). ^{13}C-labeling did not significantly impact the concentrations of EOC,
235 bacterial and fungal PLFAs, or of NLFAs compared to the ^{12}C-controls. Based on the
236 observed plant growth and ^{13}C-labeling patterns, days 1, 3, 5 and 8 were selected as time
237 points subjected to rRNA SIP.

238 **rRNA-SIP of rhizosphere microbiota**

239 SIP was conducted for rRNA extracted from both strict rhizosphere soil and
240 surrounding soil. The rRNA resolved in SIP gradients was first subjected to fingerprinting of
241 bacterial SSU rRNA amplicons. The comparison of density-resolved fingerprints from ^{13}C-
242 treatments and ^{12}C-control gradients revealed a clear labeling of bacterial T-RFs after 5 d of
243 incubation, both in rhizosphere and surrounding soil, further increasing in intensity after 8 d
244 (Fig. S1). In addition, surrounding soil rRNA extracts taken after 1 and 3 d of incubation were
245 also resolved in SIP gradients, but labeling patterns were not apparent via bacterial rRNA
246 fingerprinting for these earlier time points (data not shown).

247 Therefore, rRNA gradients of days 5 and 8 were selected for downstream sequencing
248 of bacterial and eukaryotic rRNA amplicons. First, sequencing libraries generated for light
249 rRNA fractions of the ^{12}C-treatments were used to illustrate overall community composition
250 of bacteria and microeukaryotes from different time points and compartments (Zhang and
251 Lueders, 2017). Bacterial sequencing libraries were dominated by *Actinobacteria* and
252 *Alphaproteobacteria* (Fig. 3A), with rRNA affiliated to *Marmoricola*, *Humicoccus*, and
253 *Bradyrhizobium* spp. representing some of the most abundant genus-level OTUs (~3 – 6%).
254 Microeukaryote libraries were dominated by fungal rRNA reads (40 – 48%) affiliated to the
255 *Ascomycota*, *Basidiomycota* and *Mucoromycotina* (Fig. 3B). Moreover, protists within the
256 *Ciliophora*, *Amoebozoa* and *Stramenopiles* were also of sizeable abundance (20 – 30% of all
257 eukaryotic reads, taken together).

Compared to rhizosphere soil *sensu stricto*, sequence reads of the *Deltaproteobacteria*, *Acidobacteria*, *Planctomycetes* and *Verrucomicrobia* were more abundant in the surrounding soil (Fig. 3A). Moreover, rRNA reads of the mycorrhizal *Glomeromycota* were more abundant in surrounding soil, where they represented ~5% of all eukaryotic reads (Fig. 3B). Distinctions between soil compartments were also apparent for the protists, where amoeba appeared more abundant in surrounding soil, whereas ciliates were enriched in rhizosphere rRNA, especially after 8 d (Fig. 3B).

Labeled bacterial rRNA

The taxon-specific read enrichment factors (EFs) showed that only a minor subset of the considerable bacterial diversity in the soil incorporated ^{13}C-label. Most of the more strongly labeled bacterial taxa (i.e., EF >3) showed only low relative read abundances in unlabeled rRNA. However, reads affiliated to *Opitutus* spp. (*Verrucomicrobia*) were a marked exception, as they were both, highly abundant and strongly enriched in heavy ^{13}C-rRNA (Fig. 4, Table S1). Unexpectedly, labeling after 5 d was more pronounced for a number of bacterial lineages in surrounding soil rRNA than in the strict rhizosphere. In fact, of the ~10 genera with marked labelling after 5 d, more than half showed stronger ^{13}C-enrichment in the surrounding soil. This was observed especially for *Opitutus*, but also *Mucilaginibacter*, *Ohktaekwangia* (both *Bacteroidetes*), *Sphingobium* (*Alphaproteobacteria*) and *Massilia* spp. (*Betaproteobacteria*). In contrast, reads affiliated to *Azospirillum* (*Alphaproteobacteria*), *Arthrobacter spp.* (*Actinobacteria*) and *Gemmata* (*Planctomycetes*) were more enriched in the strict rhizosphere after 5 d. On day 8, rRNA of *Kitasatospora* (*Actinobacteria*) also appeared as labeled in surrounding soil rRNA, while rRNA of *Mucilaginibacter* and *Massilia* was now also clearly labeled in the rhizosphere. rRNA of only one lineage (*Dactylosporangium*, *Actinobacteria*) showed opposite temporal dynamics, where rRNA was first enriched in ^{13}C

after 5 d, and then strongly depleted after 8 d, especially in the surrounding soil. Other lineages showed similar enrichment in both compartments, either at one (*Acidobacteria*, *Azospirillum*) or at both time points (*Rhizobacter*, *Oxalobacteraceae*). For most of the more abundant taxa, the interpretation of labeling based on EFs was consistently supported by associated T-RFs enriched in heavy ^{13}C rRNA fingerprints (Table S1, Fig. S1).

Labeled microeukaryote rRNA

rRNA of the *Glomeromycota* was most strongly ^{13}C-labeled amongst the eukaryotes (Fig. 5, Table S1). The *Paraglomerales* were the most heavily labeled AMF group and increased in labeling over time, especially in soil surrounding the strict rhizosphere. Also other AMF lineages (*Glomerales*, *Diversisporales* and *Archaeosporales*) showed notable ^{13}C-labeling. None of the abundant filamentous fungi within the *Ascomycota*, *Basidiomycota* or *Mucoromycotina* showed any ^{13}C enrichment in SIP. Only within the *Chytridiomycota*, reads affiliated to the *Rhizophydiales* and *Chytridiales* showed labeling in the rhizosphere. Also unicellular fungi were revealed to draw on rhizodeposition: rRNA of some yeasts (*Candida*, *Torulaspora* spp.) was weakly labeled in the rhizosphere after 8 d (Fig. 5). Reads affiliated to *Cryptococcus* spp. (*Tremellomycetes*, *Basidiomycota*), although being by far the most abundant yeast lineage (3 – 6%, Fig. 3B), showed no ^{13}C-enrichment.

Amongst the protists, rRNA of the oomycete *Phytophthora* spp. (*Stramenopiles*) represented the 2nd most highly labeled microeukaryote taxon in surrounding soil (after the *Paraglomerales*). In the rhizosphere, rRNA of flagellates within the *Thaumatomonadida* (*Cercozoa*) and *Apusozoa*, and of the *Leptomyxida* (*Amoebozoa*) was ^{13}C-enriched, indicative of active protistan micropredation. In contrast, flagellates within the *Bodonidae* (*Kinetoplastida*) incorporated more ^{13}C-label in soil surrounding the strict rhizosphere.

Discussion

^{13}C-labeling of rhizosphere microbiota

This study aimed to discriminate plant-derived ^{13}C-labeling patterns for the maize rhizosphere across microbial kingdoms. We also compared labeling apparent in the strict rhizosphere vs. that of surrounding soil. The dense rooting of our rhizoboxes clearly prevents the assumption that part of the investigated soil could still have represented unrooted bulk soil. Typically, soil remaining adherent to roots after extraction and manual shaking is considered as the strict rhizosphere (Andrade et al., 1997; Buddrus-Schiemann et al., 2010). However, the pronounced effect of sampling strategies on access to actual rhizosphere microbes is a matter of critical debate (Richter-Heitmann et al., 2016), and may also vary with plant and soil type. In the present work, it can be assumed that the surrounding rhizobox soil contained a larger fraction of soil less influenced by direct exudation than by AMF-mediated rhizodeposition, a compartment previously termed as the hyphosphere (Andrade et al., 1997; Kaiser et al., 2015; Qin et al., 2016).

Allocation of plant-derived C to the soil was first proven by bulk elemental analyses and by the increase in EOC over time (Fig. 1 and 2A). In our study, the strong increase of EOC until day 2, paralleled by early short-term peaks of lipid biomarkers, suggested that abundant plant-derived C was allocated to the soil within the first days of the experiment. Irrespective, the resulting transfer of label into rhizosphere microbiota seems to have been insufficient for rRNA-SIP at these early time points. While the total abundance of soil bacteria as indicated via PLFA biomarkers remained constant after day 2, the parallel increase of the NLFA biomarker for AMF together with the general fungal PLFA biomarker suggests, that the increase of total fungal biomass was mainly attributed to mycorrhizal growth. This clear increase in AMF biomass is a strong evidence for the successful establishment of

mycorrhizal associations within the growing maize roots (Kaiser et al., 2015; Qin et al., 2016).

Comparative SIP labelling across microbial kingdoms

The use of rRNA-SIP is a prime tool to trace population-specific microbial carbon flows in the rhizosphere (Haichar et al., 2016). Still, the approach has rarely been applied across microbial kingdoms in a comparative manner (Drigo et al., 2010). In the present study, labeling was traced across three relevant microbial kingdoms (bacteria, fungi, protists) and in two distinct fractions of plant-influenced soil. Several of the labeling patterns observed did not come as a surprise. Amongst the labeled bacteria, *Massilia* (Ofek et al., 2012), *Mucilaginibacter* (Madhaiyan et al., 2010), *Azospirillum* and *Arthrobacter* spp. (Babalola, 2010) are all well-known to include plant growth-promoting rhizobacteria (PGPR). Different *Sphingobacteria* and *Azospirillum spp*. have also been previously associated with rhizodeposit utilization in maize (Haichar et al., 2008; Peiffer et al., 2013). Also *Arthrobacter* spp. has been identified as a utilizer of sugars in the same soil in our previous SIP investigation of detritusphere carbon flows (Kramer et al., 2016). For all of these lineages, labelling consistent with presumed rhizodeposit usage was obtained in our study. The distinct temporal labelling pattern observed for *Dactylosporangium* spp., however, could be indicative of a stimulated usage of unlabeled soil organic matter (SOM) after prior rhizodeposit uptake, i.e. a priming effect (Kuzyakov, 2010).

However, we did not expect to find rRNA of the verrucomicrobial *Opitutus* spp. to represent the most prominently labeled bacterium in the rhizoboxes. *Opitutus* has been previously detected in the rhizosphere of maize (Correa-Galeote et al., 2016), but was originally isolated from flooded rice paddies and is known as an obligate fermenter (van Passel et al., 2011). We cannot exclude that the high water demand of the plants could have

led to the transient establishment of anoxic micro-niches in our rhizoboxes after watering. Regardless, *Opitutus* spp. was clearly identified as the most successful bacterial forager of fresh plant-derived C in our study, a finding that has not been described for any other rhizosphere system. The fact that *Opitutus* rRNA was even more clearly labeled in soil surrounding the strict rhizosphere suggests, that it must have been receiving plant-derived C via the mycorrhizal pathway.

rRNA labelling of fungi clearly substantiated the outstanding role of AMF as a conduit for fresh plant assimilates into the mycorrhizosphere (Roth and Paszkowski, 2017). A rapid transfer of plant assimilates to AMF and a direct release to soil bacteria via their hyphal network (Johnson et al., 2002) was the only conceivable route for the pronounced labeling of *Opitutus* spp. outside the strict rhizosphere after 5 d. These results are in line with the description of AMF as a "carbon bridge" between roots and the surrounding soil (Bago et al., 2003). Other bacteria for which AMF-mediated transfer of plant-derived C was suggested included *Mucilanginibacter, Ohktaekwangia,* and *Massilia* spp.. *Massilia* spp. can be very abundant in the rhizosphere of maize (García-Salamanca et al., 2013) and also *Mucilaginibacter* spp. are known as typical rhizosphere microbes and maize endophytes (Kämpfer et al., 2014). We previously identified *Mucilaginibacter* and other *Bacteroidetes* as important degraders of maize litter in the detritusphere of the same soil (Kramer et al., 2016). The present study, however, suggest that these bacteria possessed efficient ways of interacting with and accessing plant-derived C via the AMF network (Scheublin et al., 2010). We can only speculate whether chitinolytic capacities reported for members of the genus could have been involved (Yoon et al., 2012).

Amongst the filamentous saprophytic fungi, none of the abundant Ascomycota, *Basidiomycota* or even sugar fungi within the *Mucoromycotina* detected in our rRNA libraries showed ^{13}C enrichment. This was surprising, since a previous $^{13}CO_2$ pulse labeling

379 experiment conducted directly in the field suggested a prominent role of saprotrophic fungi in
380 the acquisition of root-derived C (Pausch et al., 2016), in line with the recently proposed
381 "sapro-rhizosphere" concept (Ballhausen and de Boer, 2016). The functional differences
382 observed here could potentially be attributed to the different growth stages of the investigated
383 plants (~4 weeks in rhizoboxes vs. ~3 months in the field). Thus, plant-growth dependent
384 differences in exudation and mycorrhization should be more specifically addressed when
385 investigating rhizosphere microbiome recruitment and interactions in the future (Guyonnet et
386 al., 2018).

387 Although rRNA of typical sugar fungi like *Mortierella* spp. and other hyphal
388 *Basidiomycota* and *Ascomycota* were diverse and abundant in our study (Fig. 3), they clearly
389 remained unlabeled. Different yeasts appeared as the only *Ascomycota* to receive minor ^{13}C-
390 labeling in the investigated soil. At the same time, labeling was much more pronounced for
391 members of the *Chytridiomycota*, some of which are, intriguingly, known to include AMF
392 parasites (Ross and Ruttencutter, 1977; Wakefield et al., 2010). Irrespective of the possible
393 mechanisms, our results highlight the importance of complex trans- and inter-kingdom
394 microbial carbon flows in plant-influenced soils.

395 **Micropredator labeling**

396 Except for oomycetes (*Phytophthora* spp.), some of which are known as plant-
397 pathogens (Fry, 2008), rRNA of labeled protists detected in this study all belonged to
398 microbial grazers. A distinct community of labeled protists was detected in both investigated
399 soil fractions. While amoebae were generally the most abundant protistan supergroup in light
400 rRNA libraries (Fig. 3), the active protistan community directly incorporating root-derived C
401 included mostly flagellates, especially in the strict rhizosphere. Members of the
402 *Thaumatomonadida* have been previously identified as dominant flagellates in the

403 investigated soil based on laboratory cultivation (Scharroba et al., 2012), thus their labelling
404 in the present study, albeit weak, was actually consistent. We have also recently observed
405 distinct predation patterns of amoebae on Gram-positive or –negative bacterial prey in the
406 same soil (Zhang and Lueders, 2017), so prey selection could also have contributed to the
407 trophic labeling observed. Amongst the amoebae, the facultatively mycophagous *Leptomyxida*
408 (Chakraborty and Old, 1982; Geisen et al., 2015) were also weakly ^{13}C-labeled, possibly
409 suggesting a respective fungivorous foraging on unicellular fungi or even AMF in our
410 experiment.

411 ## Conclusions

412 This study highlights the pivotal role of AMF being a major hub for translocating fresh
413 plant-derived labile carbon to soil microbes, independent from and in parallel to passive root
414 exudation (Bago et al., 2003; Kaiser et al., 2015). It is thought that a large proportion of C
415 translocated through the AMF network is transported as glycogen or triacylglycerol (Roth and
416 Paszkowski, 2017; Keymer and Gutjahr, 2018). Possibly, some of the bacteria detected as
417 highly ^{13}C-labeled in our study (e.g. *Opitutus* spp.) specialize on consuming such AMF-
418 derived C substrates.

419 Summarizing these results into a conceptual network of the flow of labeled carbon
420 between plant and microbes in the investigated rhizosphere (Fig. 6), the pivotal importance of
421 AMF as a rapid shunt of fresh plant assimilates to bacteria and also protists (*Oomycetes* and
422 *Bodonidae*) outside the strict rhizosphere is illustrated. At the same time, evidence for the
423 involvement of typical saprotrophic hyphal fungi in rhizosphere carbon flows was not
424 observed, thus challenging the proposed "sapro-rhizosphere" concept (Ballhausen and de
425 Boer, 2016). We also demonstrate that rhizodeposits are competitively utilized by specific
426 subpopulations across microbial kingdoms, but that these are also dynamic in time (Fig. 6).

Our results demonstrate how AMF-mediated carbon transfer into soil greatly extends the influence of the plant holobiont, previously thought to be mainly driven by direct root exudation. For the first time, we provide evidence for the spatial and temporal dynamics of these interactions via SIP.

Acknowledgements

This study was funded by grants of the DFG (Deutsche Forschungsgemeinschaft) to T.L., M.B. and E.K. within the Research Unit "Carbon flow in belowground food webs assessed with isotope tracers" (FOR 918). Further support was provided by the Helmholtz Society. We thank J. Moll (UFZ Leipzig) for help during the labeling experiment. Furthermore we thank L. Ruess (Univ. Berlin) and S. Scheu (Univ. Göttingen) for coordinating the Research Unit and for valuable discussions.

References

Andrade, G., Mihara, K.L., Linderman, R.G., Bethlenfalvay, G.J., 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant and Soil 192, 71-79.

Babalola, O., 2010. Beneficial bacteria of agricultural importance. Biotechnology Letters 32, 1559-1570.

Bago, B., Pfeffer, P.E., Abubaker, J., Jun, J., Allen, J.W., Brouillette, J., Douds, D.D., Lammers, P.J., Shachar-Hill, Y., 2003. Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrate as well as lipid. Plant Physiology 131, 1496-1507.

Ballhausen, M.-B., de Boer, W., 2016. The sapro-rhizosphere: Carbon flow from saprotrophic fungi into fungus-feeding bacteria. Soil Biology and Biochemistry 102, 14-17.

Bengough, A.G., 2012. Water dynamics of the root zone: Rhizosphere biophysics and its control on soil hydrology. Vadose Zone Journal 11: 2.

Berendsen, R.L., Pieterse, C.M.J., Bakker, P.A.H.M., 2012. The rhizosphere microbiome and plant health. Trends in Plant Science 17, 478-486.

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

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

Butler, J., Williams, M., Bottomley, P., Myrold, D., 2003. Microbial community dynamics associated with rhizosphere carbon flow. Applied and Environmental Microbiology 69, 6793 - 6800.

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

Correa-Galeote, D., Bedmar, E.J., Fernández-González, A.J., Fernández-López, M., Arone, G.J., 2016. Bacterial communities in the rhizosphere of amilaceous maize (*Zea mays* L.) as assessed by pyrosequencing. Frontiers in Plant Science 7, 1016.

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.

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

Drigo, B., Pijl, A.S., Duyts, H., Kielak, A.M., Gamper, H.A., Houtekamer, M.J., Boschker, H.T.S., Bodelier, P.L.E., Whiteley, A.S., Veen, J.A.v., Kowalchuk, G.A., 2010. Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2. Proceedings of the National Academy of Sciences 107, 10938-10942.

Frostegård, Å., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. Journal of Microbiological Methods 14, 151-163.

Fry, W., 2008. *Phytophthora infestans*: the plant (and R gene) destroyer. Molecular Plant Pathology 9, 385-402.

García-Salamanca, A., Molina-Henares, M.A., van Dillewijn, P., Solano, J., Pizarro-Tobías, P., Roca, A., Duque, E., Ramos, J.L., 2013. Bacterial diversity in the rhizosphere of maize and the surrounding carbonate-rich bulk soil. Microbial Biotechnology 6, 36-44.

Geisen, S., Koller, R., Hünninghaus, M., Dumack, K., Urich, T., Bonkowski, M., 2016. The soil food web revisited: Diverse and widespread mycophagous soil protists. Soil Biology and Biochemistry 94, 10-18.

Geisen, S., Tveit, A.T., Clark, I.M., Richter, A., Svenning, M.M., Bonkowski, M., Urich, T., 2015. Metatranscriptomic census of active protists in soils. ISME J 9, 2178-2190.

Guyonnet, J.P., Guillemet, M., Dubost, A., Simon, L., Ortet, P., Barakat, M., Heulin, T., Achouak, W., Haichar, F.e.Z., 2018. Plant nutrient resource use strategies shape active rhizosphere microbiota through root exudation. Frontiers in Plant Science 9, 1662.

Haichar, F.e.Z., Heulin, T., Guyonnet, J.P., Achouak, W., 2016. Stable isotope probing of carbon flow in the plant holobiont. Current Opinion in Biotechnology 41, 9-13.

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

Henkes, G.J., Kandeler, E., Marhan, S., Scheu, S., Bonkowski, M., 2018. Interactions of mycorrhiza and protists in the rhizosphere systemically alter microbial community composition, plant shoot-to-root ratio and within-root system nitrogen allocation. Frontiers in Environmental Science 6, 117.

Jeuck, A., Arndt, H., 2013. A short guide to common heterotrophic flagellates of freshwater habitats based on the morphology of living organisms. Protist 164, 842-860.

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

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

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

Kaiser, C., Kilburn, M.R., Clode, P.L., Fuchslueger, L., Koranda, M., Cliff, J.B., Solaiman, Z.M., Murphy, D.V., 2015. Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. New Phytologist 205, 1537-1551.

518 Kämpfer, P., Busse, H.-J., McInroy, J.A., Glaeser, S.P., 2014. *Mucilaginibacter auburnensis*
519 sp. nov., isolated from a plant stem. International Journal of Systematic and Evolutionary
520 Microbiology 64, 1736-1742.

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

523 Keymer, A., Gutjahr, C., 2018. Cross-kingdom lipid transfer in arbuscular mycorrhiza
524 symbiosis and beyond. Current Opinion in Plant Biology 44, 137-144.

525 Kramer, S., Dibbern, D., Moll, J., Huenninghaus, M., Koller, R., Krueger, D., Marhan, S.,
526 Urich, T., Wubet, T., Bonkowski, M., Buscot, F., Lueders, T., Kandeler, E., 2016.
527 Resource partitioning between bacteria, fungi, and protists in the detritusphere of an
528 agricultural soil. Frontiers in Microbiology 7, 1524.

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

532 Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H.,
533 Kuzyakov, Y., Pausch, J., Scheunemann, N., Schoene, J., Schmalwasser, A., Totsche,
534 K.U., Walker, F., Scheu, S., Kandeler, E., 2012. Carbon flow into microbial and fungal
535 biomass as a basis for the belowground food web of agroecosystems. Pedobiologia 55,
536 111-119.

537 Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter.
538 Soil Biology and Biochemistry 42, 1363-1371.

539 Lanzén, A., Jørgensen, S.L., Huson, D.H., Gorfer, M., Grindhaug, S.H., Jonassen, I., Øvreås,
540 L., Urich, T., 2012. CREST – Classification resources for environmental sequence tags.
541 PLoS ONE 7, e49334.

542 Madhaiyan, M., Poonguzhali, S., Lee, J.-S., Senthilkumar, M., Lee, K.C., Sundaram, S., 2010.
543 *Mucilaginibacter gossypii* sp. nov. and *Mucilaginibacter gossypiicola* sp. nov., plant-
544 growth-promoting bacteria isolated from cotton rhizosphere soils. International Journal of
545 Systematic and Evolutionary Microbiology 60, 2451-2457.

546 Mao, Y., Li, X., Smyth, E.M., Yannarell, A.C., Mackie, R.I., 2014. Enrichment of specific
547 bacterial and eukaryotic microbes in the rhizosphere of switchgrass (*Panicum virgatum*
548 L.) through root exudates. Environmental Microbiology Reports 6, 293-306.

549 Moll, J., Goldmann, K., Kramer, S., Hempel, S., Kandeler, E., Marhan, S., Ruess, L., Krüger,
550 D., Buscot, F., 2015. Resource type and availability regulate fungal communities along
551 arable soil profiles. Microbial Ecology 70, 390-399.

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

555 Ofek, M., Hadar, Y., Minz, D., 2012. Ecology of root colonizing *Massilia*
556 (*Oxalobacteraceae*). PLoS ONE 7, e40117.

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

561 Peiffer, J.A., Spor, A., Koren, O., Jin, Z., Tringe, S.G., Dangl, J.L., Buckler, E.S., Ley, R.E.,
562 2013. Diversity and heritability of the maize rhizosphere microbiome under field
563 conditions. Proceedings of the National Academy of Sciences 110, 6548-6553.

564 Philippot, L., Raaijmakers, J.M., Lemanceau, P., van der Putten, W.H., 2013. Going back to
565 the roots: the microbial ecology of the rhizosphere. Nature Reviews Microbiology 11,
566 789.

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

570 Qin, H., Brookes, P.C., Xu, J., 2016. Arbuscular mycorrhizal fungal hyphae alter soil bacterial
571 community and enhance polychlorinated biphenyls dissipation. Frontiers in Microbiology
572 7, 939.

573 Richter-Heitmann, T., Eickhorst, T., Knauth, S., Friedrich, M.W., Schmidt, H., 2016.
574 Evaluation of strategies to separate root-associated microbial communities: a crucial
575 choice in rhizobiome research. Frontiers in Microbiology 7, 773.

576 Roesch, L.F.W., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K.M., Kent, A.D., Daroub,
577 S.H., Camargo, F.A.O., Farmerie, W.G., Triplett, E.W., 2007. Pyrosequencing
578 enumerates and contrasts soil microbial diversity. ISME J 1, 283-290.

579 Ross, J., Ruttencutter, R., 1977. Population dynamics of two vesicular arbuscular
580 endomycorrhizal fungi and the role of hyperparasitic fungi. Phytopathology 67, 490-496.

581 Roth, R., Paszkowski, U., 2017. Plant carbon nourishment of arbuscular mycorrhizal fungi.
582 Current Opinion in Plant Biology 39, 50-56.

583 Ruess, L., Chamberlain, P.M., 2010. The fat that matters: Soil food web analysis using fatty
584 acids and their carbon stable isotope signature. Soil Biology and Biochemistry 42, 1898-
585 1910.

586 Sasse, J., Martinoia, E., Northen, T., 2018. Feed your friends: do plant exudates shape the root
587 microbiome? Trends in Plant Science 23, 25-41.

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

592 Scheublin, T.R., Sanders, I.R., Keel, C., van der Meer, J.R., 2010. Characterisation of
593 microbial communities colonising the hyphal surfaces of arbuscular mycorrhizal fungi.
594 ISME J 4, 752.

595 Torsvik, V., Øvreås, L., 2002. Microbial diversity and function in soil: from genes to
596 ecosystems. Current Opinion in Microbiology 5, 240-245.

597 Treonis, A.M., Ostle, N.J., Stott, A.W., Primrose, R., Grayston, S.J., Ineson, P., 2004.
598 Identification of groups of metabolically-active rhizosphere microorganisms by stable
599 isotope probing of PLFAs. Soil Biology and Biochemistry 36, 533 - 537.

600 Uksa, M., Buegger, F., Gschwendtner, S., Lueders, T., Kublik, S., Kautz, T., Athmann, M.,
601 Köpke, U., Munch, J.C., Schloter, M., Fischer, D., 2017. Bacteria utilizing plant-derived
602 carbon in the rhizosphere of Triticum aestivum change in different depths of an arable
603 soil. Environmental Microbiology Reports 9, 729-741.

604 van Passel, M.W.J., Kant, R., Palva, A., Copeland, A., Lucas, S., Lapidus, A., Glavina del
605 Rio, T., Pitluck, S., Goltsman, E., Clum, A., Sun, H., Schmutz, J., Larimer, F.W., Land,
606 M.L., Hauser, L., Kyrpides, N., Mikhailova, N., Richardson, P.P., Janssen, P.H., de Vos,
607 W.M., Smidt, H., 2011. Genome sequence of the Verrucomicrobium *Opitutus terrae*
608 PB90-1, an abundant inhabitant of rice paddy soil ecosystems. Journal of Bacteriology
609 193, 2367-2368.

610 Vandenkoornhuyse, P., Mahe, S., Ineson, P., Staddon, P., Ostle, N., Cliquet, J.-B., Francez,
611 A.-J., Fitter, A.H., Young, J.P.W., 2007. Active root-inhabiting microbes identified by
612 rapid incorporation of plant-derived carbon into RNA. Proceedings of the National
613 Academy of Sciences 104, 16970-16975.

614 Veresoglou, S.D., Chen, B., Rillig, M.C., 2012. Arbuscular mycorrhiza and soil nitrogen
615 cycling. Soil Biology and Biochemistry 46, 53-62.

616 Wakefield, W.S., Powell, M.J., Letcher, P.M., Barr, D.J.S., Churchill, P.F., Longcore, J.E.,
617 Chen, S.-F., 2010. A molecular phylogenetic evaluation of the *Spizellomycetales*.
618 Mycologia 102, 596-604.

619 Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive bayesian classifier for rapid
620 assignment of rRNA sequences into the new bacterial taxonomy. Applied and
621 Environmental Microbiology 73, 5261-5267.

622 Yoon, J.-H., Kang, S.-J., Park, S., Oh, T.-K., 2012. *Mucilaginibacter litoreus* sp. nov.,
623 isolated from marine sand. International Journal of Systematic and Evolutionary
624 Microbiology 62, 2822-2827.

625 Zhang, L., Lueders, T., 2017. Micropredator niche differentiation between bulk soil and
626 rhizosphere of an agricultural soil depends on bacterial prey. FEMS Microbiology
627 Ecology 93, fix103.
628

629

Figure legends

Fig. 1: Growth of plant biomass in ^{12}C and ^{13}C treatments (A) and ^{13}C enrichment in soil, roots and shoots of the ^{13}C-treatments (B). Vertical bars indicate standard deviation for three replicate rhizoboxes for each time point.

Fig. 2: (A) Content of extractable organic carbon (EOC) in surrounding soil of ^{12}C and ^{13}C treatments. (B) Bacterial PLFA, (C) PLFA of saprotrophic fungi, (D) mycorrhizal NLFA content in surrounding soil of ^{12}C and ^{13}C treatments. Vertical bars indicate standard deviations of triplicate measurements.

Fig. 3: Relative sequence abundance of overall bacterial (A) and microeukaryote (B) taxa in amplicon libraries of soil rRNA. Communities are shown for two time points (days 5 & 8) and two investigated soil fractions (surrounding soil, strict rhizosphere soil). Selected sub-phylum taxa mentioned in the text are highlighted.

Fig. 4: ^{13}C-labeled bacterial taxa identified in SIP after 5 and 8 days of incubation. Labeling was inferred via comparative sequencing read enrichment factors (EF) in heavy vs. light rRNA gradient fractions of ^{13}C- and ^{12}C-treatments. All bacterial taxa that showed an EF >0.5 in at least one treatment or time point were considered as ^{13}C-labeled. Other taxa identified in sequencing libraries are not shown. EFs were combined with relative read abundance of labeled taxa in heavy ^{13}C-rRNA.

Fig. 5: ^{13}C-labeled fungal and protists taxa identified in SIP after 5 and 8 days of incubation. All further details: see legend of Fig. 4.

Fig. 6: Conceptualization of plant-derived inter-kingdom microbial carbon flows as observed in the strict rhizosphere and surrounding soil of maize. Arrows indicate direct inter-population C-flows, curved arrows suggest interactions via predation.

Figure 1

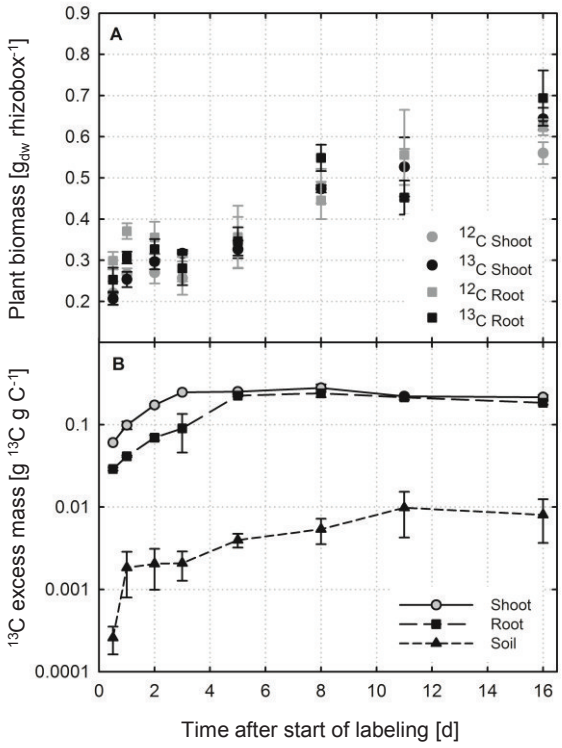

A
B
Plant biomass [g$_{dw}$ rhizobox^{-1}]
^{13}C excess mass [g ^{13}C g C^{-1}]
^{12}C Shoot
^{13}C Shoot
^{12}C Root
^{13}C Root
Shoot
Root
Soil
Time after start of labeling [d]

Figure 2

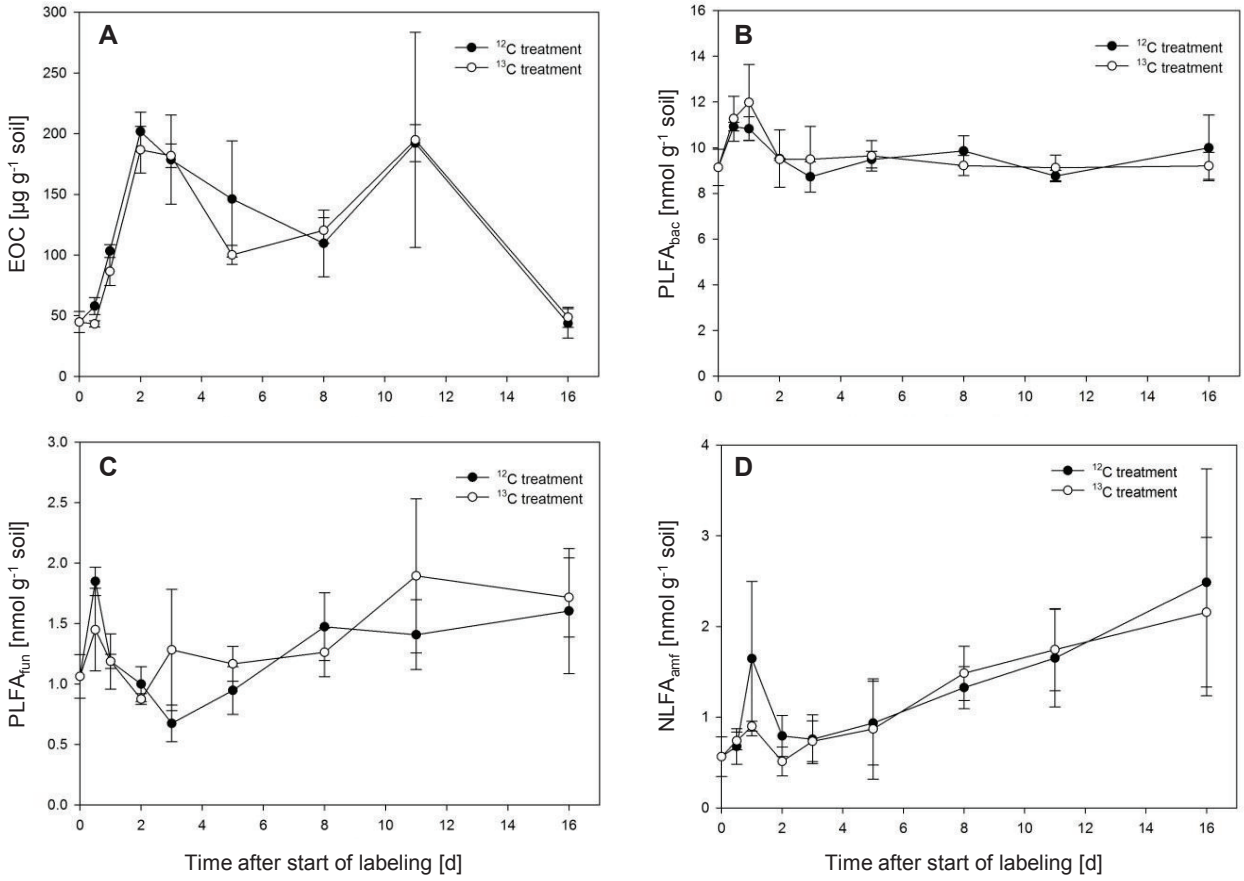

A
B
C
D
^{12}C treatment
^{13}C treatment
EOC [µg g^{-1} soil]
PLFA$_{bac}$ [nmol g^{-1} soil]
PLFA$_{fun}$ [nmol g^{-1} soil]
NLFA$_{amf}$ [nmol g^{-1} soil]
Time after start of labeling [d]
Time after start of labeling [d]

Figure 3

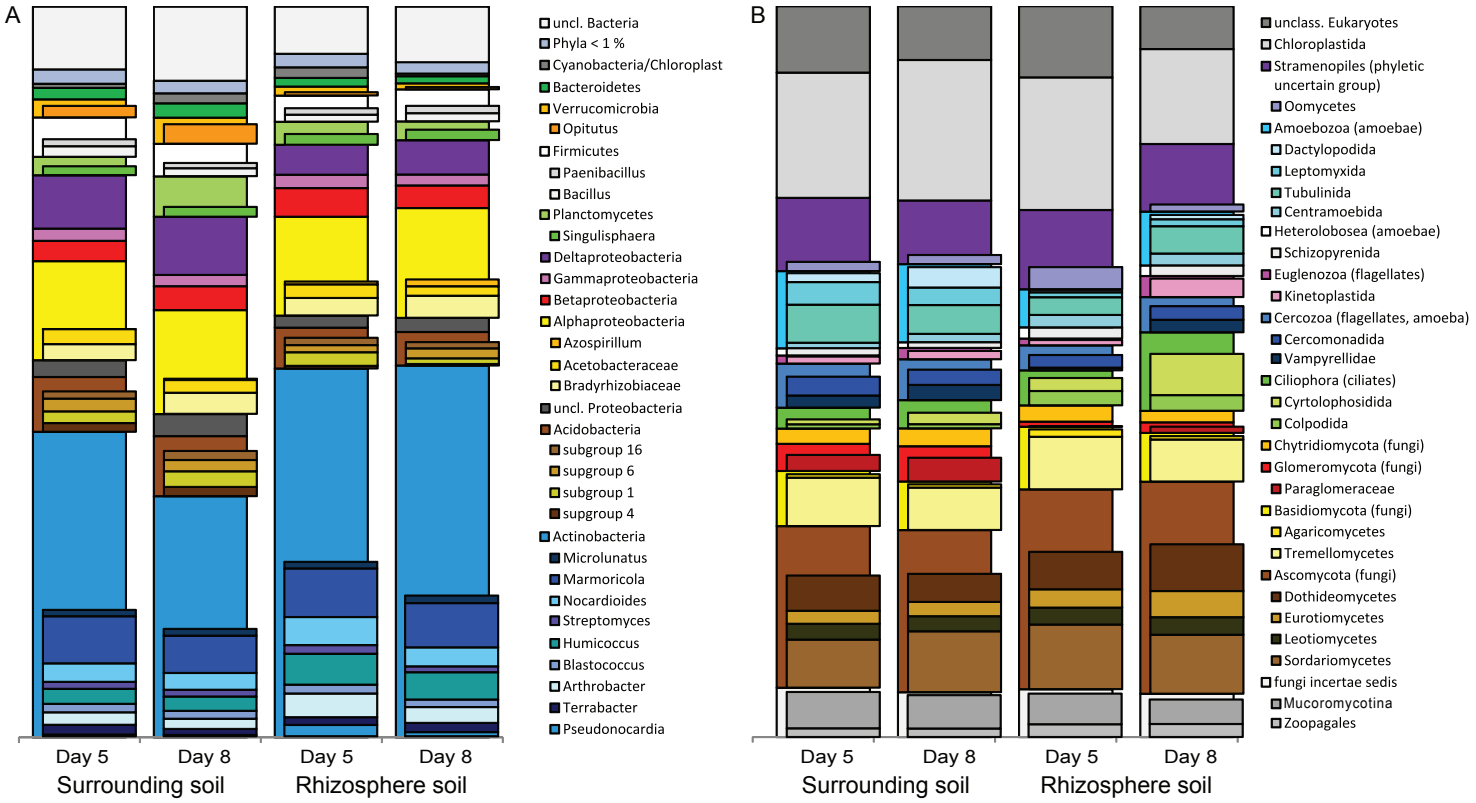

A
uncl. Bacteria
Phyla < 1 %
Cyanobacteria/Chloroplast
Bacteroidetes
Verrucomicrobia
Opitutus
Firmicutes
Paenibacillus
Bacillus
Planctomycetes
Singulisphaera
Deltaproteobacteria
Gammaproteobacteria
Betaproteobacteria
Alphaproteobacteria
Azospirillum
Acetobacteraceae
Bradyrhizobiaceae
uncl. Proteobacteria
Acidobacteria
subgroup 16
supgroup 6
subgroup 1
supgroup 4
Actinobacteria
Microlunatus
Marmoricola
Nocardioides
Streptomyces
Humicoccus
Blastococcus
Arthrobacter
Terrabacter
Pseudonocardia
Day 5
Day 8
Day 5
Day 8
Surrounding soil
Rhizosphere soil
B
unclass. Eukaryotes
Chloroplastida
Stramenopiles (phyletic uncertain group)
Oomycetes
Amoebozoa (amoebae)
Dactylopodida
Leptomyxida
Tubulinida
Centramoebida
Heterolobosea (amoebae)
Schizopyrenida
Euglenozoa (flagellates)
Kinetoplastida
Cercozoa (flagellates, amoeba)
Cercomonadida
Vampyrellidae
Ciliophora (ciliates)
Cyrtolophosidida
Colpodida
Chytridiomycota (fungi)
Glomeromycota (fungi)
Paraglomeraceae
Basidiomycota (fungi)
Agaricomycetes
Tremellomycetes
Ascomycota (fungi)
Dothideomycetes
Eurotiomycetes
Leotiomycetes
Sordariomycetes
fungi incertae sedis
Mucoromycotina
Zoopagales
Day 5
Day 8
Day 5
Day 8
Surrounding soil
Rhizosphere soil

Figure 4

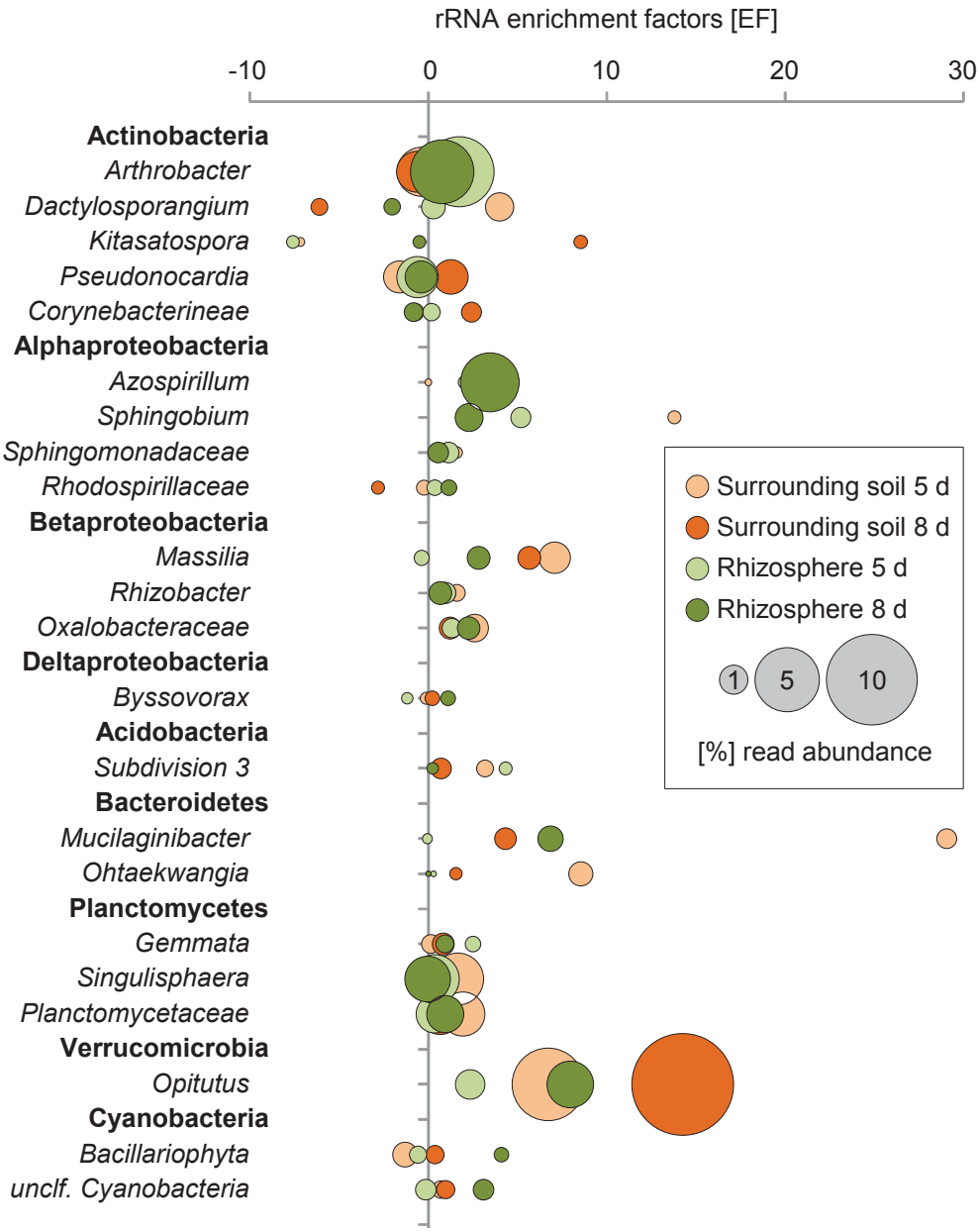

rRNA enrichment factors [EF]
-10
0
10
20
30
Actinobacteria
Arthrobacter
Dactylosporangium
Kitasatospora
Pseudonocardia
Corynebacterineae
Alphaproteobacteria
Azospirillum
Sphingobium
Sphingomonadaceae
Rhodospirillaceae
Betaproteobacteria
Massilia
Rhizobacter
Oxalobacteraceae
Deltaproteobacteria
Byssovorax
Acidobacteria
Subdivision 3
Bacteroidetes
Mucilaginibacter
Ohtaekwangia
Planctomycetes
Gemmata
Singulisphaera
Planctomycetaceae
Verrucomicrobia
Opitutus
Cyanobacteria
Bacillariophyta
unclf. Cyanobacteria
Surrounding soil 5 d
Surrounding soil 8 d
Rhizosphere 5 d
Rhizosphere 8 d
1
5
10
[%] read abundance

Figure 5

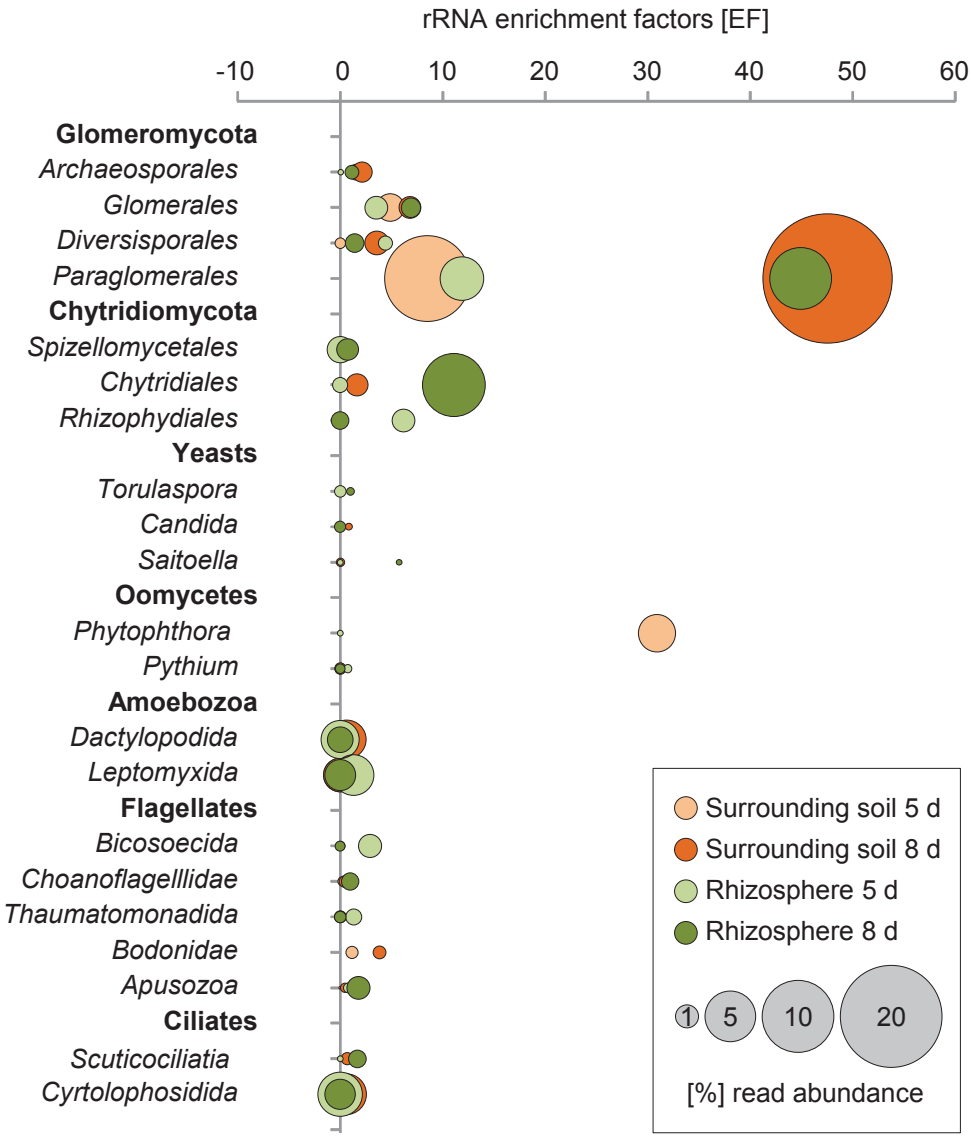
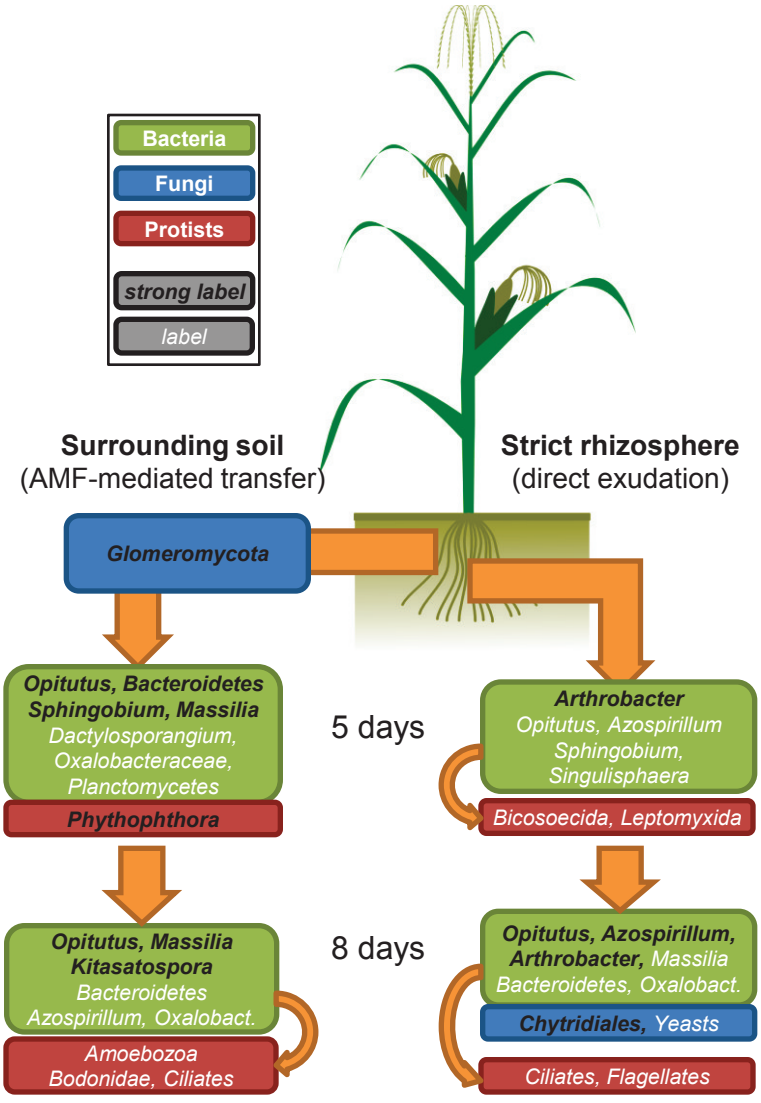

rRNA enrichment factors [EF]
-10
0
10
20
30
40
50
60
Glomeromycota
Archaeosporales
Glomerales
Diversisporales
Paraglomerales
Chytridiomycota
Spizellomycetales
Chytridiales
Rhizophydiales
Yeasts
Torulaspora
Candida
Saitoella
Oomycetes
Phytophthora
Pythium
Amoebozoa
Dactylopodida
Leptomyxida
Flagellates
Bicosoecida
Choanoflagelllidae
Thaumatomonadida
Bodonidae
Apusozoa
Ciliates
Scuticociliatia
Cyrtolophosidida
Surrounding soil 5 d
Surrounding soil 8 d
Rhizosphere 5 d
Rhizosphere 8 d
1
5
10
20
[%] read abundance

Figure 6


Bacteria
Fungi
Protists
strong label
label
Surrounding soil
(AMF-mediated transfer)
Strict rhizosphere
(direct exudation)
Glomeromycota
Opitutus, Bacteroidetes
Sphingobium, Massilia
Dactylosporangium,
Oxalobacteraceae,
Planctomycetes
Phythophthora
5 days
Arthrobacter
Opitutus, Azospirillum
Sphingobium,
Singulisphaera
Bicosoecida, Leptomyxida
Opitutus, Massilia
Kitasatospora
Bacteroidetes
Azospirillum, Oxalobact.
Amoebozoa
Bodonidae, Ciliates
8 days
Opitutus, Azospirillum,
Arthrobacter, Massilia
Bacteroidetes, Oxalobact.
Chytridiales, Yeasts
Ciliates, Flagellates

Supplementary Material for online publication only

Click here to download Supplementary Material for online publication only: Rhizo-SIP SI.pdf