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Research Article

RESEARCH ARTICLE

Micropredator niche differentiation between bulk soil and rhizosphere of an agricultural soil depends on bacterial prey

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one sentence summary: Patterns of prey selectivity and niche segregation between bacterial and protozoan micropredators in an agricultural soil are investigated using ^{13}C-labelled bacterial prey and rRNA-SIP.
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ABSTRACT

Predation is a fundamental mechanism of all food webs, but its drivers and organismic connectivities, especially at microbial level, are still poorly understood. Specifically, competitive carbon flows in the presence of multiple micropredators, as well as trophic links within and between microbial kingdoms have rarely been resolved. Here, using maize-planted agricultural soil as a model system, we have investigated the predation of amended bacterial prey by both prokaryotic and eukaryotic micropredators. We have queried how soil compartment (rhizosphere vs bulk soil) and nature of prey (Gram-positive vs Gram-negative) influence predation outcomes. We added ^{13}C-labelled biomass of *Pseudomonas putida* and *Arthrobacter globiformis* to soil microcosms and found that *P. putida* was consumed much more rapidly. Bacteria and microeukaryotes specifically responsive to the biomass amendments were identified by RNA-stable isotope probing. Amongst the bacteria, only a few myxobacteria sequestered C from *A. globiformis*, whereas a considerable diversity of predatory bacteria incorporated C derived from *P. putida*. Diverse groups of heterotrophic protists, especially amoeba including *Glaeseria*, *Hartmanella* and *Vahlkampfia* spp., were observed to incorporate ^{13}C from both amendments, but with pronounced niche differentiation between rhizosphere and bulk soil. This provides novel insights into niche partitioning between bacterial and eukaryotic micropredators in soil, driven not only by the nature of bacterial prey itself, but also by soil compartments.

Keywords: intrabacterial predation; myxobacteria; protozoa; soil microbial food web; stable isotope probing

INTRODUCTION

The large diversity of organisms in soil is of pivotal importance for carbon cycling and ecosystem functioning (Nielsen *et al.* 2011). Plant-derived carbon, including rhizodeposits and plant detritus, is a primary source of soil organic carbon. Soil bacteria, as the primary decomposers of soil organic matter, channel these resources into the soil food web, where carbon flow is controlled by complex interactions of the different decomposers and trophic levels (Handa *et al.* 2014). Despite the importance of these interactions for soil carbon fluxes, details on the identity and interactions of the microbial populations involved are still limited. Especially, the links between soil microbes and their direct predatory consumers are only poorly resolved, and population-specific details rarely find their way into food web models.

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The most important micropredators of bacteria in soil include protists, predatory bacteria and bacteriophages, with distinct predation strategies and community-level impacts (Johnke *et al.* 2014). While the importance of predation by protozoa on soil bacteria is fairly well understood (Bonkowski 2004), the role of intrabacterial predation in complex food webs is poorly investigated. Bacterial micropredators can include *Bdellovibrio* spp. (and-like-organisms, BALOs), the *Myxobacteria* (*Deltaproteobacteria*) or relatives of *Lysobacter* spp. (*Gammaproteobacteria*) (Jurkevitch *et al.* 2000; Morgan *et al.* 2010; Johnke *et al.* 2014; Seccareccia, Kost and Nett 2015). However, direct evidence for the actual activity of bacterial micropredators in complex soil systems is scarce (Lueders *et al.* 2006; Murase and Frenzel 2007; Morgan *et al.* 2010; Kramer *et al.* 2016), and competitive niche partitioning between bacterial and protistan micropredators in different soil compartments is not understood.

As a powerful tool to address such questions, stable isotope probing (SIP) of nucleic acids has been widely applied to trace organismic carbon flows in a number of soil food webs (Lueders *et al.* 2006; Murase and Frenzel 2007; Drigo *et al.* 2010; Murase *et al.* 2012; Chatzinotas *et al.* 2013; Kramer *et al.* 2016). Specifically, time-resolved SIP analyses have provided valuable information on the trophic succession and connectivities of food webs. Although the comparative labelling of both bacterial and protistan micropredators in the same soil has occasionally been observed (Lueders *et al.* 2004; Murase and Frenzel 2007; Kramer *et al.* 2016), a significant potential of SIP to resolve predatory niche partitioning in complex communities remains largely untapped.

Protists are considered as generalists in bacterial predation, but marked prey preferences have been reported for different groups (Jezbera, Hornak and Simek 2005; Bell *et al.* 2010). Bacterial micropredators also express feeding preferences. For example, preferential or obligate predation of Gram-negative bacteria has been reported for myxobacteria (Morgan *et al.* 2010) and different BALOs (Rogosky, Moak and Emmert 2006; Rotem, Pasternak and Jurkevitch 2014). Nevertheless, it remains to be shown whether such prey preferences are actually apparent in complex microbial food webs, and how they influence carbon flow under competitive micropredation. It can also be speculated that the activity of different micropredators could be dependent on soil compartments (e.g. bulk soil vs rhizosphere soil).

In a recent rRNA-SIP study investigating the flow of carbon from detritusphere substrates and plant biomass in an agricultural soil, a predatory role of myxobacteria was suggested from the observation that these bacteria showed only a late successional labelling (Kramer *et al.* 2016). At the same time, ^{13}C-labelling was also observed for members of the protozoa, mostly for amoeboid taxa. To specifically follow up on factors controlling micropredator niche partitioning in the investigated soil, we have conducted a targeted micropredator labelling experiment. We amended ^{13}C-labelled biomass of a representative Gram-positive and a Gram-negative bacterium to rhizosphere and bulk soil of an experimental maize field. We aimed to identify predatory bacteria and protozoa that were specifically responsive to the biomass amendments. We hypothesise that (i) the nature of bacterial prey is a major driver of predation outcome, and that (ii) the importance of predation by protists versus intrabacterial predation is distinct in different soil compartments (bulk soil vs rhizosphere). These insights into micropredator niche differentiation may help to better understand multitrophic connectivities in central carbon fluxes in soil.

MATERIALS AND METHODS

Soil

The soil was taken from an agricultural field located near Göttingen (Germany), where a long-term experiment has been conducted to investigate the flow of maize-derived carbon into the soil food web (Kramer *et al.* 2012; Scharroba *et al.* 2012; Pausch *et al.* 2016). A representative composite topsoil sample (0–10 cm) was taken from an area of 20 × 20 m within a plot under maize in March 2015. Soil parameters have been described previously (Kramer *et al.* 2012). Briefly, the C and N contents of the soil were 1.37% and 0.14%, respectively; soil pH was 6.0. Topsoil texture comprised 7% clay, 87% silt and 6% sand.

To obtain fresh rhizosphere soil for the SIP experiment conducted in August 2015, young maize plants were grown in a greenhouse. First, maize seeds were germinated on agar plates prepared with Miller LB broth (Sigma-Aldrich, Germany) at 30°C. Maize seedlings were then planted and grown in plastic pots (diameter = 9.5 cm, height = 14.5 cm) filled with sieved (4 mm mesh) soil from the field in a greenhouse for 12 weeks. Plants received natural sunlight and were kept at 25°C during the day and at 20°C at night. Watering was applied daily in the first 2 weeks and twice per week afterwards. Fertilisation was not applied. After 12 weeks, planted pots were densely filled by maize roots. For the SIP incubation, fresh rhizosphere soil was directly harvested by loosely shaking the roots to collect all readily detachable soil and manually removing remaining fine roots (Buddrus-Schiemann *et al.* 2010). For the bulk soil treatments, pots of soil without plants were kept next to the planted pots in the greenhouse, under identical climatic conditions and water treatment. Water content of rhizosphere and bulk soil was determined by drying ∼30 g of soil in an oven at 105°C for 48 h.

Cultivation of ^{13}C-labelled bacteria

The bacterial strains used in this experiment were the Gram-negative *Pseudomonas putida* (DSM 6125) and the Gram-positive *Arthrobacter globiformis* (DSM 20124). For both, closely related taxa are abundant in the investigated soil (Dibbern *et al.* 2014; Kramer *et al.* 2016) and can therefore be considered as representative components of the intrinsic microbial food web. Strains were originally obtained from the DSMZ (Braunschweig, Germany). Labelled bacteria were grown in M9 minimal medium, prepared with 5 x M9-Minimal salts (Serva, Germany), containing 4 g l^{-1} 99% $^{13}C_6$-glucose (Santa Cruz Biotechnology, Inc., Heidelberg, Germany) as sole carbon source. In parallel, the same strains were grown in M9 medium with unlabelled glucose (Sigma Aldrich, Germany). After ∼24 h of cultivation and two transfers, cells were collected as prey for the SIP experiment by centrifugation at 3345 rcf for 15 min, washed five times with fresh, glucose-free M9 media and finally resuspended in 15 ml M9.

Bacterial cell concentrations in the washed suspensions were determined using flow cytometry. Cell suspensions were diluted in 10-fold series and fluorescently stained with SybrGreen I (1×) for 10 min in the dark. Quantification was performed on a FC 500 cell analyser (Beckman Coulter, Germany) using a 488 nm (20 mV) laser. Instrument settings were as follows: forward scatter 178 mV, side scatter 624 mV, B530 (bandpass filter 530 nm) 397 mV, B610 572 mV, signal trigger was set on B530. All parameters were collected as logarithmic signals. BD TruCOUNT Tubes (BD Biosciences, Germany) were used as internal standard (bead concentration: 6331 ml^{-1}). Samples were run at flow rates of 30 μl min^{-1} until 200 events of the internal standard were

detected. Data analysis was performed using the CXP software (Beckman Coulter, Germany).

^{13}C-labelling of the harvested bacterial cells was determined by elementary analysis—isotope-ratio mass spectrometry (IRMS), with an elemental analyser (EURO EA, Euro Vector Instruments) coupled to an IRMS (MAT 253, ThermoFischer). Approximately 30 mg of harvested bacterial cells were weighed into tin capsules for each measurement. Each sample was measured at least twice, similarly as done before (Lueders *et al.* 2006).

Microcosm setup for SIP

The SIP microcosm experiment was designed as follows: microcosms contained 30 g of maize rhizosphere (Rh) or bulk soil (Bs), amended with same amount of either ^{13}C-labelled or unlabelled *P. putida* and *A. globiformis* for each of the eight treatments. First, for the cell amendment, 250 g of freshly retrieved rhizosphere soil or bulk soil was transferred into sterile 1000-ml beakers. 7.5 ml of bacterial cell suspensions at $\sim 9.6 \times 10^8$ cells ml^{-1}, adjusted after cell counting, were slowly added in droplets with a 1000-μl pipette while manually stirring the soil. Afterwards, amended soil was manually distributed to eight sterile 500-ml glass bottles per treatment (64 bottles in total), each with 30 g of soil, and bottles were closed with rubber stoppers and aluminium screw caps, and incubated over 16 days at 20°C. The SIP microcosms were sampled at successive time points: at time 0 (directly after bacterial amendment), after 6 h, 1 day, 2 days, 4 days, 8 days and 16 days. At each time point, 15 g of soil was sampled as duplicates from two of the eight bottles per treatment, i.e. two replicate microcosms were fully sacrificed after every second sampling time point. The two microcosms per treatment reserved for the final time point (16 days) were used for CO_2 measurement during the incubation. Control microcosms without bacterial amendments were also set up, run and sampled in parallel (data not shown). For each time point, ~2 g of fresh soil from the sampled 15 g was immediately frozen in liquid nitrogen and stored at –80°C for subsequent RNA extraction. The remaining soil was stored at –20°C.

CO_2 measurement

$^{13}CO_2$ production derived from ^{13}C-labelled bacterial biomass in microcosms was measured daily with a GC/MS (Finnigan TRACE DSQ GC/MS; Thermo Electron, Germany). Twenty-microlitre headspace gas samples were withdrawn with a gas-tight glass syringe and injected into the GC/MS. Gases were separated on a GS-Q column at 50°C (8 min) with helium as carrier gas (flow rate 3 ml min^{-1}). The molecular masses 44 Da ($^{12}CO_2$) and 45 Da ($^{13}CO_2$) were then quantified with the Trace DSQ MS detector under selected ion mode (SIM). Data were analysed using the Xclibur software (version 1.4.2; Thermo Scientific). The relative abundance of $^{13}CO_2$ in the headspace was calculated as the ratio of $^{13}CO_2$ to total CO_2 ($^{13}CO_2$ plus $^{12}CO_2$). Calibration was performed with a standard gas containing 1% CO_2 at natural $^{13}CO_2$ abundance.

RNA extraction and gradient centrifugation

Total RNA extraction and SIP analysis were done as previously described (Kramer *et al.* 2016) with minor modifications. Soil RNA for SIP was extracted from duplicate soil microcosms per time point and treatment and pooled before centrifugation. RNA extracts of day 8 were selected for ultracentrifugation based on substrate mineralisation data. Isopycnic centrifugation and gradient fractionation were done as described with 500 ng of total RNA loaded into each gradient and collection of 12 density-resolved RNA fractions (Kramer *et al.* 2016).

Fingerprinting and sequencing of rRNA

Bacterial and eukaryotic rRNA in density-resolved SIP fractions (fractions 2 to 11 of all day 8 gradients) were first analysed by terminal restriction fragment length polymorphism (T-RFLP) fingerprinting as described (Euringer and Lueders 2008; Pilloni *et al.* 2012) using bacterial (Ba27f/907r) and eukaryotic primers (Euk20f/519r). Based on rRNA fingerprinting of gradient fractions, the fractions 3, 6 and 9 of all gradients, corresponding to buoyant densities of ~1.82, 1.80 and 1.78 g ml^{-1} CsTFA, respectively, were selected as representative for 'heavy', 'medium' and 'light' rRNA and subjected to 454 amplicon sequencing. Amplicons were generated as described (Kramer *et al.* 2016) with minor modifications. Amplicons for unidirectional FLX+ sequencing were amplified using the above primers with Lib-L adapters, and multiplex identifiers attached to the forward primer. Emulsion PCR, emulsion breaking and sequencing were performed by IMGM Laboratories (Munich, Germany) on a GS FLX+ sequencer with appropriate chemistry (Roche Applied Biosystems, Penzberg, Germany). Demultiplexed and quality-trimmed reads were obtained as described previously (Pilloni *et al.* 2012), but analysed and classified using the SILVAngs data analysis platform (Pruesse, Peplies and Glöckner 2012; Quast *et al.* 2013). Default settings were used for quality control, dereplication, OTU clustering and classification, except that a minimum sequence length of 250 b was set for both bacterial and eukaryotic libraries, minimum quality score was set to 20 and classification similarity was set to 85% for eukaryotic libraries. Quantitative information on raw and trimmed reads for the different sequencing libraries generated in this study can be found in Table S1 (Supporting Information). All sequencing raw data have been deposited with the NCBI sequence read archive under the SRA accession number SRP100422 for bacterial and microeukaryotic rRNA reads.

For an overview of total rRNA community structure in the different treatments on day 8, taxon-specific relative read abundances from sequencing libraries of light ^{12}C and light ^{13}C gradient fractions were averaged. Furthermore, total rRNA community composition over all time points was monitored by T-RFLP fingerprinting of bacterial 16S rRNA and eukaryotic 18S rRNA amplicons from pooled RNA extracts of duplicate ^{12}C- and ^{13}C-microcosms for all time points, following the same procedures as described above for fingerprinting of density-resolved SIP fractions.

Calculating taxon-specific enrichment factors in labelled rRNA

To identify bacterial and eukaryotic taxa involved in the assimilation of ^{13}C from the amended bacterial biomass, sequencing read enrichment factors (EFs) in heavy rRNA fractions were inferred as described previously (Kramer *et al.* 2016). Briefly, the calculation was done as follows:

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

where ^{13C}heavy and ^{13C}light were the taxon-specific relative read abundances in heavy and light rRNA fractions of ^{13}C treatments, and ^{12C}heavy and ^{12C}light were the same for unlabelled

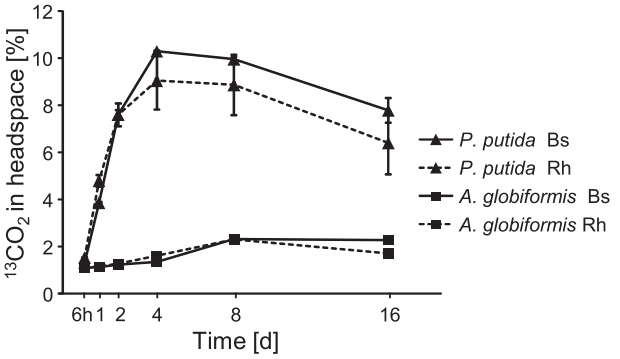

Figure 1. $^{13}CO_2$ production in rhizosphere and bulk soil microcosms amended with ^{13}C-labelled *A. globiformis* and *P. putida* during SIP incubation. Error bars represent standard error (n= 2).

treatments. EFs were calculated for all taxa with >1% read abundance in heavy rRNA fractions of at least one treatment. Bacterial and eukaryotic taxa that showed an EF > 0.5 were considered as ^{13}C-labelled (Kramer *et al*. 2016).

Statistical analysis

$^{13}CO_2$ production was compared between rhizosphere and bulk soil treatments, within *P. putida* and *A. globiformis* microcosms separately, using ANOVA. Two-way repeated measures ANOVAs were applied using the function 'anova' from the package car (Fox and Weisberg 2011) in the open source statistic program R (R Development Core Team 2013).

RESULTS

^{13}C Mineralisation during microcosm incubation

^{13}C-labelled bacterial biomass was added to the microcosms at a final concentration of ~3.8×10^7 cells per g_{dw} soil. This was intended to correspond to ~2% of the indigenous bacterial biomass of 2.1×10^9 cells per g_{dw} soil, as previously estimated for the site (Dibbern *et al*. 2014). Unlabelled bacterial biomass was amended to the microcosms at the same concentration. ^{13}C-labelled biomass was determined to be 87 and 70 atom % ^{13}C-labelled for *Arthrobacter globiformis* and *Pseudomonas putida*, respectively. Gravimetric water content of the soil after amendment was 0.22 for rhizosphere soil and 0.23 for bulk soil.

Mineralisation of ^{13}C-labelled bacterial biomass was traced over 16 days (Fig. 1) and was clearly stronger in treatments with *P. putida* than with *A. globiformis*. The evolution of $^{13}CO_2$ from amended *P. putida* in bulk soil and rhizosphere microcosms peaked at day 4, with 10.3 and 9.0 ^{13}C atom % detectable in headspace CO_2, respectively. In the *A. globiformis* treatments, mixing ratios of formed $^{13}CO_2$ were much lower, but increased slightly until day 8, with 2.3 atom % detectable in both the bulk soil and rhizosphere microcosms. ^{13}C mineralisation rate was not significantly different between bulk soil and rhizosphere microcosms over time (Fig. 1; soil effect: $F = 0.28$, $P = 0.70$, time effect: $F = 118.9$, $P < 0.001$, for *P. putida*; soil effect: $F = 11.9$, $P = 0.18$, time effect: $F = 13.1$, $P < 0.001$, for *A. globiformis*).

Community dynamics during incubation

T-RFLP analysis of total bacterial 16S rRNA was used to trace the dynamics of bacterial communities in soil microcosms over time, and also to compare communities before and after microcosm incubation as well as between treatments (Fig. S1, Supporting Information). The T-RFs of amended *P. putida* (490 bp) and *A. globiformis* (61 bp) were clearly visible and at comparable abundance directly after amendment in all treatments. The *P. putida* T-RF was in a range of 60%–71% relative abundance, while that of *A. globiformis* was between 52% and 60% in respective treatments. However, while the *A. globiformis* T-RF gradually decreased back to its initial ~10% abundance over the experiment, the T-RF of *P. putida* decreased drastically to <10% after 1 day of incubation (Fig. S1).

The comparison of initial unamended T-RF patterns to that of amended microcosms after 8 or 16 days revealed very similar overall bacterial communities, suggesting that marked long-term shifts were not induced by the amendments. Distinctions between soils were apparent, before and after the amendment, but reflected more in the relative abundances of important T-RFs than in T-RF distinctions. In contrast, microeukaryote 18S rRNA patterns were markedly distinct between rhizosphere and bulk soil microcosms (Fig. S2, Supporting Information). The amendment of bacterial prey did not induce immediate shifts in overall microeukaryotic communities. However, several gradual changes were observed, such as a consistent increase in abundance of the 273 bp T-RF in rhizosphere soils. Apart from that, rhizosphere microeukaryote communities appeared rather stable during the experiment, while specific T-RFs in the bulk soil treatments were of fluctuating abundance.

Sequencing of density-resolved rRNA

rRNA extracts from the microcosms sampled after 8 days of incubation were selected for SIP analysis based on ^{13}C mineralisation and T-RFLP patterns. To identify not only labelled taxa, but also for precise information on community assembly in the different treatments, libraries of bacterial and microeukaryotic rRNA from selected heavy, medium and light gradient fractions were sequenced. First, to reveal potential compartment effects on overall community composition, libraries from light rRNA gradient fractions were averaged between isotopic treatments (Figs 2 and 3). The abundance of bacterial taxa in libraries from light rRNA was highly reproducible, with most of the abundant taxa not showing notable distinctions between rhizosphere and bulk soil (Fig. 2). Minor exceptions included reads of *Paucimonas* spp., being consistently detected at 2%–3% abundance only in the rhizosphere. rRNA of unclassified *Gaiellales* was another example, present at consistently higher relative abundance in bulk soil (~7%) than in the rhizosphere (4%–5%).

In contrast, much more pronounced distinctions were observed for microeukaryotic communities between compartments (Fig. 3). Amongst the *Amoebozoa*, rRNA of *Glaeseria* and *Korotnevella* spp. was identified in rhizosphere or bulk soil only, respectively. rRNA of the amoeboid *Hartmannella* spp. was much higher in relative abundance in the rhizosphere (~13% vs ~3%). Other microeukaryotes enriched in the rhizosphere included protists such as *Ichthyobodo* (~9% vs. ~1%) and *Gonostomum* (10%–24% vs ~4%), as well as different fungi (*Fusarium* and *Rhizopus* spp.). In contrast, rRNA of the amoeboid *Vahlkampfia* spp. appeared highly enriched in bulk soil libraries (23%–51%), especially under *P. putida* amendment. Furthermore, ciliates within the *Cyrtolophosidida* (~5% vs ~2%), fungal *Spizellomycetales* (~3% vs ~1%) and members of the algal *Stramenopiles* (10%–16% vs ~2%) consistently showed higher relative abundance in bulk soil.

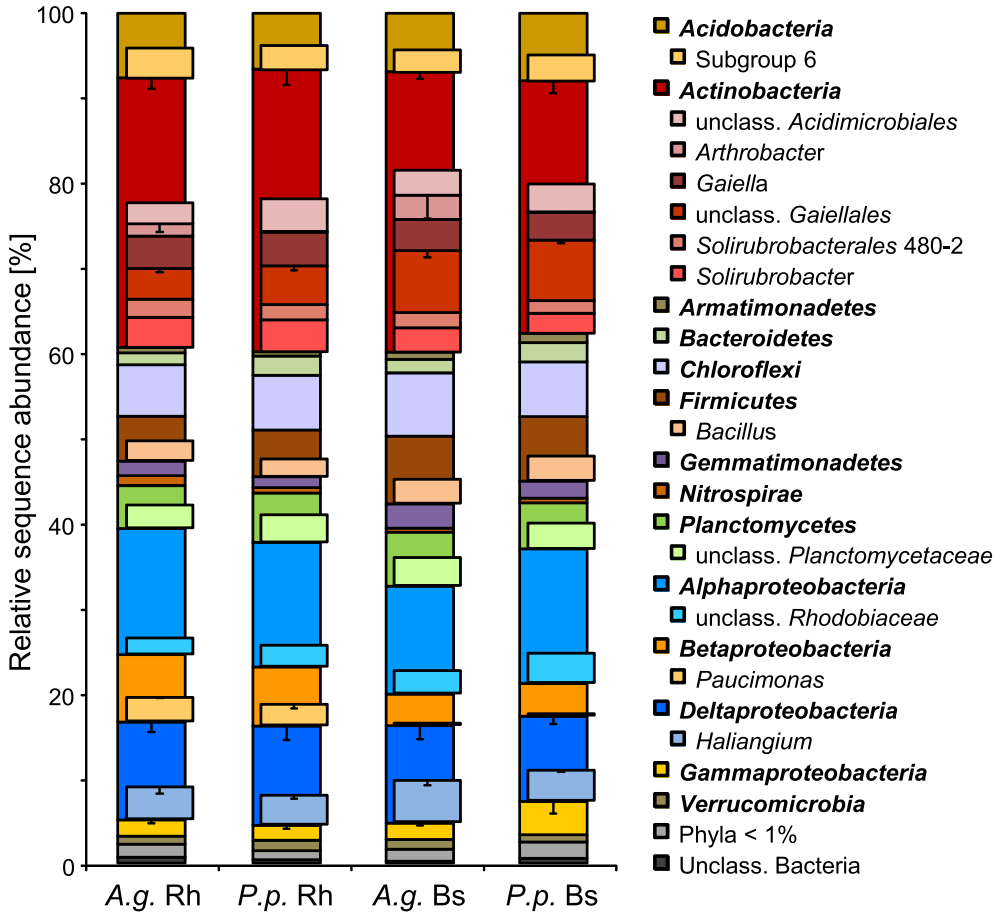

Figure 2. Relative sequence abundance of overall bacterial communities obtained by amplicon sequencing of light rRNA fractions from rhizosphere (Rh) and bulk soil (Bs) microcosms amended with *A. globiformis* (*A.g.*) or *P. putida* (*P.p.*) after 8 days of incubation. Relative sequence abundances in light fractions were averaged between ^{13}C- and ^{12}C-microcosms. Selected subphylum taxa are highlighted. Error bars (negative only) are given for selected taxa and represent standard errors ($n = 2$).

Labelled bacterial taxa

Already a first comparison of sequencing libraries across buoyant densities revealed the impact of ^{13}C-amendments in comparison to controls (Fig. S3a, Supporting Information). Not unexpectedly, heavy rRNA of *Actinobacteria* was highly dominant (>90%) in heavy ^{13}C-rRNA fractions in samples amended with *A. globiformis*. In contrast, reads of *Gammaproteobacteria* were much less enriched in heavy fractions of the ^{13}C-*P. putida* amended samples. In fact, actual reads of *P. putida* were hardly detected in these libraries. Instead, rRNA reads of the *Deltaproteobacteria* appeared clearly enriched in ^{13}C-*P. putida* treatments.

The specific bacterial populations incorporating ^{13}C from the amended biomass were identified based on the calculation of taxon-specific EFs (Kramer *et al.* 2016). ^{13}C-labelled bacterial taxa belonged to diverse bacterial phyla, mainly the *Proteobacteria*, but also the *Gemmatimonadetes*, *Chloroflexi*, *Bacteroidetes*, *Actinobacteria* and *Acidobacteria* (Fig. 4). However, as already suggested by the $^{13}CO_2$ and T-RFLP data, ^{13}C-enriched rRNA of the amended *A. globiformis* remained highly detectable after 8 days of incubation, whereas that of the *P. putida* amendment was no longer detectable. Apart from *A. globiformis*, the only intrinsic bacterial taxa that seemed to have incorporated ^{13}C-label in the respective treatments were *Haliangium* spp. and a small number of other myxobacterial lineages. Labelling of these *Deltaproteobacteria* was generally more apparent in the rhizosphere than in bulk soil.

In stark contrast, ^{13}C-label from *P. putida* was suggested to be assimilated by a considerable diversity of bacterial populations in both rhizosphere and bulk soil. Especially, rRNA of unclassified *Cystobacteraceae* (also myxobacteria) was most highly enriched in heavy rRNA from both soils. Other bacterial taxa with marked enrichment in both compartments included the gammaproteobacterial *Lysobacter* and *Arenimonas* spp., diverse myxobacteria including *Haliangium* spp., the *Bdellovibrionaceae* clade OM27, alphaproteobacterial *Sphingomonas* spp. as well as unclassified *Chloroflexi*. In addition, several taxa were found to be specifically labelled either in bulk soil or the rhizosphere, such as *Thermomonas* and *Rhodanobacter* spp. (*Gammaproteobacteria*), *Sphingobium* spp. (*Alphaproteobacteria*) and *Flavisolibacter* spp. (*Bacteroidetes*) in bulk soil, or *Sandaracinus* and *Cystobacter* spp. (*Deltaproteobacteria*) as well as unclassified *Xanthomonadaceae* (*Gammaproteobacteria*) in rhizosphere soil.

Labelled eukaryotic taxa

The composition of microeukaryotic rRNA libraries across gradient fractions suggested clear labelling patterns in SIP. rRNA of *Amoebozoa* appeared highly enriched in heavy rRNA fractions of rhizosphere samples in response to both *P. putida* and *A. globiformis* amendments, and also to a lesser extent in bulk soil for the *A. globiformis* amendment (Fig. S3b). Reads of the amoeboid *Heterolobosea* were generally more abundant in bulk soil than in

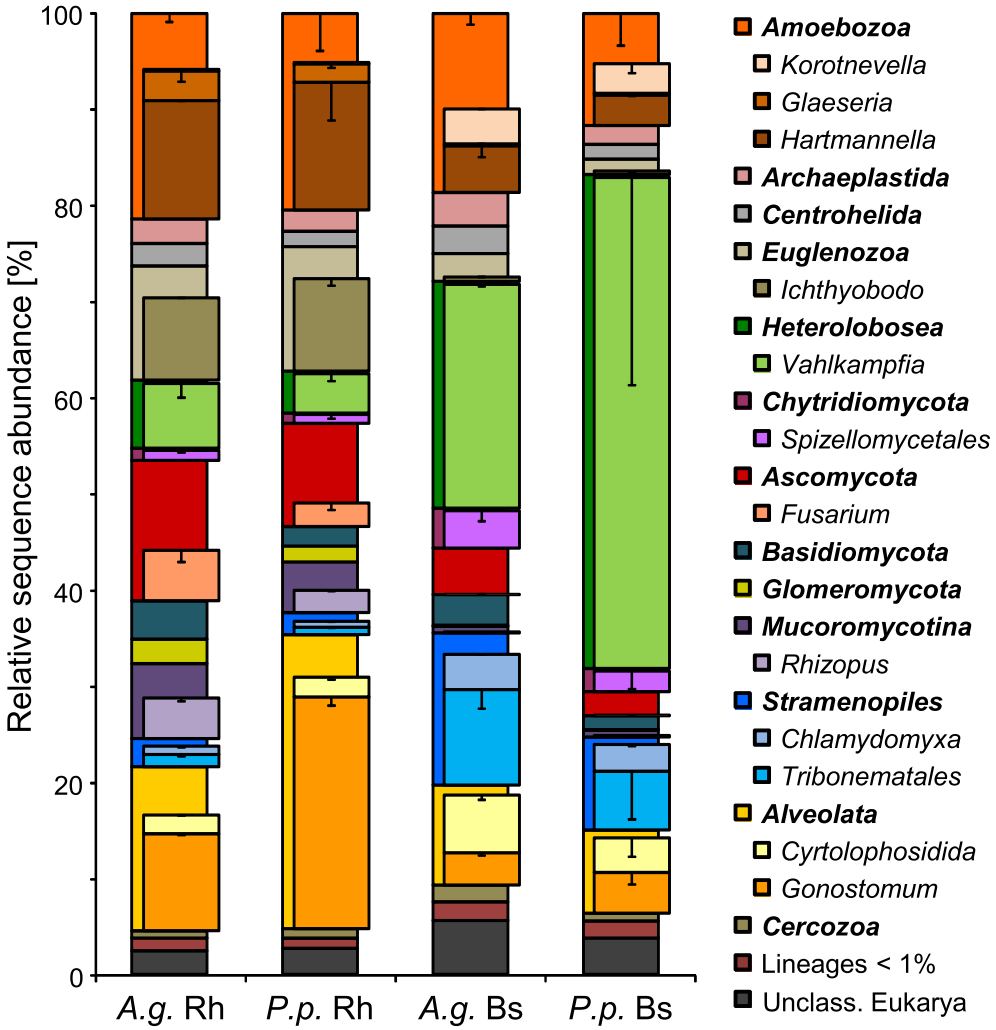

Figure 3. Relative sequence abundance for overall eukaryotic taxa obtained by amplicon sequencing of light gradient fractions from soil microcosms after 8 days of incubation. Codes and other details are as in Fig. 2.

the rhizosphere and showed enrichment in the heavy fractions especially for the *P. putida* treatment.

Taxon-specific EFs confirmed these first indications, but also revealed distinct population-specific feeding preferences (Fig. 5). rRNA of the amoeboid *Glaeseria* spp., for example, showed strong ^{13}C-enrichment under amendment of *A. globiformis* in both soils. Other amoeba including members of the *Schizoplasmodiida*, *Acanthamoeba*, *Dactylopodida* and *Vampyrellidae* showed similar labelling patterns, but less pronounced. In contrast, rRNA of *Hartmannella* spp. and the amoeboid BOLA868 lineage appeared specifically labelled under *P. putida* amendment, especially in the rhizosphere. Amoeba like *Vahlkampfia* and *Korotnevella* spp. appeared specifically ^{13}C-labelled for *P. putida* treatments only in bulk soil. Other protists found to be enriched in heavy ^{13}C-rRNA, albeit less markedly, included the flagellate *Cercomonas* spp. and the *Centrohelida* (*Heliozoa*) in bulk soil, as well as *Pseodoplatyophyra* spp. (*Ciliates*) in the rhizosphere. A choanoflagellate taxon *Salpingoeca* spp. was labelled, interestingly, for *A. globiformis* in the rhizohsphere and *P. putida* in bulk soil. Several fungal taxa also showed enrichment in heavy ^{13}C-rRNA, i.e. members of the *Mucorales* in bulk soil. An algal taxon, *Chlamydomyxa* (*Stramenopiles*), also appeared highly enriched and abundant in heavy rRNA extracted from bulk soil.

DISCUSSION

The application of nucleic acid-based SIP has unravelled microbial trophic interaction for a number of food webs in soil (Murase and Frenzel 2007; Drigo *et al.* 2010; Kramer *et al.* 2016). Here, we traced secondary trophic interactions, i.e. the consumption of bacterial prey by competing guilds of micropredators. Our results reveal marked distinctions in feeding preferences between bacterial and eukaryotic micropredators, driven not only by the nature of bacterial prey itself, but also by soil compartments.

Amendment and sequestration of bacterial prey

^{13}C-labelled cells of *Pseudomonas putida* and *Arthrobacter globiformis* were amended as representative Gram-negative and -positive bacterial prey. Both have been used previously in grazing experiments (Verhagen, Duyts and Laanbroek 1993; Eisenmann *et al.* 1998), and close relatives of both have been identified as important components of the investigated soil microbiome (Dibbern *et al.* 2014; Kramer *et al.* 2016). We are aware that this limited selection of prey will not fully reflect the variety of microbial predator–prey relationships potentially realised

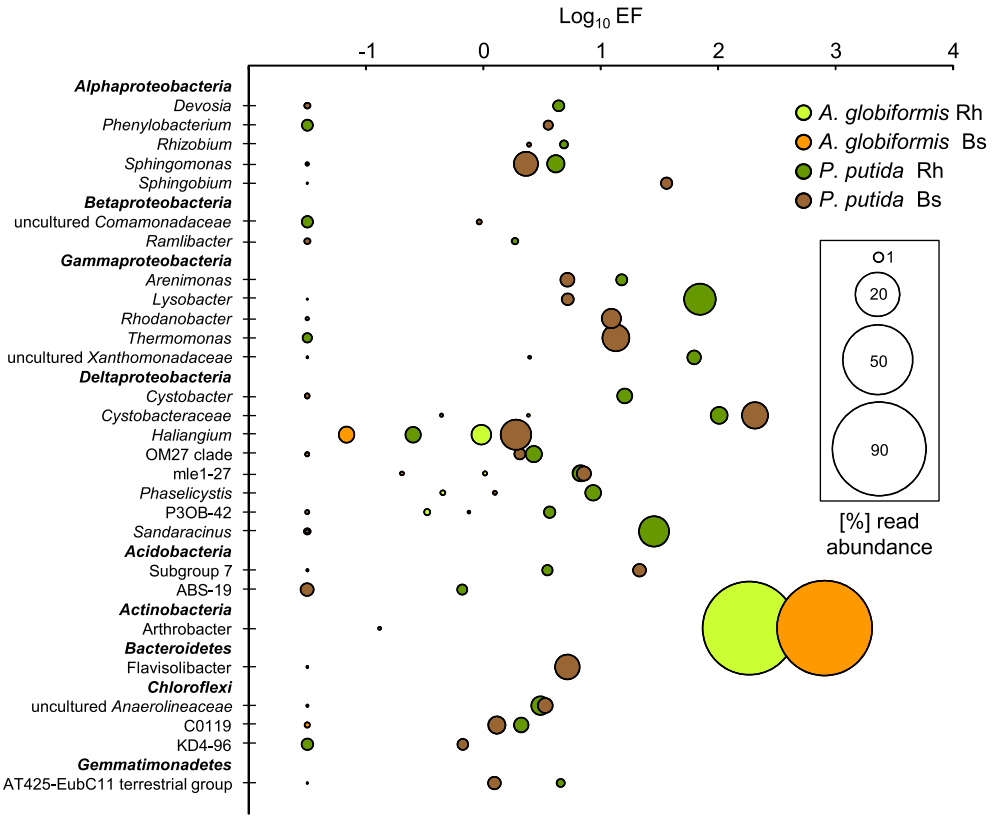

Figure 4. ^{13}C-labelled bacterial taxa identified by SIP in rhizosphere (Rh) and bulk soil (Bs) microcosms amended with *A. globiformis* and *P. putida* after 8 days of incubation. Labelling was inferred based on EF calculated from heavy versus light rRNA gradient fractions of ^{13}C- and ^{12}C-microcosms. EFs are shown in combination with relative read abundance of labelled taxa in heavy ^{13}C-RNA. The EFs of taxa with a negative read enrichment for given time points or treatments, but labelled in others, were manually set to a $\log_{10}$ of –1.5 for graphical display.

in the investigated soil. Still, we are confident that it allows addressing some fundamental principles.

The similar relative abundance of both respective T-RFs after amendment (Fig. S1) suggested that we successfully added both strains in a comparable final abundance. However, it also suggested that rRNA of the amended biomass was much more abundant in the freshly amended soil than the ~2% of total bacteria as originally intended. While this could relate to biases in extracting rRNA from intrinsic soil microbiota versus that of freshly amended cells (Wang, Hayatsu and Fujii 2012), it could also reflect a higher ribosome content of amended bacteria compared to intrinsic soil microbes, an underquantification of the amended cells, or an overestimation of the indigenous bacterial biomass. Quantitative DNA-targeted assays may help to resolve this, given that a genomic abundance of 5–7 *rrn* operons has been inferred for both *Pseudomonas* and *Arthrobacter* spp. (Stoddard *et al.* 2015). Nevertheless, we argue that the amendment had not altered indigenous bacterial communities beyond a degree that intrinsic food web functioning would no longer have been apparent, as overall community structure before the amendment and after several days remained unchanged (Fig. S1).

The more rapid mineralisation of *P. putida* versus *A. globiformis* was not unexpected. Gram-positive bacteria are generally known to be more resistant to protozoan grazing (Ronn *et al.* 2002; Jousset 2012) and bacterial micropredators preferentially feed on Gram-negatives (Rogosky, Moak and Emmert 2006; Morgan *et al.* 2010; Rotem, Pasternak and Jurkevitch 2014). Still, the drastically distinct kinetics of decline for both amendments was unexpected. We are aware that by choosing one 'late' time point (8 days) for SIP, we may have failed to detect transient early labelling of micropredators for *P. putida*. This limitation was due to a practical need to focus SIP analysis on one time point, considering that ^{13}C incorporation from *A. globiformis* may have been insufficient for trophic labelling at an earlier time of incubation. Thus, while aspects of food web dynamics will have to be addressed in future work, we are confident that our study allows addressing fundamental distinctions in micropredator prey preferences and niche segregation, as observed.

Bacterial and microeukaryotic communities in rhizosphere versus bulk soil

Total light rRNA did not suggest major distinctions in bacterial community composition for the different compartments after 8 days of incubation (Fig. 2). This was surprising, since plants are known to express major selective pressures on rhizosphere microbiomes (Reinhold-Hurek *et al.* 2015), as also previously confirmed directly for the investigated field site (Dibbern *et al.* 2014). Potentially, the young age of the maize plants (~12 weeks) was insufficient to allow for the more pronounced development of a distinct rhizosphere microbiome in our experiment. Alternatively, the fact that the rhizosphere soil used was no longer under direct root influence during SIP incubation, albeit freshly harvested, could have contributed to similar overall rRNA expression patterns.

Nevertheless, some minor distinctions in bacterial community composition were still apparent between compartments,

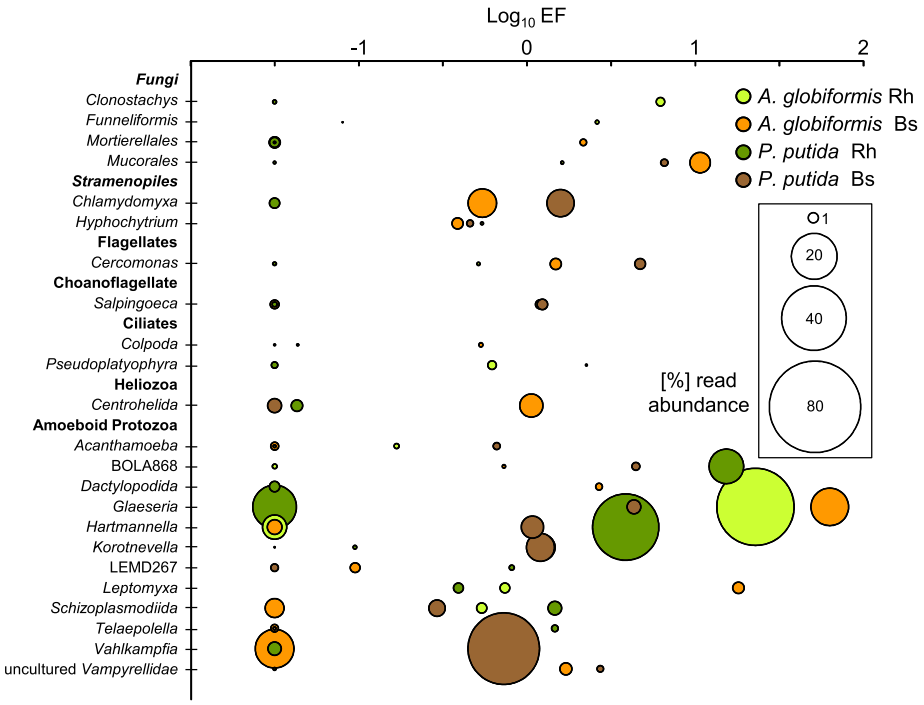

Figure 5. ^{13}C-labelled microeukaryotic taxa identified by SIP in soil microcosms after 8 days of incubation. For further details, see the legend of Fig. 4.

such as the consistent detectability of rRNA of *Paucimonas* spp. in the rhizosphere, whereas scarcely observed in bulk soil. This was consistent with our previous findings directly from the investigated field, where members of the *Oxalobacteraceae* (including *Paucimonas* spp.) were more abundant on the rhizoplane and in the rhizosphere than in bulk soil (Dibbern *et al.* 2014). Members of this genus have been previously reported to respond to plant secondary metabolites and to be of relevance in phytoremediation (Uhlik *et al.* 2013).

In contrast to bacteria, profound variability was revealed for total light rRNA of microeukaryotes between soil compartment, i.e. ciliates of the genus *Gonostomum* were preferentially found in the rhizosphere of maize, similarly as previously reported for the rhizosphere of several subtropical plants (Acosta-Mercado and Lynn 2004). Interestingly, the rhizosphere also appeared enriched in amoeboid *Hartmanella* and kinetoplastid *Ichthyobodo* spp. Both are of pathogenic concern, either as vectors for pathogenic bacteria (Dobrowsky *et al.* 2016) or directly for fish (Isaksen *et al.* 2012). While especially the former are frequently reported for agricultural soils (Takenouchi, Iwasaki and Murase 2016), our observation adds to the discussion of the rhizosphere being a reservoir not only for bacterial (Berg *et al.* 2013), but also for protistan pathogens. In bulk soil, *Vahlkampfia* spp. dominated the light rRNA libraries, especially when amended with *P. putida*. While these protozoa have been previously reported for different soils (Takenouchi, Iwasaki and Murase 2016; Tyml *et al.* 2016), the ecological relevance of such heterolobosean amoebae in soils is still poorly understood. In summary, we can assume that at least some of the original distinctions in microbial food web functioning between originally rooted and non-rooted soil were still apparent during SIP incubation.

Micropredator niche differentiation

The first intriguing finding amongst ^{13}C-labelled bacterial communities was the striking difference in diversity of labelled taxa in response to *P. putida* and *A. globiformis* amendments. Only a few selected *Myxococcales* assimilated C from *A. globiformis*. This very specific flow of ^{13}C to selected bacterial lineages strongly suggests intrabacterial predation to be involved. While the *Myxococcales* are an intensively investigated group of mostly facultative micropredators (Jurkevitch 2007), the genus *Haliangium* has not been frequently reported from soils. Originally isolated from a marine environment and considered as halophilic (Fudou *et al.* 2002), members of this genus have recently been detected in several soils (Fulthorpe *et al.* 2008; Ding *et al.* 2014) and were even discussed as biocontrol agents against fungal pathogens (Qiu *et al.* 2012). Our study now demonstrates that *Haliangium* spp. are capable of predating on Gram-positive bacteria in soil.

A much larger diversity of myxobacteria was indicated to be active in incorporating ^{13}C from *P. putida*. This included well-known soil myxobacteria such as the *Cystobacteraceae* (Dawid 2000), but also several uncultured lineages. Myxobacteria have been consistently reported to preferentially prey on Gram-negatives (Morgan *et al.* 2010). This study substantiates this preference for a considerable diversity of *Myxobacteria* in a complex natural soil microbiome. Also *Lysobacter* spp. appeared highly active in assimilating ^{13}C from *P. putida*, similarly as previously shown for a soil amended with *Escherichia coli* (Lueders *et al.* 2006). Interestingly, labelling appeared more pronounced in the rhizosphere than in bulk soil in this study. Although members of this genus are known as typical rhizosphere bacteria and also as biocontrol agents (Ciancio, Pieterse and Mercado-Blanco 2016), the predatory behaviour of *Lysobacter* spp. in rhizosphere soils, to the best of our knowledge, has not been demonstrated to date.

For both *A. globiformis* and *P. putida* amendments, an unexpected diversity of microeukaryotes showed strong ^{13}C-labelling, suggesting a substantial flow of ^{13}C to heterotrophic protists. As previously shown for the detritusphere of the investigated soil (Kramer *et al.* 2016), ^{13}C enrichment was found

mostly in amoeboid protozoa, and prey-dependent distinctions were clearly apparent. Especially, rRNA of *Glaeseria* spp. appeared strongly labelled under *A. globiformis* amendment, irrespective of soil compartment. rRNA of *Glaeseria* spp. or closely related amoeba has recently been found in a number of soils (Turner *et al.* 2013; Geisen *et al.* 2015), but their natural feeding preferences have not been elucidated. *P. putida*, in stark contrast, appeared preferentially consumed by the closely related *Hartmannella* spp. in our experiment. These are known as typical free-living soil amoebae (Takenouchi, Iwasaki and Murase 2016), but also as infectious agents in amoebic keratitis (Lorenzo-Morales *et al.* 2007). To the best of our knowledge, such marked prey-dependent niche segregation for closely related amoeba in a complex soil microbiota has not been reported to date.

One dominant amoeboid taxon with pronounced compartment-dependent activity was *Vahlkampfia* spp., which was exclusively labelled for *P. putida* in bulk soil. While possible mechanisms explaining the compartment-specific predation of this typical soil amoeba (Brown and De Jonckheere 1999) remain unclear, they could possibly reflect slight differences in soil moisture (Rodriguez-Zaragoza, Mayzlish and Steinberger 2005) between bulk soil and planted soil in our experiment. Despite our best efforts, these were possibly not fully avoided during SIP incubation.

Our results reveal pronounced niche differentiation between micropredators as driven by both bacterial prey and soil compartments. While intrabacterial predation was almost exclusive to Gram-negative prey and largely independent of soil compartment, both Gram-negative and Gram-positive bacterial prey were consumed by protistan predators, but with marked prey- and compartment-dependent niche differentiation. Thus, our first hypothesis was confirmed for both bacterial and protozoan micropredators. It has been repeatedly shown that protozoa with different prey preferences can alter the structure of soil bacterial communities (Ronn *et al.* 2002; Murase, Noll and Frenzel 2006). Although our current experiment did not include comparable w/o predation treatments, our results suggest that similar effects of shaping bacterial communities can also be assumed for bacterial micropredators. Technically, it will be a challenge to realise experimental treatments free of intrabacterial predation, at least for complex natural microbial communities. Possibly, artificial communities can offer a means to circumvent this, similarly as previously applied for other fundamental predator–prey relationships (Saleem *et al.* 2013).

In contrast to our second hypothesis, we did not observe clear distinctions between the importance of predation by protists and intrabacterial predation between soil compartments. Key predators of amended bacteria in rhizosphere and bulk soil were always represented within both pro- and eukaryotic micropredators. Our study specifically addresses the sequestration of C from bacterial prey across microbial kingdoms in soil. The labelling results and the fine taxon-level resolution of distinct micropredators obtained demonstrate the power of rRNA-SIP to elucidate microbial food webs and interaction networks (Lueders *et al.* 2016). We are aware that our approach does not allow for a strict quantitative comparison of population-level carbon fluxes, but similar labelling intensities can at least be taken as an indicator of comparable predation activities. In summary, our results provide elaborate population-level insights into microbial predation in soils. The observed patterns of prey selectivity and niche segregation may be of value to improve our understanding and the predictive modelling of microbial food webs (Rousk 2016).

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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Conflict of interest. None declared.

REFERENCES

Acosta-Mercado D, Lynn DH. Soil ciliate species richness and abundance associated with the rhizosphere of different subtropical plant species. *J Eukaryot Microbiol* 2004;**51**:582–8.

Bell T, Bonsall MB, Buckling A *et al.* Protists have divergent effects on bacterial diversity along a productivity gradient. *Biol Lett* 2010;**6**:639.

Berg G, Alavi M, Schmid M *et al.* The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. In: de Bruijn FJ (ed) Molecular Microbial Ecology of the Rhizosphere. John Wiley & Sons, Hoboken, NJ, 2013, 1209–16.

Bonkowski M. Protozoa and plant growth: the microbial loop in soil revisited. *New Phytol* 2004;**162**:617–31.

Brown S, De Jonckheere JF. A reevaluation of the amoeba genus *Vahlkampfia* based on SSU rDNA sequences. *Eur J Protistol* 1999;**35**:49–54.

Buddrus-Schiemann K, Schmid M, Schreiner K *et al.* Root colonization by *Pseudomonas* sp. DSMZ 13134 and impact on the indigenous rhizosphere bacterial community of barley. *Microbial Ecol* 2010;**60**:381–93.

Chatzinotas A, Schellenberger S, Glaser K *et al.* Assimilation of cellulose-derived carbon by microeukaryotes in oxic and anoxic slurries of an aerated soil. *Appl Environ Microb* 2013;**79**:5777–81.

Ciancio A, Pieterse CMJ, Mercado-Blanco J. Harnessing useful rhizosphere microorganisms for pathogen and pest biocontrol. *Front Microbiol* 2016;**7**:1620.

Dawid W. Biology and global distribution of myxobacteria in soils. *FEMS Microbiol Rev* 2000;**24**:403–27.

Dibbern D, Schmalwasser A, Lueders T *et al.* Selective transport of plant root-associated bacterial populations in agricultural soils upon snowmelt. *Soil Biol Biochem* 2014;**69**:187–96.

Ding G-C, Radl V, Schloter-Hai B *et al.* Dynamics of soil bacterial communities in response to repeated application of manure containing sulfadiazine. *PLoS One* 2014;**9**:e92958.

Dobrowsky PH, Khan S, Cloete TE *et al*. Molecular detection of *Acanthamoeba* spp., *Naegleria fowleri* and *Vermamoeba* (*Hartmannella*) vermiformis as vectors for *Legionella* spp. in untreated and solar pasteurized harvested rainwater. *Parasite Vectors* 2016;**9**:539.

Drigo B, Pijl AS, Duyts H *et al*. Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2. *P Natl Acad Sci USA* 2010;**107**:10938–42.

Eisenmann H, Harms H, Meckenstock R *et al*. Grazing of a *Tetrahymena* sp. on adhered bacteria in percolated columns monitored by in situ hybridization with fluorescent oligonucleotide probes. *Appl Environ Microb* 1998;**64**:1264–69.

Euringer K, Lueders T. An optimised PCR/T-RFLP fingerprinting approach for the investigation of protistan communities in groundwater environments. *J Microbiol Meth* 2008;**75**:262–68.

Fox J, Weisberg S. *An R Companion to Applied Regression*. Thousand Oaks, CA: Sage, 2011.

Fudou R, Jojima Y, Iizuka T *et al*. *Haliangium ochraceum* gen. nov., sp. nov. and *Haliangium tepidum* sp. nov.: novel moderately halophilic myxobacteria isolated from coastal saline environments. *J Gen Appl Microbiol* 2002;**48**:109–15.

Fulthorpe RR, Roesch LFW, Riva A *et al*. Distantly sampled soils carry few species in common. *ISME J* 2008;**2**:901–10.

Geisen S, Tveit AT, Clark IM *et al*. Metatranscriptomic census of active protists in soils. *ISME J* 2015;**9**:2178–90.

Handa IT, Aerts R, Berendse F *et al*. Consequences of biodiversity loss for litter decomposition across biomes. *Nature* 2014;**509**:218–21.

Isaksen TE, Karlsbakk E, Repstad O *et al*. Molecular tools for the detection and identification of *Ichthyobodo* spp. (*Kinetoplastida*), important fish parasites. *Parasitol Int* 2012;**61**:675–83.

Jezbera J, Hornak K, Simek K. Food selection by bacterivorous protists: insight from the analysis of the food vacuole content by means of fluorescence in situ hybridization. *FEMS Microbiol Ecol* 2005;**52**:351–63.

Johnke J, Cohen Y, de Leeuw M *et al*. Multiple micro-predators controlling bacterial communities in the environment. *Curr Opin Biotechnol* 2014;**27**:185–90.

Jousset A. Ecological and evolutive implications of bacterial defences against predators. *Environ Microbiol* 2012;**14**:1830–43.

Jurkevitch E. Predatory behaviors in bacteria - diversity and transitions. *Microbe* 2007;**2**:67–73.

Jurkevitch E, Minz D, Ramati B *et al*. Prey range characterization, ribotyping, and diversity of soil and rhizosphere *Bdellovibrio* spp. isolated on phytopathogenic bacteria. *Appl Environ Microb* 2000;**66**:2365–71.

Kramer S, Dibbern D, Moll J *et al*. Resource partitioning between bacteria, fungi, and protists in the detritusphere of an agricultural soil. *Front Microbiol* 2016;**7**:1524.

Kramer S, Marhan S, Ruess L *et al*. Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems. *Pedobiologia* 2012;**55**:111–9.

Lorenzo-Morales J, Martínez-Carretero E, Batista N *et al*. Early diagnosis of amoebic keratitis due to a mixed infection with *Acanthamoeba* and *Hartmannella*. *Parasitol Res* 2007;**102**:167–9.

Lueders T, Dumont MG, Bradford L *et al*. RNA-stable isotope probing: from carbon flow within key microbiota to targeted transcriptomes. *Curr Opin Biotechnol* 2016;**41**:83–9.

Lueders T, Kindler R, Miltner A *et al*. Identification of bacterial micropredators distinctively active in a soil microbial food web. *Appl Environ Microb* 2006;**72**:5342–8.

Lueders T, Wagner B, Claus P *et al*. Stable isotope probing of rRNA and DNA reveals a dynamic methylotroph community and trophic interactions with fungi and protozoa in oxic rice field soil. *Environ Microbiol* 2004;**6**:60–72.

Morgan AD, MacLean RC, Hillesland KL *et al*. Comparative analysis of *Myxococcus* predation on soil bacteria. *Appl Environ Microb* 2010;**76**:6920–7.

Murase J, Frenzel P. A methane-driven microbial food web in a wetland rice soil. *Environ Microbiol* 2007;**9**:3025–34.

Murase J, Noll M, Frenzel P. Impact of protists on the activity and structure of the bacterial community in a rice field soil. *Appl Environ Microb* 2006;**72**:5436–44.

Murase J, Shibata M, Lee CG *et al*. Incorporation of plant residue–derived carbon into the microeukaryotic community in a rice field soil revealed by DNA stable-isotope probing. *FEMS Microbiol Ecol* 2012;**79**:371–9.

Nielsen UN, Ayres E, Wall DH *et al*. Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity–function relationships. *Eur J Soil Sci* 2011;**62**:105–16.

Pausch J, Kramer S, Scharroba A *et al*. Small but active – pool size does not matter for carbon incorporation in below-ground food webs. *Funct Ecol* 2016;**30**:479–89.

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

Pruesse E, Peplies J, Glöckner FO. SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;**28**:1823–9.

Qiu M, Zhang R, Xue C *et al*. Application of bio-organic fertilizer can control *Fusarium* wilt of cucumber plants by regulating microbial community of rhizosphere soil. *Biol Fertil Soils* 2012;**48**:807–16.

Quast C, Pruesse E, Yilmaz P *et al*. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;**41**:D590–6.

R Development Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. 2013; http://www.R-project.org.

Reinhold-Hurek B, Bünger W, Burbano CS *et al*. Roots shaping their microbiome: global hotspots for microbial activity. *Annu Rev Phytopathol* 2015;**53**:403–24.

Rodriguez-Zaragoza S, Mayzlish E, Steinberger Y. Vertical distribution of the free-living amoeba population in soil under desert shrubs in the Negev Desert, Israel. *Appl Environ Microb* 2005;**71**:2053–60.

Rogosky AM, Moak PL, Emmert EAB. Differential predation by *Bdellovibrio bacteriovorus* 109J. *Curr Microbiol* 2006;**52**:81–5.

Ronn R, McCaig AE, Griffiths BS *et al*. Impact of protozoan grazing on bacterial community structure in soil microcosms. *Appl Environ Microb* 2002;**68**:6094–105.

Rotem O, Pasternak Z, Jurkevitch E. *Bdellovibrio* and like organisms.In: Rosenberg E DeLong EF, Lory S *et al*. (eds.) *The Prokaryotes: Deltaproteobacteria and Epsilonproteobacteria*, Berlin, Heidelberg: Springer, 2014, 3–17.

Rousk J. Biomass or growth? How to measure soil food webs to understand structure and function. *Soil Biol Biochem* 2016;**102**:45–7.

Saleem M, Fetzer I, Harms H *et al*. Diversity of protists and bacteria determines predation performance and stability. *ISME J* 2013;**7**:1912–21.

Scharroba A, Dibbern D, Hünninghaus M *et al*. Effects of resource availability and quality on the structure of the micro-food web of an arable soil across depth. *Soil Biol Biochem* 2012;**50**:1–11.

Seccareccia I, Kost C, Nett M. Quantitative analysis of *Lysobacter* predation. *Appl Environ Microb* 2015; **81**:7098–105.

Stoddard SF, Smith BJ, Hein R *et al*. rrnDB: improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. *Nucleic Acids Res* 2015;**43**:D593–8.

Takenouchi Y, Iwasaki K, Murase J. Response of the protistan community of a rice field soil to different oxygen tensions. *FEMS Microbiol Ecol* 2016;**92**:fiw104.

Turner TR, Ramakrishnan K, Walshaw J *et al*. Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. *ISME J* 2013; **7**:2248–58.

Tyml T, Skulinová K, Kavan J *et al*. Heterolobosean amoebae from Arctic and Antarctic extremes: 18 novel strains of *Allovahlkampfia, Vahlkampfia* and *Naegleria*. *Eur J Protistol* 2016;**56**:119–33.

Uhlik O, Musilova L, Ridl J *et al*. Plant secondary metabolite-induced shifts in bacterial community structure and degradative ability in contaminated soil. *Appl Microbiol Biot* 2013;**97**:9245–56.

Verhagen FJM, Duyts H, Laanbroek HJ. Effects of grazing by flagellates on competition for ammonium between nitrifying and heterotrophic bacteria in soil columns. *Appl Environ Microb* 1993;**59**:2099–106.

Wang Y, Hayatsu M, Fujii T. Extraction of bacterial RNA from soil: challenges and solutions. *Microbes Environ* 2012;**27**:111–21.