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Protozoa and plant growth: the microbial loop in soil revisited

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Summary

Key words: rhizosphere interactions, soil protozoa, symbiotic microorganisms, signals, auxin, carbon translocation, root architecture.

All nutrients that plants absorb have to pass a region of intense interactions between roots, microorganisms and animals, termed the rhizosphere. Plants allocate a great portion of their photosynthetically fixed carbon to root-infecting symbionts, such as mycorrhizal fungi; another part is released as exudates fuelling mainly free-living rhizobacteria. Rhizobacteria are strongly top-down regulated by microfaunal grazers, particularly protozoa. Consequently, beneficial effects of protozoa on plant growth have been assigned to nutrients released from consumed bacterial biomass, that is, the 'microbial loop'. In recent years however, the recognition of bacterial communication networks, the common exchange of microbial signals with roots and the fact that these signals are used to enhance the efflux of carbon from roots have revolutionized our view of rhizosphere processes. Most importantly, effects of rhizobacteria on root architecture seem to be driven in large by protozoan grazers. Protozoan effects on plant root systems stand in sharp contrast to effects of mycorrhizal fungi. Because the regulation of root architecture is a key determinant of nutrient- and water-use efficiency in plants, protozoa provide a model system that may considerably advance our understanding of the mechanisms underlying plant growth and community composition.

I. The Rhizosphere – Interface of Microbial and Faunal Interactions

A century ago Hiltner (1904) introduced the term 'rhizosphere' to describe the stimulation of biomass and activity of microorganism in soil around plant roots. However, even today it is often not fully acknowledged that all nutrients a plant absorbs from soil pass through a region of intense microbial and faunal activity.

The stimulation of microbial activity in the rhizosphere results from the fact that plants secrete an array of low- and high-molecular weight molecules into the soil as exudates, which may account for up to 40% of the dry matter produced by plants (Lynch & Whipps, 1990). As free-living soil microorganisms are strongly carbon limited (Wardle, 1992), a specialised microflora, typically consisting of fast growing bacteria, is triggered into activity by the carbon pulses provided as exudates (Semenov *et al.*, 1999). Root-derived carbon leads to strongly increased levels of microbial biomass and activity around roots (Alphei *et al.*, 1996) and channels energy to subsequent microfaunal grazers, where numbers of bacterial feeding protozoa and free-living nematodes may increase up to 30-fold compared with bulk soil (Griffiths, 1990; Zwart *et al.*, 1994).

Estimates of plant-below-ground investments vary widely, but even if the C-transfer to exudation was 10–20% of total net fixed carbon (Rovira, 1991), other microbial symbionts such as mycorrhizae (Söderström, 1992; Smith & Read, 1997) or N_2-fixing microorganisms (Ryle *et al.*, 1979) may each consume another 10–20% of total net fixed carbon. Although a trade-off between plant C-investment in different microbial interactions has been observed (Bonkowski *et al.*, 2001b; Rønn *et al.*, 2002; Wamberg *et al.*, 2003a,b), plants may still release up to half of their total fixed carbon to fuel microbial interactions in the rhizosphere.

It becomes immediately clear that supporting microbial interactions in the rhizosphere must be of fundamental importance for plants to justify this significant trade-off in carbon allocation, which could otherwise be used to construct light-capturing or defensive structural tissues above-ground. In particular, why are plants providing ample energy in form of exudates to a microbial community that is strongly competing with roots for available nutrients? The answer partly lies in the loop structure of the bacterial energy channel in the rhizosphere. Nutrients become only temporarily locked up in bacterial biomass near the root surface and are successively liberated by microfaunal grazing (Bonkowski *et al.*, 2000a). The interplay between microorganisms and microbivores determines the rates of nutrient cycling and strongly enhances the availability of mineral nutrients to plants (Clarholm, 1984; Ingham *et al.*, 1985; Gerhardson & Clarholm, 1986; Ritz & Griffiths, 1987; Kuikman *et al.*, 1990; Jentschke *et al.*, 1995; Alphei *et al.*, 1996; Bonkowski *et al.*, 2000b). The assumed mechanism, known as the 'microbial loop in soil' (Clarholm, 1985), is triggered by the release of root exudates from plants that increase bacterial growth in the rhizosphere. Plant available nutrients will be strongly sequestered during microbial growth (Kaye & Hart, 1997; Wang & Bakken, 1997) and would remain locked up in bacterial biomass if consumption by protozoa and nematodes would not constantly re-mobilize essential nutrients for plant uptake (Christensen *et al.*, 1992; Griffiths & Caul, 1993; Griffiths *et al.*, 1993; Bonkowski *et al.*, 2000b). Due to the relatively small differences in respect to C : N ratios between predators and bacterial prey and a relatively low assimilation efficiency, only 10–40% and 50–70% of the prey carbon will be used for biomass production by protozoa and nematodes, respectively (Griffiths, 1994; Ferris *et al.*, 1997). The excess N is excreted as ammonia and hence is readily available for other soil organisms, including plant roots (Zwart *et al.*, 1994).

In addition, the populations of soil protozoa strongly fluctuate through time (Clarholm, 1989; Christensen *et al.*, 1992; Janssen & Heijmans, 1998) and parallel to the decline in protozoan numbers their rapidly decomposable tissue may enter the detrital food-web. Although the size of most protozoa in soil may range only between 10 and 100 μm in diameter, protozoan biomass is anything but small. In most soils protozoan biomass equals or exceeds that of all other soil animal groups taken together – with the exclusion of earthworms (Sohlenius, 1980; Foissner, 1987; Schaefer & Schauermann, 1990; Schröter *et al.*, 2003). Roughly estimated, 70 and 15% of total respiration of soil animals might be attributed to protozoa and nematodes, respectively (Sohlenius, 1980; Foissner, 1987). High production rates of protozoa with 10–12 times their standing crop per year and minimum generation times of 2–4 h (Coleman, 1994) suggest a strong grazing pressure on bacterial biomass and subsequent significant effects on nutrient mineralization.

II. Microfauna and Plant Growth

Beneficial effects of protozoa on plant growth are well documented (Ekelund & Rønn, 1994; Griffiths, 1994; Zwart *et al.*, 1994). Experiments in planted microcosms provided strong evidence of the importance of protozoan grazing in the rhizosphere. Shoot biomass and amounts of shoot N mostly increased in the presence of protozoa and nematode grazers (Table 1). Microfaunal stimulation of nitrogen mineralization via the microbial loop was suggested as the main underlying mechanism (Clarholm, 1985; Griffiths, 1994; Zwart *et al.*, 1994).

Consequently, food-web models simulating N-mineralization in soil suggest protozoa and bacterivorous nematodes to be the most important contributors to nitrogen mineralization (Hunt *et al.*, 1987; De Ruiter *et al.*, 1993; Schröter *et al.*, 2003). Their indirect contributions to nutrient cycling may be even more important than their direct effects because grazing stimulates microbial mineralization processes. For

Table 1 Effects of protozoa and nematodes on plant biomass production, and total plant and shoot nitrogen contents

Effect of protozoa (% of control)			
Total plant biomass (min–max)	Total plant nitrogen (min–max)	Shoot nitrogen (min–max)	
80	20	12	Clarholm (1984)
30–78	20–45	14–48	Clarholm (1985a)
27–85	37–166	38–157	Bonkowski *et al*. (2000b)
41–47	9–17	19–30	Kuikman *et al*. (1990)
44–57	46–63	40–45	Jentschke *et al*. (1995)
43	56	58	Bonkowski *et al*. (2001a)
10–25	–16––1	–14–6	Alphei *et al*. (1996)
–5–46	–11–11	–22–13	Bonkowski *et al*. (2001b)
2–21	7–24	6–38	Kuikman *et al*. (1991)*
11	–16	–21	Kuikman *et al*. (1991)**
–22–6	nd	18–138	Elliott *et al*. (1979)
–8––2	2–21	3–22	Kuikman & van Veen (1989)
Effect of nematodes (% of control)	49		Ingham *et al*. (1985) (day 49)
58	nd	49	Ingham *et al*. (1985) (day 49)
12	nd	6	Ingham *et al*. (1985) (day 77)
–5	nd	–4	Ingham *et al*. (1985) (day 105)
15–30	10–16	11–14	Bonkowski *et al*. (2000b)
10	–5	–1	Alphei *et al*. (1996)

nd, not determined. *soil with 14–19% (v/w) moisture. **soil with 8% (v/w) moisture.

example, De Ruiter *et al.* (1993) calculated that the contribution of amoebae and nematodes to overall N mineralization in winter wheat was 18 and 5%, but their subsequent deletion from the food-web model resulted in reductions of 28 and 12% of N mineralization for amoebae and nematodes, respectively.

Although nutrient-based models appear fully sufficient to estimate the gross outcome of plant–microfauna interactions, it must be noted that the underlying mechanisms are much more complex than could have been imagined nearly 20 yr ago when the microbial loop concept was first proposed.

Roots do not only secrete carbon; the rhizodeposition of nitrogen by plants can be substantial (Høgh-Jensen & Schjoerring, 2001). By including the amount of nitrogen lost through rhizodeposition and modeling N transformations in the rhizosphere, Robinson *et al.* (1989) and Griffiths and Robinson (1992) calculated that plant-derived carbon is only sufficient to allow for recycling of the N lost from the plant by exudation rather than to support mineralization of N from soil organic matter. Therefore only a small benefit of direct microfaunal activity to the gross N nutrition of plants can be assumed (Griffiths *et al.*, 2004).

Indeed, protozoa have been found to increase plant biomass independently of nutrient contents in plant tissue (Kuikman *et al.*, 1991; Alphei *et al.*, 1996). In a laboratory experiment, even a constant surplus of nutrients did not prevent an increase of up to 60% in biomass of spruce seedlings in the presence of protozoa, but completely eliminated beneficial effects of mycorrhiza (Jentschke *et al.*, 1995).

These discrepancies in the microbial loop model suggest additional, nutrient-independent effects of protozoa on plant growth.

III. Victims and Benefactors: How Grazers Affect Bacterial Community Composition

As noted in section I, bacteria in the rhizosphere are strongly top-down regulated by grazing (Wardle & Yeates, 1993; Bonkowski *et al.*, 2000b) and there is increasing evidence that changes in the composition of rhizobacteria due to strong and selective grazing are a major determinant of microfaunal effects on plant growth (Fig. 1).

The most important bacterial grazers in soil are naked amoebae due to their high biomass and turnover and specialised feeding modes (Fig. 2). By contrast to suspension and filter feeders like bacterivorous nematodes and other protozoa, amoebae are grazing bacterial biofilms and colonies attached to soil and root surfaces and thus have access to the majority of bacteria in soil. With the aid of their pseudopodia, amoebae can reach bacterial colonies in soil pores and even inside roots inaccessible to other predators (Darbyshire & Greaves, 1973; Elliott *et al.*, 1980) and they may still continue grazing in tiny water films when other protozoa or nematodes are restricted by decreased water potential in soil (Foster & Dormaar, 1991; Ekelund & Rønn, 1994; Young & Ritz, 2000). 'Bacterial' feeding nematodes, by contrast, are able to migrate to places of high bacterial and protozoan activity (Griffiths & Caul, 1993) where they aggregate and become important grazers of

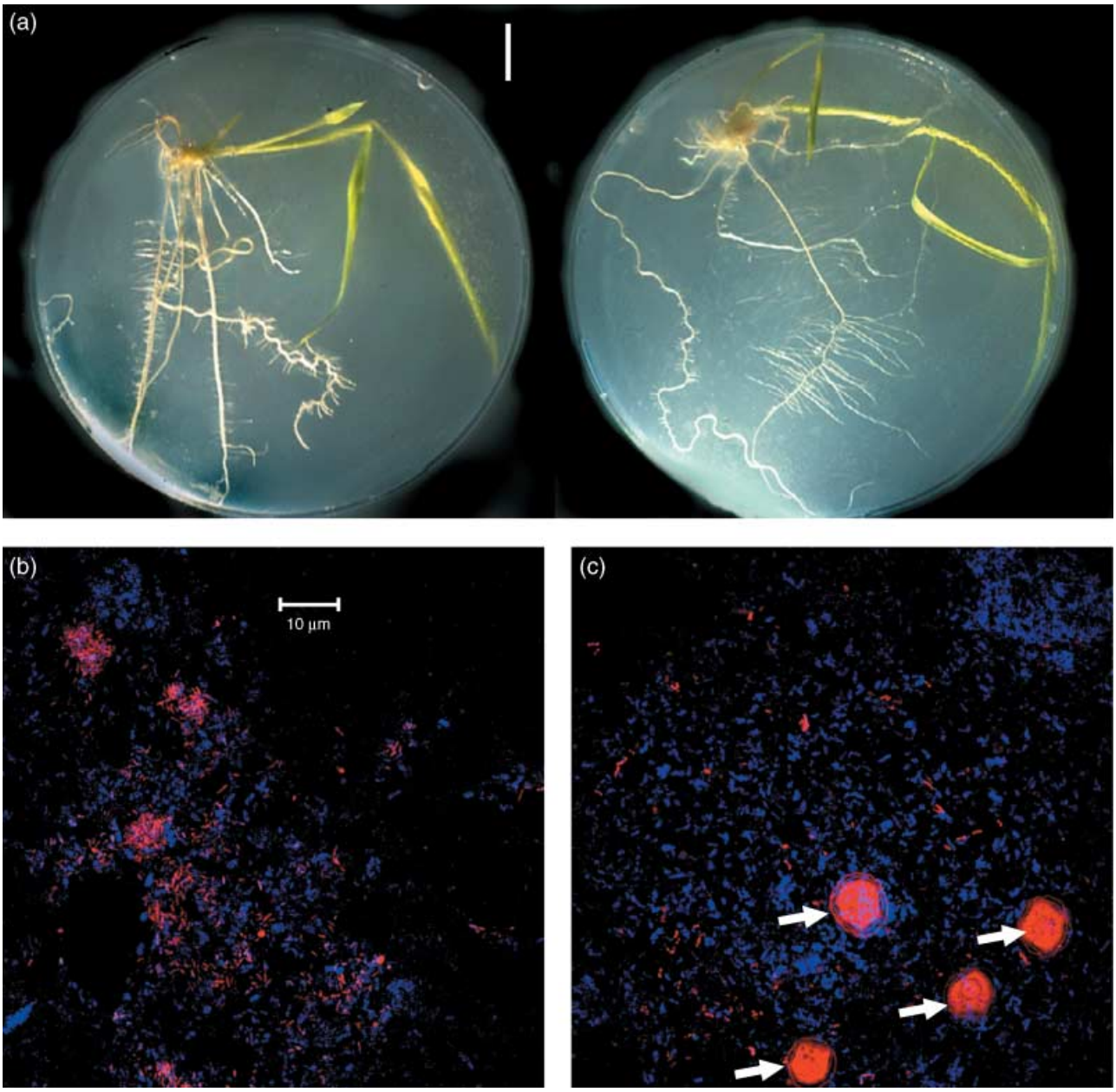


Fig. 1 Protozoan effects on root architecture and composition of bacterial communities in the rhizosphere of rice (Oryza sativa). (a,b) Differences in root architecture of 16-d-old rice seedlings growing in Petri dishes on agar inoculated with a diverse soil bacterial community in absence (left) and presence (right) of amoebae (*Acanthamoeba* sp.). The length of the white bar is 1 cm (K. Kreuzer & M. Bonkowski, unpublished). (b,c) An example for grazing-induced shifts in bacterial communities. Fluorecscent *in situ* hybridization of bacteria (red: alpha-proteobacteria, blue: eubacteria) on the agar surface near lateral roots of the plants above in absence (left) and presence (right) of amoebae. White arrows indicate red-fluorescent amoebal cysts. Please note the decrease of colonies of alpha-proteobacteria in presence of amoebae. (J. Adamczyk, M. Bonkowski, M. Wagner, unpublished.)

both bacteria and amoebae (Anderson *et al.*, 1978; Elliott *et al.*, 1980; Woods *et al.*, 1982; Alphei *et al.*, 1996; Rønn *et al.*, 1996; Bonkowski *et al.*, 2000b) and eventually become the dominant predators (Elliott *et al.*, 1980; Griffiths, 1990).

Significant changes in bacterial diversity due to protozoan grazing have been confirmed in freshwater systems (Pernthaler *et al.*, 1997; Jürgens *et al.*, 1999; Posch *et al.*, 1999) as well as in the rhizosphere of plants (Griffiths *et al.*, 1999; Bonkowski & Brandt, 2002; Rønn *et al.*, 2002). The grazing-induced changes in microbial functioning affect fundamental ecosystem properties because bacteria in soil occupy some of the most important control points for nutrient cycling and plant growth. For instance, N_2-fixing, nitrifying and denitrifying bacteria are in command of the nitrogen cycle (Mengel, 1996). Protozoan grazing does often stimulate nitrifying bacteria, presumably through predation on their faster-growing bacterial

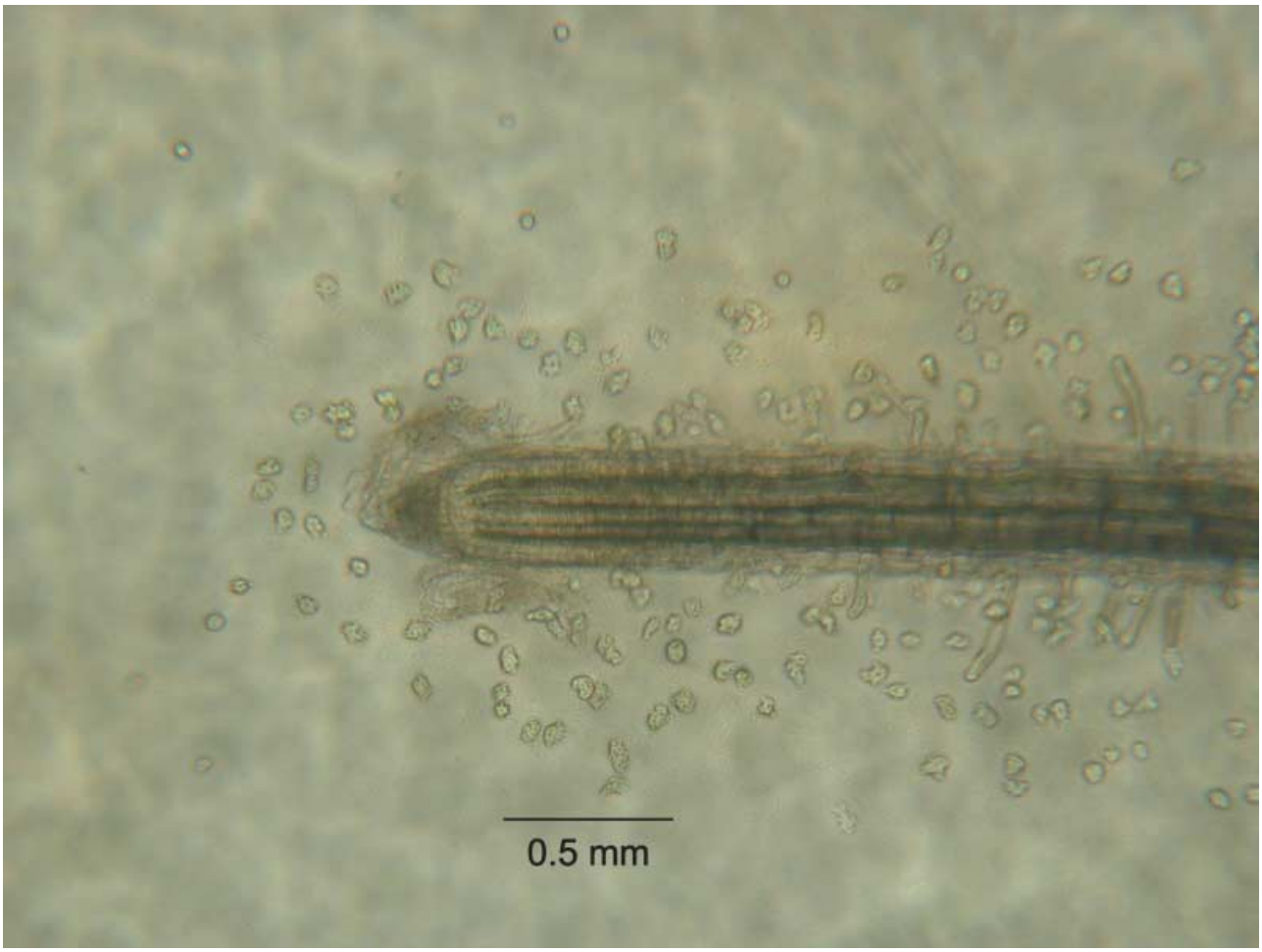


Fig. 2 Typical distribution of amoebae (*Acanthamoeba castellanii*) along a lateral root of rice (*Oryza sativa*) growing on agar.

competitors, resulting in high concentrations of NO_3^- in culture liquid and leachate of rhizosphere soil (Griffiths, 1989; Verhagen *et al.*, 1994; Alphei *et al.*, 1996; Bonkowski *et al.*, 2000b).

By contrast to interactions in freshwater systems, the three-dimensional structure of the soil habitat adds to the complexity of trophic interactions in the rhizosphere. Bacteria in biofilms on roots and on the outer zones of soil particles may receive greater grazing pressure than bacteria protected inside tiny crevices or separated by water films (Young & Ritz, 1998). In addition, temporal dynamics of bacterial activity on roots have to be considered (Semenov *et al.*, 1999). The heterogeneity of the rhizosphere in space and time needs considerably more attention in order to understand the contribution of predator–prey interactions to the dynamics of rhizosphere processes (Young & Ritz, 2000).

Bacterial communities often respond with morphological shifts to grazing which are directed either towards larger or smaller cell sizes. Protozoan grazing on bacteria commonly results in an outgrowth of filamentous bacterial cells and microcolonies that the protozoa apparently cannot effectively ingest (Bianchi, 1989; Hahn *et al.*, 1999; Jürgens & Matz, 2002). Positive feedbacks may arise through the removal of senescent bacteria and an increase in the contribution of younger strains with higher metabolic activity. Subsequent bottom-up effects occur through enhanced substrate availability, favoring those species capable of balancing their predation losses with enhanced growth rates (Posch *et al.*, 1999). However, the bigger cells of these actively growing bacterial populations seem most attractive to grazers and again receive the largest grazing pressure (Sherr *et al.*, 1992). For example, grazing of protozoa in the rhizosphere of wood barley (*Hordelymus europaeus*) resulted in a 20–40% increased respiratory quotient, qO_2 (i.e. microbial respiration per unit microbial biomass), confirming a strongly enhanced turnover of the grazed bacterial community (Alphei *et al.*, 1996) and evolution of $^{13}CO_2$ from microbial mineralization of labeled plant litter material increased up to 100% in the rhizosphere of ryegrass due to protozoan grazing (Bonkowski *et al.*, 2000b).

However, not only bacterial size and turnover matters. The fact that certain microfaunal grazers grow better on some bacterial species than on others is well established for protozoa

(Weekers *et al.*, 1993) and nematodes (Anderson & Coleman, 1981; Grewel, 1991). There is strong evidence that nematodes and protozoa use chemical clues to sense and discriminate between their bacterial prey species (Andrew & Nicholas, 1976; Snyder, 1991; Verity, 1991). In the case of protozoa a high selectivity in bacterial food choice has been confirmed (Boenigk & Arndt, 2002) and not only the feeding strategies and grazing rates may considerably differ between taxa and species, but also the sensitivity of the bacterial prey to grazing may strongly diverge and depend on the protozoan predator. For example, the same bacterial prey species may be digested differently by various protozoan grazers, and the same predator species may selectively digest variable prey (Weisse, 2002). In addition, some common pigmented soil bacteria, like *Chromobacter*, evolved secondary compounds with extreme toxicity to protozoa (Deines *et al.*, 2004).

Studies in experimental soil systems indicate that gram-positive bacteria benefit while gram-negative bacteria decrease by grazing (Griffiths *et al.*, 1999; Rønn *et al.*, 2002b). However, Rønn *et al.* (2002b) demonstrated that not all gram-negative bacteria were reduced, for example *Pseudomonas*, a typical rhizosphere colonizer, increased during grazing. Moreover, their study demonstrated that the grazing-induced changes in bacterial diversity were partly dependent on the taxa and species of protozoan grazers present. Thus, grazing of protozoa, and possibly nematodes, creates a complex top-down pressure affecting the morphological, taxonomic and functional composition of the bacterial community.

Molecular techniques, such as fluorescent *in situ* hybridization, microautoradiography (Lee *et al.*, 1999; Wagner *et al.*, 2003), stable isotope probing and metagenomics (Wellington *et al.*, 2003) now offer ways to directly visualize changes in the identity, activity, function and spatial arrangements of bacterial communities exposed to protozoan predation. They may considerably advance our understanding of the role of microfaunal predators in affecting the function and spatial organization of bacterial communities in the rhizosphere of plants.

IV. Nonmotile plants with flexible strategies

Plants are not passive recipients of nutrients, instead plants integrate information from the environment into their decisions on below-ground investments such as root production and proliferation (Huber-Sannwald *et al.*, 1997; Hodge *et al.*, 1998; Rajaniemi *et al.*, 2003), formation of symbiotic relationships with infecting microorganisms (e.g. mycorrhizal fungi, Fitter & Merryweather, 1992; Smith & Read, 1997; or N_2-fixing bacteria, Ryle *et al.*, 1979), alteration in exudation rates (Kraffczyk *et al.*, 1984; Jones & Darrah, 1995; Bonkowski *et al.*, 2001b; Wamberg *et al.*, 2003a,b), interactions with free-living bacteria (Mathesius *et al.*, 2003; Joseph & Phillips, 2003) or production of secondary compounds to defend herbivores (Baldwin & Hamilton, 2000; Cipollini *et al.*, 2003). Because root morphology is both genetically programmed and environmentally determined (Rolfe *et al.*, 1997), there must be signal transduction pathways that interpret complex environmental conditions and activate genes to enter a particular symbiosis or to form a lateral root at a particular time and place. Microbial symbionts must communicate their presence to plant hosts (Alfano & Collmer, 1996; Long, 1996; Hirsch *et al.*, 1997, 2003; Barker *et al.*, 1998) and plants need to distinguish friend (symbiont) and foe (pathogen), suggesting that the reciprocal exchange of microbial signals with roots is common (Paiva, 2000; Mathesius *et al.*, 2003). Recently, Phillips *et al.* (2003) combined these insights in the concept of 'rhizosphere control points' in order to emphasize the importance of information exchange between plants and microorganisms for gene expression patterns and resulting morphological and physiological changes in the partners.

From a microbial perspective the evolution of strategies enhancing energy transfer to the roots led to an increase in fitness of those microorganisms that influence gene regulation in plants by sending the respective signals. Indeed most of the specialised rhizosphere bacteria appear to have the potential to affect plant performance by producing hormones (Costacurta & Vanderleyden, 1995; Arshad & Frankenberger, 1998; Lambrecht *et al.*, 2000). Up to 80% of the bacteria isolated from plant rhizospheres are considered to produce auxins (Patten & Glick, 1996), and their widespread ability to produce cytokinins led Holland (1997) to suggest that cytokinins in plants may originate exclusively from microorganisms. The widespread ability of both beneficial and deleterious rhizosphere organisms to produce plant hormones and other signal molecules (Phillips *et al.*, 1999; Joseph & Phillips, 2003; Mathesius *et al.*, 2003) suggest that rhizosphere bacteria play an important role in manipulating root and plant growth (Shishido *et al.*, 1996; Rolfe *et al.*, 1997).

Recent advances in the application of microbial biosensors are expected to significantly advance our understanding of the spatial context of substrate availability and signal exchange at scales relevant to roots and microorganisms (Jaeger *et al.*, 1999; Steidle *et al.*, 2001; Leveau & Lindow, 2002).

V. Case Study: Root Foraging and Microfaunal Activity

In order to separate root foraging activity (Robinson, 1994), that is, the occupation and exploitation of organic matter by active root growth, from microfauna mediated effects on nutrient mineralization, Bonkowski *et al.* (2000b) set up a factorial experiment with ryegrass (*Lolium perenne*) and treatments of bacterivorous protozoa and nematodes where labelled plant litter was added to a soil poor in organic carbon in order to create hotspots of microbial activity.

The biomass of *L. perenne* doubled in protozoan treatments, and plant N-uptake and incorporation of ^{15}N from the labeled plant litter increased two- and threefold, respectively. Root foraging and presence of microfauna accounted for 34 and

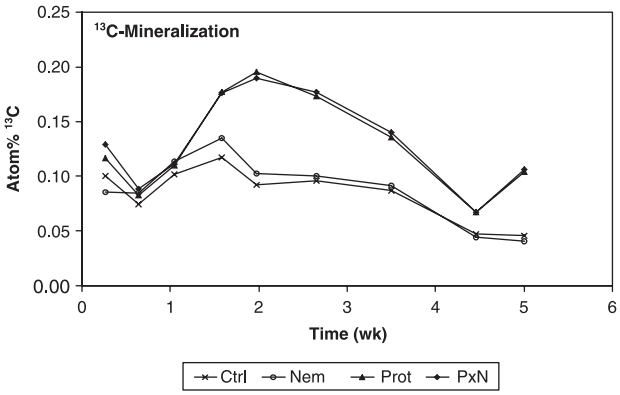


Fig. 3 Time course of organic matter decomposition (release of $^{13}CO_2$–C from labelled plant litter) in soil inoculated with a diverse soil microbial filtrate (Ctrl); and additional inoculations of bacterial-feeding nematodes (Nem); protozoa (Prot); or nematodes and protozoa (PxN). Data from Bonkowski *et al*. (2000b).

47% of variation in plant biomass, indicating that microbial–faunal interactions were a major determinant of plant growth.

The simultaneous action of root foraging and microfaunal activity led to a complex pattern of nutrient liberation in space and time. While root foraging in organic hotspots enhanced the spatial coupling of mineralization and plant uptake, microfaunal grazing increased the temporal coupling of nutrient release and plant uptake. This outcome resulted from the simultaneous interplay of three effects: First protozoan grazing strongly stimulated microbial mineralization dynamics (Fig. 3) both, by keeping the microbial community in an actively growing state and by changing the composition of the bacterial community (Griffiths *et al*., 1999). Second due to strong effects on microbial functioning (increased N mineralization) and diversity (stimulation of nitrifying bacteria), 90% of the liberated nitrogen in the rhizosphere occurred as NO_3^- and only 10% as NH_4^+. Nitrate is highly mobile in soil and plant nitrogen uptake in the presence of protozoa may even decrease due to leaching losses (e.g. Alphei *et al*., 1996) if mobilization of NO_3^- is not matched by a corresponding increase in root uptake rates. Third as a consequence of the second point, the production of significantly more roots in the presence of protozoa enabled plants to benefit from the liberated nitrogen-pool, while constant grazing pressure shifted the competition for nutrients in favour of roots (Bonkowski *et al*., 2000b).

Thus grazing on bacteria creates more complex patterns rather than simply liberating nutrients from grazed bacterial biomass. Particularly, the development of a greater root surface in the presence of protozoa, which enabled efficient nutrient uptake, is a common pattern observed that merits special attention.

VI. Friend or Foe? Microbial Signals and the Manipulation of Root Architecture

Many plant symbionts and pathogens use signals to direct plant carbon for the build-up of additional root structures. Root nodules inhabited by nitrogen-fixing bacteria (Hirsch *et al*., 1997; Mathesius *et al*., 2000), nematode-induced root galls (McKenzie Bird & Koltai, 2000), or tumors formed by *Agrobacterium* (Jameson, 2000) are well known examples. There is now increasing evidence that the effects of rhizobacteria on root architecture are controlled to a great extent by protozoan grazing (Bonkowski & Brandt, 2002; K. Kreuzer & M. Bonkowski, unpublished).

Plants develop an extensive and highly branched root system in the presence of protozoa due to a strong stimulation in lateral root production (Jentschke *et al*., 1995; Bonkowski *et al*., 2000b, 2001b; Bonkowski & Brandt, 2002; K. Kreuzer & M. Bonkowski, unpublished). These changes in root architecture correspond to hormonal effects on root growth by beneficial rhizobacteria rather than nutrient effects (Boot & Mensink, 1990; Petersen *et al*., 1996; Shishido *et al*., 1996; Rolfe *et al*., 1997; Lambrecht *et al*., 2000).

Early investigations suggested a direct release of plant hormones by amoebae (Nikolyuk & Tapilskaja, 1969). Soil amoebae grown axenically with the bacterium *Azotobacter* released phytohormones into the growth medium. Production of indolyl-3-acetic acid (IAA) related substances, the most physiological active auxins, was at a maximum in 75-d-old amoebal cultures and the biomass of pea seedlings grown in this culture fluid increased by 48%. By contrast, culture fluid of *Azotobacter* increased biomass of pea seedlings only by 3–4% (Tapilskaja, 1967).

Similarly, Bonkowski & Brandt (2002) demonstrated strong growth-stimulating effects of protozoa on the root system of water cress seedlings (*Lepidium sativum*). Already 5 d after germination the number and length of first order lateral roots were increased by factors of 4 and 5, respectively, in the presence of protozoa. A direct production of auxins by amoebae could be excluded, instead Bonkowski & Brandt (2002) demonstrated a stimulation of auxin producing bacteria that most likely was responsible for the stimulation of lateral root growth. Accordingly, the increased root surface allows more nutrients to be absorbed, but will also increase exudation rates, thereby further stimulating bacterial–protozoan interactions, as shown in Fig. 4. Thus, in addition to the stimulation of gross nutrient flows protozoa promote a mutualistic interaction between plant roots and rhizobacteria.

These results were supported in an experiment investigating protozoan effects on *Arabidopsis thaliana* plants transformed by the cytokinin-inducible ARR5-promoter-GUS construct (C. Dickler & K. Kreuzer, unpublished). As expected, root elongation and root branching nearly doubled in plants grown in the presence of amoebae (*Acanthamoeba castellanii*) compared with control plants grown in soil inoculated with a filtered soil bacterial inoculum. Simultaneously, GUS-reporter gene activity strongly increased in treatments with protozoa. The significant change in root architecture of *Arabidopsis* suggests a strong auxin effect, which presumably had to be down-regulated in the root by the auxin-antagonist

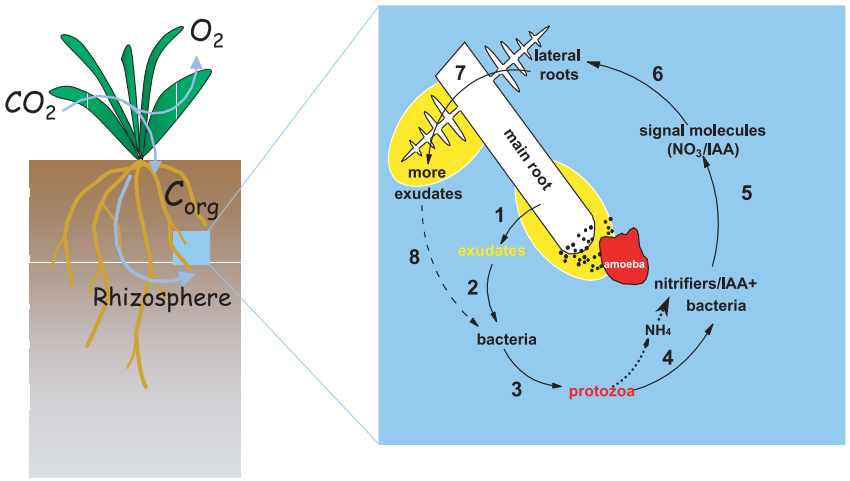


Fig. 4 A conceptual model illustrating microfaunal-induced hormonal effects on root growth, modified after Bonkowski & Brandt (2002). Root exudation (1) stimulates growth of a diverse bacterial community (2) and subsequently of bacterial-feeders such as protozoa (3). Ammonia is excreted by protozoa and selective grazing favours nitrifiers and indole-3-acetic acid (IAA+) producing bacteria (4). The release of signal molecules (5), such as NO_3 and IAA, induces lateral root growth (6), leading to release of more exudates (7), subsequent bacterial growth (8), etc.

cytokinin. Cytokinin is also important as a long-distance root-to-shoot signal communicating nitrogen availability in the rhizosphere to the shoot (Schmülling, 2002). In addition to auxins, locally high concentrations of nitrate will occur in the rhizosphere due to the stimulation of nitrifying bacteria by protozoa. Nitrate, besides being a source of nitrogen, acts as a signal for lateral root elongation (Zhang & Forde, 2000) and may aid to direct lateral root growth towards patches with high nutrient concentration (Zhang & Forde, 1998).

Recently, the role of other signal molecules, apart from hormones, in microbe-root communication has been established. Phillips *et al.* (1999) demonstrated that *Sinorhizobium meliloti* manipulates plant carbon transfer to its own benefit. The bacteria produce a signal molecule that enhances root respiration and triggers a compensatory increase in whole-plant net carbon assimilation in *Medicago sativa*. They identified the signal as lumichrome, a common breakdown product of riboflavin (Phillips *et al.*, 1999). In addition, a large proportion of the bacteria colonizing the roots of plants are capable of producing species-specific autoinducing signals to coordinate their behaviour in local rhizosphere populations, a process that has become known as quorum sensing (Dunn & Handelsman, 2002; Sturme *et al.*, 2002). Specific interactions of bacteria with plant hosts, such as nodulation (Wisniewski-Dyé & Downie, 2002) and the infection of plants by deleterious bacteria presumably depends on quorum-sensing regulation mediated by N-acyl homoserine lactone (AHL). Recently, Mathesius *et al.* (2003) demonstrated that auxin responses and investment in defence by the legume *Medicago truncatula* were directly affected by AHLs from both free-living beneficial and deleterious bacteria. Additionally, Joseph & Phillips (2003) showed that homoserine lactone, the breakdown product of AHL, leads to a strongly increased transpiration in bean (*Phaseolus vulgaris*) and speculated that the microorganisms benefit from enhanced transpiration because soil water carries mineral nutrients towards the root.

These examples give evidence of a bustling signal exchange in the rhizosphere. Several of these indirect plant–microorganism interactions could potentially be significantly influenced by bacterial grazers (Griffiths *et al.*, 2004).

VII. Carbon is the Currency: Microfauna Interactions with Mycorrhiza

The widespread symbiosis between plants and mycorrhizal fungi is regarded as mutualistic (Smith & Read, 1997). As the fungus provides its host with essential nutrients in exchange for plant carbon, both partners apparently spend resources they can afford in exchange for growth limiting nutrients. Nevertheless, theory suggests major conflicts of interest (Denison *et al.*, 2003). From a plant perspective a tight control over its carbon budget and different carbon-allocation strategies should exist; contrary, from the microbial perspective, mechanisms to increase the net efflux of carbon from roots and competition for plant C between different root colonizers are also likely to occur (Ali *et al.*, 1981; Vierheilig *et al.*, 2000; Phillips *et al.*, 2003).

In fact, not all plants benefit from arbuscular-mycorrhizal (AM) or ecto-mycorrhizal (EM) fungal colonization (Fitter & Merryweather, 1992; Jonsson *et al.*, 2001; Helgason *et al.*, 2002; Sanders, 2003). Depending on their plant host, AM species can become highly parasitic (Klironomos, 2003); also high mycorrhizal infection can be harmful to plants (Gange & Ayres, 1999; Jonsson *et al.*, 2001). Moreover, competition for plant carbon may explain mutually inhibitory effects where mycorrhizal infection prevents further root colonization by fungal and nematode pathogens (Ingham, 1988; Graham, 2001). These findings suggest that microbial root interactions might better be seen as a microbial contest to gain maximum carbon from plants.

Microfauna interactions with mycorrhiza provide a good model system to study the interplay of plants with multiple

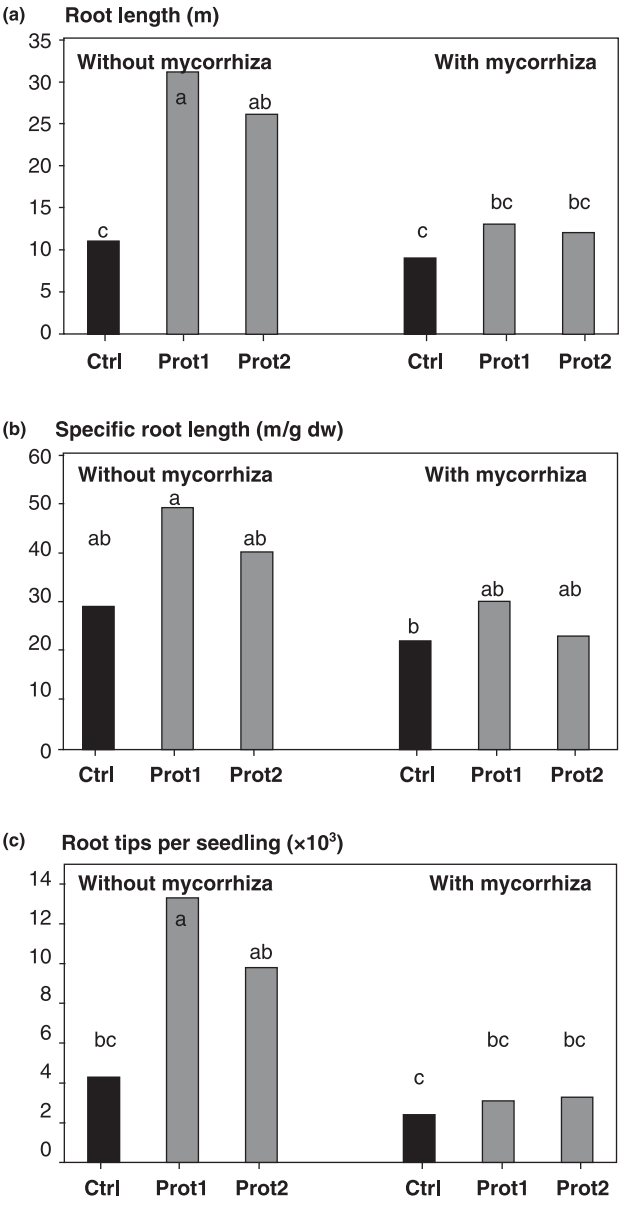


Fig. 5 Effects of protozoa on root architecture of *Picea abies* seedlings ((a) root length (b) specific root length (c) number of root tips) in treatments without and with ecto-mycorrhiza (*Lactarius rufus*). Black bars: control, Ctrl; grey bars: protozoa treatments with protozoa from soil filtrate (P1); with protozoa from cultures (P2). Different letters indicate significant differences, $P < 0.05$, Tukey-test. Data from Jentschke *et al*. (1995).

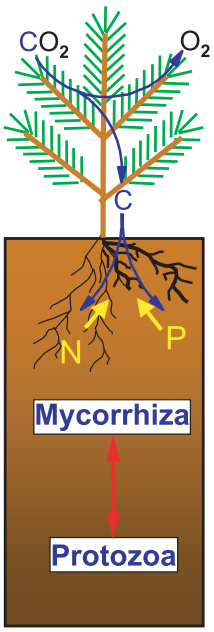
microbial root associations. As outlined above, bacteria–protozoa interactions benefit from increased root exudation and favour the development of an extensive and highly branched root system. Hyphal extensions of mycorrhizal root systems, by contrast, are commonly formed at the expense of root structures (Jonsson *et al*., 2001; Lerat *et al*., 2003). The response of plants to these conflicting demands was investigated in two experiments where nonmycorrhizal and ecto-mycorrhizal Norway spruce seedlings (*Picea abies*) were inoculated with a bacterial soil inoculum or bacterial inoculum plus protozoa (Jentschke *et al*., 1995; Bonkowski *et al*., 2001b).

In both experiments, the presence of protozoa caused the development of a highly branched root system with longer and thinner roots (Fig. 5), whereas mycorrhiza had opposite effects on root architecture (Jentschke *et al*., 1995; Bonkowski *et al*., 2001b). Presumably the plants counterbalanced contradicting effects on root growth by letting microorganisms compete one against the other. The microbial effects on root architecture were cancelled out in the combined treatment, and the performance of both microbial partners was reduced due to strong carbon limitation. The length of fungal hyphae decreased by 18% in the presence of protozoa, while the presence of mycorrhiza led to reduced numbers of bacteria (–38%) and their respective protozoan grazers (–34%), indicating a significant trade-off in plant carbon allocation between bacterial and fungal rhizosphere colonizers (Fig. 6; Bonkowski *et al*., 2001b). The plants however, took further advantage because both microbial systems differed in their abilities to mobilize essential growth-limiting nutrients: mycorrhiza strongly increased plant uptake of phosphorus, and protozoa strongly increased the mineralization of nitrogen. High leaching losses of nitrogen occurred in treatments with protozoa, but not in treatments with protozoa plus mycorrhiza. Presumably, synergistic microbial effects maximized uptake of nitrogen (+17%) and phosphorus (+55%) in the combined compared with control treatments without mycorrhiza and protozoa where the additional hyphal network increased the uptake of protozoa-mobilized nitrogen (Bonkowski *et al*., 2001b).

Rønn *et al*. (2002a) and Wamberg *et al*. (2003a,b) observed similar trade-offs in carbon allocation between microbial systems in the rhizosphere of pea plants (*Pisum sativum*) where numbers of bacteria and protozoa dropped in presence of different AM fungi. Promiscuity in respect to microbial interactions seems more common among plants than generally acknowledged. It is an open question to what extend there has been an evolutionary benefit for the plant of being able to direct its carbohydrates towards the different consumers in direct response to the needs of the plant (Griffiths *et al*., 2004).

VIII. The Plant Bridging Above- and Below-Ground Processes

The soil–root interface is the transition zone where below- and above-ground systems interact via plants. Therefore plant interactions above-ground are likely to influence processes in the rhizosphere and vice versa. For example, the amount of carbon translocated in the rhizosphere may significantly increase if plants are subjected to above-ground herbivory, and this may strongly influence the rhizosphere food-web



Interactions between protozoa and mycorrhiza

Root architecture

	Root number	Root diameter	Root tips	Root length
Mycorrhiza	–40%		–35%	–40%
Protozoa		–25%	×1.5	×1.6

Trade-off in C allocation

	Fungal hyphae	Bacteria	Amoebae
Mycorrhiza		–38%	–34%
Protozoa	–18%		

Fig. 6 Diagramatic representation of the trade-off in C-allocation between rhizosphere microfauna and mycorrhizal symbionts in *Picea abies*. Data from Bonkowski *et al*. (2001b).

structure (Mikola *et al*., 2001; Bardgett & Wardle, 2003). On the other hand, many interactions in the rhizosphere have the potential to affect food webs above-ground by changing nutrient uptake, litter quality or defence mechanisms against herbivores (Gange *et al*., 2002; Bonkowski & Scheu, 2003; Wurst *et al*., 2003).

Bonkowski *et al*. (2001a) investigated the effects of bacterial feeding protozoa and earthworms in the rhizosphere of barley on above-ground aphid performance. The biomass of barley increased by *c*. 40% in the presence of protozoa. Concomitantly, aphid numbers and biomass more than doubled on plants grown in presence of protozoa (Fig. 7). However, protozoa also increased plant reproduction (biomass of ears, number of seeds and individual seed weight). Apparently, the plants in protozoan treatments tolerated higher levels of herbivory and even increased their fitness. Effects of protozoa on plant biomass and nutrient turnover considerably exceeded effects of earthworms on most parameters measured. This indicates that, despite grass litter being added as an organic nutrient source to the experimental soil, indirect effects of bacteria-grazing protozoa were more important for plant growth and aphid performance than direct nutrient mobilization through physicochemical processes by earthworms. However, more investigations on the interactions between below- and above-ground systems are required. The presence of microfauna may often result in a dilution of nutrients in plant tissues, indicated by greater increases of plant biomass production than nitrogen uptake as seen in Table 1. This may even lead to strong negative effects on plant herbivores, as recently confirmed by M. Bonkowski, M. Omacini and H. Jones (unpublished) in a study on aphid herbivore development on the grass *Lolium multiflorum*.

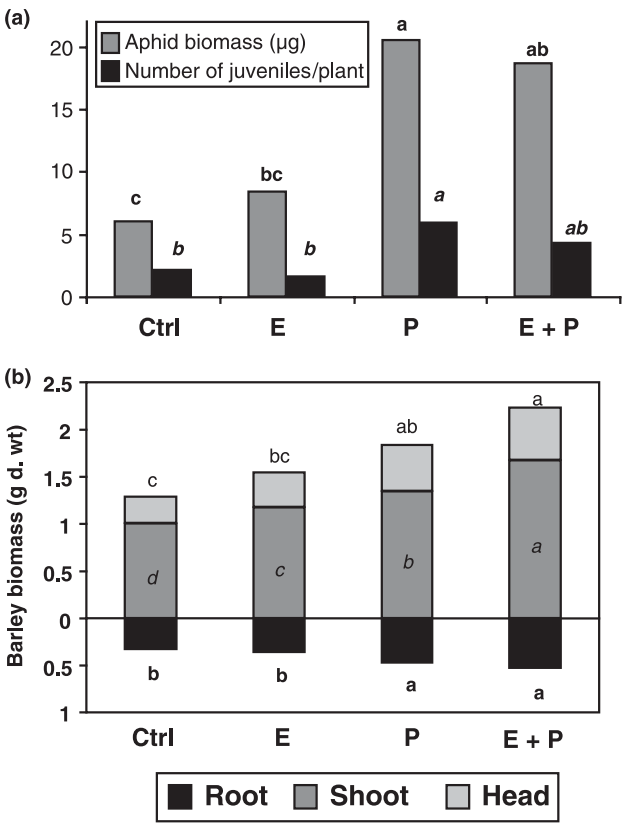


Fig. 7 Effects of protozoa and earthworms (a) on the biomass of aphids (µg), and numbers of juvenile offspring on barley plants and (b) on biomass of barley plants; animal-free control (Ctrl), earthworms (E), protozoa (P), earthworms and protozoa (E + P). Bars with the same letter are not significantly different ($P < 0.05$, Tukey-Test). Data from Bonkowski *et al*. (2001a).

IX. From Microscale to Macroscale: Interactions at the Plant Community Level

In a field situation, plants are continuously confronted to interact with their neighbours. A nutrient that escapes the rhizosphere of one plant might be easily taken up by an adjacent competitor. Under these circumstances, the costs and gains of microbial rhizosphere interactions are of fundamental importance, because even slight effects on plant competitive abilities may result in opportunity costs for affected plants and opportunity benefits for neighbouring unaffected plants (Heil & Baldwin, 2002). Under the pressure of plant competition this can result in significant effects at the plant community level (van der Heijden *et al.*, 1998; Bradford *et al.*, 2002; Klironomos, 2003; M. Bonkowski & J. Roy, unpubl.). In view of the diversity of root systems it is clear that not all plant species will interact with rhizosphere microorganisms in a similar way. Plants with a highly branched root system, as found in grasses and cereals, may respond more strongly to protozoa–bacteria interactions than plants with a large root cortex, as found in many forbs, which in turn may be stronger hosts for mycorrhiza. Moreover, plants exert species specific effects on the composition of root colonizing bacteria (Neal *et al.*, 1973; Chanway *et al.*, 1991; Wieland *et al.*, 2001), and their protozoan and nematode grazers (Geltzer, 1963; Brimecombe *et al.*, 2000). Modifications of the root environment by the rhizosphere microbial and microfaunal community need considerably more attention as driving agent for plant competition and community composition.

X. Conclusions and Future Research

The view that interactions between plants and microfauna, particularly protozoa, in the rhizosphere are solely based on the liberation of nutrients from consumed microbial biomass is rather simplistic. In recent years our perspective has profoundly changed, giving soil organisms a much more active role by interacting with, and being acted on, by living plants.

Complex microbial and faunal interactions with plant roots accompanied and shaped the evolution of land plants. This resulted in mutual interactions between plants, symbiotic microorganisms, soil animals and soil nutrients with the microfauna affecting, and being affected by, both the above- and belowground components of the plant (Griffiths *et al.*, 2004).

The recognition of bacterial communication networks in the rhizosphere (Shapiro, 1998; Taga & Bassler, 2003), the common exchange of bacterial (and other microbial and microfaunal) signals with roots (Paiva, 2000; Hirsch *et al.*, 2003; Mathesius *et al.*, 2003) and the fact that these signals are used to enhance the efflux of carbon from roots (Phillips *et al.*, 1999; Bonkowski & Brandt, 2002; Phillips *et al.*, 2003) have led to the realization that rhizosphere interactions must be seen from an evolutionary perspective where all actors basically behave in a selfish manner. In addition to the microbial interactions outlined above, a great part of soil microbial biomass is fungal and consists of a saprophytic-pathogenic continuum of fungal species. Our understanding of rhizosphere interactions will remain incomplete without considering their interactions with rhizosphere bacteria, protozoa and plant roots within the heterogeneous pore network of the soil matrix. Rhizosphere interactions are anything but co-operative and unidirectional; rather conflicting interests and reciprocal manipulations to increase own benefits seem commonplace. Microbial rhizosphere processes can strongly affect the performance of individual plants, but have the potential to shape plant successional trajectories and community composition, as well as herbivore-based food webs above-ground, and thus can influence ecosystem processes at much larger scales.

Most importantly, the microfauna has to be acknowledged as an integral driving part of rhizosphere interactions. The regulation of root architecture is a key determinant of nutrient- and water-use efficiency in plants. The finding that the effects of rhizobacteria on root architecture seem to be driven in large by protozoan grazing provides a model system with the potential to considerably advance our understanding of the mechanisms underlying plant growth regulation and 'soil fertility'. From an applied point of view, a better understanding of the role of microfauna in regulating rhizosphere processes may in many ways foster the efforts to improve crop health and productivity; may it be to assess trade-offs due to genetic manipulation of plant traits; the release of plant growth-promoting microorganisms; efforts to reduce plant herbivore-load or to restrain weedy competitors.

The examples given in this review highlight that soil, fauna, flora, root, shoot, herbivores and predators in many ways act like a single connected organism, with rhizosphere processes being virtually the basis for understanding plant ecology. Dissecting the driving mechanisms underlying these multitrophic interactions is a major challenge that has to be tackled by combined research efforts of scientists working in rather disparate fields – microbiology, (soil) animal ecology and plant physiology – in this sense rhizosphere ecology has become one of the most multifacetted and challenging frontiers in ecology.

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