Do root exudates affect nitrogen cycling through interactions with soil microbes and root herbivores?

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Marinka van Puijenbroek
Supervisors: Esther Chang and Jan Bakker

Abstract:
Plants release carbon-rich root exudates into the rhizosphere, which could have an effect on the composition and abundance of the microbial community. This process, in turn, could positively influence the growth of microbial grazers and, with that, the rate of nitrogen (N) cycling. In the first part of this review, I will discuss how root exudates affect the interactions between plants, microbes, microbial grazers and N-cycling. In general, there is a positive effect of root exudates on microbial biomass. The greater biomass of microbes is able to support higher populations of microbial grazers, which increases the rate of N-mineralisation. However, this process mainly happens on soils with enough nutrients to use the C exudates to support further growth. The second part of this review is about the effect of root herbivores on root exudation. The evidence suggests that root exudation is increased by the activities of root herbivores. Whether root herbivores also directly lead to an increase in microbial biomass is not clear as there are more factos that have influence on the size of the microbial biomass. The effect of root exudation on N-cycling should be further investigated in the future.
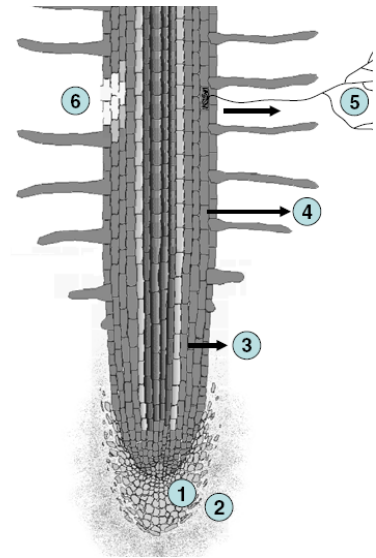
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1. Introduction

Studies on below-ground processes are very important in order to gain knowledge of the interactions between biotic and abiotic factors in the soil. For example, these interactions could have influence on the composition of the microbial and mycorrhizal communities and influence processes such as nitrogen (N) cycling. Generally, there are three kinds of interactions between plants and soil microorganisms: 1) negative interactions (*e.g.* pathogens, root herbivores); 2) positive interactions, whereby one or both of the partners receive benefits from the association (*e.g.* mycorrhizas); and 3) neutral interactions, whereby neither of the partners gain or lose from the association. An important place where interactions between microbes and plants take place is in the rhizosphere (*i.e.* the soil that is adjacent to the root). Interactions between plants and soil microorganisms are the most tightly coupled in the rhizosphere compared with the bulk soil (Bargett, 2005). Plants interact with mycorrhizal fungi in the rhizosphere in order to form symbioses and to repel pathogens in the soil, and they also have an influence on the microbial community (Bais et al., 2006; Grayston et al., 1996; Jones et al., 2008). In order to induce and support these interactions, plants exude substances from their roots. One of the major things they exude are small molecules rich in carbon (C).


Fig. 1 Schematic representation of a longitudinal section of a growing root showing the six major sites of rhizodeposition: 1 loss of root cap and border cells, 2 loss of insoluble mucilage, 3 loss of soluble root exudates, 4 loss of volatile organic C, 5 loss of C to symbionts (e.g. arbuscular mycorrhizas), and 6 loss of C due to death and lysis of root epidermal and cortical cells (from Jones, et al., 2008).

Root exudates consist mostly of small carbohydrates, like glucose and fructose, carboxylates, such as malate and fumarate, and amino acids (Grayston et al., 1996; Haase et al., 2007; Jones et al., 2008). However, root exudation is not the only process to result in rhizodeposition of C into the soil. Rhizodeposition can result from different origins (Fig. 1.): 1) root cap and border cell loss; 2) death and lysis of root cells; 3) flow of C to root-associated symbionts living in the soil (e.g. mycorrhizas); 4) gaseous losses; 5) leakage of solutes from living cells (root exudates); and 6) insoluble polymer secretion from living cells (mucilage, a gelatinous layer surrounding the root tip) (Grayston et al., 1996; Jones et al., 2008). These loss pathways are, in theory, very easily distinguishable from each other, but, it is very difficult to demonstrate and measure the different pathways experimentally. Most studies only use the amount of labelled C from the plant in the soil as a measurement for the amount of rhizodeposition (Grayston et al., 1996). This value varies from annual plants, which exude from 10% to 30 % of their total CO_2 uptake, to trees, which exude from 40% to 70% (Grayston et al., 1996; Jones et al., 2008; Singh et al., 2004). Generally, root exudates have a higher C:N-ratio ranging from 2.5 to 13 than microbial biomass, which has a ratio of 5:1 (Grayston et al., 1996).

An important consideration is to what extent rhizodeposition has an influence on the composition of the microbial community by this C subsidy. If the microbial community changes, what effect does this have on the C:N-ratio of the soil? A change in the microbial community can alter rates of N-mineralisation and N-mineralisation is a very

important process influencing the total N-uptake by plants. In this way, rhizodeposition of C-rich exudates could have an influence on the cycling of N in a system. The rate of N-cycling can also be partly regulated by the activities of bacterial-feeding fauna. Therefore, my first aim of this review is to discuss and assess the effects of root exudation on the microbial community, the microbial grazers and N-cycling (*i.e.* the microbial loop), and the interactions between these factors.

The rhizodeposition of C is influenced by a great number of factors. Where and under which circumstance does rhizodeposition occur at the highest rate? Rhizodeposition is greatest at the root tip zone (Grayston et al., 1996; Jones et al., 2008). Root exudates change both qualitatively and quantitatively with plant age. The qualities of proteins and carbohydrates released by plants have been shown to depend on the age of the plant (Grayston et al., 1996). Seedlings produce greater quantities and more diverse carbohydrates than mature trees (Grayston et al., 1996). There are more factors that influence the composition of root exudates, such as plants species, pH in the soil and temperature (Grayston et al., 1996). Not only below-ground processes have an influence on the amount of root exudates as above-ground interactions can also have an influence on below-ground processes. For example, plants have been found to exude more C into the soil when they have been defoliated (Bardgett et al., 1999b; Hamilton III and Frank, 2001; Mawdsley and Bardgett, 1997). If above-ground herbivory has an effect on the rhizodeposition of root exudates, the question arises whether below-ground herbivory could also indirectly induce rhizodeposition through changing allocation patterns? If not, perhaps below-ground herbivores can directly influence rhizodeposition processes mechanically by puncturing holes in roots during consumption. Consequently, the second topic of this review is whether root feeding organisms can stimulate root exudation and whether that can lead to a change in the soil microbial community and rates of N-mineralisation.

2. Microbial loop

N is one of the most important nutrients in the soil, and there is significant competition between plants and microbes for this element (Jones et al., 2008). The amount of available N in the soil is partly dependent on the balance between the mineralisation and immobilisation of N by the soil microbial community. This balance, in turn, is influenced by the ratio of C and N found in the soil organic matter. When plants exude C into the soil, microbes become more N-limited due to their stoichiometric needs for C and N. As a result, the microbes immobilize more N, and less N is available for plant uptake (Merckx et al., 1987; Paterson, 2003). Thus, when a plant exudes C into the rhizosphere, the conditions for the uptake of inorganic N become more unfavourable for the plant. The turnover of the microbial community is very rapid in the rhizosphere (on the order of hours to days) while plant roots have a life-span from weeks to months. The rapid microbial turnover is initiated by bacterial-feeding
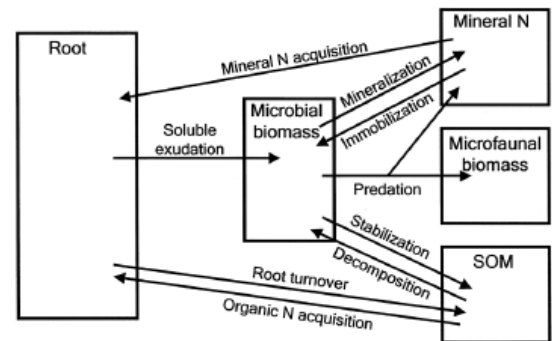
Fig. 2 Diagram illustrating the central role of the microbial biomass in mediating interactions between living roots and nutrient transformations in soil. SOM, soil organic matter (from Paterson, 2003).

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fauna. Bacteria have a C:N-ratio of 5:1, whereas the bacterial-feeding organisms have a higher C:N-ratio of 10:1. Consequently, microbial grazers ingest more N than needed and excrete this excess N, which is then available for plant use. This process is called the microbial loop (fig. 2) (Clarholm, 1985; Ingham et al., 1985; Paterson, 2003).

With respect to the microbial loop, there are a number of important questions to consider. Firstly does root exudation has an effect on microbes, and what is the effect of microbial grazers on the community? Secondly, is N mineralised by microbes or mainly mineralised by microbial grazers, and what are the consequences from different nutrient levels on the N-cycling? Finally do plants really incorporate the N that is released?
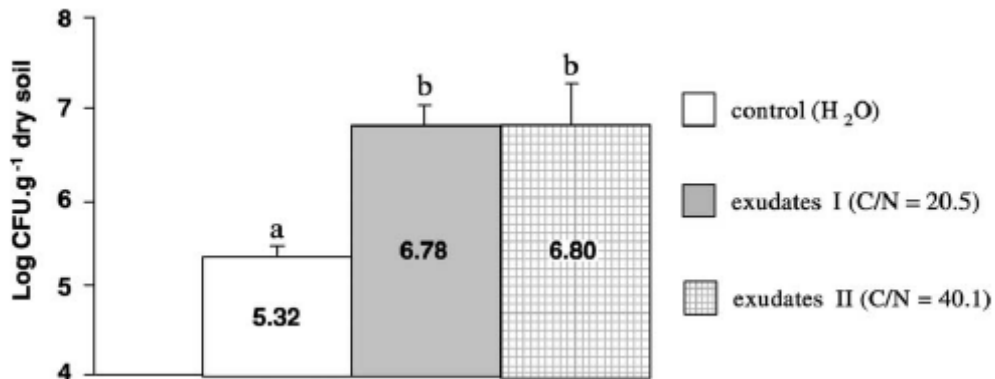
Fig. 3 Numbers of culturable heterotrophic bacteria in bulk soils enriched or not with artificial root exudates (in vitro experiment). Means (n = 4) with different letters are significantly different at P: 0,05, CFU means colony forming units (from Baudoin et al., 2003).

a) *Microbes*

Root exudates are proposed to change the biomass and composition of the microbial community. There is plenty of evidence suggesting that root exudates positively influence microbial abundance. Ingham et al. (1985) showed that the microbial biomass in the rhizosphere was always higher than in the bulk soil. Bacterial biomass and activity was higher in the presence of C-rich root exudates (Bais et al., 2006; Baudoin et al., 2003; Clarholm, 1985; Grayston et al., 1996; Merckx et al., 1987; Raynaud et al., 2006; Valé et al., 2005). Baudoin et al. (2003) shows that in the presence of artificial exudates the amount of culturable bacteria significantly higher is, than without exudates (fig. 3). Whether the composition of the microbial communities is also affected by root exudations is not as clear. There is some evidence that rhizodeposition changed the microbial community: there were more gram-negative bacteria and the abundance of the fungi increased (Butler et al., 2003). Grayston (2001) found an increase in the abundance of the *Pseudomonas sp.*, which are a nutritionally diverse group. Boudoin et al. (2003) observed a change in the microbial communities. These studies would indicate that root exudates lead to a change in the microbial community. However, the more pertinent question is whether this is accompanied by a shift in functional groups. Changes in functional group composition could give us more insight into the processes that accompany root exudation. There is not much knowledge about whether rhizodeposition can induce changes in the microbial functional groups. Unfortunately, it is technically very difficult to identify different functional groups of microbes, since these organisms

are microscopic in size and many species of bacteria and fungi are not readily culturable. Molecular methods may allow identification of some species but the function of many microbial species is unknown. In the future, there should be more attention paid to this subject.

b) *Microbial-feeding organisms*

Microbial-feeding organisms (grazers) have a high impact on the soil community. They are known to have an effect on microbial communities and on N-cycling. There are different kind of grazers including singled-celled protozoa, like amoebas, flagellates and ciliates; and microbial-feeding nematodes (Bargett, 2005; Ingham et al., 1985). The ecological niche of these grazers are all different, which would also have an influence on N-cycling.

Grazers can change both microbial biomass and activity levels. Microbial biomass has been recorded to both increase (Ingham et al., 1985) and decrease (Bardgett et al., 1999a; Djigal et al., 2004; Kuikman et al., 1990; Woods et al., 1982) when grazed. Ingham et al. (1985) found an increase in bacterial numbers when grazed, this was only consistent in the rhizosphere (fig. 4). This could imply that the rhizosphere has an tremendous effect on the bacteria, and by that an influence on the grazers. The mechanisms behind changes in microbial abundance seem to be grazer-species specific. However, all studies documented an increase in the microbial activity (Bardgett et al., 1999a; Djigal et al., 2004; Griffiths, 1994; Ingham et al., 1985; Woods et al., 1982). The increase of microbial activity can be due to multiple reasons. Ingham et al. (1985) suggested some reasons why
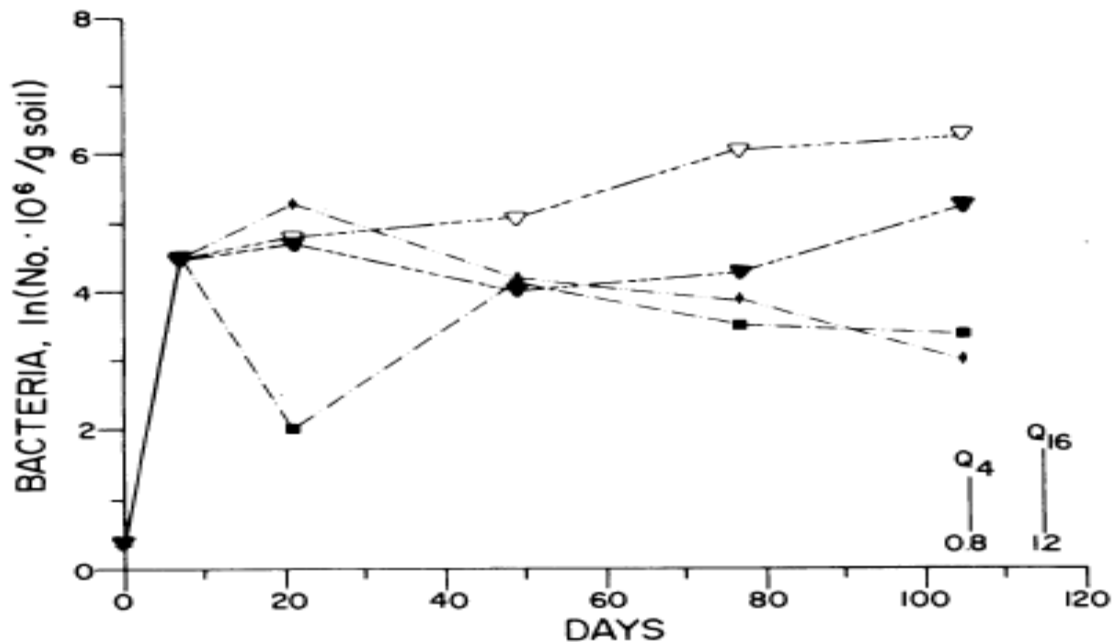
Fig. 4 Natural log (ln) of bacterial numbers in rhizospheric and non rhizospheric soil of different treatment with bacterial but without fungi. Q values are Tukey's HSD. Q_4 compares dates for same treatment, and treatments on the same date; Q_{16} compares treatments on different dates. ■—·—■ Plant + bacteria (nonrhizosphere) (pb); ▼-----▼ Plant + bacteria (rhizosphere) (pb); ♦ − ··—♦ Plant + bacteria + bacterial-feeding nematode (nonrhizosphere) (pbn_b); ▽−··—··−−▽ Plant + bacteria + bacterial-feeding nematode (rhizosphere) (pbn_b) (from Ingham et al., 1985).

nematodes can increase microbial activity: 1) 60% of the microbes that are digested through the gut of a nematode, survive ingestion and come out alive; in the gut of the nematode, they can obtain a limiting nutrient or another limiting or promoting substance; 2) the nematode can be important in dispersing microbial cells, either internally or externally, and bring them to undiscovered nutrient-rich substrates; and 3) the excretion and defecation products of nematodes may provide substrates or inorganic nutrients for bacterial growth. However, resource quality and soil conditions also have an influence on the microfaunal community. For example protozoa and nematodes need a water film to move through the soil. In absence of a water film, the abundance of microfauna will be lower. This could have an effect on the microbial community (Bardgett et al., 1999a). Grazers have a profound influence on the microbial community. The effect of the microbial fauna is dependent on the abundance of the grazers, the transportation of bacteria and N-mineralisation by the microbial fauna. The abundance of the grazers is meditated by the resource quality and soil conditions.

c) _N-cycling_
i) N-mineralisation by microbes

    The aforementioned findings clarify that root exudation has an effect on the microbial community, and that the microbial grazers also effect the microbial community. These changes in abundance or activity of microbes could have an influence on the N-mineralisation.

    The rhizodeposition of C-rich exudates into the soil can result in change of the soil C:N-ratio. Microbes have a low C:N-ratio (5:1), compared to plants and animals, which means that microbes are more N-limited. This can lead to competition with plants for N. However, there is a possibility that microbes increase rates of organic matter degradation and N-mineralisation, which can increase the availability of inorganic N in the system. Experiments conducted on the effect of root exudates on the degradation of organic matter and N-mineralisation show mixed results. Clarholm et al. (1985) discovered, using common garden experiments, that microcosms containing both plants and bacteria had increased rates of N-mineralisation. Liljeroth et al. (1994) found that there was substantial increase in the rate of soil organic matter degradation. Using a modelling approach, Raynaud et al. (2006) also predicted increased degradation rates of the organic matter. This model also predicts net immobilisation in bacterial biomass, soon after the addition of C but a few days later, there was net mineralisation. The N flux varies in time from net immobilisation to net mineralisation. On the other hand, Ingham et al. (1985) observed an increase in mineralisation rates through nematode activity, but not by microbial activity. From this, it can be concluded that the microbes immobilized the N. Decreased degradation of organic matter has also been observed (Bottner et al., 1988; Sallih and Bottner, 1988). In addition, Griffths and Robinson (1992) constructed a model, which predicted that root exudates do not stimulate the N-mineralisation because the plants acquired most of their N from the bulk soil and not from the rhizosphere.

    A possible explanation for the mixed results is that these processes are very dependent on the functional groups of microbes that are present or dominate the soil. Plant species could also make a difference in the organic decomposition rate as Liljeroth et al. (1994) found decreased mineralisation of carbon by wheat, but not with maize. Another reason could be that the mineralisation of N is not driven by microbes but by microbial grazers.

ii) N-mineralisation by microbial grazers

Grazers generally increase the mineralisation of N. The mechanism is thought to work in this way: N is immobilized by the bacteria; grazers eat the bacteria and increase rates of N-mineralisation as a consequence. Because bacteria have a C:N-ratio of 5:1, which is lower than of the grazers (C:N ratio is 10:1), grazers consume an excess of N and will excrete it as a result. Several studies have shown that there is a significant increase of the N-mineralisation when grazers are present (Bardgett and Chan, 1999; Bardgett et al., 1999a; Clarholm, 1985; Djigal et al., 2004; Ingham et al., 1985; Kuikman et al., 1990; Woods et al., 1982). The amount of N-mineralised by grazers depends mostly on the species. Kuikman, et al. (1990) observed that 20% more ^{15}N was mineralised when protozoa's were present whereas nematodes can excrete about 90% of the ingested N (Bardgett et al., 1999a). However, the increase in the amount of N mineralised is much lower.

These results demonstrate that there is always an increase in N-mineralisation through grazer activity, although there is still quite some variation in effects produced by different grazer species, different periods of time and different types of soil. Ingham et al. (1985) found that fungal-feeding nematodes had no effect on N-mineralisation, probably due to the relatively high C:N-ratio of fungi, namely 11:1, compared to bacteria. There is quite some difference in response among the different microbial-grazers. There are differences between several nematode species in their effects on the microbial community and N-mineralisation (Djigal et al., 2004). Some studies observed a higher N-mineralisation by protozoa than by nematodes (Woods et al., 1982). The effect on N-mineralisation by nematodes can also be observed to change during experiments, due to differences in life-history stage and abundance of nematodes (Ingham et al., 1985; Woods et al., 1982). The type of soil has a major influence on the grazer community. Bardgett and Chan (1999) conducted an experiment with microbial-feeding nematodes and fungal-feeding *Collembola*. When only the nematodes or *Collembola* were present, there was no effect on N-mineralisation. In the combined *Collembola*-and-nematode treatment, there was an increase in N-mineralisation. As an explanation, they argued that on acidic organic soils,
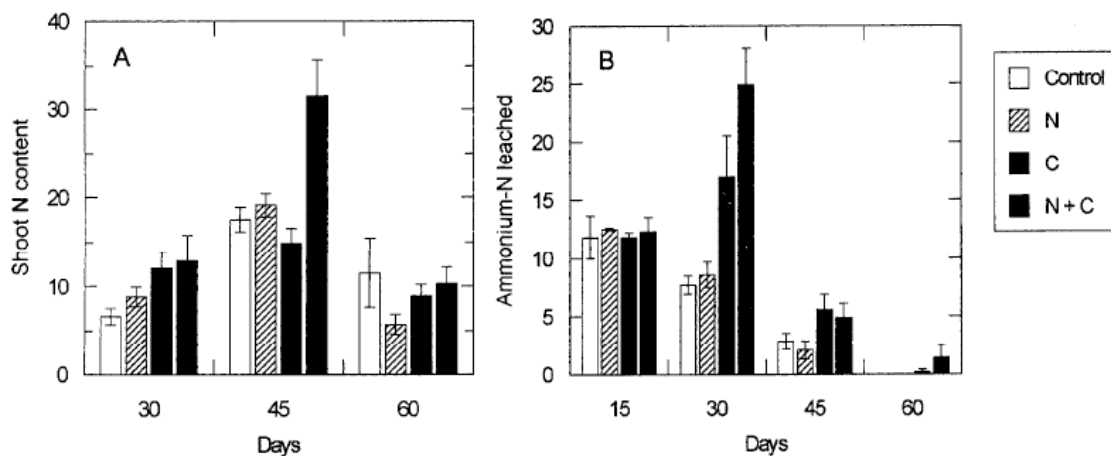
Fig. 5 Temporal changes in (A) shoot N content (mg g^{-1} dry wt), and (B) nitrogen mineralisation (measured as NHC4 –N release mg g^{-1} soil) in the different animal treatments (N = nematodes, C = Collembola, and N + C = nematodes and Collembola). Values are means ± standard error. For shoot N, *F* = 3.7 and *P* = 0.0017 for the interaction between treatment * time; for *F* = 6.5 and *P* < 0.0001 for the interaction between treatment * time. (From Bardgett and Chan, 1999)

the microbial community is fungal-dominated. When fungi are grazed by *Collembola*, the abundance of the fungi decreases. As a result, numbers of bacteria increase. This subsequently results in higher nematode abundance and the enhancement of N-mineralisation (fig. 5). These results demonstrate the functioning of the microbial loop, and the consequent enrichment of N-mineralisation. However, not only different microbial grazer species can give different rates of N-mineralisation. The total amounts of C and N available in the soil, in addition to their stoichiometric ratio, could also affect these processes. When plants grow on a nutrient-rich soil, the competition for nutrients with microbes decreases, which could additionally affect the rates of organic matter degradation and N-mineralisation.

iii) Comparison between nutrient- poor and nutrient- rich soils

Nutrient deficiency has many effects on plants. It can result in the increase of the root/shoot ratio of the plant (Valé et al., 2005), which expands the relative surface area available for nutrient acquisition. This induced root growth could be predicted to enhance the amount of rhizodeposition in the soil and decrease N-mineralisation. Remarkably, the reverse has been shown to happen: when soils have a higher N concentration, there is more rhizodeposition (fig. 6) (Bowen, 1969; Grayston et al., 1996; Liljeroth et al., 1994; Merckx et al., 1987).

Fast-growing plants grow usually on fertile soils. Valé et al. (2005) found that plants with a high shoot biomass had roots with high exudation rates and high microbial activity. The degradation of organic matter and the N-mineralisation is thought to be higher on fertile soils (Valé et al., 2005). However, Liljeroth, et al. (1994) found decreased degradation of organic matter on nutrient-rich soils. It appears that on fertile soils, there are enough nutrients, so that microbes do not need to mineralise organic matter to fulfil their needs. On nutrient-poor soils, the competition between plants and microbes is greater, thus for plants, it could be detrimental to exude a lot of substances. Therefore, plants adapted to grow on nutrient-poor soils are better off when they exude less from their roots.

Why do nutrient-rich soils result in a greater amount of root exudation? On nutrient-rich soils, there is sufficient N to fuel microbial growth.
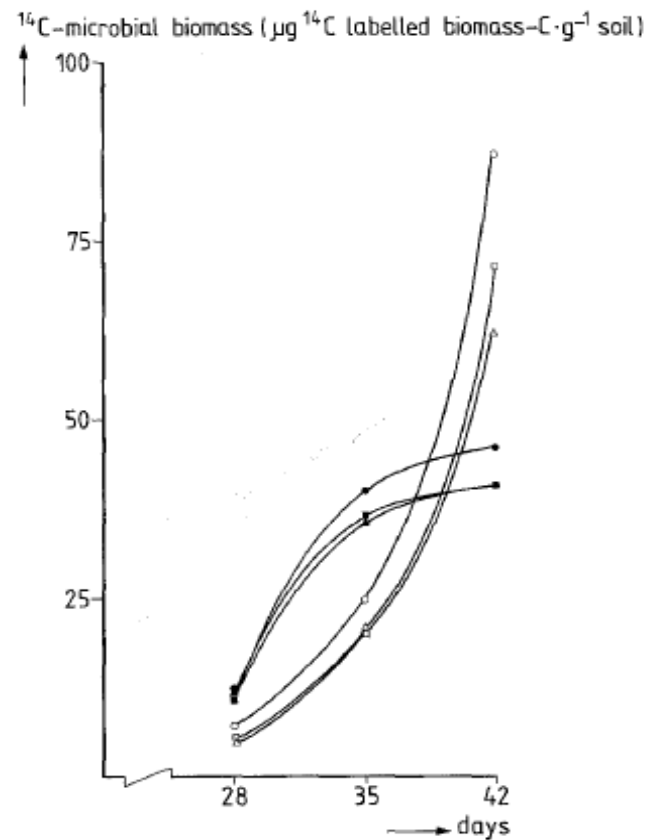

Fig. 6 Soil microbial biomass ^{14}C content during 42 days growth of maize in a sandy soil amended with mixed fertilizer at 0.5 g kg^{-1} moist soil (●■▲) and 1.0g kg^{-1}I moist soil (○□Δ). ^{14}CO$_2$ evolution from a fumigated soil sample (C_{F0-10}) was either not corrected (● ○) or corrected with $C_{uF10-20}$ (■□) or C_{F10-20} (▲Δ) and converted to biomass ^{14}C, using a k_c of 0.45.^{14}C activities were converted to weight units, using the specific activities of the roots (from Merckx et al., 1987).

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When plants exude C, they can accelerate soil nutrient cycling through microbial stimulation. However, increased mineralisation of N does not seem to account for the results in most studies (Bottner et al., 1988; Dormaar, 1990; Griffiths and Robinson, 1992; Ingham et al., 1985; Sallih and Bottner, 1988). The reason for this conundrum could be that most of the studies did not have microbial grazers in their experiments. Only the last question remains, do plants incorporate the N?

a) *Plant response*

The mineralised N can be taken up by plants but does this lead to an increase of growth, which covers the cost of producing root exudates. The shoot biomass of plants increased when grazers were present (Clarholm, 1985; Djigal et al., 2004; Ingham et al., 1985). An increase in the absolute N content was observed (Bardgett and Chan, 1999; Clarholm, 1985; Djigal et al., 2004; Ingham et al., 1985). In a classic study of Ingham et al. (1985) they fount that there was a positive effect on the total amount of N in the shoot, when bacterial-feeders where added to the system (fig. 7). There seems to be no difference between bacterial-feeders and fungal-feeders, this is because the mineralise N by the fungus was sufficient for the plant growth. That is why there is no difference between the plant, bacteria, fungus treatment and the plant, bacteria, fungus and fungal-feeding treatment (fig. 7). This means that the fungal-feeding nematodes excreted less NH_4^+-N that the bacterial-feeders. This indicates that there is an increase in the quantity of the shoots. The amount of N taken up by plants varied greatly, ranging from 75% (Clarholm, 1985) to 26% (Djigal et al., 2004) and can depend on various factors, such as plant species, grazers present, time, and soil. Time is a very important factor. Ingham, et al. (1985) found that differences in shoot biomass and shoot N content decreased over time. The life-history stage of the plant could also be an important variable.

It is difficult to assess whether the microbial loop really covers the cost of root exudation and results in a net gain for the plant. To date, there has not been a study where labelled ^{14}C was tracked through the whole system (from plant to microorganisms to grazers). Whether the uptake of N is increased by root exudation, is difficult to say. Theoretical models differ in assessments of this issue. Griffith and Robinson (1992) concluded that even if their model parameters were optimized to favour the uptake of N induced by rhizodeposition, it would account for less than 6% of the plant's N requirements. On the other hand, Raynaud, et al. (2006) concluded that all their simulations suggested that exudation can lead to an overall increase in mineral-N uptake by roots. These models are
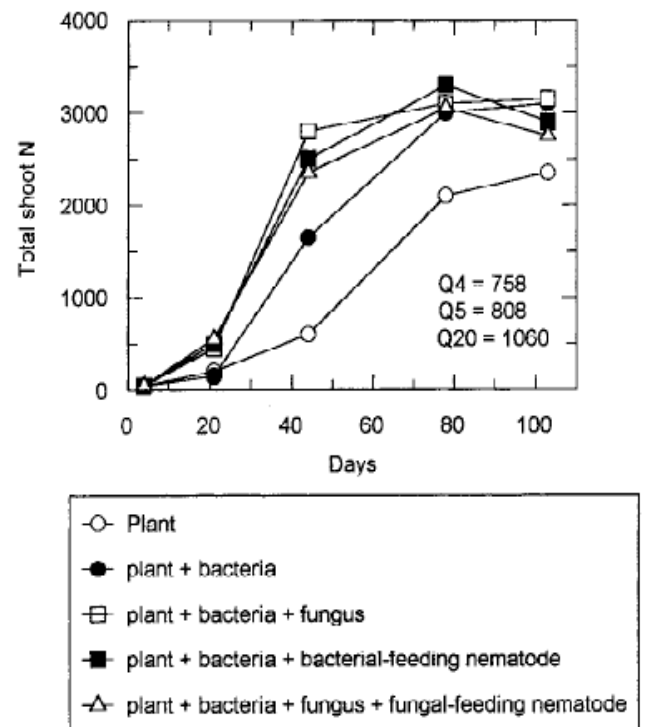
Fig. 7 Shoot nitrogen content (*µg*) of *Bouteloua gracilis* grown in microcosms with different biological assemblages. The Q_4 value represents Tukey's HSD, the difference needed for significance *P* < 0.05, between dates for the same treatment; Q_5 represents that for different treatments on the same date; and Q_{20} can be used to compare treatments on different dates (From Ingham et al., 1985.)

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useful for tracking the whole microbial loop but are greatly simplified. However, it seems plausible that rhizodeposition could lead to greater N acquisition. Root exudates promote growth of the microbial biomass, which is eaten by the microbial grazers and thus results in an increase in the mineralisation of N. This seems mainly to happen on nutrient-rich soils. The microbes in these soils are not limited by N and with stimulation of growth by root exudates, there is an acceleration in turnover. The root exudation is also greatest in rich soils. However it is important to note, that the effect of the microbial loop also is influenced by the soil microbial community, different grazer species, plant species and soil conditions. More research is needed to understand how great the influence of root exudation is on the microbial loop, and whether the microbial loop covers the cost of root exudation.

3. Root herbivores

Root herbivores include insect larvae and nematodes that eat from the roots of plants. Feeding mechanisms can differ between types of root herbivores. Insect larvae ingest entire root cells, whereas juvenile female nematodes enter the root and feed by sucking cells empty. These nematodes induce changes in the root cells to provide them with extra sugar. The nematodes become sessile and swell, after which they lay eggs. There are two types of root-feeding nematodes: cyst-nematodes (e.g. *Globodera* or *Heterodera* sp.) and root-knot nematodes (e.g. *Meloidogyne* sp.). The difference between the two groups is that whether they suck from one cell that expands or whether they merge several cells together, and how they produce their eggs (de Deyn, KNAW-NIOO, pers. comm., 2009). Root-knot nematodes cause a deformation in the roots, creating root-knots. They also increase root hair length, but decrease root hair density (Haase et al., 2007). These root herbivores are well known in agriculture as pests because they decrease plant growth (Bardgett et al., 1999a). The theory is that low amounts of root herbivory could induce leakage of small solutes whenever they puncture or eat root cells. This increase of root exudation could have an effect on the microbial community, further leading to an initiation of the microbial loop, and increased mineralisation of N. This gives some questions, firstly is there an increase of root exudation, and is this change created by mechanical leaking? Secondly does a change in root exudation lead to a change in microbial activity and microbial species composition? And at last is there a change in N-cycling and is this visible in the host plant or a neighbouring plant species?

Table 1. The effects of root-herbivores on root exudations, soil microbial community and plant growth.

Reference	Host species	Root herbivore	Effect on root exudation	Effect on soil microbial community	Effect on plant growth
Bardgett et al. (1999b)	*Trifolium repens* and *Lolium perenne*	*Heterodera trifolii* (Cyst nematode)	Excess of N from *T. repens*	15% increase of the microbial biomass	322% increase of N uptake of *L. perenne*
Denton et al. (1999)	*T. repens*	*H. trifolii* (Cyst nematode)	-	Total PLFA (active microbial biomass) was 2 to 3x higher	Decrease at high abundance, no effect at low abundance
Dromph et al. (2006)	*T. repens* and *L. perenne*	*H. trifolii* (Cyst nematode)	Excess of N from *T. repens*	-	Increase of N uptake by *L. perenne*
Grayston et al. (2001)	*Agrostis capillaris* and *T. Repens*	*Tipula paludosa* (insect herbivore)	-	Number of *Pseudonomas* were increased, yeast and fungi not effected	Shoot biomass of *T. repens* decreased, *A. cappillaris* not affected
Haase et al. (2007)	*Hordeum vulgare*	*Meloidogyne incognita* (root-knot nematode)	Increase of root exudation but only in unifected root zones	Bacteria decreased, whereas fungi increased	An increased shoot biomass and a increased N and P content.
Poll et al. (2007)	*H. vulgare*	*M. incognita* (root-knot nematode)	A decrease in root exudation	No change in microbial biomass, less ^{14}C in microbial biomass	No effect on shoot biomass, increased root biomass
Tu et al. (2003)	*Gossypium hirsutum* (Cotton)	*Rotylenchulus reniformis*	Increase in root exudation, from 8 to 69%	Microbial biomass decreased but microbial activity increased	Decreased plant growth
Wurst et al. (2006)	*Brassica oleracea*	*M. incognita* (root-knot nematode)	-	11% increase of the microbial biomass	Increased shoot biomass and N content
Yeates et al. (1999)	*T. repens*	*H. trifolii* (cyst)	No effect	1,57% of ^{14}C in microbial biomass	No effect
		Meloidogyne hapla (root-knot)	No effect	1,72% of ^{14}C in microbial biomass	No effect
		Meloidogyne trifoliaphila (root knot)	No effect	1,47% of ^{14}C in microbial biomass	No effect
		Xiphine diverscaudatum	No effect	2,45% of ^{14}C in microbial biomass	No effect
		Pratylenchus sp.	No effect	2,5% of ^{14}C in microbial biomass	No effect

a) Rhizodeposition

If rhizodeposition is enhanced by root herbivory, how great will the increase be? What kinds of substances are present in the exudates and does time influence the amount of leakage. Some studies found an increase in root exudation when root herbivores were present (Table 1) (Dromph et al., 2006; Haase et al., 2007; Tu et al., 2003; Yeates et al., 1999). However, decreases in root exudation have also been observed (Poll et al., 2007). This discrepancy is probably due to the specific host plant and root herbivore interaction, and the time scale. The amount of rhizodeposition induced by root herbivory has been noted to be 4.8 to 7.7% (Yeates et al., 1999), although Tu, et al. (2003) found an increase from 8% to 69%. The amount of root exudates leaked into the soil depends on the host plant, the root herbivore, abundance of the root herbivores and, for nematodes, on the amount of juveniles that are born. Root exudates mostly consisted of sugars, malate/fumarate and amino acids (Fig. 8) (Haase et al., 2007; Wurst et al., 2009). When nematodes were present, rhizodeposition decreased over time, and when plant age increased, the leakage of sugar and fumarate went down, but malate went up (Fig. 8) (Haase et al., 2007; Poll et al., 2007). The drop in root exudates could be caused by the change in the life-history stage of the nematodes. When nematodes have migrated into the roots and become sessile, that could slow down or stop the leakage. When root-knot nematodes form root-knots, this could also change their effect on exudation pattern (Haase et al., 2007; Poll et al., 2007). Haase, et al.(2007) found an increase in rhizodeposition at the uninfected root zones (Fig. 8). This means that root herbivory might not create mechanical leaking, but that plants increase exudation as an indirect response to herbivory. The experiments above show that there is an increase of
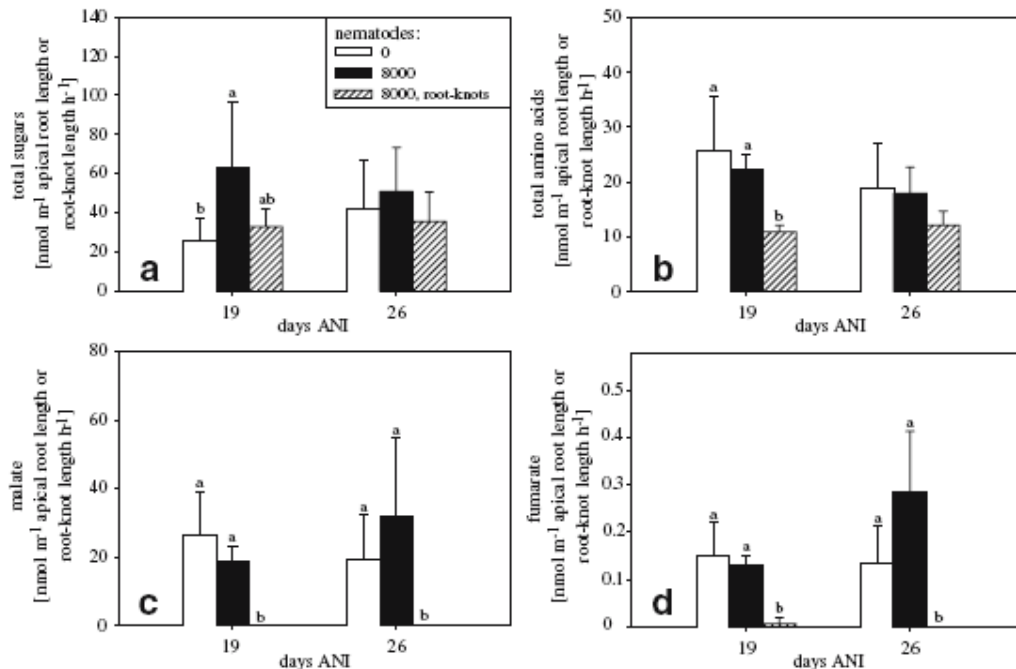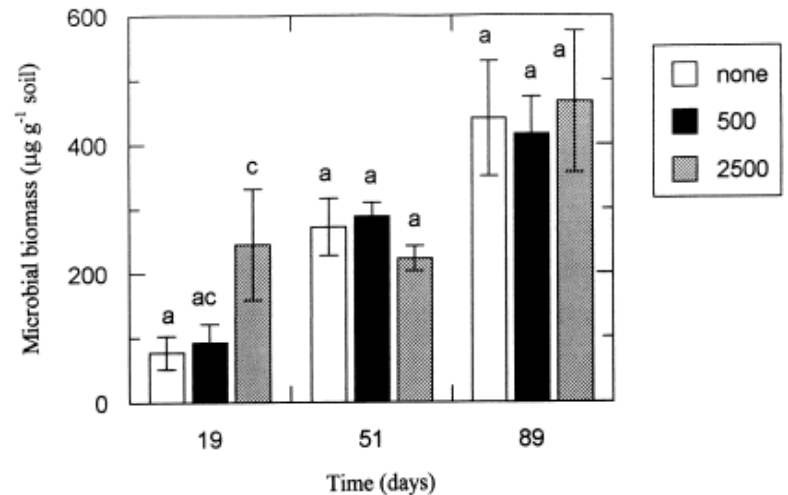

Fig. 8 Rhizodeposition of total sugars (a), total amino acids (b) and the carboxylates malate (c) and fumarate (d) from root-knots and uninfected apical root zones (0−1.5 cm behind the root tip, lateral roots) of *Hordeum vulgare* inoculated with 0 and 8,000 nematodes at different postinoculation time (days ANI = days after nematode inoculation). Presented are means±s.d.. Means within one sampling time with the same or no letters are not significantly different according to Tukey's HSD (P<0.05) (from Haase et al., 2007).

rhizodeposition, although it is not clear whether this is caused by leakage from mechanical damage. This effect may be diminished by time and depends on host species and root herbivore interactions.

b) Effect on microbes

Studies of the effects of root herbivory on the soil microbial community shows mixed results (Table 1). Some studies found an increase in the microbial biomass (fig. 9) (Bardgett et al., 1999a; Denton et al., 1999; Grayston et al., 2001; Wurst et al., 2006). Tu et al.(2003), however, did not find an increase in microbial biomass but they did find an increase in the amount of microbial activity. Yeates et al.(1999) and Grayston et al.(2001) found an increase in the microbial C content when root herbivores were present (respectively nematodes and insect herbivore). The increase of the microbial biomass or activity had a great effect of time, Denton et al. (1999) found that only in the
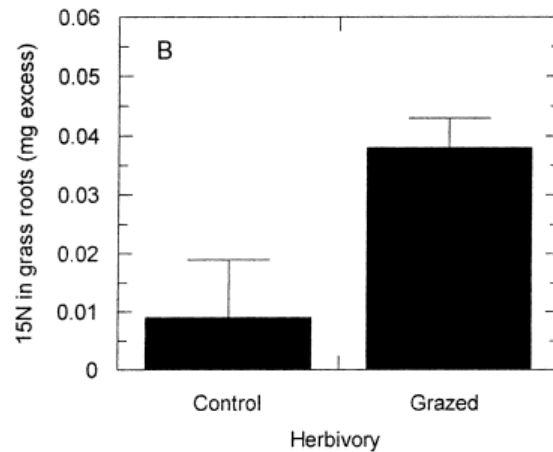

Fig. 9 Effect of different amounts of root infestation by clover cyst nematodes (none, 500 and 2500 nematodes added), over time (d), on microbial biomass. Data are means ±SE (n=18). Values with the same letter are not significantly different at the P<0.05 level (from Denton et al., 1999).

beginning of his experiment an increase in microbial biomass (fig 9.) This is in contrast with the findings of Poll et al. (2007), who found no changes in the microbial biomass and found that microbes sequestered less C when nematodes were present. Haase et al. (2007) found a decrease in the abundance of bacteria, while the abundance of fungi increased. This contrast could be caused by multiple reasons. The three reason that they both see a decrease in bacterial abundance could be: 1) there was less substrate available; 2) the increase of the antimicrobial phenolic compounds, which are increased in plant tissue as a post-infection plant defence mechanism; and 3) the effect of the gelatinous matrices, observed on the root knots surface, which protect nematode eggs against microbial attack (Haase et al., 2007). The root-knot nematodes enhance the softening and degradation of cellulose, thus changing the quality of root detritus, which could explain the increase of fungal biomass (Haase et al., 2007). The difference in effect on the microbial biomass, could be explained by the type of root herbivores (Table 1). Cyst-nematode grazing usually results in an increase in the microbial biomass, whereas grazing by root-knot nematodes usually do not have an effect. Only Wurst, et al. (2006) found that activity by the root-knot nematode, *Meloidogyne incognita*, resulted in an increase of microbial biomass, which could have resulted in an increase of fungal biomass. These findings clarify that there is much research needed on the effect of root herbivores on the soil microbial community, especially the effect of different root herbivores.

c) *N-cycling*

The increase in microbial biomass could have effect on the N-mineralisation. However there has been no studies of the effects of root herbivory on N-mineralisation. An increase in N-mineralisation could potentially cause an increase in amount of N in the plant tissue. Root herbivores are known to be deleterious for plant growth, and the experiments mentioned above are conducted with low densities of root herbivores. These densities are well below the damage threshold for plants. In spite of this, some studies still found a decreased shoot biomass with low densities of root herbivores (Grayston et al., 2001; Tu et al., 2003). Probably this was due to the abundance of the root herbivores still being high enough to cause deleterious effects for the host plant. Some studies found no effect of root herbivory (Denton et al., 1999; Poll et al., 2007; Yeates et al., 1999). However some studies observed an increase in the shoot biomass and the absolute N content (Haase et al., 2007; Wurst et al., 2006). It could be that there was an increase in the N-mineralisation. However Haase, et al. (2007) found an decrease in bacterial biomass and an increase in fungal biomass. Fungi have a higher C:N ratio than bacteria, which could result in an excess of N, which could be incorporated by plants.

Fig. 10 Effect of herbivory on nitrogen (^{15}N) transfer from the host plant to companion species (n=4, $P < 0.05$). Vertical bars indicate standard errors (from Bardgett, et al., 1999b).

Increased root exudation could also have an effect on neighbouring plant species, which could take advantage of the root exudates. Bargett, et al. (1999b) and Dromph, et al. (2006) did an experiment where *Trifolium repens* and *Lolium perenne* were grown in the same soil. They introduced a root herbivore, *Heterodera Trifolii*, to the soil. It is a specialist herbivore of *T. repens*. There was resultant flow of N from *T. repens* into the soil, which was then recycled and taken up by the neighbouring plant, *L. perenne*. There was a 322% increase in the uptake of *T. repens*-derived N (fig. 10). This process probably altered the competitive balance between the species, most likely to the detriment of the nematode infested *T. repens*, and thereby influenced the plant community structure.

These studies show that root herbivores can have a strong influence on the soil microbial community, and possibly an effect on plant community structure. It is not clear whether leakage of root exudate enhances the microbial loop. There is certainly more research needed on the effect of low-density root herbivory, on the effect of the soil microbial community, the N-mineralisation and the effect of root herbivory on N uptake of neighbouring plant species.

4. Synthesis

Root exudation leads to an increase in the microbial biomass, with the microbes immobilising N to fuel their growth. Microbes are eaten by grazers, which leads to higher microbial activity, by transport of bacteria to "new" micro sites and increased mineralisation of N by grazers. This gives us a very simplified model, which shows how the microbial loop may work. The grazers release the immobilised N sequestered in the microbial biomass and plants can then incorporate the inorganic N into their tissue. The grazers appear critical in controlling the rate of microbial turnover and nutrient cycling. This only works because the microbial community has an lower C:N ratio than the microbial grazers. Therefore has the microbial grazers an excess of N, that is the reason why the mineralise the N (fig. 11). The root exudates only accelerate the process of microbial turnover. This happens mainly in nutrient-rich soils, where there is sufficient N to fuel microbial growth. These effects are strongly contingent on the complex interactions between biotic and abiotic factors that take place in the soil.

Root herbivores induce root exudation, but whether this is due to mechanical damage of roots or due to an indirect effect, is difficult to say. The root herbivores have an influence on the microbial community, which could potentially lead to improvements in nutrient availability, and ultimately increase plant productivity. However, there is little evidence supporting this hypothesis because it seems that there are quite a lot different interactions of root herbivores with the soil microbial community. And it is not really clear what the effects on root exudation and the soil microbial community is (fig. 11). Root herbivores could also have influence on the distribution of N in the soil, which could lead to changes in plant community.
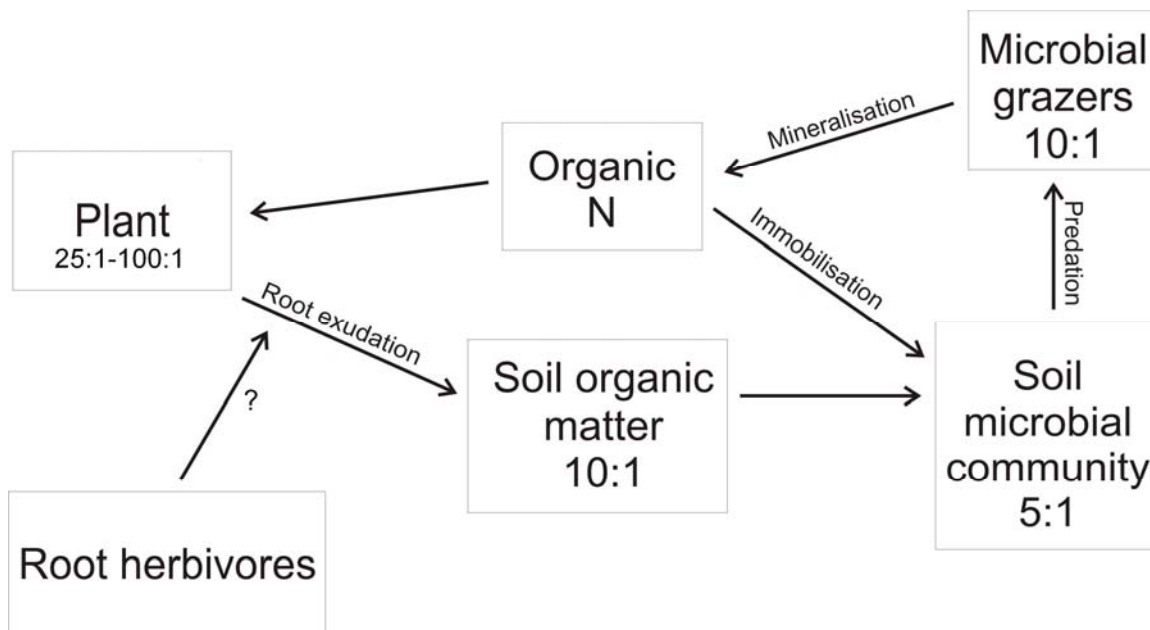
Fig. 11 This diagram shows the a part of the interactions in the rhizosphere. The microbes use the root exudates to grow, and immobilise N. The microbial grazers have a higher C:N ratio than the microbes and therefore an excess of N. The plants can accumulate more N when they exudate C, because of the fast turnover of the soil microbial community. Whether root herbivores give an increase in root exudation is not clear.

For future research, there should be more attention paid to the change in microbial functional groups caused by root exudates, and do the benefits of the microbial loop cover the cost of producing the root exudates? With respect to root herbivores, there should be more research whether the increase in root exudation leakage is caused by mechanical damage or more indirect reactions of the plant to herbivory, and what effect do they have on the soil microbial community. In addition, it should be explored whether root herbivory has an effect on the N-mineralisation. This way research could lead to a better understanding of the effect of root exudation on the interactions that happen in the rhizosphere. This review shows that the microbial loop certainly is important for the N-mineralisation, and that more research is needed about the root feeding organisms.

References

Bais,H.P., Weir,T.L., Perry,L.G., Gilroy,S., Vivanco,J.M., 2006. The Role of Root Exudates in Rhizosphere Interactions with Plants and Other Organisms. Annual Reviews of Plant Biology 57, 233-266.

Bardgett,R.D., Chan,K.F., 1999. Experimental evidence that soil fauna enhance nutrient mineralization and plant nutrient uptake in montane grassland ecosystems. Soil Biology & Biochemistry 31, 1007-1014.

Bardgett,R.D., Cook,R., Yeates,G.W., Denton,C.S., 1999a. The influence of nematodes on below-ground processes in grassland ecosystems. Plant and Soil 212, 23-33.

Bardgett,R.D., Denton,C.S., Cook,R., 1999b. Below-ground herbivory promotes soil nutrient transfer and root growth in grassland. Ecology Letters 2, 357-360.

Bargett,R.D., 2005. The Biology of Soil. Oxford University Press, Oxford.

Baudoin,E., Benizri,E., Guckert,A., 2003. Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biology & Biochemistry 35, 1183-1192.

Bottner,P., Sallih,Z., Billes,G., 1988. Root activity and carbon metabolism in soils. Biological and Fertility of Soils 7, 71-78.

Bowen,G.D., 1969. Nutrient status effects on loss of amides and amino acids from pine roots. Plant and Soil 20, 139-142.

Butler,J.L., Williams,M.A., Bottomley,P.J., Myrold,D.D., 2003. Microbial Community Associated with Rhizosphere Carbon Flow. Applied and Environmental Microbiology 69, 6793-6800.

Clarholm,M., 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biology & Biochemistry 17, 181-187.

Denton,C.S., Bardgett,R.D., Cook,R., Hobbs,P.J., 1999. Low amounts of root herbivory positively influence the rhizosphere microbial community in a temperate grassland soil. Soil Biology & Biochemistry 31, 155-165.

Djigal,D., Brauman,A., Diop,T.A., Chotte,J.L., Villenave,C., 2004. Influence of bacterial-feeding nematodes (Cephalobidae) on soil microbial communities during maize growth. Soil Biology & Biochemistry 36, 323-331.

Dormaar,J.F., 1990. Effect of active roots on the decomposition of soil organic materials. Biological and Fertility of Soils 10, 121-126.

Dromph,K.M., Cook,R., Ostle,N.J., Bardgett,R.D., 2006. Root parasite induced nitrogen transfer between plants is density dependent. Soil Biology & Biochemistry 38, 2495-2498.

Grayston,S.J., Dawson,L.A., Treonis,A.M., Murray,P.J., Ross,J., Reid,E.J., MacDougall,R., 2001. Impact of root herbivory by insect larvae on soil microbial communities. Europian Journal of Soil Biology 37, 277-280.

Grayston,S.J., Vaughan,d., Jones,d., 1996. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudations and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5, 29-56.

Griffiths,B.S., 1994. Microbial-feeding nematodes and protozoa in soil: Their effects on microbial activity and nitrogen mineralization in decomposition hotspots and the rhizosphere. Plant and Soil 164, 25-33.

Griffiths,B.S., Robinson,D., 1992. Root-induced nitrogen mineralisation: A nitrogen balance model. Plant and Soil 139, 253-263.

Haase,S., Reuss,L., Neumann,G., Marhan,S., Kandeler,E., 2007. Low-level herbivory by root-knot nematodes (*Meloidogyne incognita*) modifies root hair morphology and rhizodeposition in host plants (*Hordeum vulgare*). Plant and Soil 301, 151-164.

Hamilton III,E.W., Frank,D.A., 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82, 2397-2402.

Ingham,R.E., Trofymow,J.A., Ingham,E.R., Coleman,D.C., 1985. Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. Ecological Monographs 55, 119-140.

Jones,D.L., Nguyen,C., Finlay,R.D., 2008. Carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant and Soil 321, 5-33.

Kuikman,P.J., Jansen,A.G., van Veen,J.A., Zehnder,A.J.B., 1990. Protozoan predation and the turnover of soil organic carbon and nitrogen in the presence of plants. Biological and Fertility of Soils 10, 22-28.

Liljeroth,E., Kuikman,P., van Veen,J.A., 1994. Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organice matter at different soil nitrogen levels. Plant and Soil 161, 233-240.

Mawdsley,J.L., Bardgett,R.D., 1997. Continuous defoliation of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the composition and activity of the microbial population of an upland grassland soil. Biological and Fertility of Soils 24, 52-58.

Merckx,R., Dijkstra,A., den Hartog,A., van Veen,J.A., 1987. Production of root-derived material and associated microbial growth in soil at different nutrient levels. Biology and Fertility of Soils 5, 126-132.

Paterson,E., 2003. Importance of rhizodeposition in the coupling of plant and microbial productivity. European Journal of Soil Science 54, 741-750.

Poll,J., Marhan,S., Haase,S., Hallmann,J., Kandeler,E., Reuss,L., 2007. Low amounts of herbivory by root-knot nematodes affect microbial community dynamics and carbon allocation in the rhizosphere. FEMS Microbial ecology 62, 268-279.

Raynaud,X., Lata,J.C., Leadley,P.W., 2006. Soil microbial loop and nutrient uptake by plants : a test using coupled C:N model of plant-microbial interactions. Plant and Soil 287, 95-116.

Sallih,Z., Bottner,P., 1988. Effect of wheat (*Triticum aestivum*) roots on mineralization rates of soil organic matter. Biological and Fertility of Soils 7, 67-70.

Singh,B.K., Millard,P., Whiteley,A.S., Murrel,J.C., 2004. Unravelling rhizosphere - microbial interactions: opportunities and limitations. TRENDS in Microbiology 12, 386-393.

Tu,C., Koenning,S.R., Hu,S., 2003. Root-parasitic nematodes enhance soil microbial activities and nitrogen mineralization. Microbial Ecology 46, 134-144.

Valé,M., Nguyen,C., Dambrine,E., Dupouey,J.L., 2005. Microbial activity in the rhizosphere soil of six herbaceous species cultivated in a greenhouse is correlated with shoot biomass and root C concentrations. Soil Biology & Biochemistry 37, 2329-2333.

Woods,L.E., Cole,C.V., Elliott,E.T., Anderson,R.V., Coleman,D., 1982. Nitrogen transformation in soil as affected by bacterial-microfaunal interactions. Soil Biology & Biochemistry 14, 93-98.

Wurst,S., Langel,R., Rodger,S., Scheu,S., 2006. Effects of belowground biota on primary and secondary metabolites in *Brassica oleracea*. Chemoecology 16, 69-73.

Wurst,S., van Beersum,S., Wagenaar,R., Bakx-Schotman,T., Drigo,B., Janzik,I., Lanoue,A., van der Putten,W.H., 2009. Plant defence against nematodes is not mediated by changes i nthe soil microbial community. Functional Ecology 23, 488-495.

Yeates,G.W., Saggar,S., Hedley,C.B., Mercer,C.F., 1999. Increase in ^{14}C-carbon translocation to the soil microbial biomass when five species of plant-parasitic nematodes infect roots of white clover. Nematology 1, 295-300.