Soil biodiversity and nitrogen cycling under agricultural (de-)intensification

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Voor Jouke

Abstract

Soil biodiversity is often considered an essential element in sustaining ecosystem services in agricultural systems. Understanding functional biodiversity with respect to its impact on nitrogen mineralization and retention might, therefore, contribute to more sustainable production and prevention of environmental complications. In this thesis I determined how agricultural (de-)intensification affects soil biota abundances and diversity and I evaluated to what extent the soil biota abundances and community composition affect nitrogen cycling.

The effects of agricultural (de-)intensification (including conversion of extensively managed grassland to arable land and *vice versa*, varying crop diversity and fertilization level) on soil biodiversity across taxonomic groups were studied in a long-term field experiment. Agricultural intensification was found to have strong negative effects on larger-sized soil biota and species-poor groups of soil animals such as earthworms. Furthermore, larger-sized soil biota were strongly negatively affected by short-term consequences of agricultural intensification such as soil disturbance, whereas smaller-sized soil biota were mostly negatively affected by long-term consequences, such as reduced organic matter content. Agricultural intensification affected soil biota abundances and taxonomic diversity, but not necessarily functional diversity.

The relationship between soil biodiversity and nitrogen cycling was studied in two laboratory experiments. The difference in life-strategies between nematode species of the same trophic groups was found to be of importance for their communal effect on N mineralization. The effects of earthworms on nitrogen mineralization depended on the ecological traits of the species present, and hence, determined the outcome of their interactions. The results of the laboratory experiments indicate that the effect of soil biodiversity on ecosystem functioning is related to the diversity in ecological traits of the species present.

The relationship between soil biodiversity and nitrogen cycling under agricultural (de-) intensification was further studied in the long-term field experiment mentioned before. Agricultural de-intensification generally resulted in increased N mineralization and decreased N leaching. Increased N mineralization in de-intensified systems was related to high abundances of earthworms and soil biota in the bacterial-based food chains. Furthermore, net N mineralization increased with a higher diversity of nematode life-strategy groups. Mineralized N had a lower risk of leaching during the cropping season than mineral N applied as synthetic fertilizer, but year-round NO_3^- leaching was not dependent on the source of mineral N.

I conclude that in agricultural systems with reduced soil tillage and reduced external inputs, the abundances of certain soil biota trophic groups and diversity of life-strategy groups are promoted, which, under certain conditions, is associated with a higher nutrient use efficiency of the system.

Contents

Chapter 1

General Introduction

Introduction

The richness of the species on earth has astounded both scientists and lay people as long as man exists. Although hidden to the eye, a large part of this diversity is found belowground, which led Usher et al. (1979) to call the soil the “poor man's tropical rainforest”. While soil biodiversity is worthwhile studying for its own sake, ecologists have been intrigued by the question whether biodiversity is somehow related to ecosytem functioning. This question has become ever more urgent, as biodiversity has drastically declined in recent decades, due to human impacts such as deforestation, pollution and agricultural intensification. Soil biota are known to be of great importance for life-support functions such as soil formation, decomposition of organic matter, disease suppresiveness and nutrient availability for plant growth (Brussaard et al., 1997; De Ruiter et al., 1994; Faber et al., 2006). In conventional agricultural systems these services are partly bypassed, through the use of synthetic fertilizers and pesticides and mechanical soil tillage. Although these practices have enhanced crop production, negative consequences are the loss of nutrients and pesticides from the agroecosystem, leading to pollution of the environment. Furthermore, soil biota abundance and diversity may be reduced under intensive agricultural management, potentially leading to impaired ecosystem functioning in the longer term (Swift et al., 2004). Growing concern about the long-term negative consequences of conventional agricultural practices gave rise to political and social incentives towards long-term sustainable agriculture. Scientific research has been directed towards finding solutions that minimize external inputs and losses from the system, while maximizing the role of soil biota for agroecosystem functioning. In this context, the scientific “Stimulation Programme Biodiversity” of NWO, the Netherlands Organization for Scientific Research was started. This program has funded studies that investigate the drivers for above-ground and below-ground biodiversity and the relationships between biodiversity and ecosystem functioning. As part of this program, this thesis describes my research on the relationships between biodiversity and nitrogen cycling in agricultural soil, whereas a related project was focused on the relationship between microbiological diversity and disease suppressiveness (Garbeva et al., 2003; Garbeva et al., 2004; Garbeva et al., 2006).

Nitrogen cycling is an important process in agroecosystems, as nitrogen supply is needed for optimal crop production, whereas loss of nitrogen leads to eutrophication of the environment. Whereas conventional agricultural systems rely on artificial fertilizers, addition of organic matter in less intensive agricultural practices stimulates nitrogen mineralization by the soil biota and promotes soil biodiversity (Bloem et al., 1997). Many questions remain, however, on the role of different soil biota groups in nitrogen mineralization and the consequences for nitrogen leaching in different agroecosystems. In this thesis, I investigate the effects of agricultural

(de-)intensification on soil biota abundances and diversity, and evaluate the role of the soil biota in nitrogen cycling in (de-)intensified agroecosystems. A better understanding of the role of soil biodiversity is needed to facilitate more sustainable agriculture by enhancing ecosystem services and reducing the use of mineral fertilizers and losses to the environment.

Biodiversity and ecosystem functioning

The role of biodiversity in ecosystem functioning has been the subject of ongoing debate in ecological studies. Several hypotheses have been formulated for the relationship between biodiversity and ecosystem functioning (Brussaard et al., 2004). The rivet hypothesis poses that each species makes a significant contribution to ecosystem functioning , thus leading to enhanced ecosystem functioning with increased species diversity. In contrast, the redundancy hypothesis poses that species are redundant with respect to ecosystem functioning: as long as all functional groups are represented, ecosystem functioning is not affected by species diversity. In another hypothesis the importance of "keystone" species for ecosystem functioning is highlighted. Lastly, it can be argued that, since diversity is an abstract, aggregated entity of species (Bengtsson et al., 2000), there is no mechanistic relationship between species diversity and ecosystem functioning. Both are functions of the presence and activities of species and functional groups, and their interactions. In this hypothesis the diversity of functional groups, and functional composition, i.e. the idiosyncratic nature of species within a functional group, are important determinants of the functioning of ecosystems (Brussaard et al., 1997).

Species diversity within functional groups, however, may confer stability and resilience in the face of perturbations and, in that sense may be vital for the persistence of ecosystems and the services they provide to mankind (Folke et al., 1996; Frost et al., 1995; Giller et al., 1997). Therefore, stress and disturbance are the most likely to cause loss of functioning, if functional groups are comprised of relatively few sensitive species. For soil biota this holds to the best of our knowledge for (among others): shredders of organic matter, in particular the macrofaunal groups, with effects on decomposition; nitrogen-fixing, nitrifying and denitrifying bacteria, with effects on element cycling and greenhouse gases; and bioturbators among the macrofauna, such as earthworms, with effects on production, purification and restoration potential of soil (Brussaard et al., 1997). Since agricultural practices are known to affect the soil biota community (Swift et al., 2004), it is important to understand the measure of these effects on taxonomic and functional diversity, and the possible consequences for agroecosystem functioning.

Soil biota communities in agricultural soils under (de-)intensification

Taxonomic diversity

Agricultural ecosystems may seem simple aboveground, but they harbor a large and diverse community belowground. Abundances and number of species of soil biota generally decline with increasing organism size. Agricultural soils may contain from 8000 to 10000 different bacterial genomes (Torsvik et al., 2002), around 40 nematode genera, 20 enchytraeid genera, and up to 10 earthworm species (Cole et al., 2006). Agricultural intensification has often been found to lead to reduced abundances and diversity of the major taxa of the soil biota (Behan-Pelletier, 2003; Bouwman and Zwart, 1994; Curry et al., 2002; Grayston et al., 2004; Mäder et al., 2002; Swift et al., 2004; Van Diepeningen et al., 2006), whereas de-intensification does not always result in an (immediate) increase in biodiversity (Kleijn et al., 2001; Swift et al., 1996). In contrast, positive or neutral effects of integrated or intensive management compared to organic management have also been found (Filser et al., 2002; Freckman and Ettema, 1993; Hole et al., 2005; Porazinska et al., 1999; Wardle et al., 1999), while positive effects of arable systems compared to grassland systems have been reported, too (Griffiths and Ritz, 1988; Lagerlöf et al., 2002).

Some taxonomic groups or species may be affected more than others, leading to a different community structure and possibly altered ecosystem functioning under agricultural intensification. Effects of agricultural intensification practices such as tillage are more pronounced on macrofauna than micro-/mesofauna (Wardle, 1995). Nematode species composition was found to be different in arable systems compared to natural or grassland systems (Liang et al., 2005; Sohlenius and Sandor, 1987). The enchytraeid community can be affected by liming, reducing a key stone species (*Cognettia sphagnetorum*) and altering the species composition (Black et al., 2003; Cole et al., 2006). Similarly, agricultural intensification can change species composition of predatory mites (Jagers op Akkerhuis et al., 1988; Siepel, 1996).

Functional groups and diversity

Soil biota differ in the way they contribute to ecosystem functioning. Thus, soil biota may be classified in functional groups, which are defined as groups of species that contribute to ecosystem functioning in a similar way (Brussaard et al., 1997; Susilo et al., 2004), such as trophic groups (De Ruiter et al., 1994) and life-strategy groups. Trophic groups are formed based on the food preference and way of feeding of species, and may therefore affect ecosystem functions such as N mineralization that result from soil biota feeding. Detritus is directly consumed by bacteria and fungi, and by **detritus-feeders** such as enchytraeids and earthworms. **Bacterivores** are mainly found within the major soil biota groups of protozoans (amoebae,

flagellates) and nematodes, whereas collembolans and enchytraeids are mainly **fungivores** (De Ruiter et al., 1993). **Predators** feed on a range of soil fauna and are found among protozoans (preying on other protozoans), nematodes (preying on protozoans and other nematodes), collembolans (preying on nematodes) and mites (preying on nematodes, collembolans and other mites). **Omnivores** feed on different food sources ranging from microflora to other organisms of their own kind. Omnivores are found among nematodes and mites. **Plant parasites**, feeding on roots, are mainly found among nematodes and insects (Brussaard et al., 1997).

All trophic groups are generally present in agricultural systems, but their relative abundances may vary as a result of intensity of agricultural use. Detritus-feeding earthworms are often reduced as a result of tillage and mineral fertilizer (Lagerlöf and Andren, 1988; Wardle, 1995), whereas effects on enchytraeids may be less severe (Van Vliet et al., 1997). Bacterial and fungal feeding nematodes are usually most abundant in agricultural systems, whereas grassland systems are often found to have higher nematode plant parasite dominance (Háněl, 2003; Sohlenius and Sandor, 1987). Furthermore, omnivory is widespread and may be advantageous in arable systems, because it enables species to rapidly recover from adverse management practices (Siepel, 1996; Vreeken-Buijs et al., 1994). Predators may be stimulated by de-intensified management (Bloemers et al., 1997; Doles et al., 2001; Ferris et al., 2004; Mulder et al., 2005), but not at all times (Freckman and Ettema, 1993; Vreeken-Buijs et al., 1994; Zwart et al., 1994). Agricultural intensification may also alter trophic diversity, even to the extend that trophic diversity has been found to be higher in a conventional than in a low input system (Freckman and Ettema, 1993).

Soil biota with different life-strategies may affect ecosystem function differently. Opportunistic (r-selected) species can quickly increase with a high food availability and may therefore stimulate N mineralization at the start of the decomposition process, whereas “persisters” (K-selected species) may be able to mineralize recalcitrant material during the later stages of decomposition. Thus, a community containing species with different life-strategies (i.e. with a functional distance between species) may result in a more intensive use of the food source. Nematodes can be classified according to life-strategy on a colonizer-persister scale (cp1 – cp5). Based on this classification a maturity index can be calculated, indicating whether the community contains relatively more r- or K- selected species (Bongers, 1990). Likewise, mites can be classified in different life-strategy groups, reflecting the ability of species to cope with disturbance, colonize new substrates, and survive adverse conditions (Siepel, 1994). Agricultural intensification generally results in a reduction in the nematode maturity index (Ettema and Bongers, 1993; Freckman and Ettema, 1993; Háněl, 2003; Villenave et al., 2003), which can be explained by a sensitivity of high cp-level nematodes to disturbance and increased abundances of low cp-level nematodes following fertilization. Little research has been done on the effect of agricultural intensification on mite life-strategy groups (Siepel, 1996; Siepel and Van de Bund,

1988), but it may be expected that life-strategies resistant to disturbance and with high colonization capacity are favored in intensive agricultural systems.

Soil biota diversity and nitrogen cycling in agricultural systems

Soil biota play a crucial role in most N cycling processes, in particular N mineralization. Organisms at the base of the food web (bacteria, fungi, microbivores, earthworms) are known to have the highest impact on mineralization rates (Berg et al., 2001; De Ruiter et al., 1994; Laakso and Setälä, 1999; Whalen et al., 2000). Bacteria and fungi are directly responsible for N mineralization, by excreting enzymes that break down organic N. Depending on substrate quality, the microflora can be N limited (Bloem et al., 1997). Then microbial growth results in N immobilization. Microbivores have a C:N ratio similar to the microbial biomass and, hence, excrete N as ammonium (Bloem et al., 1997). Furthermore, microbivores may stimulate microbial turnover and activity, thereby stimulating N mineralization indirectly (Bamforth, 1985; Bloem et al., 1997; Ingham et al., 1985). N mineralization may be further enhanced by larger soil biota. In particular, earthworms are known to increase mineralization in agroecosystems (Blair et al., 1997; Brown et al., 1999). They contribute to N mineralization directly by excretion of N and indirectly by stimulating bacterial turnover and activity through gut- and cast-associated processes (Brown et al., 2004).

Increased soil biota abundances and activity can be related to increased N mineralization in agroecosystems. High microbial biomass in natural or grassed ecosystems as compared to cropped systems coincided with high N mineralization rates (Carpenter-Boggs et al., 2003; Silver et al., 2005). Similar results have been found in de-intensified cropping systems compared to intensive agricultural systems (Balota et al., 2004; Breland and Eltun, 1999; Mäder et al., 2002). Furthermore, increased microbial activity (Carpenter-Boggs et al., 2003) and increased protozoan and nematode abundances (Bloem et al., 1997; Ferris et al., 2004) in integrated cropping systems compared to conventional systems were related to increased N mineralization (De Ruiter et al., 1994). Earthworms were also found to increase nutrient availability in agroecosystems with reduced human influence and low nutrient status (Blair et al., 1997; Brown et al., 1999; Doube et al., 1997; Ruz-Jerez et al., 1992).

Functional diversity and N mineralization

Although the contribution of major soil biota groups to N mineralization is clear, many questions remain on the effects of functional and structural diversity on N mineralization (Swift et al., 2004). In experiments containing only few species, soil processes such as N mineralization were found to be augmented by the presence of soil biota communities containing representatives of

several taxonomic or trophic groups, as compared to the presence of a single group or species alone (Bardgett and Chan, 1999; Mikola and Setälä, 1998). Other studies have shown that the presence of a single keystone species is sufficient to evoke increased mineralization and plant nutrient uptake (Cragg and Bardgett, 2001; Laakso and Setälä, 1999). Studies with larger numbers of species suggest that soil biota are redundant when ecosystem functioning such as N mineralization is considered (Giller et al., 1997; Setälä et al., 2005). In laboratory cultures, N mineralization can be carried out by a single species, whereas in the field the same process will be carried out by several species of fungi, bacteria and animals (Giller et al., 1997; Swift et al., 2004). In a food web study on the role of different functional groups of soil organisms on net nitrogen mineralization, however, De Ruiter et al. (1993; 1994) found that model perturbations affecting specific functional groups often had quantitatively important effects on the simulated nitrogen mineralization. Few studies have explicitly investigated the relationship between soil biota diversity and N mineralization in agricultural systems (Swift et al., 2004). A study comparing organic and conventional farming systems indicated minimal differences in the ability of the microbial communities of the two soils to decompose organic residues (Gunapala et al., 1998). Another study, however, indicated that organic systems with higher microbial biomass and diversity decomposed plant material more completely and more efficiently (Fliessbach et al., 2000).

Nitrogen leaching

Next to N mineralization, nutrient retention is an important life support function in agroecosystems. NO_3^- leaching under precipitation surplus conditions occurs as a result of an imbalance between availability and plant uptake of nitrogen. Leached NO_3^- contaminates groundwater and may result in eutrophication of the environment. Furthermore, high levels of NO_3^- in drinking water may render it unsafe for children and elderly people. Since farmers tended to use excess NO_3^- fertilizer dressings, in order to avoid losses in crop production, NO_3^- leaching has increasingly become a problem.

Organic farming systems, where mineral fertilizers are totally banned, largely rely on N mineralization for N supply. In such systems, the use efficiency of mineralized N may be higher than that of N from mineral fertilizers since N mineralization may result in a more gradual release of nitrogen during the growing season, with less risk of NO_3^- leaching (Bloem et al., 1997; Spedding et al., 2004). Indeed, organic systems were found to have less NO_3^- leaching than conventional systems (Hansen et al., 2001; Hole et al., 2005). However, since N mineralization may continue during the fall and winter period, organic farming systems with high organic N content may have a higher risk of NO_3^- leaching during the fallow period. This may explain why negative effects of organic farm management on N leaching have also been described (Hansen et al., 2001; Hole et al., 2005).

Since soil biota play a key role in N mineralization, they may also affect N leaching. Direct effects of soil biota on N leaching have been found for certain earthworm ecological groups, which may increase N leaching through preferential flow under some circumstances (Dominguez et al., 2004; Sheehan et al., 2006). However, very little research has been directed towards understanding the role of soil biota in NO_3^- leaching.

Research aim

Soil biota play a key role in N cycling. Many questions remain, however, on the effects of soil biodiversity on N mineralization. Furthermore, more research is needed to determine the role of soil biota in agricultural soils with respect to N mineralization and N losses. The overall aim of this thesis is, therefore, to determine how agricultural (de-)intensification affects soil biota abundances and diversity, and to evaluate the role of soil biota abundances and diversity in nitrogen mineralization and retention in (de-)intensified agroecosystems. More specifically, the aims of the study were to:

- Identify the effect of agricultural (de-)intensification on soil biota abundances and diversity across different taxonomic levels;
- Investigate the relationship, if any, between soil biota abundances and diversity on the one hand and N mineralization on the other;
- Investigate the relationship, if any, between soil biota, N mineralization and N retention in agricultural soil under (de-)intensification.

Laboratory experiments were used to assess the effect of soil biota species richness within functional or taxonomic groups on N mineralization. Nematodes and earthworms were selected for these experiments, since these soil biota play key roles in N mineralization and are abundant in soil systems. The results of these studies were combined with a long-term field experiment where the relationship between soil biota diversity, N mineralization and N retention under agricultural (de-)intensification was studied. Different levels of agricultural intensification were generated by conversion of a long-term extensively managed grassland to an arable system and, *vice versa*, by re-establishment of an extensively managed grassland on formerly long-term conventionally managed arable land. Further (de-)intensification practices included reduction/increase in diversification of crops and reduction/increase in mineral N fertilization. Abundances and structural diversity of soil biota were studied in three consecutive years following conversions. Furthermore, N mineralization and N leaching were determined in these fields to study the relationship between soil biota, N mineralization and N retention.

Outline of this thesis

In chapter 2, I describe the results of a laboratory experiment on nematode species richness within the trophic group of bacterivores in relation to net nitrogen mineralization. In this microcosm experiment, we used single or two-species combinations of three bacterivorous nematode species with different life-strategies and assessed bacterial activity, biomass and mineralized N in the microcosms during and after a period of 84 days. The intricate relationship between nematode species interactions, the bacterial community and N mineralization is discussed.

In line with the previous chapter, in chapter 3 I describe a mesocosm experiment on earthworm species composition in relation to net nitrogen mineralization. Mesocosms with single species or two- or three-species combinations of three earthworm species representing different ecological groups were used to assess net N mineralization. Bacterial activity and biomass at the end of the experiment were also measured to unravel the complex relationship between earthworm species composition, the bacterial community and net N mineralization.

In chapters 4 and 5 I investigate the soil biota community under different schemes of agricultural (de-)intensification. Chapter 4 describes the effects of agricultural (de-)intensification on abundances and functional diversity of the major soil biota groups, over a period of three consecutive years. I integrate and compare effects across taxonomic levels to identify sensitive soil biota groups.

In chapter 5 I describe the effects of agricultural (de-)intensification on taxonomic diversity of the soil biota community. In line with chapter 4, I integrate and compare results on the different taxonomic groups across taxonomic levels and I synthesize the results of chapters 4 and 5 in terms of agricultural (de-)intensification effects on the soil biota group abundances, functional groups and taxonomic diversity.

In Chapter 6 I describe the effects of agricultural (de-)intensification on net N mineralization and N leaching. These results are combined with the results of chapters 4 and 5 in order to determine the relationship between soil biota abundances and diversity and N cycling. I determined N mineralization and N leaching using anion exchange resin bags, which were buried below the plow zone during the summer and winter period, for two years after conversion. This method was developed in order to gain a cumulative measurement of leached N over a longer period, while continuing conventional soil tillage practices during sampling period.

In Chapter 7 I synthesize the results from the preceding chapters. I discuss the relationship between soil biota abundance within and across taxonomic and functional groups and N mineralization. Furthermore, I discuss the relationship between the soil biota community, N mineralization and N leaching in agricultural systems under (de-)intensification. I conclude with

suggestions on the significance of soil biota abundance and diversity for N mineralization and N retention in agricultural systems.

References

Balota, E. L., A. Colozzi, D. Andrade, and R. P. Dick. 2004. Long-term tillage and crop rotation effects on microbial biomass and C and N mineralization in a Brazilian Oxisol. Soil & Tillage Research 77: 137-145.

Bamforth, S. S.. 1985. Symposium on protozoan ecology – the role of protozoa in litters and soils. Journal of Protozoology 32: 404-409.

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

Behan-Pelletier, V. M.. 2003. Acari and Collembola biodiversity in Canadian agricultural soils. Canadian Journal of Soil Science 83: 279-288.

Bengtsson, J., S. G. Nilsson, A. Franc, and P. Menozzi. 2000. Biodiversity, disturbances, ecosystem function and management of European forests. Forest Ecology and Management 132: 39-50.

Berg, M., P. de Ruiter, W. Didden, M. Janssen, T. Schouten, and H. Verhoef. 2001. Community food web, decomposition and nitrogen mineralization in a stratified Scots pine forest soil. Oikos 94: 130-142.

Black, H. I. J., V. Standen, S. Piertney, C. MacDonald, Evershed R P, and I. D. Bull 2003. The impact of environmental change on the functional role of enchytraeids in grassland. Pages 77-80 *in* W. A. M. Didden, and P. N. Van Vliet, editors. Newsletter on Enchytraeidae No.8. Proceedings of the Fifth International Symposium on Enchytraeidae. Department of Soil Quality, Wageningen University, The Netherlands.

Blair, J. M., R. W. Parmelee, M. F. Allen, D. A. Mccartney, and B. R. Stinner. 1997. Changes in soil N pools in response to earthworm population manipulations in agroecosystems with different N sources. Soil Biology & Biochemistry 29: 361-367.

Bloem, J., P. C. De Ruiter, and L. A. Bouwman 1997. Soil food webs and nutrient cycling in agroecosystems. Pages 245-278 *in* J. D. Van Elsas, J. T. Trevors, and E. M. H. Wellington, editors. Modern Soil Microbiology. Marcel Dekker, Inc., New York.

Bloemers, G. F., M. Hodda, P. J. D. Lambshead, J. H. Lawton, and F. R. Wanless. 1997. The effects of forest disturbance on diversity of tropical soil nematodes. Oecologia 111: 575-582.

Bongers, T.. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14-19.

Bouwman, L. A., and K. B. Zwart. 1994. The ecology of bacterivorous protozoans and nematodes in arable soil. Agriculture, Ecosystems & Environment 51: 145-160.

Breland, T. A., and R. Eltun. 1999. Soil microbial biomass and mineralization of carbon and nitrogen in ecological, integrated and conventional forage and arable cropping systems. Biology and Fertility of Soils 30: 193-201.

Brown, G. G., B. Pashanasi, C. Villenave, J. C. Patron, B. K. Senapati, S. Giri, I. Barois, P. Lavelle, E. Blanchart, R. J. BLakemore, A. V. Spain, and J. Boyer 1999. Effects of earthworms on plant production in the tropics. Pages 87-147 *in* P. Lavelle, L. Brussaard, and P. Hendrix, editors. Earthworm Management in Tropical Agroecosystems. CABI Publishing, Oxon, UK.

Brown, G., A. Moreno, I. Barois, C. Fragoso, P. Rojas, B. Hernandez, and J. Patron. 2004. Soil macrofauna in SE Mexican pastures and the effect of conversion from native to introduced pastures. Agriculture, Ecosystems & Environment 103: 313-327.

Brussaard, L., T. Kuyper, W. A. M. Didden, R. G. M. De Goede, and J. Bloem 2004. Biological soil quality from biomass to biodiversity - importance and resilience to management stress and disturbance. Pages 139-161 *in* P. Schjonning, S. Emholt, and B. T. Christensen, editors. Managing Soil Quality - Challenges in Modern Agriculture. CAB International, Wallingford, UK.

Brussaard, L., V. M. Behan-Pelletier, D. E. Bignell, V. K. Brown, W. Didden, P. Folgarait, C. Fragoso, D. W. Freckman, V. Gupta, T. Hattori, D. L. Hawksworth, C. Klopatek, P. Lavelle, D. W. Malloch, J. Rusek, B. Soderstrom, J. M. Tiedje, and R. A. Virginia. 1997. Biodiversity and ecosystem functioning in soil. Ambio 26: 563-570.

Carpenter-Boggs, L., P. D. Stahl, M. J. Lindstrom, and T. E. Schumacher. 2003. Soil microbial properties under permanent grass, conventional tillage, and no-till management in South Dakota. Soil and Tillage Research 71: 15-23.

Cole, L., M. A. Bradford, P. J. A. Shaw, and R. D. Bardgett. 2006. The abundance, richness and functional role of soil meso- and macrofauna in temperate grassland - A case study. Applied Soil Ecology 33: 186-198.

Cragg, R. G., and R. D. Bardgett. 2001. How changes in soil faunal diversity and composition within a trophic group influence decomposition processes. Soil Biology & Biochemistry 33: 2073-2081.

Curry, J. P., D. Byrne, and O. Schmidt. 2002. Intensive cultivation can drastically reduce earthworm populations in arable land. European Journal of Soil Biology 38: 127-130.

De Ruiter, P. C., J. Bloem, L. A. Bouwman, W. A. M. Didden, G. H. J. Hoenderboom, G. Lebbink, J. C. Y. Marinissen, J. A. De Vos, M. J. Vreeken-Buijs, K. B. Zwart, and L. Brussaard. 1994. Simulation of dynamics in nitrogen mineralization in the belowground food webs of 2 arable farming systems. Agriculture Ecosystems & Environment 51: 199-208.

De Ruiter, P. C., J. C. Moore, K. B. Zwart, L. A. Bouwman, J. Hassink, Bloem J, J. A. De Vos, J. C. Y. Marinissen, W. A. M. Didden, G. Lebbink, and L. Brussaard. 1993. Simulation of

nitrogen mineralization in the belowground food webs of 2 winter-wheat fields. Journal of Applied Ecology 30: 106.

Doles, J. L., R. J. Zimmerman, and J. C. Moore. 2001. Soil microarthropod community structure and dynamics in organic and conventionally managed apple orchards in Western Colorado, USA. Applied Soil Ecology 18: 83-96.

Dominguez, J., P. J. Bohlen, and R. W. Parmelee. 2004. Earthworms increase nitrogen leaching to greater soil depths in row crop agroecosystems. Ecosystems 7: 672-685.

Doube, B. M., P. M. L. Williams, and P. J. Willmott. 1997. The influence of two species of earthworm (*Aporrectodea trapezoides* and *Aporrectodea rosea*) on the growth of wheat, barley and faba beans in three soil types in the greenhouse. Soil Biology & Biochemistry 29: 503-509.

Ettema, C. H., and T. Bongers. 1993. Characterization of nematode colonization and succession in disturbed soil using the maturity index. Biology and Fertility of Soils 16: 79-85.

Faber, J. H., J. J. C. Van der Pol, and M. Thompson 2006. Soil health scenario's. Pages 64-78 *in* Pre-selection of scenario for cumulative risk assessment - Documentation of the first version of selection procedure including initial uncertainty evaluation. Report Deliverable D.1.2.4., Integrated Project 'NOMIRACLE' in EU 6th Framework Programme, project nr 003956.

Ferris, H., R. C. Venette, and K. M. Scow. 2004. Soil management to enhance bacterivore and fungivore nematode populations and their nitrogen mineralization function. Applied Soil Ecology 25: 19-35.

Filser, J., K. H. Mebes, K. Winter, A. Lang, and C. Kampichler. 2002. Long-term dynamics and interrelationships of soil Collembola and microorganisms in an arable landscape following land use change. Geoderma 105: 201-221.

Fliessbach, A., P. Mäder, and U. Niggli. 2000. Mineralization and microbial assimilation of C-14-labeled straw in soils of organic and conventional agricultural systems. Soil Biology & Biochemistry 32: 1131-1139.

Folke, C., C. Holling, and C. Perrings. 1996. Biological diversity, ecosystems, and the human scale. Ecological Applications 6: 1018-1024.

Freckman, D. W., and C. H. Ettema. 1993. Assessing nematode communities in agroecosystems of varying human intervention. Agriculture, Ecosystems & Environment 45: 239-261.

Frost, T. M., S. R. Carpenter, A. R. Ives, and T. K. Kratz 1995. Species compensation and complementarity in ecosystem function. Pages 224-239 *in* C. G. Jones, and J. H. Lawton, editors. Linking Species and Ecosystems. Chapman and Hall.

Garbeva, P., J. A. Van Veen, and J. D. Van Elsas. 2003. Predominant *Bacillus spp*. in agricultural soil under different management regimes detected via PCR-DGGE. Microbial Ecology 45: 302-316.

Garbeva, P., J. A. Van Veen, and J. D. Van Elsas. 2004. Assessment of the diversity, and antagonism towards *Rhizoctonia solani* AG3, of *Pseudomonas* species in soil from different agricultural regimes. FEMS Microbiology Ecology 47: 51-64.

Garbeva, P., J. Postma, J. A. Van Veen, and J. D. Van Elsas. 2006. Effect of above-ground plant species on soil microbial community structure and its impact on suppression of *Rhizoctonia solani* AG3. Environmental Microbiology 8: 233-246.

Giller, K. E., M. H. Beare, P. Lavelle, A. M. N. Izac, and M. J. Swift. 1997. Agricultural intensification, soil biodiversity and agroecosystem function. Applied Soil Ecology 6: 3-16.

Grayston, S. J., C. D. Campbell, R. D. Bardgett, J. L. Mawdsley, C. D. Clegg, K. Ritz, B. S. Griffiths, J. S. Rodwell, S. J. Edwards, W. J. Davies, D. J. Elston, and P. Millard. 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. Applied Soil Ecology 25: 63-84.

Griffiths, B. S., and K. Ritz. 1988. A technique to extract, enumerate and measure protozoa from mineral soils. Soil Biology & Biochemistry 20: 173.

Gunapala, N., R. C. Venette, H. Ferris, and K. M. Scow. 1998. Effects of soil management history on the rate of organic matter decomposition. Soil Biology & Biochemistry 30: 1917-1927.

Háněl, L.. 2003. Recovery of soil nematode populations from cropping stress by natural secondary succession to meadow land. Applied Soil Ecology 22: 255-270.

Hansen, B., H. F. Alroe, and E. S. Kristensen. 2001. Approaches to assess the environmental impact of organic farming with particular regard to Denmark. Agriculture Ecosystems & Environment 83: 11-26.

Hole, D. G., A. J. Perkins, J. D. Wilson, I. H. Alexander, F. Grice, and A. D. Evans. 2005. Does organic farming benefit biodiversity? Biological Conservation 122: 113-130.

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

Jagers op Akkerhuis, G. A. J. M., F. Deley, H. J. C. Zwetsloot, J. F. Ponge, and L. Brussaard. 1988. Soil microarthropods (acari and collembola) in 2 crop rotations on a heavy marine clay soil. Revue de Ecologie et de Biologie du Sol 25: 175-202.

Kleijn, D., F. Berendse, R. Smit, and N. Gilissen. 2001. Agri-environment schemes do not effectively protect biodiversity in Dutch agricultural landscapes. Nature 413: 723-725.

Laakso, J., and H. Setälä. 1999. Sensitivity of primary production to changes in the architecture of belowground food webs. Oikos 87: 57-64.

Lagerlöf, J., and O. Andren. 1988. Abundance and activity of soil mites (Acari) in 4 cropping Systems. Pedobiologia 32: 129-145.

Lagerlöf, J., B. Goffre, and C. Vincent. 2002. The importance of field boundaries for earthworms (Lumbricidae) in the Swedish agricultural landscape. Agriculture, Ecosystems & Environment 89: 91-103.

Liang, W. J., X. K. Zhang, Q. Li, Y. Jiang, W. Ou, and D. A. Neher. 2005. Vertical distribution of bacterivorous nematodes under different land uses. Journal of Nematology 37: 254-258.

Mäder, P., A. Fliessbach, D. Dubois, L. Gunst, P. Fried, and U. Niggli. 2002. Soil fertility and biodiversity in organic farming. Science 296: 1694-1697.

Mikola, J., and H. Setälä. 1998. Relating species diversity to ecosystem functioning: mechanistic backgrounds and experimental approach with a decomposer food web. Oikos 83: 180-194.

Mulder, C., J. Cohen, H. Setälä, J. Bloem, and A. Breure. 2005. Bacterial traits, organism mass, and numerical abundance in the detrital soil food web of Dutch agricultural grasslands. Ecology Letters 8: 80-90.

Porazinska, D. L., L. W. Duncan, R. McSorley, and J. H. Graham. 1999. Nematode communities as indicators of status and processes of a soil ecosystem influenced by agricultural management practices. Applied Soil Ecology 13: 69-86.

Ruz-Jerez, B. E., P. R. Ball, and R. W. Tillman. 1992. Laboratory assessment of nutrient release from a pasture soil receiving grass or clover residues, in the presence or absence of *Lumbricus rubellus* or *Eisenia foetida.* Soil Biology & Biochemistry 24: 1529-1534.

Setälä, H., M. P. Berg, and T. H. Jones 2005. Trophic structure and functional redundancy in soil communities. Pages 236-249 *in* R. D. Bardgett, M. B. Usher, and D. W. Hopkins, editors. Biological Diversity and Function in Soils. Cambridge University Press.

Sheehan, C., L. Kirwan, J. Connolly, and T. Bolger. 2006. The effects of earthworm functional group diversity on nitrogen dynamics in soils. Soil Biology & Biochemistry 38: 2629-2636.

Siepel, H. 1994. Life-history tactics of soil microarthropods. Biology and Fertility of Soils 18: 263-278.

Siepel, H. 1996. Biodiversity of soil microarthropods: The filtering of species. Biodiversity and Conservation 5: 251-260.

Siepel, H., and C. F. Van de Bund. 1988. The Influence of management practices on the Microarthropod community of grassland. Pedobiologia 31: 339-354.

Silver, W. L., A. W. Thompson, A. Reich, J. J. Ewel, and M. K. Firestone. 2005. Nitrogen cycling in tropical plantation forests: Potential controls on nitrogen retention. Ecological Applications 15: 1604-1614.

Sohlenius, B., and A. Sandor. 1987. Vertical-distribution of nematodes in arable soil under grass (*Festuca-pratensis*) and barley (*Hordeum-distichum*). Biology and Fertility of Soils 3: 19-25.

Spedding, T. A., C. Hamel, G. R. Mehuys, and C. A. Madramootoo. 2004. Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. Soil Biology & Biochemistry 36: 499-512.

Susilo, F. X., A. M. Neutel, M. Van Noordwijk, K. Hairiah, G. Brown, and M. J. Swift 2004. Soil biodiversity and food webs. Pages 285-306 *in* M. Van Noordwijk, G. Cadisch, and C. K. Ong, editors. Below-ground Interactions in Tropical Agroecosystems, Concepts and Models with Multiple Plant Components. CABI Publishing, Cambridge, USA.

Swift, M. J., A. M. N. Izac, and M. Van Noordwijk. 2004. Biodiversity and ecosystem services in agricultural landscapes - are we asking the right questions? Agriculture, Ecosystems & Environment 104: 113-134.

Swift, M. J., J. Vandermeer, P. S. Ramakrishnan, J. M. Anderson, C. K. Ong, and B. A. Hawkins 1996. Biodiversity and Agroecosystem Function. Pages 261-298 *in* H. A. Mooney, J. H. Cushman, E. Medina, O. E. Sala, and E. D. Schulze, editors. Functional Roles of Biodiversity: a Global Perspective. John Wiley & Sons Ltd.

Torsvik, V., L. Ovreas, and T. Thingstad. 2002. Prokaryotic diversity - Magnitude, dynamics, and controlling factors. Science 296: 1064-1066.

Usher, M.B., P. Davis, J. Harris, and B. Longstaff. 1979. A profusion of species? Approaches towards understanding the dynamics of the populations of microarthropods in decomposer communities. Pages 359-384 in: R.M. Anderson, B.D. Turner and L.R. Taylor, editors. Population Dynamics. Oxford: Blackwell Scientific Publications.

Van Diepeningen, A. D., O. J. De Vos, G. W. Korthals, and A. H. C. Van Bruggen. 2006. Effects of organic versus conventional management on chemical and biological parameters in agricultural soils. Applied Soil Ecology 31: 120-135.

Van Vliet, P. C. J., D. C. Coleman, and P. F. Hendrix. 1997. Population dynamics of Enchytraeidae (Oligochaeta) in different agricultural systems. Biology and Fertility of Soils 25: 123-129.

Villenave, C., T. Bongers, K. Ekschmitt, P. Fernandes, and R. Oliver. 2003. Changes in nematode communities after manuring in millet fields in Senegal. Nematology 5: 351-358.

Vreeken-Buijs, M. J., M. Geurs, P. C. De Ruiter, and L. Brussaard. 1994. Microarthropod biomass-C dynamics in the belowground food web of 2 arable farming systems. Agriculture, Ecosystems & Environment 51: 161-170.

Wardle, D. A.. 1995. Impacts of disturbance on detritus rood webs in agroecosystems of contrasting tillage and weed management practices. Advances in Ecological Research 26: 105-185.

Wardle, D. A., K. S. Nicholson, K. I. Bonner, and G. W. Yeates. 1999. Effects of agricultural intensification on soil-associated arthropod population dynamics, community structure, diversity and temporal variability over a seven-year period. Soil Biology & Biochemistry 31: 1691-1706.

Whalen, J. K., R. W. Parmelee, and S. Subler. 2000. Quantification of nitrogen excretion rates for three lumbricid earthworms using 15N. Biology and Fertility of Soils 32: 347-352.

Zwart, K. B., S. L. G. E. Burgers, J. Bloem, L. A. Bouwman, L. Brussaard, G. Lebbink, W. A. M. Didden, J. C. Y. Marinissen, M. J. Vreeken-Buijs, and P. C. De Ruiter. 1994. Population dynamics in the belowground food webs in two different agricultural systems. Agriculture, Ecosystems & Environment 51: 187-198.

Chapter 2

Within-trophic group interactions of bacterivorous nematode species and their effects on the bacterial community and nitrogen mineralization

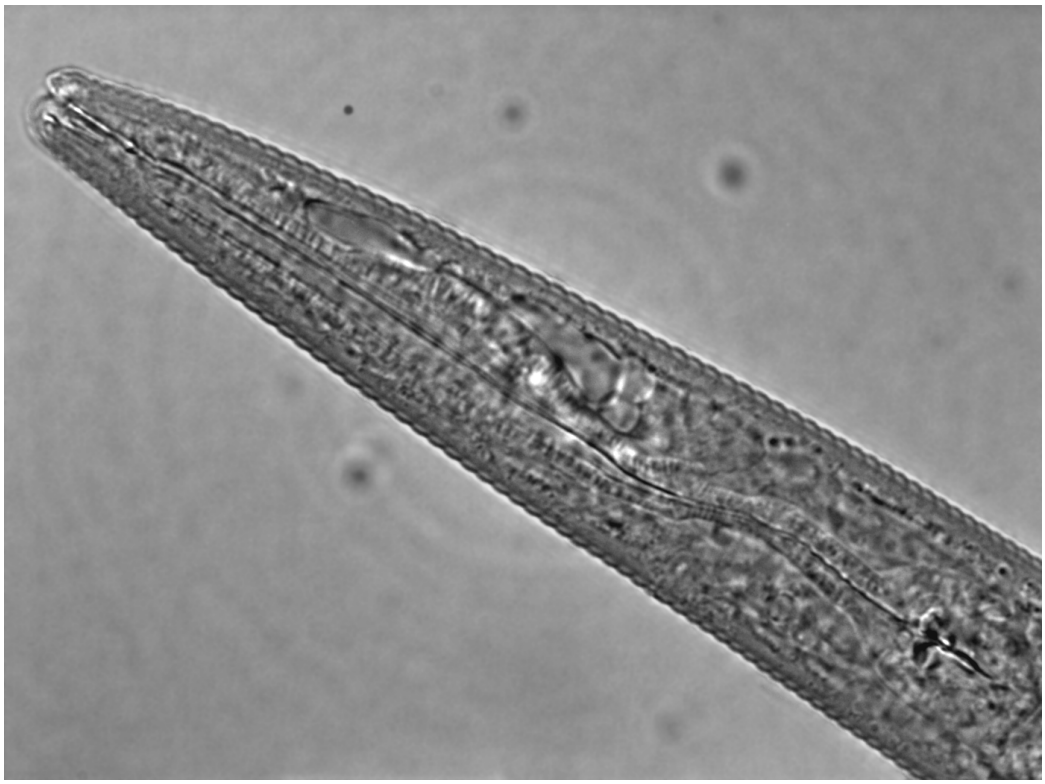

Foto: Hanny van Megen, Nematologie, Wageningen Universiteit

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Abstract

Knowledge on the interactions between organisms within trophic groups is important for understanding the role of biodiversity for ecosystem functioning. We hypothesized that interactions between bacterivorous nematodes of different life history strategies would affect nematode population development, bacterial community composition and activity, resulting in increased nitrogen mineralization. A microcosm experiment was conducted using three nematode species (*Bursilla monhystera*, *Acrobeloides nanus* and *Plectus parvus*). All the nematode species interacted with each other, but the nature and effects of these interactions depended on the specific species combination. The interaction between *B. monhystera* and *A. nanus* was asymmetrically competitive (0 , -), whereas that between *B. monhystera* and *P. parvus*, and also *A. nanus* and *P. parvus* was contramensal (+ , -). The interaction that affected microcosm properties the most was the interaction between *B. monhystera* and *P. parvus*. This interaction affected the bacterial community composition, increased the bacterial biomass and increased soil nitrogen mineralization. *B. monhystera* and *P. parvus* have the most different life history strategies, whereas *A. nanus* has a life-history strategy intermediate to *B. monhystera* and *P. parvus*. We suggest that the difference in life-history strategies between species of the same trophic group is of importance for their communal effect on soil ecosystem processes. Our results support the idiosyncracy hypothesis on the role of biodiversity in ecosystem functioning.

Introduction

The importance of soil fauna and their different soil trophic groups on ecosystem processes has been determined in many studies (Bardgett and Cook, 1998; Brussaard et al., 1997; Wardle, 2002). Only a few studies, however, have investigated the effect of within-trophic group diversity of the soil fauna on ecosystem processes (Faber and Verhoef, 1991; Laakso and Setälä, 1999; Mikola and Setälä, 1998c). Furthermore, there is hardly any knowledge on the nature of the interactions between soil organisms within trophic groups and the importance of these interactions for ecosystem processes (Wardle, 2002). Detailed knowledge on this subject is needed for a mechanistic understanding of the role of soil biodiversity in ecosystem processes.

Bacterivorous nematodes are known to contribute significantly to nitrogen mineralization, an important ecosystem process (Freckman, 1988; Ingham et al., 1985; Verhoef and Brussaard, 1990). They stimulate nitrogen mineralization directly by the excretion of nitrogen and indirectly by grazing on bacteria and protozoa, thereby stimulating bacterial turnover and production (Freckman, 1988). Different nematode species can contribute either differently (De Mesel et al., 2003) or similarly (Djigal et al., 2004; Ferris et al., 1998) to nitrogen mineralization. Nematode species can influence the population sizes of other nematodes species, which may affect the nitrogen mineralization in the system. Furthermore, interactions between species may lead to resource partitioning, and consequently a more intensive use of resources and increased nitrogen mineralization (Begon et al., 1990). The interactions between bacterivorous nematode species and their effects on nitrogen mineralization have been poorly studied. We know of only two studies that found indications of competition between bacterivorous nematode species (Anderson and Coleman, 1981; Mikola and Setälä, 1998c).

In the present study we investigated the effect of interactions between three bacterivorous nematode species (*Bursilla monhystera, Acrobeloides nanus, Plectus parvus*) on the population development of each of the individual nematode species, on bacterial biomass, growth rate and diversity, and on nitrogen mineralization. We hypothesized that the nematode species would interact through competition. The nematodes used in our experiment, although belonging to the same trophic group, differ in their life-history strategies. Nematodes can be classified according to their life-history strategies, ranging from colonizers (c), extreme r-strategists with a short generation time and high fecundity, to persisters (p), extreme K-strategists with a longer generation time and lower fecundity. Bongers (1999) scaled them accordingly on a scale of 1 to 5. *B. monhystera* is a colonizer-persister (cp)-1 nematode (value 1 on the cp scale), *A. nanus* and *P. parvus* are cp-2 nematodes (Bongers and Ferris, 1999). Of the latter two, *Plectus sp.* was found to reproduce more slowly than *Acrobeloides sp.* and *P. parvus* may therefore be scaled higher on the colonizer-persister scale relative to *A. nanus* (Ferris and Matute, 2003). Nematodes

having different life-history strategies may be able to forage on different food sources (Anderson and Coleman, 1981) and/or exploit their food at different rates. The combination of nematodes of different life strategies may, therefore, lead to a more thorough exploitation of the food source. Hence, we hypothesized that the interaction between nematodes of different cp-classes will lead to increased nitrogen mineralization.

Since the nematodes used in our experiment depend on bacteria as a food source, factors that influence the bacterial community may also influence the nematode populations and the interactions between the different nematode species. We hypothesized that the effect of interactions between nematode species on nitrogen mineralization can be explained through changes in the bacterial community. The C:N ratio of organic material in the system can also influence the bacterial biomass (Ballinger et al., 2002; Marschner et al., 2003) and may therefore influence the nematode populations and the interactions between the different nematode species. We therefore performed the experiment by adding organic material with a low (13) and a high (25) C:N ratio.

Materials and methods

Experimental setup

A microcosm experiment was carried out in audiothene (polyethylene), oxygen-permeable incubation bags. The microcosms contained 150 g fresh weight of soil, supplemented with organic material and nematodes, and were closed by heat sealing. The microcosms were incubated at 20 °C by day (16 hours) and 15 °C by night (8 hours). The experiment comprised 7 nematode treatments, consisting of 3 treatments with a single species, 3 treatments with 2 combined nematode species and a nematode-free control treatment. The treatments were applied in soil with organic matter of 2 different C:N ratios. The microcosms were sampled destructively after 14, 28, 56 and 84 days. Each treatment was replicated 4 times, so that the experiment included 7 (nematode species) x 2 (C:N ratios) x 4 (sampling times) x 4 (replicates) = 224 microcosms.

Soil

The soil used in the microcosms was taken from a humic podzol in 'De Bovenbuurt' (51°59'N, 5°40'E), which is a LTER site with grass and arable land. On 7 May 2001, soil was collected from 0-15 cm depth in the arable land. The soil was sieved (5 mm mesh size) to remove roots and coarse material, and sterilized with γ-irradiation (25 kGy).

Microbes

To re-inoculate the sterilized soil with bacteria, fungi and protozoa from the field, the method described by Bouwman et al. (1996) was used. Although protozoa were not part of this study we deliberately kept them in the system because it is impossible to exclude them from all microcosms. According to this method, 500 g soil was air-dried for 3 days, pulverized in a ball mill, moistened with tap water and incubated for 72 hours at 20 °C. This procedure was repeated three times. This 'soil powder' was used as an inoculum for bacteria, fungi and protozoa. It is known that this procedure results in the elimination of all nematodes, including eggs and dauerlarvae, whereas bacteria, fungi and protozoa remain present (Bouwman et al., 1996). In each of the 14 experimental treatments, 30 g dry weight of this 'soil powder' was thoroughly mixed with 3 kg fresh weight sterilized soil under sterile conditions and stored in a plastic bag at 20 °C for one week. Thereafter the microcosms were filled with 150 g fresh weight of this re-inoculated soil.

Organic substrate

Crop residues of 2 C:N ratio levels (C:N ratio 13 and 25) were simulated by the addition of either Lucerne (C:N ratio 13) or a combination of milled wheat straw (C:N ratio 70) and Lucerne The treatments of organic material with C:N ratio 13 received 2 mg Lucerne per g fresh weight soil, which corresponded with 64 µg N g^{-1} soil and 832 µg C g^{-1} soil. These quantities are equivalent to the addition of ca. 3000 kg C ha^{-1}, which is a common quantity used in Dutch agricultural practices.

The treatments of organic material with C:N ratio 25 received a mixture of wheat straw (2 mg g^{-1} soil and Lucerne 1.56 mg g^{-1} soil), which corresponded with 64 µg N g^{-1} soil and 1624 µg C g^{-1} soil. Thus, the total amount of nitrogen added was equal in both C:N ratio treatments. After amending the soil with organic material, analyses of pH, nitrate, ammonium, total C, total N and total P were performed. In the C:N ratio treatments 13 and 25, the pH-$CaCl_2$ was 5.88 and 6.06 respectively, N-NO_3^- was 62.9 mg kg^{-1} and 63.2 mg kg^{-1} respectively, N-NH_4^+ was 5.57 mg kg^{-1} and 11.7 mg kg^{-1} respectively, total C was 27.56 mg g^{-1} and 26.21 mg g^{-1} respectively, total N was 1.65 mg g^{-1} and 1.81 mg g^{-1} respectively and total P was 1043 mg kg^{-1} and 1132 mg kg^{-1} respectively. The addition of organic material was carried out for each experimental unit separately. The contents of each microcosm were thoroughly mixed, closed and incubated at 20 °C for one week.

Nematodes

Three bacterial-feeding nematode species were used: *Bursilla monhystera* (Buetschli, 1873), *Plectus parvus* (Bastian, 1865) and *Acrobeloides nanus* (De Man, 1880). The 7 nematode

treatments consisted of 3 treatments with a single species: *B. monhystera*, *A. nanus* or *P. parvus*; 3 treatments with 2 combined species: *B. monhystera* and *A. nanus*, *B. monhystera* and *P. parvus* or *A. nanus* and *P. parvus*; and a nematode-free control treatment. Two species, *B. monhystera* and *A. nanus*, were isolated from soil samples collected in a grassland at 'De Bovenbuurt'. *P. parvus* was obtained from existing cultures provided by the Laboratory of Nematology at Wageningen University (J. Kammenga). The nematodes were extracted from the soil samples (Oostenbrink, 1960) and cultured on proteose pepton agar (PPA) using *Acinetobacter* bacteria as a food source. Each culture was established from one individual nematode. Nematodes were extracted from the cultures by transferring the agar on top of a cottonwood filter (Hygia milac® milk filter) that was mounted in an 385 µm mesh extraction sieve and placed in an extraction dish filled with tap water. For each species used in the experiment, 0.25 ml nematode suspension was added to the soil in the audiothene bags, corresponding to a total number of 5 individuals per g soil and a soil water content of 13.4 %. After addition of the nematodes, the soil was mixed gently. The bags were closed by sealing and incubated as described above.

Measurements

Nematode counts

Nematodes were counted after 14, 28, 56, and 84 days. Nematodes were extracted from 100 g soil using an Oostenbrink elutriatior. Filters were placed in 90 ml tap water for one night to allow nematode migration through the filters. Nematodes were counted and identified to species level in 10 ml subsamples under an inverted binocular microscope at 100x and 400x magnification respectively.

Mineral nitrogen concentration

Approximately 10 g of moist soil from the experimental microcosms was weighed and dried at 40 °C. Approximately 3 g of dry soil was weighed exactly into a centrifuge tube. After adding 30 ml of $CaCl_2$, the tubes were shaken for 2 hours and subsequently centrifuged. The supernatant was poured into a tube and 5 µl 10 M HCl was added. In this solution, NO_3^- and NH_4^+ were measured using Skalar (Breda) Continuous Flow Analysis.

Bacterial biomass, growth rate and DNA analysis

Bacterial biomass, growth rate and DNA analysis were assessed in the high C:N ratio treatments only. Bacterial numbers and cell volumes were measured by confocal laser scanning microscopy and automatic image analysis, and the bacterial biomass was calculated from biovolume (Bloem et al., 1995a; Bloem et al., 1995b). The bacterial growth rate was determined by thymidine

incorporation, a measure of DNA synthesis (Michel and Bloem, 1993). These analyses were carried out for 3 of the 4 replicates, which were randomly chosen. At the end of the experiment DNA was extracted (Van Elsas and Smalla, 1995) and analyzed by denaturating gradient gel electrophoresis (DGGE) (Muyzer et al., 1993) from 2 replicates of each treatment in the high C:N ratio. This technique yields a banding pattern where the number of DNA bands reflects the number of 'species' (genotypes) of abundant bacteria, and the band-intensity reflects the relative abundance of the 'species' (Bloem and Breure, 2003; Dilly et al., 2004).

Calculations and statistics

Total mineral N was calculated as the sum of $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$. Statistical analyses were carried out using the statistical package SPSS version 10.0. If necessary, data were transformed (log or square root transformation) to meet assumptions of homogeneity of variance and normality. For analysis of nematode numbers and total mineral N one outlier in one replicate was removed to meet assumptions of homogeneity of variance and normality. The numbers of *B. monhystera*, *A. nanus* and *P. parvus* were analyzed using a four-factor ANOVA. The factors were time (destructive samplings after 14, 28, 56 and 84 days), C:N ratio (13 or 25) and the two nematode species other than the nematode species that was being tested (for *B. monhystera*: with and without either *A. nanus* or *P. parvus*; for *A. nanus*: with and without either *B. monhystera* or *P. parvus*; for *P. parvus*: with and without either *B. monhystera* or *A. nanus*). Because no adequate transformations could be found for the numbers of *P. parvus*, the Kruskal Wallis Test was applied to analyze the data for this nematode species.

Bacterial biomass, numbers and growth rates were analyzed using a four-factor ANOVA. The factors were time (destructive samplings after 14, 28, 56 and 84 days) and the three nematode species (with and without either *B. monhystera*, *A. nanus* or *P. parvus*). The number of DNA bands was analyzed using a three-factor ANOVA. The factors were the three nematode species (with and without either *B. monhystera*, *A. nanus* or *P. parvus*). The DNA banding patterns obtained using DGGE were analyzed by Principal Component Analysis using GelCompar II software (Applied Maths, Kortrijk, Belgium). The total number of nematodes and the amount of total mineral N were analyzed using a five-factor ANOVA. The factors were time (destructive samplings after 14, 28, 56, and 84 days), C:N ratio (13 or 25) and the three nematode species (with and without either *B. monhystera*, *A. nanus* or *P. parvus*). All two- and three-way interactions were tested.

Table 1. Significance of effects of time, C:N ratio, and presence of nematode species on nematode species abundances, total nematode abundance, and total mineral N. P-level results from ANOVA or non-parametric tests (*P. parvus*). P-levels of 0.05 and above are indicated as non-significant (n.s.).

Variables	**P-levels of dependents**				
	B. monhystera	***A. nanus***	***P. parvus***	**Total nematode abundance (n g^{-1})**	**Total mineral N (mg kg^{-1})**
Time	0.001	<0.001	n.s.	<0.001	<0.001
C:N ratio	<0.001	0.037	n.s.	<0.001	<0.001
B. monhystera	-	<0.001	0.026	<0.001	<0.001
A. nanus	n.s.	-	0.003	<0.001	0.053
P. parvus	<0.001	0.047	-	<0.001	<0.001
Time x C:N ratio	n.s.	n.s.	-	n.s.	n.s.
Time x *B. monhystera*	-	<0.001	-	<0.001	0.010
Time x *A. nanus*	n.s.	-	-	<0.001	n.s.
Time x *P. parvus*	n.s.	0.001	-	n.s.	n.s.
C:N ratio x *B. monhystera*	-	n.s.	-	0.001	0.001
C:N ratio x *A. nanus*	n.s.	-	-	0.026	<0.001
C:N ratio x *P. parvus*	<0.001	n.s.	-	<0.001	0.010
B. monhystera x *A. nanus*	-	-	-	<0.001	n.s.
B. monhystera x *P. parvus*	-	-	-	0.029	<0.001
A. nanus x *P. parvus*	-	-	-	n.s.	n.s.
Time x C:N ratio x *B. monhystera*	-	n.s.	-	0.037	0.001
Time x C:N ratio x *A. nanus*	n.s.	-	-	n.s.	n.s.
Time x C:N ratio x *P. parvus*	n.s	n.s.	-	n.s.	0.001
Time x *B. monhystera* x *A. nanus*	-	-	-	0.001	n.s.
Time x *B. monhystera* x *P. parvus*	-	-	-	n.s.	n.s.
Time x *A. nanus* x *P. parvus*	-	-	-	0.023	n.s.
C:N ratio x *B. monhystera x A. nanus*	-	-	-	n.s.	0.001
C:N ratio x *B. monhystera* x *P. parvus*	-	-	-	<0.001	<0.001
C:N ratio x *A. nanus* x *P. parvus*	-	-	-	n.s.	n.s

Results

Nematodes

B. monhystera

The numbers of *B. monhystera* rapidly increased with time until they stabilized at day 28 (Fig. 1a, Table 1). The presence of *A. nanus* had no effect on the numbers of *B. monhystera*. The presence of *P. parvus* had a positive effect (two-fold increase) on the numbers of *B. monhystera* in the high C:N ratio treatments, but not in the low C:N ratio treatments.

A. nanus

The numbers of *A. nanus* increased with time towards the end of the experiment (Fig. 1b, Table 1). The numbers of *A. nanus* were higher in the high C:N ratio treatments than in the low C:N ratio treatments. The presence of *B. monhystera* had a negative effect on the numbers of *A. nanus*, and this effect increased with time. The presence of *P. parvus* had a positive effect on the numbers of *A. nanus* on day 28 of the experiment.

P. parvus

The numbers of *P. parvus* decreased below the detection limit in several microcosms and numbers remained at levels lower than inoculation density in others (Fig. 1c, Table 1). The numbers of *P. parvus* did not differ between C:N ratios. The presence of *B. monhystera* and *A. nanus* had a negative effect on the numbers of *P. parvus*.

Total nematode numbers

The total nematode numbers showed the combined effects of time and C:N ratio on the individual species (Fig. 2, Table 1). The total nematode numbers increased with time and were higher in the high C:N ratio treatments than in the low C:N ratio treatments. *B. monhystera*, *A. nanus* and *P. parvus* all contributed to the total nematode numbers, though the contribution of *P. parvus* was small. The relative contribution of *B. monhystera* to the total nematode numbers was highest in the first 28 days in the high C:N ratio treatment, and the relative contribution of *A. nanus* was highest from day 56 onwards, and in the low C:N ratio treatment. The relative contribution of *P. parvus* to the total nematode numbers was lowest in the low C:N ratio treatment. The total nematode numbers were higher in the treatments with the combination of *B. monhystera* and *A. nanus* nematodes than in the treatments with the individual species only on

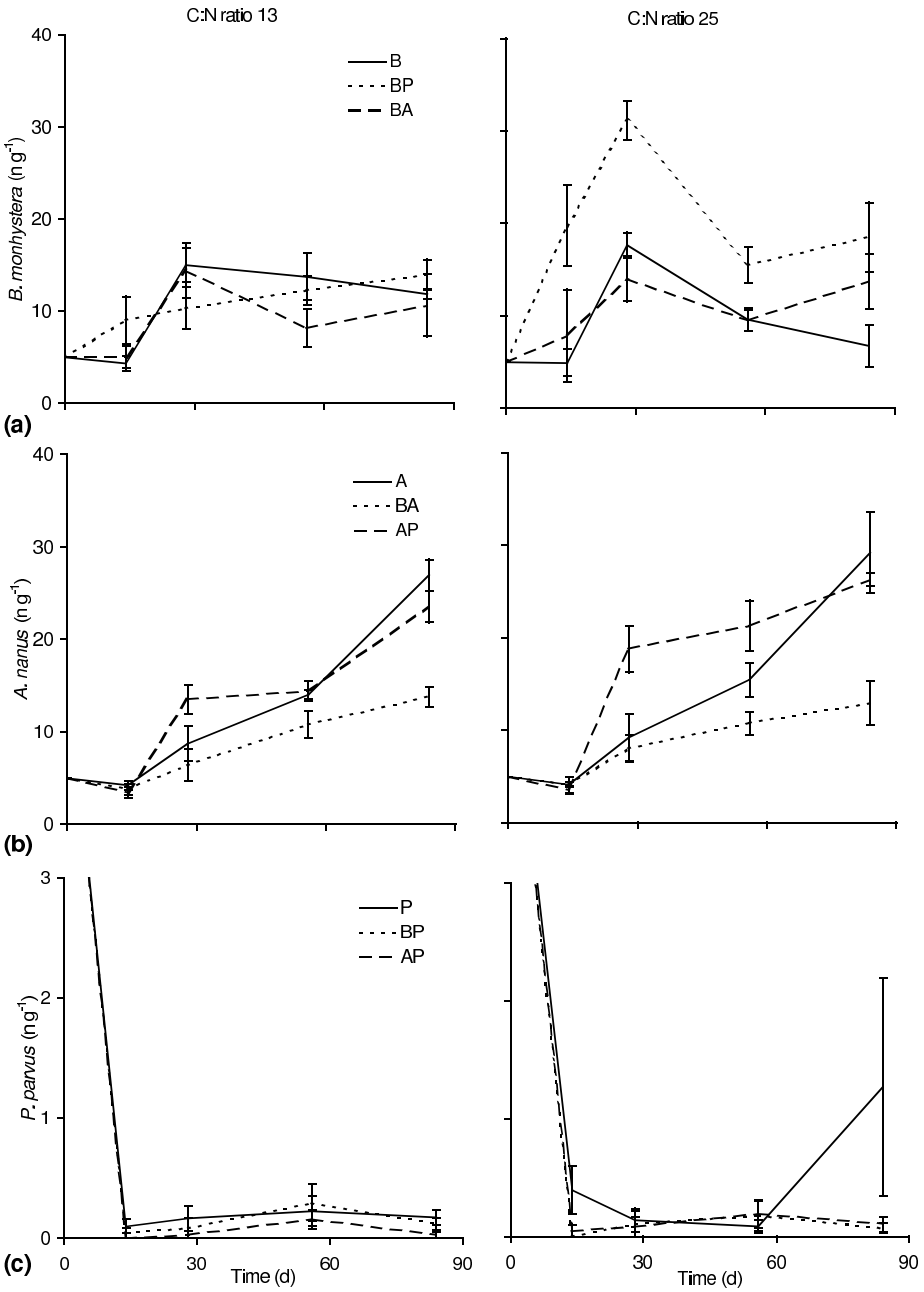


Figure 1. Nematode species abundance (mean ± SE) in the absence or presence of other nematode species at two C:N ratios. **a**. Abundance of *B. monhystera* in the absence or presence of *A. nanus* or *P. parvus*. **b**. Abundance of *A. nanus* in the presence or absence of *B. monhystera* or *P. parvus*. **c**. Abundance of *P. parvus* in the absence or presence of *B. monhystera* or *A. nanus*. Note the difference in scale of the y-axis of figure 1a and 1b with 1c. B = *B. monhystera*, A = *A. nanus*, P = *P. parvus*, BA = *B. monhystera* + *A. nanus*, BP = *B. monhystera* + *P. parvus*, AP = *A. nanus* + *P. parvus*.

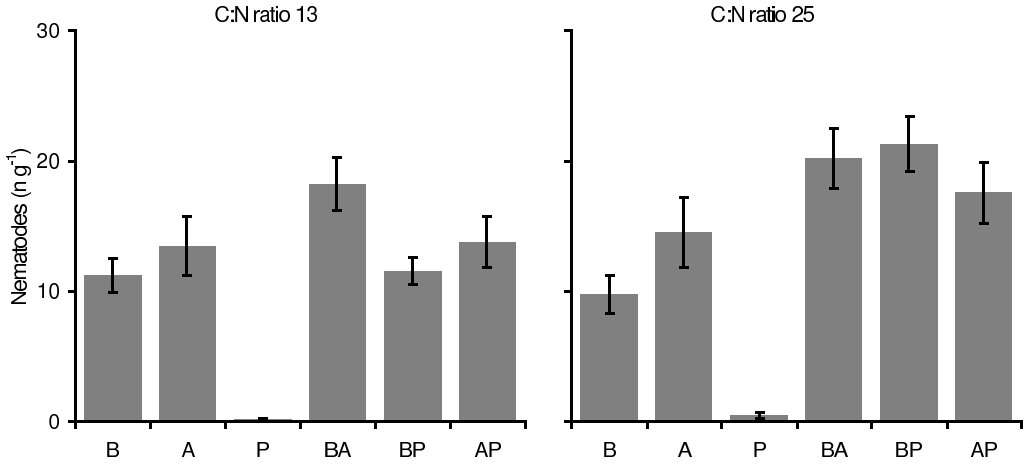


Figure 2. Total nematode abundance (mean ± SE) averaged over time in different nematode treatments at two C:N ratios. B = B. monhystera, A = A. nanus, P = P. parvus, BA = B. monhystera + A. nanus, BP = B. monhystera + P. parvus, AP = A. nanus + P. parvus.

all days except day 84. The total nematode numbers were also higher in the treatments with the combination of *B. monhystera* and *P. parvus* nematodes than in the treatments with the individual species only in the high C:N ratio treatment and in the treatments with the combination of *A. nanus* and *P. parvus* nematodes on day 28.

Bacteria

Bacterial biomass and numbers

Bacterial biomass and numbers increased with time until they stabilized at day 28 (Table 2). Determination of bacterial biomass and numbers failed at week 56. The bacterial biomass and numbers were higher than the control in the treatments with the combination of *B. monhystera* and *P. parvus* nematodes than in the treatments with the individual species only (Fig. 3a, Table 2).

Bacterial growth rate

The bacterial growth rate fluctuated with time (Table 2). It was lower at day 28 than at day 14, had increased to initial levels at day 56, and was lower again at day 84. *B. monhystera* had a

Table 2. Significance of effects of time and nematode species on bacterial biomass, bacterial numbers, bacterial growth rate (thymidine incorporation), and bacterial diversity (number of DNA bands). Bacterial characteristics were measured only in the C:N ratio 25 treatment. P-level results from ANOVA. P-levels of 0.05 and above are indicated as non-significant (n.s.).

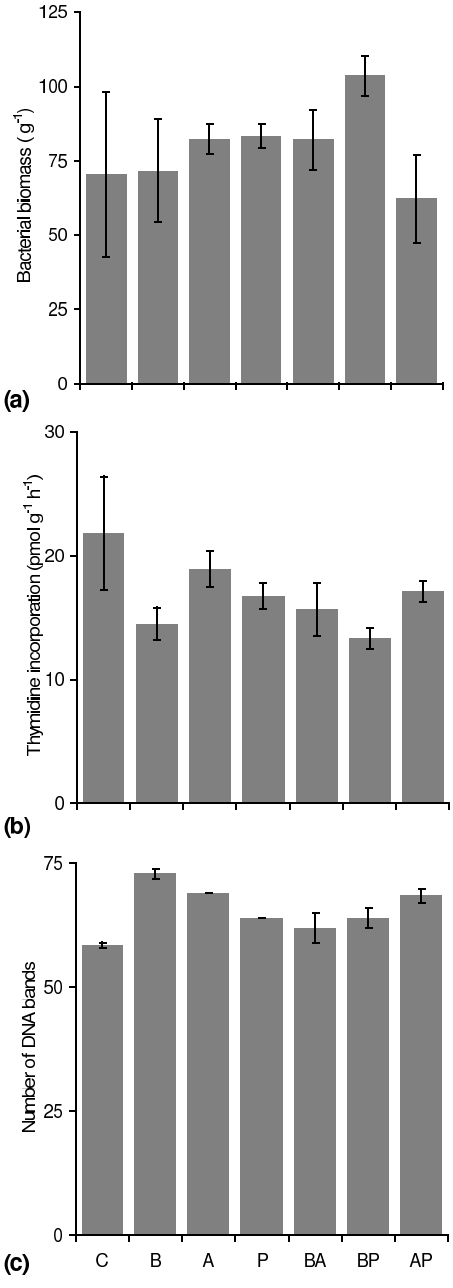
Variables	**P-levels of dependents**			
	Bacterial biomass (µg C g^{-1})	**Bacterial numbers (*10^9 g^{-1})**	**Thymidine incorporation (pmol g^{-1} h^{-1})**	**Number of DNA bands**
Time	0.008	<0.001	<0.001	-
B. monhystera	n.s.	n.s.	0.038	n.s.
A. nanus	n.s.	n.s.	n.s.	0.031
P. parvus	n.s.	n.s.	n.s.	0.007
Time x *B. monhystera*	n.s.	n.s.	n.s.	-
Time x *A. n*anus	n.s.	n.s.	n.s.	-
Time x *P. parv*us	n.s.	n.s.	n.s.	-
B. monhystera x *A. nanus*	n.s.	n.s.	0.008	<0.001
B. monhystera x *P. parvus*	0.013	0.035	n.s.	0.001
A. nanus x *P. parvus*	n.s.	n.s.	n.s.	n.s.
Time x *B. monhystera* x *A. nanus*	n.s.	n.s.	n.s.	-
Time x *B. monhystera* x *P. parvus*	n.s.	0.077	n.s.	-
Time x *A. nanus x P. parvus*	n.s.	n.s.	n.s.	-

negative effect on the bacterial growth rate (Fig. 3b, Table 2), also in treatments with the combination of *B. monhystera* and *P. parvus*. In treatments with the combination of *B. monhystera* and *A. nanus* nematodes, however, the negative effect of *B. monhystera* on the bacterial growth rate was not significant.

Bacterial DNA

The number of bacterial DNA bands, as a measure of the bacterial diversity, was in general higher in the treatments with nematodes compared with the control treatment (Fig. 3c, Table 2). The treatment with *B. monhystera* alone had the highest number of DNA bands. This positive effect of *B. monhystera* alone was reduced when *A. nanus* or *P. parvus* was also present. Numbers of DNA bands in the treatments with these combinations of nematodes were similar to the control.

The bacterial DNA banding pattern, reflecting community structure, was specific for each treatment, although the dominant species were similar in all treatments. In the treatments inoculated with *P. parvus* a specific, though not very dominant band showed up that was absent



in the other treatments. All treatments were separated by qualitative PCA, using the presence but not intensity of the bands. The X-axis accounted for 26 % of variation, and the Y-axis for 16 %. By quantitative PCA, including band intensities, only the nematode-free control treatment was separated from the other treatments (variation accounted for by X-axis: 43 %, by Y-axis: 18 %).

Nitrogen mineralization

The total mineral N concentration of the soil increased until it stabilized at day 56 (Fig. 4a, Table 1). The total mineral N concentration was higher in the low C:N ratio treatments than in the high C:N ratio treatments. In the low C:N ratio treatments, *B. monhystera*, *A. nanus* and *P. parvus* had a positive effect on nitrogen mineralization, but the effect of *A. nanus* was only marginally significant. In the high C:N ratio treatments, only *P. parvus* had a significant positive effect on nitrogen mineralization. In the low C:N ratio treatments the effects only became clear at the end of the experiment, whereas in the high C:N ratio treatments the effects were clear throughout the experiment.

In the low C:N ratio treatments, *B. monhystera* and *P. parvus* had a synergetic effect on nitrogen mineralization: the total mineral N concentration was higher in the treatments with

Figure 3. The bacterial community in different nematode treatments at day 84 of the experiment. **a**. Bacterial biomass (mean ± SE). **b**. Bacterial growth rate measured as thymidine incorporation (mean ± SE). **c**. Number of bacterial DNA bands (mean ± SE). C = control, B = *B. monhystera*, A = *A. nanus*, P = *P. parvus*, BA = *B. monhystera* + *A. nanus*, BP = *B. monhystera* + *P. parvus*, AP = *A. nanus* + *P. parvus*.

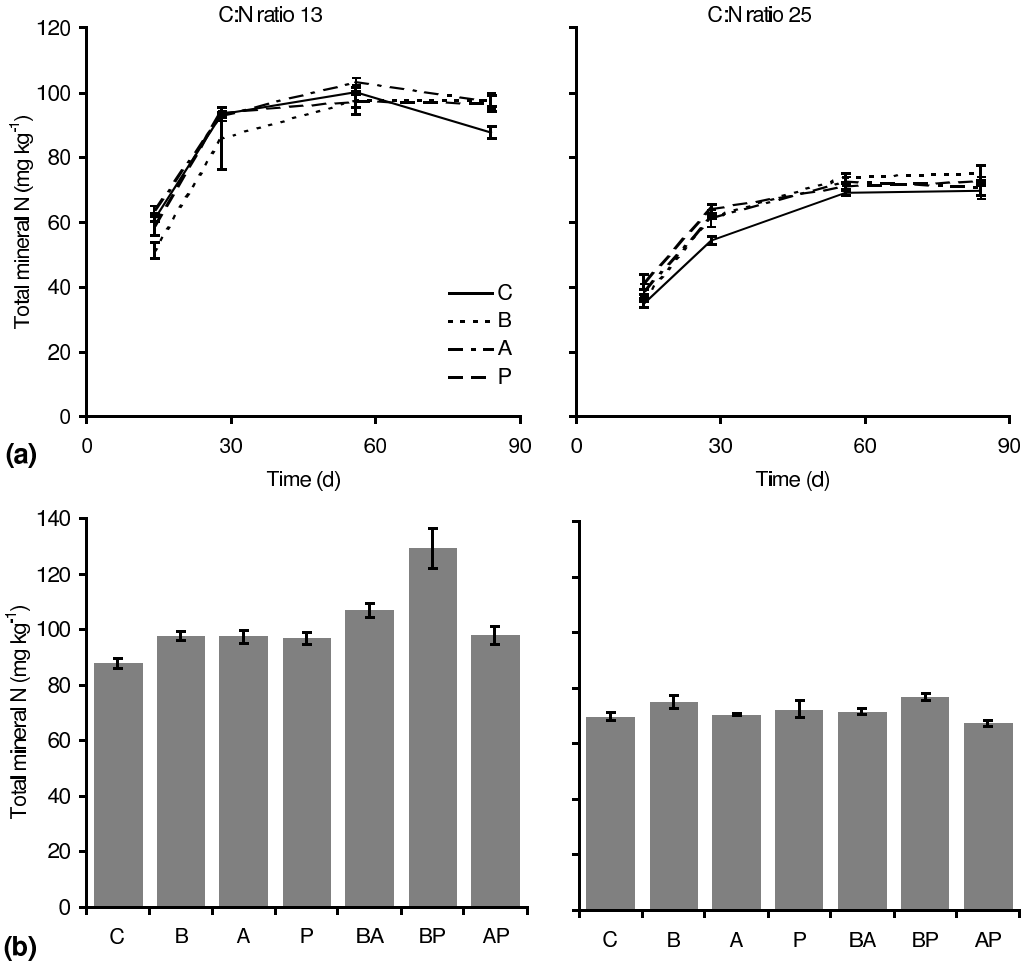


Figure 4. Mineral N (NO_3^- + NH_4^+). **a**. Mineral N (NO_3^- + NH_4^+) (mean ± SE) in the presence or absence of nematode species at two C:N ratios. **b**. Mineral N (NO_3^-+ NH_4^+) (mean ± SE) in different nematode treatments at day 84 of the experiment. C = control, B = *B. monhystera*, A = *A. nanus*, P = *P. parvus*, BA = *B. monhystera* + *A. nanus*, BP = *B. monhystera* + *P. parvus*, AP = *A. nanus* + *P. parvus*.

the combination of the species nematodes than in the treatments with the individual species only (Fig. 4b). In the high C:N ratio treatment, *B. monhystera* and *A. nanus* had a slight negative interaction effect on the nitrogen mineralization: the total mineral N concentration was lower in the treatments with the combination of the species of nematodes than in the treatments with *B. monhystera* alone and similar to the treatments with *A. nanus*.

Discussion

Nematodes

The main effects of the three investigated nematode species on each other, on bacterial variables and on nitrogen mineralization are summarized in Fig. 5. We will henceforth discuss the results in detail. *B. monhystera* numbers rapidly increased, whereas *A. nanus* numbers increased more gradually, which is in accordance with their life-history strategies (Bongers, 1990; Ferris and Matute, 2003). The numbers of *P. parvus*, for unknown reasons, increased only marginally in our experiment. Nematode numbers were generally higher in the high C:N ratio treatments than in the low C:N ratio treatments. According to the literature microfauna are generally food limited (Griffiths, 1994; Mikola and Setälä, 1998b; Wardle, 2002). This was certainly the case in our low C:N ratio treatment. In our high C:N ratio treatment such C limitation was initially absent as a result of the addition of milled wheat straw.

The three bacterivorous nematode species interacted with each other in specific ways. The presence of *A. nanus* did not have a significant overall effect on the population development of *B. monhystera*. *A. nanus* itself, on the contrary, was reduced in the presence of *B. monhystera* right from the beginning, showing that *B. monhystera* was the stronger competitor. Two other studies (Anderson and Coleman, 1981; Mikola and Setälä, 1998c) found that *Acrobeloides* sp. was reduced in competition with cp-1 nematodes (*Mesodiplogaster lheritieri* or *C. elegans*).

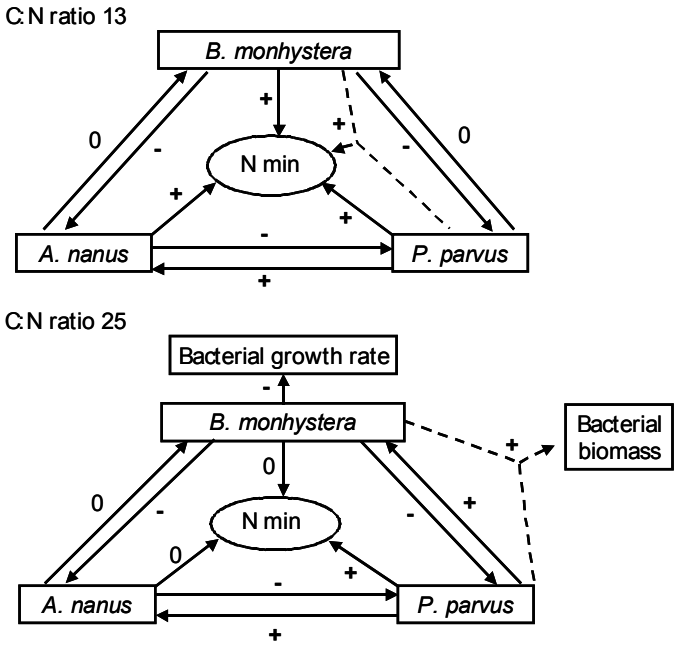


Figure 5. The interactions between the three nematode species at two C:N ratios and between nematodes and bacteria at C:N ratio 25. N min = total mineral N, + = positive effect, 0 = no effect, - = negative effect, solid lines indicate a direct effect, dotted lines indicate an interaction effect.

Even though the species did compete, they did not out-compete each other completely. Nematode numbers were higher in the treatment with the combination of both nematode species than with the individual species each, suggesting that a subtle niche segregation took place. Indeed, Anderson and Coleman (1981) found that *Acrobeloides* sp. had a wider food preference for bacterial species than *M. lheritieri.* In our case *A. nanus* may have changed to a different food source whilst *B. monhystera* was present. We suggest that, in the time period of our experiment, the stronger competitive ability of *B. monhystera* and the wider food preference for bacterial species of *A. nanus* may be general traits of cp-1 and cp-2 nematodes resp.

In the field, other factors may also influence the competitive outcome between different species of nematodes. *A. nanus* was found to have a higher resistance to predation (Mikola and Setälä, 1998c) and had a wider temperature tolerance (Anderson and Coleman, 1982). In the long term, succession can be expected to lead to a reduction of cp-1 nematodes (*B. monhystera*) in favor of cp-2 nematodes (*A. nanus*) (Ferris and Matute, 2003).

Although *P. parvus* was present in very low numbers, it had a positive effect on the numbers of both *B. monhystera* and *A. nanus*. *P. parvus*, on the contrary, was reduced in the presence of both *B. monhystera* and *A. nanus*. This (+,-) effect is called 'contramensalism' (sensu Arthur, 1986). The effect of *P. parvus* on *B. monhystera* was clear in the high C:N ratio treatment only. The presence of *P. parvus* caused an increase in nitrogen mineralization and subsequent increase in bacterial biomass and increase in *B. monhystera* numbers. It remains unclear, however, why a positive effect of *P. parvus* on bacterial biomass was not found in treatments with only *P. parvus* present. In the low C:N ratio treatment, the bacteria were probably C limited, and therefore it can be expected that the presence of *P. parvus* had no effect on bacterial growth and nematodes. Numbers of *P. parvus* were below the detection limit in some samples, and the nematodes may have died. Nitrogen could have been mineralized from dead body tissue and might have stimulated growth of the bacteria and nematodes. , The amount of nitrogen released in this way would be only $1.1*10^{-2}$ ($\mu g\ N\ g^{-1}$) (Didden et al., 1994), however, which is negligible compared to the amounts of nitrogen added (64 $\mu g\ N\ g^{-1}$) and the mineral nitrogen already present in the system (approximately 70 $\mu g\ N\ g^{-1}$).

In our study we found asymmetric competition between *B. monhystera* and *A. nanus*, and a contramensal interaction between both *P. parvus* and *B. monhystera*, and *P. parvus* and *A. nanus* (Fig. 5.) Although few studies are available on competitive interactions among soil organisms within a trophic group, asymmetric competition (Cragg and Bardgett, 2001; Huhta and Viberg, 1999) or contramensalism (Theenhaus et al., 1999) have been found for other soil organisms. Asymmetric competition between species is commonly found in aquatic and aboveground ecosystems, especially with species competing by direct interference (Begon et al., 1990). Our study provides additional evidence for belowground systems.

Bacteria

Bacterial biomass and numbers rapidly increased with time until they stabilized at day 28, probably due to food limitation. We did not find an effect of nematodes on bacterial biomass and numbers, except for the positive effect of the combination of *B. monhystera* and *P. parvus*, nor did we find an effect of nematodes on bacterial growth rate, except for the negative effect of *B. monhystera* (Fig. 3, Fig. 5, Table 2). Other studies, on the contrary, found reduced bacterial biomass and increased bacterial growth rate in the presence of nematodes (Anderson et al., 1983; Bardgett et al., 1999; Bååth et al., 1981; Mikola and Setälä, 1998b; Wardle, 2002). Not all studies agree on this subject, however, Sonneman et al. (1999) found that a natural community of nematodes had no effect on bacterial biomass, but reduced soil respiration, and increased substrate utilization. Bardgett et al. (1998) found that nematodes had a positive effect on microbial biomass, but had no effect on the numbers of culturable bacteria and microbial activity (CO_2 production). Also, Brussaard et al. (1995) found no effect of nematodes on bacterial numbers and Mikola and Setälä (1998c) found that *A. nanus* reduced microbial respiration.

The presence of nematodes generally increased the number of bacterial DNA bands. Although we tried to culture nematodes with a single bacterial species (*Acinetobacter*) prior to inoculation in our microcosms, we could not prevent some contamination with other species (data not shown). Nematodes may thus have introduced bacteria and increased diversity. It is likely, however, that any bacteria that were introduced by nematodes are common species in soil and did not add significantly to the diversity of the community that was inoculated. More likely, nematodes may have caused a measurable increase in bacterial diversity by grazing the dominant species, thereby creating more opportunity for sub-dominant species. Griffiths et al. (1999) found no effect of nematodes (*Coactadera cystilarva* and *Panagrolaimus* spp.) on the number of DNA bands, but did find clear shifts in bacterial community composition in the presence of nematodes, probably a result of selective grazing. Our results suggest that nematodes did not change the total biomass of bacteria but did have an effect on the bacterial community composition and activity. Possibly, nematodes have preferentially eaten the faster growing bacteria, shifting the community to species with lower growth rates, which may have resulted in the negative effect of *B. monhystera* on bacterial growth rate. We may conclude that the effect of nematodes on bacteria probably depends on many factors and that no clear-cut relationship exists (Bardgett et al., 1999).

Interactions between nematode species affected bacterial variables, but not in all cases. The interaction between *B. monhystera* and *P. parvus* positively affected bacterial biomass, but the interaction between *B. monhystera* and *A. nanus*, and *A. nanus* and *P. parvus* had no effect. The interaction between *B. monhystera* and *A. nanus* led to a reduced negative effect of *B. monhystera* on bacterial growth rate, but the interaction between *B. monhystera* and *P. parvus*, and *A. nanus* and *P. parvus* had no effect. Finally, the interaction between *B. monhystera* and *A.*

nanus, and *B. monhystera* and *P. parvus* led to a reduced positive effect of *B. monhystera* on bacterial diversity but the interaction between *A. nanus* and *P. parvus* had no effect.

There are only a few studies on the effects of nematode interactions on bacterial variables. Mikola and Setälä (1998c) found that *A. nanus* and *C. elegans* in monoculture did not affect bacterial biomass differently than when both species were cultured together. They found, however, that *A. nanus* in monoculture reduced microbial respiration (CO_2) more than in mixed cultures with *C. elegans,* indicating a negative interaction effect. In their system, however, these effects could be explained by the higher total nematode biomass in the *A. nanus* monoculture, whereas in our system total nematode numbers were highest in the mixed culture. Laakso and Setälä (1999) did not find effects of diversity within a trophic group on microbial biomass or respiration. Theenhaus et al. (1999) found that the interaction between two collembolan species did not affect microbial biomass, but that respiration was more reduced by collembola in two-species cultures than in one-species cultures. Cragg and Bardgett (2001) found that the presence of one collembolan species caused an increase in respiration, irrespective of the presence or absence of other collembolan species. Generally, interaction between species within a trophic group did not affect microbial biomass, but did affect respiration, positively or negatively, but no-effect cases also occurred.

The mechanism for the interaction effect we found between *A. nanus* and *B. monhystera* might be explained by assuming *A. nanus* had to shift to bacterial species of lower quality and probably lower growth rate in the presence of *B. monhystera*. If so, the bacterial community in the presence of both nematode species would be more balanced between fast and slow growing species, than when the individual species were present. The interaction between *B. monhystera* and *P. parvus*, again, is difficult to explain. The high bacterial biomass in this treatment may explain the increase of *B. monhystera* in this treatment, but the mechanism that caused the increase in bacterial biomass remains obscure.

Nitrogen mineralization

Immobilization of nitrogen occurred in all treatments until day 14, probably due to high bacterial increases. Thereafter, nitrogen mineralization occurred in all treatments until day 56, after which mineralization stopped, probably due to food limitation. All nematode species increased nitrogen mineralization. The effect was higher in the low C:N ratio treatments. Evidently, bacteria were less nitrogen limited in the low C:N ratio treatment, resulting in a lower immobilization and higher net nitrogen mineralization. In the low C:N ratio treatment the effect of nematodes became prominent during the last weeks of the experiment and in the high C:N ratio treatment during the whole experiment. This was probably caused by the fact that from the beginning

nematode numbers were higher in the high C:N ratio treatments than in the low C:N ratio treatments.

Other studies have frequently found an increased nitrogen mineralization by nematodes (Anderson et al., 1983; Bååth et al., 1981; Ferris et al., 1998; Freckman, 1988; Ingham et al., 1985; Mikola and Setälä, 1998a; Verhoef and Brussaard, 1990). This positive effect of nematodes on mineralization can be explained both by direct and indirect effects (Brussaard et al., 1995).

The interaction between nematode species affected nitrogen mineralization in only one out of the three cases present (Fig. 4, Fig. 5, Table 1). The interaction between *B. monhystera* and *P. parvus* caused an increase in nitrogen mineralization, whereas the interaction between *B. monhystera* and *A. nanus*, and *A. nanus* and *P. parvus* had no positive effect on nitrogen mineralization. We could have expected a positive interaction effect of *B. monhystera* and *A. nanus* if we bear in mind that the total number of nematodes was higher in the treatments with the combination of the species. However, the relatively small increase in total nematode numbers was not enough to result in a significant positive effect on nitrogen mineralization. The slightly negative interaction effect of *B. monhystera* and *A. nanus* on nitrogen mineralization in the high C:N ratio treatment may be linked with the positive effect of this combination on bacterial growth rate and therefore possibly higher immobilization in these treatments. The interaction effect of *B. monhystera* and *P. parvus* on nitrogen mineralization can be explained by the higher number of *B. monhystera* in this treatment, but again we remain ignorant of the exact mechanism that caused the increase in bacterial biomass and *B. monhystera* numbers.

We hypothesized that interactions between nematodes of different life-history strategies would lead to increased nitrogen mineralization. Indeed, we found that with greater differences between cp-classes (combination of *B. monhystera* and *P. parvus*) nitrogen mineralization was enhanced the most. We hypothesized that this effect would be caused by differentiation in food sources of the nematode species, which would lead to a more intensive use of the food source and consequently higher nitrogen mineralization. Although numbers of *P. parvus* were very low throughout the experiment, differentiation in food source may have taken place.

Few studies exist on the effects of interactions between soil microfaunal species within a trophic group on nitrogen mineralization, and as yet opinions on this subject differ. Most studies support the idea that species interactions within a trophic group do have an effect on nitrogen mineralization (Mikola and Setälä, 1998c; Wardle, 2002) or that species differ in their effect on nitrogen mineralization (Cragg and Bardgett, 2001; De Mesel et al., 2003; Ferris et al., 1998). Both findings support the idiosyncracy hypothesis that the effect of diversity depends on the species present and their interactions and cannot be predicted (Naeem et al., 1995). In part, the outcome of species interactions may depend on their momentary choice of microhabitat in the soil profile (or, the related substrate quality) (Faber and Verhoef, 1991). On the other hand,

Laakso and Setälä (1999) found no effect of the addition of one or five species within a trophic group, which is supportive of the redundancy hypothesis. Still, also in their experiment the effect of one collembola species was greatly different from all the others, which does support the idiosyncracy hypothesis. In accordance with these studies our results provide evidence that interactions between soil microfaunal species within a trophic group can affect the bacterial biomass and ecosystem properties, which is consistent with the idiosyncracy hypothesis. In our study, however, we investigated interactions at the very low end of the diversity spectrum, i.e. a species-poor system. In more species-rich systems the chances for presence of more redundant species will be higher.

Conclusions

We found that nematode species within the trophic group of bacterivores interacted with each other in asymmetric competition (0 , -) (*B. monhystera* and *A. nanus*) or a contramensal pattern (+ , -) (*B. monhystera* and *P. parvus*, and also *A. nanus* and *P. parvus*). These interactions affected the bacterial community and nitrogen mineralization in some, but not all treatments, depending on the species combination. The interaction that affected soil ecosystem parameters the most was the interaction between *B. monhystera* and *P. parvus*, the two species with the most different life history strategies. This finding is consistent with the hypothesis that ecological functioning of nematode communities at low species richness increases with distance in ecological traits of species present (Heemsbergen et al., 2004). We conclude that interactions between bacterivorous nematodes can influence ecosystem processes, but the effect cannot be predicted in general. At the very low number of species of the present study (1 or 2 species of nematodes from one trophic group) we found evidence for the idiosyncracy hypothesis of biodiversity.

Acknowledgements

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References

Anderson, R. V., and D. C. Coleman. 1981. Population development and interactions between two species of bacteriophagic nematodes. Nematologica 27: 6-19.

Anderson, R. V., and D. C. Coleman. 1982. Nematode temperature responses: a niche dimension in populations of bacterial-feeding nematodes. Journal of Nematology 14: 69-76.

Anderson, R. V., W. D. Gould, L. E. Woods, C. Cambardella, R. E. Ingham, and D. C. Coleman. 1983. Organic and inorganic nitrogenous losses by microbivorous nematodes in soil. Oikos 40: 75-80.

Arthur, W. 1986. On the complexity of a simple environment: competition, resource partitioning and facilitation in a two-species Drosophila system. Philisophical Transactions of the Royal Society B 313: 471-508.

Bååth, E., U. Lohm, B. Lundgren, T. Rosswall, B. Söderström, and B. Sohlenius. 1981. Impact of microbial-feeding animals on total soil activity and nitrogen dynamics: a soil microcosm experiment. Oikos 37: 257-264.

Ballinger, S. J., I. M. Head, T. P. Curtis, and A. R. Godley. 2002. The effect of C/N ratio on ammonia oxidising bacteria community structure in a laboratory nitrification-denitrification reactor. Water Science and Technology 46: 543-550.

Bardgett, R. D., and R. Cook. 1998. Functional aspects of soil animal diversity in agricultural grasslands. Applied Soil Ecology 10: 263-276.

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

Bardgett, R. D., S. Keiller, R. Cook, and A. S. Gilburn. 1998. Dynamic interactions between soil animals and microorganisms in upland grassland soils amended with sheep dung: a microcosm experiment. Soil Biology & Biochemistry 30: 531-539.

Begon, M., J. L. Harper, and C. R. Townsend 1990. . Blackwell Scientific Publications, Boston.

Bloem, J., and A. M. Breure 2003. Microbial indicators. Pages 259-282 *in* Markert, B A, Breure, A M, Zechmeister, H G, editors. Bioindicators/biomonitors - principles, assessment, concepts. Elsevier, Amsterdam.

Bloem, J., M. Veninga, and J. Shepherd. 1995a. Fully automatic determination of soil bacterium numbers, cell volumes and frequencies of dividing cells by confocal laser scanning microscopy and image analysis. Applied and Environmental Microbiology 61: 926-936.

Bloem, J., P. R. Bolhuis, M. Veninga, and J. Wieringa 1995b. Microscopic methods for counting bacteria and fungi in soil. Pages 162-173 *in* K. P. N. Alef, editors. Methods in applied soil microbiology and biochemistry. Academic Press, London.

Bongers, T.. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14-19.

Bongers, T., and H. Ferris. 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology & Evolution 14: 224-228.

Bouwman, L. A., G. H. J. Hoenderboom, K. J. Van der Maas, and P. C. De Ruiter. 1996. Effects of nematophagous fungi on numbers and death rates of bacterivorous nematodes in arable soil. Journal of Nematology 28: 26-35.

Brussaard, L., R. Noordhuis, M. Geurs, and L. A. Bouwman. 1995. Nitrogen mineralization in soil in microcosms with or without bacterivorous nematodes and nematophagous mites. Acta Zoologica Fennica 196: 15-21.

Brussaard, L., V. M. Behan-Pelletier, D. E. Bignell, V. K. Brown, W. Didden, P. Folgarait, C. Fragoso, D. W. Freckman, V. Gupta, T. Hattori, D. L. Hawksworth, C. Klopatek, P. Lavelle, D. W. Malloch, J. Rusek, B. Soderstrom, J. M. Tiedje, and R. A. Virginia. 1997. Biodiversity and ecosystem functioning in soil. Ambio 26: 563-570.

Cragg, R. G., and R. D. Bardgett. 2001. How changes in soil faunal diversity and composition within a trophic group influence decomposition processes. Soil Biology & Biochemistry 33: 2073-2081.

De Mesel, I., S. Derycke, J. Swings, M. Vincx, and T. Moens. 2003. Influence of bacterivorous nematodes on the decomposition of cordgrass. Journal of Experimental Marine Biology and Ecology 296: 227-242.

Didden, W. A. M., J. C. Y. Marinissen, M. J. Vreeken-Buijs, S. L. G. E. Burgers, R. De Fluiter, M. Geurs, and L. Brussaard. 1994. Soil meso- and macrofauna in two agricultural systems: factors affecting population dynamics and evaluation of their role in carbon and nitrogen dynamics. Agriculture, Ecosystems & Environment 51: 171-186.

Dilly, O., J. Bloem, A. Vos, and J. C. Munch. 2004. Bacterial diversity during litter decomposition in agricultural soils. Applied and Environmental Microbiology 70: 468-474.

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

Faber, J. H., and H. A. Verhoef. 1991. Functional differences between closely related soil arthropods with respect to decomposition and nitrogen mobilization in a pine forest. Soil Biology & Biochemistry 23: 15-23.

Ferris, H., and M. M. Matute. 2003. Structural and functional succession in the nematode fauna of a soil food web. Applied Soil Ecology 23: 93-110.

Ferris, H., R. C. Venette, H. R. Van der Meulen, and S. S. Lau. 1998. Nitrogen mineralization by bacterial-feeding nematodes: verification and measurement. Plant and Soil 203: 159-171.

Freckman, D. W.. 1988. Bacterivorous nematodes and organic-matter decomposition. Agriculture, Ecosystems & Environment 24: 195-217.

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., M. Bonkowski, F. Dobson, and S. Caul. 1999. Changes in soil microbial community structure in the presence of microbial-feeding nematodes and protozoa. Pedobiologia 43: 297-304.

Heemsbergen, D. A., M. P. Berg, M. Loreau, J. R. van Haj, J. H. Faber, and H. A. Verhoef. 2004. Biodiversity effects on soil processes explained by interspecific functional dissimilarity. Science 306: 1019-1020.

Huhta, V., and K. Viberg. 1999. Competitive interaction between the earthworm Dendrobaena octaedra and the enchytraeid *Cognettia sphagnetorum*. Pedobiologia 43: 886-890.

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

Laakso, J., and H. Setälä. 1999. Sensitivity of primary production to changes in the architecture of belowground food webs. Oikos 87: 57-64.

Marschner, P., E. Kandeler, and B. Marschner. 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biology & Biochemistry 35: 453-461.

Michel, P. H., and J. Bloem. 1993. Conversion factors for estimation of cell production rates of soil bacteria from thymidine and leucine incorporation. Soil Biology & Biochemistry 25: 943-950.

Mikola, J., and H. Setälä. 1998a. Productivity and trophic-level biomasses in a microbial-based soil food web. Oikos 82: 158-168.

Mikola, J., and H. Setälä. 1998b. No evidence of trophic cascades in an experimental microbial-based soil food web. Ecology 79: 153-164.

Mikola, J., and H. Setälä. 1998c. Relating species diversity to ecosystem functioning: mechanistic backgrounds and experimental approach with a decomposer food web. Oikos 83: 180-194.

Muyzer, G., E. C. De Waal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59: 695-700.

Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1995. Empirical evidence that declining species diversity may alter the performance of terrestrial ecosystems. Philisophical Transactions of the Royal Society B 347: 249-262.

Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. Pages 85-102 *in* J. M. Sasser, and W. R. Jenkins, editors. Nematology. University of North Carolina Press, Chapel Hill.

Sonneman, I., H. Dogan, A. Klein, B. Pieper, K. Ekschmitt, and V. Wolters. 1999. Response of soil microflora to changes in nematode abundance - evidence for large scale effects in grassland soil. Journal of Plant Nutrition and Soil Science 162: 385-391.

Theenhaus, A., A. Scheu, and M. Schaeffer. 1999. Contramensal interactions between two collembolan species: effects on populations development and soil processes. Functional Ecology 13: 238-246.

Van Elsas, J. D., and K. Smalla 1995. Extraction of microbial community dna from soils. Pages 1.3.3.1-1.3.3.11 *in* A. D. L. Akkermans, J. D. Van Elsas, and F. J. De Bruijn, editors. Molecular microbial ecology manual. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Verhoef, H. A., and L. Brussaard. 1990. Decomposition and nitrogen mineralization in natural and agro-ecosystems: the contribution of soil animals. Biogeochemistry 11: 175-211.

Wardle, D. A. 2002. Communities and ecosystems, linking the aboveground and belowground components. Princeton University Press, Oxford.

Chapter 3

Earthworm species composition affects the soil bacterial community and net nitrogen mineralization

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Abstract

Knowledge of the effects of species diversity within taxonomic groups on nutrient cycling is important for understanding the role of soil biota in sustainable agriculture. We hypothesized that earthworm species specifically affect nitrogen mineralization, characteristically for their ecological group classifications, and that earthworm species interactions would affect mineralization through competition and facilitation effects. A mesocosm experiment was conducted to investigate the effect of three earthworm species, representative of different ecological groups (epigeic: *Lumbricus rubellus*; endogeic: *Aporrectodea caliginosa tuberculata*; and anecic: *Lumbricus terrestris*) and their interactions on the bacterial community, and on nitrogen mineralization from 15N-labeled crop residue and from soil organic matter.

Our results indicate that *L. rubellus* and *L. terrestris* enhanced mineralization of the applied crop residue whereas *A. caliginosa* had no effect. On the other hand, *L. rubellus* and *A. caliginosa* enhanced mineralization of the soil organic matter, whereas *L. terrestris* had no effect. The interactions between different earthworm species affected the bacterial community and the net mineralization of soil organic matter. The two-species interactions between *L. rubellus* and *A. caliginosa*, and *L. rubellus* and *L. terrestris*, resulted in reduced mineral N concentrations derived from soil organic matter, probably through increased immobilization in the bacterial biomass. In contrast, the interaction between *A. caliginosa* and *L. terrestris* resulted in increased bacterial growth rate and reduced total soil C. When all three species were combined, the interaction between *A. caliginosa* and *L. terrestris* was dominant.

We conclude that the effects of earthworms on nitrogen mineralization depend on the ecological traits of the earthworm species present, and can be modified by species interactions. Knowledge of these effects can be made useful in the prevention of nutrient losses and increased soil fertility in agricultural systems, that typically have a low earthworm diversity.

Introduction

The importance of soil biota for sustainable agricultural production is increasingly acknowledged (Brussaard et al., 1997; Fragoso et al., 1997; Bardgett and Cook, 1998; Wardle, 2002). However, many questions remain on the importance of the diversity within soil biota groups for continued ecosystem functioning in agriculture (Swift et al., 2004). Earthworms are known to increase nitrogen mineralization (Blair et al., 1997; Cortez et al., 2000), and thereby can increase nutrient availability in systems with reduced human influence and low nutrient status, i.e. no tillage, reduced mineral fertilizer use and low organic matter content (Ruz-Jerez et al., 1992; Doube et al., 1997; Brown et al., 1998; Brown et al., 1999; Cortez and Hameed, 2001). The effect of earthworms on nitrogen mineralization and crop production may, however, depend on earthworm species and species interactions present in the system (Brown et al., 1999).

Earthworm species have been found to interact by competition for food (Abbott, 1980; Dalby et al., 1998; Lowe and Butt, 1999; Baker et al., 2002; Lowe and Butt, 2002) and by affecting each other's burrow system (Capowiez et al., 2001; Jégou et al., 2001). These interactions directly affected the abundances of the earthworm species populations, but may indirectly also affect nitrogen mineralization and crop production. There are indications that an increased species diversity of earthworms has a positive effect on crop production (Brown et al., 1999). Few studies, however, have directly addressed such effects of interactions between earthworm species on ecosystem processes (Wardle, 2002).

Earthworms can be classified as epigeics (living in litter or top soil layers where they forage primarily on plant residues), anecics (living in permanent deep vertical burrows in which they store plant residues that are collected from the soil surface) and endogeics (living in the soil and foraging on soil organic matter) (Bouché, 1977). Competition for food may lead to resource partitioning and a higher total abundance of the competing earthworm species compared to the single species community, cf. general ecology (Begon et al., 1990) and data for nematodes (Chapter 2). This may in turn result in increased incorporation of crop residues into the soil. Alternatively, by incorporating organic matter into the soil, epigeic and anecic species may provide an extra food source for endogeic species (Hughes et al., 1994; Jégou et al., 2001), and thus stimulate the feeding activity of endogeic species. Earthworms affect nitrogen mineralization through direct and indirect effects on the microbial community. By incorporating organic matter into the soil and by grazing on the bacterial community earthworms have been found either to enhance or decrease bacterial biomass (Ruz-Jerez et al., 1992; Scheu and Parkinson, 1994; Bohlen and Edwards, 1995; Blair et al., 1997; Brown et al., 1998; Cortez et al., 2000), and to stimulate bacterial activity (Daniel and Anderson, 1992; Ruz-Jerez et al., 1992; Wolters and Joergensen, 1992; Bohlen and Edwards, 1995). This may result in enhanced nitrogen immobilization or mineralization depending on species characteristics and substrate quality.

We performed a mesocosm experiment with three earthworm species from different ecological groups (Bouché, 1977; Perel, 1977) (*Lumbricus rubellus*, *Aporrectodea caliginosa tuberculata* and *Lumbricus terrestris*). We studied the bacterial community and soil mineral N concentrations in the presence and absence of these species and every species combination. To distinguish between mineral N derived from crop plant residues (residue-derived N_{min}) and from soil organic matter (SOM-derived N_{min}) as a result of litter feeding or soil feeding activities by the earthworms, we used ^{15}N labeled plant materials (Brown et al., 1998; Bohlen et al., 1999). We hypothesized that earthworm species of different ecological groups would specifically affect nitrogen mineralization of the crop residue and soil organic matter, and that interactions between the earthworm species would affect nitrogen mineralization, through competition and facilitation effects. We hypothesized further that the effects on nitrogen mineralization could be explained from effects on the bacterial biomass and growth rate.

Materials and methods

Experimental set-up

A mesocosm experiment was performed in PVC columns with sterilized soil amended with organic ^{15}N-labeled potato crop residue. We tested the effect of earthworm species and species interactions on soil mineral N concentrations by manipulation of three earthworm species: *Lumbricus rubellus* (Hoffmeister, 1843), *Aporrectodea caliginosa tuberculata* (Eisen, 1874), and *Lumbricus terrestris* L., in different combinations. Three single species treatments were established, four multi-species combinations (*L. rubellus* + *A. caliginosa*, *L. rubellus* + *L. terrestris*, *A. caliginosa* + *L. terrestris* and *L. rubellus* + *A. caliginosa* + *L. terrestris*) and a control free of earthworms. We analyzed earthworm species biomass and species interactions effects, using a regression analysis. The entire experiment included 8 species combinations, with earthworm biomass varying over 4 mesocosms within each treatment, equaling 32 experimental units (Table 1).

Mesocosms

Mesocosms were constructed of PVC columns (20 cm diameter, 45 cm height), which were split in two longitudinal halves and fitted together with tape. By separating the halves we could take samples from specific depths. On 12 and 13 March 2003 mesocosms were filled with the equivalent of 12 kg dry weight soil taken from a Fimic Anthrosol (FAO-UNESCO, 1988) situated in 'De Bovenbuurt' (51°59'N, 5°40'E), The Netherlands. Soil material was collected (July 2002) from 0-30 cm depth in an arable field (sandy soil, under intensive arable culture for the last 20 years, organic matter content 3.28 %). The soil was sieved over a 5 mm mesh to remove roots and coarse materials, and γ-irradiated (25 kGy) to eliminate all living organisms. The soil was air-dried and remoistened to 60% WHC. During the experiment soil moisture was kept constant gravimetrically with tap water, every week. After sterilization, drying and

Table 1. Earthworm abundance and biomass added per mesocosm. Co = control, R = *L. rubellus*, C = *A. caliginosa*, T = *L. terrestris*, RC = *L. rubellus* + *A. caliginosa*, RT = *L. rubellus* + *L. terrestris*, CT = *A. caliginosa* + *L. terrestris*, RCT = *L. rubellus* + *A. caliginosa* + *L. terrestris*.

		Count			Biomass (g)					Count			Biomass (g)		
Treatment	**Mesocosm**	**R**	**C**	**T**	**R**	**C**	**T**	**Treatment**	**Mesocosm**	**R**	**C**	**T**	**R**	**C**	**T**
Co	1	-	-	-	-	-	-	RC	17	3	1	-	2.5	0.9	-
Co	2	-	-	-	-	-	-	RC	18	1	3	-	1.3	2.3	-
Co	3	-	-	-	-	-	-	RC	19	5	4	-	4.8	3.1	-
Co	4	-	-	-	-	-	-	RC	20	2	6	-	2.2	3.8	-
R	5	2	-	-	1.9	-	-	RT	21	1	-	2	0.9	-	9.7
R	6	4	-	-	3.7	-	-	RT	22	2	-	1	2.1	-	5.7
R	7	6	-	-	5.5	-	-	RT	23	3	-	2	3.4	-	12.5
R	8	10	-	-	9.8	-	-	RT	24	5	-	1	5.4	-	5.0
C	9	-	2	-	-	1.7	-	CT	25	-	1	2	-	0.8	8.6
C	10	-	6	-	-	4.0	-	CT	26	-	3	1	-	2.2	6.2
C	11	-	8	-	-	5.8	-	CT	27	-	4	2	-	2.4	9.8
C	12	-	12	-	-	8.7	-	CT	28	-	6	1	-	3.7	3.6
T	13	-	-	1	-	-	5.5	RCT	29	3	1	2	3.3	0.7	8.3
T	14	-	-	1	-	-	5.7	RCT	30	1	3	1	0.9	2.5	6.3
T	15	-	-	2	-	-	16.0	RCT	31	5	4	2	5.4	2.9	12.5
T	16	-	-	2	-	-	9.8	RCT	32	2	6	1	1.9	3.9	4.4

remoistening, pH-H_2O equaled 6.0. Mesocosms were filled to 36 cm to ensure a constant bulk density approximating 1.11 g cm^{-3}.

Rhizon installation

Rhizons (miniature porous cups with a 2.5 mm diameter and 10 cm long hydrophilic porous polymer tube - Rhizon Soil Solution Samplers, art. number 19.21.25, Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) were installed in the mesocosms in order to repeatedly sample soil moisture. Two replicate rhizons were installed through holes in the side of each mesocosm, at 12 and 15 cm soil depth. Rhizons were installed in horizontal position in a crossed way above each other with a distance of 3 cm to minimize the influence of the replicates on each other's extraction efficiency.

Microbiota

The sterilized soil was re-inoculated with soil microbiota. For this purpose we sieved unsterilized air dried soil (from 'De Bovenbuurt' fields) with a 2 mm mesh to remove earthworm cocoons. In each mesocosm, 500 g of this inoculum soil was mixed through the sterilized soil before filling. Prior to the experiment, the mesocosms were incubated for 18 days in a climate chamber in the dark with a day/night temperature of 20/15 ºC ('day period' 16 hrs) and air humidity of 80%. The

mesocosms were covered with a plastic sheet with some holes, to prevent excess evaporation. After incubation, total soil N was 1.01 g kg^{-1}, total soil C was 19.2 g kg^{-1}, $N\text{-}NO_3$ was 39.9 mg kg^{-1} and $N\text{-}NH_4$ was 1.2 mg kg^{-1}.

^{15}N-labeled crop residue

After preincubation, potato (*Solanum tuberosum* L.) leaf and stem material ('crop residue') was added as food for the earthworms. The potato plants had been labeled with ^{15}N (Van Groenigen et al., 2005). The total N percentage of the crop residue was 1.56%, and the atomic excess of the potato crop residue for ^{15}N was 1.2368% ±0.0430%. The crop residue was dried, ground to approximately 5 cm, and remoistened before addition to the mesocosms on top of the soil. We added 75 g dry weight of crop residue to each mesocosm. This quantity was administered as ample food for the earthworms to remain vital during experimentation (Jos Bodt, personal communication, 2003).

Earthworms

After preincubation, the experiment was started by inoculating the mesocosms with earthworms. Earthworm guts were not voided since this would have reduced their vitality. The earthworm species were collected in the field prior to the experiment: *L. rubellus* and *L. terrestris* from a production grassland near Nijkerk, The Netherlands, *A. caliginosa* from a production grassland near Rheden, The Netherlands. The earthworms were kept at 15 ºC temperature on a turf medium with alder leaves (*Alnus glutinosa*) as a food source until experimentation. *L rubellus* was introduced ranging from 1 to 10 specimens per mesocosm, with a maximum total biomass of 9.8 g per mesocosm. *A. caliginosa* numbers ranged from 1 to 12 specimens with a maximum total biomass of 8.7 g per mesocosm. *L. terrestris* numbers ranged from 1 to 2 specimens with a maximum total biomass of 16.0 g per mesocosm (Table 1). In the 2- and 3-species combinations we added a maximum number of 11 earthworms per mesocosm (equivalent to 350 ind. m^{-2}), and a maximum biomass of 20.8 g per mesocosm (equivalent to 663 g m^{-2}). Earthworm densities ranged from densities comparable to those in arable soil, in the mesocosms with 1, 2 or 3 earthworms (Lee, 1985), to densities comparable to those in temperate grassland ecosystems, in the mesocosms with the maximum number of earthworms (Lee, 1985). This range was chosen with respect to the possibility of increasing earthworm densities in arable systems to densities that may be feasible under ideal circumstances.

After addition of earthworms, the mesocosms were covered with a nylon mesh to prevent animals from escaping. The mesocosms were kept in a climate chamber with a temperature of 20 ºC and a daylight period of 12 hours. The experiment was conducted March 31 until June 2 in 2003.

Measurements

Mineral N sampling with rhizons

The soil solution was sampled 14, 42 and 56 days after introductions of earthworms. The concentrations of $N\text{-}NO_3$ and $N\text{-}NH_4$ at day 14 and 42 were measured using Skalar (Breda) continuous flow analysis, those at day 56 were calculated on the basis of the data obtained from the Stable Isotope Facility (see below), due to a measuring artifact with continuous flow analysis. To distinguish between SOM-derived N_{min} and residue-derived N_{min} the samples were analyzed for ^{15}N atomic excess of mineral N. Mineral ^{15}N was determined after isolation of $N\text{-}NO_3$ and $N\text{-}NH_4$ using the micro-diffusion method of Brooks et al. (1989), modified by Sorensen and Jensen (1991). The method was identical to that described by Van Groenigen et al. (2005), except that $^{15}N\text{-}NO_3$ and $^{15}N\text{-}NH_4$ were determined simultaneously, rather than only $^{15}N\text{-}NO_3$, by adding 0.4 g Devarda's alloy and 0.4 g MgO simultaneously. The acidified filters, packed in teflon, were incubated for 7 or 8 days, dried and analyzed for ^{15}N atomic excess on an isotope ratio mass spectrometer (ANCA-IRMS, Europa Scientific Integra, UK) interfaced with a CN sample converter at the UC Davis Stable Isotope Facility, with atmospheric N_2 as a standard (0.3663% atomic excess). The ^{15}N enrichments of the samples were corrected for background abundances in the matrix solution and filter paper.
Based on the data obtained from the Stable Isotope Facility we calculated the total mineral N amounts on day 56. Since the recovery of nitrogen after preparation of the samples for ^{15}N analysis averaged 90%, we corrected the mineral N data obtained from the Stable Isotope Facility with a factor 100/90.

Harvesting of the mesocosms, total soil N, total soil C

Mesocosms were harvested randomly 62 and 63 days after the start of the experiment. Due to a failure of the climate chamber on day 61, the ambient temperature had increased to 40 ºC for 8 hours. The mesocosms were cooled at 4 ºC immediately afterwards. Earthworms had died in all treatments, and final biomass could therefore not be determined. However, in all treatments remains of earthworms of the different species were found, suggesting that earthworms had been alive in the treatments until day 61 of the experiment. We expect that a short period with high temperature did not cause major changes in bacterial biomass and diversity since 40 ºC is not high enough to kill bacteria, and it usually takes more than 8 hours to induce significant changes in bacterial biomass. Bacterial growth rate (thymidine and leucine incorporation) may be more sensitive to an increased temperature. Nevertheless, we assume that the differences between treatments were not affected by the temperature incident. Mineral N data were not affected, since all mineral N measurements were taken before the accident. Total soil N and C amounts are relatively stable parameters, that are probably not affected by a short temperature increase.
The remainders of the applied crop residue on top of the mesocosms were removed by hand. Since dead earthworm tissue was found mainly in the lower part of the soil mesocosm, soil from the upper 13 cm of the mesocosm was separated from the lower part and analyses were restricted

to the upper parts of the mesocosms. Sub-samples were dried at 40 ºC, ball-milled and approximately 40 mg was weighed out exactly in tin cups. The samples were analyzed for total soil N. Total soil C was analyzed by wet oxidation with $K_2Cr_2O_7$ (Walinga et al., 1992).

Bacterial biomass, growth rate and DNA analysis

Bacterial numbers and cell volumes at day 62 were measured by confocal laser scanning microscopy and automatic image analysis, and biomass was calculated from biovolume (Bloem et al., 1995a; Bloem et al., 1995b). The bacterial growth rate was determined by thymidine incorporation, a measure for DNA synthesis (growth rate) and leucine incorporation, a measure for protein synthesis (growth rate and biomass turnover) (Michel and Bloem, 1993). These analyses were carried out for each earthworm species combination, for three out of four randomly chosen mesocosms. From a selection of the treatments viz. the control, *L. rubellus* in single species culture, *L. rubellus* and *A. caliginosa* in mixed species culture and *L. rubellus*, *A. caliginosa* and *L. terrestris* in mixed species culture, DNA was extracted (Van Elsas and Smalla, 1995) and analyzed by denaturating gradient gel electrophoresis (DGGE) (Muyzer et al., 1993). This technique yields a banding pattern where the number of DNA bands reflects the number of 'species' (genotypes) of abundant bacteria, and the band-intensity reflects the relative abundance of the species (Bloem and Breure, 2003; Dilly et al., 2004).

Calculations and statistical analyses

Total mineral N was calculated as the sum of $N\text{-}NO_3$ and $N\text{-}NH_4$. Residue-derived N_{min} was calculated as follows:

(1) total excess ^{15}N (mg l^{-1}) = total mineral N (mg l^{-1}) *atom % ^{15}N excess / 100

(2) residue-derived N_{min} (mg l^{-1}) = total excess ^{15}N (mg l^{-1}) * 100 /atom % ^{15}N excess of the crop residue.

The SOM-derived N_{min} was calculated as the total mineral N minus the residue-derived N_{min}. Calculations of atom %^{15}N excess in mineral N were based on a natural background abundance value of 0.3663 atom % excess.

Statistical analyses were carried out using the statistical package SPSS version 11.0. Data were analyzed using backward regression with the three single species and all 2-way and 3-way interactions as input variables. In this procedure only significant parameters were retained in the model. Since the relationship between earthworm species biomass and several output parameters is regarded to be a square root rather than a linear function, square root transformations were applied in several cases, to increase model fit and to meet assumptions of homogeneity of variances and normality of data. For five variables one or two outliers were removed based on the Cook's Distance. The effect of the selected earthworm treatments on the number of bacterial DNA bands was analyzed with a one-factor ANOVA, followed by a Tukey post-hoc analysis.

Results

Mineral N derived from crop residue

The concentrations of residue-derived N_{min} in the soil solution generally increased during experimentation, with final concentrations ranging between 8 and 20 mg l^{-1}. *L. rubellus* induced an increase in residue-derived N_{min} from day 14 of the experiment onwards, with a 140% increase per 10 *g* earthworm biomass on day 56 ($P < 0.001$ on day 14, on day 42 and on day 56, Table 2, Fig. 1). *L. terrestris* enhanced residue-derived N_{min} from day 42 of the experiment onwards, with a 72% increase per 10 g earthworm biomass on day 56 ($P < 0.001$ on day 42 and on day 56, Table 2, Fig. 1). *A. caliginosa* did not affect residue-derived N_{min}, except for a small positive effect on day 14 (Table 2). No interaction effects of the earthworm species on residue-derived N_{min} were found.

Mineral N derived from soil organic matter

The concentrations of SOM-derived N_{min} in the soil solution were approximately 300 mg l^{-1}, with little variation over time. No effects of the earthworms on SOM-derived N_{min} were observed up to day 42. On day 56, *L. rubellus* and *A. caliginosa* enhanced SOM-derived N_{min}. *L. rubellus* enhanced SOM-derived N_{min} by 32% per 10 g earthworm biomass ($P < 0.001$), *A. caliginosa* enhanced SOM-derived N_{min} by 15% per 10 g earthworm biomass ($P = 0.005$) (Table 2, Fig. 2a-b).

Table 2. Statistical results of the backward regression analysis of earthworm species biomass (g) and species interaction effects on system variables. Abbreviations: N_{res}=Mineral N from the crop residue (mg l^{-1}), N_{som}= Mineral N from soil organic matter (mg l^{-1}), R = *L. rubellus*, C = *A. caliginosa*, T = *L. terrestris*, RC = *L. rubellus * A. caliginosa*, RT = *L. rubellus * L. terrestris*, CT = *A. caliginosa * L. terrestris*, RCT = *L. rubellus * A. caliginosa * L. terrestris*.

Response variable	Model coefficients	R^2	P-value
N_{res} (mg l^{-1}) Day 14	=+2.41+0.50·R +0.20·C	0.465	<0.001
N_{res} (mg l^{-1}) Day 42	=+7.74+0.94·R +0.44·T	0.529	<0.001
N_{res} (mg l^{-1}) Day 56	=+8.78+1.23·R +0.63·T	0.686	<0.001
N_{som} (mg l^{-1}) Day 14	=+328		n.s.
N_{som} (mg l^{-1}) Day 42	=+332		n.s.
N_{som} (mg l^{-1}) Day 56	=+335+34.19·√R +15.70·√C -23.66·√RC -10.44·√RT -11.21·√CT +9.30·√RCT	0.740	<0.001
Total C (g kg^{-1})	=+20.5-0.12·CT	0.258	0.004
Total N (g kg^{-1})	=+1.08		n.s.
Bacterial biomass (μg C g^{-1})	=+74.8+7.73·√RC +1.02·RT -1.26·RCT	0.546	<0.001
Thymidine incorporation (pmol $g^{-1}h^{-1}$)	=+31.6		n.s.
Leucine incorporation (pmol $g^{-1}h^{-1}$)	=+192+2.53·CT	0.277	0.009

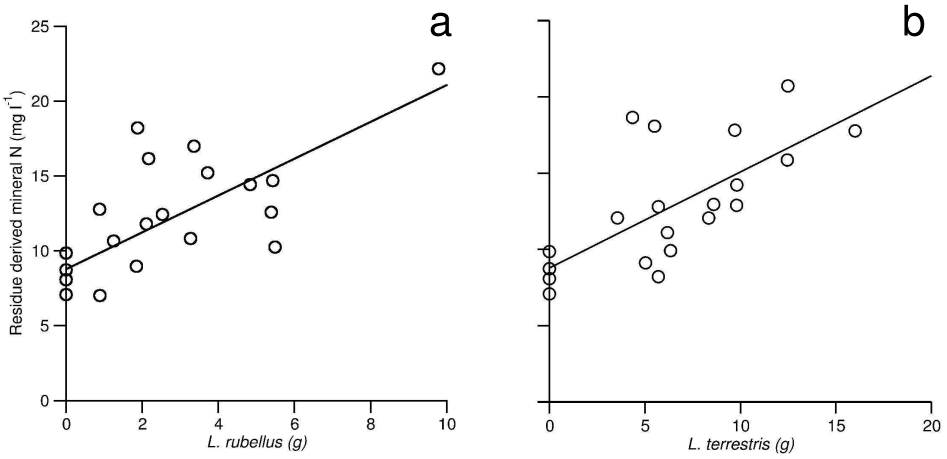

Fig. 1a,b. Partial residual plots of the effect of **a** *L. rubellus* (g) and **b** *L. terrestris* (g) on the concentrations of mineral N derived from the crop residue (mg l^{-1}) on day 56 of the experiment, as determined by backward regression analysis. For the model R^2 and significance, and the coefficients of the parameters included in the model, see Table 2.

All two-species interactions negatively affected SOM-derived N_{min} on day 56: SOM-derived N_{min} in these mixed species treatments was lower than could have been expected based on the single species treatments ($P < 0.001$ for the interaction between *L. rubellus* and *A. caliginosa,* $P < 0.001$ for the interaction between *L. rubellus* and *L. terrestris* and $P = 0.001$ for the interaction between *A. caliginosa* and *L. terrestris*, Table 2, Fig. 2c-e). When all three species were present together, however, we found a positive interaction effect on SOM-derived N_{min} ($P = 0.002$, Table 2, Fig. 2f).

Total N and total C in the soil

At the end of the experiment, total soil N averaged 1.08 g kg^{-1} and the amounts of total soil C ranged between 17 and 23 g kg^{-1}. None of the earthworm species had a main effect on total soil N or total soil C. The interaction between *A. caliginosa* and *L. terrestris*, however, negatively affected total soil C ($P = 0.004$, Table 2, Fig. 4b).

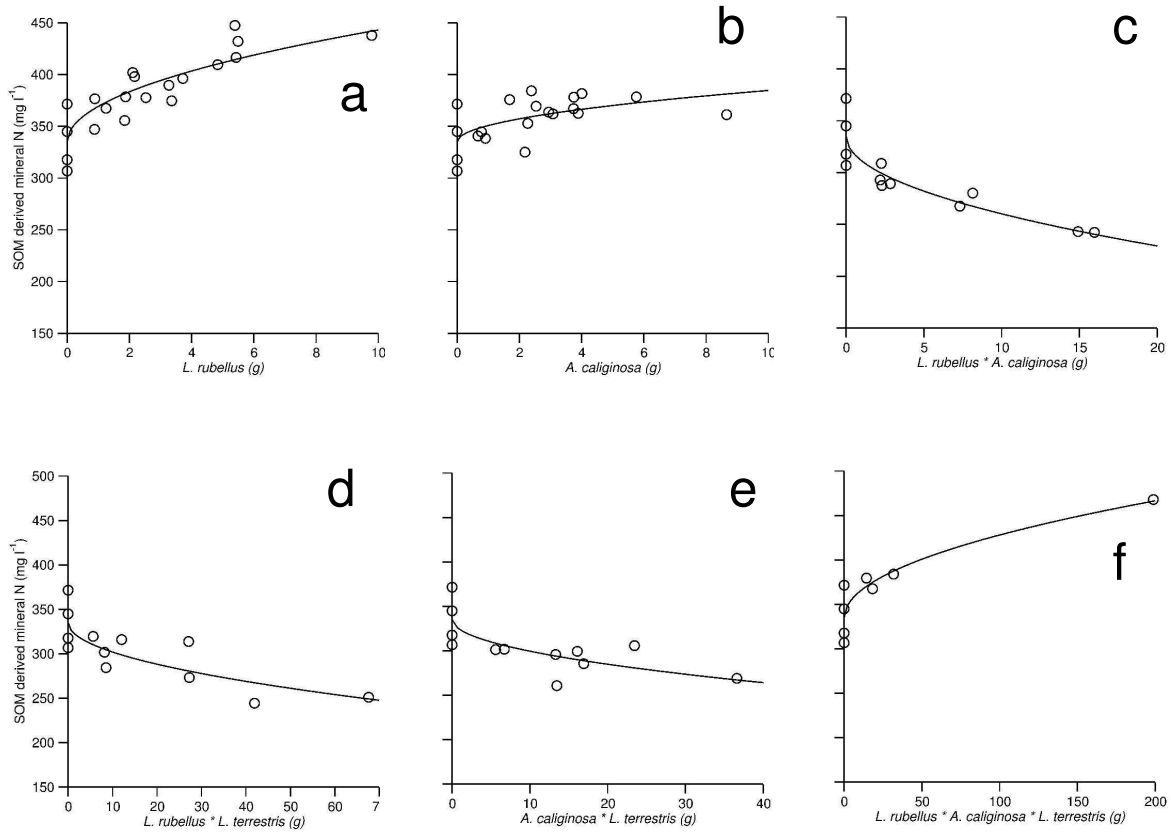

Fig. 2a-f. Partial residual plots of the effect of **a** *L. rubellus* (g), **b** *A. caliginosa* (g), and the interaction effects of **c** *L. rubellus* and *A. caliginosa* (g), **d** *L. rubellus* and *L. terrestris* (g), **e** *A. caliginosa* and *L. terrestris* (g), and **f** *L. rubellus*, *A. caliginosa* and *L. terrestris* (g) on the concentrations of mineral N derived from soil organic matter (mg l^{-1}) on day 56 of the experiment, as determined by backward regression analysis. For the model R^2 and significance, and the coefficients of the parameters included in the model, see Table 2.

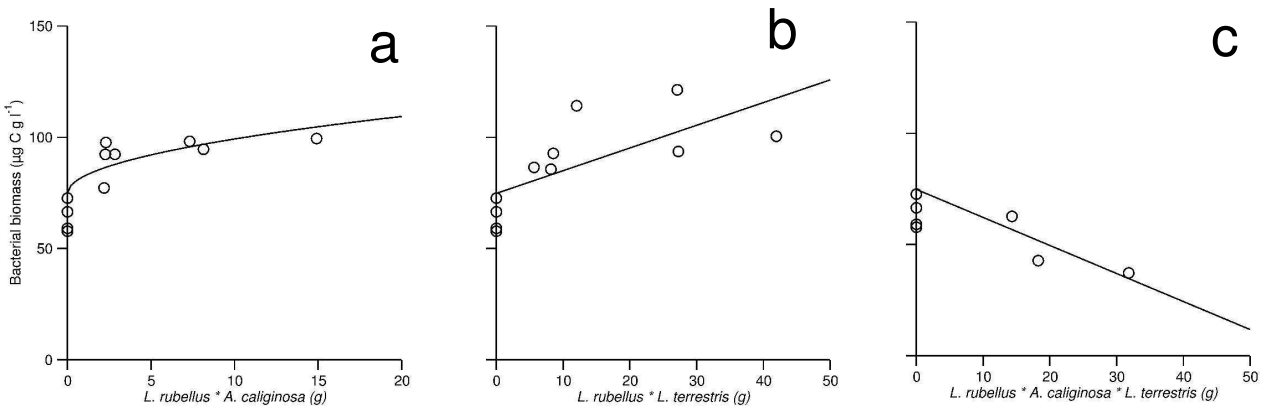

Fig. 3a-c. Partial residual plots of the interaction effects of **a** *L. rubellus* and *A. caliginosa* (g), **b** *L. rubellus* and *L. terrestris* (g), and **c** *L. rubellus*, *A. caliginosa* and *L. terrestris* (g) on the bacterial biomass (µg C g^{-1}), as determined by backward regression analysis. For the model R^2 and significance, and the coefficients of the parameters included in the model, see Table 2.

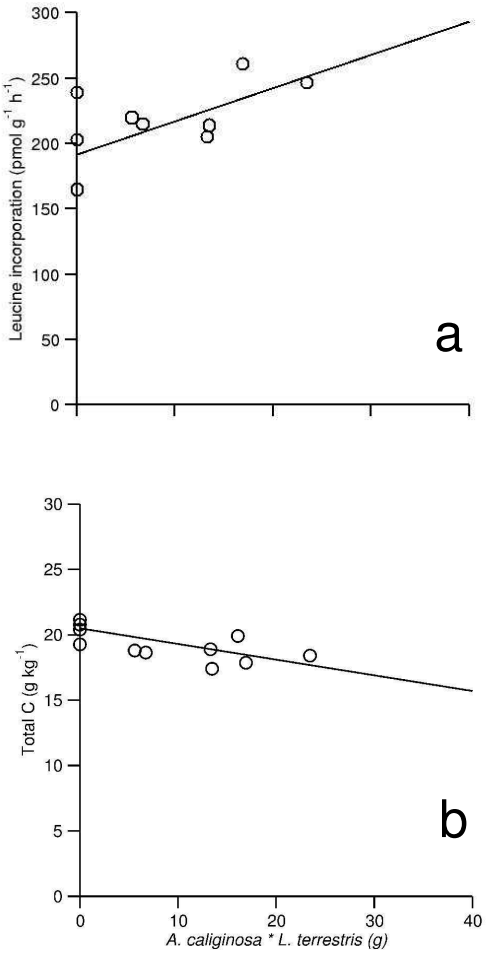

Fig. 4a,b. Partial residual plots of the interaction effects of *A. caliginosa* and *L. terrestris* (g) on **a**. the bacterial growth rate measured as leucine incorporation (pmol g^{-1} h^{-1}) and **b**. total soil C (g kg^{-1}), as determined by backward regression analysis. For the R^2 and significance of the models, and the coefficients of the parameters included in the models, see Table 2.

Bacteria

At the end of the experiment, bacterial biomass ranged between 58 and 121 µg C g^{-1} soil. None of the three earthworm species had a main effect on bacterial biomass. The two-species interactions between *L. rubellus* and *L. terrestris* ($P < 0.001$) and between *L. rubellus* and *A. caliginosa* ($P = 0.002$), however, positively affected bacterial biomass (Table 2, Fig. 3a-b). In contrast, when all three species were present together, we found a negative interaction effect on the bacterial biomass (Table 2, $P < 0.001$, Fig 3c): the bacterial biomass in this three-species treatment was lower than was expected based on the single species treatments. The interaction between *A. caliginosa* and *L. terrestris* did not affect bacterial biomass.

Bacterial growth rate measured as thymidine incorporation was on average 31.6 pmol g^{-1} h^{-1}, and measured as leucine incorporation it ranged between 146 and 261 pmol g^{-1} h^{-1}. None of the three earthworm species had a main effect on bacterial growth rate. The two-species interaction between *A. caliginosa* and *L. terrestris*, however, positively affected leucine incorporation (Table 2, $P < 0.009$, Fig. 4a).

The number of bacterial DNA bands in the selected treatments ranged between 58 and 70 bands. Earthworms had no effect on, or decreased the number of DNA bands. The number of DNA bands in the single species treatments with *L. rubellus* (70 bands) was similar to the control

treatments (70 bands). The number of DNA bands in the mixed species treatments with *L. rubellus* and *A. caliginosa* (58.7 bands), and with all three species (60.5 bands) was lower than in the control (one-factor-ANOVA, $P < 0.001$).

Discussion

We hypothesized that earthworm species of different ecological groups would affect nitrogen mineralization differently, and that earthworm species interactions would affect nitrogen mineralization through competition and facilitation effects. Our hypothesis was confirmed by the results showing that earthworm species had different effects on mineral N concentrations, agreeing with the characteristics of the ecological groups they represented. Earthworm species interactions also affected mineral N concentrations and the bacterial community, the effects being specifically dependent on the combination of species present. We will henceforth consider the effects of the different earthworm species and species combinations on the soil and bacterial parameters separately.

Earthworm species effects

L. rubellus and *L. terrestris* enhanced residue-derived N_{min} indicating that mineralization of the residue was enhanced. These results can be explained from the incorporation of plant residues into the soil by *L. rubellus* and *L. terrestris* (Bouché, 1977). *A. caliginosa* forages mainly on soil organic matter (Bouché, 1977), which explains the lack of an effect of *A. caliginosa* on residue-derived N_{min}. *L. rubellus* and *A. caliginosa* also had a positive effect on SOM-derived N_{min}, indicating that the mineralization of soil organic matter was enhanced by the burrowing and feeding of these species. Although statistically significant, the effect of *A. caliginosa* on SOM-derived N_{min} was small compared to the effect of *L. rubellus* (Fig. 2a,b), and the ecological relevance seems limited under our experimental conditions. We found no significant effect of *L. terrestris* on SOM-derived N_{min}. Possibly, the lower burrowing activity in the permanent burrow system of *L. terrestris* may not have resulted in increased SOM-derived N_{min}, in contrast to *L. rubellus* and *A. caliginosa*. Positive effects of earthworms on nitrogen mineralization are frequently found for earthworm species in general (Blair et al., 1997; Cortez et al., 2000) and were specifically described for *L. rubellus* (Brown et al., 1998; Whalen et al., 2000), and endogeic species (Villenave et al., 1999; Whalen et al., 2000). Both positive and negative effects of *L. terrestris* on nitrogen mineralization have been found (Devliegher and Verstraete, 1997; Whalen et al., 2000; Tiunov and Dobrovolskaya, 2002; Wilcox et al., 2002; Bohlen et al. 1999).

We expected that the incorporation of organic matter by *L. rubellus* and *L. terrestris* would result in increased total soil N and C amounts, and that the increased nitrogen mineralization would concur with effects on the bacterial community. We found no effects of the three earthworms species on the amounts of total soil N and C or on the bacterial community, however. In other studies a positive effect of earthworms on total soil N and C was usually observed on a longer

term than our experiment lasted (Gilot, 1997; Villenave et al., 1999). Earthworms have been found to stimulate bacterial activity (Daniel and Anderson, 1992; Ruz-Jerez et al., 1992; Li et al., 2002) and to either reduced or enhance bacterial biomass (Ruz-Jerez et al., 1992; Scheu and Parkinson, 1994; Bohlen and Edwards, 1995; Blair et al., 1997; Devliegher and Verstraete, 1997; Brown et al., 1998; Bohlen et al., 1999; Cortez et al., 2000). We agree with Bohlen et al. (1999) that no simple relationship exists between bacterial biomass and earthworms.

Species interactions

The combination of *L. rubellus* and *A. caliginosa* resulted in a reduced SOM-derived N_{min} and an enhanced bacterial biomass, indicating that immobilization of nitrogen into bacterial biomass occurred. Possibly, burrowing activity by concurring species had increased, leading to an increased availability of mineralized nitrogen for incorporation into the bacterial biomass. The combination of *L. rubellus* and *A. caliginosa* also led to a reduction in bacterial genotypes compared to *L. rubellus* in single species culture, suggesting that the induction of bacterial biomass by this treatment was accomplished by an increase in a limited number of dominant species. A similar result was found for the interaction between two nematode species (Chapter 2).

Since crop residue incorporated into the soil by *L. rubellus* may serve as an additional food source for *A. caliginosa* (Hughes et al., 1994; Villenave et al., 1999), we had expected an increased mineralization of the crop residue in the presence of the two species. The absence of an interaction effect on residue-derived N_{min} despite incorporation of crop residue into the soil by *L. rubellus* may be explained by the assumption that *A. caliginosa* did not feed on the incorporated residue after all. Alternatively, *A. caliginosa* may have affected gross mineralization of the crop residue, but net mineralization may have been limited due to immobilization of mineralized nitrogen into the bacterial biomass.

Similar to the interaction effect of *L. rubellus* and *A. caliginosa*, the combination of *L. rubellus* and *L. terrestris* resulted in a reduced SOM-derived N_{min} and enhanced bacterial biomass, indicating that immobilization of nitrogen into the bacterial biomass occurred. This effect may have been caused by an increased incorporation of the crop residue, and/or an increased burrowing activity by the concurring species. The absence of an interaction effect on residue-derived N_{min} may indicate the absence of a direct competition effect between the species. Alternatively, mineralized nitrogen from the crop residue may have been incorporated into the bacterial biomass. Evidence for interaction between *L. rubellus* and *L. terrestris* was given by Lowe and Butt (1999), who observed competition for food between these species, resulting in reduced abundances of the species. A competition effect was also observed for other earthworm species (Abbott, 1980). Our results indicate that besides affecting population densities, the interaction between *L. rubellus* and *L. terrestris* can affect the bacterial community and nutrient cycling, leading to immobilization of nitrogen.

Contrary to the previous interactions, the interaction between *A. caliginosa* and *L. terrestris* led to increased bacterial growth rate (leucine incorporation), and reduced total soil C, indicating

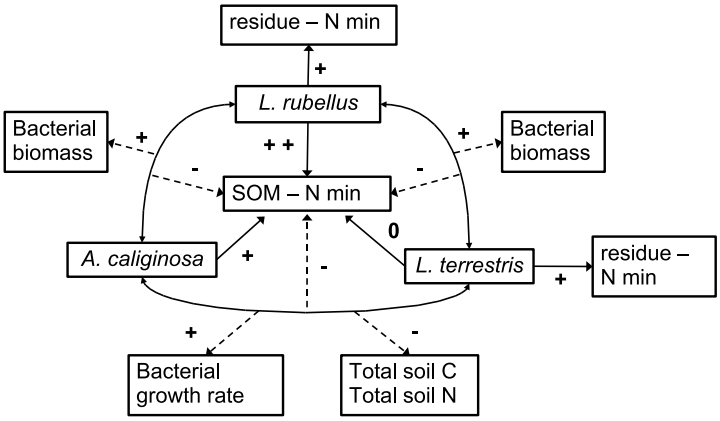

Fig. 5. Earthworm species effects (solid lines) and two-way species interactions effects (dotted lines) on concentrations of mineral N derived from the crop residue, concentrations of mineral N derived from the soil organic matter, total soil N and C, and the bacterial community. Three-way interaction effects were described in the text. Negative, positive, and strongly positive effects are indicated by -, +, and ++ respectively.

that bacterial mineralization increased in the presence of *L. terrestris* and *A. caliginosa.* These results, however, contradict the finding that SOM-derived N_{min} decreased in the presence of this species combination. This decrease in SOM-derived N_{min} was small compared to the effect of the other species combinations (Fig. 2c-d), however, and may have resulted from increased leaching of nitrogen along the permanent vertical burrows of *L. terrestris*, or from increased denitrification in the presence of *L. terrestris* (Parkin and Berry, 1999).

Although the effects of the combination of *L. terrestris* and *A. caliginosa* on bacterial growth rate and total C were small, these effects can be explained by the ecological characteristics of the species. Jégou et al. (2001) indicated that *A. caliginosa* may preferably use the burrows of *L. terrestris* and *Aporrectodea giardi*, and may use castings and burrow linings of *L. terrestris* as food sources. In our case, feeding of *A. caliginosa* on the burrow linings of *L. terrestris* may have resulted in increased bacterial growth rate. Alternatively, *A. caliginosa* may have affected the burrowing activity of *L. terrestris.* Jégou et al. (2001) found that *L. terrestris* increased its burrow length in the presence of *A. caliginosa,* and burrowing of *L. terrestris* was more superficial than when incubated alone. Possibly, an increased burrowing of *L. terrestris* may have led to increased bacterial growth rate and mineralization of total soil C.

The 3-species interaction enhanced SOM-derived N_{min} and reduced bacterial biomass, suggesting that bacterial activity increased under the 3-species combination. Indeed bacterial growth rate was highest in the three-species treatment, and total soil C second lowest. The combination of *A. caliginosa* and *L. terrestris* showed a similar high bacterial growth rate and low total C, while the two-species combinations with *L. rubellus* (with *A. caliginosa,* and with *L. terrestris*) showed opposite effects. We therefore suggest that the effect of the three-species combination was mainly

determined by the simultaneous presence of *A. caliginosa* and *L. terrestris*, while the contribution of *L. rubellus* was minimized.

Conclusions

A synthesis of the main effects of the three investigated earthworm species and the effects of species is presented in Fig. 5. We found that *L. rubellus* and *L. terrestris* enhanced mineralization of the crop residue, whereas no indication of an effect was found for *A. caliginosa*. On the other hand, *L. rubellus* and *A. caliginosa* enhanced mineralization of the soil organic matter, whereas we found no indication of an effect of *L. terrestris*. The interactions between different earthworm species affected the bacterial community and soil mineral N concentrations. Our results led to the suggestion that the interaction between *L. rubellus* and *L. terrestris,* and *L. rubellus* and *A. caliginosa* led to increased immobilization of mineral N into the bacterial biomass. On the other hand, the interaction between *A. caliginosa* and *L. terrestris* led to increased bacterial growth rate and increased mineralization of total soil C. When all three species were combined, the interaction effect of *A. caliginosa* and *L. terrestris* dominated.

Our results indicate that the effects of earthworms on nitrogen mineralization depend on the ecological traits of the earthworm species and are dependent on earthworm community composition. Similar conclusions were reached for fungal grazing collembolans (Faber and Verhoef, 1991; Cragg and Bardgett, 2001) and bacterivorous nematodes (Mikola and Setälä, 1998; Wardle, 2002; Chapter 2). Together with these findings our results support the hypothesis that there is no general rule on the effect of diversity within soil biota groups on ecosystem functioning, but that the outcome depends on the ecological traits of the species present (Heemsbergen et al., 2004; Swift et al., 2004).

The comparison of earthworm species diversity ranging from one to three species is relevant in agricultural systems where earthworm species diversity is usually below five species (Lee, 1985), and can be restricted to one species (Curry et al., 2002). The notion that earthworm communities can specifically affect soil fertility may be of great importance to increase sustainable land use in agroecosystems, as proper earthworm management may sustain crop yields whilst fertilizer inputs can be reduced (Brown et al., 2004).

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References

Abbott, I.. 1980. Do earthworms compete for food? Soil Biology & Biochemistry 12: 523-530.

Baker, G., P. Carter, V. Barrett, J. Hirth, P. Mele, and C. Gourley. 2002. Does the deep-burrowing earthworm, Aporrectodea longa, compete with resident earthworm communities when introduced to pastures in south-eastern Australia? European Journal of Soil Biology 38: 39-42.

Bardgett, R. D., and R. Cook. 1998. Functional aspects of soil animal diversity in agricultural grasslands. Applied Soil Ecology 10: 263-276.

Begon, M., J. L. Harper, and C. R. Townsend. 1990. Ecology: Individuals, Populations and Communities, second edn. Blackwell Scientific Publications, Boston.

Blair, J. M., R. W. Parmelee, M. F. Allen, D. A. Mccartney, and B. R. Stinner. 1997. Changes in soil N pools in response to earthworm population manipulations in agrocosystems with different N sources. Soil Biology & Biochemistry 29: 361-367.

Bloem, J., and A. M. Breure 2003. Microbial indicators. Pages 259-282 *in* B. A. Markert, A. M. Breure, and H. G. Zechmeister, editors. Bioindicators/Biomonitors - Principles, Assessment, Concepts. Elsevier, Amsterdam.

Bloem, J., M. Veninga, and J. Shepherd. 1995a. Fully automatic determination of soil bacterium numbers, cell volumes and frequencies of dividing cells by confocal laser scanning microscopy and image analysis. Applied and Environmental Microbiology 61: 926-936.

Bloem, J., P. R. Bolhuis, M. Veninga, and J. Wieringa 1995b. Microscopic methods for counting bacteria and fungi in soil. Pages 162-173 *in* K. P. N. Alef, editors. Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London.

Bohlen, P. J., and C. A. Edwards. 1995. Earthworm effects on N dynamics and soil respiration in microcosms receiving organic and inorganic nutrients. Soil Biology & Biochemistry 27: 341-348.

Bohlen, P. J., R. W. Parmelee, M. F. Allen, and Q. M. Ketterings. 1999. Differential effects of earthworms on nitrogen cycling from various nitrogen-15-labeled substrates. Soil Science Society of America Journal 63: 882-890.

Bouché, M. B. 1977. Strategies Lombriciennes. Pages 122-132 *in* U. Lohm, and T. Persson, editors. Soil Organisms as Components of Ecosystems. Proceedings of the VI. International Soil Zoology Colloquium. Ecological Bulletins, Stockholm.

Brooks, P. D., J. M. Stark, B. B. McInteer, and T. Preston. 1989. Diffusion method to prepare soil extracts for automated N-15 analysis. Soil Science Society of America Journal 53: 1707-1711.

Brown, G. G., B. Pashanasi, C. Villenave, J. C. Patron, B. K. Senapati, S. Giri, I. Barois, P. Lavelle, E. Blanchart, R. J. BLakemore, A. V. Spain, and J. Boyer 1999. Effects of earthworms on plant production in the tropics. Pages 87-147 *in* P. Lavelle, L. Brussaard, and P. Hendrix, editors. Earthworm Management in Tropical Agroecosystems. CABI Publishing, Oxon, UK.

Brown, G. G., P. F. Hendrix, and M. H. Beare. 1998. Earthworms (*Lumbricus rubellus*) and the fate of ^{15}N in surface-applied sorghum residues. Soil Biology & Biochemistry 30: 1701-1705.

Brown, G., A. Moreno, I. Barois, C. Fragoso, P. Rojas, B. Hernandez, and J. Patron. 2004. Soil macrofauna in SE Mexican pastures and the effect of conversion from native to introduced pastures. Agriculture, Ecosystems & Environment 103: 313-327.

Brussaard, L., V. M. Behan-Pelletier, D. E. Bignell, V. K. Brown, W. Didden, P. Folgarait, C. Fragoso, D. W. Freckman, V. Gupta, T. Hattori, D. L. Hawksworth, C. Klopatek, P. Lavelle, D. W. Malloch, J. Rusek, B. Soderstrom, J. M. Tiedje, and R. A. Virginia. 1997. Biodiversity and ecosystem functioning in soil. Ambio 26: 563-570.

Capowiez, Y., P. Monestiez, and L. Belzunces. 2001. Burrow systems made by *Aporrectodea nocturna* and *Allolobophora chlorotica* in artificial cores: morphological differences and effects of interspecific interactions. Applied Soil Ecology 16: 109-120.

Cortez, J., and R. H. Hameed. 2001. Simultaneous effects of plants and earthworms on mineralisation of ^{15}N-labelled organic compounds adsorbed onto soil size fractions. Biology and Fertility of Soils 33: 218-225.

Cortez, J., G. Billes, and M. B. Bouché. 2000. Effect of climate, soil type and earthworm activity on nitrogen transfer from a nitrogen-15-labelled decomposing material under field conditions. Biology and Fertility of Soils 30: 318-327.

Cragg, R. G., and R. D. Bardgett. 2001. How changes in soil faunal diversity and composition within a trophic group influence decomposition processes. Soil Biology & Biochemistry 33: 2073-2081.

Curry, J. P., D. Byrne, and O. Schmidt. 2002. Intensive cultivation can drastically reduce earthworm populations in arable land. European Journal of Soil Biology 38: 127-130.

Dalby, P. R., G. H. Baker, and S. E. Smith. 1998. Competition and cocoon consumption by the earthworm Aporrectodea longa. Applied Soil Ecology 10: 127-136.

Daniel, O., and J. M. Anderson. 1992. Microbial biomass and activity in contrasting soil materials after passage through the gut of the earthworm *Lumbricus rubellus* Hoffmeister. Soil Biology & Biochemistry 24: 465-470.

Devliegher, W., and W. Verstraete. 1997. The effect of *Lumbricus terrestris* on soil in relation to plant growth: effects of nutrient-enrichment processes (NEP) and gut-associated processes(GAP). Soil Biology & Biochemistry 29: 341-346.

Dilly, O., J. Bloem, A. Vos, and J. C. Munch. 2004. Bacterial diversity during litter decomposition in agricultural soils. Applied and Environmental Microbiology 70: 468-474.

Doube, B. M., P. M. L. Williams, and P. J. Willmott. 1997. The influence of two species of earthworm (*Aporrectodea trapezoides* and *Aporrectodea rosea*) on the growth of wheat, barley

and faba beans in three soil types in the greenhouse. Soil Biology & Biochemistry 29: 503-509.
Faber, J. H., and H. A. Verhoef. 1991. Functional differences between closely related soil arthropods with respect to decomposition and nitrogen mobilization in a pine forest. Soil Biology & Biochemistry 23: 15-23.
FAO-UNESCO, 1988. Soil map of the world: revised legend. FAO, Rome, Italy.
Fragoso, C., G. G. Brown, J. C. Patron, E. Blanchart, P. Lavelle, B. Pashanasi, B. Senapati, and T. Kumar. 1997. Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of earthworms. Applied Soil Ecology 6: 17-35.
Gilot, C.. 1997. Effects of a tropical geophageous earthworm, *M. anomala* (Megascolecidae), on soil characteristics and production of a yam crop in Ivory Coast. Soil Biology & Biochemistry 29: 353-359.
Heemsbergen, D. A., M. P. Berg, M. Loreau, J. R. van Hal, J. H. Faber, and H. A. Verhoef. 2004. Biodiversity effects on soil processes explained by interspecific functional dissimilarity. Science 306: 1019-1020.
Hughes, M. S., C. M. Bull, and B. M. Doube. 1994. The use of resource patches by earthworms. Biology and Fertility of Soils 18: 241-244.
Jégou, D., Y. Capowiez, and D. Cluzeau. 2001. Interactions between earthworm species in artificial soil cores assessed through the 3D reconstruction of the burrow systems. Geoderma 102: 123-137.
Lee, K. E. 1985. Earthworms: Their Ecology and Relationships with Soils and Land Use. Academic Press, London.
Li, X., M. C. Fisk, T. J. Fahey, and P. J. Bohlen. 2002. Influence of earthworm invasion on soil microbial biomass activity in a northern hardwood forest. Soil Biology & Biochemistry 34: 1929-1937.
Lowe, C. N., and K. R. Butt. 1999. Interspecific interactions between earthworms: a laboratory-based investigation. Pedobiologia 43: 808-817.
Lowe, C. N., and K. R. Butt. 2002. Growth of hatchling earthworms in the presence of adults: interactions in laboratory culture. Biology and Fertility of Soils 35: 204-209.
Michel, P. H., and J. Bloem. 1993. Conversion factors for estimation of cell production rates of soil bacteria from thymidine and leucine incorporation. Soil Biology & Biochemistry 25: 943-950.
Mikola, J., and H. Setälä. 1998. Relating species diversity to ecosystem functioning: mechanistic backgrounds and experimental approach with a decomposer food web. Oikos 83: 180-194.
Muyzer, G., E. C. De Waal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59: 695-700.
Parkin, T. B., and E. C. Berry. 1999. Microbial nitrogen transformations in earthworm burrows. Soil Biology & Biochemistry 31: 1765-1771.

Perel, T. S. 1977. Differences in lumbricid organization connected with ecological properties. Pages 56-63 *in* U. Lohm, and P. Thoeheethij, editors. Soil Organisms as Components of Ecosystems. Ecological Bulletins, Stockholm.

Postma-Blaauw, M. B., F. T. De Vries, R. G. M. De Goede, J. Bloem, J. H. Faber, and L. Brussaard. 2005. Within-trophic group interactions of bacterivorous nematode species and their effects on the bacterial community and nitrogen mineralization. Oecologia 142: 428-439.

Ruz-Jerez, B. E., P. R. Ball, and R. W. Tillman. 1992. Laboratory assessment of nutrient release from a pasture soil receiving grass or clover residues, in the presence or absence of *Lumbricus rubellus* or *Eisenia foetida*. Soil Biology & Biochemistry 24: 1529-1534.

Scheu, S., and D. Parkinson. 1994. Effects of earthworms on nutrient dynamics, carbon turnover and microorganisms in soils from cool temperate forests of the Canadian Rocky Mountains-laboratory studies. Applied Soil Ecology 1: 113-125.

Sorensen, P., and E. S. Jensen. 1991. Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene trap for N-15 determination. Analytica Chimica Acta 252: 201-203.

Swift, M. J., A. M. N. Izac, and M. Van Noordwijk. 2004. Biodiversity and ecosystem services in agricultural landscapes - are we asking the right questions? Agriculture, Ecosystems & Environment 104: 113-134.

Tiunov, A. V., and T. G. Dobrovolskaya. 2002. Fungal and bacterial communities in *Lumbricus terrestris* burrow walls: a laboratory experiment. Pedobiologia 46: 595-605.

Van Elsas, J. D., and K. Smalla 1995. Extraction of microbial community DNA from soils. Pages 1.3.3.1-1.3.3.11 *in* A. D. L. Akkermans, J. D. Van Elsas, and F. J. De Bruijn, editors. Molecular Microbial Ecology Manual. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Van Groenigen, J. W., P. J. Georgius, C. Van Kessel, E. W. J. Hummelink, G. L. Velthof, and K. B. Zwart. 2005. Subsoil ^{15}N-N_2O concentrations in a sandy soil profile after application of ^{15}N-fertilizer. Nutrient Cycling in Agroecosystems Nutrient Cycling in Agroecosystems 72: 13-25.

Villenave, C., F. Charpentier, P. Lavelle, C. Feller, L. Brussaard, B. Pashanasi, I. Barois, A. Albrecht, and J. C. Patron 1999. Effects of earthworms on soil organic matter and nutrient dynamics following earthworm inoculation in field experimental situations. Pages 173-198 *in* P. Lavelle, L. Brussaard, and P. Hendrix, editors. Earthworm Management in Tropical Agroecosystems. CABI Publishing, Oxon, UK.

Walinga, I., M. Kithome, I. Novozamsky, V. J. G. Houba, and J. J. Vanderlee. 1992. Spectrophotometric determination of organic-carbon in soil. Communications in Soil Science and Plant Analysis 23: 1935-1944.

Wardle, D. A. 2002. Communities and ecosystems, linking the aboveground and belowground components. Princeton University Press, Oxford.

Whalen, J. K., R. W. Parmelee, and S. Subler. 2000. Quantification of nitrogen excretion rates for three lumbricid earthworms using 15N. Biology and Fertility of Soils 32: 347-352.

Wilcox, C. S., J. Dominguez, R. W. Parmelee, and D. A. McCartney. 2002. Soil carbon and nitrogen dynamics in *Lumbricus terrestris*. L. middens in four arable, a pasture, and a forest ecosystems. Biology and Fertility of Soils 36: 26-34.

Wolters, V., and R. G. Joergensen. 1992. Microbial carbon turnover in beech forest soils worked by *Aporrectodea caliginosa* (Savigny) (Oligochaeta, Lumbricidae). Soil Biology & Biochemistry 24: 171-177.

Chapter 4

Agricultural (de-)intensification differentially affects abundances, functional diversity and community structure in soil micro-, meso- and macrofauna

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Abstract

Understanding the impacts of agricultural (de-)intensification on soil biota communities is useful in order to preserve and restore biological diversity in agricultural soils and enhance the role of soil biota in agroecosystem functioning. Over three consecutive years we investigated the effects of agricultural (de-)intensification, including conversion of grassland to arable land and vice versa, increased and decreased levels of mineral fertilization, and monoculture compared to crop rotation on major soil biota group abundances and functional diversity. We integrated and compared effects across taxonomic levels to identify sensitive species groups.

Conversion of grassland to arable land negatively affected both abundance and functional diversity of soil biota. Taxonomic groups with larger body size were more negatively affected than smaller-sized taxonomic groups. Conversely, re-establishment of grassland on former arable land positively affected taxonomic groups with larger body size. Further intensification of the cropping system by increased fertilization and reduced crop diversity exerted smaller and differential effects on different taxonomic groups. Conversion of grassland to arable land resulted in changed trophic group composition in earthworms and predatory mites and in a reduced life-strategy diversity in nematodes and predatory mites. Re-establishment of grassland resulted in restoration of the nematode and earthworm functional group composition to the original grassland composition. Predatory mite functional group composition, however, was still very different from the original grassland composition after three years.

Our results suggest that larger soil biota are more sensitive to agricultural intensification than smaller soil organisms. Furthermore, larger soil biota appeared to be primarily affected by short term consequences of conversion (disturbance, loss of habitat), whereas smaller soil biota were predominantly affected by long term consequences (probably loss of organic matter). Since larger-sized soil biota groups had lower species richness, we suggest that agricultural intensification exerts strongest effects on species-poor soil biota groups, thus supporting the hypothesis that biodiversity has an “insurance” function. As soil biota play an important role in agroecosystem functioning, altered soil biota abundances and functional group composition under agricultural intensification implies effects on the functioning of the system, such as nutrient mineralization.

Introduction

Agriculture intensification, including soil disturbance, increased fertilization levels and crop diversity reduction, is known to affect the soil biota community (Brussaard et al., 1990; Wardle 1995, Susilo et al., 2004; Hole et al., 2005). Understanding of the various impacts of agricultural intensification on the soil biota community is useful, in order to preserve biological diversity in agricultural soils and to promote the role of soil biota in agroecosystem functioning. Whereas in most studies agricultural impacts on only one or two taxonomic groups were investigated, we include a range of taxonomic groups, so as to integrate and compare effects across taxonomic levels and identify sensitive species groups. In this paper we describe the effects of agricultural (de-)intensification on abundances and functional diversity of higher order soil biota groups whereas effects on genera or species are described in a companion paper (Chapter 5). The concomitant investigation of intensification and de-intensification is another novel aspect of our study.

Agricultural (de-)intensification and taxonomic groups of soil biota

Agricultural intensification generally results in reduced abundances of the different soil biota orders or classes (Bouwman and Zwart, 1994; Roper and Gupta, 1995; Mäder et al., 2002). In contrast, positive or neutral effects of integrated or intensive management compared to organic management have also been found (Filser et al., 2002; Bloem and Breure, 2003; Hole et al. 2005), while positive effects of arable systems compared to grassland systems have been reported, too (Griffiths and Ritz, 1988; Lagerlöf et al. 2002). Furthermore, some taxonomic groups may be affected more than others, leading to a different community structure and possibly changed ecosystem functioning. Larger-sized taxonomic groups may be more strongly affected than smaller -sized groups (Wardle 1995), and the ratio between bacteria and fungi may change towards a more bacterial dominated system as intensification increases (Moore, 1994; Bardgett and McAlister, 1999).

Agricultural (de-)intensification and trophic groups of soil biota

Agricultural (de-)intensification may also affect functional group diversity within taxonomic groups. Functional groups can be defined as groups of species that contribute to ecosystem functioning in a similar way (Brussaard et al., 1997; Susilo et al., 2004). Among others, an important function of biota is the role of species in the food web. Although agricultural management intensification may not affect overall food web diversity (Moore, 1994), effects of agricultural intensification on abundances of soil biota trophic groups can occur through changes in resources available to soil biota. Soil biota trophic groups can be distinguished at different

taxonomic levels, ranging from protozoans to earthworms. Small **protozoans** (amoebae, flagellates) feed mainly on bacteria, whereas ciliates also feed on other protozoans (Ekelund and Rønn, 1994). Furthermore, these different groups differ in mode of feeding, pore space they can access, and moisture film they need to move around (Bamforth, 1999), and therefore amoebae, flagellates and ciliates can exert different trophic functions. All protozoan groups are usually present in agricultural ecosystems, but relative abundances may change as a result of agricultural intensification (Bamforth, 1999). The **nematode** fauna comprises a large range of trophic groups (bacterivores, fungivores, plant parasites, omnivores, carnivores) (Yeates et al., 1993). Grassland systems are often found to have higher nematode plant parasite dominance, whereas arable systems are characterized by higher bacterivore dominance (Sohlenius and Sandor, 1987; Háněl 2003). Within arable systems, conventional systems often have higher nematode plant parasite and fungivore abundances, whereas organic systems are characterized by higher bacterivore abundances (Ferris et al., 1996; Berkelmans et al., 2003). The group of **predatory mites** may exert an important role in the soil ecosystem as top predators. Within the group of predatory mites, four trophic groups can be distinguished: omnivores, parasites, general predators and microarthropod predators. Little is known of the effects of agricultural intensification on these trophic groups. Omnivory may be advantageous in arable systems and enable species to rapidly recover from adverse management practices (Vreeken-Buijs et al., 1994). **Earthworm** species differ in their preferences for food and microhabitat (Bouché, 1977) and therefore can be classified in different ecological groups. Soil tillage has been found to result in decreased epigeic and anecic earthworms, whereas endogeic earthworms are less affected (Wardle, 1995; Lagerlöf et al., 2002).

Agricultural (de-)intensification and life-strategy groups of soil biota

Besides affecting trophic groups, agricultural intensification can also affect the dominant life-strategies of soil biota groups. Nematodes can be classified according to their life-strategy on a colonizer-persister scale (cp1 – cp5), based on which a maturity index is calculated, indicating whether the system contains relatively more r- or K- selected nematodes (Bongers, 1990). Agricultural intensification generally results in a reduction in the nematode maturity index (Ettema and Bongers, 1993; Villenave et al., 2003), which can be explained by the sensitivity of high cp-level nematodes to disturbance and increased abundances of low cp-level nematodes following fertilization. Also predatory mites can be assigned to different life-strategy groups, based on reproduction type (sexual reproduction or thelytoky), development time, synchronization tactics (dormancy, diapause), and dispersal ability (wind dispersal (anemochory), or dispersal via other biota (phoresy)) (Siepel, 1994). The different groups reflect the ability of the species to cope with disturbance, colonize new substrates, and survive adverse

conditions. Therefore, agricultural intensification is likely to affect predatory mite life-strategy composition.

Agricultural intensification has been found to affect the major taxonomic group abundances and functional group composition at different taxonomic levels. Few studies, however, have directly investigated and compared these effects on abundances and functional group compositions across different taxonomic levels (Wardle, 1995).

This study

In this study we investigated the effect of agricultural (de-)intensification on major taxonomic soil biota groups, on trophic group structure of protozoans, nematodes, predatory mites and earthworms, and on life-strategy groups of nematodes and predatory mites. Agricultural intensification was investigated by studying the effect of the conversion of an extensively managed grassland to an arable system, and furthermore as the effect of increased mineral fertilization levels and reduced crop diversity. Since the reverse of intensification does not always result in restoration of the original soil biota community (Swift et al., 1996), the effects of re-establishment of extensively managed grassland on formerly long term arable land, on biodiversity were also investigated.

We hypothesized that absolute and relative abundances of taxonomic groups will change as a result of agricultural intensification, resulting in a more bacterial dominated system under intensified management and reduced abundances of larger-sized soil biota compared to smaller biota. Furthermore, we expected changes in functional group composition across taxonomic groups, with trophic group composition shifting to higher nematode bacterivore abundances and omnivorous predatory mites in arable systems, whereas litter-feeding earthworms would be reduced. Life-strategies were expected to shift towards taxa with high colonization and dispersal capacity and ability to survive unfavorable circumstances (diapause).

Materials and Methods

Experimental set-up

An experimental field ('De Bovenbuurt' 51°59'N, 5°40'E, the Netherlands) was selected, with two agricultural systems with extreme management regimes located close to each other. On the one extreme was a long term extensively managed grassland (>50 years) (long term grassland) and on the other a conventionally managed crop rotation that was converted from the grassland

around 1980 (long term arable land). In 2000 an experiment was started where part of the long term grassland was converted to different agricultural systems, and part of the long term arable land was converted to grassland, resulting in four experimental systems: long term grassland, new arable land, long term arable land and new grassland. This set-up gave us the opportunity to study agricultural intensification on the short term and long term and to study agricultural de-intensification, by comparing the different systems. Furthermore, intensification of agricultural practices was studied by establishing different management regimes on both the long term and new arable system: a crop rotation versus a monoculture of maize, and reduced versus conventional mineral fertilization.

Complete randomization of treatments was not possible, since the long term arable land and long term grassland fields were fixed. To correct for possible gradients within these two fields (approximately 2 ha), three replicate blocks on long term grassland and long term arable land were assigned, with treatments randomized (10 x12 field plots) within each block. The soil biota functional groups in long term grassland and long term and new arable systems were determined in three consecutive years, starting one year after the conversion of long term grassland in 2000. The soil biota functional groups in the new grassland were determined in year two and three after the conversion.

Description of the different systems, soil characteristics and management

Description of sites

The soil of the experimental site is a Fimic Anthrosol (FAO-UNESCO, 1988), or a loamy sand (0-10 cm: fraction > 50 µm: 89%, 16 µm - 50 µm: 7%, 2 µm - 16 µm: 5%, < 2 µm: 3%, sampled in 2002). Dominant species on the long term grassland were *Festuca rubra, Holcus lanatus, Anthoxanthum odoratum, Rumex acetosa and Ranunculus sp*. The long term arable land was under a crop rotation (oat, maize, barley, potato), with conventional fertilization levels, weed control through herbicide application and 20 cm deep tillage. At the start of the experiment in April 2000, long term grassland and long term arable land were characterized by an organic matter content of 3.9%, and 3.3% respectively, a pH (KCl) of 4.4 and 5.0 respectively, and a moisture content 28% and 16% respectively (calculated as soil water (g) * dry weight (g^{-1} *100%).

Conversions

In April 2000, part of the long term grassland was plowed (20 cm). Three plots (long term grassland) were left undisturbed. On the long term arable land, six plots were sown with a grassland species mixture containing grass, clover and herb species. Both new grassland and

long term grassland were mown and harvested two or three times in the summer, depending on the growth rate of the grass. Sods (of the upper 10 cm) of the long term grassland soil (outside the experimental plots) were applied in a corner (4 m^2) of each plot on the long term arable fields, in order to ensure the possibility of establishment of species that may be limited by dispersal.

Agricultural regimes

In the arable fields under crop rotation management, the current rotation was continued: maize (variety: Crescendo) in 2001, barley (variety: Aspen) in 2002 and potato (variety: Desiree) in 2003. In the arable fields with monoculture regime, maize was sown continuously, from 2000 onwards (variety: Crescendo in 2001, 2002, 2003). Maize seeds were coated with Groucho, a systemic insecticide formulation to reduce wireworm infestation. Reduced tillage (10 cm) and mechanical weed control instead of herbicides were applied in all experimental arable fields. All arable crops received a similar amount of organic manure (dried cow manure) in each year, and an additional dressing of mineral fertilizer for optimal production of each crop (conventional fertilization), or half of the amount needed for optimal production of the crop (reduced fertilization) (Appendix A).

Description of sampling and analysis of the different microbial and faunal groups

Samples for analysis of soil biota functional groups in the different experimental fields were taken on September 23 in 2001, September 24 in 2002, September 8 in 2003 (one, two and three years after conversion respectively). Separate samples were taken for earthworms, enchytraeids and microarthropods, and one bulk sample was taken for nematodes, protozoans, micro-organisms and abiotic analyses. All samples were taken from the upper 10 cm soil layer, except for earthworms which were sampled to 25 cm depth. Earthworms were hand sorted in the field from the soil under a 20 x 20 cm surface, and identified to species in the lab. In the year 2003 we checked the grassland fields for deep dwelling earthworms by applying formalin (0.005%) to the soil.

Enchytraeids and microarthropods were sampled in 2001 and 2003. Enchytraeids were sampled with a 5.9 cm diameter soil corer and microarthropods with a 4.7 cm diameter soil corer. Six (in 2001) and four (in 2003) replicate soil cores were taken per experimental plot. Enchytraeids were extracted using wet extraction following O'Connor (1967). Each soil sample was divided into four parts and placed on a sieve covered with a piece of cheesecloth and positioned in a water filled funnel. During 3 hours the temperature was raised to 50 °C, thus creating a temperature gradient and driving enchytraeids downwards until captured in water filled small tubes. Enchytraeids were counted and averaged per experimental plot. Microarthropods were

extracted from soil samples using a Berlese-Tullgren funnel. Each soil core was placed under a carbon lamp (50V for 3 days followed by 75V for 4 days), for top-down desiccation of the soil sample and driving the animals down until captured in 70 % alcohol. Mites and collembola were counted from each soil core under a binocular microscope; numbers were averaged per experimental plot.

Predatory mites from samples taken in 2003 were identified to species, or to genus or higher order level if identification to species was not possible (Karg, 1993). Predatory mite taxa were assigned to trophic groups and life-strategy groups (Siepel, 1994), and trophic and life-strategy diversity indices were calculated (reciprocal Simpson's index: 1/D). According to Siepel (1994), the life-strategy group without dispersal or synchronization tactics is characterized as "sexual reproduction", or "thelytoky". In our systems, however, all species exerted sexual reproduction, whereas thelytoky did not occur. Therefore, species indicated as belonging to the life-strategy group of sexual reproduction are henceforth characterized as having "no dispersal or synchronization tactics", as this explains more clearly the difference between this group and other life-strategy groups observed in our systems.

Sixty soil cores (diameter 2.2 cm, depth 10 cm) were combined into a bulk sample for determination of soil microbiota. The soil was thoroughly mixed and stored at 4 °C until analysis. Nematodes were extracted from approximately 150 g soil using an Oostenbrink elutriator (Oostenbrink, 1960). Filters were placed in 95 ml tap water for two nights to allow nematode migration through the filters. Nematodes were counted in 2.5 or 5.0 ml subsamples under an inverted binocular microscope at 100x magnification, fixed with hot 4% formaldehyde, and identified to genus according to Bongers (1988). Nematode taxa were assigned to trophic groups (Yeates et al., 1993) and life-strategy groups (c-p groups) (Bongers, 1990; Bongers et al., 1995). The trophic diversity index (reciprocal Simpson's index: 1/D) and maturity index (Bongers, 1990) were calculated.

Protozoan abundances were estimated using the most probable number method (modified from Ingham (1994)). From each of the soil samples, 0.5 g of soil was suspended in 4.5 ml soil extract (200 gram sterilized soil in 800 ml demineralized water). Eight 50 µl replicates of each soil sample dilution were further diluted 4-fold, 11 times, in 96-wells sterile microtiter plates (8 x 12). Microtiter plates were incubated at 15 °C and checked for ciliates, flagellates, amoebae, and species with both flagellate and amoeba characteristics ("amoeba-flagellates") after approximately 7 and 14 days. The trophic diversity index was calculated (reciprocal Simpson's index: 1/D).

Fungal hyphae were measured by epifluorescence microscopy using the grid intersection method. Bacterial numbers and cell volumes were measured by confocal laser scanning microscopy and automatic image analysis, and biomass was calculated from biovolume (Bloem et al., 1995a; Bloem et al., 1995b). The bacterial growth rate was determined by thymidine

incorporation, a measure for DNA synthesis (growth rate), and leucine incorporation, a measure for protein synthesis (growth rate and biomass turnover) (Michel and Bloem, 1993).

Statistical analysis

ANOVA

Statistical analyses were carried out using the statistical package SPSS version 11.0. Data were transformed (log10 or square root transformations) to meet assumptions of normality and homogeneity of variance. The effects of different land use systems on soil biota abundances functional groups in each year were analyzed with one-factor ANOVA. In this analysis long term and new grassland systems were compared with the most extensively managed long term and new arable systems (crop rotation and reduced mineral fertilization levels). Treatments were long term grassland, long term arable land and new arable land in 2001, and these treatments plus new grassland in 2002 and 2003, and were followed by a Tukey HSD post-hoc analysis.

The effects of agricultural management on soil biota abundances and functional groups were analyzed using a nested two-factor (in 2001 and 2002) or one-factor (in 2003) ANOVA design. Treatments were cropping system (monoculture versus rotation, in 2001, 2002 and 2003) and fertilization level (conventional versus reduced, in 2001 and 2002), and were nested within the factor field history (grassland or arable land). Since variation in soil biota data was expected to be high, and replications in the field were limited (n = 3), P-levels of 0.10 or less were regarded as significant. Non parametric tests were performed in case the data did not meet assumptions for ANOVA (Mann-Whitney U for comparison of two samples and Kruskal-Wallis H for comparison of more than one sample).

Redundancy Analysis (RDA)

The soil biota taxonomic and functional group composition in different land use systems and agricultural management systems in each year was analyzed with RDA (CANOCO 4.5, Biometrics, Wageningen, The Netherlands), in order to correlate community structure with environmental variables and assuming species distributions to be linear (Ter Braak and Smilauer, 2002). Biplots of environmental and species variables were produced with scaling focused on inter-species correlations. Monte Carlo permutation tests (499 random permutations, P = 0.05) were performed to establish the significance of the correlations between community structure and environmental variables. Separate analyses were done for the factors land use change and agricultural management (crop and fertilization level), explaining community structure of the different taxonomic and functional groups (taxonomic group composition, trophic group composition and life-strategy groups composition).

Results

Soil biota taxonomic groups

Land use change

Soil biota groups were generally negatively affected by conversion to arable land, but the timing of the response differed between soil biota groups (Figure 1, Appendix B). The reduction in nematodes, mesofauna and macrofauna abundances occurred immediately after conversion, whereas the reduction in protozoans and bacterial biomass was significant two and three years after conversion, respectively, and fungal biomass was not significantly affected. Bacterial growth rate, measured as leucine and thymidine incorporation, was not affected by the conversion to arable land, except for a positive effect on leucine incorporation in the third year

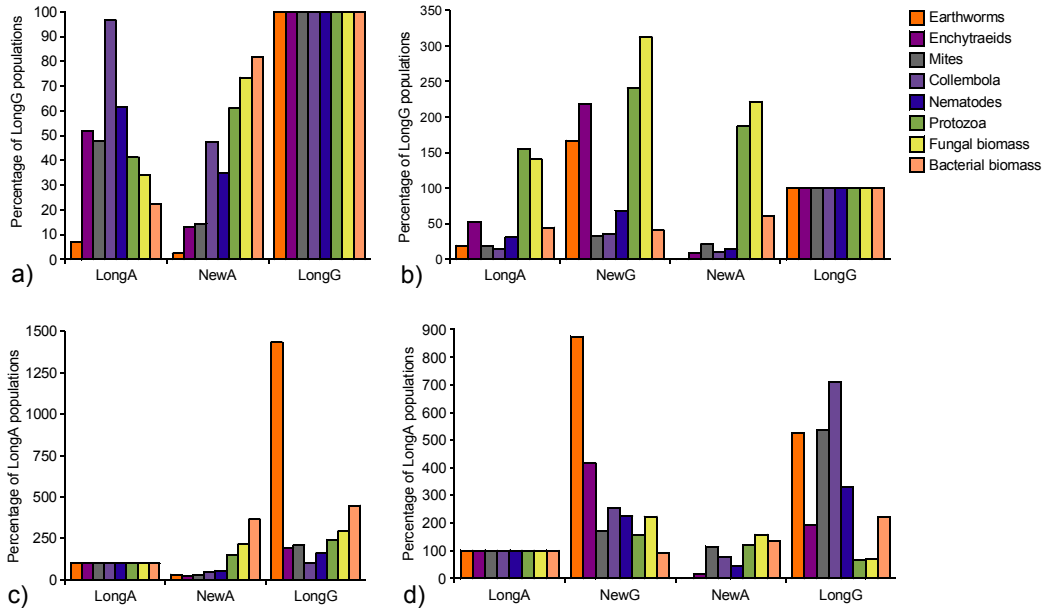

Figure 1. Relative reduction and increase of soil biota groups after land use change. Soil biota groups are presented as percentages of long term grassland populations (**a**, **b**) and as percentages of long term arable land populations (**c**, **d**), in the first year (2001) (**a**, **c**), and third year (2003) (**b**, **d**) after conversion. LongA = long term arable land, NewG = new grassland on formerly arable land, NewA = new arable land on formerly grassland, LongG = long term grassland.

after conversion. Furthermore, conversion from grassland to arable land resulted in a progressive increase of the fungal/bacterial biomass ratio that became significant in the third year of the experiment. Bacterial biomass still dominated in all systems, but the arable systems were relatively less bacterial dominated (reduced bacterial biomass compared to relatively constant fungal biomass).

A second difference in response of soil biota to conversions is apparent when the abundances after conversion are compared to abundances in the long term grassland and long term arable land (Figure 1). Larger-sized soil biota (nematodes, mesofauna, macrofauna) appeared more reduced relative to smaller biota (microflora, protozoans) after conversion of grassland to arable land. *Vice versa*, after re-establishment of grassland, larger-sized soil biota appeared to be relatively more enhanced than smaller-sized biota. The bacterial/fungal ratio was not affected by the re-establishment of grassland on formerly arable land.

Redundancy Analysis, followed by Monte Carlo permutation tests revealed that in both years the soil biota community was significantly affected by the land use system ($P = 0.026$ in 2001, $P = 0.002$ in 2003). In both years, the grassland systems were separated from the arable systems along the first axis, whereas the two field histories were separated along the second axis. These results revealed that the soil biota community composition was mostly determined by current management, and less by field history.

Agricultural management

Cropping system and fertilization level affected taxonomic groups differently, depending on the crop in the rotation and the history of the field (Table 1). In the first year after conversion (2001), a maize crop was sown in both rotation and monoculture fields. No main effects of cropping system (i.e. the previous crop) on the soil biota taxonomic groups were found in this year (data not presented). Two years after conversion (2002), all measured taxonomic groups had higher biomass, activity or abundances in the rotation barley fields compared to the monoculture maize fields. In several cases, however, these effects emerged in one of the arable systems only (long term or new arable system), or only if fertilization was also reduced. In the third year of the experiment, effects of the cropping system were only found on nematode abundances, which were reduced under the rotation potato crop compared to the monoculture maize crop. Effects of fertilization level became significant in the second year of the experiment, when reduced fertilization had a negative effect on fungal biomass and nematode abundance, but positively affected protozoans and earthworm abundances. A trend of lower protozoan abundances in the reduced fertilization fields was visible in the first year of the experiment.

Table 1. The effect of agricultural management on soil biota taxonomic groups and microbial characteristics in two years after conversion (2002, 2003). Means (n = 3) and P-levels of significance (P < 0.10) as a result of ANOVA are presented. LongA = long term arable land, NewA = new arable land on formerly grassland, Mon = monoculture, Rot = Rotation, CF = conventional fertilization, RF = reduced fertilization, Hist = field history, Fert = Fertilization. Only soil biota groups that were significantly affected by land used change are presented.

	2002								2003			
	LongA				NewA				LongA		NewA	
	Mon		Rot		Mon		Rot		Mon	Rot	Mon	Rot
Means (n = 3)	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**RF**	**RF**	**RF**	**RF**
Earthworms (n m^{-2})	13	8	13	46	0	17	4	0	4	17	0	0
Nematodes (n g^{-1})	49	41	77	49	31	34	35	29	36	23	37	10
Protozoans (n mg^{-1})	41	39	64	97	25	46	47	107	36	31	38	38
Fungal biomass (µg C g^{-1})	6	8	12	12	24	19	18	10	4	3	8	5
Thymidine incorporation (pmol g^{-1} h^{-1})	44	65	92	99	35	31	35	61	18	25	11	24
Leucine incorporation (pmol g^{-1} h^{-1})	259	349	407	437	298	231	293	349	242	316	209	24

	2002				2003	
P levels of significance (P<0.10)	**Hist**	**Crop**	**Fert**	**Crop*Fert**	**Hist**	**Crop**
Earthworms (n m^{-2})	0.090			0.096	0.001	
Nematodes (n g^{-1})	0.000	0.021	0.024			0.003
Protozoans (n mg^{-1})		0.000	0.004			
Fungal biomass (µg C g^{-1})	0.000	0.001	0.016		0.095	
Thymidine incorporation (pmol g^{-1} h^{-1})	0.001	0.016				
Leucine incorporation (pmol g^{-1} h^{-1})	0.025	0.021				

Trophic groups

Protozoans

Flagellates dominated in all systems, followed by amoebae and amoeba-flagellates, whereas ciliates constituted a very small percentage of total protozoan abundances (Figure 2, Appendix C). Conversion of grassland to arable land did not affect the dominance of small protozoans, but affected the abundances of ciliates, which were higher in number in the new arable land than in the long term grassland (Figure 2). Ciliate abundances were not affected by re-establishment of grassland on arable land. Agricultural management affected protozoan taxonomic groups, but differences in relative abundances of protozoan taxa between years were larger than differences

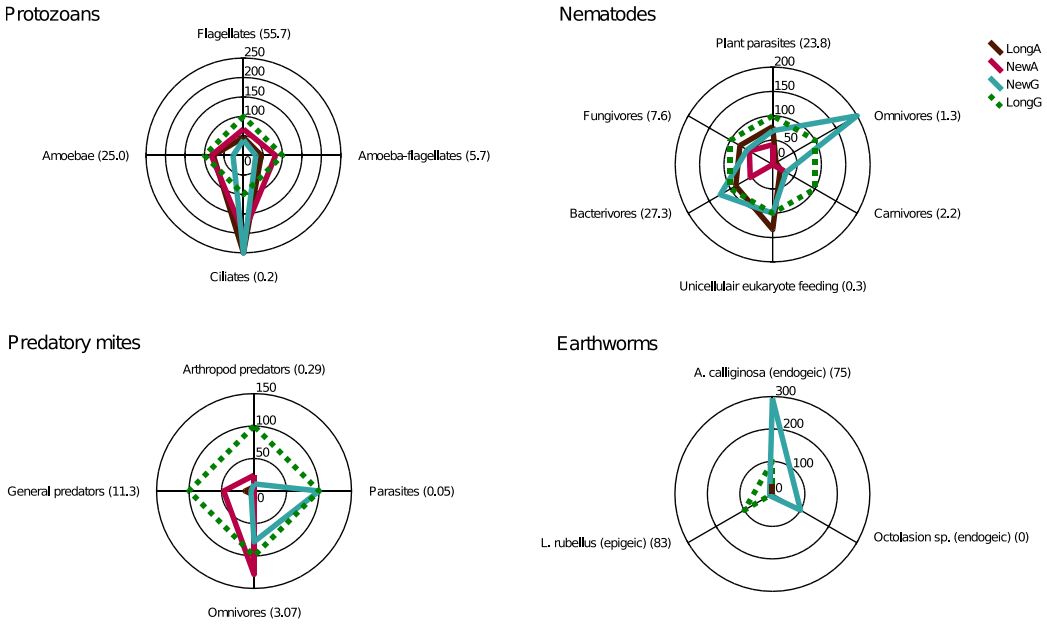

Figure 2. Land use change effects on trophic group composition of protozoans, nematodes, predatory mites and earthworms. The solid lines show relative abundances of trophic groups in the different systems compared to abundances in the long term grassland (dashed lines). Trophic group abundances in the second year after conversion (2002) or in the third year after conversion for predatory mites (2003) are presented. Absolute abundances (n m^{-2}) in the long term grassland are indicated between brackets. No earthworms of the genus *Octolasion* were found in the long term grassland, whereas in the new grassland the density was 4.2 n m^{-2}, which is indicated as 100%. LongA = long term arable land, NewG = new grassland on formerly arable land, NewA = new arable land on formerly grassland, LongG = long term grassland. Absolute abundances of all trophic groups in all years are presented in Appendix C.

between systems. Two years after conversion, small protozoans increased in the rotation barley fields compared to the monoculture maize fields, in the case of amoeba-flagellates only if fertilization was reduced (Appendix D). No such effect was found on ciliate abundances. Three years after conversion, amoeba abundances increased in the rotation potato fields compared to the monoculture maize fields. Increased fertilization had a negative effect on amoeba-flagellate abundances, whereas ciliate abundances increased in conventionally fertilized systems.

Table 2. Agricultural management effects on nematode trophic groups and the nematode maturity index in three consecutive years after conversion (2001, 2002, 2003). Numbers of unicellulair eukaryote feeding nematodes were very low and are not presented. Means (n = 3) and P-levels of significance (P < 0.10) as a result of ANOVA are presented. LongA = long term arable land, NewA = new arable land on formerly grassland, Mon = monoculture, Rot = crop rotation, CF = conventional fertilization, RF = reduced fertilization, Hist = field history, Fert = Fertilization.

	2001								2002								2003			
	LongA				NewA				LongA				NewA				LongA		NewA	
	Mon		Rot		Mon		Rot		Mon		Rot		Mon		Rot		Mon	Rot	Mon	Rot
Nematodes (n g^{-1})	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**RF**	**RF**	**RF**	**RF**
Plant parasites	26.3	27.5	19.8	27.4	9.4	15.2	11.7	9.3	27.3	22.6	16.0	18.5	15.7	16.4	12.5	9.7	15.3	6.0	18.9	2.2
Fungivores	3.5	2.6	1.9	2.9	2.7	3.6	2.2	2.8	2.0	3.8	12.8	5.9	1.8	3.1	5.5	4.2	4.9	4.8	5.3	2.0
Bacterivores	23.5	18.4	15.6	15.8	18.5	23.4	20.3	15.3	17.2	12.5	46.6	23.9	13.2	13.6	16.8	14.5	14.2	11.6	12.1	5.8
Carnivores	2.5	1.2	0.9	2.2	0.5	1.2	0.6	0.5	1.9	1.5	1.4	0.4	0.6	0.5	0.1	0.6	1.4	0.5	0.6	0.0
Omnivores	2.3	0.9	1.5	1.4	0.1	0.5	0.2	0.6	0.2	0.9	0.2	0.1	0.2	0.4	0.0	0.0	0.4	0.3	0.0	0.2
Trophic diversity (1/D)	2.6	2.3	2.5	2.5	2.2	2.4	2.1	2.5	2.2	2.4	2.3	2.3	2.3	2.5	2.6	2.5	2.8	2.7	2.5	2.4
Maturity index	2.3	2.2	2.3	2.4	1.9	2.0	2.0	2.1	2.0	2.3	1.6	1.7	2.0	2.2	1.8	1.9	2.0	1.9	1.8	1.9

	2001				2002				2003	
P-levels	**Hist**	**Crop**	**Fert**	**Crop*Fert**	**Hist**	**Crop**	**Fert**	**Crop*Fert**	**Hist**	**Crop**
Plant parasites	<0.001					0.001				0.053
Fungivores						0.001		0.068		
Bacterivores	0.094					0.007				0.029
Carnivores	0.012					0.035			0.011	
Omnivores	0.000		0.055			0.050				0.018
Trophic diversity index (1/D)	0.045		0.031							
Maturity index	0.004					<.001	0.020			

Nematodes

Conversion of grassland to arable land and vice versa did not affect the nematode trophic diversity index (1/D) (Appendix C). Bacterivorous, fungivorous and plant parasitic nematodes dominated in all systems (Figure 2). The relative abundances of the dominant groups were affected by land use change, however. In the period of three years after conversion of grassland to arable land, the relative abundances of nematode trophic groups in the new arable land (column 3 in Appendix H) changed from relative abundances resembling those in the long term grassland (column 4 in Appendix H), to relative abundances closely resembling those in long term arable land (column 1 in Appendix H). Also the abundances in the new grassland (column 2 in Appendix H) resembled those in the long term grassland in the third year after conversion. Relative abundances in arable and grassland systems changed over years, with a contrasting trend in both systems. Grassland systems were characterized by high bacterivore and fungivore percentages and low plant parasite percentages, in the first year of the experiment, but changed to high percentages of plant parasites and low percentages of bacterivores in the third year. Arable systems started with higher percentages of plant parasites than the grassland systems, but contained lower percentages of plant parasites and higher percentages of bacterivores in the third year after conversion.

Agricultural management did not significantly affect the trophic diversity index (1/D) of nematodes, except for a positive effect of reduced fertilization in the first year of the experiment (Table 2). Trophic group structure was affected, however. Barley crops had higher percentages and abundances of bacterivores and fungivores, and lower percentages and abundances of plant parasites, omnivores and carnivores than maize crops. Bacterivore and fungivore abundances were not significantly different in potato crops and maize crops, whereas plant parasite abundances were higher in the maize crops (resulting in higher percentages of bacterivores and lower percentages of plant parasites in the potato crops). The effect of fertilization level on nematode trophic group composition was less obvious. Reduced fertilization levels resulted in decreased fungivorous nematode abundances in the rotation barley fields, but slightly increased abundances in the monoculture maize fields. Omnivorous nematode abundances were reduced by increased fertilization levels

Predatory mites

The effects of land use change on the different predatory mite trophic groups were investigated three years after conversion (2003). Total predatory mite abundances were significantly reduced after conversion to arable land, and increased after re-establishment of grassland (Appendix C). A trophic diversity index (1/D) of predatory mites was not reduced after conversion of grassland to arable land in the short term, but was lowest in the long term arable land and increased after re-establishment of grassland. Furthermore, the trophic group composition of predatory mites

was affected by land use change (Figure 2, Appendix I). General predators dominated in the long term grassland and long term arable land. After conversion to arable land, omnivorous mites dominated, since all other trophic groups were severely reduced. The percentage of omnivorous mites also increased after re-establishment of grassland. Arthropod predator abundances were higher in the grassland systems, but percentages were similar in all systems. Parasitic mites occurred in the long term grassland and the new arable systems in very low abundances.

Total predatory mite abundances were not affected by the cropping system (Appendix E). The trophic group composition of predatory mites did not respond consistently to crop management and effects depended on the field history. On the new arable land, omnivorous mites dominated in the rotation potato crop, whereas general predators dominated in the monoculture maize crop. On the long term arable land, however, general predators dominated in both cropping systems. Parasitic mites only occurred in the rotation fields on the new arable land.

Earthworms

The two earthworm species present in long term grassland were severely reduced after conversion to arable land (Figure 2, Appendix C). *L. rubellus* did not occur in any of the arable systems in detectable numbers, but *A. caliginosa* was present in small numbers in the long term arable land. After re-establishment of grassland *A. caliginosa* abundances immediately increased to numbers similar to or higher than those in permanent grassland. Abundances of *L. rubellus* gradually increased in the re-established grassland, until numbers were comparable to those in the long term grassland in the third year after conversion. Of the two earthworm species, only *A. caliginosa* persisted in the arable fields (Appendix F). On the fields with arable history, abundances of *A. caliginosa* were highest in the rotation fields with reduced fertilization.

Redundancy Analysis of trophic groups

Redundancy Analyses followed by Monte Carlo permutation tests revealed that in all years the soil biota trophic group composition was significantly affected by the land use system ($P = 0.002$ in all years). In all years, the first axis separated the systems with different current management (grassland and arable systems), whereas the second axis separated the systems with different field histories. The soil biota trophic group composition was also significantly affected by the cropping system ($P = 0.020$ in 2002, $P = 0.006$ in 2003), whereas fertilization level had no statistically significant effect. The first axis separated the cropping systems (monoculture or rotation) in both years. In the second year after conversion (2002), the differently fertilized rotation systems were separated along the second axis, suggesting that fertilization affected the feeding guild composition in the rotation fields, but not in the monoculture maize fields.

Life-strategy groups

Nematodes

In all systems cp2 nematode genera dominated, followed by cp1 and cp3 genera. Conversion of grassland to arable land resulted in a decreased nematode maturity index (Table 3), resulting from reduced relative abundances of cp3 and cp4 nematodes and increased relative abundances of cp2 nematodes. After re-establishment of grassland, the nematode maturity index increased to a level where it was not statistically significantly different from the long term grassland. The effect of cropping system on nematode life-strategy groups was only significant in the second year of the experiment, when monoculture maize and rotation barley were compared (Table 2). The nematode maturity index was lower in the rotation barley fields than in the monoculture

Table 3. Land use change effects on soil biota life strategy diversity groups and indices. Means (n = 3) of the nematode maturity index **a** and predatory mite life strategy groups **b** in different land use systems, in three consecutive years after conversion (2001, 2002, 2003) are presented. Different letters indicate significant differences at P < 0.10. Please note that differences within each year are tested, not between years. LongA = long term arable land, NewG = new grassland on formerly arable land, NewA = new arable land on formerly grassland, LongG = long term grassland.

	LongA	NewG	NewA	LongG
a) Nematode maturity index		**2001**		
MI	2.4ab	ND	2.1a	2.5b
MI2_5	2.5ab	ND	2.2a	2.6b
		2002		
MI	1.7a	2.1ab	1.9ab	2.2b
MI2_5	2.3a	2.5ab	2.3ab	2.6b
		2003		
MI	1.9ab	2.2bc	1.9a	2.3c
MI2_5	2.2ab	2.4b	2.1a	2.5bc
b) Predatory mites (n m^{-2} 10^3)		**2003**		
Parasites	0.00	0.00	0.00	0.05
Facultative phoresy	0.48a	1.15ab	0.24a	1.78b
Obligate phoresy as juveniles	0.00a	0.10a	0.05a	2.88b
Obligate phoresy as adults	0.10	0.00	0.24	0.14
Obligate diapause	0.19a	3.94b	2.40b	3.07b
Facultative diapause and anemochory	0.05a	0.14a	0.00a	2.31b
No phoresy or synchronization tactics	1.20a	4.95b	0.53a	8.55b
Life strategy group diversity index (1/D)	2.36ab	2.49ab	1.95a	3.50b

maize fields, resulting from an increased abundance of cp1 and cp2 genera in rotation barley fields, whereas cp3, 4 and 5 genera were not affected. Furthermore, an effect of fertilization level became significant in the second year of the experiment, when the maturity index was higher in the reduced fertilization systems.

Predatory mites

Conversion of grassland to arable land reduced diversity in predatory mite life-strategy groups (Table 3). Re-establishment of grassland did not result in increased diversity levels, however. Diversity levels were highest in the long term grassland, and lowest in the new arable land, with intermediate levels in the long term arable land and new grassland. Land use change furthermore altered dominance of life-strategy groups (Appendix J). In long term grassland, species without dispersal or synchronization tactics dominated, followed by species with obligate phoresy as juveniles, facultative phoresy, obligate diapause and facultative diapause and anemochory. After conversion to arable land, species with obligate diapause dominated in the new arable system, since most other life-strategy groups were severely reduced or non-detectable. In contrast, the long term arable soil was dominated by species without dispersal or synchronization tactics, followed by species with facultative phoresy and obligate diapause. These three life-strategy groups also dominated in the new grassland systems, but the fraction of species with obligate diapause was larger in these systems than in the long term arable systems. A life-strategy diversity index (Simpson's index) of predatory mites was not affected by agricultural management (Appendix G). Only the species with obligate diapause was affected by agricultural management, with higher abundances under rotation barley crops than under monoculture maize crops on the new arable land, but no effect was found in long term arable land.

Redundancy Analysis of life-strategy groups

Redundancy Analysis, followed by Monte Carlo permutation tests revealed that in the first (2001) and third (2003) year after conversion life-strategy groups were significantly affected by the land use system ($P = 0.014$ in 2001, $P = 0.002$ in 2003). No significant effect was found in the second year (2002) after conversion. In the first and third year after conversion, the first axis separated the systems with different current management system (grassland or arable land), whereas the second axis separated the systems with different field histories. The soil biota life-strategy group composition was also significantly affected by the crop ($P = 0.002$ in 2002, $P = 0.006$ in 2003) and marginally affected by fertilization level ($P = 0.082$ in 2002). The first axis separated the different cropping systems (monoculture maize or rotation barley) and the second axis separated the systems with different fertilization level.

Discussion

Synopsis of results

Conversion of grassland to arable land resulted in short-term reduced richness of soil biota taxonomic groups and reduced diversity indices and changed community composition of several trophic groups and life-strategy groups. Total abundances and functional diversity of larger-sized soil biota were particularly affected. Agricultural management intensification affected trophic and life-strategy group composition, but did not affect functional diversity indices. Re-establishment of grassland resulted in effects opposite to those of conversion, although not all functional groups recovered to typical grassland diversity levels. Henceforth we will consider in more detail the effects of land use changes and management intensification on the soil biota taxonomic, trophic and life-strategy groups.

Taxonomic diversity

Soil biota taxonomic groups are still present in soil after conversion of management, although reduced

Although almost all major taxonomic groups investigated were reduced after conversion, only earthworms were reduced to non-detectable levels in the new arable land. Earthworms were found, however, in the long-term arable land, and are also reported for other arable systems (e.g. Topoliantz et al., 2000; Lagerlöf et al., 2002). Our results thus support the hypothesis that on the long term all major soil biota taxonomic groups are present in the arable soil, though generally in reduced abundances (Zwart et al., 1994; Susilo et al., 2004).

Larger-sized soil biota are more sensitive

Larger-sized soil biota (earthworms, enchytraeids, microarthropods, nematodes) appeared to be more sensitive to conversion of grassland to arable land than smaller-sized soil biota (protozoans, microflora). Our results thus agree with the review study of Wardle (1995) who indicated that larger organisms are more likely to be reduced by tillage than smaller ones. Since larger-sized organisms often occupy higher trophic levels, our data support the hypothesis that intensively managed grassland systems contain a smaller proportion of predatory species than extensively managed systems (Mulder et al., 2005). Other authors also note that relative abundances of taxonomic groups may change under intensive agricultural management (Swift et al., 1996; Susilo et al., 2004). Higher sensitivity of larger-sized soil biota may be explained by a higher sensitivity of larger organisms to soil disturbance and changed habitat (Wardle, 1995;

Vreeken-Buijs et al., 1998; Schmidt et al., 2003), higher reproduction rates of smaller organisms (Petersen, 2000) and beneficial effects of increased nutrient availability to smaller organisms (Griffiths and Ritz, 1988; Porazinska et al., 1999).

Fungal/bacterial biomass ratios are affected by grassland conversion

In contrast to the common notion that the fungal/bacterial biomass ratio is reduced in arable soil (Bardgett and McAlister, 1999; Allison et al., 2005) our results indicated an increased fungal/bacterial ratio after conversion of grassland to arable land. A reduced fungal/bacterial ratio under arable conditions is usually ascribed to the disruption of fungal hyphae by soil tillage (Brussaard et al., 1996). Possibly, our results may be explained by the fact that besides the factor of disturbance, other factors can be important to determine the fungal/bacterial ratio, such as soil organic C and N, and bulk density (Allison et al., 2005), pH and substrate quality (Blagodatskaya and Anderson, 1998) and C:N ratios of crop residues (Ferris et al., 1996).

Crop effects depend on which crop is grown in the rotation

Effects of the cropping system on the soil biota abundances depended on which crop was sown in the rotation, with positive effects of the barley crop in the rotation, but negative or neutral effects of the potato crop. Nematode abundances in particular responded rapidly to each crop, with little effect of cropping history. Our results support the conclusion of Wardle et al. (2004) that above ground biota (crops) affect belowground biota, but results depend on the context and are often unpredictable (idiosyncratic), although resource quality was found to be a consistent factor of importance. Increased abundances of soil biota under cereal crops were also found by Háněl (2003) and Jagers op Akkerhuis (1988), and may be due to the extensive root system and input of organic residue under the barley crop, compared to maize and potato crops.

Fertilization: both positive and negative effects

Conventional mineral fertilization levels were beneficial to some taxonomic groups (fungal biomass, nematodes), but had inconsistent or adverse effects on other taxonomic groups (protozoans, earthworms). Positive effects of fertilization on fungal biomass and nematodes have been found in other studies (Roper and Gupta, 1995; Verschoor et al., 2001), but neutral effects have also been described (Bardgett and McAlister, 1999; Porazinska et al., 1999). Negative effects of mineral fertilization on earthworms were found by De Goede et al. (2003), while positive effects have been described by Schmidt et al. (2003). In contrast to our results, mineral fertilization generally exerts a neutral or positive effect on protozoans (Roper and Gupta, 1995; Forge et al., 2005). As with the effect of tillage (Wardle, 1995), the effect of fertilization on soil biota taxonomic groups probably depends on abiotic conditions and food web relationships. Although mineral fertilization was not found to be harmful to all soil biota taxonomic groups,

the most sensitive group in our study (earthworms) was found to be reduced by conventional fertilization, suggesting that harmful effects may exceed beneficial effects.

Trophic diversity

Trophic diversity and composition more affected in larger-sized soil biota than in smaller-sized biota .

Similar to the effect of conversion in agriculture on total abundances of the major taxonomic groups, grassland conversions appeared to affect trophic diversity and trophic group composition of larger biota (earthworms, predatory mites) more than those of the smaller-sized biota (nematodes, protozoans). This was observed in large changes in trophic group composition in predatory mites and earthworms, whereas protozoan and nematode trophic group composition largely remained the same. These results may in part be explained by the higher taxonomic resolution of the larger-sized soil biota. Alternatively, these results may be explained by the fact that for smaller taxonomic groups most resources were still available (although reduced) after conversion, whereas predatory mites may be affected by severely reduced prey groups in the arable systems, and the habitat for earthworms species was drastically changed after conversion. Still, trophic group composition changes after agricultural intensification occurred in all taxonomic groups investigated.

Protozoans: intensification promotes ciliates but reduces small protozoans

Small protozoans (amoeba, flagellates, and amoeba-flagellates) appeared to show opposite trends compared to ciliates. The first tended to have higher abundances in the least intensive treatments (crop rotation, reduced fertilization), while the latter increased after intensification (conversion to arable land, increased fertilization). Other studies have also shown increased abundances of r-selected ciliates in arable soil under increased fertilization (Griffiths et al., 1998), whereas amoebae were found to have higher abundances in de-intensified organically enriched fields (Bloem et al., 1994). Ciliates span a wide range of r-K selected species, and the species increasing in arable soil are probably r-selected (Bamforth, 1999). Increased small protozoan abundances under the barley crop may result from increased inputs of organic matter from roots and residues. Since small protozoans, especially amoebae, considerably contribute to nutrient mineralization, increased protozoan abundances may also indicate changes in nutrient cycling.

Land use changes affect nematode trophic composition, but results vary with confounding factors

Our results indicated that land use changes affected nematode trophic group composition, as old and new arable fields and grasslands showed high resemblance, but the direction of change differed over years. Other studies found that arable systems had higher bacterivore abundances compared to grassland systems, whereas plant parasites or root-fungal feeders dominated in grassland systems (Sohlenius and Sandor, 1987; Yeates et al., 1997b). Also in our systems, relative bacterivore abundances were higher and relative plant parasite abundances lower in the arable systems in the third year of the experiment; but in the first year the opposite was found. The large differences in trophic group composition between years indicate that the relative abundances of trophic groups may be related to confounding factors that vary with time, such as the crop type and weather conditions.

Cropping system affects nematode trophic group composition, whereas fertilization has limited effects

The monoculture maize system was characterized by higher abundances of plant parasites, carnivores and omnivores, whereas bacterivorous and fungivorous nematodes were more numerous in the barley crop under rotation. Similar to our results, increased plant parasites have often been found in conventional systems, which are often characterized by lower crop diversity, compared to organically managed systems (Ferris et al., 1996; Berkelmans et al., 2003). Possibly, the maize monoculture system has selected for plant parasites resulting in increased abundances, whereas the extensive root system of barley may have stimulated microbial growth and subsequent increase in microbivorous nematodes. Furthermore, the higher carnivore and omnivore abundance in the monoculture systems may be explained by the higher predictability of the monoculture favoring some K-selected species.

Conventional fertilization levels resulted in slightly increased fungivore and omnivore abundances, but the effect was not consistent under different crops and over time. The limited effect of fertilization on nematode trophic group composition may be explained by the fact that species within trophic groups may react differently to fertilization and these opposite effects may be neutralized in the total trophic group effect (Porazinska et al., 1999, Chapter 5).

The predatory mite community shifts towards omnivores in disturbed systems

In agreement with our hypothesis, omnivorous mites were found to be least reduced after conversion of grassland to arable land, and furthermore increased in abundance and percentage after re-establishment of grassland. Omnivory may be advantageous in the disturbed systems, since these species are able to switch from one food source to another (Vreeken-Buijs et al., 1994). On the other extreme, parasites are specifically related to a host species, and a

disappearance of host species in the long term arable land may have resulted in disappearance of parasitic mites. As the soil in the potato crop is highly disturbed compared to the maize crop, this may also explain the relatively high omnivorous mite abundance under potato.

Earthworms: reduced fertilization and barley crop favor A. caliginosa

In arable systems earthworm food resources are available as crop residues for *L. rubellus* and soil organic matter for *A. caliginosa*. Despite the availability of these resources both species were severely reduced, probably due to tillage, and the abundant availability of food in field edges and adjacent grassland (experimental plots measured 120 m^2). *A. caliginosa* had higher abundances under reduced fertilization and under barley crop, indicating that mineral fertilization may be harmful to this species (De Goede et al., 2003). Other studies, however, found neutral or positive effects of mineral fertilization on (endogeic) earthworms (Schmidt et al., 2003). The barley crop may be favorable because of increased inputs of organic matter through crop residues and the extensive barley root system.

Linkage between trophic levels

Links between different trophic levels may be expected in our systems. In the new grassland system, bacterivorous nematodes increased relatively more than the bacterial biomass, indicating a higher bacterivore grazing pressure. These results agree with the higher bacterial growth rate found in these fields. Predatory mites appeared to increase relatively less in the new grassland systems, possibly explaining the higher bacterivorous nematode abundances in the new grassland systems, as these may have been less predated upon. These results indicate top-down regulation of bacteria in these fields, with possibly tri-trophic effects of reduced predatory mite abundance resulting in reduced bacterial biomass. Tri-trophic effects on the bacterial decomposition pathway as a result of agricultural intensification have been described in several studies (Wardle, 1995). However, the link between the fungal biomass and fungivore grazing is less clear in our system, possibly because fungal trophic relationships are more regulated bottom-up (Wardle and Yeates, 1993).

Life-strategy diversity

Nematode maturity index reduced in arable systems and in rotation barley fields

In agreement with our expectations, the maturity index was reduced after conversion to arable land, and under increased fertilization levels, and increased again after re-establishment of grassland. Similar results were described elsewhere (Ettema and Bongers, 1993; Bongers and Ferris, 1999). Furthermore, the cropping system affected the maturity index, but not consistently

over time. The maturity index was higher in the monoculture maize fields compared to the rotation barley fields, a result that contrasts with the study of Háněl (2003), who found a higher maturity index under wheat fields compared to maize. Other studies have found that the maturity index may decrease under the input of high quality organic residues (Ferris and Matute, 2003; Wang et al., 2003). Our results may be explained from the monoculture system being more predictable over years, whereas increased organic input under the barley crop may have stimulated enrichment opportunists and general opportunists.

Predatory mites in disturbed systems: obligate diapause and facultative phoresy

Following conversion of grassland to arable land, predatory mites with obligate diapause were less reduced relatively to other life-strategy groups. The obligate diapause can be advantageous in arable systems, since it enables the species to cope with a winter period of absence of vegetation. In our systems, the obligate diapause life-strategy group consisted of only one species (*Eupodes*). An advantage of this species in the arable fields may also be their omnivorous feeding type, as discussed before. Furthermore, our results may be explained by the fact that the species belonged to the Prostigmatida suborder, which is known to respond positively to fertilization (Behan-Pelletier, 2003). Species with facultative phoresy were also relatively less reduced in the arable systems. This is a more opportunistic life-strategy than obligate phoresy, allowing species to better adapt to new circumstances (Siepel, 1994). The dominance in the long term grassland and in the long term arable field of species without dispersal or synchronization tactics suggests that long term systems are characterized by species that are well adapted to their environment and therefore are less dependent on dispersal or synchronization tactics. Cropping system effects on life-strategy groups are not easily explained, although again the high relative abundance of obligate diapause species in the rotation potato field may be related to the omnivorous feeding type of this species.

Life-strategy diversity recovers faster in nematodes than mites

Agricultural intensification affected life-strategy group composition in both nematodes and predatory mites. A comparison between effects on both groups is hard, as nematode and predatory mite life-strategies involve different traits and are therefore characterized in different ways. Still, a difference between the taxa is seen in their response to re-establishment of grassland: the nematode maturity index increased to long term grassland levels, whereas the predatory mite community was still very different 3 years after re-establishment of grassland, and life-strategy diversity was still reduced. These results may be partly explained by the fact that most nematode species with higher cp-levels had been present in very low abundances all along in the long term arable land, and therefore could quickly increase once grassland was sown, whilst many predatory mite species had to re-colonize from outside the fields.

Conclusions

Agricultural intensification generally resulted in reduced abundances of soil biota and changed functional group compositions. Largest negative effects occurred as a result of conversion of grassland to arable land, whereas reduction in crop diversity and increased fertilization levels had smaller and less consistent effects on soil biota. The effects furthermore differed between soil biota groups. Total abundances of larger-sized soil biota appeared to be more severely affected by agricultural intensification than smaller-sized soil biota. Also functional group diversity and composition of larger-sized soil biota were more severely affected than of the smaller-sized soil biota. For protozoans and nematodes, the most significant changes after conversion of grassland to arable land occurred in total abundances, whereas smaller changes occurred in functional group compositions. Predatory mites and earthworms, however, were severely affected both in total abundance and functional group composition, as after conversion the dominance of predatory mite functional groups was changed and one earthworm functional group was lost entirely.

Our results thus indicate a higher sensitivity of larger-sized soil biota to agricultural intensification, which may partly be explained by the fact that the taxonomic richness within larger-sized soil biota groups was much smaller than within the smaller-sized soil biota groups. For predatory mites and earthworms, some functional groups were only represented by a single genus and single species respectively, whereas the dominant nematode and protozoan functional groups consisted of more species. A study on the species composition of different soil biota in these same experimental fields revealed that agricultural intensification affected nematode species composition within functional groups (Chapter 5). Therefore, as some species decrease and others increase as a result of the changes in the system, the effect on the total functional group size and composition may be limited in functional groups with higher species richness (Porazinska et al., 1999). These findings support the hypothesis that biodiversity has an "insurance" function (Ettema, 1998; Bengtsson et al., 2000).

Larger-sized soil biota (nematodes, mesofauna and macrofauna) were more severely reduced on the short term arable land than on the long term arable land, whereas for smaller-sized biota (protozoans, microflora) the opposite was observed. These results indicate that larger-sized soil biota are mostly negatively affected by the short term consequences of conversion, such as soil disturbance (Wardle, 1995), changed cropping system and absence of permanent vegetation cover, whereas the smaller biota are more negatively affected by the long term consequences of conversion, probably primarily the loss of organic matter (Yeates et al., 1997a).

As soil biota play an important role in agroecosystem functioning, changes in soil biota (functional) group composition under agricultural intensification may also imply effects on the functioning of the system. Earthworms play a key role as ecosystem engineers (Lavelle et al., 1997), which, under arable management, may partly be replaced by soil tillage. Predatory mites

may affect lower levels in the food web as they are top predators in the soil ecosystem (Ruf and Beck, 2005), but their role in arable ecosystem functioning may be limited (Laakso and Setälä, 1999). Microbiota, however, are of great importance for nutrient mineralization (e.g. Ingham et al., 1985; Bloem et al., 1997). Although functional diversity of microbiota was little affected by agricultural intensification, abundances were significantly reduced in arable fields. On average, the bacterivorous nematode abundance was reduced with 60% after conversion of long term grassland to arable land, and was 40% lower in long term arable land compared to the long term grassland. Such reductions may have significantly reduced mineralization in the arable fields. We conclude that agricultural intensification affects the soil biota community in different ways, with possible consequences for agroecosystem functioning.

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References

Allison, V. J., R. M. Miller, J. D. Jastrow, R. Matamala, and D. R. Zak. 2005. Changes in soil microbial community structure in a tallgrass prairie chronosequence. Soil Science Society of America Journal 69: 1412-1421.

Bamforth, S. S. 1999. Soil Microfauna: Diversity and Applications of Protozoa in Soil. Pages 19-25 *in* W. W. Collins, and C. O. Qualset, editors. Biodiversity in Agroecosystems. CRC Press LLC, New York.

Bardgett, R. D., and E. McAlister. 1999. The measurement of soil fungal : bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biology and Fertility of Soils 29: 282-290.

Behan-Pelletier, V. M. 2003. Acari and Collembola biodiversity in Canadian agricultural soils. Canadian Journal of Soil Science 83: 288.

Bengtsson, J., S. G. Nilsson, A. Franc, and P. Menozzi. 2000. Biodiversity, disturbances, ecosystem function and management of European forests. Forest Ecology and Management 132: 50.

Berkelmans, R., H. Ferris, M. Tenuta, and A. H. C. van Bruggen. 2003. Effects of long-term crop management on nematode trophic levels other than plant feeders disappear after 1 year of disruptive soil management. Applied Soil Ecology 23: 223-235.

Blagodatskaya, E. V., and T. H. Anderson. 1998. Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and QCO_2 of microbial communities in forest soils. Soil Biology & Biochemistry 30: 1269-1274.

Bloem, J., and A. M. Breure 2003. Microbial indicators. Pages 259-282 *in* B. A. Markert, A. M. Breure, and H. G. Zechmeister, editors. Bioindicators/Biomonitors - Principles, Assessment, Concepts. Elsevier, Amsterdam.

Bloem, J., G. Lebbink, K. B. Zwart, L. A. Bouwman, S. L. G. E. Burgers, J. A. De Vos, and P. C. De Ruiter. 1994. Dynamics of microorganisms, microbivores and nitrogen mineralisation in winter wheat fields under conventional and integrated management. Agriculture, Ecosystems & Environment 51: 129-143.

Bloem, J., M. Veninga, and J. Shepherd. 1995a. Fully automatic determination of soil bacterium numbers, cell volumes and frequencies of dividing cells by confocal laser scanning microscopy and image analysis. Applied and Environmental Microbiology 61: 926-936.

Bloem, J., P. C. De Ruiter, and L. A. Bouwman 1997. Soil food webs and nutrient cycling in agroecosystems. Pages 245-278 *in* J. D. Van Elsas, J. T. Trevors, and E. M. H. Wellington, editors. Modern Soil Microbiology. Marcel Dekker, Inc., New York.

Bloem, J., P. R. Bolhuis, M. Veninga, and J. Wieringa 1995b. Microscopic methods for counting bacteria and fungi in soil. Pages 162-173 *in* K. P. N. Alef, editor. Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London.

Bongers, T. 1988. De Nematoden van Nederland. Pirola, Schoorl, The Netherlands.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14-19.

Bongers, T., and H. Ferris. 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology & Evolution 14: 224-228.

Bongers, T., R. G. M. de Goede, G. W. Korthals, and G. W. Yeates. 1995. Proposed changes of c-p classification for nematodes. Russian Journal of Nematology 3: 61-62.

Bouché, M. B. 1977. Strategies Lombriciennes. Pages 122-132 *in* U. Lohm, and T. Persson, editors. Soil Organisms as Components of Ecosystems. Proceedings of the VI. International Soil Zoology Colloquium. Ecological Bulletins, Stockholm.

Bouwman, L. A., and K. B. Zwart. 1994. The ecology of bacterivorous protozoans and nematodes in arable soil. Agriculture, Ecosystems & Environment 51: 145-160.

Brussaard, L., J. P. Bakker, and H. Olff. 1996. Biodiversity of soil biota and plants in abandoned arable fields and grasslands under restoration management. Biodiversity and Conservation 5: 211-221.

Brussaard, L., L. A. Bouwman, M. Geurs, J. Hassink, and K. B. Zwart. 1990. Biomass, composition and temporal dynamics of soil organisms of a silt loam soil under conventional and integrated management. Netherlands Journal of Agricultural Science 38: 283-302.

Brussaard, L., V. M. Behan-Pelletier, D. E. Bignell, V. K. Brown, W. Didden, P. Folgarait, C. Fragoso, D. W. Freckman, V. Gupta, T. Hattori, D. L. Hawksworth, C. Klopatek, P. Lavelle, D. W. Malloch, J. Rusek, B. Soderstrom, J. M. Tiedje, and R. A. Virginia. 1997. Biodiversity and ecosystem functioning in soil. Ambio 26: 563-570.

De Goede, R. G. M., L. Brussaard, and A. D. L. Akkermans. 2003. On-farm impact of cattle slurry manure management on biological soil quality. NJAS Wageningen Journal of Life Sciences 51: 103-133.

Ekelund, F., and R. Rønn. 1994. Notes on protozoa in agricultural soil with emphasis on heterotrophic flagellates and their ecology. FEMS Microbiology Reviews 15: 321-353.

Ettema, C. H. 1998. Soil nematode diversity: species coexistence and ecosystem function. Journal of Nematology 30: 159-169.

Ettema, C. H., and T. Bongers. 1993. Characterization of nematode colonization and succession in disturbed soil using the maturity index. Biology and Fertility of Soils 16: 79-85.

FAO-UNESCO, 1988. Soil map of the world: revised legend. FAO, Rome, Italy.

Ferris, H., and M. M. Matute. 2003. Structural and functional succession in the nematode fauna of a soil food web. Applied Soil Ecology 23: 93-110.

Ferris, H., R. C. Venette, and S. S. Lau. 1996. Dynamics of nematode communities in tomatoes grown in conventional and organic farming systems, and their impact on soil fertility. Applied Soil Ecology 3: 161-175.

Filser, J., K. H. Mebes, K. Winter, A. Lang, and C. Kampichler. 2002. Long-term dynamics and interrelationships of soil Collembola and microorganisms in an arable landscape following land use change. Geoderma 105: 201-221.

Forge, T. A., S. Bittman, and C. G. Kowalenko. 2005. Responses of grassland soil nematodes and protozoa to multi-year and single-year applications of dairy manure slurry and fertilizer. Soil Biology & Biochemistry 37: 1751-1762.

Griffiths, B. S., and K. Ritz. 1988. A technique to extract, enumerate and measure protozoa from mineral soils. Soil Biology & Biochemistry 20: 173.

Griffiths, B. S., R. E. Wheatley, T. Olesen, K. Henriksen, F. Ekelund, and R. Rønn. 1998. Dynamics of nematodes and protozoa following the experimental addition of cattle or pig slurry to soil. Soil Biology & Biochemistry 30: 1387.

Háněl, L. 2003. Recovery of soil nematode populations from cropping stress by natural secondary succession to meadow land. Applied Soil Ecology 22: 270.

Hole, D. G., A. J. Perkins, J. D. Wilson, I. H. Alexander, F. Grice, and A. D. Evans. 2005. Does organic farming benefit biodiversity? Biological Conservation 122: 113-130.

Ingham, E. R. 1994. Protozoa. Pages 491-515 *in* R. W. Weaver, editor. Methods of Soil Analysis. Soil Science Society of America, Madison, Wisconsin, USA.

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

Jagers op Akkerhuis, G. A. J. M., F. Deley, H. J. C. Zwetsloot, J. F. Ponge, and L. Brussaard. 1988. Soil microarthropods (Acari and Collembola) in 2 crop rotations on a heavy marine clay soil. Revue de Ecologie et de Biologie du Sol 25: 175-202.

Karg, W. 1993. Acari (Acarina), Milben, Parasitiformes (Anactinochaeta), Cohors Gamasina Leach. Raubmilben. Jena, Gustav Fischer Verlag, New York.

Laakso, J., and H. Setälä. 1999. Sensitivity of primary production to changes in the architecture of belowground food webs. Oikos 87: 57-64.

Lagerlöf, J., B. Goffre, and C. Vincent. 2002. The importance of field boundaries for earthworms (Lumbricidae) in the Swedish agricultural landscape. Agriculture, Ecosystems & Environment 89: 91-103.

Lavelle, P., D. Bignell, M. Lepage, V. Wolters, P. Roger, P. Ineson, O. W. Heal, and S. Dhillion. 1997. Soil function in a changing world: the role of invertebrate ecosystem engineers. European Journal of Soil Biology 33: 193.

Michel, P. H., and J. Bloem. 1993. Conversion factors for estimation of cell production rates of soil bacteria from thymidine and leucine incorporation. Soil Biology & Biochemistry 25: 943-950.

Mikola, J., and H. Setälä. 1998. No evidence of trophic cascades in an experimental microbial-based soil food web. Ecology 79: 153-164.

Moore, J. 1994. Impact of agricultural practices on soil food web structure: Theory and application. Agriculture, Ecosystems & Environment 51: 239-247.

Mulder, C., J. Cohen, H. Setala, J. Bloem, and A. Breure. 2005. Bacterial traits, organism mass, and numerical abundance in the detrital soil food web of Dutch agricultural grasslands. Ecology Letters 8: 80-90.

O'Connor, F. B. 1967. The Enchytraeidae. Pages 213-257 *in* A. Burges, and F. Raw, editors. Soil Biology. Academic Press, London, New York.

Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. Pages 85-102 *in* J. M. Sasser, and W. R. Jenkins, editors. Nematology. University of North Carolina Press, Chapel Hill.

Petersen, H. 2000. Collembola populations in an organic crop rotation: Population dynamics and metabolism after conversion from clover-grass ley to spring barley. Pedobiologia 44: 502-515.

Porazinska, D. L., L. W. Duncan, R. McSorley, and J. H. Graham. 1999. Nematode communities as indicators of status and processes of a soil ecosystem influenced by agricultural management practices. Applied Soil Ecology 13: 69-86.

Roper, M. M., and V. Gupta. 1995. Management-practices and soil biota. Australian Journal of Soil Research 33: 321-339.

Ruf, A., and L. Beck. 2005. The use of predatory soil mites in ecological soil classification and assessment concepts, with perspectives for oribatid mites. Ecotoxicology and Environmental Safety 62: 299.

Schmidt, O., R. O. Clements, and G. Donaldson. 2003. Why do cereal-legume intercrops support large earthworm populations. Applied Soil Ecology 22: 181-190.

Siepel, H., and F. Maaskamp. 1994. Mites of different feeding guilds affect decomposition of organic-matter. Soil Biology & Biochemistry 26: 1389-1394.

Sohlenius, B., and A. Sandor. 1987. Vertical-distribution of nematodes in arable soil under grass (*Festuca-pratensis*) and barley (*Hordeum-distichum*). Biology and Fertility of Soils 3: 19-25.

Susilo, F. X., A. M. Neutel, M. Van Noordwijk, K. Hairiah, G. Brown, and M. J. Swift 2004. Soil biodiversity and food webs. Pages 285-306 *in* M. Van Noordwijk, G. Cadisch, and C. K. Ong, editors. Below-ground Interactions in Tropical Agroecosystems, Concepts and Models with Multiple Plant Components. CABI Publishing, Cambridge, USA.

Swift, M. J., J. Vandermeer, P. S. Ramakrishnan, J. M. Anderson, C. K. Ong, and B. A. Hawkins 1996. Biodiversity and Agroecosystem Function. Pages 261-298 *in* H. A. Mooney, J. H. Cushman, E. Medina, O. E. Sala, and E. D. Schulze, editors. Functional Roles of Biodiversity: a Global Perspective. John Wiley & Sons Ltd.

Ter Braak, C. J. F., and P. Smilauer 2002. CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca NY, USA.

Topoliantz, S., J. Ponge, and P. Viaux. 2000. Earthworm and enchytraeid activity under different arable farming systems, as exemplified by biogenic structures. Plant and Soil 225: 39-51.

Verschoor, B. C., R. G. M. De Goede, F. W. De Vries, and L. Brussaard. 2001. Changes in the composition of the plant-feeding nematode community in grasslands after cessation of fertiliser application. Applied Soil Ecology 17: 1-17.

Villenave, C., T. Bongers, K. Ekschmitt, P. Fernandes, and R. Oliver. 2003. Changes in nematode communities after manuring in millet fields in Senegal. Nematology 5: 351-358.

Vreeken-Buijs, M. J., J. Hassink, and L. Brussaard. 1998. Relationships of soil microarthropod biomass with organic matter and pore size distribution in soils under different land use. Soil Biology & Biochemistry 30: 97-106.

Vreeken-Buijs, M. J., M. Geurs, P. C. De Ruiter, and L. Brussaard. 1994. Microarthropod biomass-C dynamics in the belowground food web of 2 arable farming systems. Agriculture, Ecosystems & Environment 51: 161-170.

Wang, K. H., R. McSorley, and R. N. Gallaher. 2003. Effect of *Crotalaria juncea* amendment on nematode communities in soil with different agricultural histories. Journal of Nematology 35: 294-301.

Wardle, D. A. 1995. Impacts of disturbance on detritus rood webs in agro-ecosystems of contrasting tillage and weed management practices. Advances in Ecological Research 26: 105-185.

Wardle, D. A., and G. W. Yeates. 1993. The dual importance of competition and predation as regulatory forces in terrestrial ecosystems - evidence from decomposer food-webs. Oecologia 93: 306.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setala, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science 304: 1629-1633.

Yeates, G. W., R. D. Bardgett, R. Cook, P. J. Hobbs, P. J. Bowling, and J. F. Potter. 1997a. Faunal and microbial diversity in three Welsh grassland soils under conventional and organic management regimes. Journal of Applied Ecology 34: 453-470.

Yeates, G. W., S. Saggar, and B. K. Daly. 1997b. Soil microbial C, N, and P, and microfaunal populations under *Pinus radiata* and grazed pasture land-use systems. Pedobiologia 41: 549-565.

Yeates, G. W., T. Bongers, R. G. M. De Goede, D. W. Freckman, and S. S. Georgieva. 1993. Feeding habits in soil nematode families and genera - an outline for soil ecologists. Journal of Nematology 25: 315-331.

Zwart, K. B., S. L. G. E. Burgers, J. Bloem, L. A. Bouwman, L. Brussaard, G. Lebbink, W. A. M. Didden, J. C. Y. Marinissen, M. J. Vreeken-Buijs, and P. C. De Ruiter. 1994. Population dynamics in the belowground food webs in two different agricultural systems. Agriculture, Ecosystems & Environment 51: 187-198.

Appendix A. Fertilization applied in arable fields under crop rotation and monoculture management. Reduced mineral fertilization fields received half the N amount needed for optimum crop production in all years, and half the P_2O_5 and K_2O amounts for optimum crop production in 2001. Complete P_2O_5 and K_2O fertilization based on soil tests was applied in all fields in 2002 and 2003. Mon = monoculture, Rot = rotation.

				Manure		Mineral fertilizer		
Year	Field history	Crop	Fertilization	kg ha^{-1}	N	N	P	K
2001	arable (LongA)	maize (Mon)	reduced (RF)	1258	90	0	0	70
			complete (CF)	1258	90	105	13	187
		maize (Rot)	reduced (RF)	1258	90	0	0	70
			complete (CF)	1258	90	105	13	187
	grassland (NewA)	maize (Mon)	reduced (RF)	1258	90	0	0	70
			complete (CF)	1258	90	105	24	187
		maize (Rot)	reduced (RF)	1258	90	0	58	70
			complete (CF)	1258	90	105	18	187
2002	arable (LongA)	maize (Mon)	reduced (RF)	785	55	35	0	29
			complete (CF)	785	55	125	0	29
		barley (Rot)	reduced (RF)	785	55	0	0	7
			complete (CF)	785	55	55	0	7
	grassland (NewA)	maize (Mon)	reduced (RF)	785	55	35	11	195
			complete (CF)	785	55	125	11	195
		barley (Rot)	reduced (RF)	785	55	0	32	55
			complete (CF)	785	55	55	32	55
2003	arable (LongA)	maize (Mon)	reduced (RF)	1264	89	0	0	111
			complete (CF)	1264	89	88	0	111
		potato (Rot)	reduced (RF)	1264	89	36	0	0
			complete (CF)	1264	89	160	0	20
	grassland (NewA)	maize (Mon)	reduced (RF)	1264	89	0	34	145
			complete	1264	89	88	34	145
		potato (Rot)	reduced (RF)	1264	89	36	67	156
			complete	1264	89	160	67	156

Appendix B. Means (n = 3) of soil biota taxonomic groups and microbial characteristics in different land use systems, in three consecutive years after conversion (2001, 2002, 2003). LongA = long term arable land, NewG = new grassland on formerly arable land, NewA = new arable land on formerly grassland, LongG = long term grassland. Different letters indicate significant differences at P < 0.10. Please note that differences within each year are tested, not between years.

	2001				2002				2003			
	LongA	**NewG**	**NewA**	**LongG**	**LongA**	**NewG**	**NewA**	**LongG**	**LongA**	**NewG**	**NewA**	**LongG**
Earthworms (n m^{-2})	13a	92b	4a	179b	46b	233c	0a	158c	17b	146c	0a	88c
Enchytraeids (n m^{-2} 10^3)	20b	ND	5a	38b	ND	ND	ND	ND	5ab	19c	1a	9bc
Mites (n m^{-2} 10^3)	34b	ND	10a	71b	59	18	ND	33	64a	110a	73a	345b
Collembolans (n m^{-2} 10^3)	19	ND	9	19	5	6	ND	12	2ab	6b	2a	18c
Nematodes (n g^{-1})	50b	ND	29a	81c	49ab	59ab	29a	63b	23a	51b	10a	76c
Protozoans (n mg^{-1})	96	ND	143	234	97ab	60ab	107a	454b	31	49	38	20
Fungal biomass (µg C g^{-1})	9a	ND	19b	25b	12	13	10	15	3ab	7b	5ab	2a
Bacterial biomass (µg C g^{-1})	72a	ND	264b	323b	42a	46a	90b	191c	46a	42a	63a	104b
Thymidine incorporation (pmol g^{-1} h^{-1})	78	ND	53	66	99	52	61	52	25ab	38b	24ab	8a
Leucine incorporation (pmol g^{-1} h^{-1})	386	ND	343	482	437	359	349	334	316b	458c	244b	137a
Fungal : bacterial biomass ratio	0.12	ND	0.07	0.08	0.28a	0.29a	0.11b	0.08b	0.07ab	0.17a	0.08a	0.02b

Appendix C. Land use change effects on soil biota trophic groups. Means (n = 3) of protozoan (a), nematode (b), predatory mite (c) and earthworm (d) trophic groups in different land use systems, in three consecutive years after conversion (2001, 2002, 2003) are presented. Different letters indicate significant differences at P < 0.10. Differences within each year are tested, not between years. For abbreviations, see Table 1.

	2001				2002				2003			
a) Protozoans (n mg^{-1})	**LongA**	**NewG**	**NewA**	**LongG**	**LongA**	**NewG**	**NewA**	**LongG**	**LongA**	**NewG**	**NewA**	**LongG**
Flagellates	56.8	ND	93.8	107.7	25.7	22.1	37.6	55.7	13.1ab	17.8b	23.6ab	10.0a
Amoebae	6.1	ND	9.5	6.1	19.3	6.7	20.6	25.0	6.3	4.2	5.3	4.0
Ciliates	0.3	ND	0.3	0.1	0.5ab	0.5ab	0.4b	0.2a	0.5	0.4	0.5	0.2
Amoeba-flagellates	1.2	ND	12.2	7.2	2.6ab	1.8a	4.7b	5.7ab	0.9b	0.7ab	0.2a	0.2a
b) Nematodes (n g^{-1})												
Plant parasites	27.4b	ND	9.3a	23.1b	18.5ab	16.5b	9.7a	23.8b	6.0a	22.2b	2.2a	36.9c
Fungivores	2.9a	ND	2.8ab	14.0b	5.9	4.6	4.2	7.6	4.8ab	7.3b	2.0a	10.4b
Bacterivores	15.8a	ND	15.3a	39.1b	23.9ab	33.9ab	14.5a	27.3b	11.6ab	19.5bc	5.8a	24.2c
Unicellulair eukaryote feeding	0.3	ND	0.1	0.2	0.4	0.3	0.0	0.3	0.0	0.0	0.0	0.0
Carnivores	2.2b	ND	0.5a	1.8b	0.4a	0.7a	0.6a	2.2b	0.5b	0.9b	0.0a	1.4ab
Omnivores	1.4ab	ND	0.6a	3.3b	0.1a	2.6b	0.0a	1.3b	0.3a	1.5b	0.2a	2.6b
Trophic diversity index (1/D)	2.5	ND	2.5	3.0	2.3	2.4	2.5	2.8	2.7	2.8	2.4	2.7
c) Predatory mites (n m^{-2} 10^{3})												
Predatory mites total									2.0a	10.3b	3.5a	18.8b
Arthropod predators									0.29a	1.06ab	0.48a	4.37b
General predators									1.54a	5.28b	0.53b	11.3b
Omnivores									0.19a	3.94b	2.40a	3.07c
Parasites									0.00	0.00	0.05	0.05
Trophic diversity index (1/D)									1.63a	2.33b	1.88ab	2.19ab
d) Earthworms (n m^{-2})												
A. calliginosa (endogeic)	13a	92b	4a	117b	46b	221c	0a	75b	17ab	121b	0a	71ab
L. rubellus (epigeic)	0.0	0.0	0.0	62.5	0.0a	8.3a	0.0a	83.3b	0.0a	25.0b	0.0a	17ab
Octolasion sp. (endogeic)	0.0	0.0	0.0	0.0	0.0	4.2	0.0	0.0	0.0	0.0	0.0	0.0

Appendix D. Agricultural management effects on protozoan trophic groups in three consecutive years after conversion (2001, 2002, 2003). Means (n = 3) and P-levels of significance (P < 0.10) as a result of ANOVA or non-parametric tests (Amoeba-flagellates) are presented. LongA = long term arable land, NewA = new arable land on formerly grassland, Mon = monoculture, Rot = crop rotation, CF = conventional fertilization, RF = reduced fertilization, Hist = field history, Fert = Fertilization.

	2001								2002								2003			
	LongA				NewA				LongA				NewA				LongA		NewA	
	Mon		Rot		Mon		Rot		Mon		Rot		Mon		Rot		Mon	Rot	Mon	Rot
Protozoans (n mg^{-1})	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**RF**	**RF**	**RF**	**RF**
Flagellates	72	65	78	57	134	78	149	94	15	12	33	26	13	22	24	38	19	13	16	24
Amoebae	8.4	5.6	10.2	6.1	13.7	5.2	5.6	9.5	11.7	5.3	12.9	19.3	3.9	4.1	7.6	20.6	2.8	6.3	2.9	5.3
Ciliates	0.8	0.7	0.4	0.3	1.0	0.3	0.8	0.3	0.5	0.1	0.3	0.5	0.7	0.4	0.7	0.4	0.3	0.5	0.3	0.5
Amoeba-flagellates	1.2	2.2	0.9	1.2	1.6	2.5	0.6	12.2	1.1	1.5	2.1	2.6	1.5	1.4	0.9	4.7	1.0	0.9	0.3	0.2

	2001				2002				2003	
P-levels	**Hist**	**Crop**	**Fert**	**Crop*Fert**	**Hist**	**Crop**	**Fert**	**Crop*Fert**	**Hist**	**Crop**
Flagellates						0.001	0.060			
Amoebae						0.022				0.067
Ciliates			0.036		0.082					
Amoeba-flagellates			0.028	ND			0.055	0.035	0.040	

Appendix E. Agricultural management effects on predatory mite trophic groups in the third year after conversion (2003). Means (n = 3) and P-levels of significance (P < 0.10) as a result of ANOVA are presented. For abbreviations see Appendix D.

	LongA		NewA	
	Mon	Rot	Mon	Rot
Predatory mites (n m^{-2} 10^3)	**RF**	**RF**	**RF**	**RF**
Predatory mites total	1.54	2.02	1.68	3.46
Arthropod predators	0.34	0.29	0.10	0.48
General predators	1.10	1.54	1.39	0.53
Omnivores	0.10	0.19	0.19	2.40
Parasites	0.00	0.00	0.00	0.05
Trophic diversity index (1/D)	1.59	1.63	1.38	1.88

P-levels	**Hist**	**Crop**
Arthropod predators		
General predators	0.015	0.010
Omnivores		
Parasites	ND	ND
Trophic diversity index (1/D)		

Appendix F. Agricultural management effects on earthworm ecological groups in three consecutive years after conversion (2001, 2002, 2003). Means (n = 3) and P-levels of significance (P < 0.10) as a result of ANOVA or non parametric tests (*L. rubellus* in 2002) are presented. For abbreviations see Appendix D.

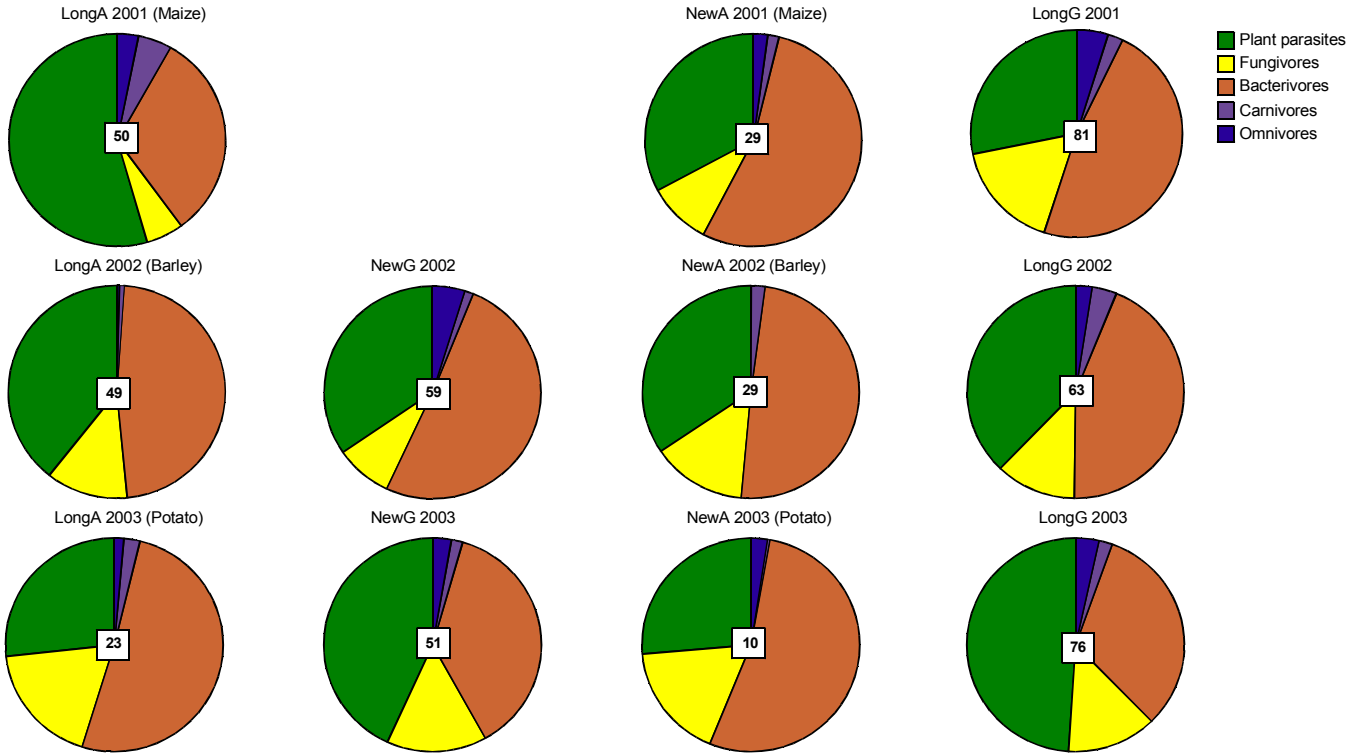
	2001								2002								2003			
	LongA				NewA				LongA				NewA				LongA		NewA	
	Mon		Rot		Mon		Rot		Mon		Rot		Mon		Rot		Mon	Rot	Mon	Rot
Earthworms (n m^{-2})	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**CF**	**RF**	**RF**	**RF**	**RF**	**RF**
A. calliginosa (endogeic)	12.5	0.0	12.5	12.5	0.0	12.5	0.0	4.2	8.3	8.3	4.2	45.8	0.0	4.2	4.2	0.0	4.2	16.7	0.0	0.0
L. rubellus (epigeic)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.2	0.0	0.0	12.5	0.0	0.0	0.0	0.0	0.0	0.0
Octolasion sp. (endogeic)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.2	0.0	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

	2001				2002				2003	
P-levels	**Hist**	**Crop**	**Fert**	**Crop*Fert**	**Hist**	**Crop**	**Fert**	**Crop*Fert**	**Hist**	**Crop**
A. calliginosa (endogeic)					0.005			0.078	0.001	
L. rubellus (epigeic)	ND	ND	ND	ND				ND	ND	ND
Octolasion sp. (endogeic)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

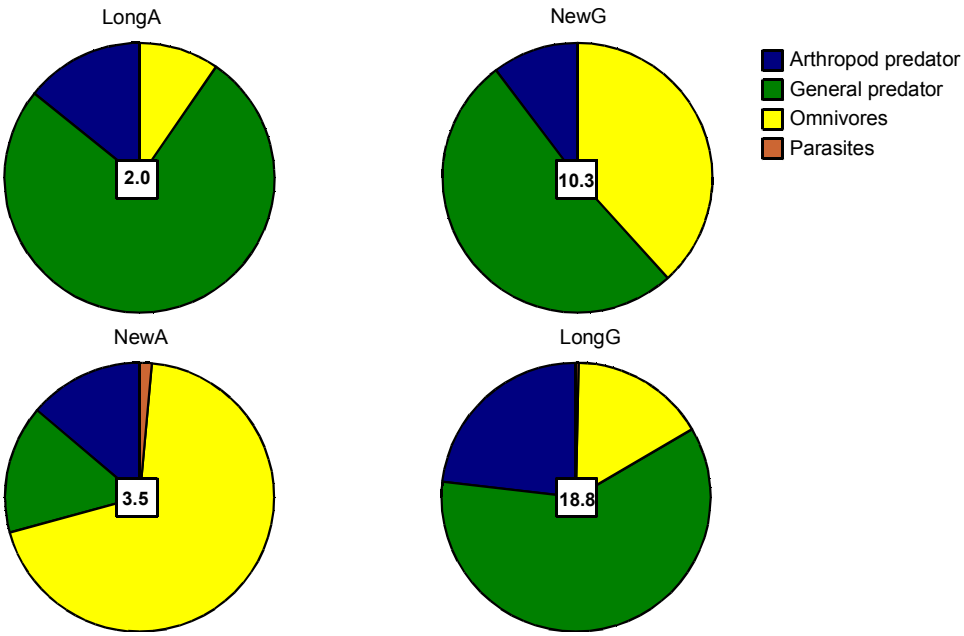
Appendix G. Agricultural management effects on the predatory mite life strategy groups in the third year after conversion (2003). Means (n = 3) and P-levels of significance (P < 0.10) as a result of ANOVA are presented. For abbreviations see Appendix D.

	LongA		NewA	
	Mon	Rot	Mon	Rot
Predatory mites (n m^{-2} 10^3)	**RF**	**RF**	**RF**	**RF**
Parasites	0.00	0.00	0.00	0.00
Facultative phoresy	0.48	0.48	0.24	0.24
Obligate phoresy as juveniles	0.05	0.00	0.14	0.05
Obligate phoresy as adults	0.24	0.10	0.29	0.24
Obligate diapause	0.10	0.19	0.19	2.40
Facultative diapause and anemochory	0.00	0.05	0.00	0.00
Thelytoky	0.00	0.00	0.00	0.00
No phoresy or synchronization tactics	0.67	1.20	0.82	0.53
Life strategy group diversity index (1/D)	2.37	2.36	2.36	1.95

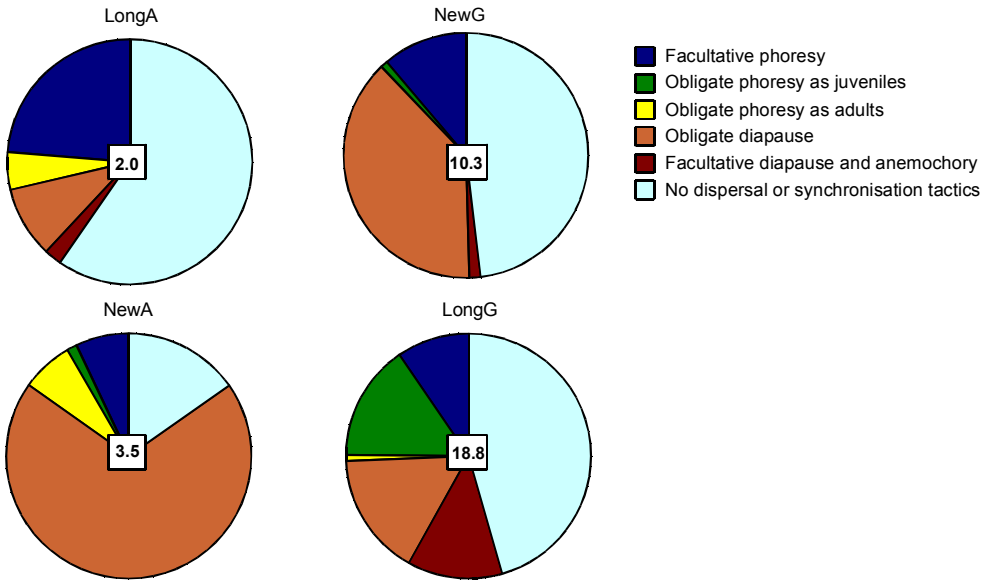
P-levels	**Hist**	**Crop**
Parasites	ND	ND
Facultative phoresy		
Obligate phoresy as juveniles		
Obligate phoresy as adults		
Obligate diapause	0.015	0.010
Facultative diapause and anemochory	ND	ND
Thelytoky	ND	ND
No phoresy or synchronization tactics		
Life strategy group diversity index (1/D)		



Appendix H. The effect of land use change on nematode trophic group composition in three consecutive years after conversion (2001, 2002 and 2003). Total nematode abundances (n g^{-1}) are indicated in the center of each pie diagram. LongA = long term arable land, NewG = new grassland on formerly arable land, NewA = new arable land on formerly grassland, LongG = long term grassland. Figures read counter clockwise.



Appendix I. The effect of land use change on predatory mite trophic group composition in the third year after conversion (2003). Total predatory mite abundances (n m^{-2} 10^{-3}) are indicated in the center of each pie diagram. LongA = long term arable land, NewG = new grassland on formerly arable land, NewA = new arable land on formerly grassland, LongG = long term grassland. Figures read counter clockwise.



Appendix J. The effect of land use change on predatory mite life strategy group composition in the third year after conversion (2003). Total predatory mite abundances (n m^{-2} 10^{-3}) are indicated in the center of each pie diagram. Parasitic mite percentages were very low and were therefore not presented in this graph. LongA = long term arable land, NewG = new grassland on formerly arable land, NewA = new arable land on formerly grassland, LongG = long term grassland. Figures read counter clockwise.

Chapter 5

Agricultural (de-)intensification differentially affects taxonomic diversity of predatory mites, enchytraeids, nematodes and bacteria

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Abstract

Agricultural intensification (including soil tillage, reduced crop diversity, increased mineral fertilization) is known to impact the soil biota community. In Chapter 4 we described the impact of agricultural intensification on total abundances and functional group structure of major soil biota groups. In this chapter we address the effects of conversion of extensively managed grassland to arable land, reduced crop diversity and increased mineral fertilization levels on taxonomic diversity at genus and species level of four major soil biota groups (predatory mites, enchytraeids, nematodes and bacteria). *Vice versa*, the effect of agricultural de-intensification (re-establishment of grassland on formerly arable land) on soil biota taxonomic diversity was also studied. We compared effects across taxonomic levels to identify sensitive species groups.

Conversion of an extensively managed grassland to an arable system on the short term had detrimental effects on taxonomic richness and diversity across taxonomic groups, with largest effects on soil biota with larger body size. Although effects of arable management were detrimental shortly after conversion, they were less so after consistent long term arable management. Restoration of grassland resulted in establishment of a species rich community of nematodes (although species were partly different), whereas predatory mite species were less successful in re-establishing and enchytraeid species were negatively affected. Agricultural management effects were less obvious than the conversion effects.

Results from this study were combined with the results in Chapter 4 on functional diversity, leading to the conclusion that agricultural intensification results in a reduction in abundance and taxonomic diversity of nematodes and bacteria, whereas functional group structure is little affected. In contrast, predatory mites and earthworms are affected both in functional group structure and taxonomic diversity. Consequences of reduced taxonomic diversity may occur as a result of reduced resilience to stress, whereas reduced total abundances of microbiota may be important for ecosystem functions such as nutrient mineralization. Furthermore, the loss of the "key" enchytraeid species *Cognettia sphagnetorum* after agricultural intensification may result in changed agroecosystem functioning. We conclude that agricultural intensification affects total abundances and taxonomic diversity of soil biota, but not necessarily the functional group diversity.

Introduction

Agricultural intensification, including soil tillage, increased mineral fertilization, and crop diversity reduction has been found to affect soil biological diversity at different levels within the soil food web, ranging from genetic diversity of bacteria to species diversity of predatory mites (Wardle, 1995; Yeates et al., 1997; Behan-Pelletier, 2003; Háněl, 2003; Garbeva et al., 2006; Van Diepeningen et al., 2006). Understanding these effects may be useful in order to preserve and restore biodiversity in agricultural soils and enhance the role of soil biota in agroecosystem functioning.

Diversity of (genera of) **bacteria** has been found to decrease as a result of agricultural intensification (Garbeva et al., 2003; Garbeva et al., 2006), although other studies suggest that agricultural intensification has limited effects on bacterial diversity (Bloem and Breure, 2003; Salles et al., 2006). Also **nematode** genus or species diversity has been found to decrease as a result of intensified agricultural management, such as conversion of grassland to arable land (Háněl, 2003), and under conventional agriculture compared to organic low-input agriculture (Freckman and Ettema, 1993; Van Diepeningen et al., 2006). Other studies, however, reported neutral effects of organic management compared to conventional management on species diversity (Ferris et al., 1996), and both negative and positive effects of increased fertilization on different species have been described (Ettema and Bongers, 1993; Porazinska et al., 1999; Sarathchandra et al., 2001; Villenave et al., 2003). The diversity of **mites** has been found to decrease as a result of agricultural intensification (Behan-Pelletier, 2003, and references therein). The species composition of the top predators, predatory mites, has been found to change in response to different cropping systems (Jagers op Akkerhuis et al., 1988). Effects of agricultural intensification on the **enchytraeid** community are little studied. The enchytraeid community has been found to be affected by liming, which reduced a key stone species (*Cognettia sphagnetorum*) and altered the species composition (Black et al., 2003; Cole et al., 2006).

While different effects of agricultural intensification on soil biota species diversity have been found, the concomitant investigation of agricultural intensification and de-intensification and the inclusion of taxonomic diversity within more than one major soil biota group are rare. Whereas effects of agricultural (de-)intensification on diversity at the level of orders or classes may be limited (Susilo et al., 2004; Chapter 4), studies of such effects at the level of genera or species may reveal changes that might otherwise be overlooked. Furthermore, such studies may clarify the absence or presence of effects on functional group diversity within major soil biota groups (Chapter 4).

Therefore, we investigated the effects of agricultural (de-)intensification (conversion of an extensively managed grassland to an arable crop rotation and *vice versa*) on species diversity of predatory mites and enchytraeids, genus diversity of nematodes and genetic diversity of bacteria.

The effects of further agricultural management (de-)intensification in terms of crop diversity and mineral fertilization on nematode genus diversity were also investigated.

In Chapter 4 we found that total abundances and functional diversity of the soil biota groups with larger body size are more negatively affected than those of smaller-sized soil biota groups (Chapter 4). We therefore hypothesised that also species diversity and community structure of larger-sized soil biota would be more negatively affected by agricultural intensification than of the smaller-sized soil biota. Furthermore, we expected that the reverse of agricultural intensification, the re-establishment of grassland on formerly arable land, would result in increased species diversity, with more positive effects on larger-sized soil biota. We expected that after conversions in agriculture, the species community would come to resemble the community of the system to which it was changed (grassland, arable land), but that effects of intensification would be quicker to become established than restorative effects (Swift et al., 1996).

Materials and Methods

Experimental setup

An experimental field ('De Bovenbuurt' 51°59'N, 5°40'E, the Netherlands) was selected, with two agricultural systems with extreme management regimes located close to each other. On the one extreme was a long term extensively managed grassland (>50 years) (long term grassland) and on the other a conventionally managed crop rotation that was converted from the grassland around 1980 (long term arable land). In 2000 an experiment was started where part of the long term grassland was converted into different agricultural regimes, and part of the long term arable land was converted into grassland, resulting in four experimental systems: long term grassland, new arable land, long term arable land and new grassland, thus giving us the opportunity to study agricultural intensification (conversion of grassland to arable land) on the short therm (new arable land), on the long term (long term arable land) and *vice versa* the effect of extensification of the arable land, by comparing the long term arable land with the new grassland and long term grassland. Further intensification of agricultural practices was studied by establishing different management regimes on both the long term and new arable system: a crop rotation versus a monoculture of maize, and reduced versus conventional mineral fertilization. Complete randomization of treatments was not possible, since the long term arable land and long term grassland fields were fixed. To correct for possible gradients within these two fields, three replicate blocks on long term grassland and long term arable land were assigned, with treatments (10 x 12 field plots) randomized within each block.

Genetic diversity of bacteria and genus diversity of nematodes were studied in long term grassland, long term arable land and new arable land in three consecutive years (2001, 2002, 2003), starting one year after the conversion of long term grassland in 2000, and in the new grassland starting two years after conversion (2002, 2003). Enchytraeid species diversity in the four experimental systems was studied in 2002, and predatory mites species diversity was studied in 2003. The effect of crop diversity reduction on nematode genus diversity was studied in three consecutive years (2001, 2002, 2003), and the effect of increased mineral fertilization on nematode genus diversity was studied in two years (2001, 2002).

Cropping systems, soil characteristics and crop management

Description of sites

The soil of the experimental site is a Fimic Anthrosol (FAO-UNESCO, 1988), or a loamy sand (0-10 cm: fraction > 50 µm: 89%, 16 µm - 50 µm: 7%, 2 µm - 16 µm: 5%, < 2 µm: 3%, measured in 2002). Dominant species on the long term grassland were *Festuca rubra*, *Holcus lanatus*, *Anthoxanthum odoratum*, *Rumex acetosa* and *Ranunculus sp*.. The long term arable land was under a crop rotation (oat, maize, barley, potato), with conventional fertilization levels, weed control through herbicide application and 20 cm deep tillage. At the start of the experiment in April 2000, long term grassland and long term arable land were characterized by organic matter contents of 3.9%, and 3.3% respectively, pH (KCl) of 4.4 and 5.0 respectively, and moisture contents of 28% and 16% respectively (calculated as soil water (g) * dry weight (g) $^{-1}$ *100%).

Conversions

In April 2000, part of the long term grassland was ploughed (20 cm). Three plots (long term grassland) were left undisturbed. On the long term arable land, three plots were sown with a grassland species mixture containing grass, clover and herb species. Both new grassland and long term grassland were mown and harvested two or three times in the summer, depending on the growth rate of the grass. Sods of the upper 10 cm of the long term grassland soil (outside the experimental plots) were applied in one corner (4 m^2) of each plot on the long term arable fields, in order to ensure the possibility of establishment of taxa, that may be limited by dispersion.

Agricultural regimes

In the arable fields under crop rotation management, the current rotation was continued: maize (variety: Crescendo) in 2001, barley (Aspen) in 2002 and potato (Desiree) in 2003. In the arable fields with monoculture regime, maize was sown continuously, from 2000 onwards. Reduced tillage (10 cm) and mechanical weed control instead of herbicides were applied in all experimental arable fields. All arable crops received a similar amount of organic manure in each

year, and an additional dressing of mineral fertilizer for optimal production of each crop (conventional fertilization) or half of the amount needed for optimal production of the crop (reduced fertilization) (Appendix A in Chapter 4).

Sampling and analysis of the different soil biota groups

Samples for analysis of taxonomic diversity of soil biota were taken from the upper 10 cm soil layer. Soil samples for identification of bacterial and nematode taxonomic diversity were taken on September 23 in 2001, September 24 in 2002 and September 8 in 2003. Sixty soil cores (diameter 2.2 cm, depth 10 cm) were combined into a bulk sample. The soil was thoroughly mixed, and stored at 4 °C until analysis. Bacterial DNA from a 2 gram subsample was extracted (Van Elsas and Smalla, 1995) and analysed by denaturating gradient gel electrophoresis (DGGE) (Muyzer et al., 1993). This technique yields a banding pattern where the number of DNA bands reflects the number of 'species' (genotypes) of abundant bacteria, and the band-intensity reflects the relative abundance of the species (Bloem and Breure, 2003; Dilly et al., 2004). Total number of DNA bands and genetic diversity (Shannon-Wiener: H') were calculated. Nematodes were extracted from approximately 150 g soil using an Oostenbrink elutriator (Oostenbrink, 1960), counted and identified to genus according to Bongers (1988). Nematode taxa were assigned to feeding groups (Yeates et al., 1993) and life strategy groups (c-p groups) (Bongers, 1990; Bongers et al., 1995).

Samples for identification of enchytraeid species diversity were taken on March 3, 2002. On each experimental plot three replicate samples were taken with a 5.9 cm diameter soil corer. Enchytraeids were extracted using wet extraction following O'Connor (1967), counted and averaged per experimental plot. Enchytraeids were determined to species or genus following Nielsen and Christensen (1959).

Samples for identification of predatory mites were taken on September 8, 2003. On each experimental plot, four replicate samples were taken with a 4.7 diameter soil corer. Microarthropods were extracted from soil samples using a Berlese-Tullgren funnel. Mites were counted and averaged per experimental plot. Predatory mites were identified to species, or to genus or family if identification to species was not possible (Karg, 1993).

Species richness and diversity indices (Shannon-Wiener: H', reciprocal Simpson's index: 1/D) of nematodes, enchytraeids and predatory mites were calculated. Both Shannon-Wiener and Simpson's indices were calculated, since the Shannon-Wiener index emphasizes the presence of rare species, whereas common species are emphasized in the Simpson's index (Magurran, 2004). Further details on sampling procedures are presented in Chapter 4.

Typifying nematode genera and enchytraeid and predatory mite species (i.e. taxa characteristic for each land use system) were calculated using the concept of constancy, fidelity and concentration of abundance (adapted from Nijboer et al., 2005). Constancy is defined as the number of occurrences of a taxon in a system divided by the number of replicate samples of the system. Fidelity is the constancy of a taxon in one system divided by the average constancy of the taxon in the systems that are compared. Concentration of abundance is the average abundance of a taxon in one system divided by the average abundance of the taxon in the systems that are compared. Long term arable land was compared with long term grassland for genera with high constancy (> 0.499), fidelity (> 1.499) and concentration (> 1.499) in either of the systems, which were the typifying genera for these systems. Similarly, new arable land was compared with long term grassland, and new grassland was compared with long term arable land.

Statistical analysis

ANOVA

Statistical analyses were carried out using the statistical package SPSS version 11.0. Data were transformed (log10 or square root transformations) to meet assumptions of normality and homogeneity of variance. The effects of different land use systems on soil biota taxonomic species richness and diversity indices in each year were analysed with one-factor ANOVA. Treatments were long term grassland, long term arable land and new arable land in 2001, and these treatments plus new grassland in 2002 and 2003, and were followed by a Tukey HSD post-hoc analysis. The effects of agricultural management on nematode genus richness and diversity were analysed using a nested two-factor (in 2001 and 2002) or one-factor (in 2003) ANOVA design. Treatments were cropping system (monoculture versus rotation, in 2001, 2002 and 2003) and mineral fertilization level (conventional versus reduced, in 2001 and 2002), and were nested within the factor field history (grassland or arable land). Since variation in soil biota data was expected to be high, and replications in the field were limited (n = 3), P-levels of 0.10 or less were regarded as significant. Non-parametric tests were performed in case the data did not meet assumptions of ANOVA (Mann-Whitney U for comparison of two samples and Kruskal-Wallis H for comparison of more than one sample).

Redundancy Analysis (RDA)

The taxonomic community structure of abundances of predatory mites, enchytraeids and nematodes in different land use systems and agricultural management systems in each year was analysed with RDA (CANOCO 4.5, Biometrics, Wageningen, The Netherlands), in order to correlate community structure with environmental variables and assuming species distributions to be linear (Ter Braak and Smilauer, 2002). Biplots of environmental and species variables were

produced with scaling focused on inter-species correlations. Species or genera were presented as vectors and environmental variables (land use system, crop, fertilization level) as centroids. In these plots the length and the slope of the vectors, and position of centroids indicate the strength of the correlation with the ordination axes and with other variables.

Monte Carlo permutation tests (499 random permutations, P = 0.05) were performed to establish the significance of the correlations between community structure and environmental variables. Separate analyses were done for the factors land use change (long term and new grassland and arable systems) and agricultural management (crop and fertilization level).

Results

Bacterial diversity

The number of DNA bands in our systems ranged between 53 and 76 (Table 1). The effect of different land use systems and land use change on bacterial genetic richness and diversity changed over years. A negative effect of conversion of grassland to arable land was found in the first year after conversion, but no significant effects were found in two following years. Re-establishment of grassland had no significant effect on bacterial genetic richness in any of the years.

Land use change effects on nematode diversity

Diversity and genus richness

The average nematode genus richness per experimental plot ranged from 19 genera in the new arable system to 29 genera in the new grassland system (Table 1). Genus richness was not statistically significantly affected by conversion of grassland to arable land, although a trend of reduced richness after conversion was visible each year. Shannon and Simpson's indices were reduced after conversion to arable land, a trend that became significant in the third year of the experiment. After re-establishment of grassland on long term arable land, genus richness and diversity indices increased to levels similar to those in long term grassland.

Nematode community structure

Furthermore, the nematode community structure was significantly affected by land use change in all years (P = 0.010 in 2001, P = 0.004 in 2002, P = 0.002 in 2003). In the first two years after conversion, the two field histories were separated along the first axis of the RDA, and the current management regimes were separated along the second axis, whereas in the third year the

Table 1. Mean (n = 3) taxonomic diversity of bacteria, nematodes, enchytraeids and predatory mites in different land use systems. Conversion of land use took place in 2000. LongA = long term arable land, NewG = new grassland on formerly arable land, NewA = new arable land on formerly grassland, LongG = long term grassland. Different letters denote significant differences within each year at P < 0.10.

	2001			2002				2003			
	LongA	**NewA**	**LongG**	**LongA**	**NewG**	**NewA**	**LongG**	**LongA**	**NewG**	**NewA**	**LongG**
Bacterial diversity											
DNA bands	53 a	56 a	70 b	73	76	76	74	62 b	60 ab	53 ab	58 a
Shannon-Wiener index (H')	3.70 a	3.71 a	3.94 b	3.90	3.98	3.98	3.86	3.75 b	3.72 ab	3.55 a	3.62 ab
Nematode diversity											
Number of genera	29	23	29	22 a	27 c	23 ab	27 bc	22 ab	29 b	19 a	23 ab
Shannon-Wiener index (H')	2.76	2.73	2.94	2.58 a	2.89 ab	2.65 ab	2.91 b	2.68 ab	3.03 c	2.41 a	2.81 bc
Simpson's index (1/D)	9.1 a	11.5 ab	14.4 b	9.2 a	14.0 ab	10.3 ab	14.1 b	11.2 ab	16.3 c	8.6 a	13.7 bc
Enchytraeid diversity											
Species richness				7.0 ab	4.3 ab	3.7 a	7.3 b				
Shannon-Wiener index (H')				1.13	0.86	0.98	1.38				
Simpson's index (1/D)				2.35	1.89	2.34	3.48				
Predatory mites diversity											
Species richness								6 a	10 b	4 a	20 c
Shannon-Wiener index (H')								1.56 b	1.72 b	0.93 a	2.43 c
Simpson's index (1/D)								4.30 b	4.35 b	2.03 a	8.55 c

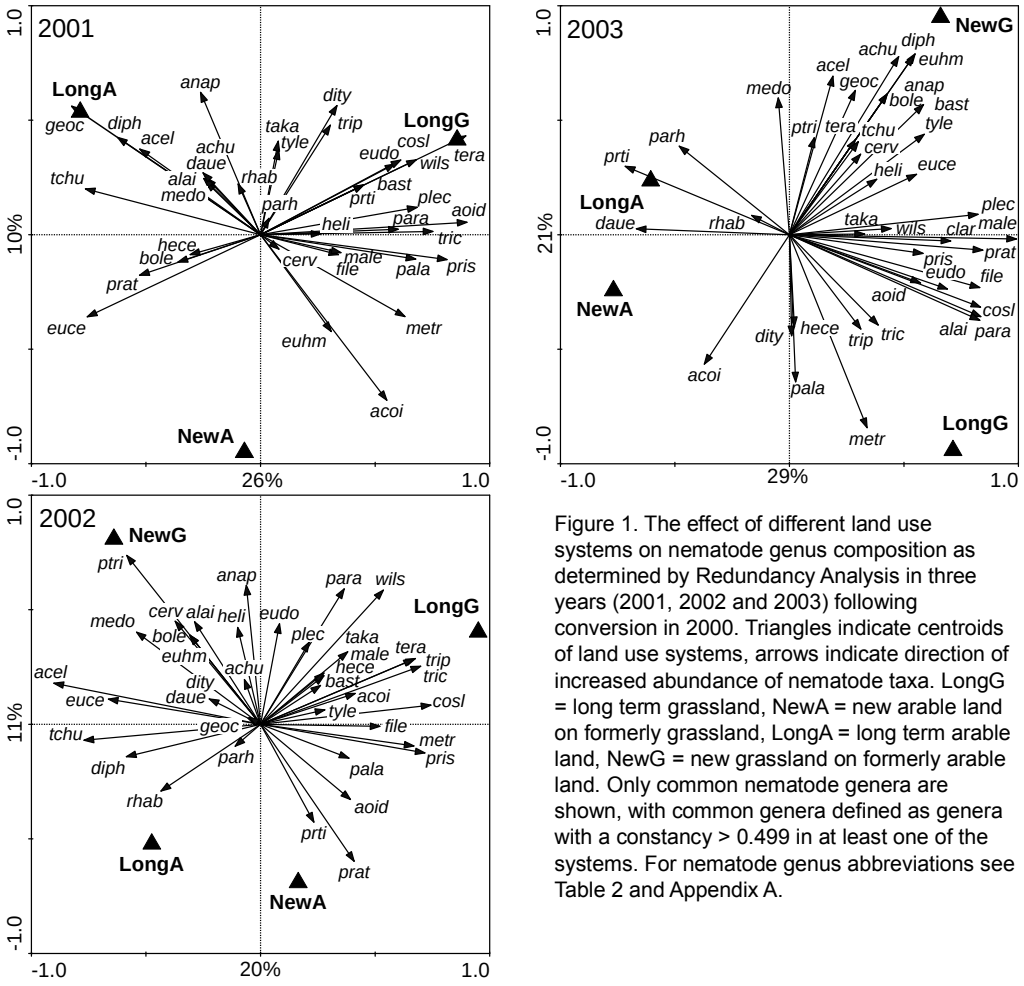

Figure 1. The effect of different land use systems on nematode genus composition as determined by Redundancy Analysis in three years (2001, 2002 and 2003) following conversion in 2000. Triangles indicate centroids of land use systems, arrows indicate direction of increased abundance of nematode taxa. LongG = long term grassland, NewA = new arable land on formerly grassland, LongA = long term arable land, NewG = new grassland on formerly arable land. Only common nematode genera are shown, with common genera defined as genera with a constancy > 0.499 in at least one of the systems. For nematode genus abbreviations see Table 2 and Appendix A.

current management regimes were separated along the first axis and the field histories along the second axis (Fig. 1). In the third year after conversion, the two arable systems were positioned closer to each other than the two grassland systems, indicating that abundances were more similar in the arable systems than in the grassland systems.

Dominance structure

Averaged over years, eight genera had a relative abundance higher than 5% on the long term grassland, designating them as dominant genera (*Aphelenchoides* (9%), *Prismatolaimus* (8%), *Filenchus* (8%), *Pratylenchus* (7%), *Paratylenchus* (6%), *Panagrolaimus* (5%), *Coslenchus* (5%) and *Ditylenchus* (5%) (Table 2, Appendix A)). After conversion of grassland to arable land,

Table 2. Absolute (n g^{-1}) and relative abundances of dominant and/or typifying nematode genera in different land use systems. Abundances and percentages are averages of three replicate fields and three consecutive years (2001 to 2003) (n=9). Dominant genera are defined as genera with a relative abundance > 5% in at least one of the systems. Typifying genera are highlighted. For explanation of the concepts of constancy and typifying genera, see the materials and methods section. For abbreviations see Table 1.

			LongA		NewG		NewA		LongG	
Genus	**Abbrev.**	**c-p/pp index**	**Abund.**	**%**	**Abund.**	**%**	**Abund.**	**%**	**Abund.**	**%**
Plant parasites										
Geocenamus	geoc	3	0.9	1.9	0.5	1.2	0.1	0.2	0.1	0.1
Helicotylenchus	heli	3	1.9	4.1	3.3	6.7	0.7	4.6	2.5	3.1
Paratylenchus	para	2	0.1	0.1	0.6	1.3	0.1	0.3	4.2	5.2
Pratylenchus	prat	3	4.4	11.0	3.8	7.7	3.3	13.7	5.1	7.2
Tylenchorhynchus	tchu	3	8.0	17.8	3.0	5.8	0.5	2.3	1.1	1.6
Root-fungal feeders										
Boleodorus	bole	2	0.1	0.1	0.5	1.2	0.0	0.1	0.0	0.0
Coslenchus	cosl	2	0.2	0.4	0.3	0.7	0.1	0.7	3.8	5.2
Filenchus	file	2	0.8	1.7	2.8	6.0	1.2	5.4	5.3	7.6
Malenchus	male	2	0.1	0.2	0.6	1.2	0.2	0.7	1.3	2.0
Fungal feeders										
Aphelenchoides	aoid	2	2.6	6.9	2.3	4.3	2.1	9.7	6.8	9.0
Diptherophora	diph	3	0.4	0.8	0.7	1.0	0.0	0.0	0.0	0.0
Ditylenchus	dity	2	1.3	4.0	1.2	2.7	0.6	3.4	3.8	5.0
Bacterial feeders										
Acrobeloides	acoi	2	1.5	5.7	1.3	2.5	3.1	14.7	2.5	3.6
Eucephalobus	euce	2	2.4	6.3	3.8	7.2	1.6	8.2	1.3	1.9
Eumonhystera	euhm	2	0.0	0.0	0.7	1.3	0.1	0.2	0.1	0.1
Metateratocephalus	metr	3	0.0	0.1	0.0	0.0	0.3	1.2	3.4	4.3
Panagrolaimus	pala	1	1.3	3.1	1.7	2.7	0.8	4.7	4.1	6.0
Prismatolaimus	pris	3	0.7	1.6	0.8	1.5	0.8	2.8	5.5	7.6
Protorhabditis	parh	1	0.9	2.6	5.5	6.2	0.4	1.4	0.7	1.0
Rhabditis	rhab	1	3.6	8.1	3.5	5.0	1.8	7.0	2.1	2.8
Teratocephalus	tera	3	0.0	0.0	0.1	0.2	0.0	0.0	2.3	2.9
Wilsonema	wils	2	0.0	0.0	0.5	0.7	0.0	0.0	0.5	0.7
Carnivores										
Tripyla	trip	3	0.1	0.2	0.0	0.0	0.0	0.0	0.6	0.9
Omnivores										
Eudorylaimus	eudo	4	0.1	0.4	0.7	1.5	0.1	0.3	1.3	1.9

six genera dominated on the new arable land, of which three were the same as those on the long term grassland (*Pratylenchus* (14%), *Aphelenchoides* (10%) and *Filenchus* (5%)) and three were different from those on the long term grassland (*Acrobeloides* (15%), *Eucephalobus* (8%) and *Rhabditis* (7%)). The dominance structure on the new arable land closely resembled the dominant genera on the long term arable land (*Tylenchorynchus* (18%), *Pratylenchus* (11%), *Rhabditis* (8%), *Aphelenchoides* (7%), *Eucephalobus* (6%), *Acrobeloides* (6%)). In contrast, the dominant genera on the new grassland system (*Pratylenchus* (8%), *Eucephalobus* (7%), *Helicotylenchus* (7%), *Protorhabditis* (6%), *Filenchus* (6%), *Tylenchorynchus* (6%) and *Rhabditis* (5%)) showed little similarity with the long term grassland system, except for the dominance of *Filenchus*.

Typifying genera

Seven typifying genera were found on the long term grassland system (*Eudorylaimus*, *Malenchus*, *Metateratocephalus*, *Paratylenchus*, *Teratocephalus*, *Tripyla* and *Wilsonema*) and two on the long term arable system (*Diphterophora* and *Geocenamus* (*Merlinius*)) (Table 2, Appendix A). After conversion of the long term grassland to new arable land, no typifying genera were found on the new arable land, indicating that the new arable land was an impoverished community derived from the long term grassland, and all genera showed either neutrality or higher incidence on the long term grassland. All typifying long term grassland genera were strongly reduced after conversion to arable land.

Typifying genera on the new grassland (*Boleodorus*, *Eumonhystera*) were different from those on the long term grassland. Still, five of the seven typifying long term grassland genera had relatively high constancy, fidelity and/or concentration levels on the new grassland, indicating that these genera increased on the new grassland, but were not (yet) as well established on the new grassland as on the long term grassland. The typifying long term arable genera were not consistently reduced after re-establishment of grassland.

Agricultural management effects on nematode diversity

Cropping system

Cropping system did not affect nematode genus richness or Shannon or Simpson's indices, except for a small positive effect of the rotation system on Simpson's index in 2002 (Table 3). Cropping system did, however, affect nematode community structure ($P = 0.002$ and 0.018 for the effect of crop in 2002 and 2003, respectively).

Fertilization

Increased fertilization levels negatively affected nematode Shannon and Simpson's indices and genus richness (Table 3; marginally significant in 2001, highly significant in 2002). This effect was especially clear on the new arable system and less clear on the long term arable system. Fertilization also affected nematode community structure (P = 0.003 and 0.036 for the effect of fertilization in 2001 and 2002, respectively).

Enchytraeid diversity

Species diversity

The average enchytraeid species richness in the second year after conversion was approximately 4 species in the transformed systems and 7 species in the long term arable and grassland systems. Conversion of grassland to arable land reduced enchytraeid species richness (Table 1). After re-establishment of grassland on the long term arable system, species richness was not significantly affected, but tended to decrease. The diversity indices showed the same trends, although not statistically significant. The decrease in species as a result of land use change became further apparent when total species richness in the different fields was compared. After conversion of grassland to arable land, six species were reduced to undetectable levels, and also after re-establishment of grassland, one species was no longer observed.

Community structure

RDA revealed that the enchytraeid community struchangnture was significantly affected by land use change (P = 0.004, Fig. 2). Long term arable systems were separated from long term grassland systems along the first axis, whereas transformed systems were separated from long term systems along

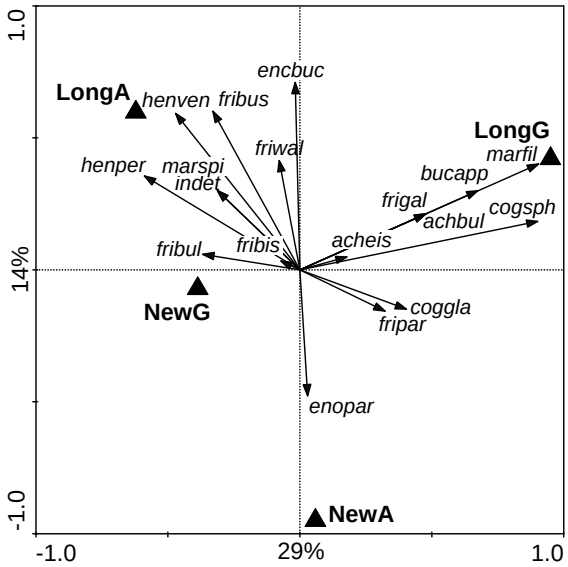

Figure 2. The effect of different land use systems on enchytraeid species composition as determined by Redundancy Analysis in the second year (2002) following conversion in 2000. For explanation of codes see Figure 1. For enchytraeid species abbreviations see Table 4 and Appendix B.

Table 3. Mean (n = 3) nematode genus richness and diversity indices under different agricultural management regimes in three years (2001 to 2003), following conversion in 2000, and results of ANOVA (factors field history (History), cropping system (Crop), and fertilization (Fert)). P-values < 0.10 are presented. LongA = Long term arable land, NewA = new arable land on formerly grassland, Mon = monoculture, Rot = rotation, CF = conventional fertilization, RF = reduced fertilization.

	2001								2002								2003			
	LongA				NewA				LongA				NewA				LongA		NewA	
	Mon		Rot		Mon		Rot		Mon		Rot		Mon		Rot		Mon	Rot	Mon	Rot
Means	CF	RF	CF	RF	CF	RF	CF	RF	CF	RF	CF	RF	CF	RF	CF	RF	RF	RF	RF	RF
Number of genera	24	25	24	29	20	24	22	23	23	25	21	22	20	23	19	23	22	22	17	19
Shannon-Wiener index (H')	2.5	2.5	2.6	2.8	2.4	2.8	2.6	2.7	2.5	2.5	2.5	2.6	2.2	2.7	2.4	2.6	2.6	2.7	2.2	2.4
Simpson's index (1/D)	7.2	7.3	8.1	9.1	8.4	13.6	9.2	11.5	7.9	6.9	8.4	9.2	5.2	10.1	8.0	10.3	10.2	11.2	6.1	8.6

	2001				2002				2003	
P-values from ANOVA	**History**	**Crop**	**Fert**	**Crop*Fert**	**History**	**Crop**	**Fert**	**Crop*Fert**	**History**	**Crop**
Number of genera	0.025						0.092		0.001	
Shannon-Wiener index (H')			0.071				0.003		0.003	
Simpson's index (1/D)	0.015		0.062			0.063	0.003	0.109	0.016	

the second axis. Most species had highest abundances in the long term grassland, or in the long term arable land, whereas abundances appeared to be low in the newly transformed systems.

Dominance structure

Two species dominated on the long term grassland (*Cognettia sphagnetorum*, 48% and *Enchytraeus buchholzia*, 21%) (Table 4, Appendix B). After conversion to arable land, 10 of the 14 grassland species were strongly reduced. *Fridericia paroniana* remained at a high abundance and dominated the new arable system (40%). Also the dominant long term grassland species (*C. sphagnetorum* and *E. buchholzia*) were relatively less reduced and remained at high relative abundances on the new arable land (22%, 11%, respectively). A similarity between the new arable land and the long term arable land was the high relative abundance of *Henlea perpusilla* (12% and 16% on the new and long term arable land respectively).

The dominance structure on the long term arable land resembled the long term grassland in the high abundance and dominance of *E. buchholzi* (59%), but differed from the long term grassland in the high relative abundances of two other species (*F. bulboides*, 11%, and *Henlea perpusilla* 16%) and the absence of *C. sphagnetorum*. The dominance structure on the new grassland largely resembled the long term arable land from which it originated, with *E. buchholzia* (70%) dominating and a high relative abundance of *H. perpusilla* (16%).

Table 4. Mean (n = 3) absolute (n m^{-2}) and relative (%) abundances of dominant and/or typifying enchytraeid species in different land use systems. Abundances and percentages are averages of three replicate fields sampled in the second year (2002) after conversion in 2000. Dominant genera are defined as genera with a relative abundance > 5% in at least one of the systems. Typifying genera are highlighted. For explanation of the concept of typifying genera, see the materials and methods section. LongA = long term arable land, NewG = new grassland on formerly arable land, NewA = new arable land on formerly grassland, LongG = long term grassland

		LongA		NewG		NewA		LongG	
Species	**Abbrev.**	**Abund.**	**%**	**Abund.**	**%**	**Abund.**	**%**	**Abund.**	**%**
Buchholzia appendiculata	bucapp	0	0.0	0	0.0	0	0.00	1926	5.80
Cognettia sphagnetorum	cogsph	0	0.0	0	0.0	354	11.11	22989	48.44
Enchytraeus buchholzia	encbuc	11671	59.4	4951	70.3	786	22.39	8645	20.65
Fridericia bulboides	fribus	1664	10.7	338	3.0	0	0.00	825	2.48
Fridericia bulbosa	fribul	328	1.4	0	0.0	170	3.21	0	0.00
Fridericia paroniana	fripar	550	1.9	136	1.2	1834	40.49	1749	3.28
Henlea perpusilla	henper	2319	16.1	943	16.2	354	11.54	472	1.42
Henlea ventriculosa	henven	1179	6.8	197	2.2	0	0.00	157	0.47
Marionia filiformis	marfil	0	0.0	0	0.0	0	0.00	3380	9.02

Typifying species

Typifying species on the long term grassland were *Cognettia sphagnetorum*, *Buchholzia appendiculata* and *Marionia filiformis*, and on the long term arable land were *Fridericia bulbosa*, *Henlea ventriculosa* and *Henlea perpusilla*. No typifying species were found on the new arable system or on the new grassland system, indicating that both systems were impoverished communities compared to the long term systems from which they originated.

Predatory mite diversity

Species diversity

Average predatory mite species richness in the third year after conversion ranged from 4 species per plot on the new arable land to 20 species per plot on the long term grassland (Table 1). Conversion of grassland to arable land resulted in reduced predatory mite species richness and diversity, to levels similar to or lower than those in the long term arable land. After re-establishment of grassland, species richness increased, but remained at approximately half of the level on the long term grassland. Diversity indices did not increase.

Community structure

RDA revealed that the predatory mite community structure was significantly affected by land use change (P = 0.002, Fig. 3). The first axis separated the grassland systems from the arable systems, whereas the second axis separated the long term grassland from the new grassland system. The two arable systems were positioned close to each other and to the origin, indicating that in the arable systems abundances of most species were low.

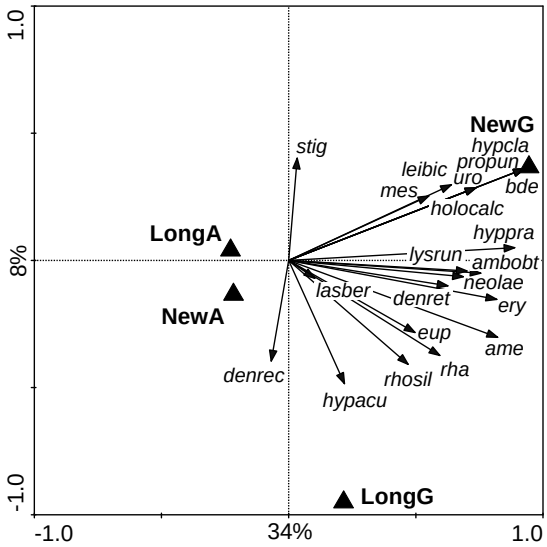

Fig. 3. The effect of different land use systems on predatory mite species composition as determined by Redundancy Analysis in the third year (2003) following conversion in 2000. For explanation of codes see Figure 1. Only common predatory mite species are shown, with common species defined as in Figure 1. For predatory mite species abbreviations see Table 5.

Dominance structure

On the long term grassland, four taxa accounted for more than 50% of the community (*Eupodes sp*. (16.4%), *Leioseius bicolor* (15%), *Hypoaspis claviger* and

Table 5. Mean (n = 3) absolute (n m^{-2}) and relative (%) abundances of common predatory mite species. Abundances and percentages are averages of three replicate fields sampled in the third year (2003) after conversion in 2000. Common species are defined as in Figure 1. Typifying genera are highlighted. For explanation of the concepts of constancy and typifying genera, see the materials and methods section. For explanation of headings, see Table 1.

		LongA		NewG		NewA		LongG	
Species	**Abbrev.**	**Abund.**	**%**	**Abund.**	**%**	**Abund.**	**%**	**Abund.**	**%**
Amblyseius obtusus	ambobt	48	4.2	240	2.8	0	0.0	2305	11.8
Ameroseius sp.	ame	0	0.0	336	4.6	0	0.0	144	0.8
Bdella sp.	bde	0	0.0	0	0.0	0	0.0	144	0.8
Dendrolaelaps rectus	denrec	0	0.0	240	2.4	192	7.0	48	0.2
Dendroseius reticulatus	denret	48	2.2	480	3.6	0	0.0	720	4.4
Erythraeidae sp.	ery	0	0.0	192	1.7	48	1.0	528	3.2
Eupodes sp.	eup	192	7.0	3939	36.4	2402	67.0	3074	16.4
Holoparasitus calcaratus	holcal	0	0.0	0	0.0	0	0.0	192	1.0
Hypoaspis aculeifer	hypacu	288	17.2	192	1.9	0	0.0	57	0.4
Hypoaspis claviger	hypcla	0	0.0	0	0.0	0	0.0	2251	13.8
Hypoaspis praesternalis	hyppra	0	0.0	192	2.8	0	0.0	815	4.8
Lasioseius berlesei	lasber	432	23.5	336	4.2	0	0.0	384	1.9
Leioseius bicolor	leibic	0	0.0	96	0.6	48	1.8	2834	15.0
Lysigamasus runcatellus	lysrun	0	0.0	48	0.3	0	0.0	192	1.3
Mesostigmata juveniles	mes	48	1.8	48	0.7	0	0.0	672	3.3
Neojordensia laevis	neolae	0	0.0	96	1.1	0	0.0	288	1.9
Protodinychus punctatus	propun	0	0.0	0	0.0	0	0.0	192	1.1
Rhagidia sp.	rha	144	6.2	576	6.5	144	3.1	528	2.8
Rhodacarellus silesiacus	rhosil	336	13.2	3170	29.4	96	3.0	720	4.3
Stigmaeidae sp.	sti	96	8.3	0	0.0	288	8.4	288	1.3
Uropoda sp.	uro	0	0.0	0	0.0	0	0.0	144	0.9

Amblyseius obtusus (11.8%) (Table 5). After conversion to arable land, absolute abundances of practically all common species declined. *Eupodes* was much less reduced compared to the other taxa and highly dominated on the new arable land (67%). Dominating species on the long term arable land (*Lasioseius berlesei* (23.5%), *Hypoaspis aculeifer* (17.2%) and *Rhodacarellus silesiacus* (13.2%)) differed from those on the systems with grassland history. After re-establishment of grassland, two species dominated (*Eupodes* (36.4%) and *Rhodacarellus silesiacus* (29.4%), which were also dominating on the long term grassland system, and on the long term arable system, respectively.

Typifying species

On the long term grassland, 15 out of 21 common species were characterised as typifying species, indicating their constant and high abundances on the long term grassland compared to the long term arable field. No typifying species occurred on the long term arable land, or on the new arable land. On the new grassland, four typifying taxa were found: *Ameroseius juveniles*, *Dendrolaelaps rectus*, Erythraeidae and *Eupodes sp.*, which were also found as typifying species on the long term grassland, except for *D. rectus*. Several other typifying long term grassland species that were not detected in the long term arable fields, appeared in the new grassland system (such as *Hypoaspis praesternalis*, *Lysigamasus runcatellus*, *Neojordensia leavis*).

Discussion

Synopsis of the results

Conversion of grassland to arable land reduced taxonomic richness and diversity and changed community structures of all investigated taxonomic groups. Taxonomic groups differed, however, in the relative reduction after conversion and in the way community structures of the different taxonomic groups were affected. The results of re-establishment of grassland ranged from negative (enchytraeids) to neutral (bacteria) to positive (predatory mites, nematodes). Agricultural management (especially fertilization) affected nematode diversity and community structure, but effects were less pronounced than the land use change effects. The different taxonomic groups will be henceforth discussed separately.

Bacterial diversity

Variability of the results may be related to ubiquity of bacteria and sensitivity to confounding factors

Bacterial genetic richness and diversity (H') were not consistently affected by land use systems and land use change. Rather, the effects seemed to vary with time, although a general trend of reduced diversity after conversion of grassland to arable land was apparent. The variability of our results may be related to the general ubiquity of bacterial “species”. Most bacterial species may be present in most soils in low numbers or in dormant condition, and may grow to larger populations under advantageous circumstances. Thus, the bacterial community structure may be able to respond quickly to altered circumstances. Besides land use type and field history, other factors may affect the bacterial community, such as weather, which differed between years. Furthermore, the arable system was part of a rotation, with different crops in each year, which may have affected the bacterial community. In the second year of the experiment, barley, which

is a Gramineae species, was sown on the arable field, which may explain the absence of any significant differences between grassland and arable systems in this year.

Bacterial sub-populations and biomass were more affected than the whole bacterial community

In another study in the same experiment, no significant differences in bacterial diversity (H') were found between the different land use systems, but the diversity of *Bacillus* and Actinomycetes was significantly and consistently higher on the fields with grassland history (Garbeva et al., 2006). Also *Burkholderia* species diversity has been found to be reduced by conversion of grassland to arable land (Salles et al., 2006). Thus, the effects of land use systems and land use change may become apparent in specific species groups, rather than when the whole bacterial community is considered without the identification of taxa. Furthermore, bacterial biomass may be an indicator of the bacterial community response to land use change. Although with a time lag, bacterial biomass was significantly reduced after conversion to arable land (Chapter 4).

Nematode diversity

Diversity and genus richness appeared to be reduced after conversion

Diversity indices of the nematode community were significantly reduced by conversion of grassland to arable land, and although genus richness was not significantly reduced, a consistent trend of approximately 18% lower genus richness on the new arable systems was found. Reduced taxonomic richness as a result of agricultural intensification was found in other studies. Genus richness was reduced from a total of 71 genera on a natural grassland to 41 genera after cultivation (Háněl, 2003). Bloemers et al. (1997) found a reduction in nematode genus richness after clearance and cultivation of a rainforest, of at most 60%. Reduction in species richness and diversity after conversion may be explained by the negative impact of soil tillage, the reduction in diversity of crops, the absence of a permanent vegetation cover and the application of inorganic fertilisers. Similarly, the removal of these factors may have resulted in the increased genus richness and diversity after re-establishment of grassland on long term arable land.

Diversity and genus richness increased after re-establishment of grassland

Similar to our results, an increase in species richness after abandonment of agricultural practices has been found (Pate et al., 2000; Háněl, 2003). Háněl (2003), however, found an increase in nematode species richness to a much lower level than those of the original natural meadows, whereas we found an increase in species richness to a level similar to the long term grassland. This difference may be related to the fact that in the previous study natural succession was allowed, whereas active sowing of grassland was practised in our research. In the same way, the absence of an increase in nematode species richness after abandonment of agricultural practices

in the study of Freckman and Ettema (1993) may be explained by the absence of active sowing of grassland in their experiment.

Dominance was partitioned over more species on the grasslands than on the arable land.

Effects of land use change on the diversity indices were more pronounced than those on genus richness. This result follows from the fact that most species were still present after conversion of grassland to arable land, but the dominances were distributed over more species on the grassland than on the arable land. The highest relative abundances on the new and long term arable systems were 15% and 18%, respectively, whereas on the long term and new grassland systems the highest relative abundances were 9% and 8%, respectively. It appeared that on the arable systems fewer species have higher dominances. Also in the study of Liang et al. (2005) dominance seemed to be partitioned over more species in the less intensive systems, whereas in the intensive systems only one species dominated.

Community structures and dominances were affected by conversion of grassland to arable land and vice versa

In the study of Liang et al. (2005) on nematode community structures in different agricultural systems, it was found that, although most species dominated in more than one system, there were clearly differences in dominance structure between agricultural systems. Also in our study, three years after conversion (but not earlier), the nematode dominance structures in both grassland systems were distinct from those in both arable systems. However, the resemblance in structures between arable systems was much higher than between those in the grassland systems. This can be explained from the reduced nematode genus richness in the arable systems, compared to the grassland systems. No new species were established in the new arable systems, but dominances of present species shifted and several species disappeared. In contrast, the new grassland systems needed to be colonized by grassland species which can be a more time-consuming and random process than local extinction. Furthermore, although the arable systems were managed exactly the same, the new grassland system, although sown with a natural grassland mixture, established a different plant community than the long term grassland system, with more clover species and a higher biomass production in the new grassland (likely due to fertile soil history).

Arable systems had no or few typifying species

Only few or no typifying species were identified on the long term and new arable systems, whereas several species were identified as typifying species of the grassland fauna. These results indicate that the arable systems were in general an impoverished community compared to the grassland systems, with hardly any common species having more constant and higher abundances in the arable systems than in the grassland systems. Furthermore, the fact that typifying arable species were not consistently reduced after re-establishment of grassland may

indicate that these species are not dependent on arable management regimes. Thus, “typical” arable species are species that are relatively less reduced, or slightly increased after conversion to arable land, but usually also occur in the grassland system. Sohlenius and Sandor (1987) found that several species had a clear preference for the barley system compared to grassland system. However, also in their experiment all species that preferred barley were also present on the grassland system.

Dominant and typifying species may (not) be linked with trophic and life strategy group composition

It can be expected that the dominant nematode species belong to the dominant trophic and life strategy groups. Indeed the dominant nematode species in our systems were bacterivores, fungivores or herbivores, the dominant trophic groups in all systems (Chapter 4). Furthermore, the dominant species belonged to cp-groups 1-3, and mostly cp-2, the cp-group that dominated in all systems. Typifying species, however, belonged to both dominant and subordinate trophic groups and cp-groups. In the long term grassland, typifying species belonged to all trophic groups but one, and to cp-groups 2-4. So, characteristic species need neither be dominant, nor belong to dominant species groups.

Generality of typifying species and possible use of indicator species

We found some similarities between our study and a study performed in Central Europe (Háněl, 2003): for example, also in the last mentioned study *Filenchus* dominated in the grassland, *Acrobeloides* in the long term arable land, and *Eucephalobus* in the abandoned arable land. Also a study performed in Central Sweden (Sohlenius and Sandor, 1987) showed some similarity with our study: *Paratylenchus* and *Wilsonema* showed a higher incidence in the grassland system in that study, and were also in our study characterized as typifying grassland species. Furthermore, *Merlinius* showed a higher incidence in the arable (barley system) in their study, and was characterized as a typifying arable species in our study. It can be concluded that although there are several species that occur as dominant species across different management systems, it seems possible to identify species that are characteristic for grassland systems or arable systems over a range of climate zones. These species could be used as indicators of different land use systems.

Nematode diversity was highest under rotation, with reduced mineral fertilization

Nematode diversity (Simpson's index) was significantly higher in the rotation fields in 2002, with the same trend occurring in other years. Genus richness, however, was not affected. These results indicate that dominances were distributed over more genera in the rotation system, but the number of genera did not increase. The different crop residues in the rotation system may stimulate different nematode genera (McSorley and Frederick, 1999), resulting in a higher diversity and a change in community composition.

Conventional mineral fertilization compared to reduced fertilization appeared to have a negative effect on nematode genus richness and diversity indices, and changed the nematode community structure. In the second year after the start of the experiment, the reduction in genus richness in conventional fertilized fields compared to reduced fertilized fields was on average 10%. Also other studies described a changed nematode community composition as a result of fertilization, as some genera were positively and others negatively affected (Ettema and Bongers, 1993, Sarathchandra et al., 2001; Villenave et al., 2003). Probably, some nematode genera were intolerant for high mineral fertilization levels, or were negatively affected by increased competition with genera that were positively affected by conventional fertilization levels. Our results support findings that de-intensified agricultural management, including reduced inputs and diversified crops, can stimulate nematode diversity and richness (Van Diepeningen et al., 2006), and indicate that strongest effects are caused by reduced mineral fertilization inputs.

Enchytraeids

In contrast to the nematode and predatory mite community, the enchytraeid community appeared to have a distinct and similarly diverse community in the long term arable field compared to the long term grassland field

Similar to the other taxonomic groups, a negative effect of conversion of grassland to arable land on the enchytraeid community was found. However, in several ways the enchytraeid community was differently affected by land use change than the other investigated taxonomic groups. The long term arable system was similar to that in the long term grassland system in species richness, with different species dominating in both systems. Furthermore, an equal number of typifying species was found in long term arable and grassland systems. Related to this, whereas re-establishment of grassland positively affected most taxonomic groups, it negatively affected the enchytraeid community. Species richness in the new grassland was as low as in the new arable system. It can be concluded that for a diverse enchytraeid community long term consistency of management is more important than the type of management (grassland or arable land) per se, and land use changes are detrimental.

Field history appeared to be a more important determinant for the enchytraeid community than the current management

Two years after conversion, the enchytraeid community in the new arable land was still very different from the community in long term arable land. Also, the enchytraeid community in the new grassland still more resembled the long term arable field from which it originated than the long term grassland. Thus, two years after conversion, field history appeared to be a more important determinant for the enchytraeid community than the current management.

Species richness was more affected than dominance structures

In contrast to the nematode community, land use change resulted in reduced enchytraeid species richness, but not in statistically significantly reduced diversity indices. This is related to the fact that after conversion of grassland to arable land, dominance was still partitioned over several species. Land use change resulted in reduction or extinction of several less common grassland species, whereas dominating species were relatively less affected.

Reduction in C. sphagnetorum in arable fields

We found that *C. sphagnetorum* was a dominant and typifying species on the long term grassland. The species was strongly reduced after conversion, and was not detected in the fields with arable history. Our results support other findings that *C. sphagnetorum* is a keystone species of acid natural ecosystems in terms of its biomass and role in soil processes (Laakso and Setälä, 1999; Cole et al., 2006). Decline of *C. sphagnetorum* abundances was found to be related to increased pH (Cole et al. 2006, and references therein), which may also explain its decline in arable fields in our study, as pH was higher in the fields with long term arable history. *C. sphagnetorum* was replaced by species from the genera *Henlea* and *Fridericia*, which agree with other findings that *C. sphagnetorum* was replaced by enchytraeids from the genus *Fridericia* in limed plots (Black et al., 2003; Cole et al., 2006).

Predatory mites

Diversity and species richness reduced after conversion

Both species richness and diversity were strongly reduced after conversion of grassland to arable land. Only one genus (*Eupodes*) remained in relatively high numbers, thus dominating the new arable land. Our results may be explained by the fact that the genus *Eupodes* belongs to the Prostigmatida suborder. Prostigmatid mites are known to respond positively to agricultural practices such as fertilization (Behan-Pelletier, 2003). Other explanations may be its omnivorous behaviour and obligate diapause life strategy (Chapter 4). Re-establishment of grassland resulted in increased species richness and diversity, but three years after conversion levels remained significantly lower than in the long term grassland.

No clear similarities between long term and new systems

Both community structure (RDA) and dominance structure analyses indicated little similarity between predatory mite communities in the long term and new arable and grassland systems. Both arable systems were characterized by low species richness and abundances, but no clear similarities in dominant species were found. Also the new grassland and long term grassland had clearly distinct community structures and dominance structures, but some similarities appeared.

Several common long term grassland species increased in abundance in the new grassland, and the genus *Eupodes* was dominant in both grassland systems. The large number of typifying species in the long term grassland in contrast to the absence of typifying species in the arable systems further indicated that long term extensive (grassland) management is an important determinant for a diverse predatory mite community. The limited number of typifying species in the new grassland indicated that restoration of such a diverse community may take several years or even decades.

Comparison of different taxonomic groups

When the four investigated taxonomic groups are compared, differences in the relative reduction after conversion of grassland to arable land appear. Bacterial species richness was reduced on average with 8%, nematode genus richness with 18%, enchytraeid species richness with 50% and predatory mite richness with 80%. Thus, the relative reduction appeared to increase with increased body size and trophic level. A similar relationship between taxonomic group body size and relative reduction was found when total biomass and abundances of different taxonomic groups were compared between the different land use systems (Chapter 4). Furthermore, in line with the above mentioned results, the largest-sized soil biota group that was investigated in these experimental fields, the earthworms, was found to be extremely reduced in species richness in short term arable land, as no earthworms were detected three years after conversion. In long term arable land one (out of two) species was consistently detected in low numbers (Chapter4).

Wardle (1995) also concluded that larger-sized soil biota are more reduced by soil tillage than smaller-sized groups. Furthermore, Buchs et al. (2003) indicated a relationship between reduced species body size and agricultural intensification for beetle species. These relationships were explained by smaller species having higher mobility and shorter reproduction periods, whereas larger species are more sensitive to mechanical disturbance and pesticides and probably modification of habitat. The same explanations may be given for our results. Furthermore, predatory mites are at the top of the soil food web and may therefore depend on the abundance of prey species that may have disappeared after conversion, whereas for bacteria, nematodes and enchytraeids (mainly feeding on organic matter, bacteria, and/or fungi) food sources are still available.

The above mentioned factors related to taxonomic group body size and place in the food web may also explain the difference in success of re-establishment of grassland on formerly arable land for nematodes and predatory mites. Nematode genera were able to quickly (within three years) colonize the new habitat, whereas the predatory mites needed more time to establish natural grassland species richness and abundances. The same holds for enchytraeids, of which the long term arable species were reduced in the new grassland, but most grassland species not yet established. Earthworm species, however, were able to establish in the new grassland, three years after conversion (Chapter 4). Possibly, the application of sods from the grassland soil has resulted in successful introduction of earthworm species in the new grassland field, in contrast to

enchytraeid and mite species. More likely, results may be explained by the fact that earthworms have higher active dispersal ability than most predatory mite and enchytraeid species. Furthermore, the two earthworm species that occurred on the long term grassland system are r-selected species.

A further difference between taxonomic groups appeared as enchytraeids established a characteristic and diverse community on long term arable land, whereas nematodes and predatory mite communities on long term arable land were impoverished communities compared to the long term grassland. This difference is hard to explain, but may for nematodes be related to the fact that they were identified to genus, whereas enchytraeids were identified to species. Otherwise, our results indicate that the long term arable system provided a niche for certain enchytraeid species, possibly related to soil abiotic conditions and/or absence of earthworm competition.

Conclusions

Conversion of a semi-natural grassland to an arable system has detrimental effects on taxonomic richness and diversity across taxonomic groups on the short term, with largest effects on the largest-sized soil biota. Effects of arable management were less detrimental in the long term. Restoration of grassland resulted in establishment of a diverse nematode community, although with a different genus composition compared to the long term grassland. However, predatory mites species richness was only partly restored after re-establishment of grassland and the enchytraeid community was even negatively affected. Agricultural management effects were less obvious than the conversion effects, with negative effects of increased fertilization rates on nematode diversity, whereas the rotation system, compared to the monoculture, had a slightly positive effect dependent on the crop in the rotation.

The results of Chapter 4 indicated that smaller soil biota (nematodes and protozoans) were primarily affected in total abundances, whereas functional group structure was less affected. Predatory mites and earthworms were affected both in total abundance and functional group structure. When the results of the both chapters are combined it can be concluded that conversion of grassland to arable land resulted in reduced nematode species richness and total abundance, but changes in functional group structure were small, whereas predatory mites and earthworms were strongly affected in both total abundance, species diversity and functional group structure. These results can be related to the taxonomic richness within the soil biota groups, which appeared to increase with smaller body size. On the one extreme, earthworm species richness consisted of only three species in the most extensive system, and as the species belong to different functional groups a reduction of one species can immediately imply a lost functional group. Nematode functional groups, however, consisted of more species, thereby maintaining the persistence of functional groups even when species richness within functional

groups was reduced. Also the largely similar relative abundances of the nematode functional groups in different systems may be explained in this way.

Species diversity has been related to ecosystem functioning in several ways (Wardle, 2002) and the reduction in taxonomic diversity in our study may therefore imply effects on agro-ecosystem functioning. Increased nematode species richness at the low end of the diversity spectrum (from one to two species) was found to affect nutrient mineralization in an idiosyncratic way, as the combination of two bacterivorous nematode species was found to increase nitrogen mineralization, whereas two other combinations had no effect (Chapter 2). Also interactions between earthworm species were found to affect bacterial nitrogen (im)mobilisation (Chapter 3). In experiments with larger species richness, however, key species (e.g. *C. sphagnetorum*) were found to be of importance for ecosystem functioning, whereas species diversity within functional groups itself was found to have little effect (Laakso and Setälä, 1999). Also in our experiment, the reduction in genera diversity of nematodes in itself may be less important for ecosystem functioning, especially since 80% of the genera richness was still present in the arable fields, and functional group composition was not affected. However, since species richness is assumed to have an "insurance function" (Bengtsson et al., 2000; Ettema, 1998), the loss in species richness in the arable fields may result in higher vulnerability to future stresses (Griffiths et al., 2000; Van der Wurff et al., 2007). Furthermore, the reduction in total abundance of the microbivores (nematodes and protozoa) in arable fields is likely to affect nutrient mineralization (Bloem et al., 1997; Ferris et al., 2004).

Enchytraeids play a role in nutrient cycling, too. *C. sphagnetorum* was recognized as a key species for ecosystem functioning (measured as primary productivity) in acid grassland or boreal ecosystems (Laakso and Setälä, 1999). The loss of *C. sphagnetorum* after agricultural intensification may indicate a loss in the enchytreaids' contribution to ecosystem functioning, although possibly other species or soil faunal groups may have partly taken over its functions. Compared to the previous groups, the predatory mites play a less important role in nutrient cycling (Laakso and Setälä, 1999). However, the strong reduction in predatory mites in the arable fields may have resulted in changed ecosystem functioning through top-down effects. Reduced predatory mite abundances concurred with increased nematode grazing pressure on the microbiota in the arable fields, which may have resulted in the reduced microbial biomass and increased microbial turnover (Chapter 4). We conclude that agricultural intensification affects total abundances, functional diversity and taxonomic diversity of different soil biota groups differently, and suggest that further research should focus on the possible consequences of these effects for agro-ecosystem functioning.

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References

Behan-Pelletier, V. M. 2003. Acari and Collembola biodiversity in Canadian agricultural soils. Canadian Journal of Soil Science 83: 279-288.

Bengtsson, J., S. G. Nilsson, A. Franc, and P. Menozzi. 2000. Biodiversity, disturbances, ecosystem function and management of European forests. Forest Ecology and Management 132: 39-50.

Black, H. I. J., V. Standen, S. Piertney, C. MacDonald, R. P. Evershed, and I. D. Bull. 2003. The impact of environmental change on the functional role of enchytraeids in grassland. Pages 77-80 *in* W. A. M. Didden, and P. N. Van Vliet, editors. Newsletter on Enchytraeidae No.8. Proceedings of the Fifth International Symposium on Enchytraeidae. Department of Soil Quality, Wageningen University, The Netherlands.

Bloem, J., and A. M. Breure 2003. Microbial indicators. Pages 259-282 *in* B. A. Markert, A. M. Breure, and H. G. Zechmeister, editors. Bioindicators/Biomonitors - Principles, Assessment, Concepts. Elsevier, Amsterdam.

Bloem, J., P. C. De Ruiter, and L. A. Bouwman 1997. Soil food webs and nutrient cycling in agroecosystems. Pages 245-278 *in* J. D. Van Elsas, J. T. Trevors, and E. M. H. Wellington, editors. Modern Soil Microbiology. Marcel Dekker, Inc., New York.

Bloemers, G. F., M. Hodda, P. J. D. Lambshead, J. H. Lawton, and F. R. Wanless. 1997. The effects of forest disturbance on diversity of tropical soil nematodes. Oecologia 111: 575-582.

Bongers, T. 1988. De Nematoden van Nederland. Pirola, Schoorl, The Netherlands.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14-19.

Bongers, T., R. G. M. de Goede, G. W. Korthals, and G. W. Yeates. 1995. Proposed changes of c-p classification for nematodes. Russian Journal of Nematology 3: 61-62.

Buchs, W., A. Harenberg, J. Zimmermann, and B. Weiss. 2003. Biodiversity, the ultimate agri-environmental indicator? Potential and limits for the application of faunistic elements as gradual indicators in agroecosystems. Agriculture, Ecosystems & Environment 98: 99-123.

Cole, L., M. A. Bradford, P. J. A. Shaw, and R. D. Bardgett. 2006. The abundance, richness and functional role of soil meso- and macrofauna in temperate grassland - A case study. Applied Soil Ecology 33: 186-198.

Dilly, O., J. BLoem, A. Vos, and J. C. Munch. 2004. Bacterial diversity during litter decomposition in agricultural soils. Applied and Environmental Microbiology 70: 468-474.

Ettema, C. H.. 1998. Soil nematode diversity: species coexistence and ecosystem function. Journal of Nematology 30: 159-169.

Ettema, C. H., and T. Bongers. 1993. Characterization of nematode colonization and succession in disturbed soil using the maturity index. Biology and Fertility of Soils 16: 79-85.

FAO-UNESCO, 1988. Soil map of the world: revised legend. FAO, Rome, Italy.

Ferris, H., R. C. Venette, and K. M. Scow. 2004. Soil management to enhance bacterivore and fungivore nematode populations and their nitrogen mineralisation function. Applied Soil Ecology 25: 19-35.

Ferris, H., R. C. Venette, and S. S. Lau. 1996. Dynamics of nematode communities in tomatoes grown in conventional and organic farming systems, and their impact on soil fertility. Applied Soil Ecology 3: 161-175.

Freckman, D. W., and C. H. Ettema. 1993. Assessing nematode communities in agroecosystems of varying human intervention. Agriculture, Ecosystems & Environment 45: 239-261.

Garbeva, P., J. A. Van Veen, and J. D. Van Elsas. 2003. Predominant *Bacillus spp*. in agricultural soil under different management regimes detected via PCR-DGGE. Microbial Ecology 45: 302-316.

Garbeva, P., J. Postma, J. A. Van Veen, and J. D. Van Elsas. 2006. Effect of above-ground plant species on soil microbial community structure and its impact on suppression of Rhizoctonia solani AG3. Environmental Microbiology 8: 233-246.

Griffiths, B. S., K. Ritz, R. D. Bardgett, R. Cook, S. Christensen, F. Ekelund, S. J. Sorensen, E. Bååth, J. Bloem, P. C. De Ruiter, J. Dolfing, and B. Nicolardot. 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship. Oikos 90: 279-294.

Háněl, L.. 2003. Recovery of soil nematode populations from cropping stress by natural secondary succession to meadow land. Applied Soil Ecology 22: 255-270.

Jagers op Akkerhuis, G. A. J. M., F. Deley, H. J. C. Zwetsloot, J. F. Ponge, and L. Brussaard. 1988. Soil microarthropods (Acari and Collembola) in 2 crop rotations on a heavy marine clay soil. Revue de Ecologie et de Biologie du Sol 25: 175-202.

Karg, W. 1993. Acari (Acarina), Milben, Parasitiformes (Anactinochaeta), Cohors Gamasina Leach. Raubmilben. Jena, Gustav Fischer Verlag, New York.

Laakso, J., and H. Setälä. 1999. Sensitivity of primary production to changes in the architecture of belowground food webs. Oikos 87: 57-64.

Liang, W. J., X. K. Zhang, Q. Li, Y. Jiang, W. Ou, and D. A. Neher. 2005. Vertical distribution of bacterivorous nematodes under different land uses. Journal of Nematology 37: 254-258.

Magurran, A. E. 2004. Measuring biological diversity. Blackwell Publishers, Malden, MA.

McSorley, R., and J. J. Frederick. 1999. Nematode population fluctuations during decomposition of specific organic amendments. Journal of Nematology 31: 37-44.

Muyzer, G., E. C. De Waal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59: 695-700.

Nielsen, C. O., and B. Christensen. 1959. The Enchytraeidae, critical revision and taxonomy of European species. Naturalis Jutlandica goede naam 8-9: 7-160.

Nijboer, R. C., P. F. M. Verdonschot, and D. C. Van Der Werf. 2005. The use of indicator taxa as representatives of communities in bioassessment. Freshwater Biology 50: 1427-1440.

O'Connor, F. B. 1967. The Enchytraeidae. Pages 213-257 *in* A. Burges, and F. Raw, editors. Soil Biology. Academic Press, London, New York.

Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. Pages 85-102 *in* J. M. Sasser, and W. R. Jenkins, editors. Nematology. University of North Carolina Press, Chapel Hill.

Pate, E., N. Ndiaye-Faye, J. Thioulouse, C. Villenave, T. Bongers, P. Cadet, and D. Debouzie. 2000. Successional trends in the characteristics of soil nematode communities in cropped and fallow lands in Senegal (Sonkorong). Applied Soil Ecology 14: 5-15.

Porazinska, D. L., L. W. Duncan, R. McSorley, and J. H. Graham. 1999. Nematode communities as indicators of status and processes of a soil ecosystem influenced by agricultural management practices. Applied Soil Ecology 13: 69-86.

Salles, J. F., E. Samyn, P. Vandamme, J. A. Van Veen, and J. D. Van Elsas. 2006. Changes in agricultural management drive the diversity of Burkholderia species isolated from soil on PLAT medium. Soil Biology & Biochemistry 38: 661-673.

Sarathchandra, S. U., A. Ghani, G. W. Yeates, G. Burch, and N. R. Cox. 2001. Effect of nitrogen and phosphate fertilisers on microbial and nematode diversity in pasture soils. Soil Biology & Biochemistry 33: 953-964.

Sohlenius, B., and A. Sandor. 1987. Vertical-distribution of nematodes in arable soil under grass (*Festuca-pratensis*) and barley (*Hordeum-distichum*). Biology and Fertility of Soils 3: 19-25.

Susilo, F. X., A. M. Neutel, M. Van Noordwijk, K. Hairiah, G. Brown, and M. J. Swift 2004. Soil biodiversity and food webs. Pages 285-306 *in* M. Van Noordwijk, G. Cadisch, and C. K. Ong, editors. Below-ground Interactions in Tropical Agroecosystems, Concepts and Models with Multiple Plant Components. CABI Publishing, Cambridge, USA.

Swift, M. J., J. Vandermeer, P. S. Ramakrishnan, J. M. Anderson, C. K. Ong, and B. A. Hawkins 1996. Biodiversity and Agroecosystem Function. Pages 261-298 *in* H. A. Mooney, J. H. Cushman, E. Medina, O. E. Sala, and E. D. Schulze, editors. Functional Roles of Biodiversity: a Global Perspective. John Wiley & Sons Ltd.

Ter Braak, C. J. F., and P. Smilauer 2002. CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca NY, USA.

Van der Wurff, A., S. Kools, M. Boivin, P. van den Brink, H. van Megen, J. Riksen, A. Doroszuk, and J. Kammenga. 2007. Type of disturbance and ecological history determine structural stability. Ecological Applications 17: 190-202.

Van Diepeningen, A. D., O. J. De Vos, G. W. Korthals, and A. H. C. Van Bruggen. 2006. Effects of organic versus conventional management on chemical and biological parameters in agricultural soils. Applied Soil Ecology 31: 120-135.

Van Elsas, J. D., and K. Smalla 1995. Extraction of microbial community DNA from soils. Pages 1.3.3.1-1.3.3.11 *in* A. D. L. Akkermans, J. D. Van Elsas, and F. J. De Bruijn, editors. Molecular Microbial Ecology Manual. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Villenave, C., T. Bongers, K. Ekschmitt, P. Fernandes, and R. Oliver. 2003. Changes in nematode communities after manuring in millet fields in Senegal. Nematology 5: 351-358.

Wardle, D. A.. 1995. Impacts of disturbance on detritus rood webs in agro-ecosystems of contrasting tillage and weed management practices. Advances in Ecological Research 26: 105-185.

Appendix A. Absolute (n g^{-1}) and relative (%) abundances of common nematode genera in different land use systems. Abundances and percentages are averages of three replicate fields and three consecutive years (2001 to 2003) (n=9). Common genera are defined as genera with a constancy > 0.499 in at least one of the systems. Typifying genera are highlighted. For explanation of the concepts of constancy and typifying genera, see the materials and methods section. LongA = long term arable land, NewG = new grassland on formerly arable land, NewA = new arable land on formerly grassland, LongG = long term grassland.

			LongA		NewG		NewA		LongG	
Genus	**Abbrev.**	**c-p/pp index**	**Abund.**	**%**	**Abund.**	**%**	**Abund.**	**%**	**Abund.**	**%**
Plant parasites										
Geocenamus	geoc	3	0.9	1.9	0.5	1.2	0.1	0.2	0.1	0.1
Helicotylenchus	heli	3	1.9	4.1	3.3	6.7	0.7	4.6	2.5	3.1
Paratrichodorus	ptri	4	0.0	0.2	1.5	2.5	0.0	0.0	0.0	0.0
Paratylenchus	para	2	0.1	0.1	0.6	1.3	0.1	0.3	4.2	5.2
Pratylenchus	prat	3	4.4	11.0	3.8	7.7	3.3	13.7	5.1	7.2
Trichodorus	tric	4	0.2	0.6	0.7	1.5	0.4	1.9	2.1	3.0
Tylenchorhynchus	tchu	3	8.0	17.8	3.0	5.8	0.5	2.3	1.1	1.6
Root-fungal feeders										
Boleodorus	bole	2	0.1	0.1	0.5	1.2	0.0	0.1	0.0	0.0
Coslenchus	cosl	2	0.2	0.4	0.3	0.7	0.1	0.7	3.8	5.2
Filenchus	file	2	0.8	1.7	2.8	6.0	1.2	5.4	5.3	7.6
Malenchus	male	2	0.1	0.2	0.6	1.2	0.2	0.7	1.3	2.0
Tylenchus	tyle	2	0.4	1.1	1.1	2.2	0.1	0.4	0.8	1.2
Fungal feeders										
Aphelenchoides	aoid	2	2.6	6.9	2.3	4.3	2.1	9.7	6.8	9.0
Aphelenchus	achu	2	0.2	0.3	1.7	3.5	0.1	0.6	0.0	0.0
Diphterophora	diph	3	0.4	0.8	0.7	1.0	0.0	0.0	0.0	0.0
Ditylenchus	dity	2	1.3	4.0	1.2	2.7	0.6	3.4	3.8	5.0

(Appendix A continued)

			LongA		NewG		NewA		LongG	
Genus	**Abbrev.**	**c-p/pp**	**Abund.**	**%**	**Abund.**	**%**	**Abund.**	**%**	**Abund.**	**%**
Bacterial feeders										
Acrobeles	acel	2	2.0	4.9	2.2	4.5	0.2	0.8	0.3	0.3
Acrobeloides	acoi	2	1.5	5.7	1.3	2.5	3.1	14.7	2.5	3.6
Alaimus	alai	4	0.7	1.3	0.9	1.7	0.1	0.3	0.9	1.3
Anaplectus	anap	2	0.3	0.7	0.6	1.3	0.0	0.1	0.3	0.4
Bastiania	bast	3	0.1	0.2	0.9	1.8	0.0	0.1	0.3	0.6
Cervidellus	cerv	2	0.2	0.6	0.9	1.5	0.3	0.9	0.3	0.4
Eucephalobus	euce	2	2.4	6.3	3.8	7.2	1.6	8.2	1.3	1.9
Eumonhystera	euhm	2	0.0	0.0	0.7	1.3	0.1	0.2	0.1	0.1
Heterocephalobus	hece	2	0.6	1.2	0.4	1.0	0.7	2.8	1.2	1.6
Metateratocephalus	metr	3	0.0	0.1	0.0	0.0	0.3	1.2	3.4	4.3
Panagrolaimus	pala	1	1.3	3.1	1.7	2.7	0.8	4.7	4.1	6.0
Plectus	plec	2	0.9	2.3	2.4	4.2	1.2	4.2	3.4	4.4
Prismatolaimus	pris	3	0.7	1.6	0.8	1.5	0.8	2.8	5.5	7.6
Pristionchus	prti	1	0.2	0.9	0.2	0.3	0.2	1.7	0.2	0.2
Protorhabditis	parh	1	0.9	2.6	5.5	6.2	0.4	1.4	0.7	1.0
Rhabditidae dauerlarvae	daue	1	0.7	1.6	0.1	0.2	0.1	0.4	0.7	0.9
Rhabditis	rhab	1	3.6	8.1	3.5	5.0	1.8	7.0	2.1	2.8
Teratocephalus	tera	3	0.0	0.0	0.1	0.2	0.0	0.0	2.3	2.9
Wilsonema	wils	2	0.0	0.0	0.5	0.7	0.0	0.0	0.5	0.7
Carnivores										
Takamangai	taka	4	0.5	1.4	0.5	1.0	0.2	0.7	0.9	1.3
Tripyla	trip	3	0.1	0.2	0.0	0.0	0.0	0.0	0.6	0.9
Omnivores										
Eudorylaimus	eudo	4	0.1	0.4	0.7	1.5	0.1	0.3	1.3	1.9
Mesodorylaimus	medo	5	0.3	0.7	0.6	1.3	0.0	0.2	0.1	0.1

Appendix B. Mean (n = 3) absolute (n m^{-2}) and relative (%) abundances of enchytraeid species in different land use systems. Abundances and percentages are averages of three replicate fields sampled in the second year (2002) after conversion in 2000. Typifying genera are highlighted. For explanation of the concept of typifying genera, see the materials and methods section. For explanation of headings, see Appendix A.

		LongA		NewG		NewA		LongG	
Species	**Abbrev.**	**Abund.**	**%**	**Abund.**	**%**	**Abund.**	**%**	**Abund.**	**%**
Achatae bulbosa	achbul	0	0.0	0	0.0	0	0.00	2083	3.04
Achatae eiseni	acheis	39	0.3	0	0.0	39	1.24	118	0.39
Buchholzia appendiculata	bucapp	0	0.0	0	0.0	0	0.00	1926	5.80
Cognettia glandulosa	coggla	0	0.0	0	0.0	79	2.47	236	0.78
Cognettia sphagnetorum	cogsph	0	0.0	0	0.0	354	11.11	22989	48.44
Enchytraeus buchholzia	encbuc	11671	59.4	4951	70.3	786	22.39	8645	20.65
Enchytronia parva	enopar	0	0.0	0	0.0	118	3.71	0	0.00
Fridericia bisetosa	fribis	550	1.9	406	3.6	118	3.85	98	0.14
Fridericia bulboides	fribus	1664	10.7	338	3.0	0	0.00	825	2.48
Fridericia bulbosa	fribul	328	1.4	0	0.0	170	3.21	0	0.00
Fridericia galba	frigal	0	0.0	0	0.0	0	0.00	354	1.06
Fridericia paroniana	fripar	550	1.9	136	1.2	1834	40.49	1749	3.28
Fridericia sp.	frisp	0	0.0	39	0.5	0	0.00	0	0.00
Fridericia waldenstroemi	friwal	131	1.0	338	3.0	0	0.00	904	3.01
Henlea perpusilla	henper	2319	16.1	943	16.2	354	11.54	472	1.42
Henlea ventriculosa	henven	1179	6.8	197	2.2	0	0.00	157	0.47
Indeterminable	indet	39	0.3	0	0.0	0	0.00	0	0.00
Marionia filiformis	marfil	0	0.0	0	0.0	0	0.00	3380	9.02
Marionia spicula	marspi	39	0.3	0	0.0	0	0.00	0	0.00

Chapter 6

Nitrogen cycling and soil biodiversity after (de-) intensification of agroecosystem management

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Abstract

Understanding functional biodiversity with respect to the regulation of nitrogen mineralization and retention is a key factor to support more sustainable production and prevent environmental complications. We studied the relationship between a range of soil biota functional group abundances and diversities and nitrogen mineralization and retention in agroecosystems after (de-)intensification. This included conversion of grassland to arable land and *vice versa*, reduced cropping diversity and reduced levels of mineral fertilization. Contributions of different soil biota groups were compared with respect to potential N mineralization (i.e. short-term mineralization of soil organic matter as quantifiable in laboratory incubations) and with respect to net N mineralization under field conditions.

Agricultural intensification generally resulted in decreased N mineralization and increased NO_3^- leaching in the second year after conversion. *Vice versa*, agricultural de-intensification resulted in increased N mineralization and decreased NO_3^- leaching. Increased N mineralization was related to both functional group abundances and functional diversity of soil biota. The relationships with taxonomic diversity were inconsistent. Key trophic groups associated with N mineralization were endogeic earthworms (*A. caliginosa*) and trophic groups in the bacterial energy channel, whereas N mineralization was negatively related to fungal biomass and nematode fungivores. In the short-term and under disturbed conditions, N mineralization can be explained by a high presence of opportunistic nematodes, whereas under field conditions N mineralization increased with a higher diversity of nematode life-strategy groups.

Whether mineralized NO_3^- is incorporated into the crop or leached out below the rooting zone depends on the cropping system. Mineral N applied as synthetic fertilizer confers a higher risk of leaching during the cropping season than mineralized N, but year-round NO_3^- leaching was not dependent on the source of mineral N. We conclude that both trophic group abundances and life-strategy diversity of soil biota significantly determine mineral N availability in extensively managed systems, and may at least in part compensate for reduced mineral N inputs from fertilizers. Such increased N mineralization may confer a low risk of NO_3^- leaching during the cropping season, as well as in systems with a high and continuous crop production.

Introduction

Biodiversity is an essential element in sustaining ecosystem services for agriculture (Altieri, 1999). Understanding functional biodiversity with respect to the regulation of nitrogen mineralization and retention is a key factor to support more sustainable production and prevent environmental complications (Swift et al., 2004). Soil biota play a key role in nitrogen cycling (Brussaard et al., 1997; Thrupp, 2004). Soil biota abundances and community composition change under agricultural (de-)intensification (Chapter 4, 5), which may imply consequences for agroecosystem functioning. However, the linkage between soil biota abundances and diversities and nitrogen cycling after agricultural (de-)intensification is still poorly understood (Swift et al., 2004). Whereas most studies have focused on the role of only one or two taxonomic groups, we studied the relationship between nitrogen cycling and a range of soil biota functional group abundances and diversities after agricultural (de-)intensification. We addressed both functional group abundances and diversity, and taxonomic diversity, so as to compare the specific contributions of different soil biota groups and diversities.

An important role of soil biota in nitrogen cycling is their contribution to N mineralization. In agroecosystems, linkages between increased microbiota abundances and N mineralization have been described frequently. The simulation model of De Ruiter et al. (1993) indicated that the *microbial biomass* was responsible for approximately 70% of N mineralization in agroecosystems. An increased microbial biomass concurred with a higher N mineralization in natural or grassed ecosystems compared to cropped systems (Carpenter-Boggs et al., 2003; Silver et al., 2005) and in de-intensified cropping systems compared to intensive agricultural systems (Balota et al., 2004; Breland and Eltun, 1999; Mäder et al., 2002). Furthermore, increased *microbial activity* was related to increased N mineralization (Carpenter-Boggs et al., 2003). Also increased microbivorous protozoan and nematode abundances were related to increased N mineralization, as a result of their grazing on the microflora (Bloem et al., 1994; Ferris et al., 2004). N mineralization may be further enhanced by abundances of larger soil biota. In particular, earthworms are known to increase mineralization in agroecosystems (Blair et al., 1997; Brown et al., 1999) due to their functioning as “ecosystem engineers” (Lawton, 1994).

Soil biota functional groups may differ in the way they contribute to N mineralization. Functional groups are defined as groups of species that contribute to ecosystem functioning in a similar way (Brussaard et al. 1997, Susilo et al. 2004). Important functional groups are *trophic groups*, indicating the food preferences of soil biota, and *life strategy groups*, which indicate the way in which soil biota cope with food availability, stress and disturbance.

Microbivorous trophic groups contribute largely to N mineralization, whereas the role of higher trophic level groups (predators, omnivores) may be limited (De Ruiter et al. 1994; Ferris et al., 2004; Laakso and Setälä, 1999b). Herbivorous trophic groups are probably less important for N mineralization (De Ruiter et al., 1993; De Ruiter et al., 1994).

Also different life-strategy groups may affect N mineralization differently. Nematodes can be classified according to their life strategy on a colonizer-persister scale (cp1 – cp5), based on which a maturity index (MI) is calculated. This index indicates whether the system contains relatively more r- or K- selected nematodes (Bongers 1990). Increased N mineralization was found to be related to nematode species combinations from different c-p classes (Chapter 2). The linkage between soil biota life-strategy and N mineralization in agroecosystems has been little studied, however.

Soil biota functional groups may contain a large diversity of genera, species, or genes, i.e. a large *taxonomic diversity*. The importance of taxonomic diversity for ecosystem functions such as N mineralization has often been debated (Swift et al. 2004). At the low end of the diversity spectrum (one up to three species), increased nematode and earthworm species richness were found to affect N mineralization (Chapter 2; Chapter 3). At the system level, however, species may be redundant in their functioning in N mineralization (Cole et al., 2006; Laakso and Setälä, 1999b; Setälä, 2005) and there is little evidence of a causal linkage between species diversity and N mineralization so far (Swift et al., 2004).

Besides N mineralization, nutrient retention is an important function for agroecosystems. NO_3^- leaching as a result of an imbalance between availability and uptake of nitrogen by plants may occur under free-draining or artificially drained water surplus conditions. A direct link between soil biota and NO_3^- leaching is not expected, except for earthworms, which may increase leaching through increased water infiltration into the soil and preferential flow (Dominguez et al., 2004). Indirectly, increased N mineralization by soil biota could lead to increased NO_3^- leaching, if the increased availability of nitrogen is not met by an increased uptake by the crop.

From a whole-system perspective, systems with increased N mineralization may require less mineral nitrogen fertilization. The nitrogen use efficiency of mineralized N in such systems may be higher than that of N from mineral fertilizers since N mineralization may result in a more gradual release of nitrogen during the growing season (Bloem et al., 1997; Swift et al., 2004). Intensified agricultural systems, with increased mineral N input and/or soil disturbance and reduced soil biota abundances were indeed found to result in increased NO_3^- leaching (Hansen et al., 2001; Hole et al., 2005). However, neutral or opposite effects of intensive agricultural management on NO_3^- leaching have also been described (Hansen et al., 2001; Hansen et al., 2001; Hole et al., 2005; Van Diepeningen et al., 2006). These results may be explained as higher mineralization and subsequent NO_3^- leaching in de-intensified systems during the fallow period between crops.

In this study, we investigated the relationship between N mineralization on the one hand, and trophic groups (bacteria, fungi, protozoans, nematodes, earthworms), life-strategy diversity (nematodes), and taxonomic diversity (bacteria and nematodes) on the other hand. To study the significance of such relationships for sustainable agriculture, we studied nitrogen cycling and soil biota in agroecosystems under different degrees of intensification, ranging from extensively managed grassland to intensively managed arable land. Intensification was studied as extensive grassland systems were converted to arable land, while de-intensification refers to arable land converted to grassland. The intensification level was varied by selecting systems that differed in crop diversity (monoculture compared to rotation management) and in the level of mineral fertilization (conventional compared to reduced fertilization). The relationship between soil biota abundances, N mineralization and NO_3^- leaching in these systems was studied.

The effects of agricultural (de-)intensification on soil biota abundances and diversity are described in Chapters 4 and 5, concluding that larger-sized soil biota were more sensitive to agricultural intensification than smaller-sized soil biota. Furthermore, it was found that agricultural intensification affected total abundances and taxonomic diversity of soil biota, but not necessarily the functional group diversity.

Since organisms at the base of the food web, and those feeding on detritus are assumed to have the highest impact on mineralization rates (Berg et al., 2001; De Ruiter et al., 1994) we expected that variation in N mineralization in our systems could be explained by the biomass of microflora, by abundances of microbivorous protozoans and nematodes, and by abundances of earthworm ecological groups. Furthermore, since the functioning of the detritivore community is related to functional distance between species (Heemsbergen et al., 2004), we expected that high N mineralization would be related to increased life-strategy diversity (thus a high maturity index, MI) of nematodes. We hypothesized that the abundances of key functional groups and functional diversity would be more explanatory for N mineralization than taxonomic diversity of species, as redundancy of species with similar functionality may occur widely in soil ecosystems (Andrén and Balandreau, 1999; Setälä, 2005). NO_3^- leaching was expected to increase in intensive agricultural systems with increased N input through mineral fertilization in the cropping season. Furthermore, a positive relationship between increased N mineralization and increased NO_3^- leaching was expected.

Materials and methods

Experimental setup

An experimental field (‘De Bovenbuurt’ 51°59’N, 5°40’E, the Netherlands) was selected, with two agricultural systems with extreme management regimes located close to each other. On the one extreme was a long-term extensively managed grassland (>50 years) ('long-term grassland') and on the other a conventionally managed crop rotation that was converted from the grassland around 1980 ('long-term arable land'). In 2000, our experiment was started when part of the long-term grassland was converted to arable land with different agricultural regimes, and part of the long-term arable land was converted to grassland, resulting in four experimental systems: long-term grassland, new arable land, long-term arable land and new grassland. Thus, relationships could be studied between N cycling and soil biota in agroecosystems under agricultural intensification (conversion of grassland to arable land) on the short-term (new arable land) and on the long-term (long-term arable land), and *vice versa* in agroecosystems under de-intensification (conversion of arable land to grassland). Further intensification of agricultural practices was studied by establishing different management regimes on both the long-term and new arable system: a crop rotation versus a monoculture of maize and reduced versus conventional mineral fertilization. Complete randomization of treatments was not possible, since the long-term arable land and long-term grassland fields were fixed. To correct for possible gradients within these two fields, three replicate blocks on long-term grassland and long-term arable land were used, with treatments (10 x 12 field plots) randomized within each block.

The effects of conversions between grassland and arable land on N cycling parameters were measured in the first year after conversion, to record any immediate effects of the conversions on N cycling. These measurements were performed in arable fields under continued conventional management (crop rotation and conventional fertilization). The relationship between N cycling and soil biota abundances and diversity in all systems was studied in the second year after conversion, as immediate effects of conversions on N cycling parameters were assumed to have disappeared.

Description of agricultural systems, soil characteristics and management

Site description

The soil of the experimental site is a Fimic Anthrosol (FAO-UNESCO, 1988), or a loamy sand (0-10 cm: fraction > 50 µm: 89%, 16 µm - 50 µm: 7%, 2 µm - 16 µm: 5%, < 2 µm: 3%, as measured in 2002). Dominant species in the long-term grassland were *Festuca rubra*, *Holcus*

lanatus, *Anthoxanthum odoratum*, *Rumex acetosa* and *Ranunculus sp.*. The long-term arable land was under a crop rotation (oat, maize, barley, and potato), with conventional fertilization levels, weed control through herbicide application, and 20 cm deep tillage. At the start of the experiment in April 2000, long-term grassland and long-term arable land had soil organic matter contents of 3.9%, and 3.3%, pH (KCl) of 4.4 and 5.0, and moisture contents of 28% and 16% (w/w), respectively.

Management conversions

In April 2000, part of the long-term grassland was ploughed to 20 cm. Three plots were left undisturbed (long-term grassland). On the long-term arable land, three plots were sown with a grassland species mixture containing grass (*Agrostis capilaris*, *Lolium perenne*, *Poa pratense*, *Festuca rubra, Holcus lanatus*, *Phleum pratense*), clover (*Trifolium repens, Lotus cornutus*) and herb species (*Rumex acetosa*, *Ranunculus sp.*, *Plantago lanceolata*, *Hypochaeris radicata*, *Achillea millefolium*). Both new grassland and long-term grassland were mown and harvested two or three times in summertime during the next years, depending on the growth rate of the grass.

Agricultural regimes

In the arable fields under crop rotation management, the current rotation was continued: maize (variety: Crescendo) in 2001 and barley (Aspen) in 2002. In the arable fields with monoculture regime, maize was sown continuously, from 2000 onwards. Conservation tillage (10 cm) and mechanical weed control were applied in all experimental arable fields. In the first year after conversion (2001) a cover crop was applied after crop harvest. All arable crops received a similar amount of organic manure in each year, and an additional dressing of mineral fertilizer for optimal production of each crop (conventional fertilization) or half of the amount needed for optimal production of the crop (reduced fertilization) (Table 1).

Experimental parameters

Total soil C and N, $N{-}NO_3^-$ and $N{-}NH_4^+$, soluble N and P

Soil samples for measurement of total soil N and C, $N{-}NO_3^-$ and $N{-}NH_4^+$, and total soluble N and P were taken on September 24 in 2002. Sixty soil cores (diameter 2.2 cm, depth 10 cm) per plot were combined into a bulk sample. The soil was thoroughly mixed, and stored at 4 °C until analysis. Sub-samples for measurement of $N{-}NO_3^-$ and $N{-}NH_4^+$, total soluble N and P were dried at 40 ºC, extracted with 0.01M Calcium Chloride and measured using continuous flow analysis (Skalar, Breda, The Netherlands). Sub-samples for analysis of total N and C were dried

Table 1. Fertilization applied in arable fields under crop rotation and monoculture management. Dried cow manure was used as organic fertilizer. Reduced fertilization fields received half the N amount needed for optimum crop production in all years, and half the P_2O_5 and K_2O amounts for optimal crop production in 2001. Complete P_2O_5 and K_2O fertilization was applied in all fields in 2002. Amounts are presented as pure N, P and K (kg ha^{-1}).

				Manure		Mineral fertilizers		
Year	Field history	Crop	Fertilization	kg ha^{-1}	N	N	P	K
2001	arable	monoculture maize	reduced	1258	90	0	0	70
			complete	1258	90	105	13	187
		rotation maize	reduced	1258	90	0	0	70
			complete	1258	90	105	13	187
	grassland	monoculture maize	reduced	1258	90	0	0	70
			complete	1258	90	105	24	187
		rotation maize	reduced	1258	90	0	58	70
			complete	1258	90	105	18	187
2002	arable	monoculture maize	reduced	785	55	35	0	29
			complete	785	55	125	0	29
		rotation barley	reduced	785	55	0	0	7
			complete	785	55	55	0	7
	grassland	monoculture maize	reduced	785	55	35	11	195
			complete	785	55	125	11	195
		rotation barley	reduced	785	55	0	32	55
			complete	785	55	55	32	55

at 40 ºC, ball-milled and analyzed for total N. Total soil C was analyzed by wet oxidation with $K_2Cr_2O_7$ (Walinga et al., 1992).

Potential N mineralization

Soil samples for measurement of potential N mineralization were taken on September 23 in 2001 and September 24 in 2002. Potential N mineralization was determined by incubating 200 g homogenized and sieved (< 2 mm) soil in 1.5 L airtight jars at 20°C in the dark for 6 weeks (Bloem et al., 1994). Results of the first week were not used to avoid effects of soil homogenization. The increase in mineral N between week 1 and week 6 was used to calculate N mineralization rate. Sub-samples of 80 g soil were extracted with 200 mL of 1 M KCl. After 1 h shaking the extracts were filtered over a paper filter. Mineral N contents (ammonium and NO_3^-) were determined by Segmented Flow Analysis (Skalar, Breda, The Netherlands). Potential N mineralization (mg kg^{-1} week) was converted to kg^{-1} ha year, using bulk density measurements and assuming all N

mineralization occurred in the upper 10 cm. Values were corrected for average yearly temperature with a factor 3, based on previous measurements (Bloem et al., 1994).

NO_3^- leaching

NO_3^- leaching was measured in the summer and winter periods starting one year after conversion in 2000 (April 2001 – September 2001, September 2001 – March 2002, March 2002 – October 2002, October 2002 – April 2003). NO_3^- leaching was measured in the four land use systems (long-term arable land, new arable land, long-term grassland, new grassland) in both years, and in the fields with different cropping systems and fertilization level in the second year. A method to measure NO_3^- leaching by incubating anion exchange resin was adapted from Dodd et al. (2000) and Wyland and Jackson (1993). Ion exchange resins have been used to measure *in situ* availability or leaching of plant nutrients at different depths (Binkley and Matson, 1983; Hatch et al., 2000; Skogley and Dobermann, 1996). The advantage of this method is the possibility to accumulate leached NO_3^- over a longer period and to continue soil tillage practices during the sampling period.

Ion exchange resins were prepared using IONAC ASB 1-P for anion exchange and IONAC C-251 for cation exchange (Sybron Chemicals, Birmingham, NJ). A mixture of these resins was used for the first sampling to collect both NO_3^- and NH_4^+ ions, whereas from September 2001 onwards only the anion resin was used to collect NO_3^-, since NH_4^+ amounts were found to be very low. In either case, for a single resin bag 45 g (± 2 g) of resin were placed in a PVC ring (7 cm in diameter, 1.6 cm in height), and both ring and resin were placed in a knotted nylon-stocking pouch to keep the resin in place within the ring. To install the resin bags in the soil profile, we excavated a 38 x 30 cm trench in each experimental field. The trenches were placed within the rows of maize, in order to cover the distance from the plant to the middle of row (38 cm, rows were at 75 cm distance). Two horizontal shafts were excavated at a depth of 35 cm along the 38 cm sides of the trench. In each shaft, three resin bags were placed horizontally under an undisturbed soil profile and soil was repacked.

At the end of the first and third incubation period resin bags were removed, and replaced with fresh ones. At the end of the second incubation period (March 2002), new trenches were excavated since different experimental fields were sampled in the second year (March 2002 – April 2003). In this year we also placed resin bags at a depth of 70 cm. Only fields with a long-term arable history were selected for this method, since higher ground water levels in the fields with grassland history rendered these unsuitable for placing resin bags at depths exceeding 35 cm. Trenches were situated in the same manner as described before. We excavated “tunnels” starting from the bottom of the 30 cm deep trenches using a soil core under an angle of 45

degrees, to a depth of 70 cm. Resin bags were placed at the bottom of these tunnels, under an undisturbed soil profile. Soil was repacked in the tunnels.

At the end of each incubation period the resin was removed from the bags and cleaned with demineralized water. We extracted NO_3^- and NH_4^+ from the resin by adding 100 mL of a 2 M KCl solution to the resin, followed by a 60 min shaking. The solution was decanted and analyzed for NO_3^- and NH_4^+ using continuous flow analysis (Skalar, Breda, The Netherlands). The results from the N obtained by the resin bags were expressed as kg N ha^{-1} for the period that the resin bags were incubated. This calculation was based on the assumption that all ions that were retained by the resin bag originated from a soil cylinder having the same diameter as the resin bag (7 cm).

Crop production

Maize was harvested starting September 23 in 2001 and September 24 in 2002. In each experimental field, fresh weight of 3 pairs of rows was determined in the field for calculation of total crop production. Sub-samples from each pair of rows were taken and stored for determination of dry weight and chemical analyses. Barley was harvested August 26, 2002. In each experimental field, fresh weights of total plants and grains of three representative 1 x 1.5 m plots were determined. Sub-samples from each plot were taken and stored for determination of dry weight and chemical analyses. After harvest of grains, crop residues were distributed evenly over the field. Sub-samples of the maize and barley crop were dried at 70 °C, ball milled and analyzed for total N. Crop production and crop N uptake were calculated as kg ha^{-1}.

Calculation of net N mineralization

Net N mineralization was calculated as the balance between the main input (fertilization, N deposition) and output of N (leached N, N uptake by the crop) in the system. Crop N uptake in the new grassland system was corrected for N fixation by clover, which was estimated to be 120 N kg ha^{-1} (Hansen and Vinther, 2001). N deposition was estimated to be 40 kg N ha^{-1} $year^{-1}$ (De Ruiter et al. 2006). Denitrification was supposed to be negligible.

Soil biota

Samples for analysis of soil biota functional groups in the different experimental fields were taken on September 24 in 2002 (two years after conversion). All samples were taken from the upper 10 cm soil layer, except for earthworms which were sampled to 25 cm depth. Earthworms were hand sorted in the field from the soil under a 20 x 20 cm surface, and identified to species in the lab. Sixty soil cores (diameter 2.2 cm, depth 10 cm) were combined into a bulk sample for determination of soil microbiota. The soil was thoroughly mixed and stored at 4 °C until analysis. Nematodes were extracted from approximately 150 g soil using an Oostenbrink

elutriator (Oostenbrink 1960), and identified to genus according to Bongers (1988). Nematode taxa were assigned to trophic groups (Yeates et al. 1993) and life strategy groups (c-p groups) (Bongers 1990, Bongers et al. 1995). The trophic diversity index (reciprocal Simpson's index: 1/D) and maturity index (Bongers 1990) were calculated. Species richness and diversity index (Shannon-Wiener: H') of nematodes were calculated.

Protozoan abundances were estimated using the most probable number method (modified from Ingham (1994)). Species with both flagellate and amoeba characteristics were assigned to the flagellates (Ekelund and Ronn 1994). The trophic diversity index was calculated (reciprocal Simpson's index: 1/D). Fungal hyphae were measured by epifluorescence microscopy using the grid intersection method. Bacterial numbers and cell volumes were measured by confocal laser scanning microscopy and automatic image analysis, and biomass was calculated from biovolume (Bloem et al. 1995a, Bloem et al. 1995b). The bacterial growth rate was determined by thymidine incorporation, a measure for DNA synthesis (growth rate), and leucine incorporation, a measure for protein synthesis (growth rate and biomass turnover) (Michel and Bloem 1993). Bacterial DNA from a 2 gram subsample was extracted (Van Elsas and Smalla 1995) and analyzed by denaturating gradient gel electrophoresis (DGGE) (Muyzer et al. 1993). Total number of DNA bands and genetic diversity (Shannon-Wiener: H') were calculated. Further details on sampling and identification of the soil biota are described in chapters 4 and 5.

Statistical analysis

ANOVA

Statistical analyses were carried out using the statistical package R. The effects of conversion of grassland to arable land (agricultural intensification) and re-establishment of grassland on former arable land (agricultural de-intensification) on N cycling parameters in the first year after conversion (2001) were analyzed by comparing the four land use systems with one-factor ANOVA (using type I sum of squares) followed by a Tukey HSD post-hoc analysis. Treatments were long-term grassland, new arable land, long-term arable land, and new grassland. Long-term and new grassland systems were compared with the conventionally managed long-term and new arable systems (crop rotation and conventional mineral fertilization levels), to determine the effect of conversions on N cycling under continued conventional management.

The effects of conversions on soil properties and N cycling parameters in the second year after conversion (2002) were analyzed in the same way. In this analysis, however, long-term and new grassland systems were compared with the most extensively managed long-term and new arable systems (crop rotation and reduced mineral fertilization levels), to determine the minimal effect of conversions on soil properties and N cycling. The effects of further intensification were

analyzed by comparing soil properties and N cycling parameters under systems with different cropping diversity (crop rotation vs. monoculture) and mineral fertilization level (reduced vs. conventional fertilization). These effects were analyzed using a nested two-factor ANOVA design. Treatments were cropping system (monoculture versus rotation) and fertilization level (conventional versus reduced), nested within the factor field history (grassland or arable land).

Backward regression analyses

The correlations between net and potential N mineralization and soil biota parameters were analyzed using backward regression. The regression analysis was performed on all experimental systems under a range of agricultural intensification management systems: long-term and new grassland systems, and long-term and new arable systems under crop rotation or monoculture, and under conventional or reduced fertilization. Experimental systems were repeated 3 times, resulting in 2 + (2 x 2 x 2) = 10 experimental systems and 30 experimental units. One experimental unit was lost due to an error during counting of protozoan numbers.

Before analysis, correlations between all input variables were calculated. In case of high correlation ($R^2 > 0.70$) between variables, one of them was selected as input variable in the model. Input variables for the model were bacterial and fungal biomass, bacterial activity (thymidine incorporation), protozoan amoebae, flagellates and ciliates, nematode bacterivores, fungivores and carnivores, the nematode trophic diversity index and nematode MI, earthworm ecological group representatives (*L. rubellus*: epigeic, *A. caliginosa*: endogeic), and bacterial and nematode diversity (Shannon diversity indices, numbers of bacterial genotypes,). Nematode omnivores were omitted due to high correlation with *A. caliginosa*. Data were checked for normality and homogeneity of variance. Transformations were not necessary.

Results

Total soil C and N, N−NO_3^-, and N−NH_4^+, soluble N and P

The effects of land use change and arable field management on soil properties are presented in Tables 2 and 3, for the second year after conversion (2002). Conversion of grassland to arable land reduced total soil N contents to levels not significantly different from those in the long-term arable land (Table 2). Re-establishment of grassland on former arable land did not (yet) affect total N content. Soil carbon contents followed the same trends as total soil N. Conversion of grassland to arable land had no effect on soil N-NO_3^- measured at crop harvest, whereas re-establishment of grassland reduced soil N-NO_3^-. Soil N-NH_4^+ and total soluble N were higher in the fields with grassland history than in the fields with arable history. Extractable P was lower in

Table 2. The effect of land use change on average soil properties (n=3) in the second year after conversion in 2000 (2002). LongA = long term arable land, NewG = resown grass/clover on formerly arable land, NewA = new arable land on formerly grassland, LongG = long term grassland. Different letters denote statistically significant differences at P < 0.05.

	LongA	NewG	NewA	LongG
Total N (g kg^{-1})	1.28 a	1.53 a	1.57 a	2.11 b
Carbon content (g kg^{-1})	21.0	23.8	24.7	27.8
N-NO_3 (mg kg^{-1})	5.4 b	0.9 a	7.9 b	5.8 b
N-NH_4 (mg kg^{-1})	1.7 a	2.9 ab	4.0 b	6.6 b
Total soluble N (mg kg^{-1})	11.2 a	9.7 a	19.2 b	22.9 b
Total soluble P (mg kg^{-1})	2.8 b	2.8 b	0.5 a	0.4 a
Water Content (%)	13.8 a	13.9 a	20.8 b	23.9 b

the fields with grassland history. Cropping system and fertilization had no significant effect on total soil N and C and NH_4^+ and total soluble P content (Table 3). Soil N-NO_3^-, total soluble N and soil water content were higher in the rotation systems (sown to barley) than in the monoculture systems (sown to maize).

NO_3^- leaching

Conversion of grassland to arable land resulted in increased NO_3^- leaching in the new arable system in the first year after conversion (summer and winter 2001-2002) (Table 4). Two years after conversion, (summer and winter 2002), no significant effect of conversion of grassland to arable land on NO_3^- leaching was found (Table 4). Re-establishment of grassland resulted in decreased NO_3^- leaching in the summer of 2001 compared to long-term arable land. Similarly, re-establishment of grassland resulted in decreased NO_3^- leaching, measured at 40 cm depth in the summer of 2002, and at 70 cm depth in both summer 2002 and winter 2002-2003.

Table 3. The effect of arable field management on average soil properties (n=3) in the second year after conversion in 2000 (2002). LongA = long term arable land, NewA = new arable land, M = monoculture maize, R = rotation, sown to barley, CF = conventional fertilization, RF = reduced fertilization, C*F = the interaction between crop and fertilization. P levels of significance (P <0.05) are presented.

	LongA				NewA				P level of sign.		
	M-CF	M-RF	R-CF	R-RF	M-CF	M-RF	R-CF	R-RF	Crop	Fert.	C*F
Carbon content (g kg^{-1})	20.0	20.6	22.3	21.0	26.6	26.1	25.1	24.7	n.s.	n.s.	n.s.
Total N (g kg^{-1})	1.30	1.29	1.39	1.28	1.77	1.75	1.82	1.57	n.s.	n.s.	n.s.
N-NO_3^- (mg kg^{-1})	3.9	2.1	6.8	5.4	6.7	5.2	10.9	7.9	0.0037	n.s.	n.s.
N-NH_4^+ (mg kg^{-1})	2.2	1.6	2.0	1.7	2.6	2.7	3.6	4.0	n.s.	n.s.	n.s.
Total soluble N (mg kg^{-1})	9.9	7.7	12.9	11.2	16.2	15.1	21.2	19.2	0.0048	n.s.	n.s.
Total soluble P (mg kg^{-1})	3.2	3.8	3.0	2.8	1.1	0.9	1.5	0.5	n.s.	n.s.	n.s.
Water Content (%)	11.2	11.3	14.0	13.8	17.7	17.1	20.6	20.8	0.0006	n.s.	n.s.

Table 4.The effects of land use change on $N\text{-}NO_3^-$ leaching, N uptake by the crop and N mineralization. LongA = long term arable land, NewG = resown grass/clover on formerly arable land, NewA = new arable land on formerly grassland, LongG = long term grassland. Different letters denote statistically significant differences at $P < 0.05$.

Year 2001- 2002	Depth (cm)	LongA	NewG	NewA	LongG
$N\text{-}NO_3^-$ leached (kg ha^{-1})					
Summer (April 2001 – Sept 2001)	40	21 b	1 a	90 c	3 a
Winter (Sept 2001 – March 2002)	40	20 a	13 a	125 b	12 a
Year-round (Sum April 2001 – March 2002)	40	41 a	14 a	215 b	14 a
N uptake					
Crop yield (oven dried, 1000 kg ha^{-1})		14.3 b	14.4 b	11.4 b	3.3 a
N removed by harvest (kg ha^{-1})		165 b	318 c	159 b	74 a
N mineralization					
Net N mineralization (kg ha^{-1} $year^{-1}$)	40	58 a	172 b	229 b	48 a

Year 2002 - 2003	Depth (cm)	LongA	NewG	NewA	LongG
$N\text{-}NO_3^-$ leached (kg ha^{-1})					
Summer (March 2002 – Oct 2002)	40	9 b	1 a	19 b	14 b
	70	12	1.8		
Winter (Oct 2002 – March 2003)	40	25	15	28	41
	70	91 b	11 a		
Year-round (Sum March 2002 – March 2003)	40	34	15	47	53
	70	102 b	13 a		
N uptake					
Crop yield (oven dried, 1000 kg ha^{-1})		3.7 a	17.3 b	2.6 a	4.3 a
N removed by harvest (kg ha^{-1})		64 a	385 b	46 a	93 a
N mineralization					
Net N mineralization (kg ha^{-1} $year^{-1}$)	40	58 a	240 b	53 a	103 a
Net N mineralization (kg ha^{-1} $year^{-1}$)	70	127 a	238 b		
Potential N mineralization (kg ha^{-1} $year^{-1}$)		57 a	75 b	103 c	139 d
Ratio potential N mineralization: net N mineralization		0.98 b	0.31 a	1.96 b	1.35 b

In the second year after conversion (2002), maize was sown in the monoculture and barley in the rotation systems. In the summer period (March till October), NO_3^- leaching was highest in the monoculture maize compared to the rotation barley, and highest in the conventionally fertilized systems compared to the reduced fertilized systems (Table 5). In the winter period (October

2002 till March 2003) neither cropping system nor fertilization had significant effects on NO_3^- leaching. Year-round NO_3^- leaching was higher in the monoculture maize fields, measured at 40 cm depth, but not significantly affected by fertilization level.

Crop yield and N removal by the crop

In the first year after conversion (2001), crop yield was lowest in the long-term grassland, but not significantly different in the new grassland and the two arable systems (sown to maize) (Table 4). N removal by the crop was highest in the new grassland and lowest in the long-term grassland (Table 4). In the second year after conversion (2002), crop yield and N depletion by the crop were highest in the new grassland, and not significantly different in the long-term grassland and the two arable systems (sown to barley) (Table 4). Crop yield and N removal by the crop were higher in the monoculture maize fields than in the rotation barley fields (Table 5). Conventional fertilization compared to reduced fertilization did not result in any significant effects on crop yield or N removal by the crop.

Net N mineralization

Conversion of grassland to arable land resulted in increased net N mineralization in the new arable land compared to the long-term grassland systems, in the first year after conversion (Table 4). In the second year after conversion, however, the difference had disappeared. N mineralization even tended to be lower in the new arable field. Re-establishment of grassland resulted in increased N mineralization in both years. Net N mineralization was not affected by the cropping system in the second year after conversion (2002 – 2003) (Table 5). Net N mineralization, determined at 0-40 cm depth, was higher in the fields with reduced fertilization.

N balances for the different land use systems and long-term and short-term arable systems are presented in figures 1, 2, and 3 respectively, indicating the relative crop N uptake compared to NO_3^- leaching, and the relative N input through mineralization compared to mineral fertilizer application.

Table 5 The effect of arable field management on N cycling parameters in the year 2002-2003 LongA = long term arable land, New A = new arable land on formerly grassland, M = monoculture maize, R = rotation, sown to barley, CF = conventional fertilization, RF = reduced fertilization, Fert = fertilization level, C*F = crop * fertilization interaction.

	Depth	Long term arable land				New arable land				P-levels of sign.		
	(cm)	M-CF	M-RF	R-CF	R-RF	M-CF	M-RF	R-CF	R-RF	Crop	Fert	C*F
$N\text{-}NO_3^-$ leached (kg ha^{-1})												
Summer (March 2002 – Oct 2002)	40	30	16	11	9	68	26	29	19	0.0022	0.0223	n.s.
	70	35	21	17	12					0.0148	0.0192	n.s.
Winter (Oct 2002 – March 2003)	40	73	32	18	25	35	62	34	28	n.s.	n.s.	n.s.
	70	159	54	39	91					n.s.	n.s.	n.s.
Year-round (Sum March 2002 – March 2003)	40	103	47	29	34	103	88	63	47	0.0302	n.s.	n.s.
	70	194	74	56	102					n.s.	n.s.	n.s.
N uptake												
Crop yield (oven dried, 1000 kg ha^{-1})		15	13	3	4	10	10	2	3	<0.0001	n.s.	n.s.
N removed by harvest (kg ha^{-1})		149	113	72	64	103	97	44	46	<0.0001	n.s.	n.s.
N mineralization												
Net N mineralization (kg ha^{-1})	40	87	85	6	58	41	110	12	53	n.s.	0.0365	n.s.
Net N mineralization (kg ha^{-1})	70	178	112	33	127					n.s.	n.s.	n.s.
Potential N mineralization (kg ha^{-1} $year^{-1}$)		50	44	66	57	83	86	114	103	0.0004	n.s.	n.s.
Ratio potential N mineralization: net N mineralization		0.6	0.5	11.4	1.0	2.0	0.8	9.6	2.0	n.s.	0.0133	n.s.

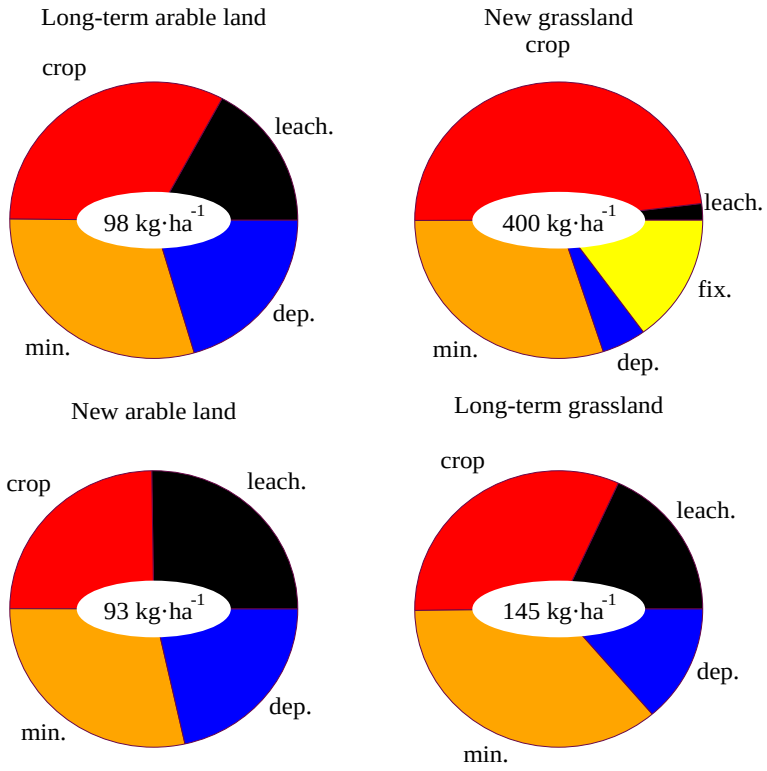

Figure 1. N balances for different land use systems. N inputs are nitrogen fixation (fix.), deposition (dep.) and mineralization (min.) and are presented in the lower half of each diagram, N outputs are crop N uptake (crop) and leached N-NO_3^- (leach.) and are presented in the upper half of each diagram. Total N input is indicated in the center of each diagram.

Potential N mineralization

In the second year after conversion (2002) potential N mineralization decreased in the order: long-term grassland > new arable land > new grassland > long-term arable land (Table 4). Conversion of grassland to arable land therefore reduced N mineralization potential, whereas re-establishment of grassland resulted in increased N mineralization potential. Potential N mineralization was on average between 0.3 and 11 times higher than net N mineralization (Table 4 and 5). The ratio between potential N mineralization and net N mineralization was lowest in the new grassland fields. Potential N mineralization was significantly higher in the rotation barley than in the monoculture maize (Table 5). Mineral fertilization level did not affect potential N mineralization. The different cropping systems did not affect the ratio between

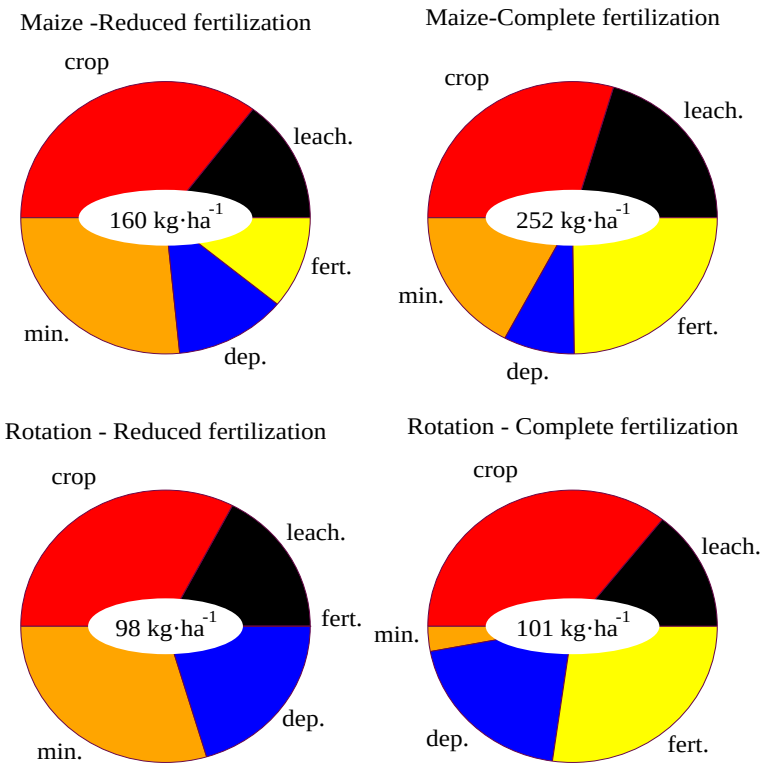

Figure 2. N balances for different arable systems on long term arable land. N inputs are fertilization (fert.), deposition (dep.), and mineralization (min.) and are presented in the lower half of each diagram, N outputs are crop N uptake (crop) and leached N-NO_3^- (leach.) and are presented in the upper half of each diagram. Total N input is indicated in the center of each diagram.

potential N mineralization and net N mineralization (Table 5). The ratio was lower in reduced fertilization fields than in conventionally fertilized fields.

Relationships between soil biota and N mineralization

Backward regression analysis of the relationship between net N mineralization and functional groups and diversity of soil biota revealed that net N mineralization was positively related to earthworm species of different functional groups (*L. rubellus, A. caliginosa*), bacterivorous nematodes, and the nematode MI (Table 6). Net N mineralization was negatively related to fungal biomass, fungivorous nematodes, and carnivorous nematodes. Net N mineralization was

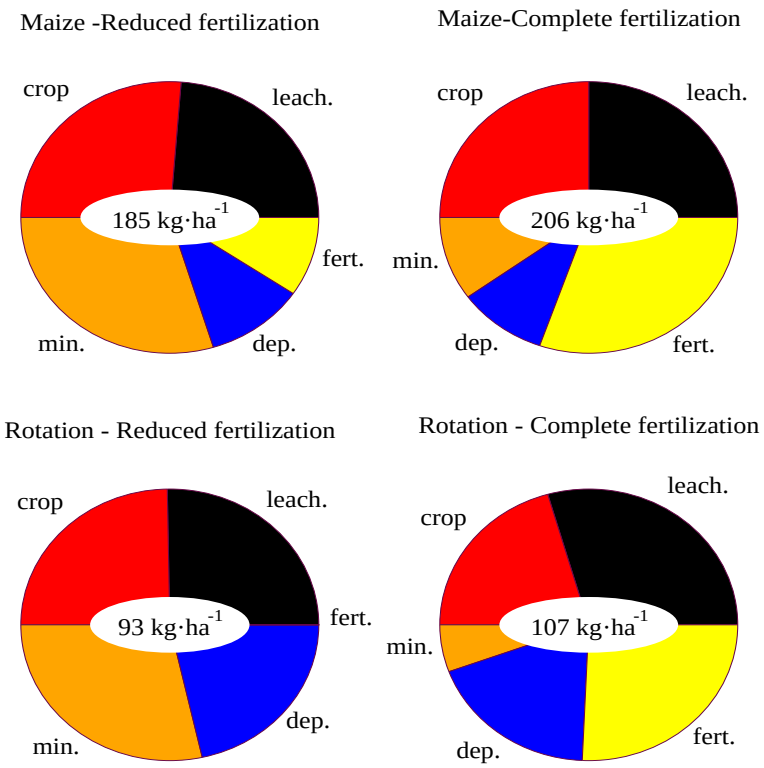

Figure 3. N balances for different arable systems on short term arable land. N inputs, N outputs, and total N input are as described in Figure 2.

positively related to the bacterial Shannon diversity index based on DNA bands (genotypes) obtained by PCR-DGGE. Total variance that could be explained by the model was 78%. Backward regression analysis of the relationship between potential N mineralization and functional groups and diversity of soil biota revealed that potential N mineralization was positively correlated to bacterial biomass, protozoan flagellates and nematode bacterivores. Potential N mineralization was negatively correlated to bacterial growth rate, nematode fungivores, and the nematode MI. Total variance explained by the model was 81%.

Table 6. Statistical results of the backward regression analysis on the relationships between net N mineralization and soil biota and potential N mineralization and soil biota parameters. All significant (P < 0.10) parameters retained in the model are presented.

Net N mineralization			
Model R^2 (adjusted): 0.78	**Estimate**	**Std. Error**	**P level**
(Intercept)	-974.12	388.95	0.022
Fungal biomass (µg C g^{-1})	-2.39	1.27	0.076
Nematode fungivores (n g^{-1})	-6.35	2.56	0.023
Nematode bacterivores (n g^{-1})	+2.04	0.89	0.034
Nematode carnivores (n g^{-1})	-19.72	10.38	0.073
Maturity Index	+128.41	39.73	0.004
A. caliginosa (n m^{-1})	+0.51	0.13	0.001
Bacterial diversity (Shannon index)	+211.95	100.53	0.049
Potential N mineralization			
Model R^2 (adjusted): 0.81	**Estimate**	**Std. Error**	**P level**
Bacterial biomass (µg C g^{-1})	+0.43	0.07	<0.001
Bacterial growth rate (pmol g^{-1} h^{-1})	-0.24	0.10	0.032
Protozoan flagellates (n mg^{-1})	+0.41	0.22	0.075
Nematode fungivores (n g^{-1})	-3.04	1.33	0.034
Nematode bacterivores (n g^{-1})	+0.64	0.36	0.089
Maturity Index	-27.45	13.81	0.062

Discussion

Synopsis of results

Agricultural intensification (conversion of grassland to arable land, monoculture, increased N fertilization) resulted in a mineralization flush in the first year after conversion, with generally decreased N mineralization rates in the second year. *Vice versa*, de-intensification (re-establishment of grassland on former arable land) resulted in increased N mineralization. In agreement with our hypothesis, increased net N mineralization was related to abundances of soil biota trophic groups, and life-strategy diversity. In contrast to our expectation, N mineralization was also related to increased bacterial genetic diversity. Henceforth, the effects of agricultural (de-)intensification on N mineralization, the relationships between N mineralization and soil biota, and the consequences for NO_3^- leaching will be discussed in detail.

Agricultural (de-)intensification effects on N mineralization

Conversion of grassland to arable land (agricultural intensification) resulted in a temporarily increased net N mineralization (i.e. only in the first year after conversion). Mineralization usually increases as a result of such a conversion, due to high amounts of plant and root residues that are decaying in the new arable system. However, after two years (2002-2003) this conversion resulted in reduced potential N mineralization and organic matter content. Also, net N mineralization tended to be lower in the new arable fields in that year. *Vice versa*, re-establishment of grassland on former arable land resulted in increased N mineralization during the first and second year. These results agree with generally observed SOM content and N mineralization to be lower in arable systems compared to grassland systems (Carpenter-Boggs et al., 2003; Parfitt et al., 2003) that may be related to reduced input of plant residues under cropped systems (Swift et al., 2004).

Further agricultural intensification included reduction in cropping diversity (monoculture maize compared to crop rotation) and increased mineral fertilization levels. Potential N mineralization was increased under the rotation barley crop, compared to the monoculture maize crop. Increased potential N mineralization under the barley crop may be related to the increased total soluble N content, as this fraction contains an easily degradable source of organic matter. Furthermore, increased potential N mineralization may be related to a higher root density and therefore higher supply of organic matter in the barley crop.

Increased mineral N fertilization resulted in decreased N mineralization. In agreement with our results, net N mineralization was found to be increased in plots receiving no fertilizer compared to soil with a fertilization history (Carpenter-Boggs et al., 2000). In contrast, a positive correlation between N fertilization and N mineralization has also been found (Carpenter-Boggs et al. 2000 and references therein). In our systems, reduction in N mineralization as a result of increased N fertilization may be related to negative effects of N fertilization on soil biota such as earthworms (Chapter 4).

N mineralization and soil biota

Trophic groups and N mineralization

In agreement with our hypothesis, net N mineralization could be explained by increased abundances of key trophic groups. N mineralization was most strongly correlated with the abundance of *A. caliginosa.* Our results are in agreement with other studies that indicate the important role of earthworms for nutrient mineralization in agroecosystems (Blair et al., 1997; Brown et al., 1998; De Goede et al., 2003). Increased N mineralization in the presence of earthworms can result from direct and indirect effects of earthworm burrowing and feeding

activity on the bacterial biomass and activity (Bohlen and Edwards, 1995; Daniel and Anderson, 1992; Ruz-Jerez et al., 1992). In contrast to *A. caliginosa*, no correlation between N mineralization and *L. rubellus* was found. *A. caliginosa* is an endogeic species foraging on soil organic matter, whereas *L. rubellus* is an epigeic species with feeding preference for plant residues at the soil surface (Bouché, 1977). *L. rubellus* hardly occurred in the arable fields, probably as a result of tillage and the absence of a permanent cover crop (Chapter 4). Consequently, the contribution of *L. rubellus* to N mineralization in our systems supposedly has been below detection.

Earthworms were not present in the laboratory incubation for potential N mineralization, and therefore a possible effect on potential N mineralization could not be demonstrated directly. Since smaller soil biota were present in the incubation, the difference between net N mineralization in the field and potential N mineralization in the incubation may indicate the net effect of earthworms on N mineralization (Table 4). In the new grassland net N mineralization was three-fold higher than potential N mineralization. Therefore, the high abundance of earthworms may have particularly stimulated N mineralization in this system. This interpretation is consistent with the results of a study in which earthworms were calculated to mineralize between 4 en 24 kg N ha^{-1} $month^{-1}$ in grasslands (between 48 en 288 kg^{-1} ha^{-1} $year^{-1}$, not corrected for temperature) (Van Vliet et al., 2007).

N mineralization was furthermore positively related to soil biota abundance in the bacterial energy channel. Potential N mineralization was correlated to the bacterial biomass and activity. In contrast, no such relationship was found with net N mineralization. Differences between correlations with net and potential N mineralization may result from the different methods that are applied to determine N mineralization. Potential N mineralization was determined by laboratory incubation, at 20°C for 6 weeks, whereas net N mineralization was calculated for field conditions during a whole year. Consequently, potential N mineralization is strongly affected by abiotic soil properties at the moment of sampling, such as the quality and content of soil organic matter, whereas net N mineralization takes into account management effects during the year. Indeed, potential N mineralization was strongly correlated to the soil organic N (R^2 = 0.86). As a result, potential N mineralization can show a high correlation with bacteria, which are directly responsible for mineralization of organic matter. The correlation between potential N mineralization and protozoan flagellates may be explained in the same way, since protozoans are also strongly affected by the soil organic matter content, whereas agricultural management had little effect (Chapter 4). The negative correlation between bacterial growth rate and N mineralization may indicate that N was immobilized in a growing bacterial biomass.

Furthermore, in agreement with other studies, net and potential N mineralization were positively related to bacterivorous nematode abundance (e.g. Bloem et al., 1994; De Ruiter et al., 1993; Ingham et al., 1985; Verhoef and Brussaard, 1990). Bacterivorous nematodes increase N

mineralization by grazing on bacterial biomass, resulting in increased bacterial activity and releasing N that would otherwise be immobilized in bacterial biomass (Bloem et al., 1997; Ingham et al., 1985). Our finding of a positive correlation between bacterivorous nematodes and bacterial growth rate (thymidine incorporation, $R^2 = 0.43$) is in agreement with this theory.

In contrast to the positive relationship with the biomass and abundances in the bacterial energy channel, net and potential N mineralization were negatively correlated with fungal biomass and fungivorous nematodes. A positive relationship with fungi was expected, since fungi mineralize N (Berg et al., 2001; De Ruiter et al., 1994; Hunt and Wall, 2002). However, since the fungi to bacteria biomass ratio in our fields was about 0.1, the role of fungi in mineralization was probably small. In general, a higher fungal biomass is found in soils with a lower N input, indicating a smaller nitrogen surplus (De Vries et al., 2006). This may be related to a lower N mineralization rate. Because of the higher C/N ratio of fungi compared to bacteria (10 vs. 4), fungivores have a smaller effect on N mineralization rate than bacterivores. In addition, fungal feeding fauna generally have a smaller biomass and lower turnover rates than bacterial-feeding fauna (Didden et al., 1994; Zwart et al., 1994).

N mineralization was furthermore negatively correlated to nematode carnivore abundance. In other studies higher trophic level organisms are usually found to be less important for N mineralization than those belonging to lower trophic levels (Berg et al., 2001; Laakso and Setälä, 1999a). In our studies, carnivorous nematode abundances were related to increased nematode plant parasite abundances, which were higher in fields with more N fertilization and less N mineralization (Chapter 4). The inverse relation between carnivorous nematode abundance and N mineralization may therefore be indirect, i.e. not causal.

Life-strategy diversity and N mineralization

In agreement with our hypothesis, the results indicated that net N mineralization was related to increased nematode life-strategy diversity, as indicated by an increased nematode MI. In contrast, potential N mineralization was negatively correlated to the nematode MI. Since potential and net N mineralization were determined on different time scales, this result may indicate the importance of different nematode life-strategies at different points in time. The negative correlation between potential N mineralization and the nematode MI may indicate the higher contribution of opportunistic nematodes to N mineralization in the short-term determination of potential N mineralization. Opportunistic (r-selected) nematodes can quickly increase after the soil homogenization that is applied prior to potential N mineralization measurement and may thus contribute more to N mineralization than “persisting” (K-selected) species. In contrast, in the long-term determination of net N mineralization, the presence of a nematode community with more diverse life-strategies (*i.e.* a higher MI) may result in a more complete N mineralization due to food partitioning. Such a result was also found in a laboratory

experiment where the combination of two nematode species from the most different cp-classes resulted in the highest N mineralization compared to combinations of species from the same cp-class or single species (Chapter 2). In agreement with these results our findings support the hypothesis that ecological functioning of soil biota communities increases with distance in ecological traits of the species present (Heemsbergen et al., 2004).

Taxonomic diversity and N mineralization

In agreement with our hypotheses, we found no relationship between N mineralization and nematode taxonomic diversity, indicating that abundances of trophic groups and functional diversity are more important for N mineralization that species diversity *per se* (Cole et al., 2006; Heemsbergen et al., 2004; Setälä, 2005). In contrast to our expectations, however, N mineralization was related to bacterial genetic diversity (Shannon index). Several studies have indicated that bacterial diversity had no measurable or consistent effect on N cycling (Degens, 1998; Griffiths et al., 2001; Gunapala et al., 1998). Possibly, the correlation between net N mineralization and bacterial genetic diversity occurred indirectly, as higher net N mineralization indicates higher food supply for the bacteria, which stimulates the presence of a higher number of bacterial genotypes. Alternatively, recent studies have indicated that nitrogen mineralization may be more dependent on bacterial species diversity than is generally assumed, since specific species are responsible for excretion of essential exoenzymes at a microsite scale (Schimel et al., 2005).

Use efficiency of mineralized N

Once N is mineralized it is at risk of leaching to below the rooting zone. NO_3^- leaching from the lower profile depth (40-70 cm in the long-term arable fields) was larger than from the upper 40 cm layer. N mineralization rates are expected to be higher in the upper layer, and therefore leaching potential from that layer is also higher. This suggests more N is immobilized by the roots in the upper 40 cm layer, whereas in the lower 30 cm all the N was lost.

Mineralized N can be incorporated into the plant biomass; the amount depends on the crop or plant community present. Our study shows examples of both increased NO_3^- leaching and increased incorporation into the plant biomass in cases of increased N mineralization. In maize on the new arable land, one year after conversion, high N mineralization was related to high NO_3^- leaching (Table 4). On the other hand, the high N mineralization in the new grassland resulted in a high N uptake by the grass, and low NO_3^- leaching. Our results indicate that a high N mineralization does not necessarily lead to increased NO_3^- leaching, provided a crop with a high nutrient uptake capacity is grown. In agreement with our results, lower leaching from

undisturbed grassland systems compared to arable systems was also indicated in other studies (Matlou and Haynes, 2006).

Mineral N from fertilizer and N that is made available through mineralization are both at risk of leaching, but the time period of increased risk differs. Mineral N fertilization is generally applied once or twice in the first half of the growing season, leading to peak concentrations of NO_3^- and consequently a high risk of leaching (Bloem et al. 1997, Van Diepeningen et al. 2006). N mineralization, however, confers a higher risk of leaching in the winter period, as mineralization continues at low rates but in the absence of a crop (Hansen et al., 2001). In our study, decreased mineral N fertilization was compensated for by increased N mineralization in three out of four systems (maize on short-term arable land, rotation barley on short-term arable land and on long-term arable land). Thus, total N available in conventionally and reduced fertilized fields was equivalent (Table 5, Fig 2, Fig. 3). In systems with increased mineral N fertilization and decreased N mineralization, NO_3^- leaching was larger in the summer. Year-round NO_3^- leaching, however, was not significantly related to the source of mineral N (Table 5, Fig. 2, Fig. 3) (Seidel et al., 2007). These results indicate that mineral N derived from fertilization is at higher risk of leaching in the summer period, whereas year-round this effect may be (partly) negated, as N mineralization confers a higher risk of leaching in the winter period, at least during our experimentation period.

Conclusions

Agricultural intensification generally resulted in decreased N mineralization and increased NO_3^- leaching, whereas de-intensification had the opposite effect. Increased N mineralization was related to both functional group abundances and functional diversity of soil biota, whereas relationships with taxonomic diversity were inconsistent. Endogeic earthworms (*A. caliginosa*) and trophic groups in the bacterial energy channel were shown to be key trophic groups for N mineralization, whereas it was negatively related to fungal biomass and nematode fungivores. Furthermore, in the short-term and under disturbed conditions, N mineralization can be explained by a high presence of opportunistic nematodes, whereas under field conditions N mineralization increased with a higher diversity of nematode life-strategy groups. Taxonomic diversity of nematodes did not contribute to N mineralization, but indications were found for a correlation between bacterial genetic diversity and N mineralization. Our results agree with studies indicating the importance for N mineralization of soil biota trophic groups (De Ruiter et al., 1994), and functional diversity within trophic groups (Heemsbergen et al., 2004; Chapter 2), whereas taxonomic diversity per se may be less important (Setälä, 2005).

Whether mineralized NO_3^- is incorporated into the crop or leached out below the rooting zone depends on the cropping system. During the cropping season mineralized N confers a lower risk

of leaching than mineral fertilizer N , but year-round NO_3^- leaching was independent on the source of mineral N due to highly variable leaching of mineralized N in the fallow period. We conclude that both trophic group abundances and life-strategy diversity of soil biota significantly contribute to mineral N availability in extensively managed systems, and can at least partly compensate for reduced mineral N inputs from fertilizers. Such increased N mineralization may confer a low risk of NO_3^- leaching during the cropping season as well as in high and continuous crop production systems.

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References

Altieri, M. A.. 1999. The ecological role of biodiversity in agroecosystems. Agriculture Ecosystems & Environment 74: 19-31.

Andrén, O., and J. Balandreau. 1999. Biodiversity and soil functioning - from black box to can of worms? Applied Soil Ecology 13: 105-108.

Balota, E. L., A. Colozzi, D. Andrade, and R. P. Dick. 2004. Long-term tillage and crop rotation effects on microbial biomass and C and N mineralization in a Brazilian oxisol. Soil & Tillage Research 77: 137-145.

Berg, M., P. de Ruiter, W. Didden, M. Janssen, T. Schouten, and H. Verhoef. 2001. Community food web, decomposition and nitrogen mineralisation in a stratified scots pine forest soil. Oikos 94: 130-142.

Binkley, D., and P. Matson. 1983. Ion-exchange resin bag method for assessing forest soil-nitrogen availability. Soil Science Society of America Journal 47: 1050-1052.

Blair, J. M., R. W. Parmelee, M. F. Allen, D. A. Mccartney, and B. R. Stinner. 1997. Changes in soil n pools in response to earthworm population manipulations in agrocosystems with different n sources. Soil Biology & Biochemistry 29: 361-367.

Bloem, J., G. Lebbink, K. B. Zwart, L. A. Bouwman, S. L. G. E. Burgers, J. A. De Vos, and P. C. De Ruiter. 1994. Dynamics of microorganisms, microbivores and nitrogen mineralisation in winter wheat fields under conventional and integrated management. Agriculture, Ecosystems & Environment 51: 129-143.

Bloem, J., M. Veninga, and J. Shepherd. 1995. Fully automatic determination of soil bacterium numbers, cell volumes and frequencies of dividing cells by confocal laser scanning microscopy and image analysis. Applied and Environmental Microbiology 61: 926-936.

Bloem, J., P. C. De Ruiter, and L. A. Bouwman 1997. Soil food webs and nutrient cycling in agroecosystems. Pages 245-278 *in* J. D. Van Elsas, J. T. Trevors, and E. M. H. Wellington, editors. Modern soil microbiology. Marcel Dekker, Inc., New York.

Bloem, J., P. R. Bolhuis, M. Veninga, and J. Wieringa 1995. Microscopic methods for counting bacteria and fungi in soil. Pages 162-173 *in* K. P. N. Alef, editors. Methods in applied soil microbiology and biochemistry. Academic Press, London.

Bohlen, P. J., and C. A. Edwards. 1995. Earthworm effects on n dynamics and soil respiration in microcosms receiving organic and inorganic nutrients. Soil Biology & Biochemistry 27: 341-348.

Bongers, T. 1988. De nematoden van nederland. Pirola, Schoorl, The Netherlands.

Bongers, T., R. G. M. de Goede, G. W. Korthals, and G. W. Yeates. 1995. Proposed changes of c- p classification for nematodes. Russian Journal of Nematology 3: 61-62.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14-19.

Bongers, T.. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14-19.

Bouché, M. B. 1977. Strategies lombriciennes. Pages 122-132 *in* U. Lohm, and T. Persson, editors. Soil organisms as components of ecosystems. proceedings of the vi. international soil zoology colloquium. Ecological Bulletins, Stockholm.

Breland, T. A., and R. Eltun. 1999. Soil microbial biomass and mineralization of carbon and nitrogen in ecological, integrated and conventional forage and arable cropping systems. Biology and Fertility of Soils 30: 193-201.

Brown, G. G., B. Pashanasi, C. Villenave, J. C. Patron, B. K. Senapati, S. Giri, I. Barois, P. Lavelle, E. Blanchart, R. J. BLakemore, A. V. Spain, and J. Boyer 1999. Effects of earthworms on plant production in the tropics. Pages 87-147 *in* P. Lavelle, L. Brussaard, and P. Hendrix, editors. Earthworm management in tropical agroecosystems. CABI Publishing, Oxon, UK.

Brown, G. G., P. F. Hendrix, and M. H. Beare. 1998. Earthworms (*Lumbricus rubellus*) and the fate of 15N in surface-applied sorghum residues. Soil Biology & Biochemistry 30: 1701-1705.

Brussaard, L., V. M. Behan-Pelletier, D. E. Bignell, V. K. Brown, W. Didden, P. Folgarait, C. Fragoso, D. W. Freckman, V. Gupta, T. Hattori, D. L. Hawksworth, C. Klopatek, P. Lavelle, D.

W. Malloch, J. Rusek, B. Soderstrom, J. M. Tiedje, and R. A. Virginia. 1997. Biodiversity and ecosystem functioning in soil. Ambio 26: 563-570.

Carpenter-Boggs, L., J. L. Pikul, M. F. Vigil, and W. E. Riedell. 2000. Soil nitrogen mineralization influenced by crop rotation and nitrogen fertilization. Soil Science Society of America Journal 64: 2038-2045.

Carpenter-Boggs, L., P. D. Stahl, M. J. Lindstrom, and T. E. Schumacher. 2003. Soil microbial properties under permanent grass, conventional tillage, and no-till management in south dakota. Soil and Tillage Research 71: 15-23.

Cole, L., M. A. Bradford, P. J. A. Shaw, and R. D. Bardgett. 2006. The abundance, richness and functional role of soil meso- and macrofauna in temperate grassland - a case study. Applied Soil Ecology 33: 186-198.

Daniel, O., and J. M. Anderson. 1992. Microbial biomass and activity in contrasting soil materials after passage through the gut of the earthworm *Lumbricus rubellus* hoffmeister. Soil Biology & Biochemistry 24: 465-470.

De Goede, R. G. M., L. Brussaard, and A. D. L. Akkermans. 2003. On-farm impact of cattle slurry manure management on biological soil quality. NJAS Wageningen Journal of Life Sciences 51: 103-133.

De Ruiter, J. G., W. A. J. Van Pul, J. A. Van Jaarsveld, E. Buijsman. 2006. Zuur- en stikstofdepositie in Nederland in de periode '81 tot 2002. Milieu en Natuur Planbureau. Rapportnummer 50003705.

De Ruiter, P. C., J. Bloem, L. A. Bouwman, W. A. M. Didden, G. H. J. Hoenderboom, G. Lebbink, J. C. Y. Marinissen, J. A. De Vos, M. J. Vreeken-Buijs, K. B. Zwart, and L. Brussaard. 1994. Simulation of dynamics in nitrogen mineralization in the belowground food webs of 2 arable farming systems. Agriculture Ecosystems & Environment 51: 199-208.

De Ruiter, P. C., J. C. Moore, K. B. Zwart, L. A. Bouwman, J. Hassink, Bloem J, J. A. De Vos, J. C. Y. Marinissen, W. A. M. Didden, G. Lebbink, and L. Brussaard. 1993. Simulation of nitrogen mineralization in the belowground food webs of 2 winter-wheat fields. Journal of Applied Ecology 30: 106.

De Vries, F. T., E. Hoffland, N. Van Eekeren, L. Brussaard, and J. Bloem. 2006. Fungal/bacterial ratios in grasslands with contrasting nitrogen management. Soil Biology & Biochemistry 38: 2092-2103.

Degens, B. P.. 1998. Decreases in microbial functional diversity do not result in corresponding changes in decomposition under different moisture conditions. Soil Biology & Biochemistry 30: 1989-2000.

Didden, W. A. M., J. C. Y. Marinissen, M. J. Vreeken-Buijs, S. L. G. E. Burgers, R. De Fluiter, M. Geurs, and L. Brussaard. 1994. Soil mesofauna and macrofauna in 2 agricultural systems - factors affecting population-dynamics and evaluation of their role in carbon and nitrogen dynamics. Agriculture Ecosystems & Environment 51: 171-186.

Dodd, M. B., W. K. Lauenroth, and I. C. Burke. 2000. Nitrogen availability through a coarse-textured soil profile in the shortgrass steppe. Soil Science Society of America Journal 64: 391-398.

Dominguez, J., P. J. Bohlen, and R. W. Parmelee. 2004. Earthworms increase nitrogen leaching to greater soil depths in row crop agroecosystems. Ecosystems 7: 672-685.

Ekelund, F., and R. Rønn. 1994. Notes on protozoa in agricultural soil with emphasis on heterotrophic flagellates and their ecology. FEMS Microbiology Reviews 15: 321-353.

Ekelund, F., and R. Rønn. 1994. Notes on protozoa in agricultural soil with emphasis on heterotrophic flagellates and their ecology. FEMS Microbiology Reviews 15: 321-353.

FAO-UNESCO, 1988. Soil map of the world: revised legend. FAO, Rome, Italy.

Ferris, H., R. C. Venette, and K. M. Scow. 2004. Soil management to enhance bacterivore and fungivore nematode populations and their nitrogen mineralisation function. Applied Soil Ecology 25: 19-35.

Griffiths, B. S., K. Ritz, R. Wheatley, H. L. Kuan, B. Boag, S. Christensen, F. Ekelund, S. J. Sorensen, S. Muller, and J. Bloem. 2001. An examination of the biodiversity-ecosystem function relationship in arable soil microbial communities. Soil Biology & Biochemistry 33: 1713-1722.

Gunapala, N., R. C. Venette, H. Ferris, and K. M. Scow. 1998. Effects of soil management history on the rate of organic matter decomposition. Soil Biology & Biochemistry 30: 1917-1927.

Hansen, B., H. F. Alroe, and E. S. Kristensen. 2001. Approaches to assess the environmental impact of organic farming with particular regard to Denmark. Agriculture Ecosystems & Environment 83: 11-26.

Hansen, J. P., and F. P. Vinther. 2001. Spatial variability of symbiotic n-2 fixation in grass-white clover pastures estimated by the n-15 isotope dilution method and the natural n-15 abundance method. Plant and Soil 230: 257-266.

Hatch, D. J., A. Bhogal, R. D. Lovell, M. A. Shepherd, and S. C. Jarvis. 2000. Comparison of different methodologies for field measurement of net nitrogen mineralization in pasture soils under different soil conditions. Biology and Fertility of Soils 32: 287-293.

Heemsbergen, D. A., M. P. Berg, M. Loreau, J. R. van Hal, J. H. Faber, and H. A. Verhoef. 2004. Biodiversity effects on soil processes explained by interspecific functional dissimilarity. Science 306: 1019-1020.

Hole, D. G., A. J. Perkins, J. D. Wilson, I. H. Alexander, F. Grice, and A. D. Evans. 2005. Does organic farming benefit biodiversity? Biological Conservation 122: 113-130.

Hunt, H. W., and D. H. Wall. 2002. Modelling the effects of loss of soil biodiversity on ecosystem function. Global Change Biology 8: 33-50.

Ingham, E. R. 1994. Protozoa. Pages 491-515 *in* R. W. Weaver, editors. Methods of soil analysis. Soil Science Society of America, Madison, Wisconsin, USA.

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

Laakso, J., and H. Setälä. 1999a. Population- and ecosystem-level effects of predation on microbial-feeding nematodes. Oecologia 120: 279-286.

Laakso, J., and H. Setälä. 1999b. Sensitivity of primary production to changes in the architecture of belowground food webs. Oikos 87: 57-64.

Lawton, J. H.. 1994. What do species do in ecosystems. Oikos 71: 374.

Mäder, P., A. Fliessbach, D. Dubois, L. Gunst, P. Fried, and U. Niggli. 2002. Soil fertility and biodiversity in organic farming. Science 296: 1694-1697.

Matlou, M. C., and R. J. Haynes. 2006. Soluble organic matter and microbial biomass C and N in soils under pasture and arable management and the leaching of organic c, n and nitrate in a lysimeter study. Applied Soil Ecology 34: 160-167.

Michel, P. H., and J. Bloem. 1993. Conversion factors for estimation of cell production rates of soil bacteria from thymidine and leucine incorporation. Soil Biology & Biochemistry 25: 943-950.

Muyzer, G., E. C. De Waal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59: 695-700.

Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. Pages 85-102 *in* J. M. Sasser, and W. R. Jenkins, editors. Nematology. University of North Carolina Press, Chapel Hill.

Parfitt, R. L., N. A. Scott, D. J. Ross, G. J. Salt, and K. R. Tate. 2003. Land-use change effects on soil c and n transformations in soils of high n status: comparisons under indigenous forest, pasture and pine plantation. Biogeochemistry 66: 203-221.

Ruz-Jerez, B. E., P. R. Ball, and R. W. Tillman. 1992. Laboratory assessment of nutrient release from a pasture soil receiving grass or clover residues, in the presence or absence of *Lumbricus rubellus* or *Eisenia foetida*. Soil Biology & Biochemistry 24: 1529-1534.

Schimel, J. P., J. Bennett, and N. Fierer 2005. Microbial community composition and soil nitrogen cycling: is there really a connection? Pages 171-188 *in* R. D. Bardgett, M. B. Usher, and D. W. Hopkins, editors. Biological diversity and function in soils. Cambridge University Press.

Seidel, K., J. Muller, M. Kayser, and J. Isselstein. 2007. The effect of fertilizer type and level of N fertilization before and after grassland renewal on N leaching losses. Journal of Agronomy and Crop Science 193: 30-36.

Setälä, H. 2005. Trophic structure and functional redundancy in soil communities. Pages 171-188 *in* R. D. Bardgett, M. B. Usher, and D. W. Hopkins, editors. Biological diversity and function in soils. Cambridge University Press.

Silver, W. L., A. W. Thompson, A. Reich, J. J. Ewel, and M. K. Firestone. 2005. Nitrogen cycling in tropical plantation forests: potential controls on nitrogen retention. Ecological Applications 15: 1604-1614.

Skogley, E. O., and A. Dobermann. 1996. Synthetic ion-exchange resins: soil and environmental studies. Journal of Environmental Quality 25: 13-24.

Swift, M. J., A. M. N. Izac, and M. Van Noordwijk. 2004. Biodiversity and ecosystem services in agricultural landscapes - are we asking the right questions? Agriculture, Ecosystems & Environment 104: 113-134.

Thrupp, L. A.. 2004. The importance of biodiversity in agroecosystems. Journal of Crop Improvement 12: 315-337.

Van Diepeningen, A. D., O. J. De Vos, G. W. Korthals, and A. H. C. Van Bruggen. 2006. Effects of organic versus conventional management on chemical and biological parameters in agricultural soils. Applied Soil Ecology 31: 120-135.

Van Elsas, J. D., and K. Smalla 1995. Extraction of microbial community dna from soils. Pages 1.3.3.1-1.3.3.11 *in* A. D. L. Akkermans, J. D. Van Elsas, and F. J. De Bruijn, editors. Molecular microbial ecology manual. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Van Vliet, P. C. J., B. Van der Stelt, P. I. Rietberg, and R. G. M. De Goede. 2007. Effects of organic matter content on earthworms and nitrogen mineralization in grassland soils. European Journal of Soil Biology 43: S222-S229.

Verhoef, H. A., and L. Brussaard. 1990. Decomposition and nitrogen mineralization in natural and agroecosystems: the contribution of soil animals. Biogeochemistry 11: 175-211.

Walinga, I., M. Kithome, I. Novozamsky, V. J. G. Houba, and J. J. Vanderlee. 1992. Spectrophotometric determination of organic-carbon in soil. Communications in Soil Science and Plant Analysis 23: 1935-1944.

Wyland, L. J., and L. E. Jackson. 1993. Evaluating nitrate recovery by ion-exchange resin bags. Soil Science Society of America Journal 57: 1208-1211.

Yeates, G. W., T. Bongers, R. G. M. De Goede, D. W. Freckman, and S. S. Georgieva. 1993. Feeding habits in soil nematode families and genera - an outline for soil ecologists. Journal of Nematology 25: 315-331.

Zwart, K. B., S. L. G. E. Burgers, J. Bloem, L. A. Bouwman, L. Brussaard, G. Lebbink, W. A. M. Didden, J. C. Y. Marinissen, M. J. Vreeken-Buijs, and P. C. De Ruiter. 1994. Population dynamics in the belowground food webs in two different agricultural systems. Agriculture, Ecosystems & Environment 51: 187-198.

Chapter 7

General Discussion

Introduction

Abundance and diversity of species in agricultural soils is vast (Brussaard et al., 1997). Soil biota are known to be of great importance for life-support functions such as decomposition of organic matter, cycling of nutrients, soil formation and nutrient availability for plant growth (Brussaard et al., 1997; De Ruiter et al., 1994; Faber et al., 2006). In intensive agriculture, however, these functions are partly bypassed by practices such as synthetic fertilization and soil tillage. Negative consequences of intensified agricultural practices include impoverished biodiversity and increased losses of nutrients to the environment. These have resulted in political and social incentives towards long-term sustainable agriculture, with reduced external inputs. In these systems, higher nutrient use efficiency may be obtained by a more prominent role of soil biota (Brussaard et al., 2007). Therefore, increased understanding of the way and measure in which agricultural practices affect the soil ecosystem, as well as the role of soil biota for ecosystem functioning is important for long-term sustainable agricultural production.

In this thesis, I aimed to determine how agricultural (de)-intensification affects soil biota abundances and diversity, and to evaluate the possible role of soil biota abundances and diversity in nitrogen mineralization and retention in (de)-intensified agroecosystems. I expected biodiversity to be decreased as a result of increased agricultural intensification, and increased under de-intensification practices. I hypothesized that a higher functional diversity of soil biota confers an increased nitrogen mineralization in de-intensified systems. I expected that systems with reduced external inputs and increased soil biota diversity would show increased nitrogen use efficiency. Therefore, I studied functional and taxonomic diversity in a range of (de-)intensified agroecosystems, while the function of soil biota abundances and diversity for nitrogen mineralization was studied both in these systems and in two laboratory experiments. I will henceforth discuss the results against the background of increased scientific knowledge on the relationship between biodiversity and ecosystem functioning in agroecosystems.

Soil biota diversity and ecosystem functioning

The importance of soil biodiversity for agroecosystem functioning has been subject of ongoing scientific debate (Brussaard et al. 2007, Ritz, 2005). Several hypothetic relationships have been formulated (Brussaard et al., 2004; Chapin et al., 2000). Manifestations of all forms of relationships are present, indicating that there is no general relationship between biodiversity and function (Ritz, 2005). Still, conclusions may be drawn on specific components of the biodiversity-ecosystem function relationships, such as the relationship between soil biodiversity and nitrogen cycling (Swift et al., 2004).

Redundancy at the higher species diversity level

Although the role of key functional and taxonomic groups such as bacteria, microbivores and earthworms for nitrogen cycling has frequently been established (Brussaard et al., 1997; De Ruiter et al., 1994), recent studies suggest that species diversity within functional groups has no major influence on ecosystem functioning in field soils (Setälä et al., 2005). At the coarse level of trophic groups, redundancy may be limited (Hunt and Wall, 2002), but at the level of species functional redundancy seems to be common in soil ecosystems (Bradford et al., 2002; Cole et al., 2006; Griffiths et al., 2001; Setälä et al., 2005; Van der Wurff et al., 2007). Functional redundancy among soil biota can be explained by the high degree of generalism (omnivory). Omnivorous species are not specialized in a certain food source and can therefore easily replace lost species, irrespective of what their diet may have been (Setälä et al., 2005). Generalism in soil ecosystems is stimulated by the small spatial scale and heterogeneous structure of soils, which implies limited movement of species. Species have to ”eat what they get”, leading to ubiquity of omnivory (Andren et al., 1999; Setälä et al., 2005).

The importance of redundancy among soil biota is also clear from this thesis. Although abundances of most trophic groups declined after intensification of agricultural systems, all trophic groups among smaller soil biota were still present in the most intensively managed systems (Chapter 4). Species diversity within trophic groups, however, was reduced after agricultural intensification (Chapter 5). These results indicate that even though species are lost from the system, other species may compensate in number, resulting in little effect on net diversity of functional groups. Furthermore, N mineralization was not related to species diversity of nematodes as a whole, but it was related to bacterivorous and fungivorous nematode abundances, indicating that as long as the functional group is present in sufficient abundance, reduction in species diversity is less important.

Relationship with resistance and resilience against stress

Although soil biota communities can be redundant at the species level with regard to ecosystem functioning, increased soil biodiversity may confer an insurance against ecosystem malfunctioning under stress or disturbance in the future (Andren et al., 1995; Bengtsson et al., 2000; Brussaard et al., 2007; Ettema, 1998). Van der Wurff et al. (2007) indicated that although redundancy occurred among nematode species in contaminated sites, those communities were at increased risk when exposed to additional disturbance. Similar results were found for the bacterial community (Griffiths et al., 2000), although Griffiths et al. (2001) indicated that these effects may have been caused by changes in the specific properties of the community, rather than by species diversity *per se*. In line with the above mentioned studies, the reduced taxonomic diversity that was found in intensified agricultural systems in my studies (Chapter 5) may confer

an increased risk of reduced ecosystem functioning in cases of future disturbance and stress, e.g. resulting from global change agents.

Vulnerability of species-poor groups

Furthermore, although redundancy may be common for species-rich soil biota, which are usually found among the smaller soil biota (Chapter 4, 5), species losses in species-poor functional or taxonomic groups are at a higher risk of affecting ecosystem functioning (Brussaard et al., 1997; Brussaard et al., 2004; Wardle, 2006). Such species-poor assemblages occur (among others) among litter transformers and ecosystem engineers in the macrofauna (Brussaard et al., 1997). In line with these studies, my studies indicate that reduced diversity in species-poor taxonomic groups can affect agroecosystem functioning. Earthworm species diversity in my field systems was limited to 3 species (Chapter 4). Decreased species diversity, as occurred under agricultural intensification, immediately implied the loss of an ecological (functional) group. My laboratory study indicated that this loss may have consequences for N cycling (Chapter 3). In addition, reduced species diversity of predatory mites rigorously changed the composition of trophic and life-strategy groups (Chapter 4, 5). Although the link between predatory mite diversity and ecosystem functioning was not studied, my results indicate that reduced species diversity in this group resulted in reduced functional diversity and therefore was likely to have impaired soil ecosystem functioning .

Functional diversity: distance in ecological traits of species

The importance of functional diversity for ecosystem functioning, in contrast to species diversity per se, has been highlighted by Heemsbergen et al. (2004). In the latter study ecological function was enhanced with increasing distance in ecological traits of species present. Such results may be explained as species with different traits can be complementary and niche differentiation may take place. Also in plant communities evidence was found for a positive effect of species diversity on ecosystem functioning through a “complementarity effect” of species with different traits (Loreau and Hector, 2001). In agreement with these findings, results presented in this thesis show an effect of increased functional diversity on (agro)ecosystem functioning. Both my lab studies and field experiment show that increased life-strategy diversity of nematodes was related to increased N mineralization (Chapter 2, 6). Also earthworms with different ecological traits interacted to affect N mineralization (Chapter 3). Thus, besides the presence and abundance of key trophic groups, also the functional diversity within these groups can affect N mineralization.

Earthworm diversity too low to make a difference in our arable systems

Although functional diversity of both nematodes and earthworms could be related to changes in N cycling (chapter 2, 3), functional diversity was not realized for both groups in our arable systems. Considering nematodes, all different life-strategy groups occurred in our agricultural systems. Considering earthworms, however, only one species (*A. caliginosa*) was present in most of our arable systems. Higher earthworm diversity only occurred in extensively managed grassland systems. In the arable systems, increased cropping system diversity and decreased mineral N fertilization slightly enhanced the abundance of the one existing species, but did not (yet) result in increased earthworm diversity within or across functional groups. In agreement with our finding, the abundances of epigeic and anecic species was generally very low in arable systems in the Netherlands (Rutgers et al., 2006). To enhance (the role of) earthworm functional diversity in agricultural systems, systems without tillage and with a high input of crop residues throughout the year are probably more appropriate.

Mineralization and nutrient retention

The different chapters of my thesis show that increased abundances of soil biota trophic groups and increased soil biota functional diversity can lead to increased N mineralization in agricultural systems. Once nitrogen is mineralized, it can be used for crop N uptake or it may be leached to the groundwater. The fate of mineral N depends (among others) on the timing of release, the location of release and the crop. Mineral N that originates from organic matter may be less prone to leaching than N applied as mineral fertilizer, since the former is released steadily during the growing season, whereas the latter is applied at a few discrete points in time at the beginning of the growing season. On the other hand, increased N mineralization may lead to increased N leaching in the winter period, as N mineralization continues – be it at low rates - while crop N uptake is negligible. Therefore, year-round N leaching may not be dependent on the source of mineral N (Seidel et al., 2007). The results presented in this thesis indeed show that increased N mineralization in combination with reduced mineral N fertilization can result in decreased N leaching during the summer period. This positive effect of N mineralization may be partly negated, however, as N leaching during the fallow winter period may be higher in arable systems with increased N mineralization. Still, in systems with a year-round grass crop, the highest N mineralization coincided with the lowest N leaching. Although no general rule on the relationship between soil biota diversity, N mineralization and N leaching can be based on this research alone, my research does indicate that the contribution of soil biota to N mineralization can be related to decreased N leaching, provided a crop with a sufficient N uptake capacity is present year-round.

Conclusions

In this thesis, I aimed to determine the effects of agricultural (de)-intensification on soil biota abundances and diversity, and to evaluate the role of soil biota abundances and diversity in nitrogen mineralization and retention in (de-)intensified agroecosystems. The results presented in this thesis show that agricultural intensification reduces soil biota abundances and functional and taxonomic diversity across taxonomic groups, with largest effects on larger-sized species-poor soil biota groups. Redundancy appears to be common for species-rich soil biota groups, whereas reduced species diversity in species-poor soil biota groups, such as earthworms and predatory mites, may lead to impaired ecosystem functioning. In de-intensified agroecosystems, enhanced N mineralization is related to increased abundances of key trophic groups and functional diversity within these groups. This enhanced N mineralization may (partly) compensate for reduced mineral N inputs from fertilizers in more sustainable agroecosystems. In such systems, increased N mineralization may confer a lower risk of NO_3^- leaching during the cropping season or with a high and continuous crop production. I conclude that de-intensified agricultural systems, including reduced external inputs and no soil tillage, promote the role of key functional and taxonomic groups and functional diversity, which can lead to a higher nutrient use efficiency of the system.

References

Andren, O., J. Bengtsson, and M. Clarholm 1995. Biodiversity and species redundancy among litter decomposers. Pages 177-188 in H. P. Collins, G. P. Robertson, and M. J. Klug, editors. The Significance and Regulation of Biodiversity. Kluwer, Dordrecht.

Andren, O., L. Brussaard, and M. Clarholm. 1999. Soil organism influence on ecosystem-level processes bypassing the ecological hierarchy? Applied Soil Ecology 11: 177-188.

Bengtsson, J., S. G. Nilsson, A. Franc, and P. Menozzi. 2000. Biodiversity, disturbances, ecosystem function and management of European forests. Forest Ecology and Management 132: 39-50.

Bradford, M. A., G. M. Tordoff, T. Eggers, T. H. Jones, and J. E. Newington. 2002. Microbiota, fauna, and mesh size interactions in litter decomposition. Oikos 99: 317-323.

Brussaard, L., P. de Ruiter, and G. Brown. 2007. Soil biodiversity for agricultural sustainability. Agriculture, Ecosystem & Environment 121: 233-244.

Brussaard, L., T. Kuyper, W. A. M. Didden, R. G. M. De Goede, and J. Bloem 2004. Biological soil quality from biomass to biodiversity - importance and resilience to management stress and disturbance. Pages 139-161 in P. Schjonning, S. Emholt, and B. T. Christensen, editors. Managing Soil Quality - Challenges in Modern Agriculture. CAB International, Wallingford, UK.

Brussaard, L., V. M. Behan-Pelletier, D. E. Bignell, V. K. Brown, W. Didden, P. Folgarait, C. Fragoso, D. W. Freckman, V. Gupta, T. Hattori, D. L. Hawksworth, C. Klopatek, P. Lavelle, D. W. Malloch, J. Rusek, B. Soderstrom, J. M. Tiedje, and R. A. Virginia. 1997. Biodiversity and ecosystem functioning in soil. Ambio 26: 563-570.

Chapin, F., E. Zavaleta, V. Eviner, R. Naylor, P. Vitousek, H. Reynolds, D. Hooper, S. Lavorel, O. Sala, S. Hobbie, M. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. Nature 405: 234-242.

Cole, L., M. A. Bradford, P. J. A. Shaw, and R. D. Bardgett. 2006. The abundance, richness and functional role of soil meso- and macrofauna in temperate grassland - A case study. Applied Soil Ecology 33: 186-198.

De Ruiter, P. C., J. Bloem, L. A. Bouwman, W. A. M. Didden, G. H. J. Hoenderboom, G. Lebbink, J. C. Y. Marinissen, J. A. De Vos, M. J. Vreeken-Buijs, K. B. Zwart, and L. Brussaard. 1994. Simulation of dynamics in nitrogen mineralization in the belowground food webs of 2 arable farming systems. Agriculture Ecosystems & Environment 51: 199-208.

Ettema, C. H.. 1998. Soil nematode diversity: species coexistence and ecosystem function. Journal of Nematology 30: 159-169.

Faber, J. H., J. J. C. Van der Pol, and M. Thompson 2006. Soil health scenario's. Pages 64-78 in . Pre-selection of scenario for cumulative risk assessment - Documentation of the first version of selection procedure including initial uncertainty evaluation. Report Deliverable D.1.2.4., Integrated Project 'NOMIRACLE' in EU 6th Framework Programme, project nr 003956.

Griffiths, B. S., K. Ritz, R. D. Bardgett, R. Cook, S. Christensen, F. Ekelund, S. J. Sorensen, E. Bååth, J. Bloem, P. C. De Ruiter, J. Dolfing, and B. Nicolardot. 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship. Oikos 90: 279-294.

Griffiths, B. S., K. Ritz, R. Wheatley, H. L. Kuan, B. Boag, S. Christensen, F. Ekelund, S. J. Sorensen, S. Muller, and J. Bloem. 2001. An examination of the biodiversity-ecosystem function relationship in arable soil microbial communities. Soil Biology & Biochemistry 33: 1713-1722.

Heemsbergen, D. A., M. P. Berg, M. Loreau, J. R. van Hal, J. H. Faber, and H. A. Verhoef. 2004. Biodiversity effects on soil processes explained by interspecific functional dissimilarity. Science 306: 1019-1020.

Hunt, H. W., and D. H. Wall. 2002. Modelling the effects of loss of soil biodiversity on ecosystem function. Global Change Biology 8: 33-50.

Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412: 72-76.

Ritz, K. 2005. Underview: origins and consequences of below-ground biodiversity. Pages 381-401 in R. D. Bardgett, M. B. Usher, and D. W. Hopkins, editors. Biological Diversity and Function in Soils. Cambrigdge University Press, UK.

Rutgers, M., A. J. Schouten, and J. J. Bogte 2006. Data obtained from the database of the Netherlands Soil Monitoring Network (LMB). in L. Van Hulst, and C. Speijers, editors. Het Globe Programma: Bodem. Globe Nederland, SME Advies. SME Advies, Utrecht.

Seidel, K., J. Muller, M. Kayser, and J. Isselstein. 2007. The effect of fertilizer type and level of N fertilization before and after grassland renewal on N leaching losses. Journal of Agronomy and Crop Science 193: 30-36.

Setälä, H., M. P. Berg, and T. H. Jones 2005. Trophic structure and functional redundancy in soil communities. Pages 236-249 in R. D. Bardgett, M. B. Usher, and D. W. Hopkins, editors. Biological Diversity and Function in Soils. Cambridge University Press.

Swift, M. J., A. M. N. Izac, and M. Van Noordwijk. 2004. Biodiversity and ecosystem services in agricultural landscapes - are we asking the right questions? Agriculture, Ecosystems & Environment 104: 113-134.

Van der Wurff, A., S. Kools, M. Boivin, P. van den Brink, H. van Megen, J. Riksen, A. Doroszuk, and J. Kammenga. 2007. Type of disturbance and ecological history determine structural stability. Ecological Applications 17: 190-202.

Wardle, D. A.. 2006. The influence of biotic interactions on soil biodiversity. Ecology Letters 9: 870-886.

Summary

Soil biota play a key role in life-support functions such as decomposition of organic matter and nitrogen cycling. In intensive agriculture, this function is partly bypassed by the use of synthetic fertilizers and soil tillage. Negative consequences of these agricultural practices include decreased soil biota diversity and nutrient losses to the environment. Therefore, increased research attention has recently been directed towards finding long-term sustainable solutions in agriculture. Higher nutrient use efficiency in agro-ecosystems may be obtained by a more prominent role of soil biota. Soil biota enhance nitrogen mineralization and may therefore reduce dependency on external inputs. Many questions remain, however, on the potential effects of soil biodiversity on N mineralization. Furthermore, more research is needed to determine the way and measure in which agricultural practices affect the soil ecosystem. In this thesis I aimed to determine how agricultural (de-)intensification affects soil biota abundances and diversity, and to evaluate the role of soil biota abundances and diversity in nitrogen mineralization and retention in (de-)intensified agroecosystems.

Chapter 1 of this thesis describes the available information on the relationship between biodiversity and (agro-)ecosystem functioning. Recently, the hypothesis that the diversity of functional groups is an important determinant of the functioning of ecosystems has received much attention. Taxonomic diversity within functional groups may confer resistance against future disturbance and stress. Abundances and taxonomic diversity of soil biota usually decrease in intensified agricultural systems. Furthermore, agricultural intensification may result in altered community structure and relative abundances of soil biota trophic groups and life-strategy groups (i.e. functional groups). Major soil biota groups such as microflora (decomposers), microbivores (grazers and browsers) and earthworms (ecosystem engineers) are known to enhance N mineralization. Once N is mineralized it is at risk of leaching out to below the rooting zone. However, many questions remain on the effects of functional and taxonomic diversity on N mineralization and the relationships with N leaching. The aims and outline of this thesis are presented at the end of chapter 1.

Chapter 2 describes a laboratory experiment on species interactions within the trophic group of bacterivorous nematodes and their effect on N mineralization. In this microcosm study, I used single or two-species combinations of three bacterivorous nematode species with different life-strategies, ranging from extremely opportunistic (*Bursilla monhystera)* to slightly opportunistic (*Plectus parvus*), while *Acrobeloides nanus* has an intermediate position. Nematode population development, bacterial activity and biomass, and mineralized N in the microcosms were assessed during and after a period of 84 days. All the nematode species interacted with each

other, but the nature and effects of these interactions depended on the specific species combination. The combination of nematodes with the most diverse life-strategies (*B. monhystera* and *P. parvus*) resulted in increased bacterial biomass and increased N mineralization, whereas the two other combinations (*B. monhystera* and *A. nanus*, *A. nanus* and *P. plectus*) had no effect. The results indicated that the difference in life-strategies between species of the same trophic group is of importance for their communal effect on soil ecosystem processes. At the very low number of species of the present study (1 or 2 species of nematodes from one trophic group), these results support the idiosyncracy hypothesis of biodiversity.

In line with the previous chapter, **chapter 3** describes a mesocosm experiment on the relationships between earthworms species diversity and N mineralization. I studied three earthworm species, representative of different ecological groups (epigeic, i.e. surface-living and litter-feeding: *Lumbricus rubellus*; endogeic, i.e. sub-surface-living and soil organic matter-feeding: *Aporrectodea caliginosa tuberculata*; and anecic, i.e. deep-borrowing and litter-feeding: *Lumbricus terrestris*) and their interactions on the bacterial community, and on N mineralization from 15N-labeled crop residue and from soil organic matter. The epigeic and anecic species enhanced N mineralization of the applied crop residue, whereas the epigeic and endogeic species enhanced mineralization of the soil organic matter. Furthermore, the combination of epigeic and endogeic, and epigeic and anecic species resulted in increased N immobilization, whereas the interaction between endogeic and anecic species resulted in increased C mineralization. I concluded that the effects of earthworms on nitrogen mineralization depend on the ecological traits of the earthworm species present, and on the interactions between those species. In agreement with the previous chapter, these results support the hypothesis that the effect of species diversity on ecosystem functioning depends on the ecological traits of the species present.

The impact of agricultural (de-)intensification on soil biota diversity is studied in chapters 4 and 5. In **chapter 4** we assessed the effects of agricultural (de-)intensification, including grassland conversion and restoration, cropping system simplification and increased mineral fertilization, on abundances and *functional* diversity of soil biota. Over three consecutive years, soil biota abundances were negatively affected by agricultural intensification, whereas de-intensification had the opposite effect. Furthermore, functional diversity of larger-sized soil biota was negatively affected, but effects on functional diversity of smaller-sized soil biota were less consistent. Larger-sized soil biota appeared to be primarily affected by short-term consequences of conversion (disturbance, loss of habitat), whereas smaller-sized soil biota were predominantly affected by long-term consequences (probably loss of organic matter). Since larger-sized soil biota had lower species richness, I suggest that agricultural intensification exerts strongest effects on species-poor soil biota groups, thus supporting the hypothesis that biodiversity has an "insurance" function.

The effects of agricultural (de-)intensification on *taxonomic* diversity of soil biota were assessed in **chapter 5**. In line with the previous chapter, agricultural intensification was found to have negative effects on taxonomic richness and diversity across taxonomic groups, with largest effects on the largest-sized soil biota. Restoration of grassland (agricultural de-intensification) had positive effects on nematode diversity, but less clear effects on predatory mites and enchytraeid diversity. The results of chapter 4 and 5 taken together, I concluded that agricultural intensification affects total abundances and taxonomic diversity of soil biota, but not necessarily the functional group diversity.

The relationship between soil biota diversity, N mineralization and N leaching in agricultural systems under (de-)intensification was addressed in **chapter 6**. Contributions of different soil biota groups to potential N mineralization and net N mineralization were compared, using regression analysis. Agricultural intensification generally resulted in decreased N mineralization and increased NO_3^- leaching in the second year after conversion, whereas de-intensification had opposite effects. Increased N mineralization was related to both functional group abundance and functional diversity of soil biota. The relationships with taxonomic diversity were inconsistent. Key trophic groups for N mineralization were endogeic earthworms (*A. caliginosa*) and trophic groups in the bacterial energy channel. Furthermore, net N mineralization increased with a higher diversity of nematode life-strategy groups. Mineralized N had a lower risk of leaching during the cropping season than mineral N applied as synthetic fertilizer, but year-round NO_3^- leaching was not dependent on the source of mineral N. I concluded that both trophic group abundances and life-strategy diversity of soil biota contribute significantly to mineral N availability in extensively managed systems, possibly leading to increased nitrogen use efficiency.

In **chapter 7** I discussed the main results of this thesis in the light of increased scientific knowledge on the function of soil biota diversity for nitrogen cycling in agroecosystems. In agreement with the notion that redundancy appears to be common in soil ecosystems, we found evidence that smaller-sized species-rich soil biota groups are functionally redundant against disturbances caused by agricultural intensification. However, reduced species diversity in intensively managed systems may confer decreased resistance and resilience against future stress and disturbances. Species losses in species-poor soil biota groups such as earthworms appear to be at larger risk of affecting ecosystem functioning. Furthermore, the importance of functional diversity within key trophic groups for ecosystem functioning has been indicated in this thesis. However, in tilled agricultural systems such diversity is not likely to be realised for earthworm species. Increased N mineralization in systems with increased soil biota diversity may be related to decreased N leaching, provided a crop with a high N uptake capacity is present year-round.

Overall, the results presented in this thesis indicate that agricultural intensification reduces soil biota abundances and diversity across taxonomic groups, with largest effects on larger-sized species-poor soil biota groups. In de-intensified agricultural systems, abundances of key trophic groups as well as functional diversity within these groups contribute to increased N mineralization. I conclude that in agricultural systems with reduced soil tillage and reduced external inputs, the abundances of key soil biota trophic groups and diversity of life-strategy groups are promoted, which, under certain conditions, is associated with a higher nutrient use efficiency of the system.

Samenvatting

Bodemorganismen spelen een sleutelrol in de recycling van nutriënten door hun bijdrage aan de afbraak van organisch materiaal (decompositie), het beschikbaar maken van voedingstoffen (mineralisatie) die benut kunnen worden voor plantengroei en bioturbatie van de bodem. In de intensieve landbouw wordt deze rol ten dele overgenomen door het gebruik van kunstmest en grondbewerking. Deze landbouwpraktijken hebben het nadelige gevolg dat onder andere de diversiteit aan bodemorganismen afneemt, en nutrientenverliezen, met name stikstof, naar het milieu toenemen. Daarom hebben wetenschappers zich recentelijk in toenemende mate gericht op het creëren en ondersteunen van vormen van landbouw die op de lange termijn duurzaam zijn. Een efficiëntere benutting van nutrienten in agro-ecosystemen kan wellicht bereikt worden wanneer de bodemgemeenschap een grotere rol in de nutriëntencyclus gaat spelen. Bodemorganismen kunnen verhogen de mineralisatie van stikstof verhogen en zouden daardoor het gewas minder afhankelijk van externe toevoer van nutriënten kunnen maken. Er zijn echter nog veel vragen, bijvoorbeeld omtrent het mogelijke effect van bodembiodiversiteit op stikstofmineralisatie. Ook is er meer onderzoek nodig om te bepalen hoe en in welke mate landbouwpraktijken het bodemecosysteem beïnvloeden. Het doel van dit proefschrift was om te bepalen hoe intensivering en extensivering van landbouwsystemen de aantallen en diversiteit van bodemorganismen beïnvloeden, en om na te gaan op welke manier de diversiteit aan bodemorganismen een rol kan spelen in de mineralisatie en retentie van stikstof in landbouwsystemen onder intensivering en extensivering.

Hoofdstuk 1 van dit proefschrift geeft een samenvatting van de beschikbare informatie over het verband tussen bodembiodiversiteit en het functioneren van landbouw-ecosystemen. Er is recentelijk veel aandacht voor de hypothese dat het functioneren van ecosystemen voor een belangrijk deel bepaald wordt door de verscheidenheid (diversiteit) aan functionele groepen. Bovendien kan de taxonomische (bijv. soort of geslacht) diversiteit *binnen* een functionele groep ervoor zorgen dat het systeem beter weerstand kan bieden tegen toekomstige verstoringen en stress. De taxonomische diversiteit en aantallen bodemorganismen zijn doorgaans verlaagd in intensieve landbouwsystemen. Intensivering van landbouw kan bovendien leiden tot een veranderde samenstelling van de bodemgemeenschap en tot veranderingen in relatieve aantallen van trofische- en levensstrategie groepen van bodemorgansismen (i.e. functionele groepen). Het is bekend dat N-mineralisatie verhoogd wordt door de aanwezigheid van dominante bodemdiergroepen zoals de bacteriën en schimmels (organische stof-afbrekers), microbivoren (consumenten van bacteriën en schimmels) en regenwormen (organische stof-afbrekers, grondmengers, gangengravers: “ecosysteem–ingenieurs”).

Gemineraliseerde N loopt echter het risico uit te spoelen tot buiten het bereik van plantenwortels. Ondanks onze aanzienlijke kennis ten aanzien van de rol van bodemorganismen in de nutrientenkringloop in het bodemecosysteem bestaan er nog belangrijke vragen over de effecten van functionele en taxonomische diversiteit op stikstofmineralisatie en de relatie met stikstofuitspoeling. Hoofdstuk 1 sluit af met de doelstellingen en hoofdstukindeling van dit proefschrift.

Hoofdstuk 2 beschrijft een laboratoriumexperiment over soorteninteracties binnen de trofische groep van bacterivore nematoden, en de effecten daarvan op N-mineralisatie. In dit microcosmosexperiment heb ik gebruik gemaakt van monoculturen en twee-soortencombinaties van drie bacterivore nematodesoorten met verschillende levensstrategieën variërend van extreem opportunistisch (*Bursilla monhystera*) tot enigszins opportunistisch (*Plectus parvus*), terwijl *Acrobeloides nanus* een tussenpositie inneemt. Gedurende en na afloop van een periode van 84 dagen, zijn de groei van de nematodenpopulaties, de bacteriële activiteit, bacteriële biomassa en de gemineraliseerde N gemeten. Alle combinaties van nematodensoorten vertoonden interacties, maar de aard en het effect van deze interacties hing af van de specifieke combinatie. De combinatie van nematodesoorten met de meest verschillende levensstrategieën (*B. monhystera* en *P. parvus*) resulteerde in verhoogde bacteriële biomassa en N-mineralisatie, terwijl twee andere combinaties (*B. monhystera* en *A. nanus*, *A. nanus* en *P. parvus*) geen effect hadden. Deze resultaten geven aan dat het verschil in levensstrategieën tussen soorten van dezelfde trofische groep van belang is voor het gezamenlijke effect op bodemecosysteemprocessen. Bij het lage aantal soorten dat in deze studie gebruikt is (combinaties van 1 of 2 nematodesoorten uit dezelfde trofische groep) ondersteunen onze resultaten de hypothese dat de effecten van soortendiversiteit uniek zijn voor elke soortencombinatie en afhangen van de ecologische eigenschappen van de aanwezige soorten.

In lijn met het voorafgaande hoofdstuk beschrijft **hoofdstuk 3** een potexperiment over het verband tussen diversiteit aan regenwormsoorten en N-mineralisatie. Ik heb drie soorten regenwormen bestudeerd, die ieder een verschillende ecologische groep vertegenwoordigen (strooiselbewoners: soorten die leven nabij het bodemoppervlak en zich voeden met gewasresten, m.n. *Lumbricus rubellus*; bodembewoners: soorten die leven in de bodem en zich voeden met bodem organisch materiaal, m.n. *Aporrectodea caliginosa*; en pendelaars: soorten die diep graven en naar het bodemoppervlak komen om zich te voeden met gewasresten, m.n. *Lumbricus terrestris*). De gevolgen van soort-specieke interacties tussen deze drie regenwormsoorten zijn vastgesteld voor de bacteriële gemeenschap, de mineralisatie van bovengrondse gewasresten die met ^{15}N gelabeld waren, en de mineralisatie van bodemorganisch materiaal. De strooiselbewoner en pendelaar verhoogden de mineralisatie van de bovengrondse gewasresten, terwijl de strooiselbewoner en bodembewoner de mineralisatie van bodemorganisch materiaal verhoogden. De combinatie van de strooiselbewoner en de bodembewoner, en de combinatie van de strooiselbewoner en de pendelaar resulteerde in

verhoogde N-immobilisatie, terwijl de combinatie van de bodembewoner en de pendelaar leidde tot verhoogde C-mineralisatie. De conclusie is dat de effecten van regenwormen op N-mineralisatie afhangen van de ecologische eigenschappen van de soorten, en van de interacties tussen die soorten. In overeenstemming met hoofdstuk 2 ondersteunen deze resultaten de hypothese dat het effect van soortendiversiteit op ecosysteemfunctioneren afhangt van de ecologische eigenschappen van de aanwezige soorten.

Effecten van landbouwkundige intensivering en extensivering op de diversiteit van de bodemgemeenschap heb ik bestudeerd in de hoofdstukken 4 en 5. In **hoofdstuk 4** heb ik in een proefveld de effecten van landbouwkundige intensivering (m.n. omzetting van grasland naar akker, vereenvoudiging van de teelt en verhoogde minerale bemesting) en extensivering (m.n. herinzaai van gras op akker, ruime rotatie, beperkte of geheel afwezige minerale bemesting) op aantallen en *functionele* diversiteit van de bodemgemeenschap bepaald. Gedurende drie achtereenvolgende jaren namen de aantallen bodemorganismen af ten gevolge van landbouwkundige intensivering, terwijl extensivering juist het tegenovergestelde effect had. Ook de functionele diversiteit van de grotere bodemorganismen nam af, terwijl de effecten op de functionele diversiteit van de kleinere bodemorganismen minder eenduidig waren. Grotere bodemorganismen leken vooral te lijden aan de korte-termijn gevolgen van intensivering (bodembewerking, verlies van habitat), terwijl de kleinere bodemorganismen vooral werden benadeeld door de lange-termijn gevolgen (waarschijnlijk verlies van organisch materiaal). De soortenrijkdom aan grotere bodemorganismen was relatief laag. Daarom concludeer ik dat landbouwkundige intensivering de meest nadelige invloed heeft op met name de relatief soortenarme functionele diergroepen. Dit ondersteunt de hypothese dat biodiversiteit de functie van een “verzekering” kan hebben.

De effecten van landbouwkundige intensivering en extensivering op *taxonomische* diversiteit van bodemorganismen is bepaald in **hoofdstuk 5**. In lijn met het voorafgaande hoofdstuk heb ik gevonden dat landbouwkundige intensivering een nadelig effect heeft op de taxonomische rijkdom en diversiteit binnen verschillende bodemdiergroepen (roofmijten, potwormen, nematoden, bacteriën) waarbij de grotere bodemdieren het meest nadelig beïnvloed werden. Herinzaai van gras op voormalige akker (landbouwkundige extensivering) had een positief effect op de nematodendiversiteit, maar het effect op roofmijten en potwormen was minder eenduidig. Hoofdstuk 4 en 5 samengenomen concludeer ik dat landbouwkundige intensivering een negatief effect heeft op de totale aantallen bodemorganismen en taxonomische diversiteit, maar niet noodzakelijkerwijs op de functionele diversiteit.

Het verband tussen bodembiodiversiteit, N-mineralisatie en N-uitspoeling in landbouwkundige systemen onder intensivering en extensivering wordt behandeld **hoofdstuk 6**. Ik heb de bijdragen van de verschillende bodemdiergroepen aan de potentiële N-mineralisatie en netto N-mineralisatie vergeleken met behulp van regressie-analyse. Landbouwkundige intensivering

resulteerde over het algemeen in een verlaagde N-mineralisatie en verhoogde NO_3^--uitspoeling, twee jaar na het begin van het veldexperiment, terwijl extensivering juist het tegenovergestelde effect had. Verhoogde N-mineralisatie ging samen met zowel grotere abundantie van functionele groepen als met een grotere functionele diversiteit van bodemdiergroepen. Het verband met taxonomische diversiteit was echter minder eenduidig. Trofische groepen die essentieel waren voor de N-mineralisatie waren bodembewonende regenwormen (*A. caliginosa)* en verschillende trofische groepen in de bacteriële voedselketen. Verder nam de N-mineralisatie toe met een hogere diversiteit aan levenstrategiegroepen van nematoden. Gemineraliseerde N liep een minder hoog risico op uitspoeling gedurende de gewasgroeiperiode dan N die gegeven werd als kunstmest. Maar gezien over een heel kalenderjaar was de N uitspoeling niet afhankelijk van de bron (gemineraliseerde N of kunstmest). Geconcludeerd kan worden dat zowel de abundantie van trofische bodemorganismengroepen als ook de diversiteit aan levenstrategieën significant bijdragen aan het beschikbaar komen van minerale N in extensieve landbouwsystemen, hetgeen mogelijk kan bijdragen aan een hogere stikstofbenutting van het landbouwkundig systeem.

In **hoofdstuk 7** heb ik de belangrijkste resultaten van dit proefschrift bediscussieerd in het licht van de toegenomen wetenschappelijk kennis over de functie van bodembiodiversiteit voor de stikstofkringloop in landbouwecosystemen. Recentelijk onderzoek wijst erop dat redundantie veelvuldig voorkomt in de bodem (d.w.z. dat verschillende soorten eenzelfde functie vervullen en dus redundant zijn met betrekking tot die functie). In overeenstemming met dit idee heb ik gevonden dat kleinere, soortenrijke bodemdiergroepen een bepaalde mate van functionele redundantie vertonen als reactie op verstoring door landbouwkundige intensivering: ondanks het verdwijnen van soorten blijft de functie van de groep behouden. Verlaagde of lage soortendiversiteit in intensief beheerde systemen kan echter wel leiden tot een verlaagde weerstand en een verminderd herstelvermogen ten aanzien van toekomstige stress en verstoring van het systeem. Wanneer soorten verloren gaan uit soortenarme bodemdiergroepen, zoals regenwormen, brengt dat het risico met zich mee dat het direct van invloed is op het functioneren van het ecosysteem. Verder is het belang van functionele diversiteit binnen essentiële trofische groepen aangetoond in dit proefschrift. Echter, in landbouwsystemen met standaard grondbewerking is het niet waarschijnlijk dat functionele diversiteit ook gerealiseerd wordt voor regenwormen. Verhoogde N-mineralisatie in systemen met een verhoogde bodembiodiversiteit gaat gepaard met verlaagde uitspoeling, mits een gewas met hoge stikstofopname jaar-rond aanwezig is.

Samenvattend tonen de resultaten van dit proefschrift aan dat landbouwkundige intensivering de aantallen en diversiteit van bodemdieren verlaagt, waarbij de grotere en soortenarme diergroepen het sterkste worden beïnvloed. In extensieve landbouwsystemen dragen essentiële trofische groepen en de functionele diversiteit binnen deze groepen bij aan een verhoogde N-mineralisatie. Daarom zullen in landbouwsystemen met verminderde grondbewerking en

verlaagde externe inputs de aantallen van essentiële trofische soorten toenemen, wat onder bepaalde omstandigheden gepaard gaat met een hogere stikstofbenutting van het systeem.

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Curriculum Vitae

Maria Bredina Postma-Blaauw was born on October 17th, 1975, in Enschede, The Netherlands. In 1994 she completed her secondary education (VWO) at the "Ichthuscollege" in Enschede. In the same year she started her study of Biology at Wageningen University. In 1998 she wrote an MSc thesis on the above-ground and belowground relationships between ants, nematodes and above-ground herbivores entitled "Spatial mosaics and vegetation diversity in grassland ecosystems: Interacting effects of ants, nematodes and above-ground herbivores". In 1999 she accomplished an internship at the Natural Resource Ecology Lab, Colorado State University, Fort Collins, USA. She finished her studies "with honors" in 1999. In 2000 she started her Phd study on soil biodiversity and nitrogen cycling under agricultural (de-)intensification at the department of Soil Quality at the Wageningen University. She completed her research in April 2008 with the defense of this thesis.

PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of Literature (4.2 ECTS)

- The significance of faunal diversity in agricultural soil for disease suppressiveness and nutrient retention (2000)

Laboratory Training and Working Visits (0.6 ECTS)

- Collembola identification training course; VU, Amsterdam (2000)

Post-Graduate Courses (7.0 ECTS)

- Applied soil ecology; PE (1999)
- Interactions between plants and attacking organisms: mechanisms, genetics, ecology and evolution; PE (2000)
- Functional biodiversity for sustainable crop protection; PE&RC (2001)
- How to manage diversity in living systems?; PE&RC and WIAS (2002)
- Soil ecology: linking theory to practice; PE&RC, FE and SENSE (2003)

Competence Strengthening / Skills Courses (2.6 ECTS)

- Advanced statistics; PE&RC (2001)
- Endnote; Wageningen University Library (2001)
- Techniques for writing and presenting a scientific paper; SENSE (2002)

Discussion Groups / Local Seminars and Other Scientific Meetings (9.1 ECTS)

- Agro-biodiversity, crop protection and adaption
- Crop and weed ecology
- Bioavailability lunches and seminars of Soil Quality

PE&RC Annual Meetings, Seminars and the PE&RC Weekend (0.9 ECTS)

- PE-days (2000)
- PE-days (2002)
- PE-days (2003)

International Symposia, Workshops and Conferences (9.6ECTS)

- International colloquium on soil zoology; Ceske Budejovice, Czech Republic (2000)
- World conference on natural resource modelling (2000)
- NWO Symposium biodiversity (2001)
- NWO Symposium, “Biodiversity in disturbed ecosystems” (2001)
- KLV-studiedag “Ecologisering Bedrijfssystemenonderzoek” (2001)
- Symposium international fertiliser society (2001)
- NWO Symposium, biodiversity: driving force of life; Den Haag, the Netherlands (2002)
- XIVth International colloquium on soil zoology and ecology; Rouen, Mont Saint Aignan, France (2004)
- CONSIDER-workshop 3: environmentally-friendly agriculture and soil biodiversity; Frick, Switzerland (2004)

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