Plant and soil community assembly in secondary succession on ex-arable land

Fundamental and applied approaches

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Contents

You could be my unintended,
Choice to live my life extended,
You could be the one I'll always love.

Matthew Bellamy, Muse

Summary

Agricultural land is converted into semi-natural ecosystems in order to counteract the current loss of species-rich grasslands and heathlands. However, efforts to restore such plant communities on former agricultural land have shown variable success. Secondary succession is the process of species replacements which begins with some biological legacy following an initial disturbance (e.g. agricultural practices). Much is still unknown about how ecosystem development proceeds during secondary succession, especially about the role of interactions between plants and soil organisms. Soil organisms can play an important role in structuring plant communities. Directly, through beneficial or harmful effects on seedling establishment, plant growth and survival, and indirectly, through their effect on carbon and nutrient availability, which, in turn, influences plant growth and competition. Plants, in turn, provide the basic resources for soil organisms and this interdependence of plants and soil organisms can result in plant-soil feedbacks. Plants can influence the composition of their associated soil community, which, subsequently, can affect plant performance. So far, studies on plant community development following land abandonment have paid little attention to temporal changes in soil communities, the interrelationships between soil and plant community development and the potential use of soil organisms to restore species-rich grasslands and heathlands. The objectives of my thesis are 1) to analyse diversity patterns and changes in the composition of soil communities following land abandonment and how this relates to plant community development; 2) to study the role of plant-soil feedback in plant community development and successional replacements; and 3) to test the effects of soil community manipulations on restoration of species-rich grasslands.

Soil-dwelling nematodes are among the most numerous soil organisms in agricultural and grassland soils. They display high taxonomic richness, inhabit different trophic positions within soil food webs and are used as indicators for changes in ecosystem functioning. In a chronosequence consisting of former agricultural fields that differed in time since abandonment and spanning a range of 34 years, we analyzed soil nematode community composition and studied the relationship with plant community development. We showed that the plant and nematode communities do not necessarily develop in parallel towards the same reference system and that successful restoration of plant communities does not imply successful restoration of soil communities. Within the same chronosequence, three former agricultural sites (early, mid, late) as well as a reference heathland were selected and sampled in detail for soil mites and nematodes. From each site, samples were taken at different spatial locations, and at four different times throughout the growth season. We showed that diversity of the soil community differed between sites, but also depended on the spatial scale of the sampling regime and on the group of soil organisms considered. The data indicated that successional changes in the soil nematode community composition were mainly due to gradual shifts in dominance patterns in response to altered environmental conditions between sites, while successional changes in the soil mite community depended most on colonisation from local species pools. The data also revealed that the trophic structure of the soil food web initially changed following land abandonment. However, this was followed by a phase in which little change occurred, possibly due to retarded soil organic matter accumulation.

In microcosm experiments, we studied the role of plant-soil feedbacks in secondary plant community succession. In an experiment in which we constructed model systems of plants and soils associated to different successional stages, we showed that irrespective of abiotic soil conditions, negative plant-soil feedback effects enhance succession in early stages, while positive plant-soil feedback effects stabilise succession by enhancing the growth of slow-growing late-successional plant species. Positive plant-soil feedback effects were strongest in late-successional soil and these effects enhanced plant community evenness. In a second experiment, we studied for a range of early-successional plant species how they changed soil microbial communities and how these changes affected competitive interactions between early- and mid-successional plant species. We showed that microbe-mediated plant-soil feedback effects enhance replacement of early-successional plant species by mid-successional species. Interestingly, early-successional plant-soil feedback effects provided biotic legacies, which influenced dominance patterns of mid-successional plant species.

To test the potential of soil community manipulations as a management strategy in restoration of species-rich grasslands, two field experiments were set up. In one experiment, we tested the effect of carbon substrates (to induce microbial N-immobilisation) and top soil removal on plant and soil community development. To test the relative importance of soil nutrient status and dispersal limitation, the treatments were applied both with and without sowing later-successional plant species. The results show that seed addition is more important than soil fertility reduction measures for restoration of plant communities. Apparently, initial stages of secondary vegetation succession are determined by plant species arrival rather than by abiotic factors. In a second experiment, which was set up on an ex-arable site from which the top soil had been removed (the receptor site), we tested whether simultaneous introduction of later-successional plants and soil organisms can influence the direction of plant community development towards a later-successional 'target' system. Plants and soil organisms were collected from a mature, species-rich *Cirsio-Molinietum* fen meadow (the donor site) and introduced 1) by spreading hay and soil, independently or combined; or 2) by transplanting intact turfs. We could not demonstrate that introduction of later-successional soil organisms facilitate the establishment of later-successional plant species. Unfavorable soil conditions at the receptor site may have limited survival of later-successional soil organisms in the turfs and may have precluded successful establishment of the soil organisms at the receptor site.

In conclusion, microcosm experiments show that there is strong potential for interdependence in rate and direction of plant and soil community development in secondary grassland succession following land abandonment. Feedback between plants and soil organisms can strongly affect plant competitive interactions and successional replacements at small spatial and temporal scales. Through ecological legacies, such feedback effects may affect long-term plant community composition patterns. However, at the field scale level, plant and soil communities may develop independent of each other to a great extent, and other factors, especially the order of plant species arrival, may overrule the effects of soil organisms in plant community succession. We know little about the required conditions for successful establishment of later-successional soil organisms and, so far, we could not demonstrate that introduction of soil organisms is an effective measure to improve grassland restoration.

Chapter 1

General introduction

Framework

Species-rich, semi-natural grasslands and heathlands have long been part of the agricultural landscape of Western and Central Europe. Since the Middle Ages these systems have had an important role in agricultural systems on Pleistocene sandy soils, which were used for sheep grazing and sod cutting. As a result of the introduction of artificial fertilisers, intensification of agricultural practices, land use changes and increased levels of acidifying and nitrifying atmospheric deposition (RIVM 2002), the distribution of species-rich grasslands and heathlands declined dramatically in the twentieth century (De Smidt 1979). Nowadays, these systems are regarded as an internationally endangered habitat type (Webb 1998), which urgently need restoration and conservation. Conversion of arable land into semi-natural systems has been suggested as one way of counteracting current loss of species-grasslands and heathlands (Hansson and Fogelfors 1998, Ejrnæs et al. 2003, Walker et al. 2004). Current overproduction at the world market and other crises in European agriculture have resulted in large areas of former agricultural land becoming available for restoration.

In spite of many restoration efforts, much is still unknown about how ecosystem development proceeds in secondary succession, especially concerning interactions between the above- and belowground subsystem (De Deyn 2004). For successful restoration of degraded ecosystems, an integrated above-belowground approach may be required (Wardle 2002, Bardgett et al. 2005a). Precisely, it is crucial to understand how ecosystems are assembled, i.e. how the species that make up a particular community arrive in an area, survive, and interact with other species (Temperton et al. 2004). Accordingly, ecosystem restoration provides an acid test for our understanding of how communities assemble, as 'each time we undertake restoration we are seeing whether, in the light of our knowledge, we can recreate ecosystems that function, and function properly' (Bradshaw, 1987). In other words: verification by experimentation. In this thesis, I use restoration of species-rich grasslands on former agricultural land as a model to disentangle the interplay between plant and soil communities in secondary succession, using both descriptive and experimental, and both fundamental and more applied approaches.

Restoration of species-rich grasslands and heathlands on former agricultural land

Plant communities

In this thesis, I focus on former agricultural lands on Pleistocene sandy soils in the central and eastern part of the Netherlands, which are typically 'managed' by low-intensive grazing of natural and introduced vertebrate herbivores, such as roe deer, fallow deer, red deer, horses and Scottish Highland cattle. Grazing prevents persistent establishment of shrubs and trees, and policy makers and nature managers ultimately aim at development towards species-rich grassland and heathland plant communities (*Galio hercynici-Festucetum ovinae* and *Genisto anglicae-Callunetum*) (Bal et al. 2001). During the initial stages of secondary succession following land abandonment, plant communities will be dominated by fast-growing arable weeds that germinate from the local seed bank, such as *Viola arvensis* and *Polygonum aviculare* and by pioneer species, such as *Conyza*

canadensis and *Chenopodium album* that colonise from the surrounding area (Fig. 1.1; Degn 2001, Baer et al. 2002). Figure 1.2 shows a theoretical step-by-step pathway of secondary plant community succession when soil fertility is gradually reduced by grazing or mowing. Weeds and pioneer species will be replaced by ruderal plant communities, characterised by species such as *Elytrigia repens*, *Cirsium arvense* and *Tanacetum vulgare*. Ruderal plant communities, in turn, will be succeeded by mid-fertile grassland communities that consist of species such as *Holcus lananatus* and *Trifolium repens*.

Figure 1.1 Plant community dominated by arable weeds and pioneer species two years after land abandonment, Assel, the Netherlands. Flowering *Viola arvensis* in May. The remnants of the former crop (*Zea mays*) are still visible (left photograph) and dominance of *Conyza canadensis* in late summer (right photograph).

Continuing fertility reduction will result in relatively, low-fertile grassland communities, which are characterised by species such as *Agrostis capillaris*, *Hypochaeris radicata*, *Rumex acetosella* and *Campanula rotundifolia*. Finally, these low-fertile grassland communities will be succeeded by oligotrophic mattgrass swards, characterised by species such as *Arnica montana*, *Festuca ovina*, *Nardus stricta* and *Potentilla erecta* and by *Calluna vulgaris*-dominated heathland communities. Current levels of acidifying and nitrifying atmospheric deposition may preclude successional development towards these 'target' communities (Bobbink et al. 1998). Top soil removal may be an effective measure to circumvent adverse effects of atmospheric deposition. Also, when applied immediately after land abandonment, top soil removal may cause a successional short-cut by providing suitable conditions for 'instantaneous' establishment of later-successional soil communities (Fig. 1.2). However, a prerequisite is that these later-successional species are readily available from the seed bank or are introduced by dispersal.

Constraining factors

So far, restoration of species-rich grassland on former agricultural land has shown variable success. Three major factors have been put forward that can constrain plant community development towards later-successional stages. First, after cessation of agricultural practices, soils are

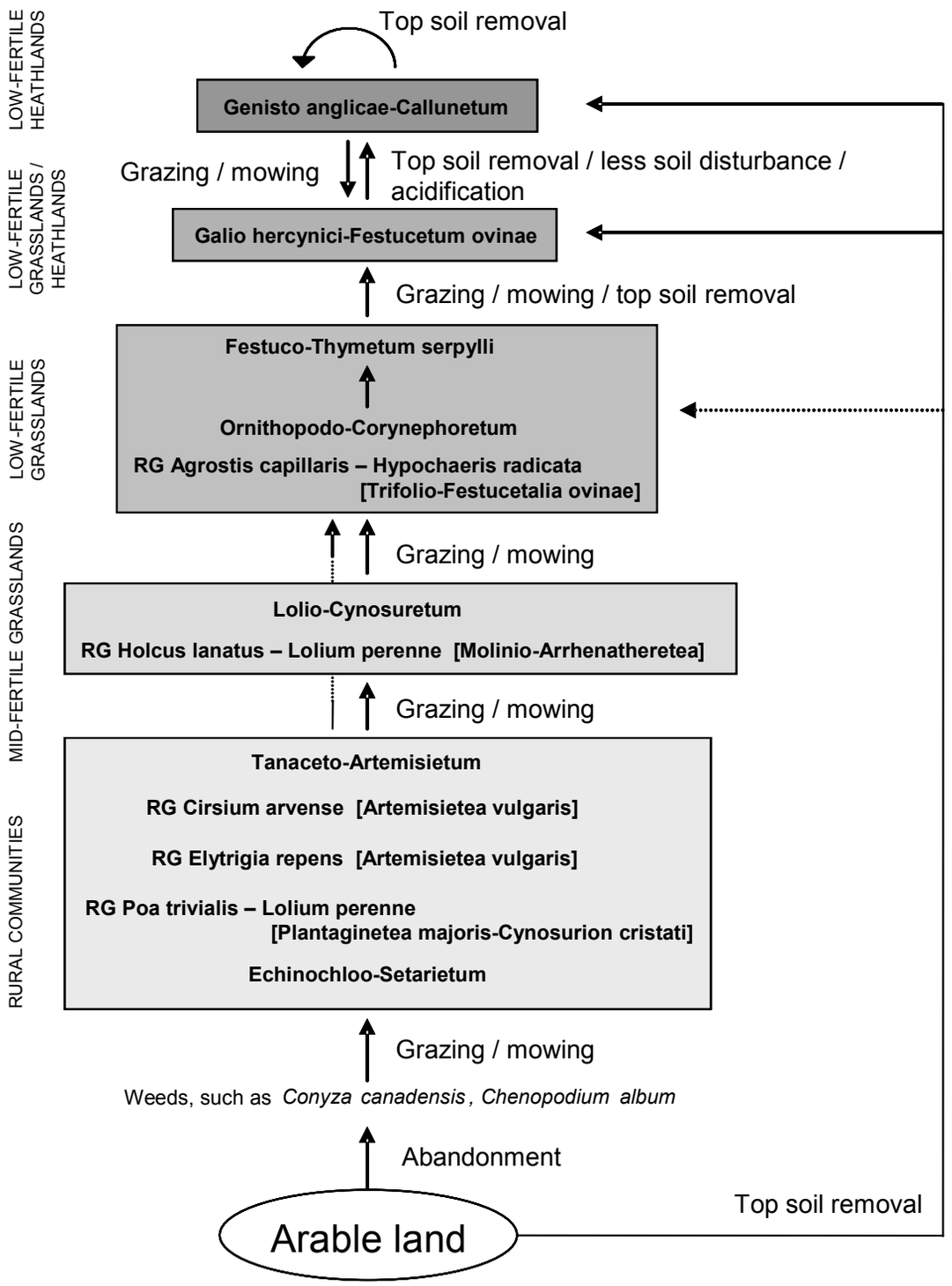

Figure 1.2 Theoretical one-way pathway of secondary plant community succession on former agricultural land for Pleistocene, dry, sandy soils in the Netherlands subject to soil fertility reduction by hay making or grazing, or by top soil removal. Based on Stortelder et al. (1999) and Schaminée et al. (1996, 1998, 2000). Boxes show scientific names for plant associations. RG = 'Frame community' (characteristic species of lower taxonomic levels are missing while the community can clearly be designed to a higher syntaxonomic level).

characterised by high nutrient availability resulting from excessive application of fertilisers. High soil fertility favours the competitive ability of fast-growing, early-successional, weedy species over slow-growing later-successional species (McLendon and Redente 1992, Marrs 1993). Once these plants have established, such early-successional plant communities may be difficult to be invaded by later-successional species and can persistent for a long term. Therefore, they can retard or prevent successional replacements towards later-successional plant communities.

Second, the success of restoration of species-rich grasslands and heathlands is often hampered by the absence of seeds of later-successional plant species (Bakker and Berendse 1999). After decades or centuries of agricultural practice, most later-successional species are eliminated from the viable seed bank (Bekker et al. 1997). Hence, subsequent species establishment and restoration of later-successional plant communities will depend on the dispersal of plant propagules from local and regional species pools (Bakker et al. 2000, Zobel et al. 1998). However, dispersal possibilities are generally poor and colonisation of later-successional plant species is usually low (Van Diggelen et al. 1997, Verhagen et al. 2001).

Third, the development of the soil community may be a crucial factor in the rate and direction of plant community succession (De Deyn et al. 2003). Soil organisms affect plant competitive interactions and replacement through direct interactions with the root system (herbivores, pathogens, mutualists) and indirectly through effects on decomposition and mineralisation processes. Moreover, soil nutrient effects on plant competition depend on the interaction with soil organisms (De Deyn et al. 2004a). In turn, soil community development is affected by changes in vegetation composition through plant species-specific differences in both the quantity and quality of resources that they return to the soil (Wardle 2002, Wardle et al. 2004a), indicating a strong potential for successional plant-soil feedbacks. However, after cessation of agricultural practices, plant and soil communities may differ in the time they need to convert towards a more naturally composed soil community. In general, changes in soil communities are slow and the development of the soil community may lag behind changes in the plant community (Wardle et al. 1999, Korthals et al. 2001). So far, temporal interactions between plant and soil communities and the potential effects on rate and direction of secondary plant community succession remain scarcely tested experimentally and, therefore, make up the primary research subject of this thesis.

Community assembly in secondary succession

Succession can be defined as species replacement over time. Traditionally, the concept of succession has been applied to plants. Two seminal views have dominated the succession debate during the last century. First, Clements (1916, 1928) developed a concept of succession that was highly deterministic and directional towards a stable 'climax' system that is no longer subject to succession. Emphasising the autogenic processes, this deterministic concept of succession suggests that there can be multiple situations from which succession starts. This may be a reasonable assumption for fields on which agricultural practices have been abandoned. However, this concept also predicts that within a particular biogeographical range, succession on those fields

would result in a predictable convergence of all plant communities towards one type of vegetation. Clement's concept has later been modified by Odum (1969), who proposed the holistic view that during succession, both changes in biotic communities, not necessarily plant communities, and in associated ecosystem processes arise from relationships and interactions within a community, resulting in a predictable trajectory towards an ultimate climax state. Second, Gleason (1917, 1926) developed a more individualistic concept of succession, which describes succession as an unpredictable process that is dependent on the properties of individual plant species, and strongly dependent on the historic or starting conditions (historically contingent). In contrast to Clements, Gleason noted that each assemblage of species is independent of other assemblages and that there is no reason for predictable development in plant communities. Egler's (1954) concept of 'initial floristic composition' suggests that after land abandonment, the species that are initially present or that arrive early can influence the course of succession for a long time. By chance, the species initially present differ from site to site, resulting in low predictability of plant community composition. The succession concepts of deterministic, individualistic and initial floristic composition remained alive during the 20th century (Crawley 1993) and they are still debated. The current view is that these different successional developments may each act at different levels of abstraction. For example, deterministic and individualistic succession can act simultaneously depending on whether plant species composition is considered at the taxonomic or functional level (Fargione et al. 2003, Fukami et al. 2005).

The research area of community assembly focuses on how species interactions could influence the composition of species occurring together (or not) (Keddy and Weiher 1999). In restoration ecology, an important question is if there are rules governing the assembly of plant communities (Temperton et al. 2004). In other words, to what extent are changes in plant species composition predictable and can rate and direction of changes in species composition be manipulated? While the concept of succession focuses on the trajectory of species replacement, community assembly focuses more on the end state of species composition. However, both concepts are closely related and ask what happens when you start a community from scratch (Young et al. 2001, Chase and Leibold 2003). The individualistic succession concept of Gleason implies that there are no rules in community assembly and, therefore, practically excludes any measure involving manipulations of the biotic or abiotic environment as restoration tool, except for active introduction of the desired species. The deterministic succession concept, however, suggests that community assembly is restricted by species interactions (competition, herbivory, predation, mutualism, etc.) and by interactions with the abiotic environment (Diamond 1975, Keddy 1992, Keddy and Weiher 1999). This provides ample opportunities for manipulation of the temporal changes in plant community composition. Practices involving alteration of abiotic soil conditions, aboveground herbivores, as well as active introduction of plant species have been shown to be successful in accelerating plant community succession following land abandonment. Manipulations of direct and indirect plant-soil organism interactions may also influence vegetation succession, but this has been largely overlooked in programmes for restoration of plant communities. An important reason may be that assembly of soil communities and the consequences for secondary succession are poorly understood.

Successional changes in soil communities and their effects on plant community dynamics

Soil communities consist of a huge variety of species, both taxonomically and functionally. Traditionally, there is considerable knowledge on the composition and structure of soil communities and the functioning of soil ecosystem processes in agricultural soils (Hooper et al. 2000). More recently, recognition is growing that soil organisms can also play an important role in the structuring of plant communities. This can be either directly, through beneficial or harmful effects on seedling establishment, plant growth and survival (e.g. Bruehl 1987, Brown and Gange 1990, Van der Putten et al. 1993, Smith and Read 1997), and indirectly, through their role in carbon and nutrient cycling (Bardgett and Shine 1999, Wardle 2002, Hättenschwiler et al. 2005). However, how the soil subsystem changes during secondary succession following abandonment of agricultural land and how these changes affect the plant community development or, vice versa, how plant community succession affects the soil subsystem, remains largely unknown (Ehrenfeld et al. 2005). In this thesis, I particularly focus on 1) successional changes in diversity, as well as in taxonomic and functional composition of the soil community in secondary vegetation succession; and 2) the effect of feedback interactions between plants and soil communities on the rate and direction of plant species replacements.

The soil food web

Indirect effects of soil organisms on plant community interactions through their role in nutrient cycling and carbon flow are regulated by the decomposer or soil food web, which includes organisms differing in their function and the trophic level they occupy (Wardle 2002). The basic resource input into the soil food web consists of detritus, i.e. dead organic matter, and plant roots (Fig. 1.3). Litter transformers, such as earthworms and enchytraeids, distribute organic matter in the soil, reduced the size of the detritus and make it available to the primary decomposers (Swift et al. 1979). In terrestrial ecosystems, the primary decomposers consist of bacteria and fungi, which are directly responsible for the decomposition of organic matter and the mineralisation of nutrients which are required for plant growth. Soil micro-organisms are fed upon by secondary decomposers, i.e. microbial feeding organisms, such as protozoa, nematodes, Collembola and mites, which in turn are fed upon by predaceous soil organisms (Fig. 1.3). Secondary decomposers and soil organisms of higher trophic levels can affect nutrient mineralisation through modifying the biomass, composition and activity of the primary decomposers or directly by consuming detritus and releasing organic nutrients (Mikola et al. 2002).

In secondary succession following abandonment of agricultural land, release from agricultural practices and changes in plant species composition are suggested to result in an increase in the total quantity of soil organic matter, while the quality or decomposability is suggested to decrease, i.e. a shift from labile to more recalcitrant organic matter (Knops and Tilman 2000, Baer et al. 2002). As a result, during transition from a high-input agricultural system towards a low-input, semi-natural system, a shift is expected from fast-cycling to slow-cycling decomposition, i.e. from a bacterial-dominated soil food web to a fungal-dominated soil food web (Moore et al.

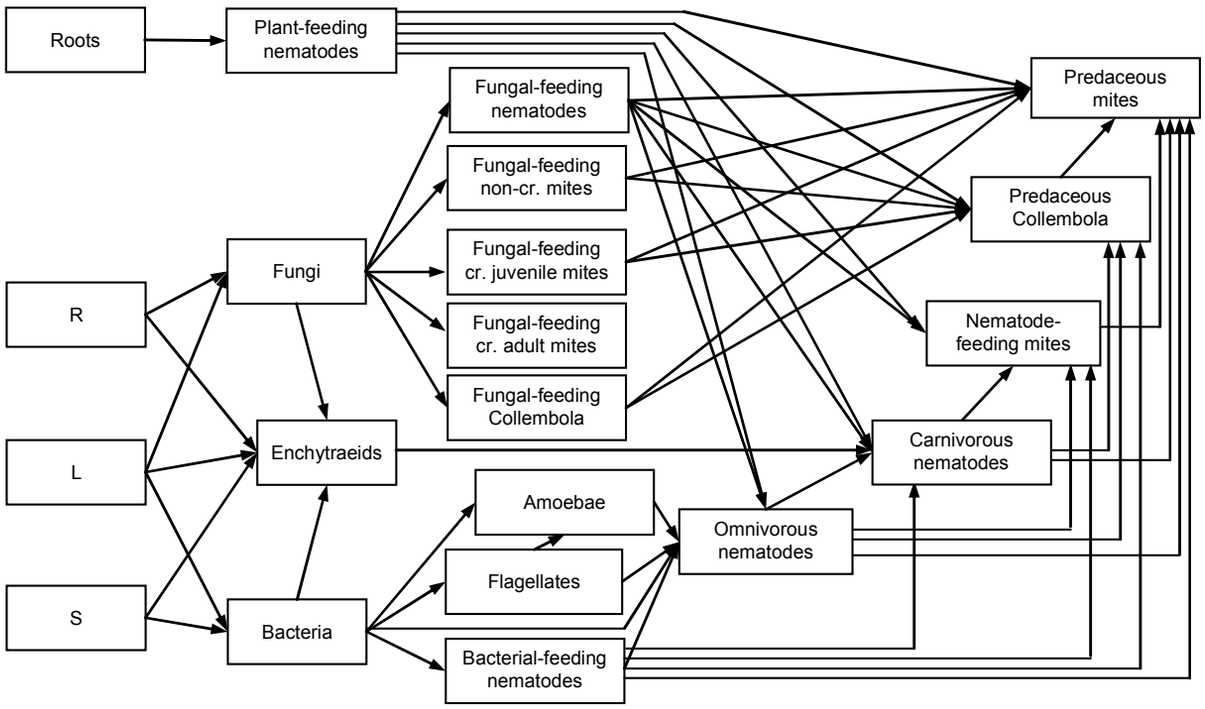

Figure 1.3 Soil food web diagram (Holtkamp et al., in preparation). Arrows represent feeding links and point towards the consumer. TL = Trophic Level, R = recalcitrant organic matter, L = labile organic matter, S = water soluble sugars, and cr = cryptostigmatic (= Oribatida).

1996, Bardgett and Cook 1998). Rapid decomposition of labile organic material is mainly accounted for by opportunistic bacteria, whereas the contribution of slower-growing fungi is more important in the protracted decomposition of recalcitrant organic material (Beare et al. 1992). Changes in the basal part of the soil food web may trickle-up to higher trophic levels, and decomposition by bacteria may affect a chain of higher trophic level organisms that differ from those associated to decomposition by fungi (Fig. 1.3; Brussaard et al. 1996). In secondary succession, additional to the bottom-up effects of changes in resource availability, physical changes in the soil, such as release from agricultural disturbance, may alter the trophic structure of the soil food web.

Soil nematodes and their indicative value

Living soil organisms are reliable bio-indicators, as they provide a good reflection of the soil system and the ecological services and ecosystem functioning of the system. Throughout this thesis, soil nematodes will be used as indicators for changes in human impact (including land use change) (Freckman and Ettema 1993), ecosystem functioning (Bongers and Ferris 1999), and successional changes in the soil subsystem (Háněl 2003). Nematodes are among the most numerous soil organisms in agricultural and grassland soils with densities up to 3-4 million m^{-2} (Bardgett et al. 1999). Importantly, soil nematodes also display high taxonomic and functional richness within grassland systems (i.e. Yeates et al. 1997). Based on the morphology of the mouth parts (Fig. 1.4) soil nematodes can be attributed to one of the following feeding habit

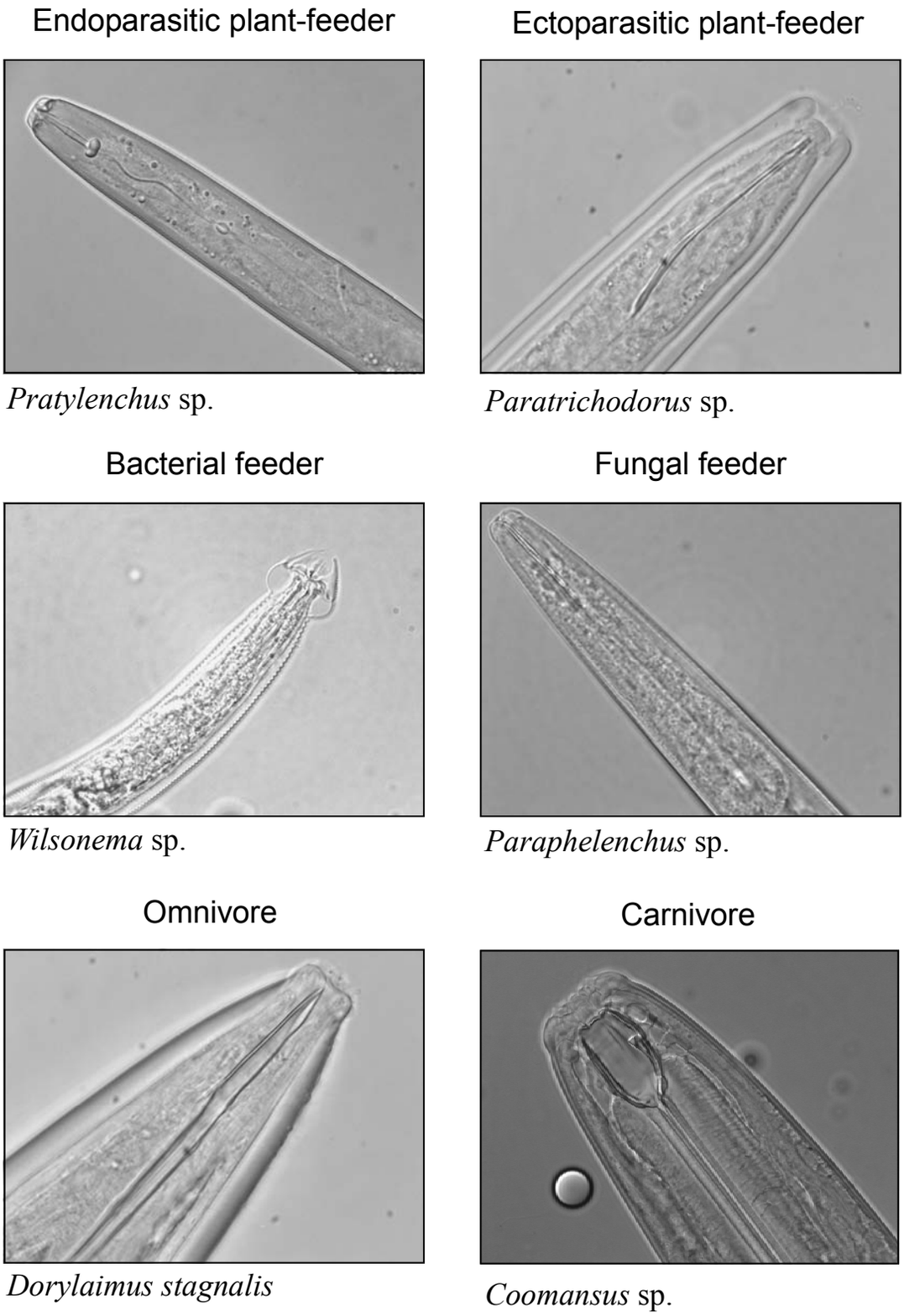

Figure 1.4 Mouth morphology of endoparasitic, ecto-parasitic, bacterial-feeding, fungal-feeding, omnivorous and carnivorous nematodes. Photo credits: Hanny B.B. van Megen, Laboratory of Nematology, Wageningen University.

classes (Yeates et al. 1993a): 1) endoparasitic plant-feeders, 2) ecto-parasitic plant feeders, 3) bacterial feeders, 4) fungal feeders, 5) omnivores, and 6) carnivores. Ectoparasitic nematodes feed on outer cortical cells and root hairs, while endoparasitic plant feeders enter the plant root and feed on deep cell layers. Omnivorous nematodes feed on bacteria, amoebae, flagellates and bacterial-feeding, fungal-feeding, and plant-feeding nematodes (Yeates et al. 1993a, Didden et al.1994), wile carnivorous nematodes feed on other groups of nematodes and on other organisms such as enchytraeids (Yeates et al. 1993a, Didden et al. 1994; Fig. 1.3). Entomopathogenic nematodes are not considered in this thesis.

In high-input, physically disturbed agricultural systems, the soil nematode community is dominated by plant- and bacterial feeding nematodes (Hendrix et al. 1986). Nematode populations can respond relatively fast to cessation of agricultural practices (Háněl 2003). During secondary succession, a shift from bacterial-dominated decomposition to fungal-dominated decomposition, altered abiotic soil conditions (Yeates et al. 1997, Verschoor et al. 2001) and changes in plant species composition and diversity (Korthals et al. 2001) are expected to affect both the taxonomic and the trophic composition of the soil nematode community. Plant community development, in turn, may depend on soil nematode community composition (De Deyn et al. 2003), which may lead to complex plant-soil feedback interactions. In secondary succession, the reciprocal relationship between plant community development and the composition of plant-feeding nematode communities has been studied extensively (Verschoor 2001, Verschoor et al. 2001, Verschoor et al 2002), while the relationship with other groups of nematodes and their taxonomic diversity remains largely unknown.

Plant-soil organism interactions and the concept of plant-soil feedback

Plant community composition can be influenced by interactions in the soil food web, i.e. through the role of soil organism decomposition of organic matter and mineralisation or immobilisation of plant-available nutrients (Bradford et al. 2002, Wardle et al. 2004a). In addition to these indirect plant-soil organism interactions, plant growth and competition, and, hence, plant community interactions are directly affected by soil organisms via interactions within the root system. The soil organisms involved can be either beneficial or harmful to the plant. Mutualistic soil organisms, such as N-fixing bacteria and mycorrhizal fungi, can affect plants positively through the role they play in supplying resources. Mycorrhizal fungi form an intimate symbiosis with roots of almost all grassland species (Smith and Read 1997). The prevalent forms in grasslands are arbuscular mycorrhizae (AM) (Stanton 1988). AM fungi can increase uptake of nutrients and water by increasing the absorptive area of the root via fungal hyphae that extent the root surface. The presence of the fungal associates may lead to improved performance in times of stress, for example when resources are limiting (Smith and Read, 1997), or if the plant is attacked by pathogenic fungi (e.g. Newsham 1995, West 1997) or insect herbivores (Gange 2001). Other soil organisms, such as microbial pathogens, and herbivorous nematodes and insect larvae can negatively affect plant performance (Van der Putten et al. 1988, Brown and Gange 1992). Root herbivorous insects, for example, consume root biomass and increase the vulnerability of the plant to attack by other pathogens (Masters and Brown 1997).

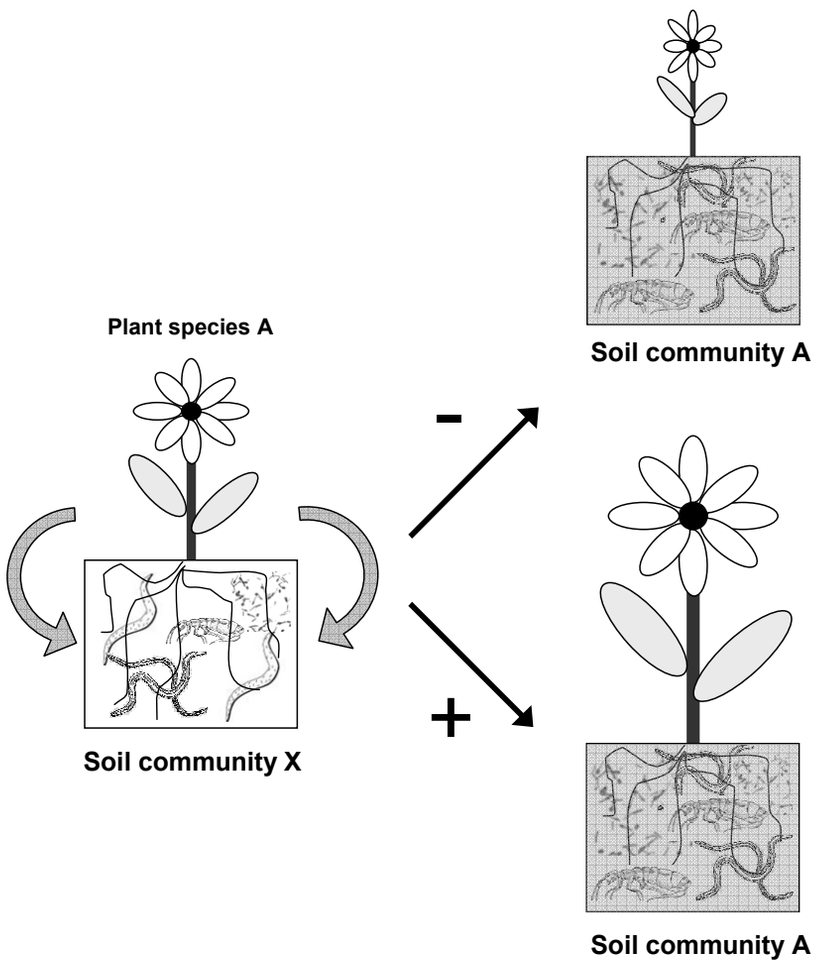

Figure 1.5 Diagram showing the concept of plant-soil feedback for plant species A, which influences soil community X in a species-specific way, resulting in soil community A. Subsequent plant growth may increase (positive plant-soil feedback) or decrease (negative plant-soil feedback).

Interactions between plants and the soil around their roots are not uni-directional and depend on feedback mechanisms. Feedback between plants and soils can operate through physical, chemical or biological pathways (Ehrenfeld et al. 2005). The latter entails indirect effects through plant-specific litter input and nutrient depletion (Berendse 1998) and direct effects through plant-specific accumulation of root-associated soil organisms. In this thesis, the focus will be on direct, biological plant-soil feedback, hereafter referred to as 'plant-soil feedback' (Bever et al. 1997). Specifically, plants have different abilities to influence their own performance by changing the density and composition of their associated soil community (Van der Putten et al. 1993, Bever 1994, Callaway et al. 2004b, Bartelt-Ryser et al. 2005). For example, through exudation of specific organic compounds, plant roots form the major source of carbon on which soil micro-organisms depend. The response of soil microbial communities to root exudation has been shown to depend upon both the amount and the composition of root exudates (Grayston et al. 1998, Griffiths et al. 1999), which can be highly specific for a given plant species (Whipps 2001). Soil communities that are initially similar may therefore differentiate in response to different plant species (Westover et al. 1997).

The dynamic inter-relationship between the composition of plant and soil communities as described by plant-soil feedback involves a two-step process: 1) a plant changes the composition of the root-associated soil community; and 2) this change in turn affects plant performance. Plant-soil feedback can be positive or negative (Fig. 1.5). Moreover, plant growth may be differentially affected by species-specific changes in soil community composition by heterospecific predecessors or neighbours. In plant communities the outcome of the balance between positive and negative feedback with the soil community for a particular species relative to that of other species will determine its competitive ability and resulting abundance in the future plant community (Bever et al. 1997, Van der Putten and Peters 1997). Hence, plant-soil feedback effects may play a major role in plant species coexistence, diversity and successional replacements. The role of the initial soil community composition in plant-soil feedback interactions, whether plant-soil feedback contributes to the rate and direction of plant community dynamics, and how plant-soil feedback interacts with other factors that structure plant communities remains largely unknown.

Soil biodiversity

The taxonomic diversity of soil communities on a square meter basis is several orders of magnitude higher than that of aboveground communities (Heywood 1995, Wardle 2006). High soil biodiversity is usually explained by the heterogeneous soil habitat, both spatially and temporally (Ettema and Wardle 2002), which may partly result from heterogeneous input of plant carbon substrates (Hooper et al. 2000). This heterogeneity provides unprecedented potential for niche partitioning and habitat specialization, thereby allowing species coexistence and favouring biodiversity (Bardgett 2002). After cessation of agricultural practices and at the start of secondary succession, soil biodiversity is relatively low (Bargett and Cook 1998). Particularly soil mites, which are the most abundant and diverse group of micro-arthropods and inhabit a range of feeding habitats, have been suggested to be very sensitive to agricultural disturbance (Siepel and Van de Bund 1988) and are negatively associated with low levels of organic matter as occur in agricultural soils (Petersen and Luxton 1982). After land abandonment, release from physical and chemical disturbance and a build-up in the amount, complexity and diversity of organic matter are expected to result in an increase of soil mite diversity with successional time (Maraun and Scheu 2000, Bardgett and Shine 1999). Therefore, the phenomenon of secondary succession provides great potential for studying soil diversity patterns and the underlying factors that control them. Similar to succession theories, traditionally, soil organisms have received little attention in soil biodiversity studies (Wall et al. 2005) and soil biodiversity only recently became fully recognised as an important factor in ecosystem functioning (Brussaard et al. 1997, Heemsbergen et al. 2004, Bardgett et al. 2005b, De Deyn and Van der Putten 2005, Usher and Coleman 2006).

Plant biodiversity studies have shown that patterns in secondary succession depend on the species that are initially present, their persistence or extinction, and on the colonisation and establishment of new species from local or regional species pools (Egler 1954). Similar patterns can be expected for soil organisms. However, spatial and temporal scales of dispersal and colonisa-

tion differ between plant and soil organisms, as well as between phylogenetic groups of soil organisms. This may result in different successional diversity patterns for plants and soil organisms. A better understanding of the mechanisms that drive soil diversity patterns can enhance our understanding of the relationship between soil and plant diversity. So far, this relationship appears to be rather idiosyncratic (De Deyn and Van der Putten 2005, and references therein).

Research objective and outline of the thesis

The objectives of this thesis are 1) to analyse diversity patterns and changes in the taxonomic and trophic composition of soil communities in relation to secondary plant community succession; 2) to study the role of plant-soil feedback in plant community interactions and successional replacements; and 3) to test the effects of soil community manipulations, both direct and indirect, on restoration of species-rich grasslands on dry sandy soils. My approach to obtain these objectives encompasses descriptive and empirical field studies and glasshouse microcosm experiments.

The successional development of soil nematode communities, described in terms of taxa and feeding groups, and the relationship between successional trajectories of soil nematode and plant communities was studied in a chronosequence of abandoned agricultural fields (**Chapter 2**). A chronosequence can be defined as 'a sequence of soils developed on similar parent materials that are under the influence of constant or ineffectively varying climate and biotic factors, whose differences can thus be ascribed to the lapse of differing increments of time since the initiation of soil formation' (Stevens and Walker 1970). This means that if several sites can be accurately aged with respect to time since land abandonment, then the plant communities in the different stages of development can be used to compose a successional series. In collaboration with Annemieke van der Wal (NIOO-KNAW), 26 former agricultural fields in the central and eastern part of the Netherlands were selected, ranging in time since abandonment from 1 to 34 years (Table 2.1). As a comparison, the chronosequence included present agricultural fields and three semi-natural reference sites: two *Calluna* heathlands and one mattgrass sward. Both for plant and nematode communities, their successional development in similarity to reference communities (both the semi-natural reference sites and a theoretical reference obtained from literature) was analysed. From the chronosequence, three former agricultural sites differing in time since abandonment ('early', 'mid' and 'late') and one reference site were subsequently selected (Table 3.1) and sampled in detail for soil mites and nematodes using a spatio-temporal design (**Chapter 3**). Mite and nematode communities were analysed taxonomically and according to their feeding group composition. We compared their taxon and feeding group composition and tested the role of historical factors (i.e. site), soil properties, and seasonal fluctuations using a multivariate variance partitioning technique. We also analysed successional soil diversity patterns within samples (α-diversity), between samples (β-diversity) and within field sites (γ-diversity). The results are discussed in the context of deterministic and historically contingent succession theories.

In glasshouse experiments, the role of plant-soil feedbacks in secondary plant community succession was studied. We established model ecosystems of plants and soils from different successional stages using soils from 'early', 'mid', and 'late' fields from the chronosequence (Table 4.1), to examine whether plant-soil feedback retards or enhances ecosystem development during secondary succession (**Chapter 4**). Experimental plant communities were grown twice on the same soil and plant performance in the second growth period was used to assess the soil feedback effect. Feedback effects were related to the successional origin of the soils. In a second microcosm experiment, we tested the effect of microbial-mediated plant-soil feedback on the competitive ability of six early-successional plant species and the legacy effects of these feedbacks for later-successional plant communities (**Chapter 5**). Feedback effects were determined both when plants were grown with conspecific and with heterospecific neighbours. In a third stage of the experiment, microbial inocula were used to test if soil micro-organisms were involved in the feedback effects. Contingency effects of plant-soil feedback in early-successional stages on plant community composition in mid-successional stages were analysed by comparing the performance of mid-successional species in relation to the history of plant-soil feedback.

Finally, to test the potential of soil community manipulations in restoration of species-rich grasslands, two field studies were performed in collaboration with the State Forestry Service (Staatsbosbeheer). A 3-year field experiment, which was set up in close collaboration with Annemieke van der Wal, was established on a recently abandoned ex-arable field in Assel, the Netherlands. We tested the effect of carbon substrates (to induce microbial N-immobilisation) and top soil removal, on plant community development and soil communities of bacteria, fungi and nematodes (**Chapter 6**). To test the importance of differences in substrate quality, both straw and wood amendments were used. The treatments were applied both with and without sowing a mixture of later-successional plant species, to test the relative importance of soil nutrient status and dispersal limitation as controlling factors in secondary succession following abandonment of agricultural land. We combined the field experiment with a short-term glasshouse study. A second field experiment was established on a topsoil-removed former agricultural site in Lievelde, the Netherlands. The experiment had been established in 2001 and was designed to test the effects of soil organism and plant propagule introductions on the rate and direction of plant community succession (**Chapter 7**). Two alternative approaches were used: 1) single or combined spreading of hay and soil; and 2) transplantation of intact turfs including vegetation and soil, which could function as 'stepping stones'. Material for the treatments was collected from a nearby *Cirsio-Molinietum* fen meadow (the donor site), which can be classified as 'target site'. Plant community composition was analysed in four subsequent years. The soil nematode community was used as an indicator for soil community development. We also compared plant and soil community development at the transplanted turfs with developments at the donor site from which they originated. The implications of the results of the different experiments for grassland restoration are discussed in **Chapter 8**.

Chapter 2

Successional trajectories of soil nematode and plant communities in a chronosequence of ex-arable lands

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Abstract

Conversion of arable land into semi-natural grassland or heathland is a common practice for restoring and conserving plant diversity. However, little is known about the effectiveness of land conversion for restoring and conserving taxonomic and functional diversity in the soil. We studied soil nematode community development in a chronosequence of abandoned fields and related this to plant community development. The taxonomic and functional composition of the soil nematode community was analyzed to detect changes in soil food web structure, using semi-natural sites and theoretical plant and soil communities as references.

While plant communities clearly developed towards the semi-natural references, there was less direction in succession of nematode taxa. The number of fungal feeding nematodes increased after land abandonment. Numbers of omni-carnivorous nematodes expanded only during the first years, after which there were no substantial changes for the next three decades. Plant communities on the ex-arable fields developed towards the theoretical reference plant associations *Galio hercynici-Festucetum ovinae* and *Genisto anglicae-Callunetum*. Nematode communities developed away from a theoretical community indicative of arable land, but there was no clear development towards a theoretical (semi-)natural reference. Our results show that restoration and conservation of plant communities is of limited indicative value for developments belowground: successful restoration of plant diversity does not necessarily imply successful restoration of belowground diversity. Assessing the impact of conservation measures on restoring soil biodiversity requires information on belowground community composition of semi-natural areas in order to establish proper references for restoration sites.

Keywords *Above-belowground linkages, biodiversity restoration and conservation, functional diversity, land use change, taxonomic diversity*

Introduction

A major tool for biodiversity conservation in industrialized countries is the restoration of former natural ecosystems on abandoned agricultural land. Large areas of former production land are becoming available for restoring species rich semi-natural grassland or heathland and their specific above- and belowground biodiversity (Brussaard et al. 1996, Walker et al. 2004). On sandy soils in The Netherlands, policy makers and nature managers aim to (re)create open, species-rich, low-fertile grasslands which are of high conservation value because of their endangered flora and fauna. However, in spite of these restoration and management efforts, much is still unknown about how ecosystem development proceeds, and especially whether aboveground developments are indicative for restoring and conserving communities and processes in the soil.

Considerable attention has been paid to the role of abiotic soil properties in vegetation succession on abandoned land (e.g. Hansson and Fogelfors 1998, Degn 2001, Critchley et al. 2002, Ejrnæs et al. 2003). Other studies strongly suggested a profound role of soil fauna as a driver of

secondary plant succession following land abandonment (Brown and Gange 1992, De Deyn et al. 2003). Belowground fauna and microorganisms affect plant community composition both directly and indirectly (Wardle et al. 2004a). Direct effects can be attributed to altered competitive ability of plants that accumulate parasites, pathogens and root herbivores or mutualistic symbionts (Johnson et al. 1991, Van der Putten et al. 1993, Klironomos 2002). Indirectly, decomposers influence plant performance by releasing nutrients from soil organic matter (Wardle 2002).

As plant species differ in both the quantity and quality of root exudates and litter, they can have profound effects on the composition and functioning of the soil community (Thornton and Matlack 2002, Wardle 2002). During secondary succession, readily available resources become depleted, so that plant communities become dominated by slow-growing species with poorly degradable litter (Bardgett and McAlister 1999). As a result, the soil microbial community will change from a bacterial dominated to a more fungal dominated community (Swift et al. 1979, Klein et al. 1995). It has been proposed that belowground responses to ecosystem restoration are slower than aboveground due to differential response rates between plants and soil organisms (Korthals et al. 2001, Hedlund et al. 2003).

The aim of the present study was to analyze if secondary succession and conservation of plant communities are indicative of soil community conservation. We used a 34-year old chronosequence of ex-arable fields (Van der Wal et al. 2006b). In order to determine potential shifts in soil community composition and functioning, we analyzed the taxonomical and functional composition of the soil nematode community. Soil nematodes include herbivores, bacterivores, fungivores and omni-carnivores that feed at different trophic levels in the soil food web. Changes in their community composition is indicative of changes in environmental conditions (Bongers and Ferris 1999, Wasilewska 1994 Thornton and Matlack 2002, Wall et al. 2002). Nematode populations can respond relatively fast to land abandonment (Háněl 2003), influenced by e.g. variation in C:N ratios of input materials (Ferris and Matute 2003). Therefore, we expect soil nematodes to be a suitable species group for indicating taxonomic and functional changes in the soil food web.

We tested the hypotheses that (i) Plant community composition and soil nematode community composition both follow successional trajectories towards non-cultivated semi-natural reference systems. (ii) During secondary succession the dominance in the nematode community will change from bacterial-feeders to fungal-feeders, and (iii) There will be a positive relationship between plant and nematode communities in their similarities to semi-natural reference sites. We will discuss if the success of plant community restoration and conservation may be indicative of the success of restoration and conservation of the soil community.

Table 2.1 Site descriptions.

Site	Field age	Lat.	Long.	Soil texture	Plant association[1]	No. of plant species	Ellenberg fertility score
Agri I		52.03	6.05	loamy very fine sand	'Wheat'		
Agri II		51.11	6.27	sand/coarse sand	'Wheat'		
Agri III		52.10	6.30	sand/coarse sand	'Maize'		
A	1	52.21	5.82	coarse sand	*Echinochloo-Setarietum*	17	6.2
B	1	52.00	5.75	coarse sand	*Matricaria recutita-Spergula arvensis*—[*Aperion spicae-venti*] [2]	21	5.1
C	1	52.00	5.75	coarse sand	Sclerantho annui-Arnoseridetum typicum	19	5.8
D	2	52.04	6.05	very fine sandy loam	*Lolio-Cynosuretum typicum*	17	6.0
E	2	52.03	6.04	very fine sandy loam	*Plantagini-Lolietum perennis*	22	5.7
F	5	52.08	5.98	coarse sand	*Elymus repens* - [*Artemisietea vulgaris*] [2]	23	5.0
G	5	52.08	5.96	coarse sand	Echio-Melilotetum	15	4.0
H	5	52.13	6.28	coarse sand	*Urtica dioica* - [*Galio-Urticetea*] [2]	15	7.1
I	7	52.02	5.99	coarse sand	*Hieracio-Holcetum mollis*	13	3.8
J	7	52.00	6.00	loamy fine sand	*Hieracio-Holcetum mollis*	13	3.9
K	8	52.03	6.04	loamy coarse sand	*Hieracio-Holcetum mollis*	28	3.3
L	8	52.01	5.99	coarse sand	*Dauco-Melilotion*	21	4.3
M	8	52.01	6.00	very fine sandy loam	*Lolio-Cynosuretum typicum*	23	6.5
N	8	52.06	5.75	coarse sand	*Echio-Verbascetum typicum*	24	5.0
O	8	52.11	6.27	sand/coarse sand	*Urtica dioica* - [*Galio-Urticetea*] [2]	8	8.1
P	9	51.98	5.52	coarse sand	*Echio-Verbascetum typicum*	18	4.2
Q	12	52.02	6.01	loamy coarse sand	*Echio-Melilotetum*	19	6.2
R	13	52.02	5.99	coarse sand	Ornithopodo-Corynephoretum	21	3.5
S	13	52.06	5.95	coarse sand	*Agrostis capillaris-Hypochaeris radicata* - [*Trifolio-Festucetalia ovinae*] [2]	15	4.1
T	13	52.01	5.99	loamy coarse sand	*Nanocyperion flavescentis* / *Valeriano-Filipenduletum*	24	5.0
U	13	52.04	5.77	coarse sand	*Tanaceto-Artemisietum agrostietosum*	16	4.5
V	15	51.99	5.79	coarse sand	*Tanaceto-Artemisietum agrostietosum*	18	4.4
W	16	52.02	6.03	very fine sandy loam	*Hieracio-Holcetum mollis*	30	4.4
X	18	52.07	5.74	coarse sand	*Echio-Verbascetum typicum*	27	5.7
Y	21	52.03	5.80	sand/coarse sand	*Plantagini-Festucion*	28	4.1
Z	34	52.06	6.00	coarse sand	*Galio hercynici-Festucetum ovinae*	12	3.9
Semi-natural site I		52.27	5.73	Sand	*Galio hercynici-Festucetum ovinae*	26	1.6
Semi-natural site II		52.06	5.94	coarse sand	*Genisto anglicae-Callunetum*	7	1.0
Semi-natural site III		52.06	5.75	coarse sand	*Genisto anglicae-Callunetum typicum*	6	1.1

Field age = years since abandonment. Lat. = latitude (°N), Long. = longitude (°E). [1] According to Schaminée et al. (1996, 1998). [2] 'Frame community' (characteristic species of lower syntaxonomic levels are missing while the community can clearly be designed to a higher syntaxonomic level).

Study sites and methods

Sites attributes

We studied plant and soil nematode community development on a chronosequence of 26 ex-arable fields within a geographical region in The Netherlands on sandy or sandy loam glacial deposits. Besides soil type and time after abandonment, additional site selection criteria were similarity in management practices and restoration targets. Three agricultural fields (two cropped with barley (*Hordeum vulgare*), one with maize (*Zea mays*)) were also included, as well as three semi-natural sites (two *Calluna*-heathlands, one matt-grass sward). All 26 ex-arable sites had been previously cultivated with arable crops (maize, cereals) and were more than 1 ha in size. Most sites were located within National Park Veluwezoom in central Netherlands. Four sites were situated about 40 km eastwards, near Vorden and two sites 35 km northwards, but all these sites were within the same region and on the same soil material (Table 2.1). Altitude ranged from 10 to 80 m above sea level. Plant communities were the result of natural colonization and all sites were managed by low-intensive grazing of natural and introduced vertebrate herbivores (roe deer, fallow deer, red deer, horses, Scottish Highland cattle).

Sample collection

Soil

Within each field we established a 50 x 50 m² plot in an area with homogeneous vegetation cover minimally at 20 m from the edge of the field. In April 2003, in each plot 80 soil cores (Ø 3.4 cm, 10 cm depth) were collected according to a stratified, W-shaped pattern across the plot, bulked, and stored at 4 °C until analysis. After homogenizing and sieving (Ø 4 mm), one sub-sample of 100 g was collected to extract nematodes. Soil water content was determined by drying a separate sub-sample at 70 °C until constant weight.

Plant community

In July 2003, in every field at five stratified random positions along the transect in a 1 m² quadrat the estimated percentage cover of each vascular plant species was recorded. The data from the five quadrats were aggregated in order to obtain one vegetation record per field, representative for the 50 x 50 m² plot from which the soil samples were taken. Using the vegetation data, for each field the plant association was determined using the program SynBioSys (Hennekens et al. 2001). Ellenberg fertility scores (Ellenberg et al. 1991) were calculated by the program Turboveg (Hennekens and Schaminée 2001).

Soil nematode community

Nematodes were extracted by Oostenbrink elutriators (Oostenbrink 1960). Nematodes present in 10% of the extracted soil were heat-killed and fixed (35% formaldehyde diluted to 4%), after which a minimum of 150 nematodes were identified to family or genus level according to Bongers (1988). The group *Dorylaimoidea* (*sensu* Jairajpuri and Ahmad 1992) was used to specify a het-

erogeneous group of dorylaimids comprising *Dorylaimidae*, *Qudsianematidae*, *Thornenematidae* and *Aporcelaimidae*.

Data analysis

Nematode feeding groups and faunal profiles

Nematodes were allocated to feeding groups according to Yeates et al. (1993a). We distinguished plant feeders, bacterial feeders, fungal feeders, and omni-carnivores, and expressed their numbers per 100 g dry soil. For each site, proportion of fungal feeders was calculated as the number of fungal feeders divided by the sum of fungal and bacterial feeders. The Channel Index (CI), a weighted ratio of opportunistic bacterial and fungal feeding nematodes (characterized by short generation time, small eggs and high fecundity) was calculated according to Ferris and Matute (2003). A low CI indicates that bacterial decomposition predominates; a high CI indicates fungal decomposition.

Response to time since abandonment

Responses of the plant community and soil nematode community to time since abandonment were analyzed by detrended correspondence analysis (DCA) using the program CANOCO, version 4.5 (Ter Braak and Smilauer 1998-2002). DCA is an ordination technique used to find a configuration of samples in the ordination space, so that the distances between samples in this space do correspond best to the dissimilarities in their species composition. The analyses were carried out with log-transformed species cover or abundance. Since rare species may have large effects on the analysis, their influence was reduced by 'down weighting' them. Correspondence of the first two DCA axes, Ellenberg fertility scores, and abundance of nematode feeding groups to field age was calculated by the Pearson correlation coefficient using STATISTICA (release 6.1, Statsoft, Inc.). We used non-parametric Kruskal-Wallis tests (significance level $P = 0.05$) to test differences between chronosequence sites on the one hand and arable sites and/or semi-natural sites on the other.

Similarity to semi-natural reference sites

Similarities of both plant and nematode communities to the semi-natural sites were calculated using Sørensen's quantitative index CN (Magurran 1988), hereafter referred to as Sørensen's similarity (CN). CN incorporates both species occurrence and abundance, and was calculated as $CN = 2\,jN/(aN + bN)$ where aN is the total species cover at site A, bN is the total species cover at site B and jN is the sum of the lower of the two cover estimates for species which occur in both sites. The relationship between similarity and time since abandonment was determined using linear regression.

Similarity to theoretical references

Vegetation classifications of Schaminée et al. (1996, 1998) were used to derive reference communities appropriate to geographical district, site management and 'targets' set by biodiversity conservation policy. We used matgrass sward, *Galio hercynici-Festucetum ovinae* [*Nardo-Galion*], and dry heathland, *Genisto anglicae-Callunetum* [*Calluno-Geniston*], as theoretical reference

vegetation. As nematode references we used clusters from Van der Waarden et al. (2002). We selected nematode communities of 'heathlands, dunes and drifting sands' as reference community, which was the best representative reference cluster present in the database. As this classification includes a wider, and somewhat different variety of systems than the actual theoretical reference for our chronosequence, we also included the cluster 'agricultural field on sand or peat' to represent a theoretical reference for the start of the chronosequence. Analogous to similarity measures to the semi-natural sites, for all chronosequence sites Sørensen's similarity (*CN*) to the theoretical references was calculated for both plant and nematode communities. The relationship between similarity and time since abandonment was determined using linear regression.

Relationship between nematode and plant communities

To relate soil nematode community development to plant community development, first the relationship between the relative rates of both successional processes was analyzed. We used linear regression to determine whether there was a relationship between relative (scaled from 0 to 1) Sørensen's similarity (*CN*) to the semi-natural sites for both plant and nematode communities. We also calculated plant community and nematode similarities of each individual ex-arable site to all other ex-arable sites, resulting in two similarity matrices (*sensu* Lepš et al. 2001) that were used to correlate similarity in plant community composition to similarity in nematode community. Since, we had $n(n\text{-}1)/2$ pairs in the analysis, but only n independent observations, a non-parametric Mantel test was used to test this relationship (Sokal and Rohlf 1981).

Results

Response to time since abandonment

Plant species and community development

A total of 121 plant species were found at the 26 ex-arable sites, comprising 10 woody species, 22 grasses, 8 legumes and 81 other forbs. Arable weeds and pioneer species, such as *Apera spica-venti*, *Conyza canadensis* and *Viola arvensis* dominated the youngest fields. At the oldest fields later-successional species, such as *Agrostis capillaris*, *Deschampsia flexuosa* and *Rumex acetosella* were most abundant. The semi-natural sites were dominated by *Calluna vulgaris* and *Molinia caerulea*. The sites represented 17 different plant associations, ranging from agricultural communities (*Echinochloo-Setareitum*) to low-fertile grassland communities (*Galio hercynici-Festucetum ovinae*) (Table 2.1). Ellenberg fertility scores ranged from 3.3 to 8.1, but the relationship with field age was not significant ($R^2 = 0.13$, $P = 0.07$). Ellenberg fertility scores were lowest for the semi-natural sites (1.0 - 1.6). Plant species richness of the ex-arable sites varied from 8 to 30 species per 5 m^2. DCA analysis (Fig. 2.1a) showed clear separation of the ex-arable sites from the semi-natural sites. First axis sample scores for the ex-arable sites showed a strong positive relationship with field age ($R^2 = 0.48$, $P < 0.001$).

Nematode densities, taxa, feeding groups and community development

Total nematode density ranged from about 1000 to more than 6000 individuals per 100 gram dry soil, but was not affected by time since abandonment (R^2 = 0.004, P = 0.73). Total densities of nematodes in the arable or semi-natural fields did not differ from those in abandoned fields (Kruskal-Wallis test, P = 0.58 and P = 0.50, respectively). Nematodes were classified into 34 different taxa, mostly to family or genus level (Appendix 2A). The youngest fields were mainly dominated by plant-feeding *Dolichodoridae* and bacterial-feeding *Rhabditidae*, whereas in older fields fungal-feeding *Aphelenchoides* and omnivorous *Dorylaimoidea* species became more abundant. Only for density of the fungivorous genus *Aphelenchoides* there was a positive relationship with field age (R^2 = 0.19, P = 0.03). DCA analysis (Fig. 2.1b) showed that abandoned fields differed from the two semi-natural heathland sites but there was no clear pattern with time since abandonment. Scores from the first axis were not related to field age (R^2 = 0.01, P = 0.59), but there was a positive relationship for scores from the second axis (R^2 = 0.34, P = 0.002).

Densities of plant feeding nematodes in the ex-arable fields ranged from 200 to more than 2000 individuals per 100 g dry soil, but did not change significantly along the chronosequence (R^2 = 0.005, P = 0.75, Fig. 2.2a). Splitting up plant-feeding nematodes into sedentary parasites, migratory endoparasites, semi-endoparasites, ectoparasites and epidermis and root hair feeders, did not change these results (data not shown). Densities of bacterial feeders, on average the most abundant group, varied from 400 to 2800 individuals per 100 g dry soil, but did not show any relationship with time since abandonment (R^2 = 0.08, P = 0.17, Fig. 2.2b). There was a positive relationship (R^2 =0.21, P = 0.02) between the total density of fungal feeding nematodes and

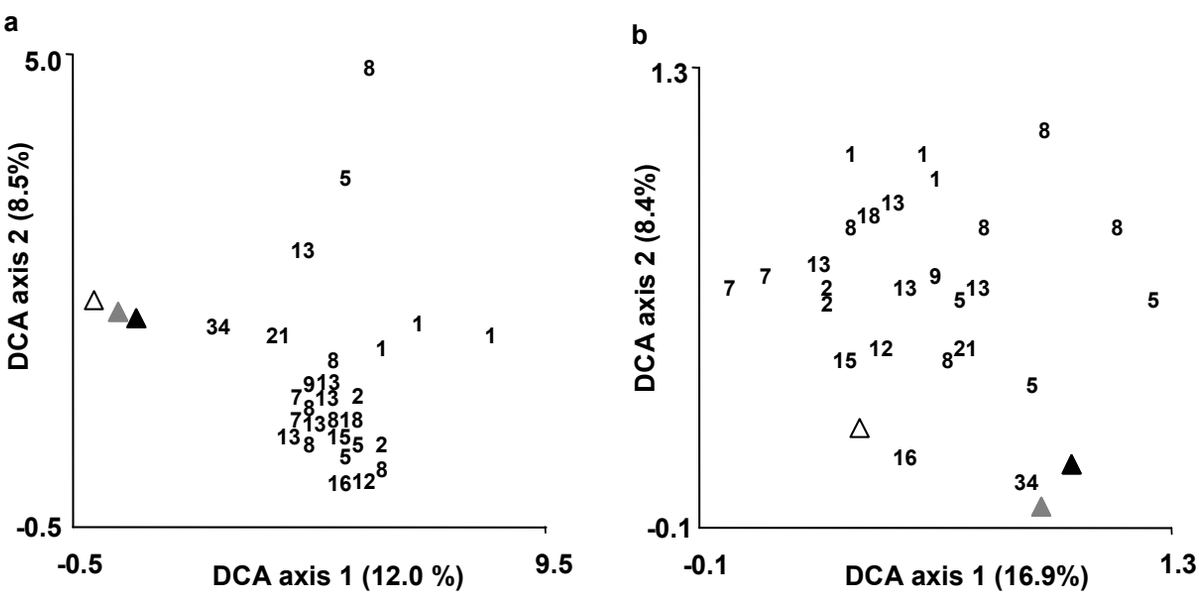

Figure 2.1 Ordination of the ex-arable sites and semi-natural sites by detrended correspondence analysis (DCA) for plant community composition (a) and soil nematode community (b). Each number represents one field site and indicates the years since abandonment. Percentages along the axes correspond to the amount of explained variability in species/taxa composition. △ = semi-natural site I, ▲ = semi-natural sites II, ▲ = semi-natural sites III.

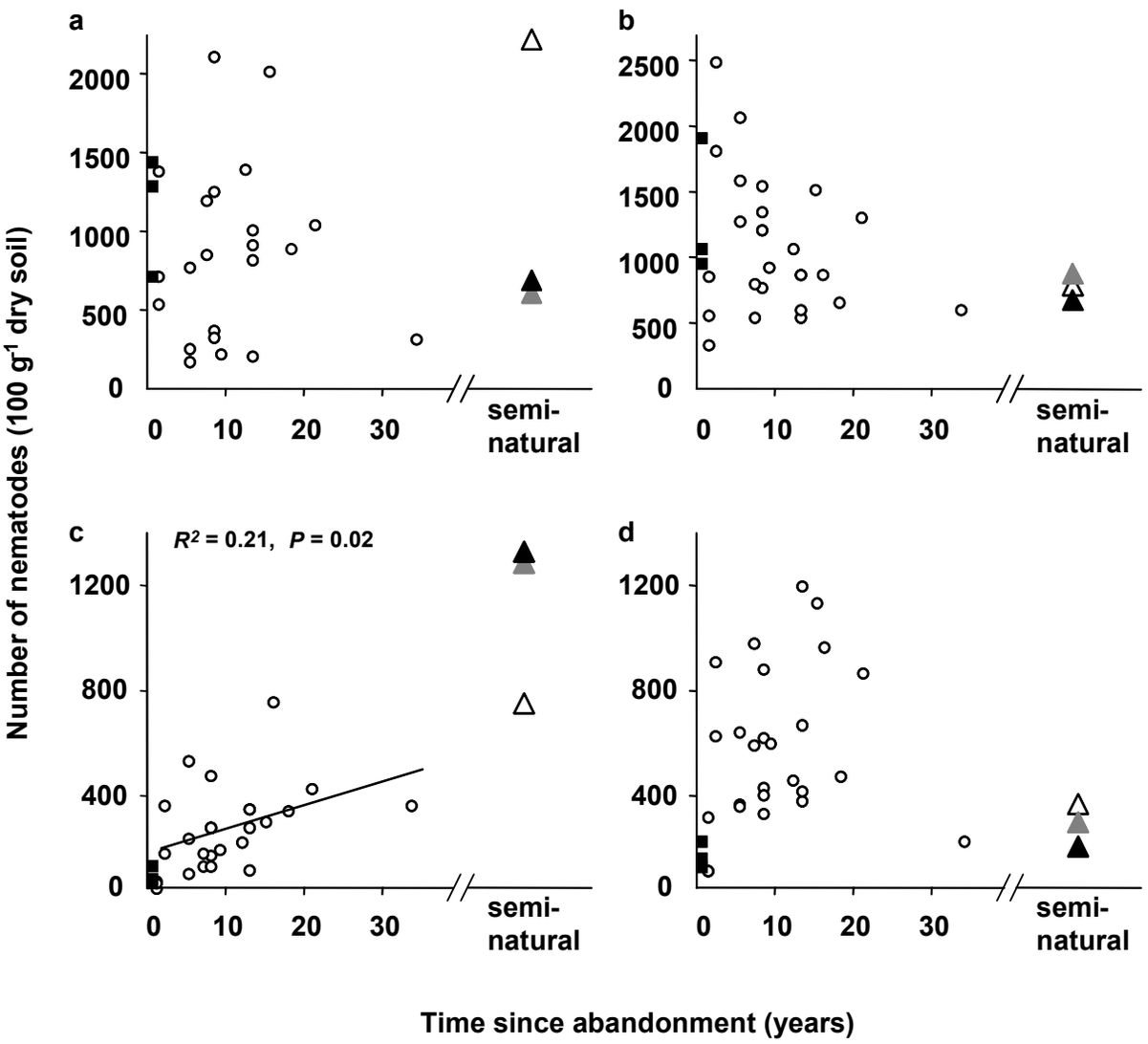

Figure 2.2 Temporal changes in total densities of nematode feeding groups (100 g^{-1} dry soil). R^2 and P values are presented for significant linear regressions. Arable sites and semi-natural sites were excluded from regression analysis. (a) Plant feeders, (b) bacterial feeders, (c) fungal feeders, (d) omni-carnivores. For reasons of visualization some data were slightly offset to avoid overlapping data points. n = arable field, ■ = ex-arable field, △ = semi-natural site I, ▲ = semi-natural sites II, ▲ = semi-natural sites III.

field age, although there was substantial variation between fields of similar age (Fig. 2.2c). In the heathlands (semi-natural sites II and III) densities of fungal feeders were approximately twice as high as numbers in the ex-arable sites. The relationship between fungal feeders and time after abandonment could be considerably enhanced by using the ratio between fungal feeders and total microbivorous (fungal feeding plus bacterial feeding) nematodes (R^2 = 0.50, P < 0.001). The Channel Index (CI) showed a positive relationship with field age (R^2 = 0.22, P = 0.02), indicating a change from bacterial to fungal decomposition. Highest densities of omni-carnivorous nematodes were found in sites of intermediate age, but there was no relationship with field age (Fig. 2.2d). Density of omni-carnivores in arable sites was lower than in the chronosequence

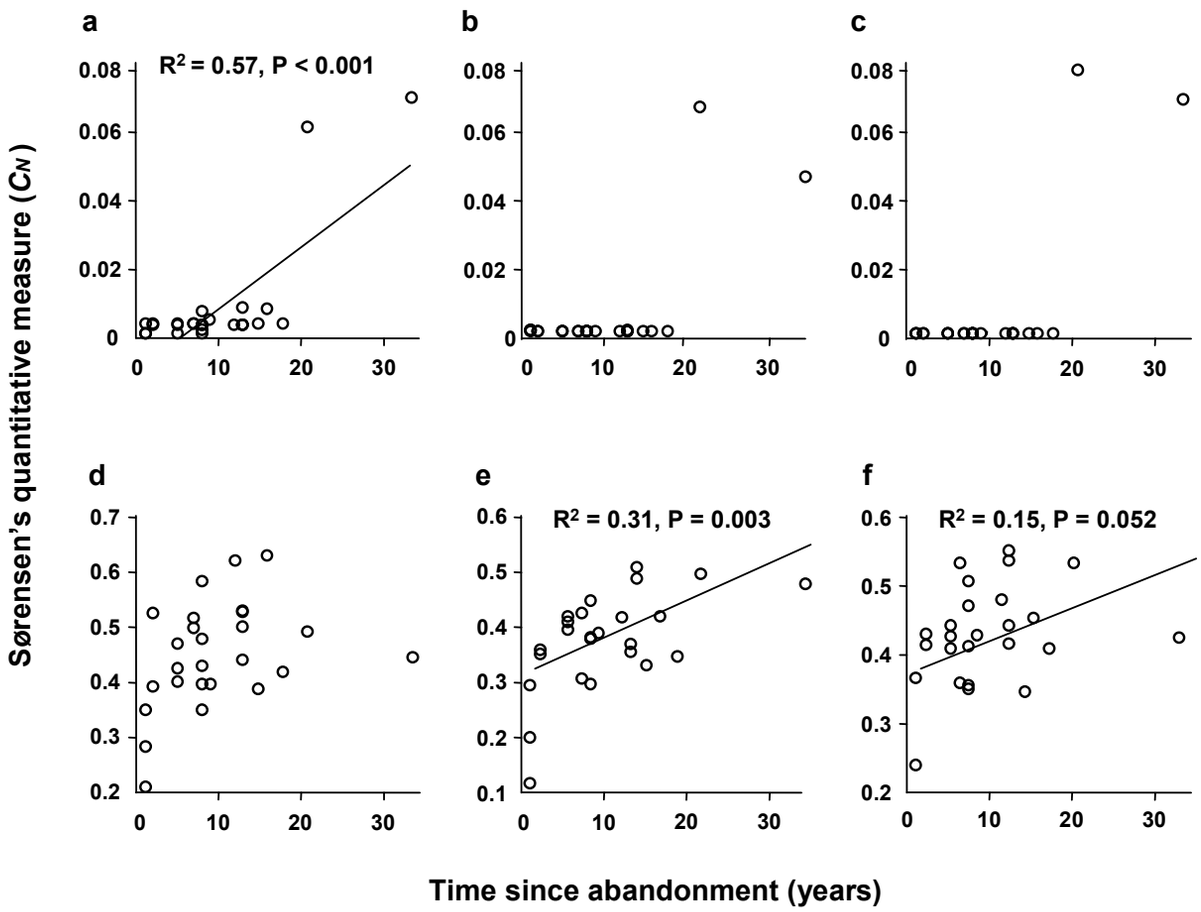

Figure 2.3 Change in Sørensen's similarity (*CN*) of plant (a-c) and nematode community composition (d-f) to semi-natural site I (a,d), to semi-natural site II (b,e) and to semi-natural site III (c,f). Linear regression terms were only included when significant. R^2 and *P* values are presented for significant linear regressions.

sites (Kruskal-Wallis test, $P = 0.02$). The density of omni-carnivorous nematodes in the oldest field and in the semi-natural sites was relatively low.

Similarity to references

Semi-natural sites

Overall, when compared to semi-natural reference sites, Sørensen's similarity (*CN*) in plant community composition of abandoned fields was low. However, there was a positive relationship between plant community similarity to semi-natural site I (matgrass sward) and field age ($R^2 = 0.57$, $P < 0.001$, Fig. 2.3a). Significance was, to a large extent, determined by the two oldest fields (Fig. 2.3a), but the relationship remained significant when these fields were excluded from the analysis ($R^2 = 0.21$, $P = 0.03$). Except for the two oldest fields of the sequence, similarity in plant community composition to semi-natural sites II and III (heathlands) was close to zero (Fig. 2.3b,c). On the other hand, for nematodes, similarity to semi-natural site II increased significantly with time since abandonment ($R^2 = 0.31$, $P = 0.003$, Fig. 2.3e). Similarity of nematode

community composition to semi-natural site I did not increase significantly with field age (R^2 = 0.12, P = 0.08, Fig. 2.3d). Increase with field age in similarity of nematode community composition to semi-natural site III was marginally significant (R^2 = 0.15, P = 0.052, Fig. 2.3f). Therefore, nematode community composition developed towards a different reference site than plant community composition.

Theoretical references

Similarity of plant community composition to the theoretical reference types *Galio hercynici-Festucetum ovinae* and *Genisto anglicae-callunetum* increased significantly with field age (R^2 = 0.69, P < 0.001 and R^2 = 0.67, P < 0.001, Fig. 2.4a,b). There was no relationship between similarity of the nematode community composition to the reference cluster 'heathland, dunes and drifting

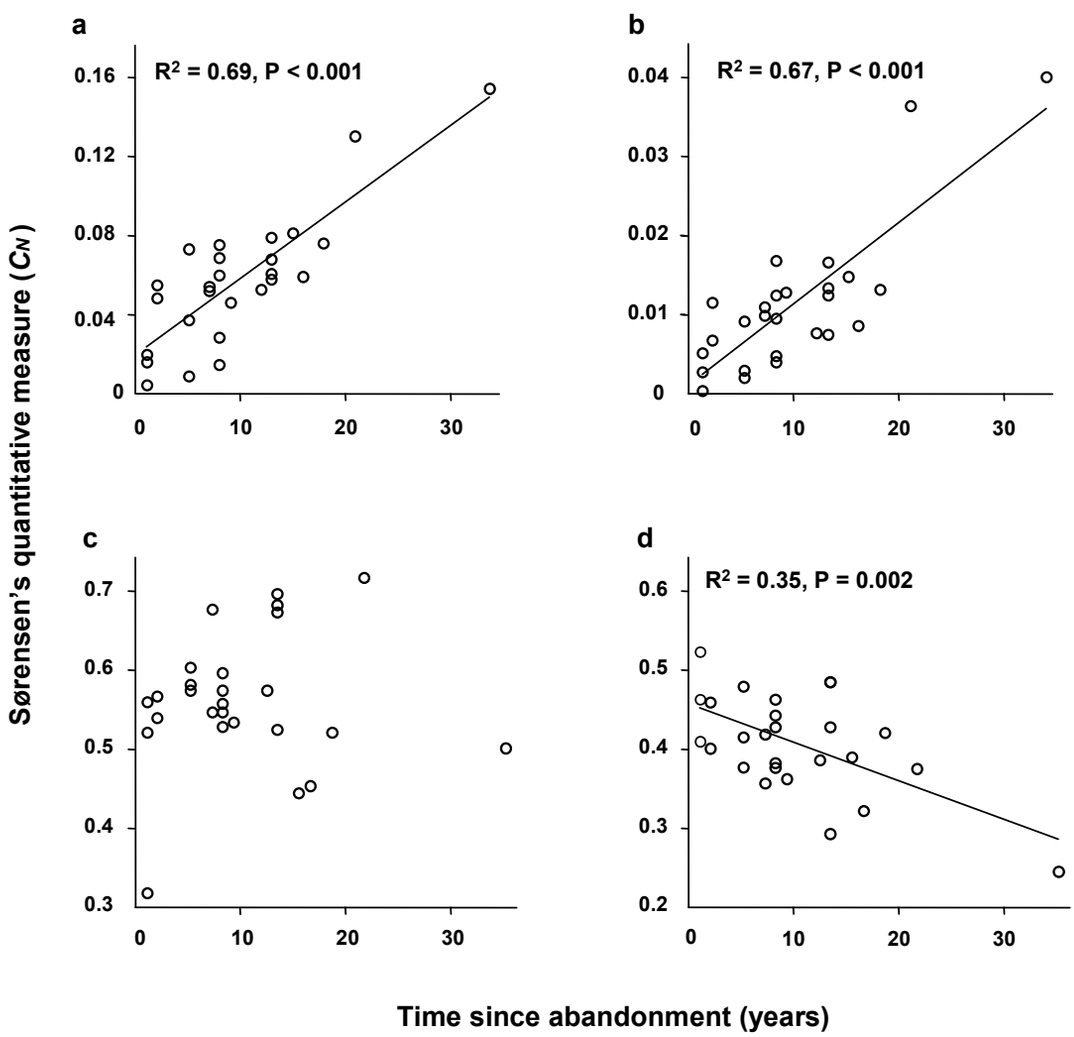

Figure 2.4 Change in Sørensen's similarity (*CN*) of plant community composition (a) to theoretical reference *Galio hercynici-Festucetum ovinae* and (b) to theoretical reference *Genisto-anglicae-Callunetum,* and change in Sørensen's similarity (*CN*) of soil nematode community (c) to reference cluster 'dunes, heathlands, drifting sands' and (d) to reference cluster 'agricultural field sand / peat. Linear regression terms were only included when significant.

sands' and time since abandonment (R^2 = 0.01, P = 0.58, Fig 2.4c). However, similarity to the reference cluster 'agricultural field on sand / peat' (theoretical starting point of the sequence) decreased significantly with time since abandonment suggesting that the composition of the nematode community drifted away from that of an agricultural community (R^2 = 0.35, P = 0.002, Fig. 2.4d).

Relationship between nematode and plant community

The analysis of the relationship between plant and nematode communities based on their similarities relative to the semi-natural sites (Sørensens' similarities scaled from 0-1; see Methods) was constrained because plant similarities showed a significant regression against semi-natural site I, while nematode similarities showed a significant regression against semi-natural site II only (Figs. 2.3a and e, respectively). For all but the two oldest fields, relative nematode similarity to the three semi-natural sites was always higher than relative plant community similarity.

The alternative approach of correlating the similarities of nematodes (and plants) in one field with nematodes (and plants) in all other fields (i.e. within-sequence similarities) resulted in a sig-

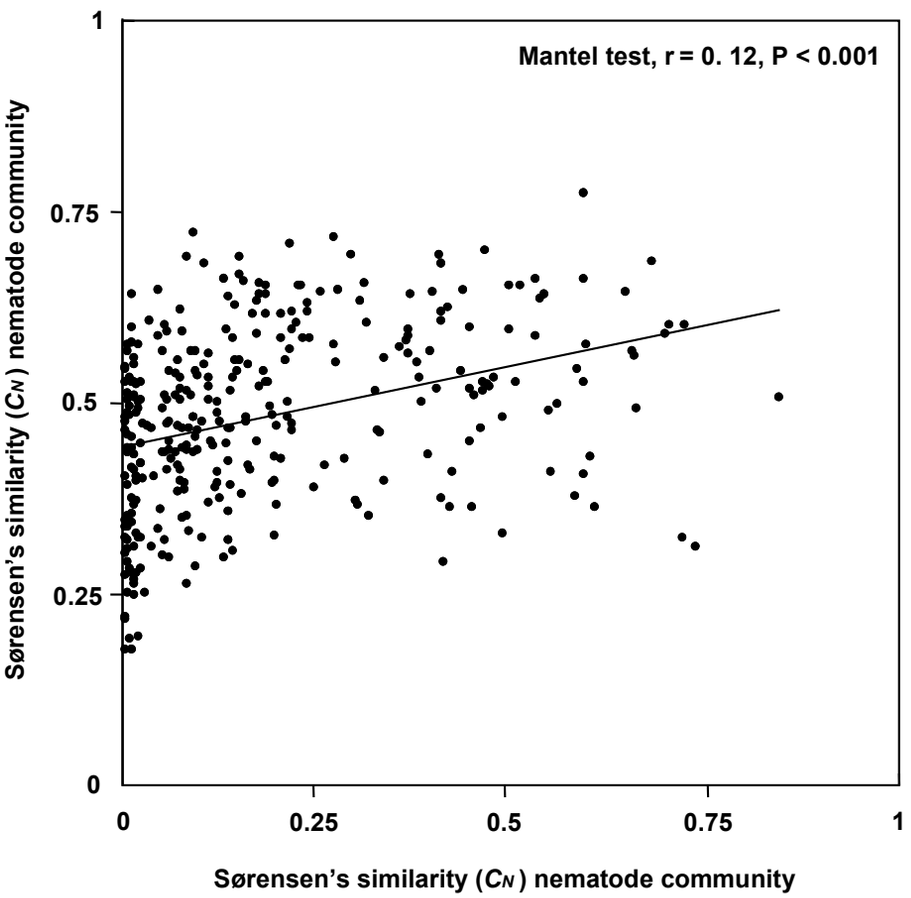

Figure 2.5 Relationship between similarity in nematode community and similarity in plant community for ex-arable sites. Sørensen's similarity (*CN*) of each ex-arable site to all other ex-arable sites was calculated.

nificant positive relationship between plant and nematode similarities ($r = 0.12$, $P < 0.001$, Mantel test, 1000 iterations, Fig. 2.5).

Discussion

Response to time since abandonment

Plants are commonly used as indicators for restoration success (Bakker and Berendse 1999, Critchley et al. 2004). However, since changes in (functional) soil animal composition and food web structure may have severe implications for ecosystem functioning and resistance to stress and invasions (Laakso et al. 2000, Steiner and Leibold 2004), examining the success of ecosystem restoration may be more comprehensive when the development of soil communities is integrated. In this study we quantified the temporal developments in plant and soil nematode communities after land abandonment and analyzed whether plant community development is indicative for soil community restoration. While DCA analysis indicated at a clear secondary succession of plant community development in the chronosequence, sites of the same age were quite variable. These site-specific differences may be due to differences in botanical legacy (Jensen et al. 2001), propagule availability (Bakker and Berendse 1999, Pywell et al. 2002), assembly rules (White and Jentsch 2004), stochasticity (Bradshaw 1983), or other factors some of which may be related to the site history.

Compared to the plant communities, the succession trajectory for soil nematode community composition was less clear. The colonization abilities for soil nematodes are generally limited (Ettema and Bongers 1993, Yeates and Van der Meulen 1996), so that nematode community development may be even more sensitive to initial composition (Egler 1954) than plant community development. Indeed, nematode succession patterns were more characterized by shifts in dominance patterns than by colonization of new taxa (Appendix 2A). Poor dispersal capacity may, therefore, limit the response of nematode assemblages to new plant communities during secondary succession.

Successional changes in nematode community composition also could be obscured by site-specific variability at the time of abandonment as a consequence of historical differences in crop rotation, fertilization and other management practices. However, we observed clear successional pathways when considering changes in functional diversity of nematodes along the chronosequence. Knowledge of nematode feeding habits is still evolving (Yeates et al. 1993b, Okada et al. 2005), but the information provided by Yeates et al. (1993a) is quite robust and powerful, so that slight alterations in allocating feeding patterns to nematode taxa will have minor effects on our conclusions.

Agricultural practices favor bacterial-feeding nematodes relative to fungal feeders (Hendrix et al. 1986, Villenave et al. 2001) and nematode densities and the Channel Index suggested a shift from bacterial to fungal based decomposition pathways after land abandonment. As the total number of bacterial feeding nematodes itself did not change the basal part of the detritus food

web seems to expand after stopping agricultural practices. The pattern in nematode densities mirrored the pattern in microbial biomass, where bacterial biomass remained fairly constant and fungal biomass rapidly increased in the first years after land abandonment (Van der Wal et al. 2006b). Our results confirm that the trophic structure of the nematode community provides an integrated measure of the status of the other groups on which they feed (Ritz and Trudgill 1999).

Omnivory has been suggested as one of the key aspects of food web complexity (Polis and Strong 1996). In our study the abundance of omni-carnivorous nematodes did not change along the chronosequence, although their abundance initially increased after land abandonment. This suggests that the first years after abandonment the soil food web becomes more complex and that subsequently no substantial changes in soil food web structure occur over a time period of at least three decades.

Similarities to references

Plant community similarity showed a positive relationship with the mattgrass sward, but not with the two heathlands. If succession to mattgrass sward is a stage in succession to heathland (Schaminée et al. 1996), a chronosequence of 3-4 decades does not allow testing successional development towards heathland vegetation in the Netherlands. As the similarity of the plant community to the theoretical 'target' plant communities *Galio-hercynici-Festucetum* and *Genisto-anglicae-Callunetum* increased with time, the sites developed towards the references aimed at by policy and nature management. Therefore, considering vegetation development, land abandonment is a useful method of restoring habitats for these types of semi-natural plant communities, as also found for other systems (Ejrnæs et al. 2003, Hansson and Fogelfors 1998). The observed low degree of similarity (<16%) is because the theoretical references contain all species expected, but in reality these species do not all co-occur at a special scale sampled for this study.

As for plant similarities, the nematode community similarity with semi-natural sites showed a positive relationship with field age for only one of the three reference sites, however, nematode and plant similarities correlated with different reference sites. Soil community development is, therefore, not necessarily mirrored by plant community development. We used the nematode cluster system described by Van der Waarden et al. (2002), but the clusters to be compared with are less well developed for nematodes than for plants. Our best theoretical reference for nematodes included all dry sandy habitats (dunes, heathlands and drifting sands) probably limiting to observe a positive relationship between similarity to the theoretical reference and time since abandonment. Strong decrease in similarity to the agricultural reference cluster indicated a temporal change in nematode community structure after abandonment, which was less well detectable in DCA of the sites from the chronosequence. Therefore, in spite of an evident change in the nematode communities along the chronosequence, the direction of succession was far less clear than for plant communities. Insufficient knowledge on nematode community composition in semi-natural reference sites, different successional pathways belowground, or a strong and

long-lasting legacy effect of agricultural practices on the soil community may all have contributed to our results.

Relationship between nematode and plant community development

Our results support the hypothesis that similarity in plant community composition and soil nematode community composition correspond (Fig. 2.5). However, there were substantial site-specific differences in the relationship between nematode and plant community development. Opposite to our expectations, nematode and plant community development did not proceed in tandem. While the similarity of the plant community to the semi-natural sites was highest in the two oldest fields of the chronosequence, similarity for the nematodes was highest in sites with intermediate lengths of abandonment. For all but the oldest fields relative nematode community similarity to the semi-natural sites was higher than relative plant community similarity. This does not support a time lag in belowground community development (Korthals et al. 2001). However, time lags may especially appear on a time scale of several years (Korthals et al. 2001), or they may appear only when enhancing the resolution of the nematode identification.

Implications for restoration and conservation of grassland communities

The success of ecological restoration is usually valued by easily determinable community components (notably high profile conservation plant species). We show that plant and soil nematode communities do not necessarily develop in parallel towards the same reference system. Moreover, in the process of restoration different system components may need different length of time to recover (Parker and Picket 1997). Therefore, whether or not conservation fails may depend on which indicators to be used.

Theoretical plant communities, based on large numbers of field data resulted in a better relationship between plant community development and time after land abandonment than real reference sites. Theoretical nematode communities are poorly available, which may be even worse for other soil taxa. This needs improvement, in order to enhance our understanding of belowground successional patterns and to evaluate the conservation success of soil biodiversity and soil ecosystem functioning. The traditional botanical focal point of restoration ecology (Young 2000) should be complemented by incorporating the belowground biota in order to improve conservation biology.

Acknowledgements

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Supplementary material

Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.biocon.2005.06.005.

Appendix 2A Density of nematode taxa and feeding habits to which the taxa were allocated.

Appendix 2A Density of nematode taxa (number of individuals per 100 g dry soil) and feeding habits to which the taxa were allocated (Yeates et al. 1993a). 1 = Plant feeders, 2 = bacterial feeders, 3 = fungal feeders, 4 = omni-carnivores. Natural = Semi-natural reference sites.

	Feeding habit	Site																																		
		Agri I	Agri II	Agri III	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	Natural I	Natural II	Natural III
					Field age																															
					1	1	1	2	2	5	5	5	7	7	8	8	8	8	8	9	12	13	13	13	13	15	15	18	21	34			
Heterodera	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	77	136	29	111	0	106	0	0	0	0	202	0	0	0	0	0	0	0
Meloidogyne	1	41	0	0	14	13	37	372	218	0	0	0	0	42	123	15	750	0	0	83	42	0	22	39	133	640	282	164	321	0	86	123	127
Rotylenchus	1	0	0	0	0	0	0	0	0	42	0	0	57	0	0	0	68	0	0	0	63	42	0	20	17	67	81	49	0	0	542	0	0
Pratylenchus	1	500	239	1080	857	144	134	248	182	14	103	83	71	84	25	139	307	100	83	110	21	85	32	157	66	0	161	246	0	55	57	0	0
Ditylenchus	1	0	0	50	0	0	12	0	73	0	0	0	28	42	0	0	0	14	0	0	21	0	0	20	17	0	0	0	0	14	57	82	0
Helicotylenchus	1	0	85	0	0	0	0	165	691	28	17	166	468	251	540	15	205	14	55	14	507	445	54	20	17	135	1291	0	99	177	998	0	0
Dolichodoridae	1	14	887	0	400	316	256	786	109	70	68	193	43	0	74	15	341	29	111	0	296	148	0	39	99	270	0	98	49	0	57	0	0
Trichodorus	1	0	0	0	0	0	0	0	0	0	0	248	0	0	0	0	0	0	415	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paratylenchus	1	0	0	0	0	0	0	289	582	42	0	55	43	125	590	0	170	186	166	0	0	42	22	157	116	472	242	278	25	0	0	0	0
Criconematidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42
Tylenchidae	1	95	102	327	129	276	134	455	945	98	34	56	170	668	762	154	613	0	332	55	359	276	119	489	382	235	1453	84	519	109	457	472	530
Rhabditidae	2	378	478	955	257	289	73	0	364	141	51	497	43	104	0	93	239	129	277	28	169	42	87	98	100	34	121	65	148	0	86	0	21
Acrobeloides	2	297	85	75	0	171	122	1199	509	268	257	414	71	418	270	170	443	243	138	206	296	64	119	215	216	236	81	33	222	95	228	267	403
Acrobeles	2	27	0	201	143	53	37	0	0	268	445	221	14	63	0	62	34	100	28	96	0	0	54	59	66	0	0	180	247	0	0	0	0
Chiloplacus	2	0	34	0	0	0	0	0	0	0	0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cervidellus	2	108	0	0	0	0	12	248	36	0	68	83	57	84	98	62	68	143	28	14	42	127	43	137	66	0	40	16	99	136	0	41	42
other Cephalobidae	2	257	239	729	157	289	146	661	836	211	325	248	113	84	246	416	682	257	221	206	486	170	151	235	216	1011	363	229	222	0	171	0	64
Wilsonema	2	0	0	0	0	0	0	0	36	14	68	83	0	42	74	123	0	172	83	28	0	21	43	0	0	0	0	33	25	27	57	21	21
other Plectidae	2	41	239	50	71	158	37	661	255	367	342	442	312	146	123	401	68	300	360	440	148	254	151	215	349	270	161	229	371	109	143	267	233
Alaimidae	2	0	34	126	14	39	0	0	0	14	17	28	0	0	49	0	0	29	304	55	0	21	11	59	0	34	121	0	74	55	0	82	21
Teratocephalus	2	0	0	0	0	0	0	0	0	155	68	28	0	0	49	46	0	0	111	0	63	0	65	0	0	0	81	0	74	191	86	164	64
Bastiana	2	0	0	0	0	0	0	0	0	0	0	138	0	0	0	46	0	0	55	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metateratocephalus	2	0	0	0	29	0	0	0	0	14	154	248	28	0	0	0	0	0	194	0	21	0	0	0	0	135	40	0	0	109	200	144	21
Panagrolaimidae	2	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aphelenchoides	3	81	34	25	14	39	37	331	145	56	120	386	99	146	197	123	409	71	55	110	190	297	87	274	199	270	646	262	198	327	742	1210	1273
Aphelenchus	3	0	0	0	0	0	0	0	0	0	86	110	0	0	25	0	0	14	0	0	0	0	0	0	0	0	40	0	25	0	0	41	42
Diphterophora	3	14	0	0	0	0	0	0	0	14	0	0	0	0	25	15	34	14	194	55	0	21	0	39	50	0	40	49	173	0	0	21	0
Dorylaimoidae	4	54	68	100	29	171	49	661	291	113	154	221	227	334	295	108	443	272	498	289	254	551	206	294	183	741	282	295	519	164	228	62	170
Tylencholaimus	4	14	0	0	0	0	0	0	0	0	0	0	28	355	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prismatolaimus	4	68	34	50	29	26	0	248	327	155	171	221	71	293	98	170	170	29	249	289	190	614	130	274	66	34	605	164	321	41	143	144	127
Pristionchus	4	0	0	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mononchidae	4	0	0	25	0	105	37	0	0	85	0	55	28	0	25	0	0	14	55	0	0	42	32	59	66	101	0	0	0	0	0	0	0
Monhysteridae	4	0	0	0	29	0	0	0	0	0	17	0	170	0	0	0	0	0	0	14	0	0	0	0	83	270	81	0	25	0	0	0	0
Tripyla	4	0	0	0	0	0	0	0	0	0	0	0	57	0	0	62	0	0	28	0	0	0	0	39	0	0	0	0	0	0	0	0	0

Chapter 3

Diversity patterns and community development of soil mites and nematodes during secondary grassland succession

Paul Kardol, Jeffrey S. Newton, T. Martijn Bezemer, Mark Maraun, Annemieke van der Wal and Wim H. van der Putten

Abstract

Soil communities play an important role in ecosystem development. However, little is known on how and at what spatial and temporal scales soil communities develop. Here, in a spatio-temporal design, we compared composition and diversity patterns of two groups of soil organisms (mites and nematodes) at four stages along a chronosequence from abandoned arable land to heathland, which is a possible end-point reference. For both groups of organisms we determined soil α-diversity, β-diversity and γ-diversity and we used partial ordination analyses to determine the role of site, soil properties, and season in taxon and feeding-group composition. For oribatid mites, the patterns of α- and γ-diversity developed similarly along the chronosequence. In contrast, this did not apply to nematodes. When succession proceeded, oribatid mite β-diversity decreased, whereas nematode β-diversity increased. The spatio-temporal diversity patterns after land abandonment suggest that community development for oribatid mites depends predominantly on colonisation of new taxa, whereas community development for nematodes depends on shifts in dominance patterns. This would imply that on old-fields diversity patterns of oribatid mites are controlled by dispersal, whereas diversity patterns of nematodes are controlled by changing abiotic or biotic soil conditions. Both for mites and nematodes, site, as well as the intersection between site and soil properties were the best predictors for taxon and feeding-group composition. Site effects point at autogenic factors or historical contingency that drive soil community development, while the intersection between site and soil properties indicates that successional changes in community composition are environmentally-controlled.

Keywords *Biodiversity, chronosequence, community assembly, ex-arable land, partial ordination, soil communities, spatio-temporal scale*

Introduction

A comprehensive recognition of the biological component of the soil subsystem is necessary for understanding ecosystem processes during land use change and to improve our ability to restore endangered ecosystems (Wolters et al. 2000, Bardgett et al. 2005a, Kardol et al. 2006). In recent years, our understanding of the role of soil organisms and diversity in ecosystem functioning has tremendously improved (e.g. Fitter et al. 2005, Wall et al. 2005). However, much remains unknown about the factors that explain the structure, composition and diversity of soil communities, especially concerning their temporal and spatial development (Ettema and Wardle 2002). Importantly, factors explaining soil community diversity and composition may vary across spatial and temporal scales (Bardgett et al. 2005b, Zaitsev et al. 2006). In this study, we compared composition and diversity patterns of soil mite and nematode communities in secondary grassland succession, using a spatio-temporal design. In grasslands, mites and nematodes comprise the majority of soil organisms with respect to density, diversity and function (Petersen and Luxton 1982, Seastedt 1984, Stanton 1988).

The most compelling explanation for the shear amount of biological diversity found in the soil, perhaps, lies in the extremely heterogeneous habitat in which soil organisms dwell, both spatially

and temporally (Ettema and Wardle 2002). This heterogeneity provides unprecedented potential for niche partitioning and habitat specialization, thereby allowing species coexistence and favouring biodiversity (Bardgett 2002). In secondary succession following cessation of agricultural practices, release from physical and chemical disturbance may enable soil community diversity to increase (Maraun and Scheu 2000). Build-up in the amount, complexity and diversity of organic matter as driven by changes in the vegetation (Bardgett and Shine 1999) may further enhance diversity. Changes in plant community composition, increased plant species heterogeneity and organic matter input to the soil may enhance spatial and seasonal variation in soil microhabitats and food resources (Hansen and Coleman 1998). Increased habitat heterogeneity may enhance species turnover between spatially or temporally separated communities. Within-ecosystem estimates of diversity comprise numerous organismal and ecological entities across a wide range of spatial and temporal scales. So far, multiple-scale diversity patterns for soil organisms are poorly understood (De Deyn and Van der Putten 2005).

Whether successional changes in ecological communities are deterministically controlled by changes in the abiotic environment (Clements 1916) or are historically contingent (Gleason 1927) has been debated for almost a century. So far, soil communities have received little attention in this discussion. The deterministic succession concept predicts a progressive and environmentally directed change in soil community composition, whereas the historical contingency concept predicts that community development depends primarily on initial site conditions, such as the biotic legacy of the former land use. During secondary succession, changes in community composition can depend on colonisation of new species, as well as on changes in the relative abundance of resident species in response to changes in soil properties (Belyea and Lancaster 1999). So far, it remains unknown whether deterministic or historically contingent succession prevails in soil community succession. Importantly, functional types, feeding types and phylogenetic groups of soil organisms may experience variable colonisation abilities and may differ in how they respond to changes in biotic and abiotic soil properties and plant community composition (Scheu and Schulz 1996, Wardle and Van der Putten 2002). Analogous to secondary plant community succession (Fukami et al. 2005), temporal patterns in the taxonomic composition of soil organisms may be historically contingent and depend predominantly on the initial composition and on dispersal from external species pools, while temporal patterns in the 'functional' composition may depend more on changes in soil properties.

We studied soil mite and nematode community development during four subsequent seasons at three stages along a 22-year old chronosequence of abandoned agricultural fields, which we called 'early', 'mid' and 'late'. As a possible end-point reference, we included a semi-natural heathland, which was the predominant ecosystem type before the land became cultivated. After cessation of agricultural practices, we expected community diversity for mites and nematodes to increase. We distinguished three types of diversity (Whittaker 1960, 1972): α-diversity as a measure of diversity within samples, β-diversity as a measure of dissimilarity between samples within a site, and γ-diversity as a measure of diversity within sites. We compared successional patterns of α-, β-, and γ-diversity for mites and nematodes. Additionally, we expected both mite and nematode community composition to develop towards the reference heathland. We described

successional patterns both in taxon and feeding-group composition for mites and nematodes. Explicitly, we tested the explanatory power of historically contingent factors (e.g. site), soil properties, and seasonal fluctuations using partial ordination analyses.

Methods

Study area and sampling

Soil samples were collected in 2004 from three former agricultural sites, which were abandoned in 2002 (early-successional), 1995 (mid-successional) and 1982 (late-successional). A semi-natural heathland was selected as a reference system. Sites were located within the same geographical region in the central part of the Netherlands on well-drained, sandy soils originating from former glacial deposits (Table 3.1). The sites were managed by low-intensive grazing of natural and introduced vertebrate herbivores. Within each site a 50 x 50 m^2 plot was chosen in an area at a minimum distance of 20 m from the edge. In April, June, September and November 2004, at each site, four soil samples of 25 x 25 x 10 cm^3 each were collected from four random positions at minimally 5 m distance from each other. This resulted in 4 (sites) x 4 (seasons) x 4 (replicates) = 64 samples. Within each soil sample a sub-sample of 10 cm Ø and 10 cm depth was taken for determination of the soil mite community. The remaining part of the sample was sieved through a 4 mm Ø mesh and homogenized, before collecting sub-samples for isolating soil nematodes and analyses of soil properties.

Soil properties

We distinguished three fractions of soil organic matter (SOM): water soluble sugars (S), labile organic matter (LOM), and recalcitrant organic matter (ROM). LOM is the fraction soluble in acid hydrolysis (26 n H_2SO_4) and ROM is calculated as [total SOM - LOM - S] (Rovira and Vallejo 2002). Soil moisture content was determined after drying at 75 °C for 48 hrs. Mineral N was extracted by shaking 10 g soil (dry weight) with 50 ml 1M KCl for 2 h, after which NH_4^+ and NO_3^- concentrations were determined colorimetrically using a Traacs 800 auto-analyzer (TechniCon Systems, Inc.) . Net N-mineralization or -immobilization was determined after an incubation period of 3 weeks as the difference between soil mineral N (NH_4^+ + NO_3^-) at the end and start of the incubation period. Total organic C was determined by the Walkley-Black potassium dichromate-concentrated sulfuric acid oxidation procedure (Nelson and Sommers 1982). Content of total N and total P was measured by digestion of samples with a mixture of H_2SO_4-Se and salicyclic acid (Novozamsky et al. 1984). Soil respiration was determined by measuring the amount of CO_2 production in 40 g fresh soil incubated for 48 hrs at 20°C (Van der Wal et al. 2006b). CO_2-concentrations (1 ml headspace gas) were analyzed using a gas chromatograph (Carlo Erba GC 6000) equipped with a hot wire detector (HWD 430). Helium was used as carrier gas.

Table 3.1 Site description of the early-, mid- and late-successional former agricultural sites and of the heathland, which served as an end-point reference for secondary succession. Data for soil properties are mean ± s.e (N = 16). Different letters denote significant differences between sites (Kruskal-Wallis test with multiple comparisons of mean ranks, $P < 0.05$). Lat. = latitude (°N), Long. = longitude (°E).

Site	Abandoned	Lat.	Long.	Soil texture*	Plant association*	Ellenberg fertility score*
Early	2002	52.21	5.82	Coarse sand	*Echinochloo-Setarietum*	6.2
Mid	1995	52.01	5.99	Coarse sand	*Dauco-Melilotetum*	4.3
Late	1982	52.03	5.80	Sand / coarse sand	*Plantagini-Festucion*	4.1
Reference	-	52.06	5.75	Coarse sand	*Genisto anglicae-Callunetum typicum*	1.1

* from Kardol et al (2005).

Table 3.1 *Continued.*

Site	Total SOM (kg C m^{-2} 10 cm depth)	C:N	pH	Total N (kg m^{-2} 10 cm depth)	Total P (kg ha^{-1} 10 cm depth)
Early	2.73 ± 0.02^{a}	22.40 ± 0.54^{bc}	5.44 ± 0.11^{b}	0.12 ± 0.004^{a}	981 ± 32^{b}
Mid	2.22 ± 0.05^{a}	14.79 ± 0.18^{a}	5.14 ± 0.11^{b}	0.15 ± 0.005^{ab}	825 ± 15^{b}
Late	3.78 ± 0.11^{b}	21.92 ± 0.48^{b}	5.51 ± 0.13^{b}	0.17 ± 0.005^{bc}	461 ± 17^{a}
Reference	5.84 ± 0.04^{c}	27.16 ± 1.05^{a}	4.01 ± 0.10^{a}	0.22 ± 0.016^{c}	148 ± 10^{a}

Mites

The samples included the organic layer and the top layer of the mineral horizon. Mites were extracted using Tullgren funnels (Van Straalen and Rijninks 1982) for a period of three weeks to ensure optimal extraction. Emerging mites and other soil animals were collected in Gisin-medium (750 ml 95% alcohol, 250 ml ethyl ether, 30 ml glacial acetic acid, 3 ml 40% formaldehyde). Prior to counting, mites were manually separated from other soil animals and soil particles. We classified all individuals into feeding-groups: 1) predatory Prostigmata + Mesostigmata (Smith et al. 1998); 2) nematophagous Uropodina (Walter and Proctor 1999); 3) fungal-feeding Astigmata (O'Connor 1984); 4) fungal-feeding Oribatida adults; and 5) fungal-feeding Oribatida juveniles (Smith et al. 1998). We distinguished multiple groups of fungal-feeding mites because of their different growth rates and predator-prey relationships (*sensu* Hunt et al. 1987). Adult oribatid mites were identified to species or genus level. Juvenile oribatid mites could not be identified.

Nematodes

Nematodes were extracted from the soil by Oostenbrink elutriators (Oostenbrink 1960). Nematodes present in 10% of the extracted soil were heat-killed and fixed (35% formaldehyde diluted to 4%), after which a minimum of 150 nematodes were identified to family or genus level. The group *Dorylaimoidea* was used to specify a heterogeneous group of omnivorous dorylaimids comprising *Dorylaimidae*, *Qudsianematidae*, *Thornenematidae* and *Aporcelaimidae*. Nematodes were classi-

fied into feeding-groups according to Yeates et al. (1993a): 1) endoparasitic plant-feeders; 2) ectoparasitic plant-feeders; 3) bacterial-feeders; 4) fungal-feeders; 5) omnivores; and 6) predators.

Data analyses

For all univariate analyses, the assumption of normality was checked with a Kolmogorov-Smirnov test and the assumption of homogeneity of variances with Levene's test. If the assumptions were not met, data were log(x+1)-transformed (except otherwise mentioned). If the assumptions were still not met after transformation, data were analyzed using non-parametric Kruskal-Wallis tests. Differences between sites in their soil properties were tested using Kruskal-Wallis tests with multiple comparisons of mean ranks for individual comparisons ($P < 0.05$). Differences between sites in the total density of mites and nematodes and in the density of different feeding-groups were tested using one-way Analysis of Variance (ANOVA) with site as fixed factor, followed by a Tukey posthoc test for individual comparisons.

For diversity measurements and taxon composition analyses of mites we used taxonomic data of adult Oribatida, which are known to be highly sensitive to environmental changes (Behan-Pelletier 1999). For nematodes we used taxonomic data over all feeding groups. Oribatid mite and nematode α-diversity were calculated as the Shannon Index, $H' = -\Sigma\, p_i \ln p_i$, where $p_i = n_i / N_i$, n_i = the density of the i-th taxon, and N = the total density. For each site, differences in α-diversity between seasons were analyzed using one-way ANOVA with season as fixed factor. Because α-diversity was not affected by season, we assumed the 16 samples collected from each site (4 seasons x 4 replicates) to be independent replicates. Therefore, between-site comparisons were tested using one-way ANOVA with each individual sample as a replicate (N = 16) and site as fixed factor followed by a Tukey posthoc test for individual comparisons. As a measure of oribatid mite and nematode β-diversity, we calculated the inverse of the percentage similarity index (equation 1), where x_i and y_i is the relative density (N_i/N, $N = \Sigma\, N_i$) of the i-th taxon in the two communities compared (Lepš 2001).

$$PS = \sum_i \min(x_i y_i) \qquad \text{[Equation 1]}$$

We calculated β-diversity for each site as the mean percentage dissimilarity from each sample with all other samples collected from that site (N = 16). Differences in β-diversity between sites were tested using ANOVA with site as fixed factor and Tukey posthoc tests for individual comparisons. We compared successional α- and β-diversity patterns between oribatid mites and nematodes by testing the interaction effect between site and phylogenetic group (oribatid mites or nematodes) in a two-way ANOVA. To achieve homogeneity of variances, diversity measures were divided by the mean value of the respective phylogenetic group prior to analysis. For each site, γ-diversity was measured as total number of taxa observed across all samples (N = 16). Univariate analyses were performed using STATISTICA (release 7.1, Statsoft, Inc., Tulsa, Oklahoma, USA).

Taxon and feeding-group composition of nematodes and oribatid mites across the 64 samples were examined using multivariate Principal Component Analysis (PCA). Following Borcard et al. (1992) we used canonical ordination to partition the variation in oribatid mite and nematode taxon and feeding-group composition into independent variance components. The explanatory power of three groups of variables was tested in partial Redundancy Analysis (RDA): 1) site (early-, mid-, late-successional, reference), 2) season (April, June, September, November) and 3) soil properties. The number of soil properties had to be reduced in order to avoid overestimating the explained variation. Therefore, we first performed a PCA analysis including the following soil properties: pH, total N, NO_3^-, NH_4^+, N-mineralization, total P, CO_2-respiration, C:N-ratio, S, LOM, ROM, and total SOM (Appendix 3C). Then, we used the sample scores of the first three PCA axes, which cumulatively explained more than 99.9% of the variance (axis: 96.1%; axis 2: 2.4%; axis 3: 1.4%) as soil properties in RDA. Variance partitioning was performed by partialling out each of the groups of variables as covariables at a time and comparing the resulting percentage of variance explained by the partial RDA with the one obtained with the full RDA model (Ter Braak and Verdonschot 1995). All numerical data used in PCA and RDA were log-transformed to reduce differences in density between collected taxa. Multivariate analyses were performed using CANOCO, version 4.5 (Ter Braak and Šmilauer 1998-2002).

Results

Density patterns

Total mite density ranged from 116000 individuals per m^2 in the heathland reference to 229000 in the late-successional site. Densities of all mite feeding-groups significantly differed among the sites (Table 3.2). However, successional patterns were not consistent across feeding-groups (Table 3.2). The density of fungal-feeding Prostigmata was lowest in the reference site, whereas the density of fungal-feeding oribatid mites (both juveniles and adults) was lowest in the early-successional site. Oribatid mite densities did not differ among the mid-successional, late-successional and reference site. The most abundant oribatid mite taxa were Brachychthonioidea, Suctobelbidae spp. and *Tectocepheus velatus* (Appendix 3A). The density of nematophagous Uropodina increased from the early- towards the late-successional site. Uropodina densities in the reference site did not differ significantly from those in the mid- and late-successional site. Densities of fungal-feeding Astigmata and predatory Mesostigmata did not show clear successional trends.

Total nematode density was significantly lower in the reference site than in the early-, mid-successional sites ($\pm$ 1500 x 10^3 versus >3000 x 10^3 individuals per m^2) (Table 3.3). Similar to mites, successional patterns of nematodes differed among feeding-groups. The density of ecto-parasitic plant-feeders decreased from the early-successional site towards the reference site. This decrease could be related to a strong decline in the density of Dolichodoridae (Appendix 3B). In contrast, bacterial-feeders increased in density from the early- towards the late-successional site, but were lowest in the reference site (Table 3.3). Compared to the former agricultural sites, the numbers of bacterial-feeding *Acrobeles* and Cephalobidae were particularly low in the reference

Table 3.2 Density of mites (10^3 ind. m^{-2} at 0-10 cm depth) observed in the early-, mid- and late-successional sites and in the reference heathland. Data are mean ± s.e. Different letters denote significant differences between means based on one-way ANOVA followed by Tukey posthoc tests or Kruskal-Wallis test* with multiple comparisons of mean ranks, $P < 0.05$.

Site	Total	Predatory Prostigmata	Predatory Mesostigmata	Nematophagous Uropodina[1, *]	Fungal-feeding Astigmata*	Fungal-feeding Oribatida adults	Fungal-feeding Oribatida juveniles*
Early	180 ± 26 ^{ab}	157 ± 23 ^{c}	10 ± 5 ^{ab}	1 ± 2 ^{a}	11 ± 11 ^{a}	0.1 ± 0.1 ^{a}	2 ± 3 ^{a}
Mid	150 ± 34 ^{a}	105 ± 32 ^{b}	8 ± 2 ^{a}	25 ± 20 ^{ab}	2 ± 2 ^{ab}	15 ± 6 ^{b}	20 ± 5 ^{b}
Late	229 ± 48 ^{b}	154 ± 40 ^{bc}	16 ± 4 ^{b}	207 ± 199 ^{c}	5 ± 8 ^{ab}	14 ± 8 ^{b}	38 ± 11 ^{b}
Reference	116 ± 25 ^{a}	55 ± 13 ^{a}	8 ± 2 ^{a}	66 ± 32 ^{bc}	0.3 ± 0.4 ^{b}	24 ± 8 ^{b}	29 ± 9 ^{b}
ANOVA / Kruskal-Wallis test							
$F_{3,60}$ / $H_{3,64}$	7.63	19.71	4.96	36.4	11.40	36.98	39.63
P	< 0.01	< 0.01	< 0.01	< 0.01	0.01	< 0.01	< 0.01

[1] Unit = ind. m^{-2} 10 cm depth.

Table 3.3 Density of nematodes (10^3 ind. m^{-2} at 0-10 cm depth) observed in the early-, mid- and late-successional sites and in the reference heathland. Data are mean ± s.e. Different letters denote significant differences between means based on one-way ANOVA followed by Tukey posthoc tests or Kruskal-Wallis test* with multiple comparisons of mean ranks, $P < 0.05$.

Site	Total	Endoparasites*	Ectoparasites	Bacterial-feeders	Fungal-feeders	Omnivores	Predators*
Early	3121 ± 592 ^{a}	168 ± 57 ^{a}	1012 ± 217 ^{a}	1244 ± 219 ^{b}	168 ± 46^{a}	150 ± 48 ^{a}	134 ± 95 ^{b}
Mid	3839 ± 7150 ^{a}	154 ± 111 ^{ab}	834 ± 149 ^{ab}	1760 ± 306 ^{c}	360 ± 80 ^{bc}	403 ± 118 ^{b}	12 ± 9 ^{a}
Late	4132 ± 758 ^{a}	306 ± 156 ^{a}	525 ± 191 ^{bc}	1973 ± 226 ^{c}	416 ± 97 ^{c}	544 ± 110 ^{b}	35 ± 22 ^{a}
Reference	1533 ± 287 ^{b}	39 ± 34 ^{b}	342 ± 96 ^{c}	533 ± 98 ^{a}	265 ± 56 ^{ab}	216 ± 63 ^{a}	7 ± 7 ^{a}
ANOVA / Kruskal-Wallis test							
$F_{3,60}$ / $H_{3,64}$	30.9	23.64	12.65	32.62	8.67	20.83	27.03
P	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

site (Appendix 3B). The density of fungal-feeding nematodes increased from the early- to the late-successional site, but it was at an intermediate level in the reference site (Table 3.3). The density of predatory nematodes, dominated by Mononchidae, was highest in the early-successional site, but did not differ among the other sites (Table 3.3; Appendix 3B). Densities of omnivores and endoparasitic plant-feeders did not show clear successional trends.

Diversity patterns

α-Diversity

In total 8956 oribatid mites (adults) belonging to 35 taxa (Appendix 3A) and 9609 nematodes belonging to 41 taxa (Appendix 3B) were identified. For oribatid mites, α-diversity differed significantly among the sites ($H_{3,64}$ = 42.36, P < 0.01), however, not among seasons (data not shown). α-Diversity was higher in the mid-successional and reference sites than in the early- and late-successional sites (Fig 3.1a). As for oribatid mites, nematode α-diversity differed signifi-

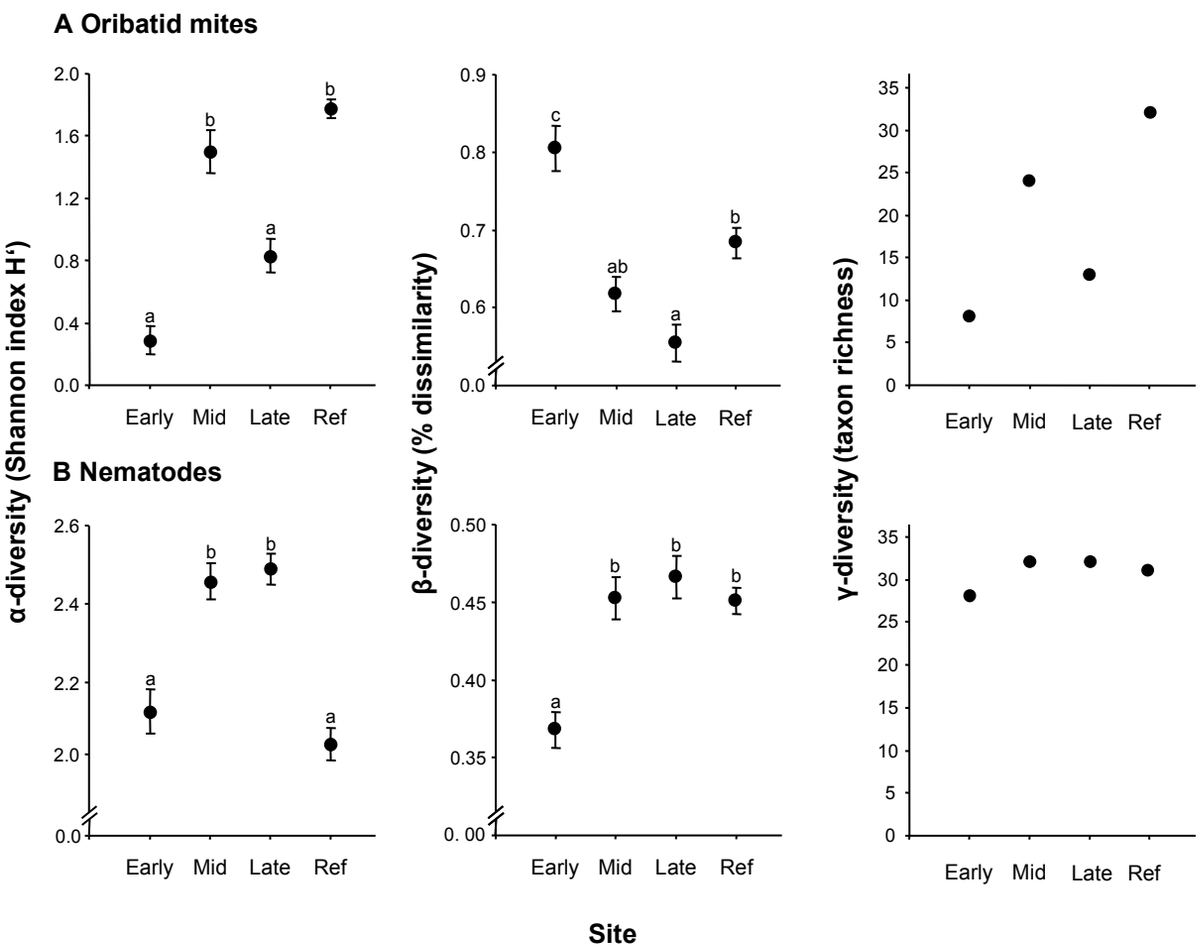

Figure 3.1 α-Diversity (measured as Shannon Index H'), β-diversity (measured as the percentage dissimilarity between samples), and γ-diversity (measured as taxon richness) for oribatid mites (a) and for nematodes (b) in the early-, mid- and late-successional sites and in the reference heathland site. Data for α- and β-diversity are mean ± s.e. Different letters denote significant differences between sites (oribatid mites: Kruskal-Wallis, P < 0.05; nematodes: ANOVA, Tukey posthoc tests, P < 0.05).

cantly among sites ($F_{3,60}$ = 24.78, P <0.01) and not among seasons (data not shown). However, successional α-diversity patterns for oribatid mites and nematodes did not develop in parallel (site x phylogenetic group interaction: $F_{1,3}$ = 31.15, P < 0.01; Figs. 3.1a,b). For nematodes, α-diversity was higher in the mid- and late-successional sites than in the early-successional and reference sites (Fig. 3.1b). For both oribatid mites and nematodes, Shannon Index *H'* patterns (α-diversity) were similar according to patterns of species richness (data not shown).

β-Diversity

β-Diversity of oribatid mites differed significantly among the sites ($F_{3,60}$ = 14.14, P < 0.01) and decreased from the early- towards the late-successional site, while being intermediate in the reference site (Fig. 3.1a). Nematode β-diversity also differed significantly among the sites ($F_{3,60}$ = 18.24, P < 0.01), however, in a different way than observed for oribatid mites (site x phylogenetic group interaction: $F_{1,3}$ = 42.37, P < 0.01; Figs. 3.1a,b). Nematode β-diversity in the early-successional site was significantly lowest, but did not differ between mid- and late-successional sites and the reference site (Fig. 3.1b).

γ-Diversity

For oribatid mites, γ-diversity, measured as taxon richness, differed strongly among sites, ranging from 8 taxa in the early-successional site to 32 taxa in the reference site (Fig. 3.1a). For nematodes, γ-diversity was relatively consistent among the sites and ranged from 28 taxa in the early-successional site to 32 taxa in the mid- and late-successional sites (Fig. 3.1b). The γ-diversity pattern for oribatid mites reflected the pattern of α-diversity (Fig. 3.1a), while the γ-diversity pattern for nematodes did not reflect the pattern of α-diversity and appeared more comparable to the β-diversity pattern (Fig. 3.1b).

Taxon and feeding-group composition

Principal Component Analysis (PCA) showed that taxon composition of oribatid mites developed uni-directionally from the early- towards the late-successional site (Fig. 3.2a). The one late-successional sample that clustered together with the early-successional samples was collected from an area which was highly disturbed by wild boars (*Sus scrofa*) (P. Kardol, pers. obs.). Samples from the reference heathland site were clearly separated from samples from the former agricultural sites. For nematodes, samples from the early-successional site and from the reference site clustered apart from each other and apart from the mid- and late-successional sites (Fig. 3.2b). Samples from the mid- and late-successional sites showed substantial overlap and did not separate in the PCA. For oribatid mites and for nematodes the first two PCA axes accounted for 49.4% and 37.6% of the total variance, respectively. PCA revealed indistinct patterns according to the season in which the samples were taken (Figs. 3.2a,b).

PCA on feeding-group composition revealed less clear separation of sites than PCA on taxon composition (Figs. 3.2b,d). For mites, the first two PCA axes explained 47.1% of the total variance. The early-successional site was separated from the heathland reference site. The other sites did not separate in the PCA. For nematodes, the first two PCA axes explained 82.3% of the to-

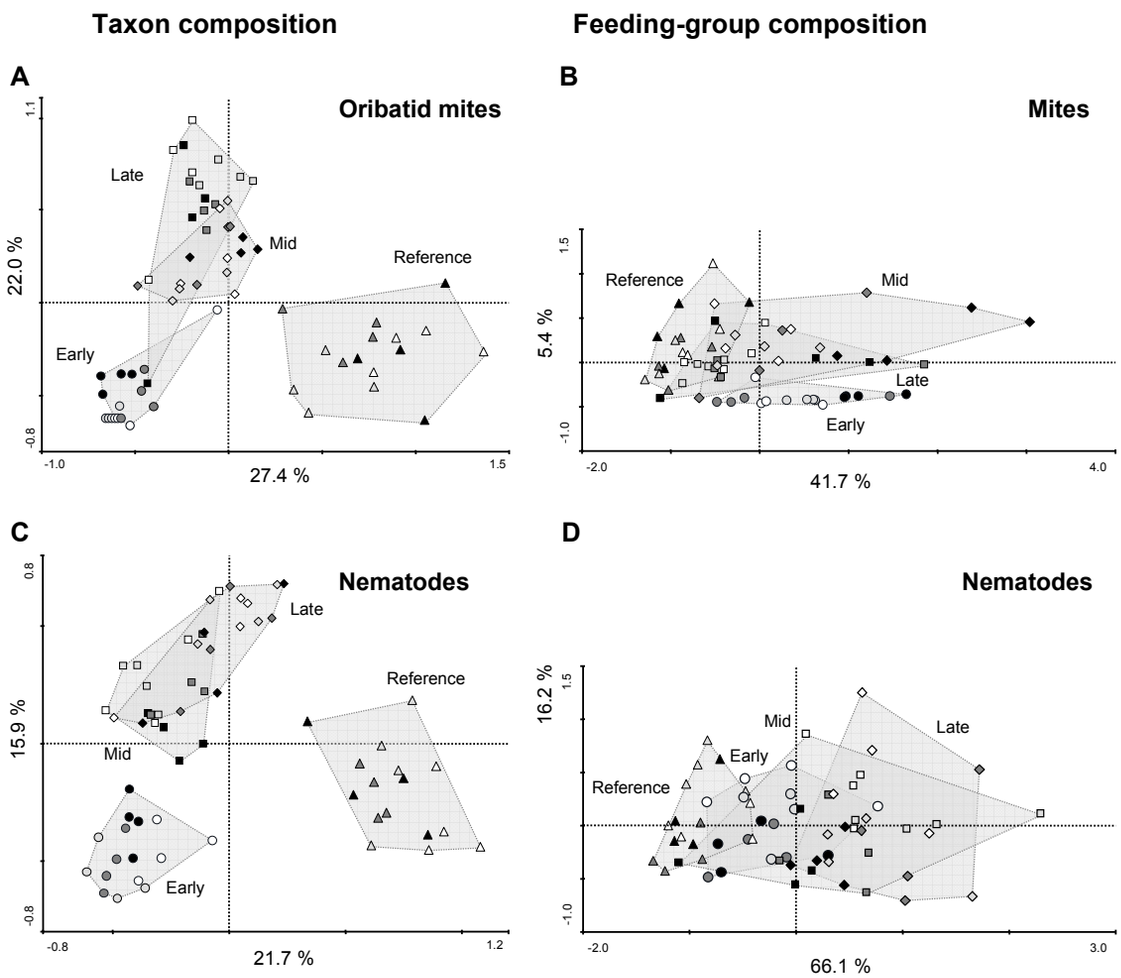

Figure 3.2 Sample plot of principal component analysis (PCA) of taxa composition of oribatid mites (a) and nematodes (c) and of feeding-group composition of mites (b) and nematodes (d). Each symbol represents one sample. Envelops are drawn around samples from the same sites. Symbols for sites: circles = early-successional site, diamonds = mid-successional site, squares = late-successional site, triangles = reference site. Colors for seasons: white = April, light grey = June, dark grey = September, black = November. Percentages along the axes correspond to the amount of explained variability in taxon or feeding-group composition.

tal variance. Opposite to the mites, for nematodes the late-successional site was separated from the heathland reference site. Both mites and nematodes showed no consistent patterns in seasonal clusters (Figs. 3.2c,d).

The total amount of variation in taxon composition explained by site, soil properties, and season tended to be higher for oribatid mites than for nematodes (54.5% versus 45.8%), while the reverse was observed for feeding-group composition (53.8% versus 59.2%). The relative importance of site, soil properties, and season in determining taxon and feeding-group composition was assessed using a RDA variance partitioning procedure. First, we checked the explanatory power of each of the variable groups independently. Both for (oribatid) mites and nematodes, each of the variable groups explained a significant amount of variation in community composi-

tion ($P < 0.05$, Monte Carlo Permutation tests), which enabled us to include all groups in the partitioning procedure. Both for mites and nematodes, site had the largest unique contribution to the total amount of explained variation in taxon and feeding-group composition (Fig. 3.3). The unique contribution of site was higher for feeding-group than for taxon composition, being significant in all cases. In contrast, for both oribatid mites and nematodes, the unique contribution of soil properties was higher for taxon composition than for feeding-group composition. Soil properties contributed significantly to the amount of explained variation in taxon composition of oribatid mites only (Fig. 3.3a). High inter-correlations between site and soil properties made that a substantial percentage of variation (16.5-26.0%) could not be ascribed exclusively to either site or soil properties. The amount of variation in community composition uniquely explained by season was significant, except for the taxon composition of oribatid mites (Fig. 3.3a). The unique contribution of season was higher for feeding-group composition than for taxon composition (3.6% and 5.5% versus 9.5% and 10.4% for mites and nematodes, respectively). For mites the seasonal effect on feeding-group composition could be attributed to the tendency of seasonal increase in predatory Prostigmata and decrease in nematophagous Uropodina (Appendix 3A). For nematodes seasonal fluctuations in feeding-group composition were primarily due to high variability in the density of predatory nematodes (Appendix 3B).

Discussion

Diversity patterns

The successional patterns of oribatid mites and nematodes as observed in a chronosequence of abandoned arable lands reveal that the response of soil biodiversity to land use differs between phylogenetic groups. Moreover, successional patterns of soil biodiversity were not consistent across levels of scale (α-, β- or γ-diversity). While the taxon α- and γ-diversity of oribatid mites increased with time since abandonment, suggesting colonisation-dependent succession, the taxon γ-diversity of nematodes remained quite constant, suggesting that nematode community development was strongly dependent on historical contingency. The development of β-diversity, which is the sum of plot diversity in space and time (i.e. season), showed that nematode community composition became more heterogeneous, whereas, concurrently, oribatid mite communities became more homogeneous when secondary succession proceeded.

After agricultural disturbance, oribatid mite community development may depend mainly on colonisation of new taxa (Scheu and Schulz 1996, Gormsen et al. 2006, Zaitsev et al. 2006; Appendix 3A). In our chronosequence, the relatively low numbers of oribatid mites in the early-successional site were reflected in relatively low α-diversity and patchy distribution over space (samples within fields) and time (season) and contributed to dissimilarity between local communities, as indicated by high β-diversity. Parthenogenetic reproduction and short development periods of oribatid mites (Norton 1994) may have facilitated rapid local population growth in the first years. Thereafter, passive and active dispersal (Norton 1994) may have resulted in an increasingly homogeneous distribution of oribatid mites, hence a decline in β-diversity. Therefore, when assembly time increases, the importance of the local species pool for mite commu-

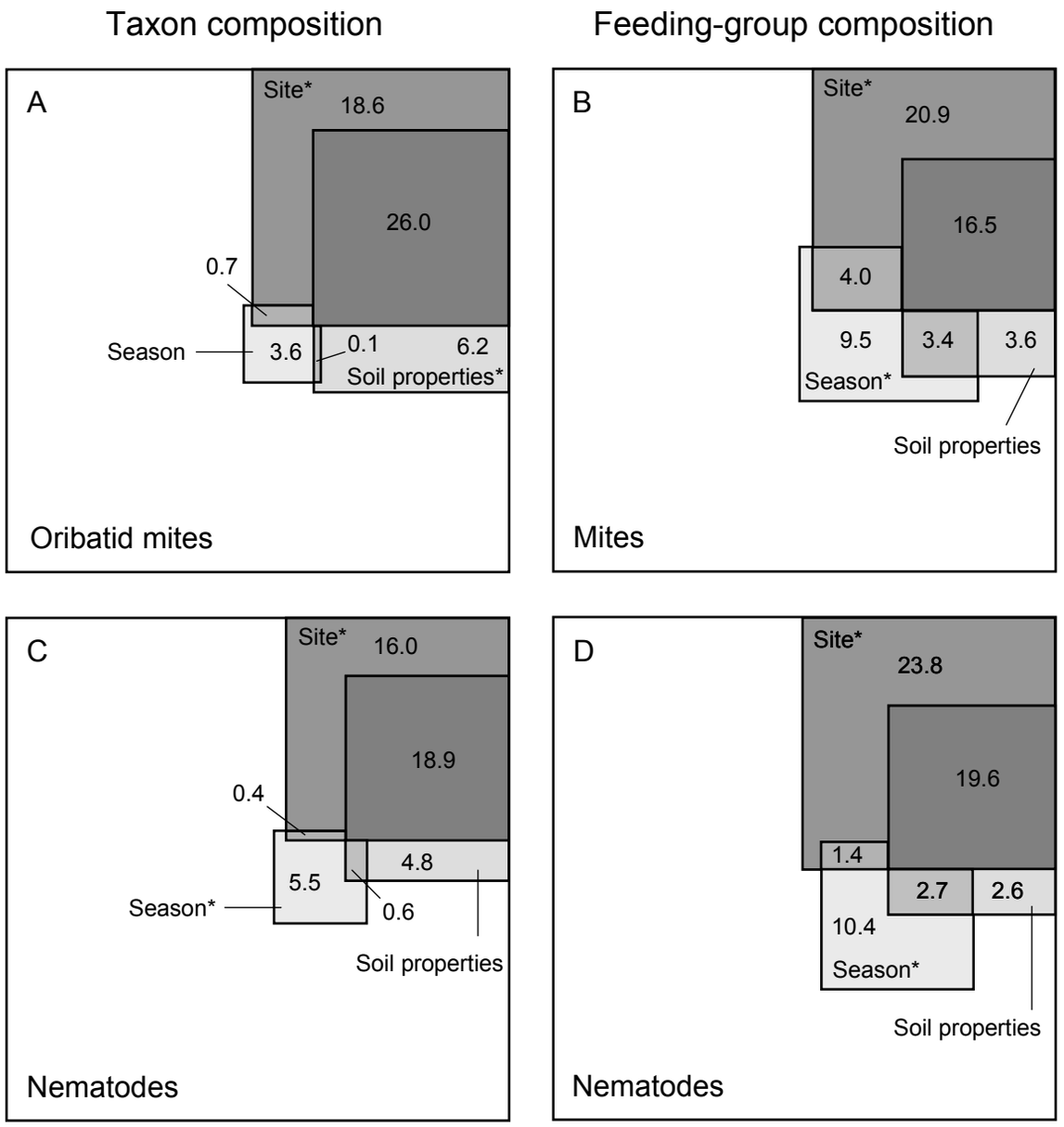

Figure 3.3 Variance partitioning (RDA) of taxa composition of oribatid mites (a) and nematodes (c) and of feeding-group composition of mites (b) and nematodes (d). The numbers list the percentage variance accounted for by site, soil properties, and their intersections. The area of each cell is proportional to the variance accounted for by that component. The area of the white square that is not covered by any of the other squares or rectangles is proportional to the unexplained variance. * Indicates a significant unique contribution (RDA, Monte Carlo permutation tests).

nity diversity may decrease, whereas the importance of the regional species pool may increase (Mouquet et al. 2003) when secondary succession proceeds on abandoned arable land.

Opposite to what we observed for oribatid mites, nematode community development in secondary succession was mostly due to shifts in dominance patterns (Appendix 3B; Kardol et al. 2005), as is also supported by the relatively constant γ-diversity. Following land abandonment,

starting from a relatively homogeneous 'agricultural' nematode community, passive, stochastic dispersal and poor colonisation abilities (Ettema and Bongers 1993) combined with diversifying responses to increasing botanical heterogeneity potentially stimulate spatial segregation of nematodes. This conclusion is supported by an increase in β-diversity with proceeding secondary succession. Most likely, differences in initial abundance and disparate dispersal abilities both will have contributed to the divergence between successional diversity patterns of oribatid mites and nematodes. Alternatively, differences in successional β-diversity patterns between oribatid mites and nematodes may be explained by the level of taxonomic resolution, differences in feeding-specificity, or differences in the perception of spatial scales and environmental heterogeneity (Klironomos et al. 1999).

In contrast to our expectations and to previous observations (Scheu and Schulz 1996, Zaitsev et al. 2006), we found lower oribatid mite α-diversity in the late- than in the mid-successional site. This pattern could not be explained by numerical abundances as observed by others (Mulder et al. 2005). Instead, differences between sites in α-diversity were reflected in γ-diversity patterns, which may indicate that community diversity is primarily constrained by colonisation from the species pool present in the immediate vicinity. To test this hypothesis we collected four random oribatid mite samples in the surrounding forest of each of the sites (according to the procedure described in the methods section) and counted the number of taxa found in these surrounding habitats (i.e. the local species pool) and the number of taxa in common with those observed in the studied sites (Appendix 3D). In support of our hypothesis, more taxa were found in the surrounding forest of the mid-successional site than in the surrounding forest of the late-successional site. However, less than 30%, respectively 40% of taxa found in the surrounding forest were also observed in the mid- and late-successional site, so that the regional species pool of oribatid mites stretches further than the surrounding forest. The forest that surrounded the early-successional site had relatively high γ-diversity. Whether this indicates at dispersal limitations or limited availability of suitable micro-habitats (Petersen and Luxton 1982) needs further experimental testing.

Soil nematode α-diversity may be controlled by within-site constraints rather than that it would depend on the species pool. Nematode α-diversity was lower in the early-successional and the reference sites than in the mid- and late-successional sites, whereas there was less difference among sites in γ-diversity. Low α-diversity in the early-successional site may be related to monotonous plant species distributions and uniform soil conditions in the pioneer habitat. Similarly, low α-diversity in the reference site could be related to the low botanical diversity in the *Calluna-vulgaris*-dominated heathland (Kardol et al. 2005), for example owing to limited micro-habitats (Wasilewska 1995). However, in other studies plant-nematode diversity relationships have found to be neutral (Korthals et al. 2001) or negative (Wardle et al. 1999). Therefore, nematode diversity could also be a reflection of the traits or characteristics of the plant species, rather than of plant diversity per se (Wardle et al. 2003, De Deyn et al. 2004b). In the reference site, low total density of nematodes may have contributed to low α-diversity measurements.

We used α-, β- and γ-components at particular scales, whereas diversity measurements can be applied to different spatial scales (Whittaker et al. 2001). Depending on the scale considered, environmental heterogeneity that is responsible for the bulk of diversity can be interchanged between the different diversity components (Loreau 2000, Veech 2005). Specifically, dispersal of organisms between spatial units acts as a homogenizing force, which tends to reduce the β-component of diversity but at the same time, potentially, increases the α-component.

Taxon and feeding-group composition

Densities of mite feeding-groups differed among sites, however, there were no consistent successional patters when comparing feeding-groups. Fungal-feeding oribatid mites showed very low densities in the early-successional site, while densities of predatory Prostigmata and Mesostigmata showed less variation across the chronosequence. Because mites are low in abundance after agricultural disturbance (Zaitsev et al. 2006), such inconsistency may be related to dispersal and colonisation abilities (Houck 1994). Following land abandonment, due to limited mobility, oribatid mites may colonise gradually, whereas, phoretic predatory mites (Schulz 1991) may colonise early-successional sites more rapidly and stochastically (Scheu and Schulz 1996). This colonisation pattern may explain the variable densities that we observed across the chronosequence. Probably, when succession at one site would be monitored over time, less variable patterns would be observed. In contrast to our findings, Wardle et al. (1995) showed that in a three-year succession of sawdust oribatid mites were particularly rapid colonisers, suggesting that soil community development depends on context.

The composition of nematode feeding-groups may reflect changes in environmental conditions (Bongers and Ferris 1999, Verschoor et al. 2001) and soil food web structure (Ferris and Matute 2003). However, successional patterns for nematode feeding-groups were highly variable, and our findings did not support a successional build-up in soil food web structure or complexity in secondary succession, as observed in primary succession (Neutel et al. 2001). Illustratively, predatory nematodes, comprising the highest trophic level among soil nematodes and recognized as indicative for soil food web complexity (Ferris and Matute 2003), were most abundant in the early-successional site. Apparently, for those groups of soil organisms present at the start of secondary succession, changes in feeding-group composition are mainly due to shifts in dominance patterns (Hedlund et al. 2003) rather than a build-up in complexity (Neutel et al. 2001). Our results strongly suggest that secondary succession in soil communities differs from primary succession in that the legacy effect of former land use and regional species pools have overwhelming effects on soil community development and that these effects depend on the soil organisms considered.

Taxon composition of oribatid mites and nematodes showed clustering of samples corresponding to site origin. For mites, taxon composition developed uni-directionally from the early- to the late-successional site, however, such a development was less evident for nematodes. For none of the species groups a development towards the reference site could be detected, which raises questions about the use of an ecosystem from which ex-arable land has been derived as a

reference for ecosystem development following land abandonment. On the other hand, when considering the composition at the level of feeding-groups, there was far less evidence of clustering of sites. This suggests that there are only few changes in soil food web structure during the first decades after land abandonment at the level of soil invertebrates. Interestingly, such a 'stabilisation phase' has been suggested for primary decomposers as well (Van der Wal et al. 2006b). Within feeding-groups large numbers of species may be functionally redundant (e.g. Setälä et al. 2005) and our results suggest that at this time scale of secondary succession changes in taxon composition are having little impact on functioning of the soil communities. More detailed food web studies are necessary to analyze how the functioning of the whole soil food web changes when secondary succession proceeds. Alternatively, low numbers of feeding-groups compared to numbers of taxa may have resulted in reduced discriminative power of the PCA analyses on feeding-groups.

The largest part of explained variation in taxon and feeding-group composition of both mites and nematodes could be attributed to site and to the intersection between site and soil properties. If soil properties are a function of successional time (e.g. described by the factor 'site' in our study) alone, theoretically, the factors time and soil properties on their own should not explain any variance in addition to the portion of explained variance shared by the two factors. Alternatively, when soil properties vary within sites (e.g. spatial heterogeneity), soil properties, site or both factors may explain a proportion of the variance in addition to the proportion that they share. The high proportion of variance explained by the intersection of site and soil properties indicates at strong environmentally-controlled successional changes in soil community composition (Scheu and Schulz 1996). The unique contribution of site on soil community development could be interpreted as a direct effect of successional time, e.g. autogenic factors not mediated through other variables, such as changes in soil properties, or as an effect of historical factors, such as between-site differences in former arable crops, initial organic residues or fertilisation regime. Alternatively, non-measured variation in soil properties or other environmental conditions could be attributed to site effects.

Controlling factors in successional community changes may depend on the phylogeny of the organisms (Scheu and Schulz 1996) or on the level of community organization. In plant community assembly, taxonomic community succession may be historically contingent while trait-based community succession being determined by environmental changes (Fukami et al. 2005). For soil communities, however, we showed that soil properties (i.e. environmental variables) were less predictive in explaining 'trait-based' feeding-group composition than in explaining taxon composition. The relatively high contribution of soil properties in explaining variation in taxon composition suggests limited functional redundancy at the taxonomic level at which the soil organisms were identified. Feeding-group composition of soil organisms may depend largely on the organic matter status of the soil (Peterson and Luxton 1982). Therefore, relatively small within-sites differences in quantity and quality of organic matter may explain the low unique contribution of soil properties in explaining feeding-group composition (Wardle 2002). Seasonal effects, particularly apparent in feeding-group composition should be considered separately

from successional community changes. The significant explanatory power of season in community composition emphasizes the need of multi-seasonal sampling in soil community studies.

Conclusions

Our study shows that the estimation of successional soil diversity development can depend on the diversity scale (α- versus β- versus γ-diversity) and the group of organisms considered (oribatid mites versus nematodes). These scale and phylum-dependent differences limit the capacity to generalize predictions of multi-scale diversity patterns across groups of organisms (De Deyn and Van der Putten 2005). We conclude that soil community development in the case of (oribatid) mites may be limited by dispersal from the local species pool, whereas nematode community development may be limited by abiotic or biotic conditions within the soil subsystem. In addition to historical and environmental factors as our results indicate, successional changes in soil community composition and diversity may be affected by resource availability, interspecific competition and predators (Maraun et al. 2001, Mulder et al. 2005). Therefore, for a more complete understanding of successional changes in the soil system, future studies need to elucidate indirect interactions and bottom-up versus top-down forces in soil community structure (Hedlund et al 2000, Wardle et al. 2003, 2005) and link the corresponding composition and diversity patterns to functioning.

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Supplementary material

Appendix 3A List of mites taxa and their densities.

Appendix 3B List of nematode taxa (assigned to feeding-groups).

Appendix 3C Principal component analyses of environmental soil properties.

Appendix 3D Oribatid mite γ-diversity in the ex-arable sites and in the heathland and in the surrounding forest of each site.

Appendix 3A List of mite taxa and their density (10^3 ind. m^{-2} 10 cm depth) observed at the early-, mid- and late-successional sites and at the reference site in April, June, September and November. Names are in accordance with Subias (2004). Data are mean ± s.e. N = 4.

	Early-successional site				Mid-successional site				Late-successional site				Reference site			
	April	June	Sept	Nov	April	June	Sept	Nov	April	June	Sept	Nov	April	June	Sept	Nov
Prostigmata	1382 ± 139	1580 ± 61	1284 ± 346	2038 ± 52	983 ± 159	679 ± 89	1301 ± 468	1233 ± 402	1061 ± 128	1173 ± 195	1299 ± 292	2612 ± 365	679 ± 100	451 ± 236	480 ± 96	591 ± 179
Mesostigmata (excl. Uropodina)	36 ±5	101 ± 18	85 ± 15	166 ± 82	57 ± 6	104 ± 13	66 ± 8	110 ± 32	161 ± 48	109 ± 14	141 ± 33	215 ± 40	91 ± 29	70 ± 11	88 ± 18	58 ± 18
Uropodina	-	-	0.3 ± 0.3	-	4 ± 2	1 ± 0.5	4 ± 4	0.3 ± 0.3	19 ± 12	47 ± 39	9 ± 3	8 ± 0.5	12 ± 4	5 ± 3	4 ± 2	6 ± 4
Astigmata	317 ± 174	108 ± 54	13 ± 5	-	42 ± 18	25 ± 9	1 ± 0.5	0.6 ± 7	158 ± 158	51 ±47	2 ± 2	0.3 ± 0.3	2 ± 2	2 ± 1	8 ± 8	2 ± 2
Oribatida (juveniles)	0.3 ± 0.3	0.6 ± 0.5	0.6 ± 0.5	0.3 ± 0.3	189 ± 22	149 ± 35	108 ± 25	164 ± 106	104 ± 27	156 ± 36	253 ± 145	56 ± 9	314 ± 114	137 ± 62	223 ± 65	289 ± 51
Oribatida (adults)																
Achipteria coleoptrata	-	-	-	-	0.3 ± 0.3	3 ± 2	-	-	-	-	-	-	4 ± 4	0.6 ± 0.4	-	-
Banksinoma lanceolata	-	-	-	-	-	3 ± 3	-	-	-	-	-	-	11 ± 4	-	10 ± 2	7 ± 3
Brachychthonioidea	31 ± 31	-	6 ± 4	2.2 ± 1.9	35 ± 20	26 ±11	104 ± 47	81 ± 24	407 ± 141	238 ± 60	82 ± 59	383 ± 108	122 ± 27	61 ± 34	41 ± 22	118 ± 68
Carabodes labyrinthicus	-	-	-	-	-	-	-	-	-	-	-	-	14 ± 14	6 ± 4	-	0.3 ± 0.3
Chamobates cuspidatus	-	-	-	-	-	-	-	-	-	-	-	-	4 ± 4	6 ± 4	-	-
Eupelops plicatus	-	-	-	-	5 ± 5	-	0.3 ± 0.3	0.3 ± 0.3	2 ± 1	2 ± 1	2 ± 1	-	0.3 ± 0.3	-	0.3 ± 0.3	0.3 ± 0.3
Galumnidae spp.	-	-	-	-	13 ± 11	5 ± 2	15 ± 5	20 ± 8	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	7 ± 3	10 ± 2	2 ± 2	1 ± 1	5 ± 5
Hemileius initialis	-	-	-	-	-	-	-	-	-	-	-	-	1 ± 1	-	0.6 ± 0.5	0.3 ± 0.3
Hypochthoniella minutissima	-	-	-	-	-	-	-	-	-	-	-	-	48 ± 32	4 ± 3	28 ± 26	45 ± 25
Hypochthonius rufulus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3 ± 0.3
Liacarus coracinus	-	-	-	-	-	-	0.6 ± 0.7	-	-	-	-	-	6 ± 3	-	3 ± 2	2 ± 1
Liebstadia similis	-	-	-	-	4 ± 4	0.3 ± 0.3	4.8 ± 2.6	8 ± 3	-	-	-	-	-	-	0.3 ± 0.3	-

Appendix 3A *Continued.*

	Early-successional site				Mid-successional site				Late-successional site				Reference site			
	April	June	Sept	Nov	April	June	Sept	Nov	April	June	Sept	Nov	April	June	Sept	Nov
Metabelba pulverulenta	-	-	-	-	0.3 ± 0.3	-	2 ± 2	-	-	-	-	-	-	-	-	-
Microppia minus	0.6 ± 0.4	0.3 ± 0.3	0.3 ± 0.3	-	-	2 ± 4	0.3 ± 0.3	-	-	-	-	3 ± 2	11 ± 6	31 ± 18	-	56 ± 32
Microtritia minima	-	-	-	-	-	-	-	-	-	-	-	-	3 ± 2	1 ± 2	3 ± 3	4 ± 1
Minunthozetes pseudofusiger	-	-	-	-	1 ± 1	3 ± 2	-	-	-	-	-	0.3 ± 0.3	0.3 ± 0.3	-	-	-
Murcia trimaculatas	-	-	-	0.3 ± 0.3	40 ± 9	24 ± 12	4 ± 2	28 ± 18	0.6 ± 0.7	0.3 ± 0.3	0.3 ± 0.3	-	-	-	0.3 ± 0.3	0.3 ± 0.3
Nothrus silvestris	-	-	-	-	-	-	-	2 ± 2	-	-	-	-	4 ± 3	3 ± 3	6 ± 4	12 ± 3
Odontocepheus elongatus	-	-	-	-	0.3 ± 0.3	-	-	-	-	-	-	-	0.6 ± 0.4	0.6 ± 0.5	3 ± 3	4 ± 3
Ophidiotrichus tectus	-	-	-	-	-	-	2 ± 1	-	-	-	-	-	-	-	10 ± 10	-
Oppiella nova	5 ± 5	0.6 ± 0.5	5 ± 5	6 ± 3	66 ± 64	1 ± 0.3	5 ± 5	7 ± 7	6 ± 5	11 ± 10	31 ± 17	12 ± 3	-	-	-	0.3 ± 0.3
Oribatella sp.	-	-	-	-	0.3 ± 0.3	-	-	-	-	-	-	-	-	0.6 ± 0.7	-	-
Oribatula tibialis	-	-	-	-	-	-	-	-	-	-	-	-	0.6 ± 0.5	-	-	-
Phthiracaridae sp.	-	-	-	-	-	-	-	-	-	-	-	-	5 ± 5	5 ± 5	2 ± 2	3 ± 2
Platynothrus peltifer	-	-	-	-	26 ± 12	38 ± 9	6 ± 3	22 ± 15	4 ± 2	35 ± 30	15 ± 4	5 ± 4	12 ± 6	1 ± 0.3	8 ± 4	12 ± 7
Punctoribates punctum	-	-	-	-	14 ± 9	3 ± 2	0.3 ± 0.3	2 ± 2	-	-	-	-	3 ± 3	-	-	-
Ramusella clavipectinata	-	-	-	-	-	-	4 ± 4	-	-	-	-	-	-	-	-	-
Rhysotritia duplicata	-	-	-	-	-	-	-	-	-	-	-	-	19 ± 6	3 ± 1	4 ± 2	6 ± 3
Scheloribatidae	-	-	-	-	12 ± 8	12 ± 9	-	-	-	-	-	0.3 ± 0.3	0.3 ± 0.3	3 ± 2	1 ± 0.5	0.6 ± 0.5
Scutovertex minutus	-	-	-	-	5 ± 1	4 ± 1	4 ± 1	4 ± 3	0.3 ± 0.3	2 ± 1	4 ± 4	0.3 ± 0.3	-	-	-	-
Suctobelbidae spp.	0.6 ± 0.5	-	-	-	-	2 ± 1	0.6 ± 0.5	-	9 ± 6	11 ± 8	13 ± 7	11 ± 6	95 ± 39	31 ± 12	29 ± 12	72 ± 42
Tectocepheus sarekensis	17 ± 17	-	-	-	35 ± 6	32 ± 4	33 ± 10	25 ± 11	74 ± 13	24 ± 9	76 ± 21	46 ± 18	4 ± 3	12 ± 13	20 ± 10	46 ± 27
Tectocepheus velatus	0.6 ± 0.5	-	-	-	-	1 ± 2	-	-	-	-	-	-	11 ± 11	21 ± 14	-	-
Transoribates lagenula	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3 ± 0.3	1 ± 2
Zygoribatula cognata	-	-	-	-	-	-	-	-	-	0.3 ± 0.3	0.3 ± 0.3	-	-	-	1 ± 1	-

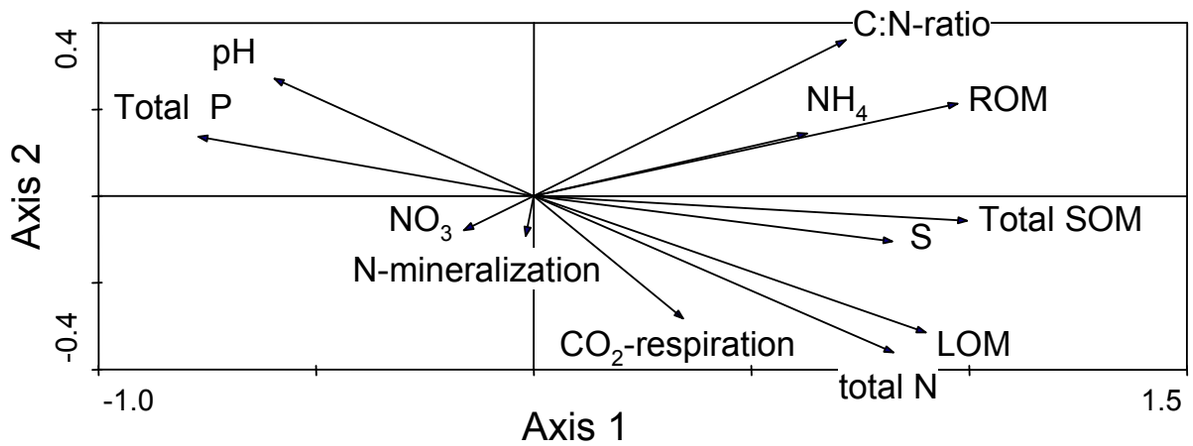
Appendix 3B List of nematode taxa (10^3 ind. m^{-2} 10 cm depth) observed at the early-, mid- and late-successional sites and at the reference sites in April, June, September and November. Data are mean ± s.e. N = 4. Names are in accordance with Bongers (1988). The taxa were assigned to trophic groups according to Yeates et al. (1993a).

	Early-successional site				Mid-successional site				Late-successional site				Reference site			
	April	June	Sept	Nov	April	June	Sept	Nov	April	June	Sept	Nov	April	June	Sept	Nov
Endoparasitc plant-feeders																
Ditylenchus	6 ± 6	5 ± 5	15 ± 15	5 ± 5	-	9 ± 9	-	19 ± 14	-	30 ± 11	8 ± 8	-	37 ± 14	2 ± 2	3 ± 3	9 ± 4
Helicotylenchus	-	-	-	-	82 ± 64	7 ± 8	-	-	199 ± 97	54 ± 33	14 ± 9	12 ± 12	-	8 ± 5	-	7 ± 8
Heterodera	-	-	-	-	137 ± 137	9 ± 9	-	29 ± 23	-	-	-	-	-	-	-	-
Meloidogyne	-	-	5 ± 5	-	-	-	-	-	18 ± 13	72 ± 30	496 ± 263	283 ± 132	79 ± 43	8 ± 6	-	-
Rotylenchus	-	-	-	-	10 ± 7	-	34 ± 34	56 ± 30	-	-	-	-	-	-	-	-
Pratylenchus	221 ± 82	179 ± 50	140 ± 58	97 ± 14	48 ± 33	48 ± 16	5 ± 6	122 ± 66	20 ± 20	-	9 ± 9	7 ± 7	-	-	-	2 ± 2
Ectoparasitic plant-feeders																
Dolichodoridae	916 ± 128	1080 ± 133	911 ± 103	488 ± 82	234 ± 51	419 ± 107	384 ± 53	484 ± 169	130 ± 40	208 ± 143	183 ± 52	132 ± 48	-	6 ± 5	-	5 ± 5
Ecphyadophora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12 ± 12	-
Criconematidae	-	-	-	-	-	-	-	6 ± 6	-	-	-	-	34 ± 19	43 ± 21	14 ± 10	16 ± 7
Hemicyclyophora	-	-	-	-	-	-	-	-	-	7 ± 7	-	-	-	-	-	-
Paratylenchus	9 ± 6	10 ± 6	-	-	347 ± 76	310 ± 99	70 ± 70	453 ± 316	10 ± 6	124 ± 59	482 ± 304	123 ± 108	-	3 ± 2	3 ± 3	7 ± 5
Trichodorus	-	20 ± 20	6 ± 7	-	-	-	-	-	13 ± 14	-	-	-	-	-	-	-
Tylenchidae	61 ± 14	40 ± 15	465 ± 291	39 ± 13	90 ± 23	310 ± 58	163 ± 100	65 ± 29	249 ± 102	233 ± 58	156 ± 52	51 ± 11	243 ± 67	209 ± 135	376 ± 66	398 ± 116
Bacterial-feeders																
Acrobeles	500 ± 106	434 ± 77	592 ± 112	418 ± 124	146 ± 42	237 ± 110	502 ± 151	109 ± 52	585 ± 183	360 ± 108	336 ± 133	570 ± 78	-	1 ± 2	22 ± 10	7 ± 5
Acrobeloides	74 ± 13	44 ± 21	50 ± 17	113 ± 33	361 ± 11	538 ± 95	301 ± 102	428 ± 60	226 ± 761	281 ± 98	789 ± 295	258 ± 29	90 ± 39	121 ± 50	217 ± 110	183 ± 67
Alaimidae	3 ± 4	10 ± 6	83 ± 43	23 ± 13	33 ± 6	23 ± 8	45 ± 24	69 ± 24	27 ± 16	36 ± 23	9 ± 9	41 ± 20	-	-	14 ± 9	11 ± 6
Bastiania	-	4 ± 4	-	-	11 ± 7	55 ± 26	-	-	90 ± 22	20 ± 13	-	12 ± 12	-	5 ± 6	-	11 ± 7
Bunonema	-	-	-	-	-	7 ± 7	6 ± 6	-	-	-	-	-	-	-	6 ± 6	10 ± 10
Cervidellus	25 ± 18	21± 15	34 ± 7	17 ± 8	98 ± 52	398 ± 190	153 ± 55	110 ± 92	207 ± 59	654 ± 282	274 ± 116	183 ± 44	23 ± 8	4 ± 3	81 ± 40	5 ± 5
Chiloplacus	-	-	-	-	7 ± 7	7 ± 7	-	-	-	6 ± 6	-	-	-	-	-	3 ± 4
other Cephalobidae	256 ± 52	435 ± 54	115 ± 49	198 ± 32	493 ± 137	744 ± 209	308 ± 82	193 ± 75	238 ± 25	205 ± 78	293 ± 32	67 ± 20	5 ± 3	10 ± 3	-	2 ± 3

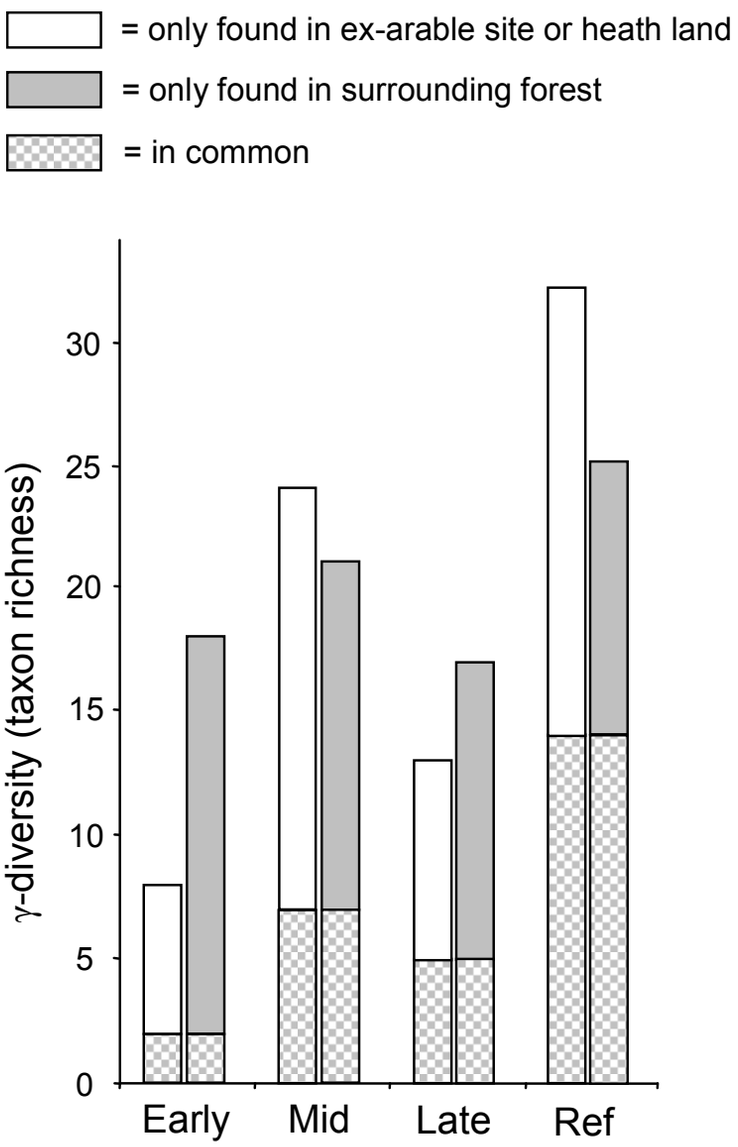
Appendix 3B *Continued.*

	Early-successional site				Mid-successional site				Late-successional site				Reference site			
	April	June	Sept	Nov	April	June	Sept	Nov	April	June	Sept	Nov	April	June	Sept	Nov
Cylindrolaimus	-	-	-	-	-	-	-	-	-	-	-	5 ± 5	-	-	-	-
Panagrolaimidae	--	46 ± 29	76 ± 34	29 ± 11	-	16 ± 5	-	6 ± 6	-	6 ± 6	-	-	-	-	-	-
Plectidae	343 ± 137	210 ± 60	90 ± 12	262 ± 105	173 ± 70	236 ± 63	157 ± 55	183 ± 46	415 ± 95	182 ± 25	194 ± 80	247 ± 28	210 ± 63	78 ± 11	116 ± 28	60 ± 31
Rhabditidae	99 ±89	81 ± 50	102 ± 30	116 ± 32	259 ± 57	91 ± 44	65 ± 27	68 ± 26	33 ± 17	80 ± 30	288 ± 89	23 ± 18	-	21 ± 22	3 ± 3	2 ± 2
Teratocephalidae	19 ± 10	15 ± 15	6 ± 7	31 ± 23	50 ± 29	63 ± 64	6 ± 6	87 ± 80	19 ± 14	46 ± 23	34 ± 14	15 ± 10	205 ± 98	234 ± 33	215 ± 40	101 ± 45
Wilsonema	-	-	-	4 ± 4	45 ± 30	34 ± 26	132 ± 66	15 ± 9	167 ± 85	89 ± 46	133 ± 111	153 ± 60	3 ± 3	12 ± 11	12 ± 12	30 ± 13
Fungal-feeders																
Aphelenchoides	110 ± 49	64 ± 51	82 ± 28	119 ± 26	144 ± 49	235 ± 26	95 ± 24	92 ± 34	296 ± 81	90 ± 33	123 ± 59	38 ± 24	436 ± 39	150 ± 6	206 ± 21	199 ± 59
Aphelenchus	10 ± 10	10 ± 10	13 ± 13	81 ± 25	15 ± 10	145 ± 40	61 ± 28	59 ± 20	91 ± 68	221 ± 47	127 ± 69	121 ± 51	4 ± 3	2 ± 2	3 ± 3	2 ± 3
Diphterophoridae	59 ± 40	15 ± 15	76 ± 58	33 ± 17	70 ± 41	178 ± 56	214 ± 49	130 ± 29	100 ± 34	284 ± 101	72 ± 14	100 ± 23	-	9 ± 5	35 ± 7	13 ± 5
Omnivores																
Diplogasteridae	-	-	5 ± 5	-	-	-	-	-	-	-	-	-	-	-	-	-
Neodiplogasteridae	-	-	11 ± 7	-	-	-	-	-	-	-	-	-	-	-	-	-
Prismatolaimus	6 ± 6	-	18 ± 12	12 ± 4	51 ± 29	62 ± 62	105 ± 16	56 ± 16	79 ± 54	157 ± 88	230 ± 89	76 ± 26	44 ± 10	52 ± 14	53 ± 28	37 ± 22
Achromadoridae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pristionchus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Monhysteridae	9 ± 6	-	77 ± 46	-	-	8 ± 8	27 ± 21	20 ± 195	39 ± 16	12 ± 12	17 ± 10	18 ± 13	23 ± 16	3 ± 2	6 ± 6	2 ± 2
Thornenematidae	-	-	-	-	-	7 ± 8	-	-	-	11 ± 12	-	-	-	11 ± 8	-	-
Dorylaimoidea others	75 ± 16	119 ± 33	161 ± 55	106 ± 23	188 ± 47	577 ± 147	275 ± 63	238 ± 80	336 ± 53	340 ± 47	365 ± 93	496 ± 60	91 ± 26	83 ± 38	319 ± 99	142 ± 20
Predators																
Mononchidae	96 ± 47	107 ± 29	291 ± 173	39 ± 11	5 ± 5	-	-	22 ± 10	51 ± 32	-	-	18 ± 13	2 ± 2	1 ± 2	-	-
Tobrilus	-	-	-	-	-	9 ± 9	-	-	-	7 ± 7	-	-	-	5 ± 2	12 ± 12	-
Tripyla	-	4 ± 5	-	-	17 ± 6	-	-	-	28 ±14	37 ± 17	-	-	-	8 ± 7	-	-

Appendix 3C Principal component analysis (PCA) of environmental soil properties and the cumulative percentage of explained variance for the first four PCA axes. SOM = soil organic matter, LOM = labile organic matter, ROM = recalcitrant organic matter, S = the fraction of sugars that is soluble in water.



Appendix 3D Oribatid mite γ-diversity (measured as total taxon richness) in the ex-arable sites and in the heathland (sum of samples collected in April, June, September, and November 2004; total N = 16) and in the surrounding forest of each site (samples collected in February 2006; N = 4). Indicated are the number of taxa exclusively found in the ex-arable site or heathland, the number exclusively found in the surrounding forest and the number of taxa found in both systems.



Chapter 4

Temporal variation in plant-soil feedback controls succession

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Abstract

Soil abiotic and biotic factors play key roles in plant community dynamics. However, little is known about how soil biota influence vegetation changes over time. Here, we show that the effects of soil organisms may depend on both the successional development of ecosystems and on the successional position of the plants involved. In model systems of plants and soils from different successional stages, we observed negative plant-soil feedback for early-successional plant species, neutral feedback for mid-successional species, and positive feedback for late-successional species. The negative feedback of early-successional plants was independent of soil origin, while late-successional plants performed best in late- and worst in early-successional soil. Increased performance of the subordinate, late-successional plants resulted in enhanced plant community diversity. Observed feedback effects were more related to soil biota than to abiotic conditions. Our results show that temporal variations in plant-soil interactions profoundly contribute to plant community assemblage and ecosystem development.

Keywords *Above-belowground interactions, biodiversity, ecosystem restoration, plant community composition, plant-specific effects, secondary succession, soil communities*

Introduction

A highly debated issue in ecology is which factors explain temporal changes in the structure and composition of natural vegetation (Tilman 1988, Schulze and Mooney 1993, Grime 2001, Van der Maarel 2004). The holistic view of ecosystem development suggests plant communities to develop towards a stable climax stage determined by community-controlled changes in the abiotic environment (Clements 1916, Odum 1969). On larger temporal and spatial scales, variation in the abiotic environment is an excellent predictor of plant community composition (Huston 1994). Locally, soil organisms can be important drivers of vegetation changes (Brown and Gange 1992, Van der Putten et al. 1993, Bradford et al. 2002, Wardle 2002, De Deyn et al. 2003). However, it has remained unknown whether successional changes in soil communities enhance or retard vegetation changes, and whether biotic plant-soil feedbacks contribute to ecosystem development on a longer term (Bever 2003, Wardle et al. 2004a).

Soil contains a large diversity of organisms, some of which are selectively enumerated in the rhizosphere depending on plant identity (Kowalchuck et al. 2002). The soil organisms that have increased in abundance can negatively or positively influence plant performance, plant growth and competitiveness, which is how soil organisms may directly or indirectly alter plant community composition (Van der Putten et al. 1993, Bever et al. 1997, Van der Putten et al. 1997, Bartelt-Ryser et al. 2005). The interactions between plants and their biotic and abiotic soil environment are called plant-soil feedback (Bever et al. 1997, Ehrenfeld et al. 2005). Theory suggests that plant community succession will depend on the direction and magnitude of plant-soil feedbacks, which have been predicted to vary along environmental gradients (Reynolds et al. 2003). However, these theoretical predictions have never been tested experimentally. So far, plant-soil feedback has been studied exclusively as plant performance in one or two species

mixtures in soil conditioned by conspecifics and heterospecifics (Van der Putten et al. 1993, Bever 1994, Klironomos 2002).

In the present study we examined whether plant-soil feedback retards or enhances ecosystem development from relatively unstable, early-successional stages towards relatively stable, later-successional stages of secondary succession on old fields. We grew mixed plant communities on early-, mid- and late-successional soils to test the hypothesis that in mixed plant communities the direction and magnitude of soil feedback effects depend both on plant succession class and the successional stage of the soil. The aim of our study was to determine how short-term plant influences on soil properties may influence succession and ecosystem development on a longer term.

Secondary succession is widespread and takes place at relatively high rates compared to primary succession (Walker 1999), providing an excellent model to study temporal ecosystem changes. For example, some studies have shown how soil fauna may contribute to succession and plant species diversity by selective inhibition of specific plant species thereby indirectly favoring others (Brown and Gange 1992, De Deyn et al. 2003, Schädler et al. 2004). In microcosms, we inoculated soils from different successional origins into sterilised soil to standardise abiotic conditions and established mixtures of plant species from three succession classes. After one growth period, shoot biomass was determined for each of the plant species and the soils were re-planted with the same plant species mixtures as before. Plant performance in the second growth period was used to assess the soil feedback effect. We show that the effect of plant-soil feedback depends on both the successional development of the ecosystem and on the succession class of the plants involved.

Material and methods

Experimental design

Soil was collected from a series of recently abandoned ex-arable fields to semi-natural grasslands and heathlands (Kardol et al. 2005, Van der Wal et al. 2006b). All fields were located on sandy or sandy loam glacial deposits in the central part of the Netherlands. We selected five early-successional fields (abandoned 1 or 2 years ago), five mid-successional fields (abandoned 7 to 9 years ago), and five late-successional fields (abandoned more than 20 years ago or semi-natural mattgras sward / heathland). Within each successional stage the five replicates corresponded in vegetation composition as much as possible (Table 4.1). Fields were managed by low-intensive grazing of natural and introduced vertebrate herbivores. Within each individual field a 50 x 50 m^2 plot was chosen in an area with a homogeneous vegetation cover and at minimum 20 m from the field edge. In April 2003, 80 soil cores of Ø 3.4 cm and 10 cm deep were collected from each plot according to a stratified pattern. The soil samples were bulked and stored at 4 °C to serve as a single replicate. The soil of each field was homogenised and sieved (Ø 4 mm) to remove roots and stones. Average abiotic and microbial soil properties for early, mid and late soil inocula are shown in Appendix 4A and Appendix 4B in the Supplementary material.

Table 4.1 Descriptions of the fields from which soil was collected (Kardol *et al.* 2005, Van der Wal *et al.* 2006b).

Succession stage	Latitude (°N)	Longitude (°E)	Field age*	Plant association (Schaminée *et al.* 1996, 1998)
Early	52.21	5.82	1	*Echinochloo-Setarietum*
	52.00	5.75	1	*Matricaria recutita-Spergula arvensis – [Aperion spicae-venti]*†
	52.00	5.75	1	*Scleranthο annui-Arnoseridetum typicum*
	52.04	6.05	2	*Lolio-Cynosuretum typicum*
	52.03	6.04	2	*Plantagini-Lolietum perennis*
Mid	52.02	5.99	7	*Hieracio-Holcetum mollis*
	52.00	6.00	7	*Hieracio-Holcetum mollis*
	52.03	6.04	8	*Hieracio-Holcetum mollis*
	52.01	5.99	8	*Ornithopodo-Corynephoretum*
	51.98	5.52	9	*Festuco-Galietum typicum*
Late	52.03	5.80	21	*Plantagini-Festucion*
	52.06	6.00	34	*Galio hercynici-Festucetum ovinae*
	52.27	5.73	-	*Galio hercynici-Festucetum ovinae*
	52.06	5.94	-	*Genisto anglicae-Callunetum typicum*
	52.06	5.75	-	*Genisto anglicae-Callunetum typicum*

* Years since abandonment. † Frame community (characteristic species of lower syntaxonomic levels are missing while the community can clearly be designed to a higher syntaxonomic level).

In a greenhouse, we established microcosms (∅ = 17 cm, h = 18 cm) of a mixture of sterilised mineral soil and non-sterilised field soil (6:1 w:w). Mineral soil (0.0 mg/kg NH_4, 9.2 mg/kg NO_3, 34.4 mg/kg P-Olsen, and < 1% organic matter) originated from an ex-arable field abandoned 8 years ago and located in the same area as the other fields. After removing the 'organic' top layer (approximately 40 cm) the mineral soil was collected from a 20 cm deep layer, sterilised by γ-irradiation (25 kGy), homogenised and sieved. In order to prepare the soil inoculations, sterilised soil was carefully mixed with early-, mid- or late-successional soil. In total 45 microcosms (3 succession stages x 5 replicate fields x 3 microcosms per replicate field) were established. All microcosms were randomly distributed over trolleys that were shifted position six times a week to avoid effects of microclimate differences within the greenhouse. Initial soil moisture level was set at 15 % (w:w). Each microcosm was planted with a mixed community composed of grasses and forbs typical of recently abandoned ex-arable fields (early succession), ex-arable fields in transition to species-rich grassland (mid succession), or species-rich grasslands (late succession). We used two grass and two forb species characteristic for each of the succession stages: *Apera spica-venti* (L.), *Echinochloa crus-galli* (L.) (early-successional grasses) and *Conyza canadensis* (L.), *Rumex obtusifolius* (L.) (early-successional forbs); *Agrostis capillaris* (L), *Bromus hordeaceus* ssp. *hordeaceus* (L.) (mid-successional grasses) and *Hypochaeris radicata* (L.), *Achillea millefolium* (L.) (mid-successional forbs); *Festuca filiformis* (Pourr.), *Nardus stricta* (L.) (late-successional grasses) and *Campanula rotundifolia* (L.), *Arnica montana* (L) (late-successional forbs). The seeds were obtained from the fields or provided by a specialised supplier. Seeds were sown on glass beads, moistened with demineralised water and placed in a germination cabinet (16/8 L/D photo regime, 18/22 ºC). Because not all species started to germinate immediately, one

week after germination seedlings were placed in a climate chamber at 4ºC with light according to day/night regime, until transplanting to ensure that all species were of comparable size at the start of the experiment. Each microcosm was planted with one individual of each of the 12 species. Seedlings were planted at random in fixed positions and each of the three microcosms per replicate had a different plant configuration to minimise positioning effects. The first week of the experiment dead seedlings were replaced. Throughout the experiment, light regime was minimally 16 hours of light per day and natural daylight was supplemented with metal halide lamps (225 $mmols^{-1}m^{-2}$ PAR) to ensure minimum light supply and a L/D temperature regime of 21/16 ºC. Plants were watered six times a week and the initial soil moisture level was re-set twice a week by weighing. Every week plants received 50 ml 0.25 strength Hoagland nutrient solution (Hoagland and Arnon 1950). Seedlings recruiting from propagules present in the soil inocula were removed manually.

Plant measurements

Plants were grown for five months (period 1) after which the shoots were clipped at soil surface. Shoot dry weights of individual plant species were determined after drying for 48h at 70ºC. After the first harvest the soil of each microcosm was homogenised while removing main roots, node-bearing plant fragments and rhizomes to prevent vegetative re-growth. Only a small proportion of the total root biomass produced in the first growth period was transferred to the second growth period. Then, each soil was replanted with seedlings of the same twelve species to determine plant community feedback using the same randomization procedure as in period 1. Five months later (period 2) shoots were clipped and shoot dry weights were measured.

Data analysis

Differences in physical, chemical and microbial characteristics between early, mid and late succession fields were analysed using Tukey's hsd posthoc tests after one-way Analysis of Variance (ANOVA). To avoid pseudo-replication biomass data of the three microcosms per replicate were averaged prior to analyses. Plant evenness was calculated as Simpson's evenness index (Simpson 1949) ($SIEI = 1/\Sigma\, p_i^2 \times 1/S$, where p_i represents the proportional contribution of the ith species to the total shoot biomass produced and S the number of species present). To test plant community feedback in relation to soil inocula, shoot biomass and evenness were analysed using two-way ANOVA with soil origin and growth period as fixed factors. The assumption of normality was tested with Kolmogorov-Smirnov procedures (Lilliefors). Homogeneity of variances was checked with Levene's test. To achieve normality and homogeneity of variance shoot biomass data were square-root transformed prior to analysis. For shoot biomasses, the statistical differences between groups were analysed using Tukey's hsd post-hoc tests. To test for effects of depletion of nutrients by plants in the first growth period on biomass production in the second growth period, the relationship between shoot biomass in the first and in the second period was determined using linear regression. The relationship between shoot biomass of late-successional plant species and the sum of shoot biomasses of early- and mid-successional species was also determined using linear regression. The difference

of this relationship between the first and the second growth period was tested by a Student's t-test on the regression coefficients. Regression analyses were done with data obtained from all individual microcosms (N = 45). ANOVA and regressions were performed using STATISTICA (release 7.1, Statsoft, Inc.). Effects of soil origin and effects of fungal and mycorrhizal biomass (NLFA concentration) in the soil inocula on plant community composition were tested using Redundancy analysis (RDA) on individual shoot biomass with Monte Carlo permutation tests (999 unrestricted permutations). The effect of soil origin was also tested with fungal and mycorrhizal biomass as covariables. RDA analyses were performed using CANOCO, version 4.5 (Ter Braak and Šmilauer 1998-2002).

Results and discussion

Plants from different successional classes varied in their feedback response to soil inocula and the outcome of plant-soil feedback interactions depended on the successional stage of the soil. In the second growth period, early-successional species had significantly lower shoot biomass than in the first period (Fig. 4.1a, Table 4.2). Shoot biomass of mid-successional species did not differ between the growth periods (Fig. 4.1b, Table 4.2), whereas late-successional species produced significantly more shoot biomass in the second than in the first period (Fig. 4.1c, Table 4.2). In the second growth period, early- and mid-successional plant species were unaffected by soil origin, whereas late-successional species produced significantly more shoot biomass in late than in early soil (Fig. 4.1a-c, Table 4.2). Therefore, early-successional plant species experienced negative feedback independent of soil origin, whereas mid-successional species had a neutral feedback and late-successional species a positive feedback. The positive feedback of the late-successional species was highest in the late-successional soil.

Plant-soil feedbacks can operate through pathways involving abiotic or biotic processes (Ehrenfeld et al. 2005). We first tested whether the observed enhanced performance of the late-successional plant species in the second period may have been due to relaxed competition from

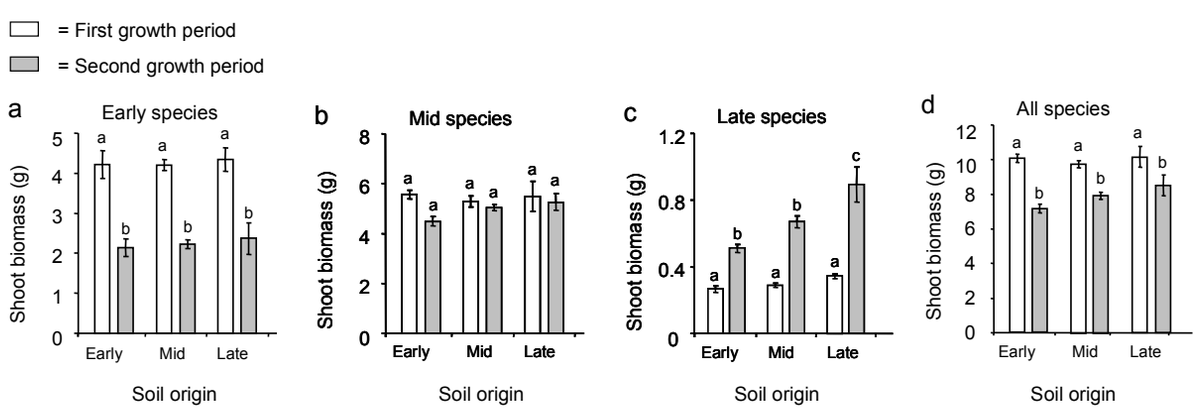

Figure 4.1 Shoot biomass for classes of early- (a), mid- (b) and late-successional (c) plant species and total shoot biomass (d) from microcosms inoculated with early, mid and late soil inocula. Data are mean values ± s.e. N = 5. Different letters denote significant differences (Tukey's hsd posthoc tests after two-way ANOVA for shoot biomass with soil origin and growth period as fixed factors, $P < 0.05$).

Table 4.2 Two-way ANOVA for shoot biomass with soil origin and period as fixed factors.

		Total biomass		Biomass early species		Biomass mid species		Biomass late species	
	df	F	*P*	F	*P*	F	*P*	F	*P*
Soil origin	2	2.14	0.14	0.23	0.80	0.55	0.58	11.51	< 0.01*
Period	1	50.84	< 0.01*	80.06	< 0.01*	3.76	0.06	99.78	< 0.01*
Soil origin x period	2	1.71	0.2	0.03	0.98	5.07	0.35	5.07	0.01*
Error (mean squares)	24	0.67		0.38		0.53		0.017	

* Indicates significant effect.

the early- and mid-successional plant species. When such effects would be operative, there should be a negative relationship between biomass of late- and that of early- and mid-successional plant species. However, the soil origin effect on performance of late-successional species was irrespective of biomass production of early- and mid-successional species. There was no relationship between the sum of shoot biomasses of early- and mid-successional species and shoot biomass of late-successional species in the first period (R^2 = 0.008, r = -0.09, P = 0.55), while in the second period there was a positive relationship (R^2 = 0.11, r = 0.33, P = 0.02). The regression coefficient of this relationship was significantly higher in the second than in the first growth period (Student's t-test, P = 0.02). Moreover, there was a significant soil origin x period interaction for late-successional species only (Table 4.2). Therefore, we did not find any support for relaxed competition driving the observed results. Alternatively, enhanced performance of the late-successional species may have been due to positive feedback from soil organisms.

The model communities showed a successional shift from early- to late-successional plant species. The contrasting effects between early and late species, especially in the response to soil origin, strongly suggest that soil feedback effects depend on the successional position of the plant species involved. Total shoot biomass in the second period was lower than in the first period, but did not depend on soil origin (Fig. 4.1d, Table 4.2). The observed shifts in biomass production could have been the result of altered soil community composition, as well as nutrient depletion. With respect to nutrient depletion, the ample supplied nutrients will have minimized differences in nutrient availability between both growth stages. Moreover, when nutrient depletion in the first growth phase would explain plant performance in the second growth phase, biomass production in phase 1 should be inversely related to biomass production in phase 2. We tested this possibility by regression analysis with data obtained from all individual microcosms (R^2 = 0.056, r = 0.23, P = 0.12) between microcosm biomass in phase 1 and phase 2, the feedback effect does not appear to be due to nutrient depletion. Therefore, our results point at a feedback effect from the soil community, rather than from changes in soil nutrient availability.

Soil nutrient status might have been changed by adding the soil inocula. Plant available nitrogen and phosphorus did not differ between early, mid and late soil inocula, but organic matter

content and total P differed along the successional gradient (Appendix 4A). However, as we added the field soil according to a 1:6 dilution with sterilized substrate and nutrients were added throughout the experiment, the experimental conditions will have strongly minimised effects of initial differences in abiotic soil properties. While we have diluted differences in soil abiotic conditions between the succession stages and overwhelmed possible mineralization differences by nutrient supply, the soil biota may have become propagated because of the substrate provided by the plants roots. Therefore, over time, effects of soil biota may have become more important while effects of initial abiotic differences may have become less contributing to the observed feedback effects. Our experimental design did not allow us to distinguish between intrinsic and plant-induced changes in soil communities.

In the field soil, fungal biomass was lowest in early- and highest in late-successional soil inocula as were NLFA concentrations that indicate biomass of arbuscular mycorrhizal fungi (Olsson 1999) (Appendix 4B). On the other hand, bacterial biomass did not differ between the inocula. The potential role of initial differences in fungal and mycorrhizal abundance in the soil inocula was tested using multivariate analyses. RDA on shoot biomasses showed a highly significant soil origin effect on plant species composition (Table 4.3). Soil origin explained 33.1% and 26.7% of the variance in the first and second growth period, respectively. The amount of variance explained by fungal biomass and NLFA concentration was lower (approximately 15%). When the effect of fungal biomass was removed from the analysis, the effect of soil origin on shoot biomass composition was still significant in both growth periods. However, when the effect of NLFA concentration was removed from the analysis, the effect of soil origin was significant in the first, but not in the second period (Table 4.3). This suggests that particularly during the second growth period, the effects of soil origin on variation in plant growth could have been related to the status of mycorrhizal fungi in the soils. Feedback effects through altered mycorrhizal associations may have been underestimated because of the nutrient supply (Treseder 2004).

Table 4.3 Results of redundancy analysis (RDA) for shoot biomasses in the first and second growth period. Explanatory variables tested: soil origin (early, mid and late), fungal biomass[1] and mycorrhizal biomass (NLFA)[1] of the soil inocula. The effect of soil origin was also tested with fungal biomass and NLFA as covariable. Shown are trace (sum of all canonical eigenvalues, i.e. % explained variance), F- and *P*-values (Monte Carlo permutation tests). * Indicates significant effect.

Variable	Covariable	Growth period 1			Growth period 2		
Soil origin	-	0.33	2.97	< 0.01*	0.27	2.18	< 0.01*
Fungal biomass	-	0.15	2.35	0.01*	0.15	2.21	0.03*
NLFA	-	0.14	2.18	0.03*	0.17	2.69	0.02*
Soil origin	Fungal biomass	0.20	1.67	0.03*	0.22	1.92	0.02*
Soil origin	NLFA	0.26	2.43	< 0.01*	0.19	1.60	0.06

[1] Recalculated from van der Wal et al. (2006b).

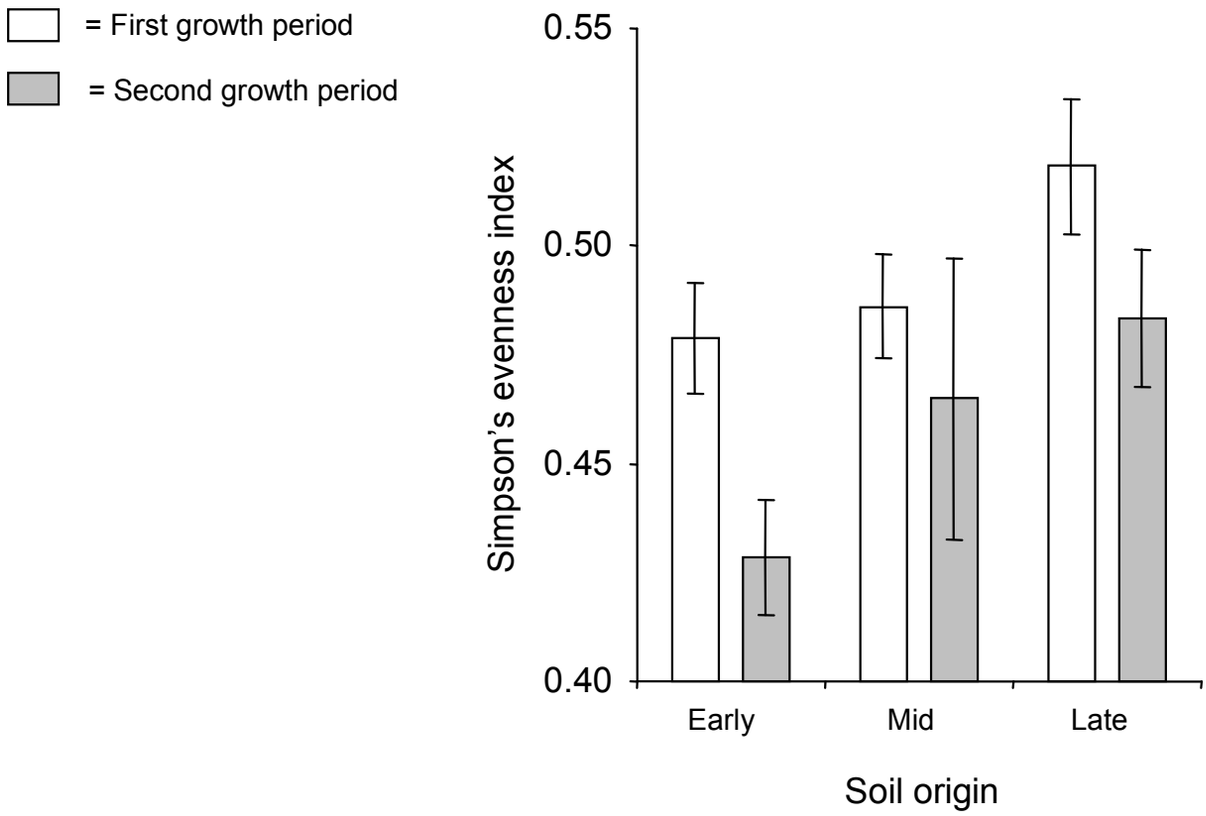

Figure 4.2 Simpson's evenness of plant communities inoculated with soil from early, mid and late succession origin. Data are mean values ± s.e.m.. N = 5. Two-way ANOVA revealed significant effects of growth period ($F_{1,24}$ = 6.09, P = 0.02) and soil origin ($F_{2,24}$ = 3.66, P = 0.04) on plant community evenness. The interaction term was not significant ($F_{1,24}$ = 0.44, P = 0.64).

Changes in plant community composition are usually well correlated with changes in soil conditions such as pH, organic matter, weathering processes, and nutrient availability (Wardle et al. 2004b). Previous studies, therefore, emphasised that plant succession is well predictable by abiotic soil properties (Tilman 1988, Grime 2001). However, the changes in abiotic conditions act on longer time scales than changes in biotic conditions. It may take decades, centuries or even millennia for abiotic soil conditions to change (Wardle et al. 2004b), while biotic feedback effects can develop in periods ranging from months to decades (Van der Putten et al. 1993, Bever et al. 1997). Our experimental set-up did not allow litter deposition and the development of soil organic matter content. Our results, therefore, suggest that on short time scales ($\leq$ decades) plant succession may depend more on accumulation of harmful and beneficial organisms in the rhizosphere (Van der Putten et al. 1993, Bever 2003) than on altered abiotic soil properties. In our study we treated the soil merely as a 'black box' and the results are due to a net effect of growth promoting and growth inhibiting soil organisms. Future studies are needed to unravel the contribution of the various soil organisms in order to explain their role in the observed feedback effects.

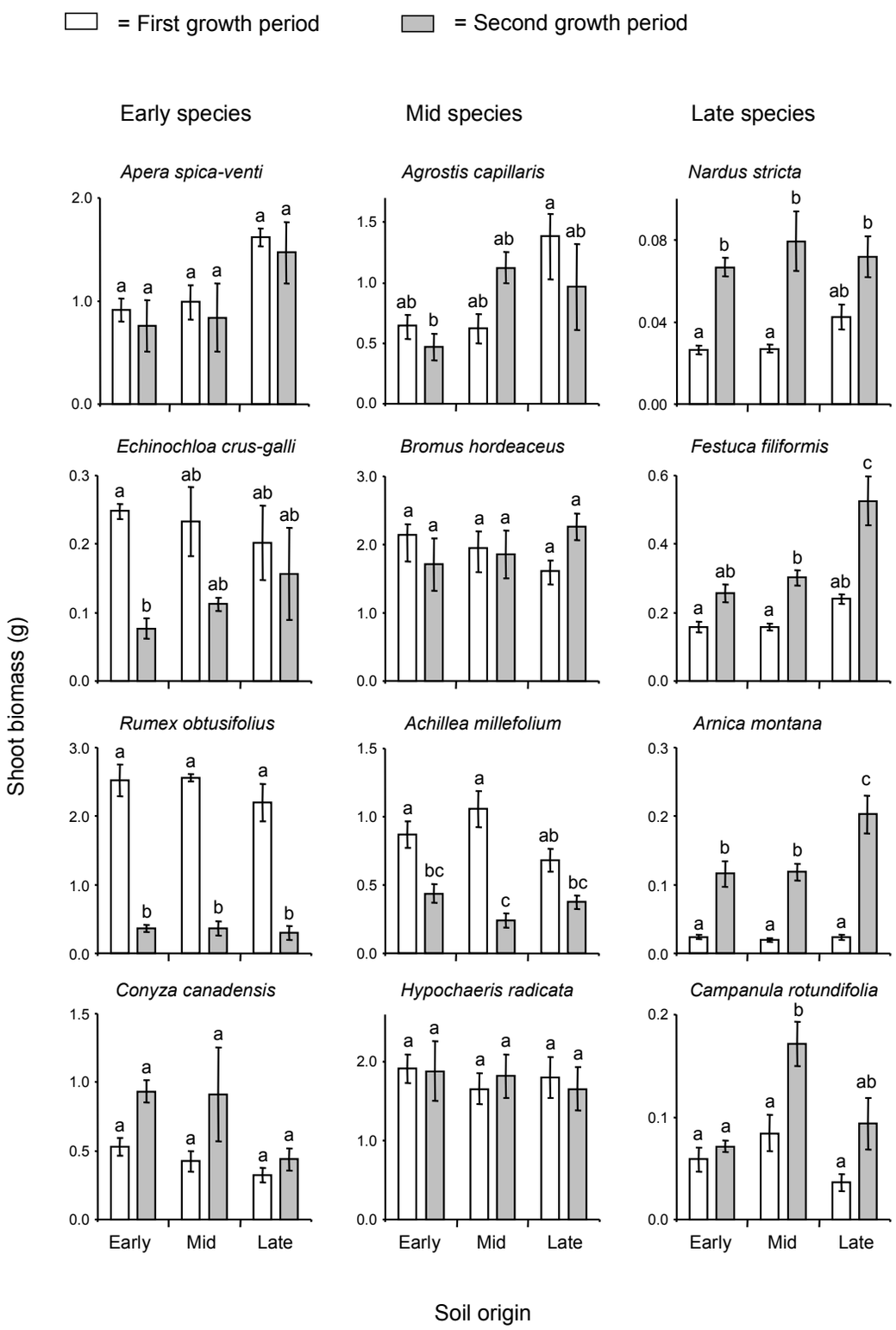

Figure 4.3 Shoot biomass for individual early- (left column of panels), mid- (middle column of panels) and late- (right column of panels) successional plant species for early, mid and late soil inocula. N = 5. Data are mean values ± s.e.. Different letters denote significant differences (Tukey's hsd posthoc tests after two-way ANOVA for shoot biomass with soil origin and growth period as fixed factors, $P < 0.05$).

Fast growth, poor defense and other traits of early secondary succession plant species (ruderals, pioneers) (Grime 2001), potentially make them particularly vulnerable to natural enemies and therefore to negative plant-soil feedback (Price 1984, Bever 2003, Reynolds et al. 2003). Positive plant-soil feedback as observed for late-successional plant species may be due to accumulation of beneficial soil organisms in their rhizosphere, gaining differential access to nutrient pools (Reynolds et al. 2003, Ehrenfeld et al. 2005), for example, as our results indicate, by mycorrhizal associations. Late-successional plant species are generally thought to interact positively with mycorrhizal fungi and to depend more on these associations than early species do (Janos 1980, Smith and Read 1997).

So far, conceptual models to predict the role of plant-soil feedback in plant species replacements (Bever 2003, Reynolds et al. 2003) suggest that positive plant-soil feedback plays a central role in early-successional communities, where host-specific pathogens are supposed to be typically absent, whereas negative plant-soil feedback prevails during later stages. Our study confirms that the direction of feedback between plant and soil varies during secondary succession, but the variation is opposite to the conceptual model of Reynolds et al. (2003). The negative feedback in early stages might explain why dominance of the early colonizing plant species is temporary (Van der Putten et al. 1993, Packer and Clay 2000, Klironomos 2002), whereas positive feedback of the late-successional plant species may contribute to their persistence (Bever 2003).

In our experiment, during both growth periods plant community evenness was lowest in early- and highest in late-successional soil (Fig. 4.2). This was due to increased performance of the subordinate, late-successional plant species in late soil, while biomass production of the early- and mid-successional species was not affected by soil origin. The general pattern of our results for the three plant succession classes is supported by the performance of individual plant species (Fig. 4.3). Nevertheless, some distinctive species-specific effects may point at plant species-specific variation in feedback effects. For example, the early-successional species *Conyza canadensis* had neutral soil feedback instead of negative (Fig. 4.3). Interestingly, *C. canadensis* is non-native, so that this pattern of feedback corresponds with the neutral feedbacks reported for other exotic plant species (Klironomos 2002, Reinhart et al. 2003), suggesting that invasive species may have become released from their native soil pathogens.

Previously, belowground communities have been proposed to enhance the rate of vegetation succession (Brown and Gange 1992, Van der Putten et al. 1993, De Deyn et al. 2003), whereas aboveground herbivory has been suggested to retard succession in high-productive systems and to enhance succession in low-productive systems (Bardgett and Wardle 2003). Interestingly, our results suggest that during succession from high- to low-productive systems, negative plant-soil feedback enhances succession in early stages (while aboveground herbivory might retard succession), while positive plant-soil feedback retards succession in later stages (and aboveground herbivory could enhance succession). Indeed, succession entails an interacting complex of processes, some of which counteract one another (Odum 1969, Price 1984). Counteracting or non-additive effects of above- and belowground communities on the rate of

ecosystem development from fast recycling in early developmental stages to slow recycling in later, mature stages, provide a challenging framework for future research. Moreover, stage-dependent plant-soil feedbacks may have important implications for predicting the rate of succession and thereby for the practice of ecological restoration (Young et al. 2005). Current nature management is almost exclusively focused on managing soil fertility and aboveground interactions (mainly vertebrate herbivores), while these effects may be opposite to the effects by the (often overlooked) soil community. Therefore, an integrated above-belowground approach will be required (Wardle 2002). Our framework of stage-dependency and shifts in the direction of plant-soil feedback effects can be used to further develop theory on drivers of community assembly and ecosystem succession and should improve our ability to understand, predict, and counteract effects of human-induced land-use changes on the functioning and development of ecosystems.

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Supplementary material

The following supplementary material is available online from www.Blackwell-Synergy.com:

Appendix 4A Abiotic characteristics of the soil inocula.

Appendix 4B Microbial characteristics of the soil inocula.

Appendix 4A Abiotic characteristics of early, mid and late soil inocula, recalculated from van der Wal et al. (2006b). Data are mean values ± s.e.m. F and *P* values after one-way ANOVA with soil origin (early, mid, late) as fixed factor. Different letters denote significant differences between soils (Tukey's hsd posthoc tests, $P < 0.05$).

Soil origin	Organic matter (%)	C:N ratio	pH	Mineral-N (mg kg^{-1})	N-mineralisation (mg kg^{-1})	P total (mg kg^{-1})	Available P (P-CaCl) (mg kg^{-1})
Early	5.3 ± 0.5 [a]	19.2 ± 2.5	5.2 ± 0.2	6.0 ± 1.3	20.2 ± 5.5	842 ± 22 [a]	3.8 ± 2.8
Mid	3.7 ± 0.1 [a]	18.3 ± 1.9	5.2 ± 0.2	9.9 ± 2.2	21.7 ± 1.9	580 ± 53 [b]	2.3 ± 1.2
Late	8.1 ± 0.7 [b]	26.2 ± 2.2	4.5 ± 0.3	9.5 ± 1.0	17.4 ± 3.1	201 ± 65 [c]	1.1 ± 0.6
ANOVA							
$F_{2,12}$	19.83	3.76	2.65	1.89	0.32	40.60	0.54
P	< 0.01	0.054	0.11	0.19	0.73	<0.01	0.59

Appendix 4B Microbial characteristics of early, mid and late soil inocula, recalculated from van der Wal et al. (2006b). Data are mean values ± s.e.m. F and *P* values after one-way ANOVA with soil origin (early, mid, late) as fixed factor. Different letters denote significant differences between soils (Tukey's hsd posthoc tests, $P < 0.05$). † Indicates mycorrhizal biomass.

Soil origin	Bacterial biomass (μg C g^{-1})	Fungal biomass (μg C g^{-1})	NLFA 16:1W5 (μg g^{-1})†
Early	37.6 ± 11.1	14.8 ± 2.7 ^{a}	0.5 ± 0.2
Mid	38.0 ± 6.1	23.2 ± 2.1 ^{ab}	2.6 ± 0.8
Late	36.8 ± 8.6	39.8 ± 9.2 ^{b}	4.3 ± 1.7
ANOVA			
$F_{2,12}$	0.01	5.11	3.08
P	0.99	0.02	0.08

Chapter 5

Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly

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Abstract

Plant-soil feedback affects performance and competitive ability of individual plants. However, the importance of plant-soil feedback in historical contingency processes and plant community dynamics is largely unknown. In microcosms, we tested how six early-successional plant species of secondary succession on ex-arable land induced plant-specific changes in soil community composition. Following one growth cycle of conditioning the soil community, soil feedback effects were assessed as plant performance in soil of their own as compared to soil from a mixture of the other five early-successional species. Performance was tested in monocultures and in mixed communities with heterospecific competition from mid-successional species. The role of soil micro-organisms was determined by isolating the microbial component from the soil community, re-inoculation of micro-organisms into sterilized substrate and analyzing plant biomass responses of the early- and mid-successional species.

Plant-soil feedback responses of the early-successional species were negative and significantly increased when the plants were grown in a competitive environment with heterospecifics. In monocultures, three early-successional species experienced negative feedback in soil with a history of conspecifics, while all early-successional species experienced negative feedback when grown with interspecific competition. Interestingly, the non-native forb *Conyza canadensis* showed the weakest soil feedback effect. Biomass production of the early-successional plant species was profoundly reduced by the microbial inocula, most strongly when exposed to inocula of conspecific origin. Molecular characterization of the fungal and bacterial rhizosphere communities revealed a relationship between plant biomass production and the composition of the dominant fungal species. Furthermore, our results show that in early secondary succession, the early-successional plant species induce changes in the soil microbial community composition, that cause historical contingency effects in dominance patterns of mid-succession plant communities.

We conclude that feedback between early-successional plant species and soil micro-organisms can play a crucial role in breaking dominance of early-successional plant communities. Moreover, the influences on soil micro-organism community composition influenced plant community dynamics in the mid-successional plant communities. These results shed new light on how feedback effects between plants and soil organisms in one successional stage result into a biotic legacy effect, which influences plant community processes in subsequent successional stages.

Keywords *Community assembly, biotic legacy, competition, DGGE, ex-arable land, historical contingency, micro-organisms, plant-soil feedback, secondary succession*

Introduction

Nutrient and light availability are major driving forces of plant species replacements along successional trajectories (Tilman 1988, Grime 2001). At local scales, the interactions between plants and the soil community may strongly influence interspecific plant competition (Van der Putten

and Peters 1997) resulting in enhanced rates of community development (Brown and Gange 1992, Van der Putten et al. 1993, Olff et al. 2000, De Deyn et al. 2003). Soil community influences on plant community development may result from direct interactions between plant roots and pathogens (Bruehl 1987), herbivores (Brown and Gange 1990) and mutualists (Smith and Read 1997), as well as from indirect interactions with decomposing and mineralizing soil organisms (Bardgett and Shine 1999, Wardle et al. 2003, Hättenschwiler et al. 2005) or from belowground trophic chain reactions (Strong et al. 1996). Moreover, plants may influence their own performance by changing the composition of their associated soil communities (Van der Putten et al. 1993, Bever 1994). Here, we explore how these feedback effects may work out in early secondary succession and how they may contribute to historical contingency effects in later stages of plant community development.

The concept of ecological plant-soil feedback provides a powerful framework to study temporal plant community dynamics (Bever et al.1997, Bever 2003). Plant-soil feedback involves two steps. First, plants change the composition of their associated soil community (Chanway et al. 1991, Kowalchuk et al. 2002). Second, these plant-induced changes in the soil community affect subsequent plant performance (Bever et al. 1997, Thrall et al. 1997). Through its effect on the composition of the soil community, a given plant species may either increase its own growth rate relative to other plants species (positive feedback) or decrease its own growth rate (negative feedback).

Although the nature of plant-soil feedback is still largely unknown, most evidence points towards plant-specific accumulation of beneficial or harmful micro-organisms in the rhizosphere (Van der Putten 2003). Positive plant-soil feedback would be expected to lead to monocultures and to slow down the rate of succession (Bever 2003, Bonanomi et al. 2005). However, there is growing evidence that soil community feedback is commonly negative (Bever 1994, Bever et al. 1997, Olff et al. 2000, Klironomos 2002) and that negative feedbacks may affect interspecific interactions (Van der Putten and Peters 1997, Bonanomi and Mazzoleni 2005). Depending on the dominance position of the involved plant species in the community, negative plant-soil feedback may either favor or disrupt species coexistence (Dobson and Crawley 1994, Bever 2003, Reynolds et al. 2003). Theoretical studies show that negative plant-soil feedback can contribute to the coexistence of competitive plant species that compete for the same soil and light resources (Bever et al. 1997, Bever 2003, Bonanomi et al. 2005). Empirical studies emphasize the potential role of plant-specific accumulation of soil-borne pathogens and negative feedbacks in species replacements (Van der Putten et al. 1993, Van der Putten and Peters 1997).

Plant-induced changes in the soil community have been shown to be responsible for reduced growth of early-successional species (Keever 1950, Van der Putten et al. 1993, Bever 1994). However, the role of such feedbacks in temporal plant community dynamics has received little attention (Bardgett et al. 2005a, Bartelt-Ryser et al. 2005, Kardol et al. 2006). So far, it is unclear how plant-specific changes in soil communities contribute to plant community dynamics and the rate of successional replacements, as well as how long these effects remain operational (Van der Putten 2003). When plant-soil feedback reduces the competitive ability of an early-

successional plant species, this may strongly affect the outcome of interspecific competition with later successional species (Bever 2003). However, the influence of an early-successional species on the soil community composition could also result in a biotic legacy effect that influences plant community dynamics and patterns of plant dominance in later successional stages.

One of the critical steps in understanding the process of succession is to analyze how plant-soil feedback may influence plant community interactions. We constructed microcosm ecosystems composed of plant species characteristic for early and mid secondary succession stages on ex-arable land. In order to provide all early-successional plant species with the same competitive environment, and also to obtain a succession scenario that is realistic in field conditions, we used a mixture of four mid-successional plant species and analyzed how interspecific competition influenced plant-soil feedback effects. Initial plant communities on fertile, ex-arable land are dominated by arable weeds established from the seed bank and long-distance dispersed pioneer species. These species are characterized by fast growth rates (Grime 2001, Chapin et al. 1994) and the low diversity early succession plant communities, often consisting of locally-single species patches, may provide conditions that are typical for pathogens to flourish (Herms and Mattson 1992, Coley et al. 1985, Burdon 1987). Little is known about the relationship between plant successional type and feedback with the soil community (Jarosz and Davelos 1995, Reynolds et al. 2003, Van der Putten 2003).

In a greenhouse experiment using a two-phase feedback approach (Bever et al. 1997) we tested first the hypothesis that in secondary succession, species-specific plant-induced changes in soil community composition reduces the competitive ability of early-successional species (Van der Putten and Peters 1997, De Deyn et al. 2003). Second, we tested the hypothesis that soil micro-organisms are involved in these plant-soil feedbacks. This hypothesis was tested by isolating the microbial component of the soil community (Klironomos 2002, Bezemer et al. 2005) and re-inoculating the micro-organisms into sterilized soil. We also studied the effects of the soil micro-organisms on other, early- and mid-successional plant species. The fungal and bacterial components of the rhizosphere community were characterized molecularly by Denaturing Gradient Gel Electrophoresis (DGGE) in order to detect plant species-specific changes in the dominant bacterial and fungal taxa present in the rhizosphere. Finally, we analyzed contingency effects of plant-soil feedback in early-successional stages on plant community composition in mid-successional stages by comparing the performance of the mid-successional plant species in relation to the history of plant-soil feedback.

Methods

Soil

In February 2005, the soil for the experiment was obtained from the upper 15 cm of a maize (*Zea mays* L.) field in Renkum, the Netherlands (52°00' N, 5°45' E). It was a coarse loamy sand, neutral in pH and rich in available nitrogen and phosphorus (Appendix 5A), which is a usual start situation for secondary succession on ex-arable land (Van der Wal et al. 2006b). The soil

was sieved using a 10 mm mesh in order to remove stones and large organic particles, hand-mixed and stored in polypropylene bags outdoors (frost-free) until the experiment was set-up. Before the start of the experiment, five sub-samples from the soil were collected to determine soil moisture content after drying at 75 °C for 48 hours, NH_4 and NO_3 using KCl-extraction, available P using $CaCl_2$-extraction and pH in 1:2.5 dry soil:water suspensions.

Plant species

As early-successional plant species, we used three grasses, *Alopecurus geniculatus* L. (marsh foxtail), *Apera spica-venti* L. (loose silkybent), and *Poa annua* L. (annual bluegrass) and three forbs, *Capsella bursa-pastoris* L. (shepherd's purse), *Conyza canadensis* L. (canadian horseweed), and *Viola arvensis* Murray (european field pansy). As mid-successional plant species, we used the grasses, *Agrostis capillaris* L. (common bent) and *Anthoxanthum odoratum* L. (sweet vernal grass), as well as two forbs, *Achillea millefolium* L. (common yarrow) and *Plantago lanceolata* L. (narrowleaf plantain). All species are typical for early- and mid-successional stages of secondary succession after land abandonment on sandy or sandy loam glacial deposits in the Netherlands (Kardol et al. 2005).

Seeds

Plant seeds of *C. canadensis* and *A. spica-venti* were obtained from two ex-arable fields located in the same geographical region (Assel, 52°21' N, 5°82' E; Renkum 52°00' N, 5°45' E). Seeds of the other plant species were provided by specialized suppliers (Cruydt-hoeck, Groningen, the Netherlands; Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany). Seeds were sterilized with a 0.1% chloride solution for three minutes and sown on glass beads, moistened with demineralized water and germinated in a cabinet of 16/8 hr L/D photo regime and 18/22 °C. One week after germination, seedlings were placed in a climate chamber at 4 °C with light according to day/night regime until transplanting to ensure all species were of comparable ontogenetic state at the start of the experiment.

Experiment 1a

In order to create different soil histories, we established microcosms with monocultures of each of the early-successional plant species to obtain 'home soils' and with mixtures of the five remaining early-successional plant species to obtain 'foreign soils'. Each treatment was replicated 5 times. Experiment 1a was duplicated to allow us to test the treatment effects (home soil, foreign soil) in two competitive environments in experiment 1b. So, the experimental design of experiment 1a involved 6 species x 2 designs (monocultures and mixtures) x 5 replicates x 2 duplicates = 120 microcosms. The microcosms were containers of 18 x 18 x 18 cm^3 filled with the field soil (15% w:w) planted with 25 seedlings of an early-successional species to create home soil or 5 seedlings of each of the five remaining plant species (adding up to a total of 25 seedlings) to create foreign soil. Each of the replicates of the mixed plant communities had a randomly defined plant configuration to avoid positioning effects. Microcosms were randomly placed on trolleys in a greenhouse. Light regime was minimally 16 h/d of light, and natural day-

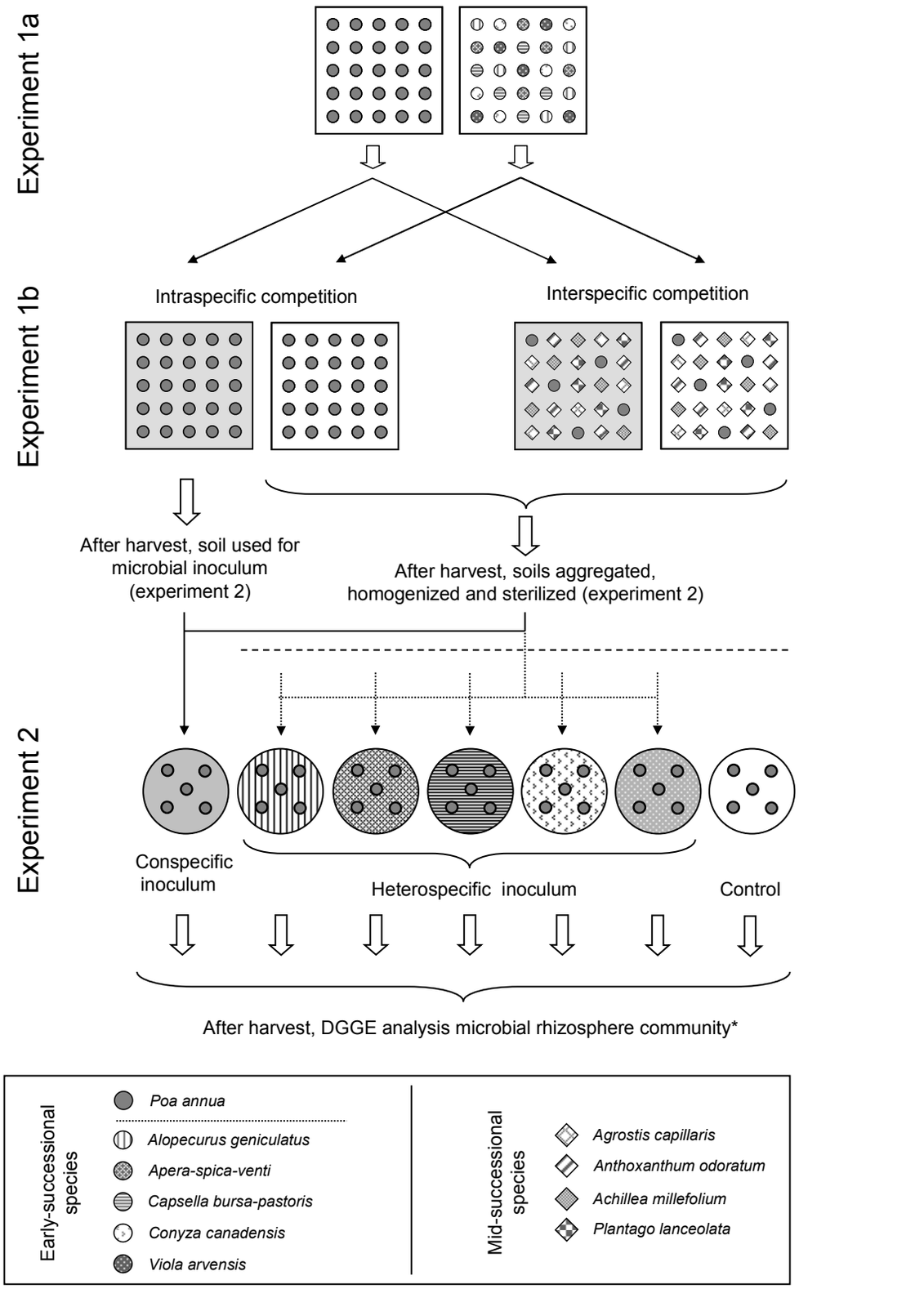

Figure 5.1 Example of the full experimental design for one of the early-successional plant species, *Poa annua*. Shown are diagrammatic microcosms of a single replicate. * DGGE analyses were not performed for the other plant species.

light was supplemented with metal halide lamps (225 μmols^{-1}m^{-2} photosynthetically active radation) to ensure minimum light supply and a L:D temperature regime of 21:16 °C. The positions of the trolleys were shifted three times a week to minimize effects of microclimate differences within the greenhouse. In the first week of the experiment, plants that died were replaced. Plants were watered daily with demineralized water and twice a week initial soil moisture level was re-set by weighing. After two months plants were harvested by clipping the shoots at soil surface. Shoot dry weight of each microcosm was determined after drying at 70 °C for minimally 72 hours and shoots of the mixed plant communities were sorted, dried and weighed to species. After harvest the soil of each separate microcosm was homogenized while removing node-bearing plant fragments and rhizomes to prevent re-growth. The roots were left in the soil as to preserve the microbial communities that had been developed in the rhizosphere.

Experiment 1b

In order to determine the soil feedback effect on the six early-successional plant species seedlings of each species were planted in their home and foreign soil originating from experiment 1a. For each of the six early-successional species, feedback effects were tested in two competitive environments: 1) in monocultures and 2) in mixed communities with heterospecifics (Fig. 5.1). Monocultures were composed of 25 seedlings of an early-successional species. Mixed communities were composed of 5 seedlings of an early-successional species plus 5 seedlings each of four mid-successional plant species. Figure 5.1 shows a diagram of the experimental set-up for *Poa annua*. The experimental design involved 6 early-successional plant species x 2 soil histories (home and foreign) x 2 competitive environments (monocultures providing intraspecific competition and mixed communities with heterospecifics providing interspecific competition) x 5 replicates = 120 microcosms. Each of the replicates of the mixed plant communities had a randomly defined plant configuration to avoid positioning effects. Three weeks after establishing the experiment, in each microcosm shoot length of 5 early-successional individuals was measured. In the monocultures of each replicate, we selected the individuals at positions that corresponded with the positions of their conspecifics in the mixed community of that replicate. Plants were grown for two months and harvested as in experiment 1a.

Experiment 2

In order to single out the contribution of the microbial soil community to the observed plant-soil feedback patterns in experiment 1b, we collected the soil micro-organisms from the monocultures on home soils of each early-successional plant species. The experimental design involved 10 species (6 early-successional species and 4 mid-successional species) x 7 treatments (6 early-successional inocula and 1 control treatment) x 5 replicates = 350 pots. Microbial inocula of each early-successional plant species were made by adding 15 l tap water to 15 kg home treatment soil (Bezemer et al. 2005). These mixtures were left for 5 h to enable large particles to sink, after which the supernatant was sieved through a 75-mm mesh, followed by two sieves of 45mm and two sieves of 30 mm. This method omitted the micro-arthropods and nematodes, as

well as mycorrhizal fungi (Ames *et al.* 1987), but let most other micro-organisms in the suspension pass through.

Soils on which either in the first or the second stage of experiment 1 mixed communities had been grown (Fig. 5.1) were aggregated and node-bearing plant fragments and rhizomes were removed. The aggregated soil was homogenized, sterilized by g- irradiation (25 kGy) and a subsample was analyzed for nutrients (Appendix 5B). Pots of 11 cm high, Ø 9-13 cm (basis-top) were filled with 900 g of sterilized soil and monocultures of all early- and mid-successional species were established (5 seedlings per pot). At the second, third and fourth day after planting, the pots were injected with a total of 145 ml microbial inoculum originating from soil of one of the six early-successional species or, as a control, 145 ml of tap water. Plants were grown as in experiment 1. After two months the plants were harvested by clipping the shoots at soil surface and roots were washed from the soil. Shoot and root dry weights were determined after drying at 70 °C for minimally 72 hours. Soil chemical analyses were made for the original six soil batches (Appendix 5C), as well as for the soil inocula (Appendix 5B). We analyzed NH_4, NO_3, available P, K and Mg. Microbial inocula were checked for the presence of nematodes, which were not found.

We analyzed the molecular community profiles of the soil micro-organisms in the inocula, as well as in the rhizosphere soil of *P. annua* plants in experiment 2, as this early-successional species showed strong feedback and inoculum effects. We used a culture-independent molecular approach, involving direct DNA isolation, PCR of fungal 18S rRNA and bacterial 16S rRNA genes, and Denaturing Gradient Gel Electrophoresis (DGGE) (Muyzer et al. 1993, Kowalchuk and Smit 2004). After the harvest of experiment 2, pieces of young roots of *P. annua* with the remaining adhering soil were collected for all treatments. The roots were chopped into 1 cm pieces and homogenized in order to obtain rhizosphere samples. DNA was extracted from 0.25 g sub-samples of *P. annua* rhizosphere as well as from 0.25 ml sub-samples of the microbial inocula using a Power Soil Kit (Mo BIO Laboratories, Carlsbad, California, USA) and used for Polymerase Chain Reactions (PCR). Fragments of bacterial 16S ribosomal DNA were amplified with the primers 968-CG and 1378r (Heuer et al. 1997, 1999). Fragments of fungal 18S ribosomal DNA were amplified with the primers Fr1GC and FF390 (Vainio and Hantula 2000). For bacterial DNA we used the Acter 5535 program and for fungal DNA the fun47-40 touch-down cycling program (both programs from PTC-200, MJ Research, Biorad Veenendaal, the Netherlands, Appendix 5D). Amplification reactions were performed in a volume of 25 µl and consisted of 15nmol/L each primer, approximately 50 ng of environmental template DNA, 1 U Expand High Fidelity DNA polymerase (Boehringer, Mannheim, Germany) and the manufacturer's recommended nucleotide concentrations and buffer conditions. PCR products were used for DGGE analysis, using the methods of Muyzer et al. (1993) for bacterial products and the method of Kowalchuk and Smit (2004) for fungal products. Linear gradients from 45 to 65% and from 40% to 55% denaturant were used for bacterial and fungal products, respectively.

Data analysis

Plant-soil feedback of the early-successional plant species was calculated as $100*(B_{home} - B_{foreign})/(B_{foreign})$, where B_{home} is shoot biomass in an individual replicate home soil and $B_{foreign}$ is the mean shoot biomass of all five replicates in foreign soil of that particular early-successional plant species. Feedback responses were calculated using data of experiment 1b, both for monocultures and for early-successional plant species in the mixed plant communities. The same formula was used to calculate feedback effects after three weeks of experiment 1b substituting plant length for biomass. Feedback responses were analyzed using maximum likelihood estimation (type III, PROC MIXED (SAS, Cary, North Carolina, USA) with design (monoculture or heterospecific competition), plant guild (grass or forb), and design X plant guild as fixed factors, and plant species nested within plant guild and design X species(plant guild) as random factors. Because the variance components of the random effects were not significant, only effects of fixed factors are reported and discussed. The measures of feedback response met the parametric assumption of normality (Kolmogorov-Smirnov tests, $P > 0.05$). The assumption of homogeneity of variances was met at the $P > 0.01$ level (Cochran's, Hartley's & Bartlett tests). In separate analyses, for each plant species, differences in feedback responses between monocultures and mixed plant communities were analyzed using one-way GLM with design as fixed factor. t-Tests were used to analyze if feedback responses differed from zero. To rule out that feedback responses may have been affected by differences in biomass production between home and foreign soil treatments in the first stage of the experiment (Appendix 5E), the difference in biomass production between home and foreign treatments in experiment 1a was used as a covariate in the PROC MIXED model. Further, we used linear regression to check for relationships between shoot biomass in the first and the second stage of the experiment. Principal Component Analysis (PCA) and Redundancy Analysis (RDA) (499 unrestricted permutations, CANOCO, V. 4.5, Ter Braak and Šmilauer 1998-2002) were used to test contingency effects as a result of the different early-successional plant species that had been grown on the soils in the first stage of the experiment. We analyzed the effects of soil histories caused by the early-successional plant species on the composition of mid-successional plant species in the second stage of the experiment. Because the identity of the competing early-successional species changed in unison with the treatments (i.e. the soil history), RDA was carried out both with and without adding shoot biomass of the competing early-successional species as covariate. Similarly, we tested the effect of total shoot biomass production in the first or second stage of the experiment. For PCA and RDA biomass data for each individual microcosm were used.

For each plant species, effects of microbial inocula (experiment 2) were calculated as inoculum effect = $100*(B_i - B_c)/(B_c)$, where B_i is total root + shoot biomass of each replicate with microbial inoculum added and B_c is mean total root + shoot biomass of the five replicate sterilized controls. For early-successional plant species, inocula effects were analyzed using maximum likelihood estimation (type III, PROC MIXED) with inoculum species (i.e. the early-successional plant species from which the inoculum originated) as fixed factor, and plant species and plant species X inoculum species as random factors. Contrasts between inocula from conspecific and heterospecific origin were tested within the observed levels of the random effect

(narrow inference space), i.e. within the species levels. For mid-successional plant species, inocula effects were analyzed utilizing two distinctive procedures. First, we used a GLM with plant guild (grass or forb), inoculum guild (originating from early-successional grass or early-successional forb), plant species nested within plant guild, and inoculum species nested within inoculum guild as fixed factors. The following interactions were included: plant guild X inoculum guild, plant guild X inoculum species(inoculum guild), and inoculum guild X plant species (plant guild). Second, we used maximum likelihood estimation (type III, PROC MIXED) with plant guild, inoculum guild and their interaction as fixed factors, and plant species nested within plant guild and inoculum guild X plant species(plant guild) as random factors. In separate analyses, for each plant species, statistical differences between inocula were analyzed using Tukey's hsd post-hoc tests after one-way GLM with inoculum origin as fixed factor. The measures of inoculum effects met the parametric assumptions of normality (Kolmogorov-Smirnov tests, $P > 0.05$) and homogeneity of variances (Cochran's, Hartley's & Bartlett tests, $P > 0.05$). For each plant species inocula effects were tested to differ from zero with Bonferroni-adjusted t-tests.

Bacterial and fungal DGGE gels were read into the ImageMaster Elite program (Version 4.20, Amersham Pharmacia Biotech, Piscataway, New Jersey, USA). Matching of bands was performed in reference to a hypothetical composite lane containing bands at all positions found across the entire dataset. Comparisons of bacterial and fungal DGGE profiles used Pearson's similarity indices for each pair-wise lane comparison, taking both band number and intensity into account after background subtraction and signal normalization using the ImageMaster Elite Database program (Version 2.0, Amersham Pharmacia Biotech, Piscataway, New Jersey, USA). PCA was carried out on bacterial and fungal DGGE profiles. Effects of inoculum origin on bacterial and fungal rhizosphere community composition were tested in RDA with Monte Carlo permutation tests. The relationship between microbial rhizosphere community composition and biomass production of *P. annua* (root, shoot and total) was determined by linear regression analysis using the first and second axis sample scores derived from PCA carried out on bacterial and fungal DGGE profiles.

Results

Feedback effects (experiment 1)

Plant-soil feedback responses of the early-successional species were generally negative and significantly increased when the plants were grown in a competition with heterospecifics (design: $F_{1,4} = 25.80$, $P < 0.007$; Fig. 5.2). Over all plant species, plant-soil feedback reduced biomass production by 16.8 % in monocultures and by 67.1 % when grown with heterospecific competition. Individual species differed somewhat in their responses. Three of the six early-successional plant species experienced a negative feedback (t-tests, $P < 0.05$, Appendix 5F) from their soil community when plants were grown in monocultures (Fig. 5.2). For *A. geniculatus*, *C. canadensis* and *V. arvensis* the feedback response in monocultures did not differ significantly from zero (t-tests, $P > 0.05$, Appendix 5F). When grown in competition with heterospecifics, all early-successional species experienced a negative feedback (t-tests, $P < 0.05$, Appendix 5F) from their

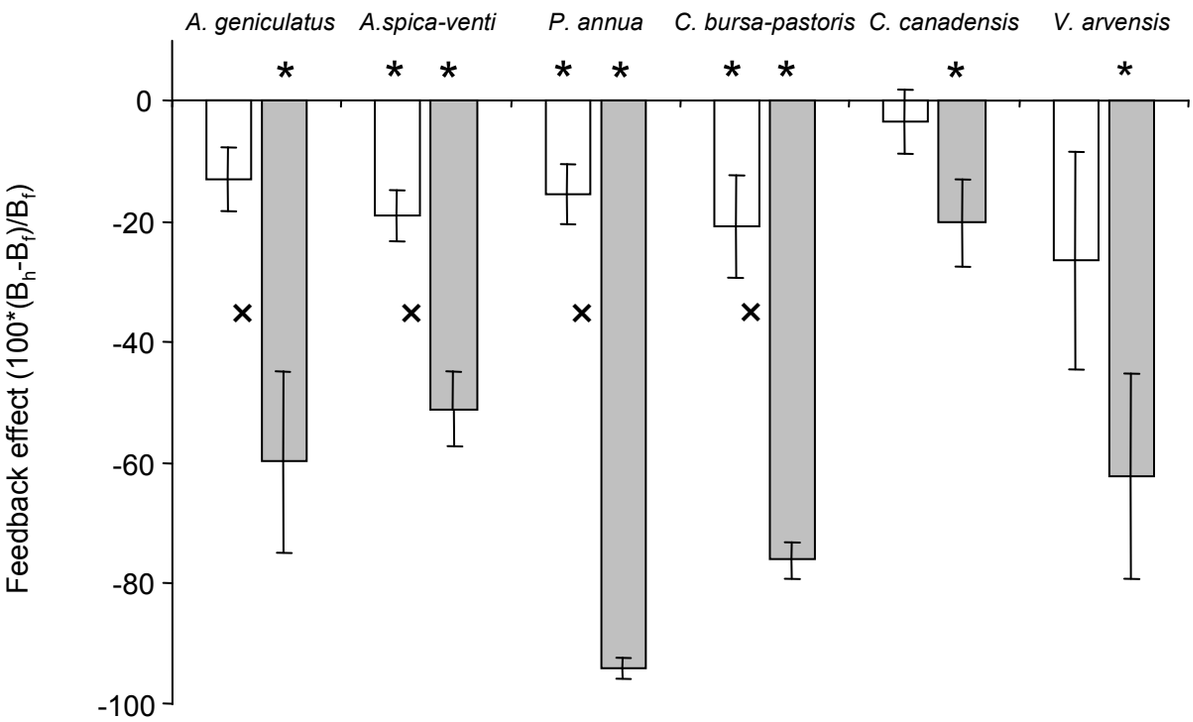

Figure 5.2 Change in shoot biomass production of early-successional plant species when grown in soil previously colonized by conspecifics (home soil) as compared to soil in which heterospecific species have been growing (foreign soil). White bars indicate feedback effects in monocultures and grey bars indicate feedback effects of the early-successional species in mixed communities with mid-successional species (e.g with heterospecific competition). B_h = biomass in home soil, B_f = mean biomass in foreign soil. Data are mean ± s.e. * Indicates feedback effects significantly different from zero (t-tests, $P < 0.05$). Ð Indicates significant difference (One-way ANOVA / Mann-Whitney U-test, $P < 0.05$) between feedback effects in monocultures and in mixed communities.

soil community. For *A. geniculatus*, *A. spica-venti*, *P. annua*, and *C. bursa-pastoris*, the soil feedback was significantly more negative when they were grown in a competitive environment then when grown as mono-specific stands (Fig. 5.2, Appendix 5G). Forbs and grasses did not differ in their feedback responses (plant guild: $F_{1,4} = 0.26$, $P = 0.64$), and there was no design X plant guild interaction ($F_{1,4} = 1.00$, $P = 0.37$).

After the first three weeks of growth the negative feedback effects were already detectable and these effects were independent of heterospecific competition (data not shown). For all treatments, total shoot biomass per microcosm was higher in the first stage of experiment 1 than in the second stage (Appendix 5E). There was no relationship between shoot biomass in the first and the second stage of the experiment for any of the plant species (for all microcosms $R^2 = 4x10^{-5}$, $P = 0.94$), which reduces the likelihood that the observed feedback effects were due to nutrient depletion. Moreover, adding the difference in biomass production between home and foreign treatments in the first stage of the experiment (Appendix 5E) as a covariate to the GLM model did not qualitatively affect our results (data not shown). Therefore, the observed plant-soil feedback effects appeared to be strongly due to interactions of the plants with pathogenic or root-feeding rhizosphere organisms.

Moreover, results from the feedback experiment showed that the history of early-successional plant species effects on soil community development in the first stage of the feedback experiment influenced the performance of the mid-successional plant species in the mixed communities (Fig. 5.3). Although, no species were consistently lost with respect to soil history (data not shown), principal component analysis (PCA) revealed clear clustering of samples of mid-successional plant species based on the early-successional species that had previously been grown on the soils (Fig. 5.3). The first and second PCA axis explained 61% and 26% of the variation in composition (i.e. biomass distribution) of the mid-successional species. The mid-successional grasses *A. capillaris* and *A. odoratum* performed less well on soils with a history of early-successional grasses, indicated by their negative correlations to soils originating from early-successional grasses and positive correlations to soils originating from early-successional forbs (Appendix 5H). Redundancy analysis (RDA) showed that the contingency effect of soil history on mid-successional plant composition was highly significant ($F = 23.19$, $P = 0.002$). These shifts in community composition could not be fully described to differences in total biomass production. Adding total shoot biomass in the first or the second stage of the experiment (Appendix 5E) as covariates in RDA resulted in a reduction in the sum of all canonical eigenvalues from 0.718 to 0.661 and 0.497, respectively. Adding the shoot biomass production of the competitive early-successional species in second stage of the experiment (Appendix 5E) resulted in a sum of all canonical eigenvalues of 0.602. When using one or combinations of these covariates, soil history still explained a highly significant amount of the variation ($P = 0.002$), indicating legacy effects of changes in soil biological properties as affected by the early-successional species influencing the dynamics of mid-successional plant communities. In contrast to the effects of monoculture soils, the soils of the mixed communities of early-successional plant species (e.g. foreign treatments) did not reveal any distinct clustering in PCA, and RDA did not show a significant effect of soil history (data not shown).

Inoculum effects (experiment 2)

Inoculum effects for early-successional plant species were generally neutral to strongly negative (Fig. 5.4, Appendix 5I) and the plants were affected by microbial inocula from conspecific origin more than by inocula from heterospecific origin (Fig. 5.4, Table 5.1). Irrespective of con- and heterospecificity, inocula effects could not be attributed to the specific origin and neither was the variance component of the random plant species effect significant (Table 5.1). However, the variance component of the random plant species X inoculum species interaction was highly significant (Table 5.1). Therefore, comparisons between conspecific and heterospecific inoculum origin were tested within plant species. The complementary contrast analyses showed that five of the six species were stronger affected by inocula from conspecific origin than by inocula from heterospecific origin (Table 5.1). For example, the microbial inoculum from conspecific origin reduced biomass of *A. geniculatus* by about 60% compared to the control without inoculum, whereas the microbial inocula from the other plant species (heterospecific origin) reduced biomass of *A. geniculatus* no more than 30-40%. The microbial inoculum of *V. arvensis* caused 65% conspecific reduction in biomass production, whereas the heterospecific inocula were not affecting plant biomass. Opposite to all other plant species, heterospecific microbial inocula

positively influenced biomass production of *C. bursa-pastoris*, while the conspecific inoculum had a neutral effect (Fig. 5.4, Appendix 5I). The non-native forb *C. canadensis* did not differ in its response to inocula from conspecific or heterospecific origin.

Effects of microbial inocula originating from early-successional plant species on the performance of mid-successional plant species differed strongly between grasses and forbs (GLM, plant guild: $F_{1,15} = 117.13$, $P < 0.0001$), although there was also inter-species variation within plant guilds (GLM, plant species(plant guild): $F_{2,15} = 12.40$, $P < 0.0001$). Furthermore, irrespective of the species origin (GLM, inoculum species(inoculum guild): $F_{4,15} = 0.25$, $P = 0.91$), inocula from early-successional grasses showed stronger effects than inocula from early-successional forbs (inoculum guild: $F_{1,15} = 29.54$, $P < 0.001$). However, grasses and forbs differed in their responses to inoculum guild. Supporting the results from the feedback experiment (Fig. 5.3, Appendix 5H), biomass production of the mid-successional grasses *A. capillaris* and *A. odoratum* was reduced significantly more by inocula from early-successional grasses than from early-successional forbs (Fig. 5.5). In contrast, the mid-successional forbs *A. millefolium* and *P. lanceolata* were far less influenced by the microbial inocula and they did not respond differently to the inocula from early-successional grasses and forbs (Fig. 5.5), as indicated by the significant interaction between plant guild and inoculum guild (GLM, $F_{1,15} = 5.81$, $P = 0.0177$). The nested interaction terms plant guild X inoculum species(inoculum guild) and inoculum guild X plant spe-

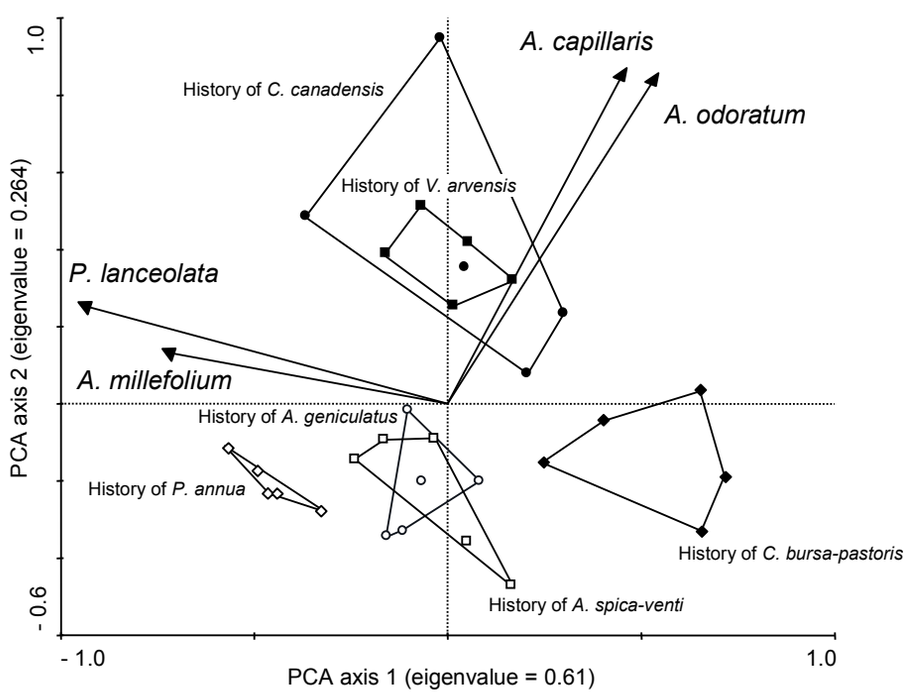

Figure 5.3 Ordination diagram (species-samples biplot) of PCA for mid-successional plant species (grasses: *A. capillaris*, *A. odoratum*; forbs: *P. lanceolata*, *A. millefolium*) grown in soil with histories of early-successional plant species. Each data point represents one microcosm. Open symbols for soils with histories of grasses (○ = *A. geniculatus*, □ = *A. spica-venti*, ◇ = *P. annua*) and solid symbols for soils with histories of forbs (● = *C. canadensis*, ■ = *C. bursa-pastoris*, ◆ = *V. arvensis*). Envelopes are drawn around samples with replicate soil histories. Eigenvalues along the axes indicate the amount of explained variability in species composition.

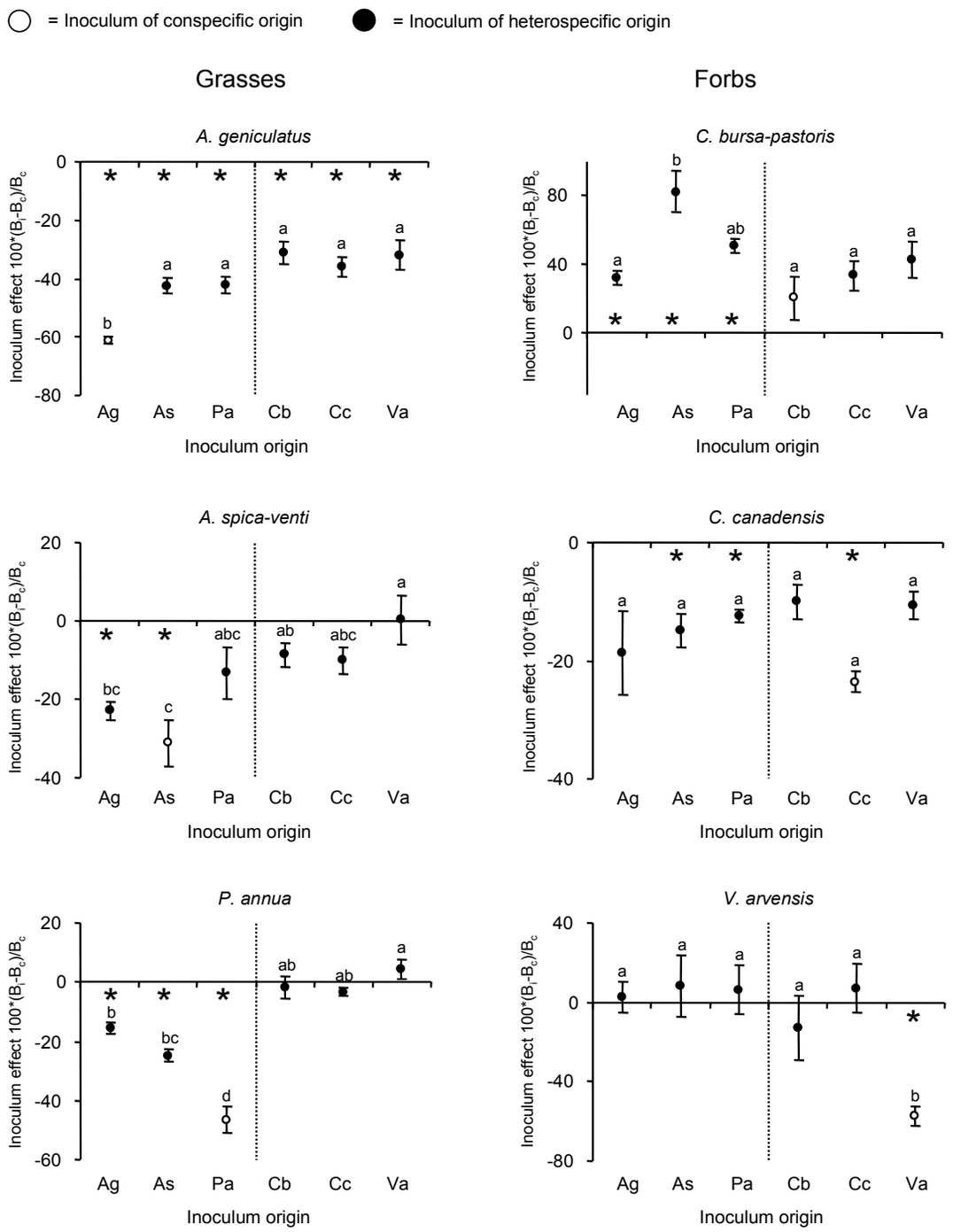

Figure 5.4 Biomass production of early-successional grasses (left column of panels) and forbs (right column of panels) in soil with microbial inocula relative to soil without these inocula. The inocula originated from soils with histories of conspecifics (white symbols) and heterospecifics (black symbols), as indicated by first letters of genus and species names (Ag = *A. geniculatus*, As = *A. spica-venti*, Pa = *P. annua*, Cb = *C. bursa-pastoris*, Cc = *C. canadensis*, Va = *V. arvensis*). B_i = biomass with inoculum added, B_c= mean biomass in sterilized control. Data are mean ± s.e. * Indicates inoculum effect significantly different from zero (Bonferroni-adjusted t-tests, $P < 0.05$). Different letters denote significant differences between means (One-way ANOVA, Tukey posthoc tests, $P < 0.05$). Dotted lines divide between inocula originating from grasses (Ag, As, Pa) and forbs (Cb, Cc, Va).

Table 5.1 Results from PROC MIXED analysis of effects of inoculum origin on plant biomass production relative to the sterilized control treatment (i.e. inoculum effect).

Effect	DF_{num}	DF_{denom}	F-value	*Z*	*P*
Inoculum species	5	25	0.21		0.9538
Plant species				1.47	0.0714
Plant species X inoculum species				3.03	< 0.0001
Conspecific-heterospecific origin contrasts					
A. geniculatus	1	25	10.04		0.0040
A. spica-venti	1	25	5.89		0.0228
P. annua	1	25	23.39		< 0.0001
C. bursa-pastoris	1	25	11.56		0.0023
C. canadensis	1	25	1.39		0.2487
V. arvensis	1	25	55.52		< 0.0001
Overall	6	25	18.31		< 0.0001

Notes: Inoculum species was included as fixed factor in the model, plant species and plant species X inoculum species as random factors. Contrasts between inocula from conspecific and heterospecific origin were tested within the observed levels of the random plant species effect.

cies(plant guild) were not significant (GLM, $F_{4,15} = 0.42$, $P = 0.79$ and $F_{2,15} = 2.44$ $P = 0.09$, respectively). Although the results indicate at selective legacy effects of the early-successional species on the mid-successional species, some prudence with respect to generalization may be appropriate. Actually, the PROC MIXED procedure, including plant species nested within plant guild as random factor, revealed only marginally significant effects of plant species and inoculum species on the inoculum effect of mid-successional plant species, whereas the interaction between plant guild and inoculum guild was not significant (Appendix 5J).

DGGE analysis

Similarity in microbial composition between inoculum samples and the respective rhizosphere samples was low (mean Pearson's index = 0.59 ± s.d. 0.04 for fungal profiles and 0.49 ± s.d. 0.03 for bacterial profiles), indicating at shifts in dominant species in the microbial communities since the start of the experiment. DGGE on PCR products of amplified microbial rRNA from the inoculum samples produced 3 to 7 detectable bands for fungi and 7 to 14 bands for bacteria. Similarity between inoculum samples was significantly higher for bacteria than for fungi (mean Pearson's index = 0.96 ± s.d. 0.02 and 0.80 ± s.d. 0.15, respectively; Mann-Whitney U-test, Z = -3.96, P < 0.01). DGGE profiles for *P. annua* rhizosphere samples contained 3 to 13 fungal bands and 2 to 17 bacterial bands. PCA of bacterial and fungal rhizosphere patterns revealed high within-treatment variation (Appendix 5K). The bacterial profiles did not reveal any clustering of samples and strongest variation was observed in the control samples. Inoculum origin explained 17.9% of the variation in bacterial rhizosphere profiles, but this was not significant (RDA, Monte-Carlo permutation tests for all canonical axes, F-ratio = 1.018, P = 0.41). PCA on fungal profiles showed weak clustering of the control samples, however, inoculum samples could not be distinctly separated from each other (Appendix 5K). Inoculum origin explained

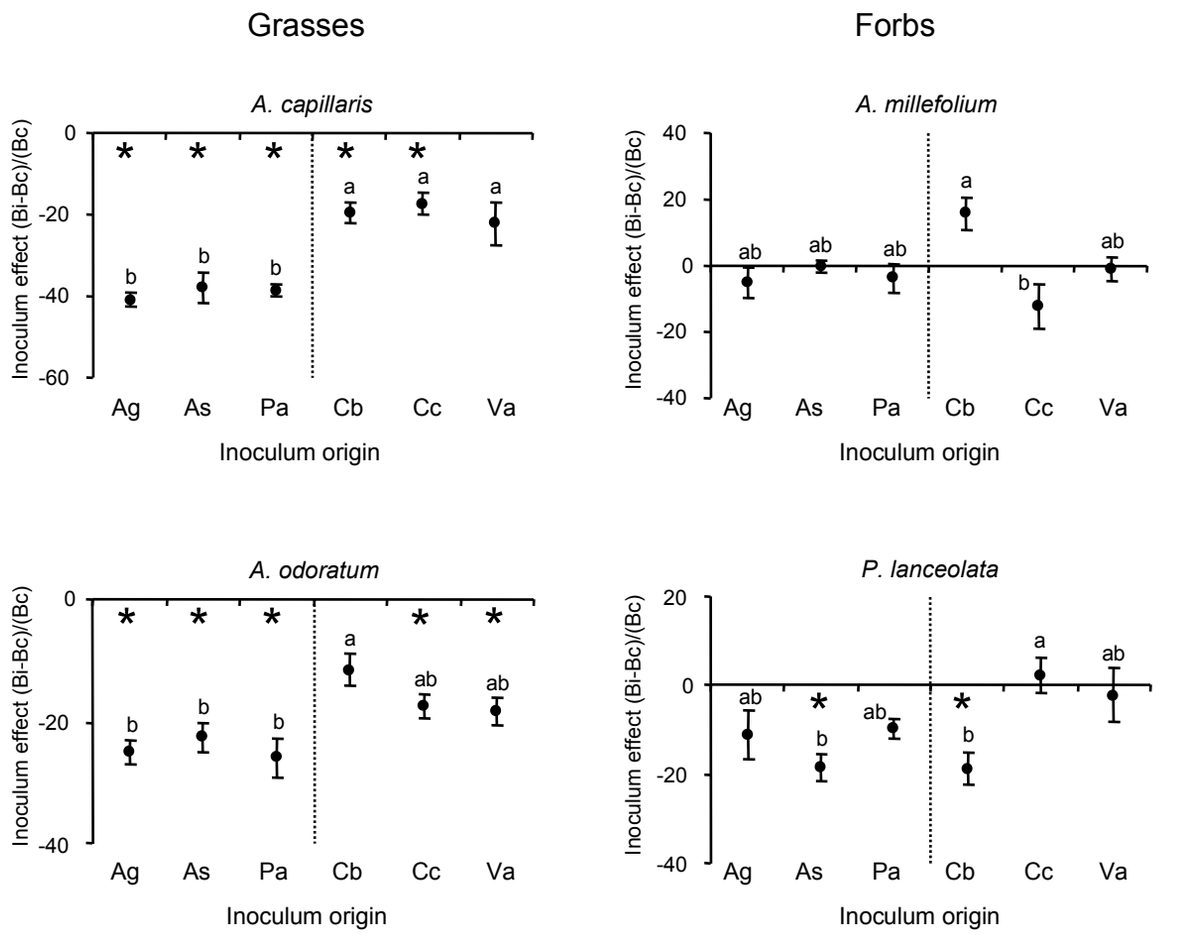

Figure 5.5 Biomass production of mid-successional grasses (left row of panels) and forbs (right row of panels) in soil with microbial inocula relative to soil without these inocula. The inocula originated from soils with histories of early-successional plant species, as indicated by the first letters of genus and species names (Ag = *A. geniculatus*, As = *A. spica-venti*, Pa = *P. annua*, Cb = *C. bursa-pastoris*, Cc = *C. canadensis*, Va = *V. arvensis*). B_i = biomass with inoculum added, B_c= mean biomass in sterilized control. Data are mean ± s.e. * Indicates inoculum effect significantly different from zero (Bonferroni-adjusted t-tests, $P < 0.05$). Different letters denote significant differences between means (One-way ANOVA, Tukey posthoc tests, $P< 0.05$). Dotted lines divide between inocula originating from grasses (Ag, As, Pa) and forbs (Cd, Cc, Va).

20.7% of the variation in fungal rhizosphere profiles, which was not significant (RDA, Monte-Carlo permutation tests for all canonical axes, F-ratio = 1.220, P = 0.15).

PCA clustering revealed a significant relationship between fungal rhizosphere community DGGE and total biomass production of *P. annua* for the first axis (Table 5.2). This indicates that the observed microbial inocula effects can be due to changes in the presence or abundance of dominant fungal species in the rhizosphere. However, the relationship between shoot biomass and PCA sample scores from both the first and the second axes was significant, while this was not the case for root biomass. There was no relationship between bacterial rhizosphere community DGGE clustering and biomass production of *P. annua* (Table 5.2).

Table 5.2 Relationship between biomass production of *P. annua* (root, shoot, and total) and microbial rhizosphere community composition.

	Fungal profile				Bacterial profile			
	First PCA axis		Second PCA axis		First PCA axis		Second PCA axis	
Biomass	R^2	P-value	R^2	P-value	R^2	P-value	R^2	P-value
Root	0.040	0.25	0.006	0.65	0.006	0.66	0.006	0.66
Shoot	0.189	0.01	0.174	0.01	0.003	0.93	0.063	0.15
Total	0.130	0.03	0.084	0.09	0.001	0.87	0.036	0.28

Note: Tested with linear regression using first and second axis sample scores derived from PCA on bacterial and fungal DGGE profiles.

Discussion

Biotic plant-soil feedback effects are known to influence plant performance and competitive ability (Van der Putten et al. 1993, Bever 1994, Van der Putten and Peters 1997). Our results show that plant-soil feedback dynamics in pioneer stages of secondary succession result in a historical contingency effect that can enhance the rate of succession to later-successional plant communities. The legacy effects of the early-successional plant species on the soil microbial community have long-lasting effects, which may influence plant performance and population dynamics in further stages of secondary succession. Therefore, plant community dynamics at any stage of ecosystem development may in part reflect soil community influences from the past. These results are of major importance to better understand the dynamics in natural plant communities.

Biomass production of early-successional plants was significantly reduced in soil that had been cultured by conspecifics compared to soil that had been cultured by heterospecifics. These negative plant-soil feedback effects could arise when the presence of specific plant roots or root exudates cause selective increase in the density of selective, or host-specific pathogens. These pathogens will have overwhelmed potential beneficial effects of rhizobacteria and arbuscular mycorrhizal fungi. For common grassland species, such feedbacks from the soil community to individual plant species have been suggested to result from build-up of pathogenic soil bacteria and fungi (Bever 1994, Mills and Bever 1998, Westover and Bever 2001, Holah and Alexander 1999). The 'blackbox approach' of our feedback experiment did not allow us to identify the components of the soil community responsible for the observed biomass patterns. However, consistent with previous studies, also in our experiment the plant-soil feedback effects were most likely attributable to plant-specific shifts in microbial soil communities, as the results of the microbial inocula (experiment 2) were quite consistent with the feedback responses in whole soil (experiment 1). This does not exclude the role of other agents, such as nematodes (De Deyn et al. 2003, Olff et al. 2000) or other invertebrates (Gange and Brown 2002, De Deyn et al. 2003), but it shows that in soil different taxonomic groups of organisms may have similar effects.

Alternatively, the observed plant-soil feedback effects could have been the result of changes in abiotic soil properties (Berendse 1998). However, starting from soil with low organic matter content minimized potential differences in decomposition and mineralization. Moreover, differences in plant composition and biomass production between home and foreign soil treatments in the first stage of the experiment (Appendix 5E) could not explain observed reductions in biomass production. Additionally, nutrient concentrations in the inocula were low and the amount of nutrients added by the inocula relative to the total amount of available nutrients was marginal (< 5%, Appendix 5C). This supports our hypothesis that plant-specific changes in soil micro-organisms communities were involved in the observed feedback effects rather than changes in the availability of soil nutrients (Bezemer et al. 2006).

Molecular analysis of rhizosphere community of *Poa annua* pointed at plant-specific shifts in the fungal soil community as influenced by plant history. DGGE analyses were variable within treatments, but they suggested a relationship between plant biomass production and the composition of the dominant fungal species that were present in the rhizosphere. However, the causality needs further experimental exploration, which should also explain why the relationship between biomass production and the composition of the fungal rhizosphere community was stronger for shoot biomass than for root biomass. Bacterial profiles did not show any clear patterns and, moreover, microbial community profiles of the rhizosphere showed low similarities with the corresponding inocula profiles. Both fungal and bacterial DGGE profiles of the sterilized control treatments contained detectable bands as well, which is due to recolonization of the sterilized soil by airborne micro-organisms. Part of the observed variation in rhizosphere profiles, therefore, will not have been due to the inocula treatments. Furthermore, DGGE profiles may not include all micro-organisms present (Van der Wal et al. 2006b) and they do not discriminate between pathogens and non-pathogens. Therefore, classical isolation and enumeration studies are needed as a follow-up in order to detect candidate pathogens.

Our results show that the soil pathogens were selective, but probably not strongly host-specific. The (partial) biomass reduction of the early-successional species *Alopecurus geniculatus*, *Apera spica-venti*, *Poa annua* and *Conyza canadensis* by heterospecific inocula suggests that there were also more generalist pathogens involved. Non-specific plant-pathogen associations may manifest themselves frequently aboveground (Burdon 1987) and for soil pathogens it has been suggested that they were predominantly generalistic (Jarosz and Davelos 1995). Our inoculation experiment clearly shows that the observed effects were due to combinations of more and less selective (or host specific) effects, which differed among plant species. This conclusion is supported by the differential responses of the early-successional plant species to heterospecific inocula. These results might have been, at least in part, the result of plant-specific differences in the susceptibility to non-specific soil pathogens (Burdon 1987). Interestingly, the only species that did not respond disproportionately to its own soil community was *C. canadensis*, which is a non-native plant species originating from northern America. In another study, non-native plant species have shown neutral to positive soil feedback effects (Klironomos 2002), so that our results support the possibility of release from soil-borne enemies of exotic plant species in their non-native range (Klironomos 2002, Callaway et al. 2004b).

For *Capsella bursa-pastoris* we observed marked discrepancy between its feedback effect in experiment 1 and the respective inoculum effects in experiment 2. *C. bursa-pastoris* showed a strong negative feedback effect whereas the conspecific microbial inoculum did not result in reduced biomass production. Apparently, the feedback effect of *C. bursa-pastoris* may not have been caused by plant-specific accumulation of pathogenic soil bacteria and fungi. Most likely, other candidates, such plant-parasitic nematodes (Van der Putten et al. 1993, Olff et al. 2000), pathogenic effects caused by mycorrhizal fungi (Bever 2002b) or synergistic effects between different groups of pathogens (De Rooij-van der Goes et al. 1995) have been involved in the feedback response of *C. bursa-pastoris*. We did not establish if the remaining roots may have released growth reducing allelopathic compounds (Singh et al. 1999). Alternatively, some of the potential pathogens may have been susceptible to the procedure of collecting and inoculating the soil microbial inocula. Since nutrient availability was high (Appendix 5C), we do not assume competition for nutrients between plants and soil micro-organisms (Kaye and Hart 1997) to have influenced the results of our experiment.

Earlier studies on plant-soil feedback (Van der Putten et al. 1993, Bever 1994, Klironomos 2002) paid little attention to the implications of early-successional plant-soil feedback effects for long-term plant community assembling processes. Our results show two possible mechanisms by which early-successional plant soil-feedbacks can affect plant succession. First, negative plant-soil feedbacks were most pronounced when plants were grown in competition with heterospecifics, pointing at soil pathogens involved as an apparent factor in interspecific plant competition thereby enhancing competitive displacement of early-successional species by their successors (Van der Putten and Peters 1997). Enhanced feedback effects in competition with heterospecifics could not be explained by differences in total biomass production or production of the heterospecifics. Our experimental design did not allow us to ascribe differences in feedback responses of early-successional species between monocultures and mixed communities to interspecific competition from mid-successional species in particular. Enhanced feedback effects due to competitive interactions, and subsequent changes in dominance patterns, may be observed within successional stages as well.

Second, we showed that the legacy effect of feedback between plants and soil biota have much longer lasting effects on plant community development than previously supposed (Van der Putten et al. 1993, De Deyn et al. 2003). In the feedback experiment plant history had a significant effect on dominance patterns of the mid-successional species in the mixed plant communities. For example, mid-successional grasses performed less well when preceded by monocultures of early-successional grasses. We could not consider effects of soil history on mid-successional species separate from the actual presence of the early-successional species. However, the corresponding responses of the mid-successional plant species to the microbial inocula from monocultures of the early-successional plant species showed that soil micro-organisms may, at least in part, explain the ecological legacy effects of the soil (Swanson and Franklin 1992). Both the inoculation experiment and the feedback experiment indicate that among an array of plant-specific effects, the legacy effects of early-successional grasses on mid-successional grasses through soil microbial community development were more pronounced than effects on forbs.

However, other studies show plant-induced effects on soil community composition for forbs as well (De Deyn et al. 2004b, Van Ruijven et al. 2005), and when using mixed model analysis with random factors the grass effects were only marginally significant, indicating that more studies are needed in order to allow more general conclusions on guild shifts in ecosystem succession.

The potential of applying the concept of microbe-mediated plant-soil feedback in restoration ecology remains tentative (Young et al. 2005, Kardol et al. 2006). Agricultural history may strongly affect initial composition of the soil community at the start of land abandonment (Girvan et al. 2004). Variability in agricultural land use may therefore give rise to disparate plant-soil feedbacks for early-successional plants and, hence, variable effects on later-successional plant community development. While specific plant properties can have a selective effect on the organisms they accumulate in their rhizosphere (Kowalchuk et al. 2002), the abundance and composition of the soil community can be considered as the regional species pool that strongly affects the local community structure in the rhizosphere (De Ridder-Duine et al. 2005). Selective amplification of the soil community by plant roots consequently influences plant-soil feedback effects to both the offspring of the initial colonizer and to the later succession species. In contrast to primary succession, for secondary succession after land abandonment this suggests potential strong legacy effects of the former land use.

Conclusion

Traditionally, life history traits, such as propagule arrival and establishment, growth and longevity, have been assumed to sufficiently account for the pattern and endpoint of successional change without invoking an important role for any biological interactions (Egler 1954, Noble and Slatyer 1980, Walker et al. 1986). Biological interactions were proposed to be more important in determining the rate of succession than its final outcome (Walker and Chapin 1987, Kardol et al. 2006). Here, we show that plant-soil feedback effects in early stages of secondary succession enhance the rate of species replacement and, most importantly, that they provide a biotic legacy effect resulting in altered patterns of dominance in mid succession plant communities. Our results imply that stochastic colonization of early-successional plant species (i.e. initial species composition), through ecological legacy of plant soil-feedbacks, could lead to historical contingent effects in later successional plant community assembly (White and Jentsch 2004). Further studies are needed to determine how these historical contingency effects influence not only rate, but also direction of succession.

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Supplementary material (*Ecological Archives*)

Appendix 5A Abiotic soil characteristics at the start of the feedback experiment.

Appendix 5B Available nutrients in the sterilized soil and in the microbial inocula originating from soil in which monocultures of early-successional plant species had been grown in experiment 1.

Appendix 5C Nutrient availability of soils in which monocultures of early-successional plant species had been grown in experiment 1.

Appendix 5D Temperature touch-down cycling programs for bacterial and fungal PCR.

Appendix 5E Shoot biomass per pot for monocultures and mixed communities in experiment 1.

Appendix 5F Results of t-tests to analyze if feedback responses of early-successional plant species in monocultures and in mixed communities in competition with later-successional species differed from zero.

Appendix 5G Results of GLM testing the difference in feedback effects of early-successional plant species in monocultures and in mixed communities in competition with later-successional species.

Appendix 5H Factor loadings of PCA for mid-successional plant species grown in soil with histories of early-successional plant species.

Appendix 5I Results of t-tests to analyze if inocula effects for early-successional plant species and mid-successional plant species differed from zero.

Appendix 5J Results from PROC MIXED analysis of effects of inoculum origin on plant biomass production of mid-successional plant species relative to the sterilized control treatment.

Appendix 5K Ordination diagram of PCA on bacterial and fungal DGGE profiles of *P. annua* rhizosphere samples.

Appendix 5A Abiotic soil characteristics at the start of the feedback experiment. Data are mean ± s.e.

Moisture (%)	14.56	±	0.15
pH	6.57	±	0.02
NH_4^+ (mg / kg)	0.58	±	0.01
NO_3^- (mg / kg)	99.51	±	2.17
Available P (mg / kg)	15.71	±	0.93

Appendix 5B Available nutrients in the sterilized soil (mg per pot) and in the microbial inocula (mg added per pot) originating from soil in which monocultures of early-successional plant species had been grown in experiment 1.

Nutrients	Sterilized soil	Inoculum origin					
		A. geniculatus	*A. spica-venti*	*P. annua*	*C. canadensis*	*V. arvensis*	*C. bursa-pastoris*
NH_4^+	21.72	0.00	0.00	0.00	0.00	0.00	0.00
NO_3^-	0.00	0.01	0.00	0.02	0.00	0.09	0.01
P	0.67	0.03	0.03	0.03	0.03	0.03	0.03
K	11.91	0.09	0.11	0.16	0.07	0.14	0.11
Mg	28.22	0.43	0.47	0.46	0.36	0.44	0.45

Appendix 5C Nutrient availability (mg/kg) of soils in which monocultures of early-successional plant species had been grown in experiment 1.

	Plant species					
Nutrients	*A. geniculatus*	*A. spica-venti*	*P. annua*	*C. canadensis*	*V. arvensis*	*C. bursa-pastoris*
NH_4^+	5.24	3.64	3.47	1.41	2.49	2.99
NO_3^-	7.54	1.64	1.69	0.81	19.11	9.16
P	0.56	0.88	0.20	0.71	0.88	0.80
K	12.24	20.94	57.94	13.97	12.48	8.25
Mg	41.85	44.00	66.43	37.92	29.09	39.73

Appendix 5D Temperature touch-down cycling programs for bacterial and fungal PCR.

Bacterial PCR					Fungal PCR			
1	94 °C	2 min			1	94 °C	4 min	
2	92 °C	30 s			2	92 °C	30 s	
3	65 °C	1 min			3	55 °C	1min	
4	68 °C	2 min			4	68 °C	2 min	
5	go to 2	1 time			5	go to 2	1 time	
6	92 °C	30 s			6	92 °C	30 s	
7	63 °C	1min			7	53 °C	1min	
8	68 °C	2 min			8	68 °C	2 min	
9	go to 6	1 time			9	go to 6	1 time	
10	92 °C	30 s			10	92 °C	30 s	
11	61 °C	1min			11	51 °C	1min	
12	68 °C	2 min			12	68 °C	2 min	
13	go to 10	1 time			13	go to 10	1 time	
14	92 °C	30 s			14	92 °C	30 s	
15	59 °C	1min			15	49 °C	1min	
16	68 °C	2 min			16	68 °C	2 min	
17	go to 14	1 time			17	go to 14	1 time	
18	92 °C	30 s			18	92 °C	30 s	
19	57 °C	1min			19	47 °C	1min	
20	68 °C	2 min			20	68 °C	45 s	1 s / cycle
21	go to 18	1 time			21	go to 18	31 times	
22	92 °C	30 s			22	68 °C	10 min	
23	55 °C	1min						
24	68 °C	45 s	1 s / cycle					
25	go to 22	24 times						
26	68 °C	10 min						

Appendix 5E Shoot biomass (g dry weight) per pot for monocultures and mixed communities in experiment 1. Data are mean ± s.e..

				Plant species											
Exp.	Treatment	Design		*A. geniculatus*	*A. spica-venti*	*P. annua*	*C. bursa-pastoris*	*C. canadensis*	*V. arvensis*
1a		Monocultures[1]		22.36 ± 1.05	15.14 ± 0.73	25.00 ± 1.00	24.91 ± 0.62	13.16 ± 0.44	17.51 ± 1.06
		Mixed communities[2]		23.37 ± 0.71	23.45 ± 0.63	23.32 ± 0.95	20.92 ± 0.75	21.90 ± 0.77	22.06 ± 0.55
1b	Home soil	Monocultures		8.33 ± 0.51	6.44 ± 0.33	7.67 ± 0.45	6.31 ± 0.68	6.18 ± 0.33	6.35 ± 1.55
	Foreign soil	Monocultures		9.58 ± 0.40	7.95 ± 0.30	9.08 ± 0.64	7.97 ± 0.73	6.40 ± 0.15	8.63 ± 0.46
	Home soil	Mixed comm..	Early sp.	0.18 ± 0.07	0.76 ± 0.10	0.10 ± 0..03	0.68 ± 0.09	0.60 ± 0.05	0.36 ± 0.16
			Mid sp.	9.42 ± 0.30	6.62 ± 0.66	10.63 ± 0.46	7.54 ± 0.50	12.90 ± 1.28	12.49 ± 0.36
			Total	9.62 ± 0.37	10.39 ± 0.71	10.72 ± 0.45	8.22 ± 0.57	13.49 ± 1.25	12.85 ± 0.45
	Foreign soil	Mixed comm.	Early sp.	0.45 ± 0.17	1.56 ± 0.40	1.58 ± 0.16	2.85 ± 0.34	0.75 ± 0.04	0.95 ± 0.16
			Mid sp.	10.93 ± 0.57	8.27 ± 0.46	9.59 ± 0.40	6.59 ± 0.50	8.57 ± 0.70	9.93 ± 0.53
			Total	11.38 ± 0.58	9.83 ± 0.27	11.17 ± 0.47	9.44 ± 0.53	9.32 ± 0.69	10.89 ± 0.53

Exp. = Stage of the feedback experiment.
[1] Resulted in 'home soil' treatments in experiment 1b.
[2] Resulted in 'foreign soil' treatments in experiment 1b.

Appendix 5F Results of t-tests to analyze if feedback responses of early-successional plant species in monocultures and in mixed communities in competition with later-successional species differed from zero. N = 5. Shown are t-values and P-values.

	Monocultures		Mixed communities	
Plant species	t-value	P-value	t-value	P-value
A. geniculatus	-2.47	0.069	-3.96	0.017
A. spica-venti	-4.54	0.011	-8.18	0.001
P. annua	-3.12	0.035	-52.35	<0.001
C. bursa-pastoris	-3.01	0.040	-24.67	<0.001
C. canadensis	-0.67	0.537	-2.79	0.049
V. arvensis	-1.47	0.216	-3.67	0.021

Appendix 5G Results of GLM testing the difference in feedback effects of early-successional plant species in monocultures and in mixed communities in competition with later-successional species.

Plant species	$F_{1,8}$ / Z[1]	P-value
A. geniculatus	2.19	0.03
A. spica-venti	18.12	<0.01
P. annua	219.61	<0.01
C. bursa-pastoris	36.91	<0.01
C. canadensis	3.47	0.10
V. arvensis	3.00	0.18

[1] Feedback effects for *A. geniculatus* were analyzed using a non-parametric Mann-Whitney U-test.

Appendix 5H Factor loadings of PCA (i.e. correlation coefficient between treatments and species) for mid-successional plant species (grasses: *A. capillaris*, *A. odoratum*; forbs: *P. lanceolata*, *A. millefolium*) grown in soil with histories of early-successional plant species.

	Mid-successional plant species			
Soil history	*A. capillairis*	*A. odoratum*	*A. millefolium*	*P. lanceolata*
A. geniculatus	-0.255	-0.246	0.042	0.018
A. spica-venti	-0.146	-0.298	0.254	-0.153
P. annua	-0.348	-0.537	0.151	0.817
C. bursa-pastoris	0.065	0.246	-0.532	-1.001
C. canadensis	0.370	0.448	0.124	0.105
V. arvensis	0.305	0.387	-0.040	0.223

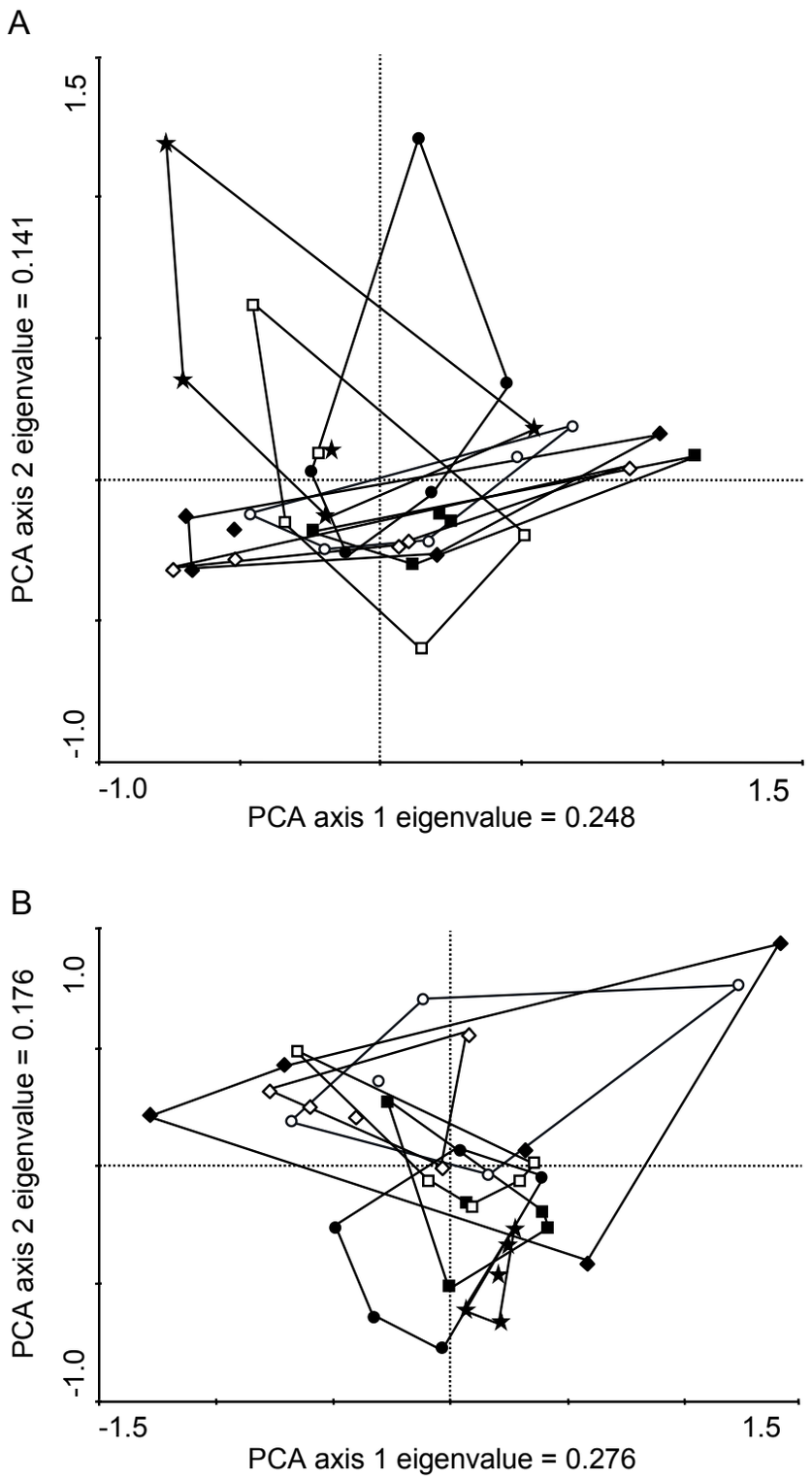
Appendix 5I Results of t-tests to analyze if inocula effects for early-successional plant species and mid-successional plant species differed from zero. Inocula effect were calculated as biomass production in soil with microbial inocula relative to soil without these inocula. N = 5. The inocula originated from soils with histories of the early-successional species *A. geniculatus*, *A. spica-venti*, *P. annua*, *C. bursa-pastoris*, *C. canadensis*, and *V. arvensis*.

	Inoculum origin											
	A. geniculatus		*A. spica-venti*		*P. annua*		*C. bursa-pastoris*		*C. canadensis*		*V. arvensis*	
Plant species	t-value	P-value	t-value	P-value	t-value	P-value	t-value	P-value	t-value	P-value	t-value	P-value
A. geniculatus	-45.80	<0.001	-16.19	<0.001	-14.23	<0.001	-8.31	0.001	-11.23	<0.001	-6.44	0.003
A. spica-venti	-9.78	<0.001	-5.31	0.006	-2.03	0.0112	-3.02	0.039	-2.92	0.043	0.03	0.978
P. annua	-7.80	0.001	-11.10	<0.001	-10.44	<0.001	-0.49	0.652	-2.27	0.086	1.26	0.277
C. bursa-pastoris	7.42	0.002	15.22	<0.001	12.64	<0.001	1.62	0.180	3.91	0.017	3.13	0.052
C. canadensis	-2.61	0.059	-5.22	0.006	-11.38	<0.001	-3.31	0.030	-13.92	<0.001	-4.54	0.011
V. arvensis	0.83	0.467	0.54	0.617	0.51	0.635	-0.80	0.470	0.57	0.596	-11.93	<0.001
A. Capillaris	-22.98	<0.001	-10.12	<0.001	-26.85	<0.001	-8.25	0.001	-6.03	0.004	-4.26	0.013
A. odoratum	-12.98	<0.001	-9.61	<0.001	-7.94	0.001	-4.442	0.012	-9.46	<0.001	8.26	0.001
A. millefolium	-1.09	0.338	-0.16	0.884	-0.86	0.440	3.26	0.0031	-1.82	0.142	-0.27	0.799
P. lanceolata	-2.03	0.112	-6.35	0.0003	-4.32	0.0012	-5.31	0.006	-0.62	0.569	-0.35	0.747

Appendix 5J Results from PROC MIXED analysis of effects of inoculum origin on plant biomass production of mid-successional plant species relative to the sterilized control treatment. Effects were tested for the grasses *A. capillairs and A. odoratum*, and the forbs *A. millefolium* and *P. lanceolata*. The inocula originated from soils with histories of the early-successional grasses *A. geniculatus*, *A. spica-venti*, *P. annua* and the early-successional forbs *C. bursa-pastoris*, *C. canadensis*, and *V. arvensis*. Plant guild (grass or forb), inoculum guild (originating from ealy-successional grass or from early-successional forb) and their interaction were included as fixed factor in the model, and plant species nested within plant guild and the interaction inoculum guild X plant species (plant guild) as random factors.

Effect	DF_{num}	DF_{denom}	F-value	z	*P*
Plant guild	1	2	9.45		0.0915
Plant species(plant guild)				0.79	0.2153
Inoculum guild	1	2	12.12		0.0735
Plant guild X inoculum guild	1	2	2.38		0.2626
Inoculum guild X plant species(plant guild)				0.61	0.2715

Appendix 5K Ordination diagram (sample plot) of PCA on bacterial (A) and fungal (B) DGGE profiles of *P. annua* rhizosphere samples. Scores are shown for inoculum treatments (origin: ○ = *A. Geniculatus*, □ = *A. spica-venti,* ◇ = *P. annua*, ● = *C. canadensis*, ■ = *C. bursa-pastoris,* ◆ = *V. arvensis*) and for the sterilized control treatment (★). Each point repesents one sample. Eigenvalues along the axes indicate the amount of explained variability in species composition.



Chapter 6

Seed addition outweighs soil fertility reduction measures in plant community development during ecosystem restoration on ex-arable land

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Abstract

Ecological restoration of species-rich ecosystems on ex-arable land is constrained by multiple limiting factors. Usually, these factors are studied one by one, whereas little is known on their interactions and hierarchies. We performed a 3-year field experiment and a short-term microcosm study to test the response of plant community development to soil fertility reduction measures, sowing mid-successional plant species and their combined effects. In order to reduce soil fertility we added carbon amendments (wood, straw), or removed the top soil (TSR) and we measured the response of plants and belowground communities of micro-organisms and nematodes.

TSR reduced soil fertility and plant biomass, and effectively suppressed arable weeds. TSR effects lasted for the entire three years. However, TSR created a harsh environment for the establishment of mid-succession plant species, resulting in low total plant species cover and ineffectiveness of sowing. Addition of straw influenced plant community composition only when combined with sowing mid-successional plant species. However, these changes in community composition could be attributed to different dominance patterns of the sown species; mid-successional species were not favored at the cost of early-successional species. Mid-successional plants also established successfully without carbon amendments. Carbon amendments, particularly straw, resulted in short-term reduction of plant biomass, suggesting a temporal decrease in plant-available nutrients. We could not demonstrate microbial nutrient immobilization, probably because the time interval of our measurements exceeded the duration of immobilization. In the microcosm experiment, carbon amendments enhanced numbers of bacterial and fungal feeding nematodes. However, in the field experiment, carbon amendments did not affect microbial biomass and did not appear to affect higher trophic levels of the soil food web, e.g. microbial feeding nematodes.

We conclude that in ex-arable soils, on a short term, seed limitation constrains ecosystem restoration more than high soil fertility. Sowing seeds was more effective to establish mid-successional plant communities than adding straw, wood, or removing the top soil. Once established, priority effects may prevail and prevent replacement of later-successional plant communities through invasions by early-successional weeds. Top soil removal is relatively expensive and resulted in loss of most of the soil organisms and relatively sparse vegetation cover.

Keywords *Above-belowground linkages, bacteria, biomass production, carbon amendments, fungi, land use change, microbial community, nematodes, propagule availability, soil fertility, soil food web, vegetation composition*

Introduction

In industrialized countries, a common practice to counteract the loss of natural habitats is conversion from high-input arable systems into low-input, semi-natural grassland systems (Brussaard et al. 1996, Walker et al. 2004). A major limitation for re-establishing species-rich vegetation is the high nutrient availability of these ex-arable soils, which results in initial domi-

nance of fast growing annual weeds and tall forbs (Marrs 1993, Hanson and Fogelfors 1998). Alternative to gradually removing or concentrating nutrients by grazing of hay making (Bakker and Berendse 1999), nutrients may be discarded by removing the entire top soil (Marrs 1985, Van Diggelen et al. 1997), or immobilized by soil microorganisms (e.g. Blumenthal et al. 2003). Another major constraint of grassland restoration is the absence of seeds of later-successional 'target' plant species (Bakker and Berendse 1999). Moreover, plant community responses to nutrient availability may depend on species availability (Forster and Dickson 2004). Therefore, grassland restoration may require an integrated approach and it may be necessary to influence major limiting factors in conjunction in order to effectively direct ecosystem development towards a reference system (Martin and Wilsey 2006).

After agricultural land abandonment, restoration projects aim at the development of low-fertile, later-successional plant communities which commonly occurred until the first half of the 20th century (Verhagen et al. 2001). The outcome of competition between fast-growing, early-successional weedy plant species and slower growing, later-successional grassland species depends on the availability of nitrogen in the soil (McLendon and Redente 1992, Marrs 1993). High nitrogen availability after cessation of agricultural practices favors the competitiveness of early-successional species over later-successional ones (Bazzaz 1979), which slows down plant community succession. Therefore, reducing nutrient availability is a widely applied management tool when restoring former semi-natural species-rich grasslands. Removal of the organic top layer down to the mineral subsoil has been proven effective in long-term reduction of plant-available nutrients and biomass production (Marrs 1985, Verhagen et al. 2001). However, removal of the upper soil layer is rather drastic and expensive (Aerts et al. 1995). Moreover, top soil removal also may lead to unwanted side effects, such as concomitant removal of soil biota and the dormant viable seed bank (Marrs 1985, Pywell et al. 2002), whereas the mineral subsoil is a harsh environment for seed germination.

Alternatively, soil nutrient availability may be reduced by microbial immobilization (McLendon and Redente 1992, Paschke et al. 2000, Blumenthal et al. 2003, Eschen et al. 2007). As carbon availability often restricts microbial biomass production (Wardle 1992), excess of nitrogen could be made limiting by adding organic carbon (Zink and Allen 1998, Grönli et al. 2005), which would lead to a reduction of plant-available nitrogen (Morgan 1994, Wilson and Gerry 1995, Schmidt et al. 1997, Averett et al. 2004, Corbin and D'Antonio 2004). Opportunistic, fast growing micro-organisms may be particularly stimulated by carbon amendments (Van der Wal et al. 2006a). However, due to their high turnover rates, their effect on nitrogen immobilization may be transient (Reever Morghan and Seastedt 1999, Huddleston and Young 2005). The groups of micro-organisms involved and the temporal extent of immobilization effects may depend on the substrate quality (higher or lower C:N ratio; i.e. recalcitrant or labile) (Török et al. 2000, Van der Wal et al. 2006a). Microbial control of immobilization and mineralization of plant-available nutrients may also depend on top-down effects of soil organisms occupying higher trophic levels of the soil food web, such as nematodes (Wardle, 2002, Wardle et al. 2005).

After decades or centuries of agricultural practice, most later-successional species are eliminated from the viable seed bank (Bekker et al. 1997). Hence, in addition to soil fertility, subsequent ecosystem restoration on ex-arable land will also depend on seed dispersal from external species pools (Bakker et al. 1996, Zobel et al. 1998). However, dispersal possibilities are generally poor and colonization and establishment of later-successional plant species is generally low (Van Diggelen et al. 1997, Verhagen et al. 2001). Moreover, plant community assembly can depend strongly on the sequence of plant species arrival (Fattorini and Halle 2004, Foster and Dickson 2004). Therefore, in case of temporal N-immobilization through carbon amendments, it may be critical at the same time to introduce seeds of later-successional species.

Our central hypothesis was that soil fertility reduction should be combined with introduction of later-successional species to enhance vegetation succession after land abandonment. In a 3-year field experiment and a short-term microcosm study, we examined the effect of carbon amendments and top soil removal with and without sowing later-successional plant species on plant community development and belowground communities of bacteria, fungi and nematodes. To test the effect of difference in substrate quality we used both wood and straw amendments. We tested the hypotheses that (i) Carbon amendment enhances microbial biomass, immobilizes plant-available nutrients and reduces plant biomass. We expected these effects to last for a relatively short period (< 1 year). (ii) Top soil removal reduces plant-available nutrients and plant biomass production for a longer term (> 3 years). (iii) Changes in the microbial community will influence secondary consumers of the soil food web, i.e. microbe-feeding nematodes. (iv) Sowing later-successional species is more effective for plant community restoration when combined with nutrient immobilization. (v) Sowing combined with carbon amendments is more effective than with top soil removal.

Methods

Site description and design of the field experiment

To examine effects of carbon amendments, top soil removal, and sowing mid-successional species on plant community succession and belowground developments, we conducted a 3-year field experiment on an ex-arable site in Assel, the Netherlands (52°21' N, 5°82 E). The site was located on coarse sandy glacial deposits and had previously been cultivated with maize (*Zea mays*). After abandonment in autumn 2002, the site was grazed by red deer and rabbits. In May 2004, we set up a field experiment with four replicate blocks (Appendix 6A). Within each block, eight treatment plots of 4 x 4 m^2 were separated by 3 m wide border rows. Blocks were located at 5 m distance from each other.

We established the following treatments: control (C), addition of straw (S), addition of wood fragments (± 1-5 cm) (W), and top soil removal (TSR). All treatments were applied in combination with and without sowing mid-successional plant species. Wheat straw (*Triticum* spp.) and birch wood (*Betula pendula*) was obtained from local suppliers and was evenly distributed over the appropriate treatments to obtain final concentrations of 2 mg C g^{-1} dry soil (*sensu* Schutter

and Dick 2001). Straw and wood amendments were mechanically disked into the top 10 cm of the soil; plots without wood or straw addition were also disked. The TSR treatments were established adjacent to the other treatments (Appendix 6A) in an area where the organic top layer was removed to a depth of 40-50 cm, exposing the mineral soil. TSR plots were not disked. The treatments were randomly applied to the plots.

For sowing we used mixtures of four perennial grasses (*Agrostis capillaris* L. (common bent), *Anthoxanthum odoratum* L. (sweet vernal grass), *Briza media* L. (quaking grass), *Festuca ovina* L. (sheep's fescue)) and four perennial forbs (*Achillea millefolium* L. (common yarrow), *Hypochaeris radicata* L. (hairy catsear), *Plantago lanceolata* L. (narrowleaf plantain), *Rumex acetosella* L. (common sheep sorrel)). All species are characteristic for mid-successional stages of secondary succession after land abandonment on sandy or sandy loamy soils in this region (Kardol et al. 2005). The seeds, provided by a specialized supplier (Cruydt-hoeck, Groningen, the Netherlands), were sown at densities of approximately 500 seeds (grasses) or 150 seeds (forbs) per m^2 (Van der Putten et al. 2000). The seeds were mixed with sand to facilitate equal sowing. After establishment, all plots were raked and rolled to bury the seeds.

Plant community

In July / August 2004, 2005 and 2006, the percentage cover of each vascular plant species was recorded in the inner 2 x 2 m^2 of each plot (Appendix 6B). The percentage cover of *Conyza canadensis*, which was the dominant weedy species at the start of the experiment, was used as indicator for weed suppression. Every year at peak standing biomass (August) the aboveground biomass was determined by clipping four (or two, 2004) 25 x 25 cm^2 subplots within each plot (Appendix 6B). At peak standing biomass in 2005 and 2006, in the center of each 25 x 25 cm^2 subplot a soil sample of Ø 5 cm and 10 cm depth was taken to determine root biomass. Roots were washed from the soil over a sieve (2 mm mesh). Above- and belowground standing biomass of each subplot was determined after drying shoot and root material at 70 °C for minimally 72 hours.

Abiotic soil parameters, bacteria, fungi and nematodes

In May 2004, immediately after establishment of the treatments, 2005 and 2006, from each plot 15 random soil samples of Ø 3.4 cm and 10 cm depth were collected, bulked, mixed, sieved using a 4 mm mesh and stored at 4°C until analysis. Subsamples were taken for physical, chemical and microbial soil properties and for isolating soil nematodes.

Mineral N was extracted by shaking 10 g soil (dry weight) with 50 ml 1 M KCl and available P and K by shaking 10 g soil (dry weight) with 100 ml 0.01 M $CaCl_2$, after which NH_4^+, NO_3^-, and PO_4^{3-}-concentrations were determined colorimetrically using a Traacs 800 auto-analyzer. Soil samples were analyzed for potential available P, P-Olsen by extraction with sodium bicarbonate (Olsen et al. 1954). The pH was measured in a 0.01 M $CaCl_2$ solution. Total N and total P were measured by digestion of samples with a mixture of H_2SO_4-Se and salicyclic acid. Total organic

carbon was determined by the Walkley-Black potassium dichromate-concentrated sulfuric acid oxidation procedure (Nelson and Sommers, 1982). Soil organic matter (SOM) was fractioned into a labile pool (extraction with 26 N H_2SO_4) and a recalcitrant pool (residual material after digestion). Soil moisture content was determined as weight loss after drying at 105 °C for minimally 24 hours.

Bacterial numbers were determined by microscopical counting of cells stained by 40,6-diamidino-2-phenylindole (DAPI) (Porter and Feig 1980), but with modifications allowing use of soil samples (Van der Wal et al. 2006a). Bacterial cells were counted using a Leitz epifluorescence microscope at 10 x 100 magnification. Ergosterol was used as an indicator of fungal biomass (Van der Wal et al. 2006b) and was measured using a disruptive extraction without saponifcation (Gong et al. 2001). Nematodes were extracted from the soil by Oostenbrink elutriators (Oostenbrink 1960) and 10% of the nematodes from the extracted soil samples were heat-killed and fixed using 35% formaldehyde diluted to 4%. Of each sample, a minimum of 150 nematodes were identified to family or genus level. The group *Dorylaimoidea* was used to specify a heterogeneous group of omnivorous dorylaimids comprising *Dorylaimidae*, *Qudsianematidae*, *Thornenematidae* and *Aporcelaimidae* (Jairajpuri and Ahmad 1992). Nematodes were allocated to feeding groups according to Yeates et al. (1993). We distinguished endo-parasitic plant feeders, ecto-parasitic plant feeders, root-hair feeders, bacterial feeders, fungal feeders and omnicarnivores, and expressed their numbers per 100 g dry soil.

Microcosm experiment

Parallel to the field experiment, we set up a microcosm experiment to examine short-term effects of carbon amendments on plant and soil nematode community development under controlled conditions. One week after we established the field experiment, random soil samples (5 cm ∅, 10 cm depth) were collected within the border rows of the plots. Soil samples were bulked for each of the two areas (TSR and non-TSR). Soil was homogenized and sieved (∅ 4 mm) to remove roots and stones. Part of the non-TSR soil was enriched with 1 cm pieces of straw (S) or with small pieces (< 0.5 cm^3) of wood (W) to obtain soil carbon concentrations of 2 mg C g^{-1} dry soil. Non-enriched, disked soil served as a control (C).

In a greenhouse, we established microcosms of 18 x 18 x 18 cm^3 filled with the field soils. Each treatment (C, W, S, and 'TSR') was replicated five times. The microcosms were randomly distributed over trolleys that were shifted position three times a week to avoid effects of microclimate differences within the greenhouse. Initial soil moisture level was set at 10 % (w:w). Each microcosm was planted with a mixed community composed of the same eight mid-successional plant species that had been sown in the experimental field. Seeds were sown on glass beads, moistened with demineralised water and placed in a germination cabinet (16/8 L/D photo regime, 18/22 ºC). Because not all species germinated at the same time, one week after germination seedlings were placed in a climate chamber at 4ºC with light according to day/night regime, until transplanting. This procedure ensured that all species were of comparable size and ontogenetic state at the start of the experiment. Each microcosm was planted with one individual of

each of the eight species. Seedlings were planted in fixed positions and each of the replicates had a different plant configuration to minimize positioning effects. The first week of the experiment dead seedlings were replaced. Throughout the experiment, light regime was minimally 16 hours of light per day and natural daylight was supplemented with metal halide lamps (225 $mmols^{-1}m^{-2}$ PAR) to ensure minimum light supply and the L/D temperature regime was 21/16 ºC. Plants were watered three or four times a week, depending on the demand. The initial soil moisture level was re-set twice a week by weighing. Seedlings originating from propagules present in the field soil were removed manually.

After 56, 128, and 198 days, shoots were clipped at 2 cm above the soil surface, sorted into species and weighted after drying at 70 ºC for minimally 72 hours. After 253 days, shoots were clipped at the soil surface and roots were washed from the soil over a sieve (2 mm mesh). Shoots were sorted to species, dried and weighted. Because root biomass could not be sorted to species, for each microcosm the total root biomass was determined. At the first, second and final harvests, three soil cores of ∅ 1 cm and 15 cm depth were collected from each microcosm. Per microcosm the soil cores were bulked and NH_4^+ and NO_3^- content was determined as described for the field experiment. Soil samples from the first and second harvest were also analyzed for available phosphate (P-Olsen). At the final harvest, five soil cores of ∅ 1 cm and 15 cm depth were collected in each microcosm, bulked and used for nematode extraction according to the procedure as described for the field experiment.

Data analysis

Microcosm experiment

For all univariate analyses (both field and microcosm experiment), the assumption of normality was checked with Kolmogorov-Smirnov tests and the assumption of homogeneity of variances with Cochran C, Hartley, Bartlett tests. If the assumptions were not met, data were transformed prior to analysis and when the assumptions were still not met a non-parametrical test was used.

Biomass production at the four consecutive harvests was analyzed using repeated measures ANOVA with treatment (C, W, S, 'TSR') as fixed factor and harvest as repeated measure. Contrasts were specified to compare treatment effects within each harvest. Differences between treatments in cumulative total shoot biomass (i.e. the sum of harvest 1-4), total root biomass and cumulative shoot biomass of the individual species were analyzed using one-way ANOVA followed by Tukey posthoc tests for individual comparisons or using a non-parametric Kruskall-Wallis test with multiple comparisons of mean ranks for individual comparisons. Prior to analysis, data on total biomass were log-transformed.

Differences between treatments (C, S, W) in the density of different nematode feeding groups were analyzed using one-way ANOVA with treatment as fixed factor, followed by Tukey posthoc tests for individual comparisons. Data on ecto-parasitic plant feeders, root-hair feeders and bacterial feeders were square-root transformed. Numbers of endo-parasitic plant feeders were close to zero, did not differ between treatments and are not presented. 'TSR' was excluded from

ANOVA because of the different initial soil condition. Effects of carbon amendments on NH_4^+ content and P-Olsen were analyzed using repeated measures ANOVA with treatment (C, W, S) as fixed factor and harvest as repeated measure. For NH_4^+ content, contrasts were specified to compare treatments within harvests. NO_3^- concentrations at harvest 2 and 4 were close to zero and were not analyzed. NO_3^- content at harvest 1 was analyzed using one-way ANOVA with treatment (C, W, S) as fixed factor. Contrasts were specified for individual comparisons.

Field experiment

Responses of the plant community to carbon amendments, top soil removal and sowing mid-successional plant species were analyzed using Principal Component Analysis (PCA). PCA is an ordination technique used to find a configuration of samples in the ordination space, so that the distances between samples in this space correspond best to the dissimilarities in their species composition. The analysis was carried out with log-transformed species cover data.

Shoot and root biomass production were analyzed using two-way repeated measures ANOVA with treatment (C, W, S, TSR) and sowing as fixed factors and year as repeated measure. Repeated measures ANOVA was followed by contrast analyses for comparisons between treatments. Analyses were performed both including and excluding TSR treatments. Shoot biomasses were log-transformed. Total cover of sown plant species and the proportion of cover by sown species in the total cover were analyzed using one-way repeated measures ANOVA with treatment as fixed factor and year as repeated measure, separately for sown and unsown treatments. Data on total cover of sown species were log-transformed. Log-transformed data on the percentage cover of *C. canadensis* were analyzed using two-way repeated measurement ANOVA with treatment (C, W, S) as fixed factor and year (2004, 2005) as repeated measure. TSR treatments were excluded from the analysis because the cover of *C. canadensis* in these treatments was negligible (0-1%). The year 2006 was excluded from the analyses, because by that time *C. canadensis* disappeared almost completely from the experimental field site. Within-year contrasts between treatments were specified separately for sown and unsown treatments.

To compare intensity and persistence of effects of carbon amendments on plant community development between sown and unsown treatments, we calculated dissimilarity in plant species composition for wood and straw treatments to the respective sown and unsown control treatments. We calculated dissimilarity to the control as Euclidean Distance (*ED*, equation 1) based on percentage cover data.

$$ED = \sqrt{\sum_{i=1}^{n} (p_i - q_i)^2} \qquad \text{[Equation 1]}$$

With n the species number, p_i the percentage cover of the i-th species in plant community p and q_i the percentage cover of the i-th species in plant community q. In general, dissimilarity among treatments was higher in unsown than in sown treatments. Therefore, to compare the dissimilarity to the control between sown and unsown treatments, ED were standardized by dividing distance measures by the mean ED among the respective control treatments. The standardized dissimilarities were analyzed using two-way repeated measures ANOVA with treatment (W, S) and

sowing as fixed factors and year as repeated measure. Data were log-transformed. T-tests were used to analyze if dissimilarity to the control for straw and wood treatment differed from 1, *i.e.* if dissimilarity to the control differed from average within-control dissimilarity.

Bacterial numbers, ergosterol content, density of nematode feeding groups and abiotic soil parameters were analyzed using two-way repeated measures ANOVA with treatment (C, W, S) and sowing as fixed factors and year as repeated measure. Contrasts were specified for individual comparisons. Data on the number of ecto-parasitic plant feeders, root-hair feeders, potassium, and soil NH_4^+ content were log-transformed. Data on omni-carnivores were square-root transformed. Because NH_4^+ content was (close to) zero in 2004 and 2005 in all treatments, these data were not analyzed. NH_4^+ content in 2006 was analyzed using two-way ANOVA with treatment (C, W, S) and sowing as fixed factors. Because of the different initial soil condition in the TSR treatments, biotic and abiotic soil parameters were analyzed separately from the other treatments, using one-way repeated measures ANOVA with sowing as fixed factor and year as repeated measure. In separate repeated measures ANOVA's we tested the single effect of top soil removal on biotic and abiotic soil parameters. Effects of addition of wood and straw on temporal changes in nematode taxa and feeding group composition were tested using Redundancy Analyses (RDA) with Monte Carlo permutation tests according to Lepš and Šmilauer (2003). Across non-TSR treatments, relationships between the number of bacteria and the number of bacterial feeding nematodes and between fungal biomass and fungal feeding nematodes were tested using linear regression, separately for each year. Linear regressions were also used to test the relationship between microbial parameters and soil moisture content.

Multivariate analyses were performed using the program CANOCO, version 4.5 (Ter Braak and Šmilauer 1998-2002), univariate analyses using STATISTICA (release 7..1, Statsoft, Inc., Tulsa, Oklahoma, USA).

Results

Microcosm experiment

The pattern of shoot biomass production over time was strongly affected by addition of carbon amendments and 'top soil removal' (Fig. 6.1). There was an overall effect of treatment ($F_{3,16}$ = 471.16, $P < 0.001$) and harvest ($F_{3,48}$ = 16.91, $P < 0.001$), but treatment effects also differed between harvests (harvest x treatment: $F_{3,48}$ = 50.14, $P < 0.001$; Fig. 6.1). At the first harvest after 56 days, shoot biomass in wood treatments was reduced by approximately 65% and in straw treatments by more than 80%. However, at the second, third and fourth harvests there were no significant differences in shoot biomass between wood, straw and control treatments, revealing a short-term reduction in biomass production of less than four months. Total shoot biomass (i.e. the sum of harvest 1-4) was not affected by carbon amendments. Total root biomass after the final harvest was higher in straw treatments than in the control and wood treatments. Shoot biomass in 'TSR' treatments was significantly lowest at all harvest dates (Fig. 6.1). Moreover,

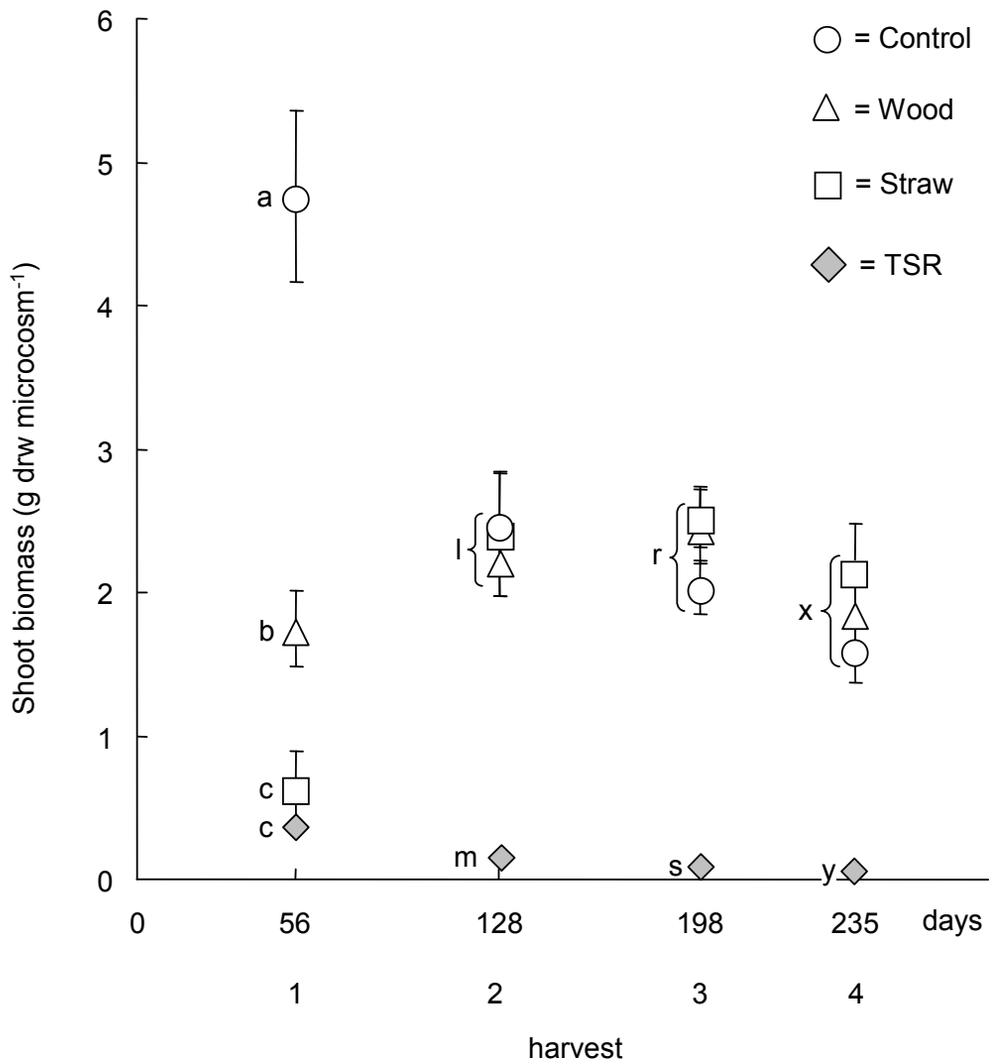

Figure 6.1 Shoot biomass (mean ± s.e) in control, wood, straw and 'top soil removal' treatments at four consecutive harvests in the microcosm experiment. Different letters denote significant differences between treatments according to contrast analyses after repeated measures anova ($P < 0.05$), separately for harvest 1 (a,b,c), harvest 2 (l,m), harvest 3 (r,s) and harvest 4 (x,y).

total shoot and root biomass after the final harvest in 'TSR' treatments were strongly reduced (Fig. 6.2), revealing a longer-term reduction (more than eight month) in biomass production.

While total shoot biomass per microcosm was not affected by carbon amendments, individual plant species responded differently to addition of straw or wood (Appendix 6C), pointing at a shift in plant community composition. For example, total shoot biomass of *Agrostis capillaris* was reduced by addition of wood, but not by addition of straw. In contrast, shoot biomass of *Rumex acetosella* and *Festuca ovina* was enhanced by addition of straw, but not by addition of wood. Shoot biomass of *Briza media* and *Hypochaeris radicata* was reduced both in wood and straw treatments. Shoot biomass of *Anthoxanthum odoratum* and *Plantago lanceolata* was not affected by carbon amendments.

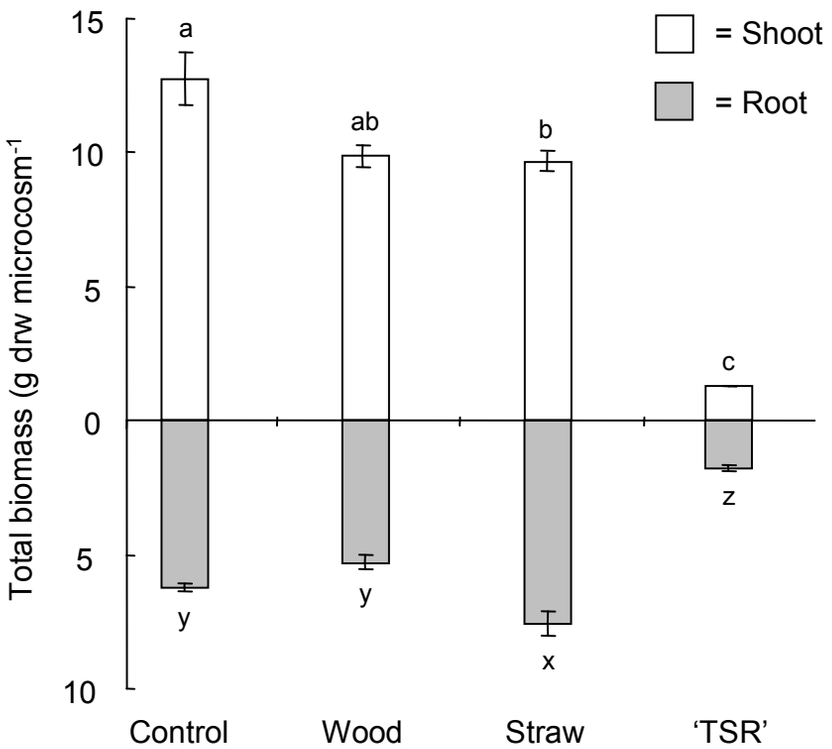

Figure 6.2 Sum of shoot biomass (harvest 1-4) and root biomass in control, wood, straw and 'top soil removal' ('TSR') treatments in the microcosm experiment. Date are mean ± s.e. Shoot biomass ($H_{3,20}$ = 15.83; P = 0.001) and root biomass ($F_{3,16}$ = 78.38; P < 0.001) differs between treatments. Different letters denote significant differences according to multiple comparisons of mean ranks (Kruskal-Wallis test) for shoot biomass and according to Tukey posthoc tests after ANOVA for root biomass.

Effects of carbon amendments on numbers of nematodes differed markedly among feeding groups (Table 6.1). Numbers of bacterial feeders were significantly enhanced in straw and wood treatments. Numbers of fungal feeders were higher in wood than in straw treatments, but in both treatments they did not differ from the control. The fungal feeder *Tylencholaimus* sp. was exclusively found in wood treatments. Omni-carnivores were not affected by carbon amendments. Root hair feeders (*Tylenchidae*) were significantly enhanced by carbon amendments and densities were particularly high in straw treatments. Numbers of ecto-parasitic plant feeders were lower in straw treatments than in wood treatments. Except for ecto-parasites, nematode numbers were extremely low in the 'TSR' treatment.

In spite of the reduced initial plant biomass, we did not find direct evidence for temporal immobilization of plant available nutrients by carbon amendments. Overall, NH_4^+ content was low (< 8 mg kg^{-1}). The effect of carbon amendments on soil NH_4^+ content changed over time (interaction treatment x harvest: $F_{4,24}$ = 3.02, P = 0.004) and contrast analyses revealed significantly enhanced levels of NH_4^+ contents in wood and straw treatments at harvest 2. At harvest 1 and 4 there were no differences between treatments (results not shown). NH_4^+ content in 'TSR' treatments was three- to five-fold lower than in other treatments. Soil NO_3^- content at harvest 1 was significantly enhanced in straw treatments, but not in wood treatments (Tukey posthoc tests

Table 6.1 Densities of nematodes per feeding group (number per 100 g dry soil) in control, straw and wood treatments in the microcosm experiment. Data are mean ± s.e. Results from ANOVA indicate overall treatment effects. Within columns, different letters denote significant differences between means based on one-way ANOVA followed by Tukey posthoc tests ($P < 0.05$). 'TSR' was excluded from ANOVA (see Methods).

Treatment	Ecto-parasites	Root-hair feeders	Bacterial feeders	Fungal feeders	Omni-carnivores
Control	22 ± 11 ^{ab}	226 ± 105 ^{c}	399 ± 32 ^{b}	34 ± 4 ^{ab}	314 ± 34 ^{a}
Straw	70 ± 27 ^{a}	1442 ± 288 ^{a}	769 ± 106 ^{a}	26 ± 13 ^{b}	306 ± 37 ^{a}
Wood	11 ± 4 ^{b}	670 ± 157 ^{b}	659 ± 60 ^{a}	72 ± 19 ^{a}	363 ± 91 ^{a}
ANOVA					
$F_{2,12}$	4.16	16.30	10.39	4.12	0.33
P	0.04	< 0.001	0.002	0.04	0.72
'TSR'	32 ± 7	5 ± 2	113 ± 28	15 ± 1	69 ± 10

after ANOVA; overall treatment effect: $F_{2,12} = 13.14$, $P < 0.001$). NO_3^- content at harvest 2 and 4 was close to zero in all treatments. NO_3^- content in 'TSR' treatments was below the detection limit. Potential available P decreased from harvest 1 to harvest 2 ($F_{2,12} = 6.97$, $P = 0.021$), but did not differ between treatments ($F_{2,12} = 1.87$, $P = 0.20$). P-Olsen in 'TSR' treatments was on average 13-fold lower than in other treatments.

Field experiment

Plant community development

Sowing mid-successional plant species markedly affected plant species composition, whereas the effect of carbon amendments was far less discriminative (Fig. 6.3). The strongest development in plant community composition took place from year one to year two. Further differences between the second and the third year were relatively small. In non-TSR treatments, vegetation in unsown plots was characterized by dominance of annual weedy species, in the first year mostly *Conyza canandensis* and *Digitaria ischaemum*, and in the second and third year mostly *Elytrigia repens*, *Cerastium fontanum* subsp. *vulgare* and *Apera spica-venti*. In the first year, the sown species were not yet fully established and the sown treatments were dominated by annual weeds as well. However, after the first year, sown treatments were strongly dominated by the sown, mid-successional species (Table 6.2). The proportion sown species of the total species cover was 94 to 99% and this was not affected significantly by carbon amendments (data not shown). However, the total cover of sown plant species, which may exceed 100% due to overlapping shoots, was significantly enhanced by addition of straw, but not by addition of wood (Table 6.2).

There was relatively little plant cover in TSR treatments, as indicated by their position close to the crossing of the first two PCA axes (Fig. 6.3). In unsown treatments total species cover increased from 1% in the first year to 5% in the second year and to 5-10% in the third year,

Table 6.2 Mean (± s.e.) total cover of sown, mid-successional plant species for control, straw, wood and top soil removal (TSR) treatments in the field experiment and F- and *P*-values of repeated measures ANOVA for main treatment effects. Repeated measures ANOVA's were performed separately for unsown and sown treatments. Different letters denote significant overall differences between treatments (contrast analyses, $P < 0.05$).

					Year 1	Year 2	Year 3
	$F_{3,12}$	*P*					
Unsown	4.52	0.024	Control	a	-	-	0.5 ± 0.3
			Wood	a	-	0.2 ± 0.2	2.3 ± 1.4
			Straw	a	0.1 ± 0.1	-	4.9 ± 4.2
			TSR	b	0.2 ± 0.1	2.1 ± 1.4	7.3 ± 2.2
Sown	140	< 0.001	Control	y	4.4 ± 1.3	75.6 ± 4.2	104 ± 2.9
			Wood	xy	3.6 ± 0.6	83.8 ± 6.9	108 ± 5.8
			Straw	x	5.4 ± 1.2	105 ± 4.4	109 ± 2.4
			TSR	z	6.1 ± 0.8	18.1 ± 1.1	18.1 ± 1.4

whereas in sown treatments total species cover increased from 5-10% in the first year to 15-20% in the second and third year. In the second and third year, the plant community in sown treatments consisted completely of sown species. However, also in the unsown plots these mid-successional species were relatively abundant (Table 6.2). In unsown plots, across years, the cover of mid-successional species was higher in TSR treatments than in non-TSR treatments.

In the first year, the percentage cover of dominant weedy species *Conyza canandensis* was significantly suppressed by straw addition ($F_{1,21} = 87.9$, $P < 0.001$), but not by wood ($F_{1,21} = 1.23$, $P = 0.27$). With straw the percentage cover was < 2%, whereas the percentage cover in control plots and plots with wood ranged from 5 to 25%. In the second year, there were no differences between control, straw and wood plots. In the first year, when the sown species had not yet fully established, sowing did not affect the percentage cover of C. *canadensis* ($F_{1,22} = 0.00$, $P = 0.99$). But in the second year, the percentage cover of *C. canadensis* was significantly reduced by sowing ($F_{1,22} = 72.44$, $P < 0.001$). The cover of *C. canadensis* was approximately 4% in unsown treatments and close to zero in sown treatments. There was no interaction between carbon amendments and sowing ($F_{1,18} = 1.44$, $P = 0.26$). In the third year, the percentage cover of *C. canadensis* was close to zero in all treatments. In TSR treatments the cover of *C. canadensis* never exceeded 1%.

In absence of sowing mid-successional plant species, dissimilarity in plant community composition between control and straw treatments was significant in the first year, after which the composition converged towards the control (Fig 6.4a). A similar pattern was observed for the wood treatment. In contrast, in presence of sowing, straw treatments remained significantly dissimilar from the control also in the second and third year (Fig. 6.4b), indicating a continued effect of straw amendments on plant community composition, probably due initial changes in plant com-

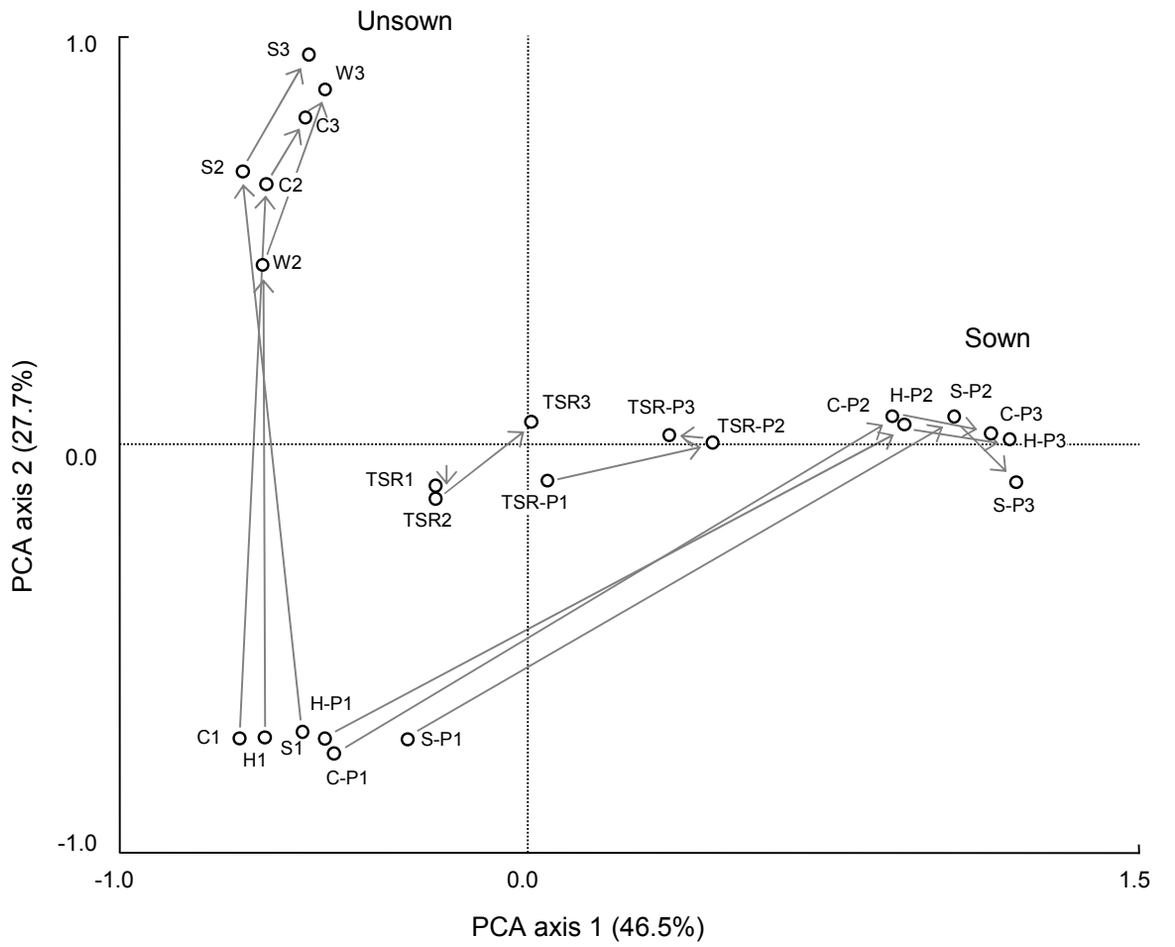

Figure 6.3 Ordination diagram of PCA for plant species composition in the field experiment. For each year centroid scores (*i.e.* weighted means of sample scores of samples that belong to the particular treatment) are shown for control (C), wood (W), straw (S), and top soil removal (TSR). 'P' in treatment codes indicates treatments including sowing mid-successional plant species. Numbers in treatment codes indicate the consecutive years of recording. Arrows indicate the temporal change in plant species composition for each treatment. Percentages along the axes correspond to the amount of explained variation in plant species composition.

munity composition. Wood treatments were not significantly different from the control (Fig. 6.4). Indeed, the dissimilarity of straw treatments tended to be more affected by sowing than the dissimilarity of wood treatments, as indicated by the marginally significant interaction between treatment and sowing (treatment x sowing: $F_{1,12} = 4.62$, $P = 0.052$). Overall, dissimilarity in plant community composition of straw and wood treatments to the control was higher in sown than in unsown treatments ($F_{1,12} = 13.02$, $P < 0.004$) and dissimilarity was higher for straw than for wood treatments ($F_{1,12} = 9.21$, $P = 0.010$).

Shoot and root biomass

Shoot and root biomass differed significantly between years and the significant year x treatment interaction (Appendix 6D) indicates that the effect of carbon amendments changed over the

years. In sown plots, straw addition significantly reduced shoot biomass in the first year, but significantly increased shoot biomass thereafter (Fig. 6.5). In unsown plots, straw addition did not affect shoot biomass. Both in sown and unsown plots wood addition did not affect shoot biomass. In sown plots, straw addition increased root biomass in the second and third year, while wood addition increased root biomass only in the third year. In unsown plots in the second year, root biomass was reduced by adding wood or straw, however, biomass was unaffected in year three. Across all years, root and shoot biomass in TSR treatments were lower than in any other treatment (Fig. 6.5).

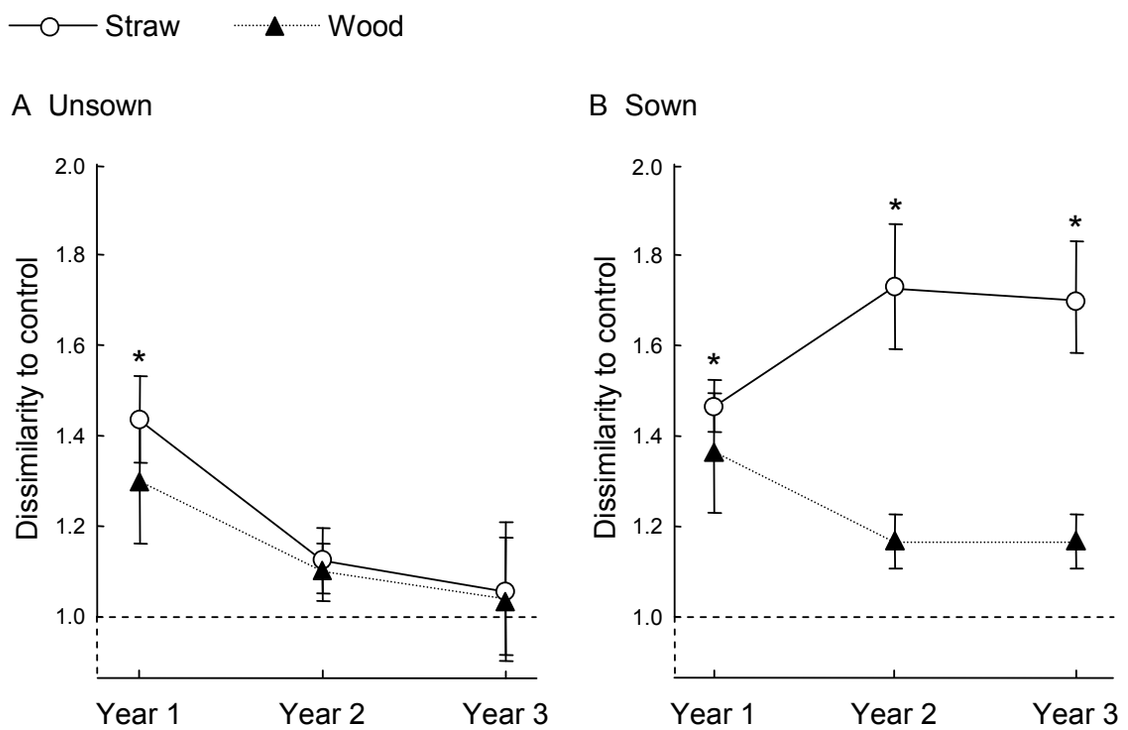

Figure 6.4 Changes in dissimilarity in plant species composition of straw and wood treatments to the control in the field experiment, calculated as standardized Euclidean Distance (mean ± s.e.) for unsown treatments (a) and sown treatments (b) in year 1, year 2 and year 3 of the field experiment. * indicates dissimilarities significantly different from 1, i.e. treatments being different from the control (t-test, $P < 0.05$).

Bacteria, fungi and nematodes

Bacterial numbers and fungal biomass were not affected by carbon amendments (Table 6.3). Bacterial numbers were higher in the second year than in the first and the third year, while fungal biomass increased after the first year. Across years, bacterial numbers were strongly related to soil moisture content (linear regression: R = 0.44, $P < 0.001$), which was not the case for fungal biomass (R = 0.14, $P = 0.24$). On average, bacterial numbers and fungal biomass in TSR treatments were two-fold and 14-fold lower, respectively, than in non-TSR treatments (data not shown). Bacterial numbers were not affected by sowing mid-successional plant species, neither in non-TSR treatments (Table 6.3) nor in TSR treatments ($F_{1,6} = 0.15$, $P = 0.70$). Sowing mid-successional plants significantly enhanced fungal biomass in TSR treatments ($F_{1,6} = 12.44$, $P = 0.012$), but not in non-TSR treatments (Table 6.3).

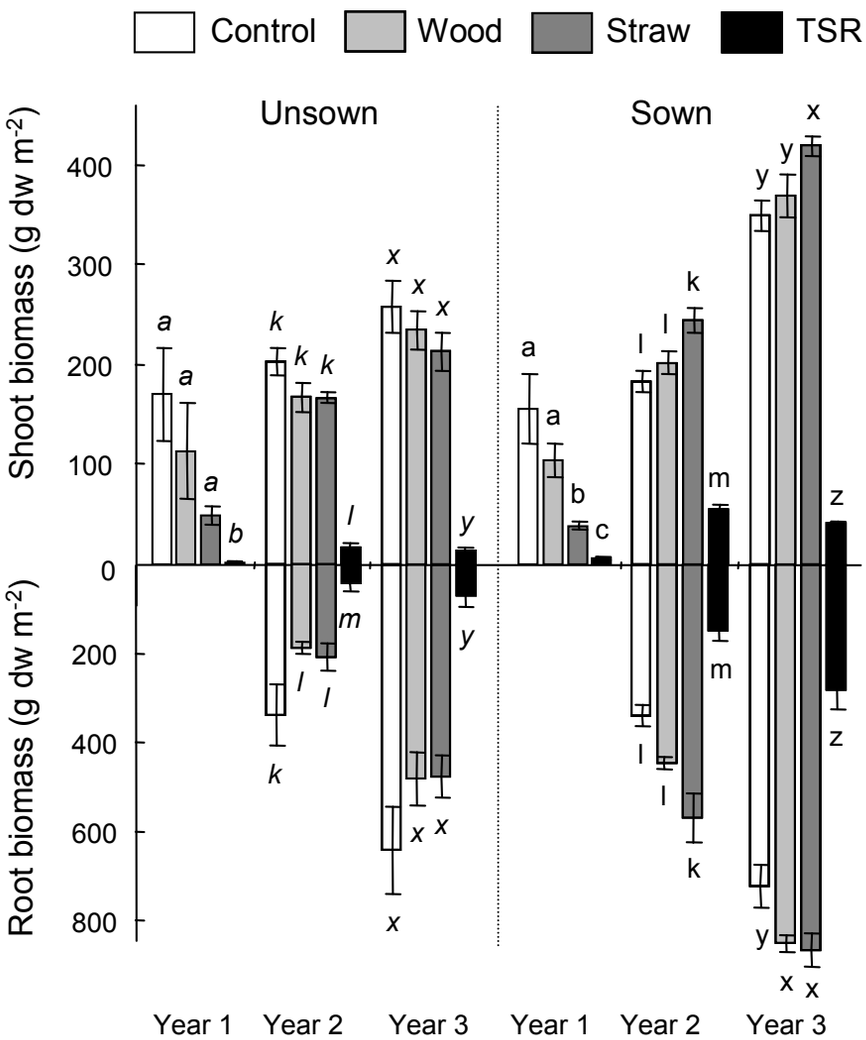

Figure 6.5 Shoot and root biomass (mean ± s.e.) in control, wood, straw and top soil removal (TRS) treatments in year 1, year 2 and year 3 of the field experiment for unsown treatments (left) and sown treatments (right) in the field experiment. Within the two sowing treatments, different letters denote significant within-year differences between treatments according to contrast analyses after repeated measures ANOVA ($P < 0.05$).

Across non-TSR treatments, numbers of bacteria were not related to bacterial feeding nematode abundance and fungal biomass was not related to fungal feeding nematode abundance (linear regression, $P > 0.05$). Corresponding to the neutral effects on bacteria and fungi, numbers of bacterial and fungal feeding nematodes were not affected by addition of wood or straw (Table 6.4). Numbers of nematodes belonging to other feeding groups were not affected by carbon amendments. Two years after the experiment was established, root hair feeders were significantly more abundant when mid-successional plant species were sown. Nematodes of other feeding groups were not affected by sowing. Independent of treatments, numbers of omnivores-carnivores and number of plant-parasitic, bacterial feeding and fungal feeding nematodes decreased after the first or second year (Table 6.4; Appendix 6E). For both sown and unsown treatments, temporal changes in taxa or feeding group composition of the soil nematode com-

Table 6.3 Results of two-way repeated measures ANOVA for bacterial numbers and ergosterol content in the field experiment with treatment (control, straw, wood) and sowing as fixed factors and year as repeated measure factor. Mean (± s.e.) bacterial numbers (times 10^8 per g dry soil) and ergosterol content (mg kg^{-1} dry soil) are shown for each year. Different letters denote significant differences between years after contrast analyses ($P < 0.05$). Top soil removal treatments were analyzed separately (see Methods).

			Bacterial numbers		Ergosterol	
Source		df	F	*P*	F	*P*
Between subjects						
Treatment (T)		2	1.58	0.23	0.57	0.57
Sowing (S)		1	1.18	0.29	0.14	0.71
T x S		2	0.02	0.98	1.01	0.38
Error		18				
Within subjects						
Year		2	77.70	< 0.001	19.92	< 0.001
	Mean ± s.e		*Bacterial numbers*		*Ergosteraol*	
	Year 1		*5.2 ± 0.4* ^{b}		*0.58 ± 0.07* ^{b}	
	Year 2		*12.2± 0.8* ^{a}		*0.91 ± 0.09* ^{a}	
	Year 3		*5.4 ± 0.4* ^{b}		*0.84 ± 0.08* ^{a}	
Year x T		4	1.79	0.15	2.15	0.09
Year x S		2	1.72	0.19	1.72	0.19
Year x T x S		4	0.67	0.62	1.40	0.25
Error		48				

munity were not affected by carbon amendments (RDA with Monte Carlo permutation tests, data not shown). Top soil removal reduced total numbers of nematodes on average 15-fold and numbers remained low across the years. In TSR treatments, numbers of nematodes were not affected by sowing mid-successional plant species (Appendix 6E).

Abiotic soil parameters

We did not observe changes in plant available nutrients by carbon amendments in the field experiment. Top soil removal led to long-term reduction in soil fertility. Across years, NO_3^- content was not affected by carbon amendments ($F_{1,18} = 14.93$, $P = 0.077$). NH_4^+ content was close to zero in the first and second year and was not affected by carbon amendments in the third year ($F_{2,18} = 0.89$, $P = 0.43$). In non-TSR treatments, available and potential available P decreased over time ($F_{2,36} = 83.79$, $P < 0.001$ and $F_{2,36} = 67.70$, $P < 0.001$, resp.), but were not affected by carbon amendments ($F_{2,18} = 0.41$, $P = 0.67$ and $F_{2,18} = 0.08$, $P = 0.92$, resp.). K increased by addition of straw (contrast with control: $F_{2,36} = 30.21$, $P < 0.001$), but not by addition of wood. Total N and total P, pH, and SOM were not affected by carbon amendments. Across years, NO_3^- content, available P and potential available P, K, total N, total P, and SOM were reduced by top soil removal, whereas NH_4^+ content was not affected. Sowing mid-

Table 6.4 Results of two-way repeated measures ANOVA for nematode feeding groups in the field experiment with treatment (control, straw, wood) and sowing as fixed factors and year as repeated measure. Contrasts were specified for significant terms. Different letters denote significant differences after contrast analyses ($P < 0.05$). Top soil removal treatments were analyzed separately (see Methods).

		Endo-parasites		Ecto-parasites		Root-hair feeders		Bacterial feeders		Fungal feeders		Omni-carnivores	
Source	df	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Between subjects													
Treatment (T)	2	0.96	0.40	0.28	0.76	0.01	0.99	0.43	0.66	0.49	0.62	0.16	0.86
Sowing (S)	1	0.58	0.46	0.69	0.42	2.48	0.13	0.03	0.86	1.69	0.21	0.71	0.41
T x S	2	0.05	0.95	0.14	0.87	0.26	0.77	0.09	0.91	1.22	0.32	0.08	0.92
Error	18												
Within subjets													
Year	2	73.45	< .001	40.55	< .001	5.78	0.006	14.75	< .0001	23.42	< 0.001	7.51	0.002
Year 1		*a*		*a*		-		*a*		*a*		*a*	
Year 2		*b*		*b*		-		*b*		*b*		*a*	
Year 3		*b*		*b*		-		*b*		*c*		*b*	
Year x T	4	1.45	0.24	2.05	0.11	1.13	0.36	2.36	0.07	1.24	0.31	1.73	0.17
Year x S	2	1.16	0.32	1.34	0.27	13.61	< 0.001	0.11	0.90	2.31	0.11	0.14	0.87
Sowing (Year 1)						*0.28*	*0.60*						
Sowing (Year 2)						*0.46*	*0.50*						
Sowing (Year 3)						*11.91*	*0.003*						
Year x T x S	4	0.94	0.45	0.77	0.55	0.48	0.75	0.27	0.89	0.22	0.93	0.19	0.94
Error	48												

successional plant species resulted in lower NO_3^- (second and third year: $F_{1,18} = 16.38$, $P < 0.001$ and $F_{1,18} = 7.04$, $P < 0.016$, resp.) and NH_4^+ content ($F_{1,18} = 24.48$, $P < 0.001$). Appendix 6F shows a complete overview of physical, chemical and microbial soil parameters in the field experiment.

Discussion

In support of our hypothesis, removal of the organic top layer substantially reduced plant biomass throughout the duration of the field experiment. Our results suggest a strong and lasting reduction in plant available nutrients and early-successional, annual weeds were almost completely absent. However, bacterial numbers and fungal biomass, as well as numbers of nematodes were very low and barely increased during the first three years, showing that top soil removal eliminated almost the entire soil community. After three years, the sown plots were far from species-rich semi-natural grasslands, probably because the bare soil was inhospitable for plant species to establish. This suggests that low plant species cover in the TSR plots was only partly due to absence of a seed bank (e.g. Verhagen et al. 2001).

Reduced biomass in treatments with straw and wood in the early phases of the microcosm and field experiments, as well as weed suppression in field plots with straw suggested a temporal decrease in plant-available nutrients due to carbon addition. Previous grassland restoration studies have shown that addition of sugar or sawdust promotes microbial immobilization of plant-available nutrients (Wilson and Gerry 1995, Reever Morghan and Seastedt 1999, Bear et al. 2003, but see Wilson et al. 2004). However, in our study, concentrations of mineral-N in the microcosm and field experiment did not reveal N-limitation. Probably, our measurements would have been more sensitive when determining N-immobilization or mineralization in the absence of living plants (Van der Wal et al. 2006a). Mineral-N concentrations in the soil are affected by rates of plant uptake, loss (e.g. denitrification) and input (N-mineralization) and may not represent the overall quantity of N available to plants (Maron and Jefferies 1999). Nevertheless, our shoot biomass results suggest that short-term N-immobilization may have occurred at the start of the experiments, however, occurring between the start and collecting the first soil samples.

The efficacy and persistence of microbial N-immobilization through carbon amendments depend on the composition (sucrose, sawdust, wood chips, etc.), quality (i.e. C:N ratio) (Török et al. 2000, Van der Wal et al. 2006a) and quantity of the added substrates (Blumenthal et al. 2003). Effectiveness of labile substrates, such as sugar and sawdust, has been shown to range from a few days (Bjarnason 1987, Degens 1998), two or three months (Reever Morghan and Seastedt 1999, Huddleston and Young 2005) to over one year (Blumenthal et al. 2003, Eschen et al. 2007). We chose more recalcitrant substrates, such as wood and straw, not only because they are inexpensive and easily available for nature managers, but also because these substrates potentially show more persistent effects. However, this was not the case here. We could also not demonstrate differences between wood and straw. Neither of the substrates had any effect on bacteria and fungal biomass in the field experiment one or two years after applying the treatments.

The short-term reduction in plant biomass, observed both in the microcosm and the field experiment, may reflect microbial responses to easy available carbon sources on the surface of the wood and straw fragments (Daniel 2003). More than two years after applying the treatments, large pieces of straw and wood were still present in the field soil (P. Kardol, pers. obs.). The actual breakdown of lignin present in wood and straw may take many years and after utilization of the easy available fraction, the remaining recalcitrant carbon may not be readily accessible for opportunistic soil micro-organisms, and can only be decomposed by slow-growing rot fungi, resulting in a slow, but long-lasting N-immobilization (Van der Wal et al. 2006a). Alternatively, we cannot rule out that effects of carbon amendments other than N-immobilization, such as direct chemical inhibition, or immobilization of nutrients other than N, could have played a role in plant responses (Blumenthal et al. 2003).

Bottom-up control of secondary consumers (Wardle et al. 1995) suggests that changes in microbial biomass would be reflected in the levels of their consumers, such as microbial feeding nematodes. Higher numbers of bacterial feeding nematodes in straw and wood treatments under controlled conditions in the microcosm experiment may indicate a trophic relationship between primary and secondary decomposers. However, in the field experiment, we did not find evidence that addition of carbon, providing the primary resource input for the soil food web (Wardle 2002), trickles-up to higher trophic levels. Moreover, along with observations of soil micro-organisms, one and two year after carbon addition in the field experiment, bacterial and fungal feeding nematodes were unresponsive to carbon amendments. Also omni-carnivores were not affected by carbon amendments. While microbial communities could have increased and decreased again within the first year of the experiment resulting in no net changes, potential responses in the nematode community were expected to last longer (Wardle 2002). Therefore, our results indicate that addition of straw or wood under field conditions does not lead to trophic cascades or structural changes in the soil food web (Wardle et al. 1995) on a 2-year timescale. Remarkably, in the microcosm experiment, the number of root-hair feeding nematodes increased in wood, and particularly in straw treatments. They may have responded to higher root biomass in the straw treatment, although root biomass in the wood treatment did not differ from the control. Probably, root hair feeders, which are more loosely associated with plant roots than other plant-parasitic nematodes, also may have fed on fungal hyphae (Okada et al. 2002), which could have been more abundant in carbon amended treatments.

Without management practices, after abandonment of high-fertile arable systems the plant community is strongly dominated by annual weeds, inhibiting colonization and establishment of 'desirable' grassland species (Hansson and Fogelfors 1998). We showed that soil fertility reduction, either by top soil removal or by addition of straw or wood substrates, reduces biomass production, potentially releasing later-successional grassland species from competitive suppression by 'unwanted', early-successional weeds. However, measures to reduce soil fertility applied alone appeared to be ineffective in enhancing establishment of later-successional plant species. Restoration of species-rich grassland on ex-arable lands has been suggested to be impeded both by high soil fertility and by seed limitation (Bakker and Berendse 1999). If true, we would expect that establishment of later-successional species in absence of fertility reduction measures would

be ineffective. Indeed, sowing mid-successional plant species was highly successful when simultaneously straw or wood was added. Nevertheless, these results did not support our hypothesis that soil fertility reduction should be combined with introduction of later-successional species to enhance vegetation succession after land abandonment: the sown species also established successfully in absence of wood or straw addition.

The sown species strongly decreased the abundance of unsown weedy plant species and comprised over 90% of the total plant cover in the third year of the experiment. Although addition of straw resulted in lasting changes in plant community composition only when simultaneously applied with sowing mid-successional plant species (Fig. 6.4), these changes could be attributed to different dominance patterns of the sown species, rather than to successional changes in community composition. This is also indicated by species-specific responses to carbon addition of these species in the microcosm experiment. Overall, our results suggest that restoration of species-rich grasslands after land abandonment is strongly constrained by recruitment limitation (Poschlod et al. 1998, Pywell et al. 2002), rather than by high initial soil fertility. Therefore, we conclude that in early stages of secondary succession after land abandonment biotic constraints outweigh abiotic constraints.

In summary, temporal soil fertility reduction by microbial N-immobilization will be ineffective in restoring species-rich grasslands when community assembly is recruitment limited and dispersal of 'target' plant species from local or regional populations is slow (Hutching and Booth 1996, Pywell et al. 2002). Top soil removal reduces soil fertility and suppresses weeds for a longer period enabling later-successional species to disperse and become established over time. However, this will be a long-term process (up to decades) and the first years after application, vegetation cover will be scarce. Importantly, our study shows that restoration of species-rich grasslands can be much less constrained by high soil fertility than previously thought (e.g. Marrs 1993, Pywell et al. 1994). Moreover, we propose that, irrespective of abiotic soil characteristics, reducing recruitment constraint by artificial introduction of later-successional species immediately after land abandonment is a highly effective measure for short-term restoration of species-rich plant communities. Sowing is low in costs and labor, and once established, priority effects may prevail and prevent replacement of later-successional plant communities through invasions by early-successional weeds (Young et al. 2001, Chase 2003, Ejrnæs et al. 2006; but see Tilman 1997), resulting in lasting suppressing of 'unwanted' species and a persistent successional shift in plant community composition.

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Supplementary material

Appendix 6A Diagram of the experimental design of the field experiment.

Appendix 6B Diagram showing the positions within a field plot where shoot and root biomass samples were collected and where vegetation recordings were made.

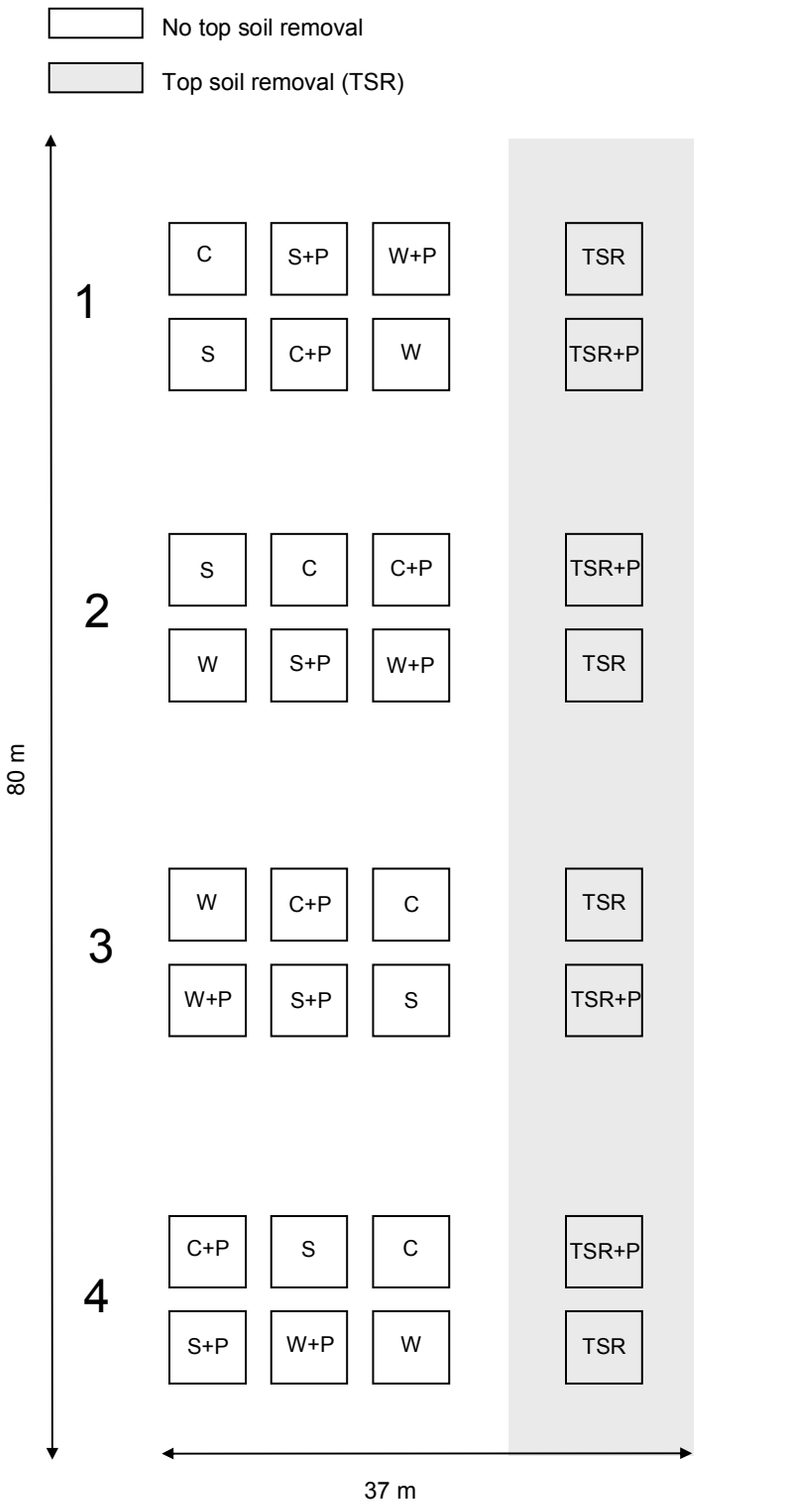
Appendix 6C Table showing the sum of shoot biomass of individual plant species for control, wood, straw and 'top soil removal' treatments in the microcosm experiment.

Appendix 6D Table showing the results of two-way repeated measures anova for shoot and root biomass in the field experiment.

Appendix 6E Figure showing the densities of nematodes per feeding group in control, straw and wood treatments in the field experiment.

Appendix 6F Table showing the physical, chemical and microbial soil parameters for control, wood, straw and top soil removal treatments in the field experiment.

Appendix 6A Diagram of the experimental design of the field experiment.

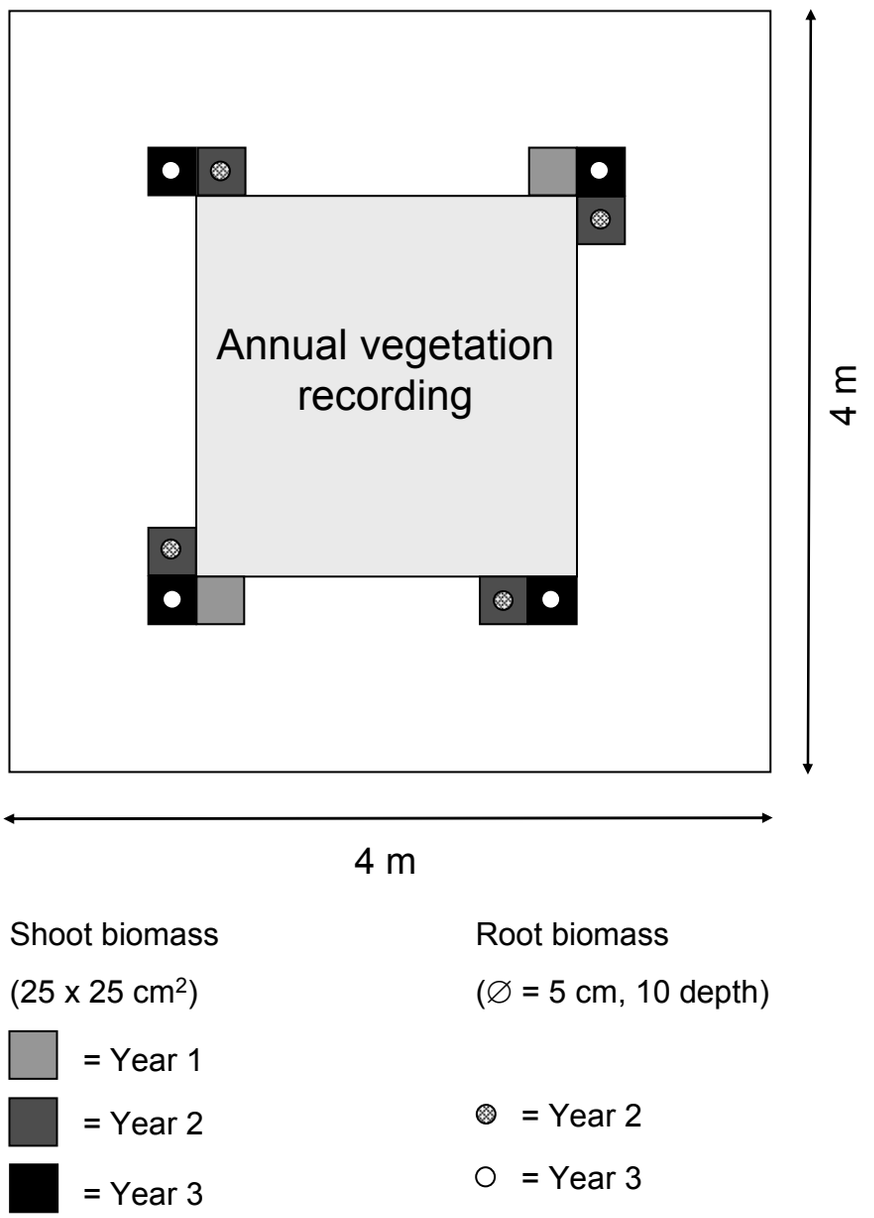


C = Control TSR = Top soil removal

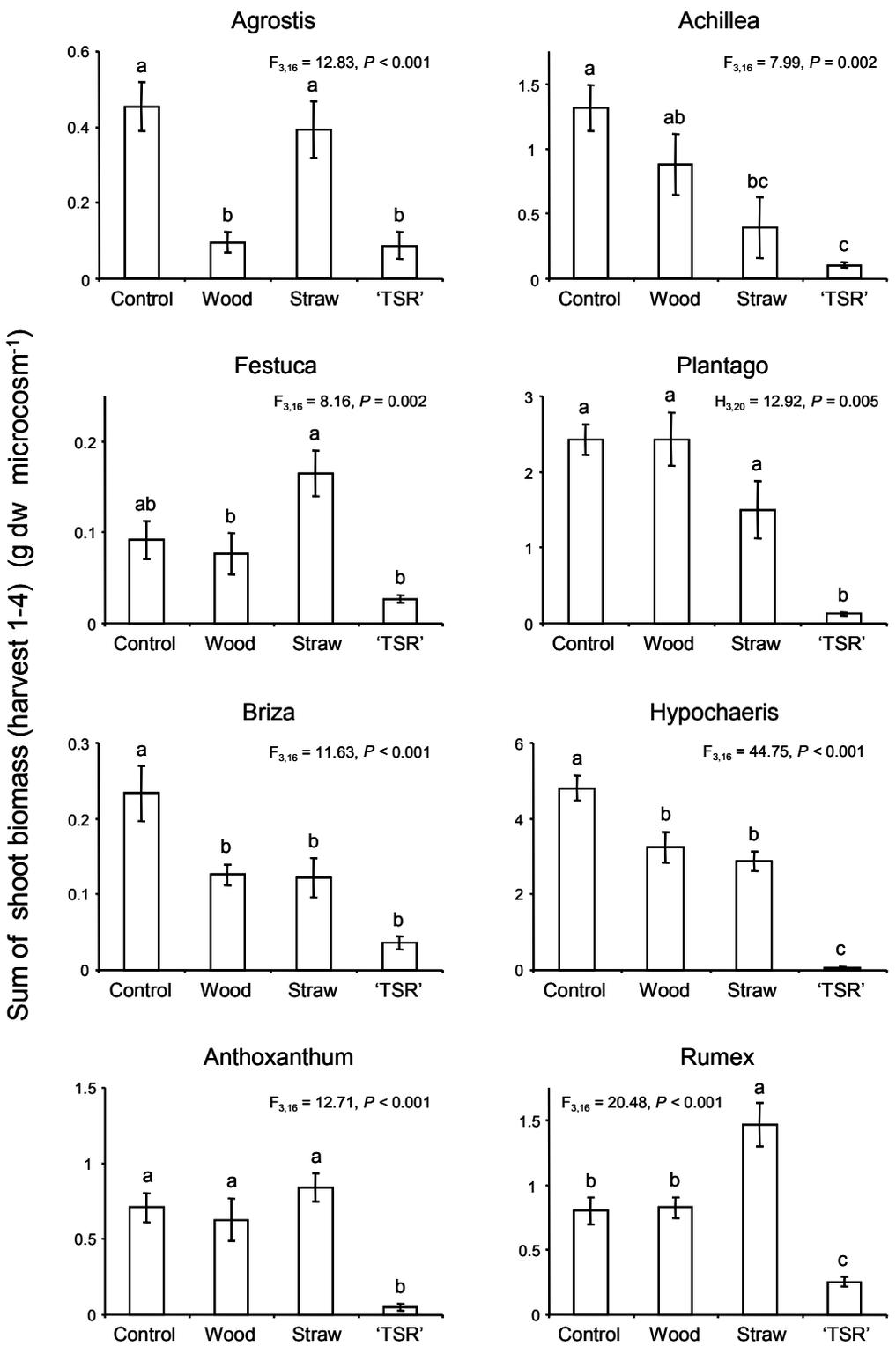
W = Wood addition +P = sowing mid-successional plant species

S = Straw addition

Appendix 6B Diagram showing the positions within a field plot where shoot and root biomass samples were collected and where vegetation recordings were made.



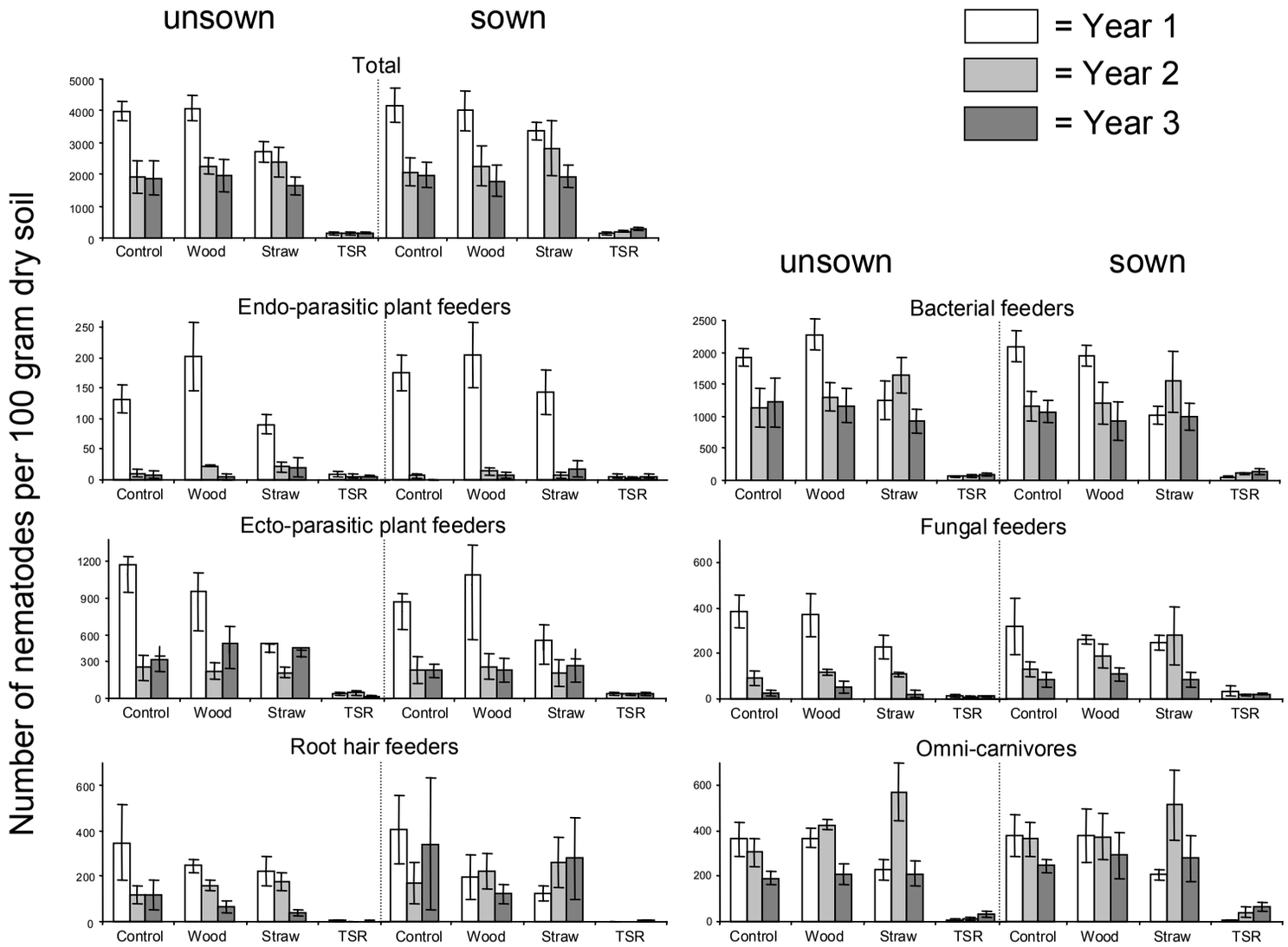
Appendix 6C Sum of shoot biomass (harvest 1-4) of individual plant species for control, wood, straw and 'top soil removal' ('TSR') treatments in the microcosm experiment. Data are mean ± s.e. The statistical results indicate overall treatment effects. Different letters denote significant differences according to Tukey posthoc tests after ANOVA or according to multiple comparisons of mean ranks (Kruskal-Wallis test).



Appendix 6D Results of two-way repeated measures ANOVA for shoot biomass and root biomass in the field experiment with treatment (control, straw, wood and top soil removal (TSR)) and sowing as fixed factor and year as repeated measure. Excluding TSR treatments from the analysis did not qualitatively affected the results. Shoot biomass was measured in the first, second and third year. Root biomass was measured in second and third year.

	Shoot biomass			Root biomass		
Source	df	F	*P*	df	F	*P*
Between subjects						
Treatment (T)	3	69.7	< 0.001	3	49.6	< 0.001
Sowing (S)	1	10.3	0.003	1	68.6	< 0.001
T x S	3	4.0	0.019	3	7.9	< 0.001
Within subjects						
Year	2	153.8	< 0.001	1	156.1	< 0.001
Year x T	6	15.3	< 0.001	3	8.8	< 0.001
Year x S	2	0.8	0.45	1	3.9	0.06
Year x T x S	48	1.5	0.18	24	0.2	0.88

Appendix 6E Densities of nematodes per feeding group in control, straw and wood treatments in the field experiment for year 1, year 2 and year 3. Data (mean ± s.e.) are shown for sown and unsown plots.



Appendix 6F Physical, chemical and microbial soil parameters for control, wood, straw and top soil removal treatments in the field experiment. Data are mean ± s.e. N = 5.

			Moisture (%)	pH	NH_4 (mg kg^{-1})	NO_3 (mg kg^{-1})	P-Olsen (mg kg^{-1})	P-CaCl (mg kg^{-1})	K (mg kg^{-1})
Unsown	Year 1	Control	10.2 ± 0.8	4.58 ± 0.09	0.10 ± 0.04	0.10 ± 0.10	120 ± 11	9.68 ± 0.37	37.7 ± 3.6
		Wood	11.2 ± 0.3	4.63 ± 0.13	0.06 ± 0.06	0.03 ± 0.03	132 ± 7	8.32 ± 1.40	38.3 ± 1.6
		Straw	10.6 ± 0.9	4.52 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	138 ± 10	10.07 ± 0.94	67.6 ± 4.0
		TSR	8.1 ± 0.4	4.60 ± 0.04	0.00 ± 0.00	0.07 ± 0.05	16 ± 8	0.03 ± 0.00	14.1 ± 0.8
	Year 2	Control	15.2 ± 1.4	4.62 ± 0.08	0.44 ± 0.19	1.45 ± 0.24	115 ± 13	7.93 ± 0.39	39.7 ± 2.7
		Wood	15.7 ± 0.3	4.61 ± 0.10	0.12 ± 0.05	0.81 ± 0.15	119 ± 7	7.17 ± 0.98	38.7 ± 2.1
		Straw	15.9 ± 1.0	4.58 ± 0.09	0.12 ± 0.07	1.41 ± 0.43	122 ± 7	7.90 ± 0.38	59.8 ± 6.8
		TSR	10.7 ± 0.2	4.63 ± 0.05	0.08 ± 0.08	0.08 ± 0.05	15 ± 8	0.04 ± 0.03	11.8 ± 0.7
	Year 3	Control	2.5 ± 0.4	4.58 ± 0.10	2.39 ± 0.20	0.58 ± 0.18	108 ± 15	7.37 ± 0.66	51.8 ± 6.6
		Wood	2.7 ± 0.2	4.63 ± 0.11	2.30 ± 0.52	0.32 ± 0.11	113 ± 6	6.05 ± 0.99	45.0 ± 3.9
		Straw	2.6 ± 0.5	4.58 ± 0.10	2.05 ± 0.16	0.38 ± 0.08	115 ± 5	6.90 ± 0.34	65.5 ± 7.5
		TSR	4.7 ± 0.4	4.56 ± 0.04	1.43 ± 0.11	0.26 ± 0.16	13 ± 9	0.12 ± 0.05	13.6 ± 0.8
Sown	Year 1	Control	10.5 ± 0.6	4.49 ± 0.06	0.00 ± 0.00	0.06 ± 0.06	131 ± 11	8.77 ± 0.36	32.0 ± 1.6
		Wood	11.0 ± 0.6	4.67 ± 0.11	0.00 ± 0.00	0.06 ± 0.04	134 ± 8	8.45 ± 1.71	36.5 ± 2.9
		Straw	10.3 ± 0.9	4.79 ± 0.13	0.03 ± 0.02	0.13 ± 0.13	125 ± 16	8.78 ± 1.60	63.6 ± 4.5
		TSR	7.9 ± 0.4	4.57 ± 0.02	0.00 ± 0.00	0.10 ± 0.04	11 ± 4	0.02 ± 0.00	12.0 ± 0.6
	Year 2	Control	15.3 ± 0.8	4.50 ± 0.06	0.27 ± 0.11	0.59 ± 0.13	119 ± 7	7.36 ± 0.28	36.9 ± 4.3
		Wood	16.4 ± 0.6	4.69 ± 0.08	0.14 ± 0.04	0.38 ± 0.06	122 ± 5	6.84 ± 1.35	36.6 ± 1.2
		Straw	16.2 ± 1.2	4.76 ± 0.08	0.09 ± 0.03	0.48 ± 0.12	112 ± 13	6.69 ± 1.11	47.8 ± 1.7
		TSR	10.6 ± 0.7	4.58 ± 0.01	0.27 ± 0.18	0.11 ± 0.09	8 ± 1	0.02 ± 0.00	13.7 ± 2.4
	Year 3	Control	3.0 ± 0.3	4.51 ± 0.06	1.51 ± 0.05	0.36 ± 0.04	117 ± 10	6.22 ± 0.14	49.3 ± 3.7
		Wood	3.2 ± 0.4	4.65 ± 0.10	1.32 ± 0.10	0.11 ± 0.06	114 ± 6	5.76 ± 1.00	38.0 ± 2.0
		Straw	3.2 ± 0.5	4.78 ± 0.11	1.32 ± 0.10	0.14 ± 0.08	105 ± 16	5.89 ± 0.92	58.7 ± 2.6
		TSR	4.4 ± 0.3	4.56 ± 0.02	1.08 ± 0.12	0.00 ± 0.00	9 ± 3	0.07 ± 0.02	15.5 ± 2.0

Appendix 6F *Continued.*

			Total N (mg kg^{-1})	Total P (mg kg^{-1})	Total organic matter (%)	Labile organic C (g kg^{-1})	Bacteria (10^8 ind. g^{-1})	Ergosterol (mg kg^{-1})
Unsown	Year 1	Control	953 ± 55	731 ± 58	3.47 ± 0.36	6.69 ± 0.32	4.4 ± 0.9	0.82 ± 0.18
		Wood	966 ± 51	679 ± 30	3.73 ± 0.13	7.70 ± 0.54	5.8 ± 2.0	1.03 ± 0.23
		Straw	962 ± 57	748 ± 68	3.64 ± 0.27	7.60 ± 0.24	5.7 ± 2.0	0.56 ± 0.03
		TSR	325 ± 17	139 ± 22	1.82 ± 0.14	5.38 ± 0.25	2.4 ± 0.4	0.03 ± 0.00
	Year 2	Control	885 ± 77	687 ± 61	3.34 ± 0.33	6.44 ± 0.35	11.7 ± 1.6	0.91 ± 0.07
		Wood	1038 ± 38	692 ± 28	3.84 ± 0.17	5.91 ± 0.54	12.8 ± 1.0	1.11 ± 0.16
		Straw	1098 ± 105	756 ± 104	3.75 ± 0.37	6.43 ± 0.41	15.1 ± 2.8	1.33 ± 0.22
		TSR	312 ± 18	136 ± 30	1.95 ± 0.08	5.26 ± 0.10	6.1 ± 1.1	0.04 ± 0.01
	Year 3	Control				8.94 ± 1.26	7.2 ± 1.0	0.80 ± 0.12
		Wood				8.14 ± 0.51	5.8 ± 0.3	1.14 ± 0.21
		Straw				9.53 ± 0.36	7.0 ± 0.7	1.01 ± 0.04
		TSR				7.80 ± 0.34	2.2 ± 0.4	0.05 ± 0.03
Sown	Year 1	Control	885 ± 59	611 ± 57	3.71 ± 0.30	6.66 ± 0.51	5.2 ± 0.9	0.56 ± 0.05
		Wood	993 ± 42	684 ± 39	3.87 ± 0.14	7.96 ± 0.17	6.5 ± 0.2	0.68 ± 0.11
		Straw	875 ± 50	617 ± 52	3.55 ± 0.39	6.49 ± 1.07	9.4 ± 1.2	0.84 ± 0.11
		TSR	340 ± 15	109 ± 6	1.76 ± 0.11	4.66 ± 0.30	2.9 ± 0.7	0.02 ± 0.01
	Year 2	Control	933 ± 86	669 ± 77	3.66 ± 0.29	5.88 ± 0.43	13.3 ± 1.6	1.02 ± 0.17
		Wood	1002 ± 54	674 ± 28	3.93 ± 0.13	6.58 ± 0.57	15.2 ± 1.7	1.27 ± 0.16
		Straw	921 ± 84	633 ± 58	3.71 ± 0.45	6.01 ± 0.79	15.6 ± 0.9	1.35 ± 0.09
		TSR	280 ± 36	94 ± 7	1.83 ± 0.18	5.11 ± 0.47	6.2 ± 1.2	0.13 ± 0.05
	Year 3	Control				8.64 ± 0.77	7.4 ± 0.7	1.10 ± 0.04
		Wood				9.69 ± 0.55	4.6 ± 0.8	1.21 ± 0.04
		Straw				8.56 ± 0.45	6.2 ± 1.2	0.97 ± 0.07
		TSR				8.67 ± 0.35	2.6 ± 0.7	0.17 ± 0.04

Chapter 7

Complementary effects of soil organism and plant introductions in restoration of species-rich grassland communities

Paul Kardol, T. Martijn Bezemer and Wim H. van der Putten

Photo credit: Wim H. van der Putten

Abstract

Soil organisms can strongly affect competitive interactions and successional replacements of grassland plant species. However, introduction of soil organisms as management strategy in restoration of species-rich grasslands has not been experimentally tested yet. We tested the hypothesis that simultaneous introduction of plant propagules and soil organisms originating from later-successional, species-rich *Cirsio-Molinietum* fen meadow(the donor site) enhances plant species development towards the 'target' grassland community and stimulates the development of the soil community. We used soil nematodes as indicator for soil community development. We established a 5-year field experiment at a top-soil removed, ex-arable site (the receptor site) and introduced plant propagules and soil organisms by 1) spreading hay and soil, independently or combined, and 2) transplanting intact turfs. Our results show that spreading hay from the donor site affected plant community composition, whereas spreading soil from the donor site did not have an additional effect. Soil spreading did not affect the soil nematode community composition. Introduction of soil organisms by means of turf transplantation was not successful either. Unfavorable soil conditions (e.g. low soil organic matter content and seasonal fluctuations in soil water level) at the receptor site may have limited nematode survival in the turfs and may have precluded successful establishment of the later-successional soil organisms at the receptor site. Differences in abiotic soil conditions between the donor site and the receptor site could also explain why plant species composition at the transplanted turfs became less similar to the donor site from which they originated and why most plant species did not expand into the receiving plots. Probably, introduction of soil or turf from mid-successional stages may be more effective than soil and turfs from 'target' stages. Further research should focus on the required conditions for establishment of soil organisms on ex-arable land in order to make use of their contribution to grassland restoration and conservation.

Keywords *Above-belowground linkages, hay spreading, plant-soil organism interactions, nematodes, secondary succession, soil spreading, turf transplantation, principal response curves (PRC)*

Introduction

Conversion of agricultural land into species-rich grasslands is a common practice for restoring plant species diversity. After agricultural land abandonment, the major abiotic constraint for development of species-rich grasslands is high soil fertility. As plant species composition is highest at intermediate soil fertility (Grime 1997), the majority of restoration projects on ex-arable land are treated by addition of carbon substrates (e.g. Blumenthal et al. 2003), top soil removal (Marrs 1985, Van Diggelen et al. 1997), grazing or hay making (e.g. Milchunas and Lauenroth 1993, Bakker and Ollf 1995, Pywell et al. 2002) in order to reduce soil fertility. Also the absence of propagules of later-successional 'target' species may be a major constraint when restoring species-rich grasslands (Bakker and Berendse 1999). So far, nature restoration and conservation have made little use of recent studies showing that on smaller spatio-temporal scales, interactions with soil organisms can strongly affect competitive interactions and successional replace-

ments of plants (De Deyn et al. 2003, De Deyn et al. 2004a, Kardol et al. 2006, Kardol et al. 2007).

Soil organisms can affect plant community composition both directly and indirectly (Wardle et al. 2004a). Direct effects can be attributed to altered competitive ability of plants that accumulate parasites, pathogens and root herbivores or mutualistic symbionts, such as mycorrhizal fungi (Johnson et al. 1991, Van der Putten et al. 1993, Klironomos 2002). Indirectly, decomposers affect plant community interactions by releasing nutrients from litter, root exudates and soil organic matter (Wardle 2002). In secondary grassland succession, soil organisms selectively suppress plant species from production grasslands, thereby enhancing the relative abundance of later-successional plant species. Within each grassland stage, dominant plant species are suppressed more by root herbivores than subordinate plant species, resulting in enhanced evenness of plant community composition (De Deyn et al. 2003). On ex-arable land, soil organisms play a similar role in speeding up initial succession and slowing down succession when time after abandonment proceeds. This has been shown both by applying soil insecticides at early secondary succession stages (Brown and Gange 1992) and by testing soil feedback effects along a 34-year old chronosequence of ex-arable lands (Kardol et al. 2006). However, these results have not yet been tested as management strategy in restoration of species-rich grasslands.

As plant and soil communities are mutually dependent of each other (Wardle 2002, Bever 2003), it is likely that synchronised introductions of plant propagules and soil organisms are required to successfully establish their mutual relationship. Different approaches can be used to introduce later-successional plant propagules and soil organisms. First, hay and soil originating from a reference habitat (the donor site) can be spread at the restoration site (the receptor site). Seeds present in hay collected from species-rich grassland may ensure introduction of desired species from local provenance (e.g. Patzelt et al. 2001, Kiehl and Wagner 2006), however the precise seed content of the hay may be difficult to predict. Spreading a thin layer of top soil obtained from a 'target' habitat may introduce the desired soil organisms. Yet, establishment of later-successional soil communities may not be evident (Pywell et al. 2007), since this has been suggested to depend strongly on the initial site conditions and could be inhibited by the resident soil community at the receptor site (De Deyn 2004). Probably, elimination of the resident soil community by top soil removal (Chapter 6) could enhance establishment of introduced soil organisms.

Alternative to hay and soil spreading, intact turfs obtained from a 'target' habitat, containing both soil and vegetation could be introduced. These turfs could function as stepping stones for later-successional plants and soil organisms (Bullock 1998). Established turfs may provide a local pool from which plant species can spread by seeds into the receptor site (Pywell et al. 1995), or plant species may gradually colonise the receptor site by vegetative growth and, thereby, enhancing the area of later-successional 'target' habitat. Moreover, turfs could conserve soil abiotic conditions better than the hostile conditions caused by drought and high temperatures present at the soil surface. So far, studies involving turf transplantation, as well as studies involving addition of top soil, evaluated the effects on ecosystem restoration by measuring changes in plant

species composition and chemical or physical soil conditions (Pywell et al. 1995, Bullock 1998, Standen and Owen 1999, Bruelheide and Flintrop 2000, Kailová 2000, Vécrin and Muller 2003), whereas concomitant effects on soil organisms, most essential for successful establishment of later-successional plant-soil organism relationships and integral part of ecosystem restoration, are usually ignored (but see Pywell et al. 2007).

In the present study, we tested the hypothesis that simultaneous introduction of plant propagules and soil organisms originating from later-successional, species-rich *Cirsio-Molinietum* fen meadow (i.e. the donor site) enhances plant species diversity and community development towards the 'target' fen meadow plant community, and stimulates the development of the soil community. We used soil nematodes as indicator for soil community development. Nematodes are abundant, trophically diverse, sensitive to disturbances, and indicative of ecosystem functioning (Bongers and Ferris 1999, Ferris et al. 2001) and successional changes in the soil subsystem (Háněl 2003, Kardol et al. 2005). We set up a 5-year field experiment at an ex-arable field site from which the top-soil had been removed (i.e. the receptor site) and tested our hypothesis using two alternative approaches to introduce plant propagules and soil organisms: 1) spreading hay and soil, independently or combined, and 2) transplanting turfs as 'stepping stones'. We also compared plant and soil community development at the transplanted turfs with the donor site and assessed 'turf survival'. Finally, we compared effects of top soil removal with no top soil removal.

Methods

Site description and experimental design

A 5-year field experiment was carried out on a former agricultural field in Lievelde, in the central-east of the Netherlands (52°01 N, 5°36 E). The field site was located on sandy deposits and had been cultivated, most recently by maize (*Zea mays*). In winter 2000/2001, 50 cm of the top soil had been removed down to the mineral subsoil in order to decrease soil fertility. In summer 2001, in collaboration with Land and Water Management Service (Dienst Landelijk Gebied) and State Forest Service (Staatsbosbeheer), we set up an experiment in which we examined effects of introducing later-successional plant species and soil organisms by 1) spreading hay, 2) spreading soil, 3) spreading soil and hay, and 4) transplantation of intact turfs, containing both soil and vegetation. Material for the hay (i.e. fresh harvested shoots and seed capsules), soil and turfs was obtained from a reference, or donor site located 250 m from the receptor site (Appendix 7A). The field experiment also included control plots where no further actions had been taken. The five treatments were carried out in a randomised block design with five replicates of each treatment.

The donor site (Appendix 7B) was a species-rich *Cirsio-Molinietum* fen meadow with plant association *Cirsio dissecti-Molinietum* (Schaminée et al. 1996). The vegetation was dominated by *Anthoxanthum odoratum*, *Carex panicea*, *Centaurea jacea, Dactylorhiza maculata*, *Succisa pratensis* and *Lysimachia vulgaris* (Jongejans 2004). Early August, hay was collected after mowing and drying for

three days and soil and turfs of 25 x 25 x 10 cm^3 were collected from the upper 10 cm of a 2 x 5 m^2 randomly chosen area. Hay and soil treatments were applied by spreading the material homogeneously over the plots, which were scarcely covered by vegetation at the time of application. Soil was spread as 2.5 l m^{-2}, hay was spread so that 50-75 % of the soil surface was covered and turfs were placed at a density of 1 m^{-2} (Appendix 7B).

Plots were 5 x 5 m^2 and separated by 1 m border rows. Owing to slight differences in elevation, soil moisture differed across the field and we placed two blocks 35 m from the other three blocks in order to include some of the environmental heterogeneity in our experimental design. Additionally, we installed five 5 x 5 m^2 plots within the field margin where the top soil had not been removed ('no top soil removal', NTSR) (Appendix 7A). To compare plant and soil nematode community development at the transplanted turfs with plant community development at the donor site, we installed five plots of 0.75 x 0.75 m^2 (i.e. the aggregated size of nine transplanted turfs) at the donor sites, adjacent to the area from which the turfs had been excavated. The receptor site and the donor site were mown each year at end of August after which the hay was removed.

Plant community

From August 2003-2006, every year the percentage cover of each vascular plant species was recorded in the inner 3 x 3 m^2 of each plot. For turf transplantations, vegetation recordings were made separately for the inner nine turfs (hereafter referred to as 'transplanted turfs') and for the area in between the turfs (hereafter referred to as 'turf receiving' treatments) (Appendix 7B). Also from the 0.75 x 0.75 m^2 plots at the donor site, the vegetation was recorded annually.

Soil properties

In August 2003, from each plot, nine soil samples of ∅ 3.5 cm and 10 cm depth were collected according to a grid pattern, bulked, homogenised and stored at 4°C until analysis. For turf transplantations, soil samples were collected from the centre of the inner nine turfs, as well as from nine positions in-between the turfs corresponding to the pattern of the nine soil samples in the other plots (Appendix 7B). Also from the plots at the donor site, nine soil samples were collected. For each plot, the nine soil samples were bulked. Soil samples from 2003 were analysed for ergosterol to determine fungal biomass (Stahl and Parkin 1996, Van der Wal et al. 2006b), chemical properties and nematodes.

Soil samples for ergosterol and chemical properties were sieved through a 4 mm mesh prior to analysis. Ergosterol was measured using a disruptive extraction without saponifcation (Gong et al. 2001). For each sample, the net mineralization or immobilization of N was determined after incubation of 50 g fresh soil at 20°C for six weeks, as the difference between initial and final amount of mineral N (NH_4^+ + NO_3^-). Soil mineral N was extracted by shaking 10 g of soil (dry weight) with 50 ml KCl, after which NH_4^+- and NO_3^--concentrations were measured colorometrically using a Traacs 800 auto-analyser. Soil samples were analysed for potential available P

by extraction with sodium bicarbonate (Olsen et al. 1954). Total N and P were measured by digestion of samples with a mixture of H_2SO_4-Se and salicyclic acid. Soil organic matter content was measured as the loss of material on ignition at 400-450°C. The pH was measured in 1:2.5 (dry weight) soil:water suspensions. Soil water content was determined as weight loss after drying at 70°C for 72 hours.

Nematodes were extracted from the soil by Oostenbrink elutriators (Oostenbrink 1960) after which 10% of the nematodes from the extracted soil samples were heat-killed and fixed using 35% formaldehyde diluted to 4%. Of each sample, a minimum of 150 nematodes were identified to family or genus level. The group *Dorylaimoidea* was used to specify a heterogeneous group of omnivorous dorylaimids comprising *Dorylaimidae*, *Qudsianematidae*, *Thornenematidae* and *Aporcelaimidae* (Jairajpuri and Ahmad 1992). Nematodes were allocated to feeding groups according to Yeates et al. (1993a). We distinguished plant feeders, bacterial feeders, fungal feeders and omnicarnivores, and expressed their numbers per 100 g dry soil.

Data analysis

For all univariate analyses, the assumption of normality was checked with Kolmogorov-Smirnov tests and the assumption of homogeneity of variances with Cochran C, Hartley, Bartlett tests. If the assumptions were not met, data were log(x+1) transformed prior to analysis. If the assumptions were still not met data were analysed using a non-parametric Kruskal-Wallis test. Soil chemical properties, moisture content, ergosterol content, the total number of nematodes and numbers of nematodes within feeding groups were analysed using one-way ANOVA with treatment (control, hay, soil, hay + soil, turf receiving, transplanted turfs, 'no top soil removal' and donor site) as fixed factor. Statistical differences between treatments were analysed using Tukey's hsd post-hoc tests. Species richness was measured as the number of species per plot and analysed by repeated measures ANOVA with treatment (control, hay, soil, hay + soil, turf receiving, and 'no top soil removal') as fixed factor and year as repeated measure. A contrast was specified to analyse the difference in species richness between 'no top soil removal' and top soil-removed treatments. Univariate analyses were performed using STATISTICA (release 7.1, Statsoft, Inc., Tulsa, Oklahoma, USA). The response of the taxonomic composition of soil nematodes to the treatments was analysed by principal component analysis (PCA). The analysis was carried out with square-root transformed abundance data. Differences in taxonomic composition among control, hay, soil, hay + soil and turf receiving treatments were tested in redundancy analysis (RDA) with Monte Carlo permutation tests (999 unrestricted permutations).

We analysed effects of the experimental manipulations on plant community development using redundancy analysis (RDA) and principal response curves (PRC) according to Lepš and Šmilauer (2003). RDA is a constrained form of principal component analysis in which plant species composition is explained by environmental variables (i.e. the experimental treatments). We tested the overall treatment effect on plant community development by including all treatment x year interactions as explanatory variables in RDA. In detailed analyses, we separately tested the effect of each treatment (hay, soil, hay + soil, and turf receiving) on the temporal changes in

plant species composition by testing the explanatory power of each particular treatment x year interaction, while defining the remaining treatment x year interactions, except the control x year interaction, as covariables (Lepš and Šmilauer 2003). Significance of treatment x year interactions was tested with Monte Carlo permutation tests restricted to split-plot design to reflect the repeated measurements (999 permutations).

PRC is an extension of RDA that extracts the complexity of time-dependent community-level responses into an easy-to-interpret graphic form, expressing treatments as deviations from a reference treatment (Van den Brink and Ter Braak 1999). PRC first accounts for variation in plant species composition due to time, and then attributes the remaining variation to the experimental manipulations. We generated PRC diagrams by plotting the first principal component of the treatment effects against time (i.e. years 2003-2006). PRC diagrams were generated by comparing the treatment to 1) the control and 2) the donor site. The latter were generated both including and excluding the 'no top soil removal' treatments. To compare in the turf receiving treatments the community composition of the transplanted turfs relative to the other area within the plot, a separated PRC diagram was generated including data of the control treatment, and separately transplanted turfs and area between the turfs, using the donor site as the reference treatment. We tested the significance of the first and of higher order PRCs by Monte Carlo permutations tests (999 permutations restricted to split-plot design to reflect the repeated measurements). We interpreted the directional changes in plant community composition by integrating the response of the individual plant species in the PRC diagrams, using a species weight diagram showing the affinity of the plant species with the response indicated in the diagram (Lepš and Šmilauer 2003). All multivariate analyses were performed using CANOCO, version 4.5 (Ter Braak and Šmilauer 1998-2002).

PRCs were generated to analyse deviations in plant community development relative to the control or the donor site. However, since plant communities of the transplanted turfs originated from the donor site, we were also explicitly interested in the similarity between these two plant communities over time. Therefore, we calculated Sørensen's quantitative index CN (Magurran 1988), hereafter referred to as Sørensen's similarity (CN). CN incorporates both occurrence and abundance of every species, and was calculated as $CN = 2\,jN/(aN + bN)$ where aN is the total species cover at site A, bN is the total species cover at site B and jN is the sum of the lower of the two cover estimates for species which occur in both sites. For each year, we calculated the similarity between the transplanted turfs and the plots at the donor site as well as among the plots at the donor site. For each of the two data sets we performed a linear regression analysis. To analyse if the similarity between transplanted turfs and the plots at the donor site differed from the similarity among plots at the donor site, we tested the null hypotheses that the intercepts and slopes of the regression analyses did not differ (t-tests).

Results

Plant community

Differences in plant community development could be explained significantly by the experimental treatments (RDA with Monte Carlo permutation tests: F = 1.67, P = 0.03). Detailed analyses showed that hay and hay + soil spreading (F = 3.31, P = 0.01 and F = 2.70, P = 0.03, respectively) significantly explained variation in plant community development, whereas soil spreading alone and turf transplantation did not (F = 1.92, P = 0.09 and F = 1.30, P = 0.25, respectively). Plots with hay and hay + soil had higher abundance of grasses and other monocot species, such

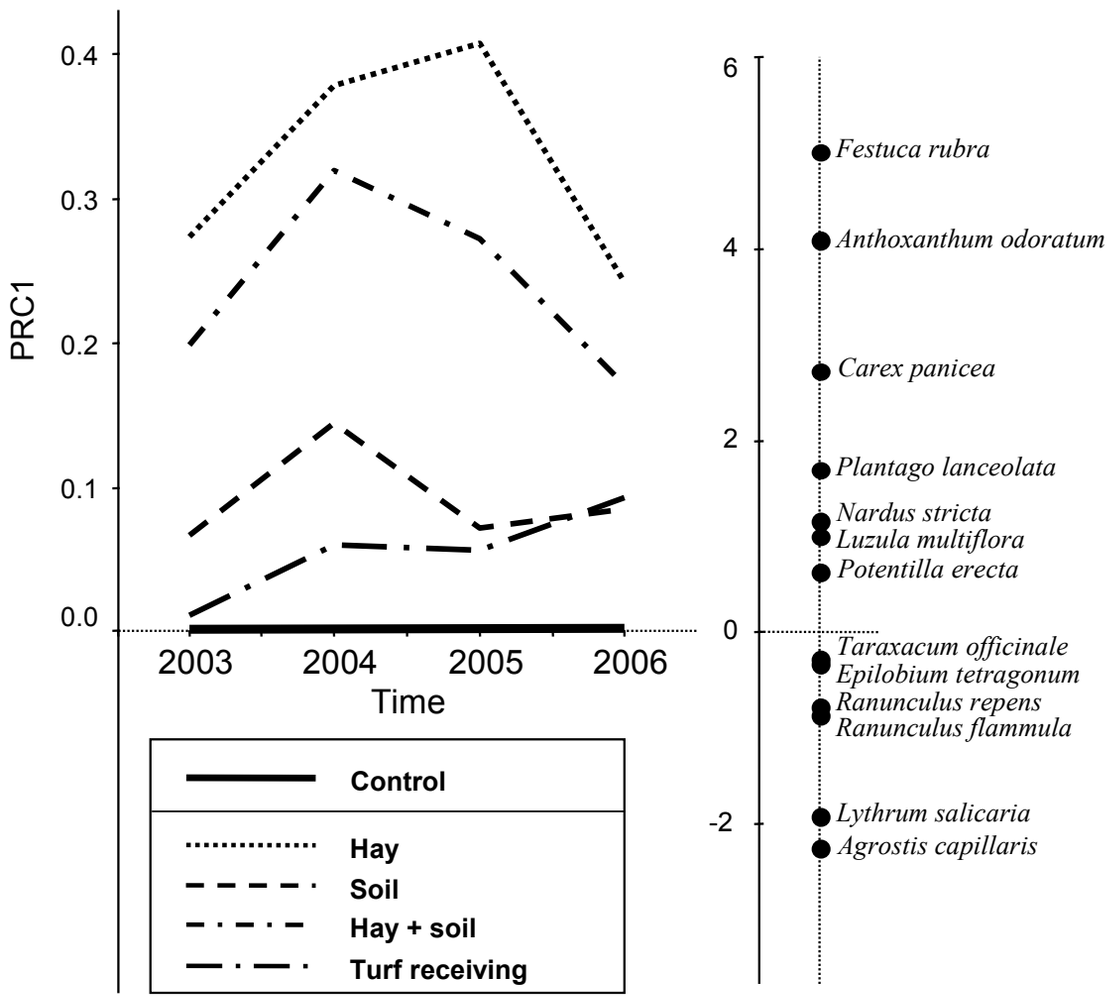


Figure 7.1 Principal response curves (PRC) for hay, soil, hay + soil, and turf receiving treatments for the first RDA axis (eigenvalue = 0.07, F = 10.21, P = 0.001). The diagram shows the deviation in plant community development relative to the control, which is represented as a horizontal line along the time axis. Second and subsequent axes of RDA were not significant. The vertical 1-D plot at the right side of the diagram is a species weight diagram showing the relative abundance of each species compared to the control. A positive score indicates an increase in abundance, while a negative score indicates a decline. For clarity, only species with the best fit to the first ordination axis are shown (Lower Axis Minimum Fit 5).

as *Festuca rubra*, *Anthoxanthum odoratum* and *Carex panicea*, whereas control plots had higher abundance of *Agrostis capillaris* and *Lythrum salicaria* (Fig. 7.1). However, for hay and hay + soil spreading, there was no apparent directional development in plant community composition away from the control treatment. Instead, plant community composition converged to the control after 2005 (Fig. 7.1). The differences in PRC scores between the treatments in the year 2003 should be attributed to divergence in plant community development during the first two years after the treatments had been installed in 2001.

Plant community composition of all treatments (including the control) at the receptor site tended to converge towards the donor site (Fig. 7.2). Hay and hay + soil treatments were most

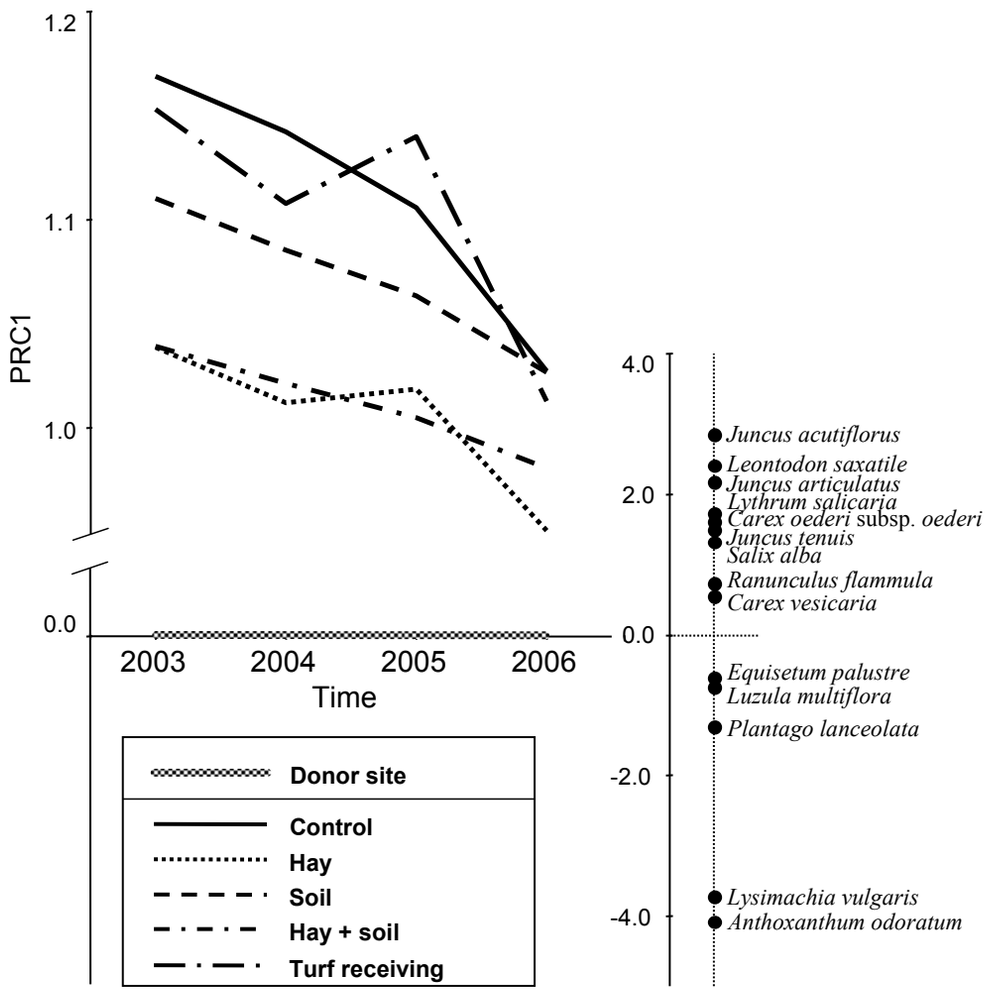


Figure 7.2 Principal response curves (PRC) for control, hay, soil, hay + soil, and turf receiving treatments for the first RDA axis (eigenvalue = 0.16, F = 30.91, P = 0.001). The diagram shows the deviation in plant community development relative to the donor site, which is represented as a horizontal line along the time axis. Second and subsequent axes of RDA were not significant. The vertical 1-D plot at the right side of the diagram is a species weight diagram showing the relative abundance of each species compared to the donor site. A positive score indicates an increase in abundance, while a negative score indicates a decline. For clarity, only species with the best fit to the first ordination axis are shown (Lower Axis Minimum Fit 15).

similar to the donor site. Nevertheless, five years after initiating the treatments, the overall difference between the top soil-removed receptor site, irrespective of treatments, and the donor site was substantial (indicated by the high scores on the y-axis). The donor site was characterised by higher abundance of *Anthoxanthum odoratum*, *Lysimachia vulgaris*, *Plantago lanceolata* and *Luzula multiflora*, while the top soil-removed receptor site was characterised by higher abundance of *Leontodon saxatile*, *Lythrum salicaria*, and different rushes (*Juncus sp*). Irrespective of treatments, some rare plant species, which could be defined as 'target species' for restoration of species-rich grasslands, such as *Campanula rapunculus*, *Centaurium erythraea*, *Dactylorhiza maculata*, *Danthonia decumbens*, *Nardus stricta* and *Carex oederi* subsp. *oederi* established at the receptor site. The 'no top soil removal' treatments differed considerably from the other treatments and were far more different

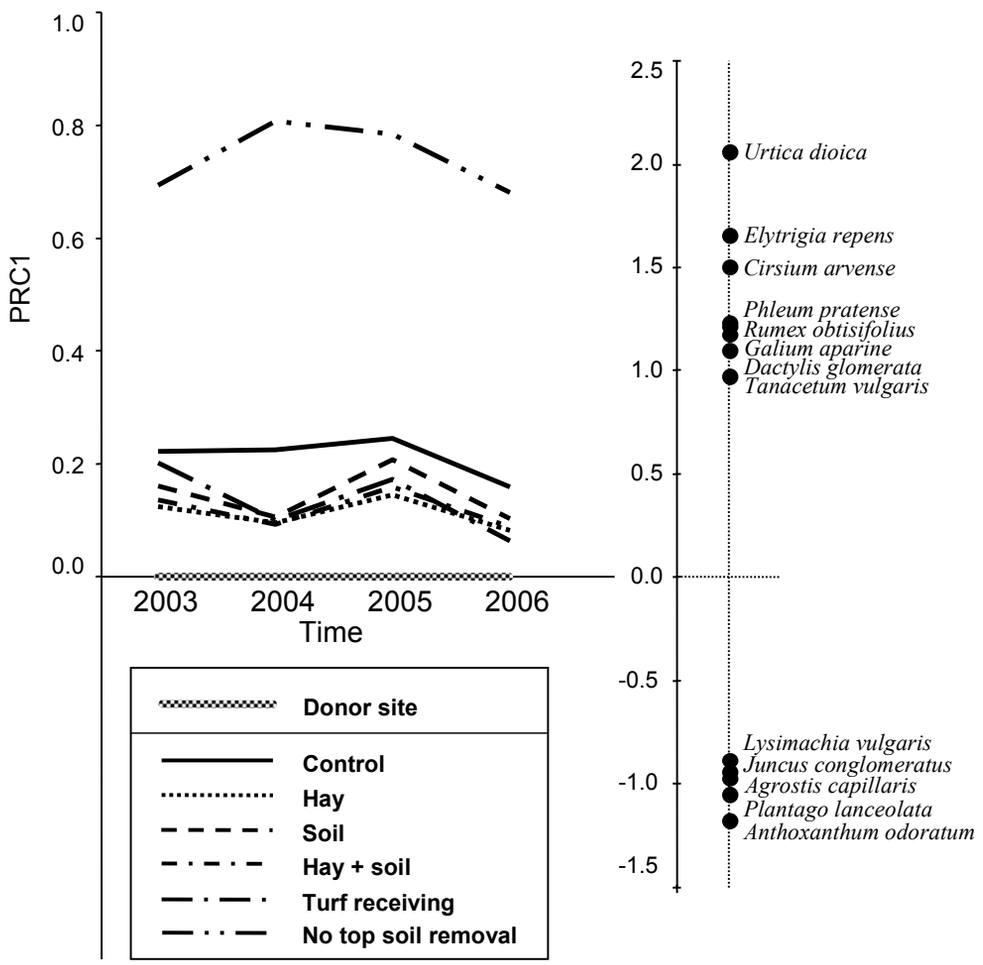


Figure 7.3 Principal response curves (PRC) for control, hay, soil, hay + soil, turf receiving and 'no top soil removal' treatments for the first RDA axis (eigenvalue = 0.24, F = 41.84, P = 0.001). The diagram shows the deviation in plant community development relative to the donor site, which is represented as a horizontal line along the time axis. Second order PRC (RDA: eigenvalue = 0.17, F = 36.98, P = 0.002) is not shown. Third and subsequent axes of RDA were not significant. The vertical 1-D plot at the right side of the diagram is a species weight diagram showing the relative abundance of each species compared to the donor site. A positive score indicates an increase in abundance, while a negative score indicates a decline. For clarity, only species with the best fit to the first ordination axis are shown (Lower Axis Minimum Fit 15).

from the donor site than all the top soil-removed treatments (Fig. 7.3). The 'no top soil removal' treatments had few species in common with the top soil-removed site and were dominated by ruderal, nitrofilous species, such as *Urtica dioica*, *Elytrgia repens* and *Cirsium arvense*.

Plant community composition of the transplanted turfs tended to diverge from the donor site (Fig. 7.4) and most plant species of the transplanted turfs did not expand outside the turfs (P. Kardol, personal observation). For *Lysimachia vulgaris*, which was among the dominant plant species both at the donor site and the transplanted turfs, we could detect spread from the turfs into the whole plot. Probably, this was also the case for *Anthoxanthum odoratum*. However, for *A. odoratum* we could not exclude arrival through dispersal from adjacent plots, since across years the cover of *A. odoratum* in the turf receiving treatments did not significantly differ from the control

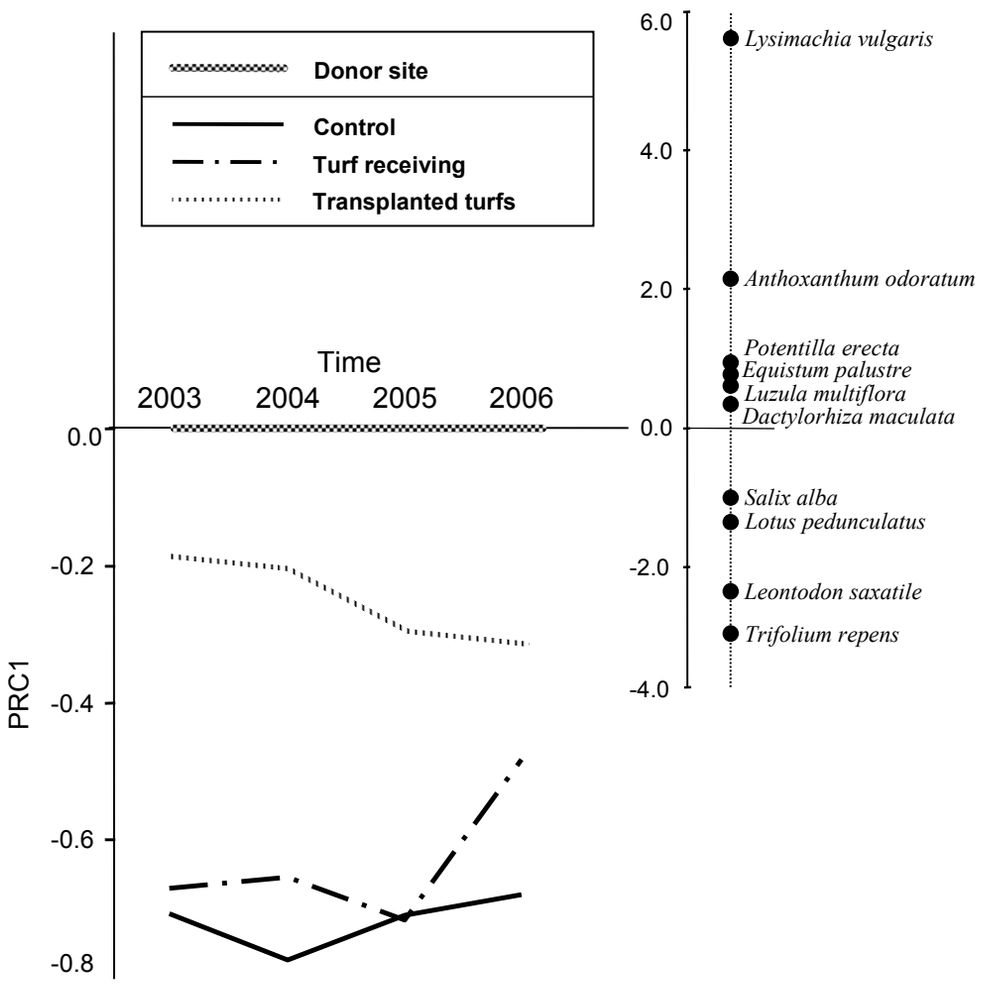


Figure 7.4 Principal response curves (PRC) for control, turf receiving treatments and the transplanted turfs for the first RDA axis (eigenvalue = 0.32, F = 36.88, P = 0.001). The diagram shows the deviation in plant community development relative to the donor site, which is represented as a horizontal line along the time axis. Second order PRC (RDA: eigenvalue = 0.07, F = 9.03, P = 0.008) is not shown. Third and subsequent axes of RDA were not significant. The vertical 1-D plot at the right of the diagram is a species weight diagram showing the relative abundance of each species compared to the donor site. For clarity, only species with the best fit to the first ordination axis are shown (Lower Axis Minimum Fit 20).

(Repeated measures ANOVA, $F_{1,8}$ = 3.00, P = 0.12). Other species which were present at the turfs at the time of transplantation, such as *Dactylorhiza maculata* and *Potentilla erecta*, had difficulties to persist in the turfs and where not able to spread into the surrounding area. In contrast, species such as *Leontodon saxatile*, *Lotus pedunculatus*, *Ranunculus repens* and *Trifolium repens* invaded from the whole plot into the turfs. The similarity in plant community composition of the turfs to the plots at the donor site was significantly lower than the similarity among the plots at the donor site (Intercept: t = 3.63, P < 0.001) and the difference increased over time (Slope: t = 2.79, P < 0.01) (Fig. 7.5). As regards the plant community, this indicates a limited 'survival' of the turfs. In 2006, the turf receiving treatments and the transplanted turfs converged to each other (Fig. 7.4) and the turf receiving treatment converged to the donor site (Fig. 7.2 and Fig. 7.4). However, the first years after establishment, the transplanted turfs appeared to become more similar to the whole plot than *vice versa* (Fig. 7.4). This suggests that convergence of the turf receiving treatments to the donor site in 2006 was not necessarily attributable to the turf transplantation.

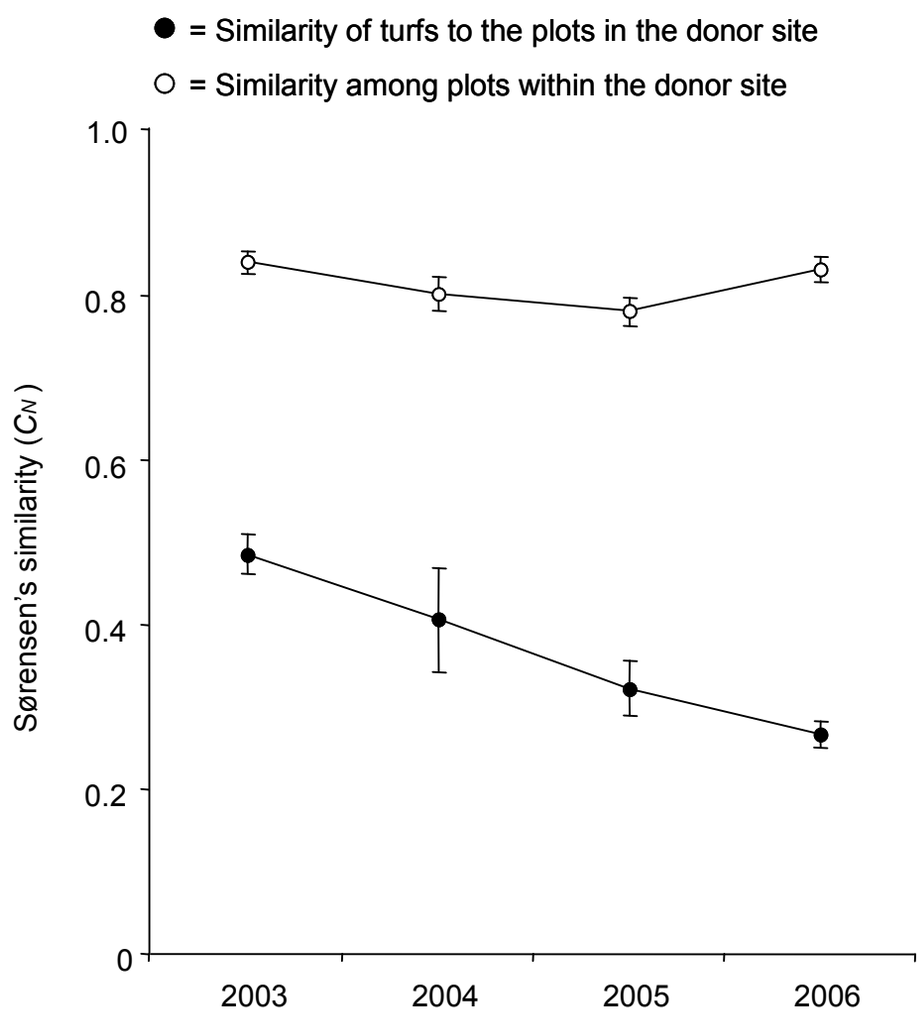


Figure 7.5 Temporal change in plant community similarity of the transplanted turfs to the plots at the donor site and among the plots at the donor site, calculated as Sørensen's similarity (*CN*). Data are mean ± s.e. (n = 5).

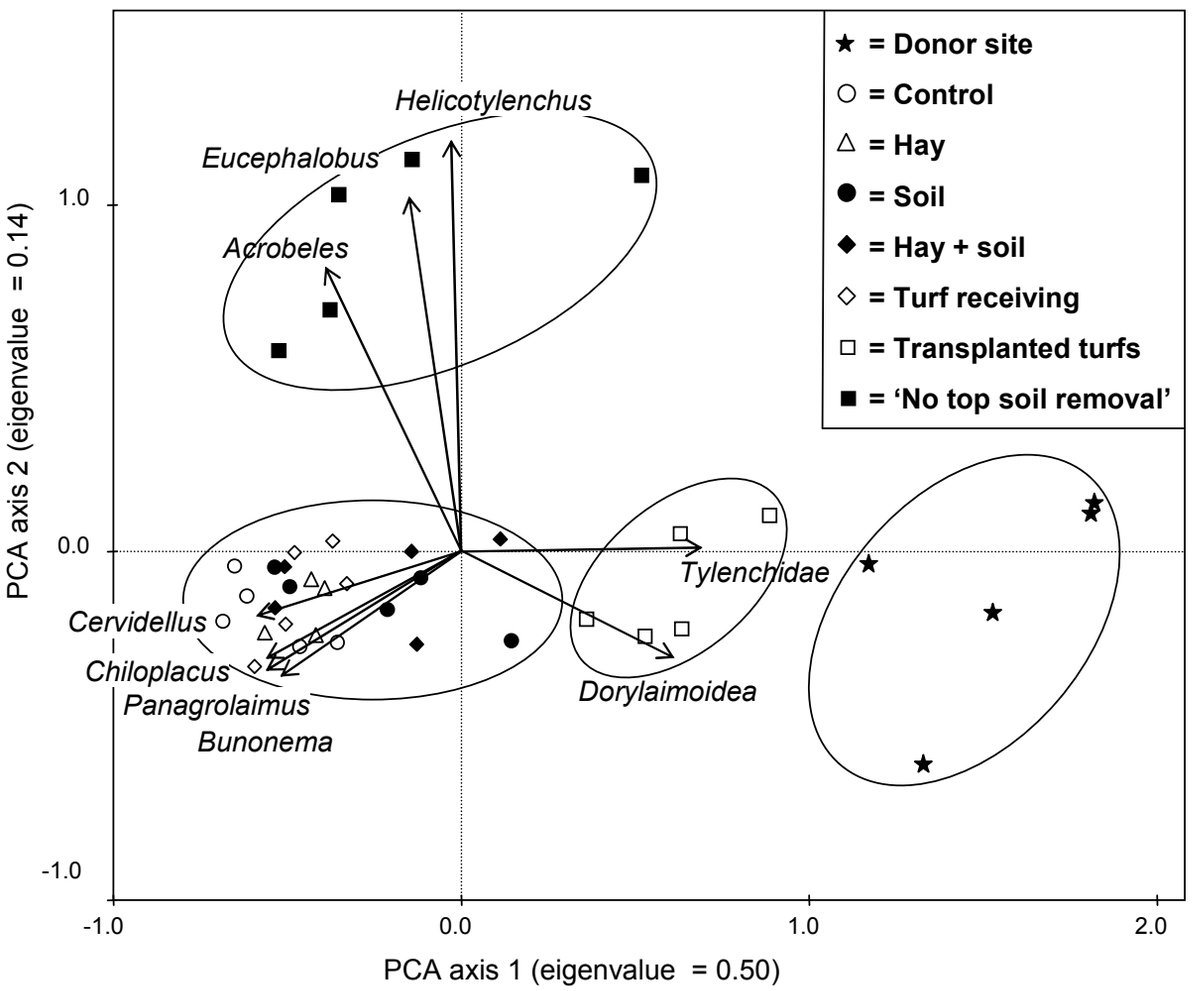


Figure 7.6 Ordination diagram (species-samples biplot) of PCA for the soil nematode community. Each data point represents one treatment plot. Ovals are drawn around clusters of samples from replicate treatments. Samples from the control, hay, soil, hay + soil and turf receiving treatments could not be clustered separately. Eigenvalues along the axes indicate the amount of explained variability in species composition. For clarity, only nematode taxa with the best fit range are shown.

Across years, a total of 87 plant species were found. Species richness in hay, soil, hay + soil and turf receiving treatments did not differ from the control (Tukey posthoc tests, $P > 0.05$). Species richness in 'no top soil removal' treatments was significantly lower than in the top soil-removed treatments, averaging 20 and 8 species per plot, respectively (Contrast analysis after ANOVA: $F_{4,29} = 42.57$, $P < 0.001$). Overall, species richness significantly increased over time (Year: $F_{3,72} = 56.61$, $P < 0.001$). However, the species richness did not increase in all treatments, especially not in the 'no top soil removal' treatment, as indicated by the significant treatment x year interaction ($F_{15,72} = 11.25$, $P < 0.001$).

Soil properties

The total number of soil nematodes ranged from approximately 2000 per 100 g dry soil in treatments at the top soil-removed receptor site to more than 16000 at the donor site. The total

Table 7.1 Soil characteristics (0-10 cm) at the donor site and at the treatment plots in August 2003. Moisture content is also given for August 2005. For turf transplantation, data are presented for the receiving plots (turf receiving) and for the transplanted turfs. NTSR = 'No top soil removal'. SOM = Soil organic matter. Data are mean ± s.e. Different letters denote significant differences between means based on one-way ANOVA followed by Tukey posthoc tests or Kruskal-Wallis test* with multiple comparisons of mean ranks ($P < 0.05$).

	N-mineralization (N mg kg^{-1})	P-Olson (mg kg^{-1})	N total (g kg^{-1})*	P total (mg kg^{-1})*	% SOM	pH	% Moisture [2003]	% Moisture [2005]	Ergosterol (mg kg^{-1})*
Donor site	14.84 ± 1.83 ^{a}	2.92 ± 0.32 ^{b}	2.77 ± 0.06 ^{a}	239 ± 6 ^{b}	6.28 ± 0.17 ^{a}	4.87 ± 0.03 ^{d}	37.2 ± 0.6 ^{a}	37.3 ± 0.9 ^{a}	1.03 ± 0.03 ^{ab}
Control	3.29 ± 0.86 ^{b}	9.24 ± 4.15 ^{b}	0.21 ± 0.04 ^{c}	72 ± 15 ^{c}	1.14 ± 0.15 ^{c}	6.28 ± 0.09 ^{a}	19.8 ± 1.7 ^{b}	22.1 ± 1.1 ^{bc}	0.41 ± 0.17 ^{bc}
Hay	1.10 ± 0.32 ^{b}	4.36 ± 1.36 ^{b}	0.21 ± 0.03 ^{c}	47 ± 6 ^{c}	1.04 ± 0.15 ^{c}	6.33 ± 0.05 ^{a}	20.8 ± 2.8 ^{b}	21.6 ± 1.5 ^{c}	0.43 ± 0.17 ^{bc}
Soil	1.46 ± 0.51 ^{b}	5.39 ± 1.86 ^{b}	0.24 ± 0.02 ^{c}	57 ± 9 ^{c}	1.19 ± 0.13 ^{c}	6.15 ± 0.04 ^{a}	20.6 ± 1.3 ^{b}	23.7 ± 1.2 ^{bc}	0.34 ± 0.04 ^{c}
Hay + soil	2.64 ± 1.27 ^{b}	6.42 ± 1.69 ^{b}	0.25 ± 0.03 ^{c}	73 ± 13 ^{c}	1.23 ± 0.10 ^{c}	6.14 ± 0.04 ^{a}	21.0 ± 2.5 ^{b}	22.0 ± 2.1 ^{c}	0.35 ± 0.10 ^{c}
Turf receiving	2.26 ± 0.74 ^{b}	5.08 ± 1.66 ^{b}	0.22 ± 0.01 ^{c}	64 ± 10 ^{c}	1.10 ± 0.08 ^{c}	6.25 ± 0.05 ^{a}	18.5 ± 1.0 ^{b}	19.2 ± 1.3 ^{c}	0.15 ± 0.04 ^{c}
Trans-planted turfs	1.87 ± 0.66 ^{b}	3.33 ± 0.63 ^{b}	1.44 ± 0.06 ^{b}	137 ± 5 ^{b}	3.94 ± 0.16 ^{b}	5.34 ± 0.02 ^{c}	30.4 ± 2.3 ^{ab}	34.5 ± 1.5 ^{ab}	1.56 ± 0.26 ^{a}
NTSR	41.49 ± 12.07 ^{a}	93.16 ± 10.36 ^{a}	2.86 ± 0.27 ^{a}	933 ± 72 ^{a}	6.77 ± 0.63 ^{a}	5.78 ± 0.04 ^{b}	26.6 ± 2.3 ^{b}	29.5 ± 2.7 ^{b}	072 ± 0.05 ^{bc}
One-way ANOVA									
$F_{7,32}$	19.60	14.03	129.3	124.7	77.03	108.9	11.09	17.15	11.84
P	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

number of nematodes in 'no top soil removal' plots and in the transplanted turfs was approximately 6000-7000 per 100 g dry soil. For none of the nematode feeding groups we observed differences in abundance among control, hay, soil, hay + soil, and turf receiving treatments (Appendix 7C). Numbers of plant feeders and omni-carnivores in the transplanted turfs were intermediate between numbers in the donor site and in turf receiving treatments. The 'no top soil removal' treatments were characterised by high numbers of bacterial feeders and relatively low numbers of fungal feeders (Appendix 7C).

The taxonomic composition of the nematode community did not differ among control, hay, soil, hay + soil and turf receiving treatments (RDA, Monte Carlo permutation tests for all canonical axes, $F = 1.01$, $P = 0.44$, Fig. 7.6). The first ordination axis, which explained 50% of the variability in taxon composition, revealed clear separation of the donor site and the treatments at the receptor site. The transplanted turfs clustered separately from the donor site and appeared to develop 'towards' the surrounding communities at the top soil-removed soil. Root-hair feeding *Tylenchidae* and omnivorous *Dorylaimoidae* were particularly abundant at the donor site, while bacterial feeding *Cervidellus*, *Chiloplacus*, *Panagrolaimus* and *Bunonema* were more abundant at the receptor site. The second ordination axis explained 14% of the total variability and separated the 'no top soil removal' treatments from the treatments at the top soil-removed site. Separation of 'no top soil removal' treatments could be contributed to high abundance of plant-feeding *Helicotylenchus* and bacterial-feeding *Eucephalobus* and *Acrobeles*.

Abiotic soil properties in hay, soil, hay + soil and turf receiving treatments did not differ from the control or from each other (Table 7.1). Compared to 'no top soil removal', the treatments at the top soil-removed receptor site were characterised by lower N-mineralization, P-availability, total N, total P and soil organic matter content and soil moisture content. The donor site was characterised by low pH, high organic matter content, high soil moisture content and relatively low P-availability. Values of chemical soil properties of the transplanted turfs, were generally in between the values of the donor site and the top soil-removed site. Ergosterol content, as indicator of fungal biomass, was particularly high in the transplanted turfs.

Discussion and conclusion

We expected that introduction of later-successional soil organisms into the top soil-removed site will facilitate the establishment of plant species from the donor site. However, our expectation was not confirmed in this 5-year field experiment. Spreading hay from the donor site affected plant community composition, whereas spreading soil from the donor site did not have an additional effect. Soil spreading did not affect the soil nematode community composition. This could have been due to the adverse abiotic conditions on the bare soil surface. However, introduction of soil organisms by means of turf transplantation was not successful either. Therefore, the establishment of later-successional soil organisms into top soil-removed restoration sites seems severely constrained by unknown environmental factors, whereas introductions of invertebrates and soil microorganisms were successful in sterilised soil in greenhouse conditions (De Deyn et al. 2003, Kardol et al. 2006).

Soil community composition is known to depend strongly on environmental conditions (Scheu and Schulz 1996, Bongers and Ferris 1999). As most of the soil food web had been removed together with the top soil, chemical, physical, and hydrological soil conditions at the receptor site may well have been responsible for the poor establishment of the introduced soil organisms (Pywell et al. 2007). We recorded a more than five-fold higher amount of soil organic content matter at the donor site than in the top soil-removed receptor site. This will have limited resource input into the soil food web, as well as the amount of available habitats for primary decomposers and their predators (Bardgett 2002). Indeed, bacterial and fungal feeding nematodes, as well as omni-carnivores were significantly lower in the receptor site than in the donor site and were not affected by spreading soil from the donor site. Initial scarce vegetation cover could have resulted in insufficient or poor quality host plants. Numbers of plant-feeding nematodes were very low in all top soil-removed treatments.

Numbers of nematodes in the transplanted turfs decreased when compared to the donor site. Moreover, the taxonomic composition of the nematode community in the turfs developed towards the receptor site. This suggests limited survival of the nematodes from more mature ecosystems within the isolated turfs. This might be due to intrinsic loss through habitat fragmentation (Hanski 1991, Lawton 1995, Gonzalez et al. 1998, Rantalainen et al. 2006). In those conditions it is to be expected that the nematode community becomes dominated by bacterial and fungal feeding nematodes, which are of low trophic level positions (Wright and Coleman 1993, Hoyle 2004). However, all feeding types of nematodes decreased in abundance just about the same extent (Appendix 7C). Moreover, soil nematodes act on small spatial scales relative to the turf size (25 x 25 x 10 cm^3) (Ettema and Wardle 2002, Ettema and Yeates 2003) and are probably not particularly sensitive to reductions in habitat size (Rantalainen 2004). Therefore, the size of the turfs per se is not expected to have affected soil nematodes. Instead, changes in environmental conditions that result form the edge-area ratio of the turfs (Saunders et al. 1991) could have caused reduction in nematode abundance. Particularly, the turfs were placed in mineral soil, which is poorly buffered to seasonal fluctuations in soil water level. In summer, this resulted in dehydration of the turfs (P. Kardol, personal observation), which could have had direct or indirect detrimental effects on soil nematodes (Lindberg et al. 2002).

Our results also suggest that the turfs did not function as a pool from which nematodes could colonise the top soil-removed receptor site. Other studies have suggested that many soil organisms can survive on bare, mineral soil at least for one or two years (Siiri-Pietikäinen et al. 2003). There are different reasons as to why we did not record nematode dispersal out the turfs. First, colonization of the receptor site by nematodes from the transplanted turfs depends mainly on active locomotion, and this may result in slow colonization rates (Warwick 1984). Moreover, in our study, unfavorable soil conditions and differences in substrate type could have led to limited survival of soil nematodes outside their home habitat (i.e. the turf). It is also possible that due to a mismatch in abiotic soil conditions between the transplanted turf and the receptor site, nematodes did not disperse from the turf. Alternatively, disturbance of turfs, by lifting, transport and relaying, may have suppressed soil organisms and could have contributed to the decline of soil nematodes originally present at the turfs. In an analogous experiment, where differences in

abiotic soil conditions between donor and receptor site were smaller, colonization of soil mites from transplanted turfs was not successful either (Gormsen et al. 2006). Therefore, in order to enhance the use of soil organisms for nature restoration, their transplantation, establishment and proliferation needs to be improved.

Plant species composition at the transplanted turfs became less similar to the donor site and most plant species did not expand into the receiving plots. This indicates that also for the plant community there was a mismatch between the donor site and the receptor site. Apparently, abiotic or biotic soil conditions of the mineral soil at the receptor site were unfavorable for the plant species at the turfs. The inherent time-lag in the response of plant communities to environmental change may have tempered the rate of diversification between the transplanted turfs and the donor site. As discussed for soil organisms, fluctuating soil water levels at the receptor site may have disfavored some plant species at the turfs. Unfortunately, we did not record the plant species composition of the turfs at the time of transplantation. Moreover, the depths of the turfs (10 cm) could have damaged deep rooting species (Bullock 1998), such as the orchid *Dactylorhiza maculata*, which was present at the turfs the first year after transplantation (P. Kardol, personal observation), but has not been observed after.

Hay spreading has been recommended as a successful method to introduce later-successional plant species on a variety of soil types (Manchester et al. 1999, Patzelt et al. 2001, Hölzel and Otte 2003, Kiehl and Wagner 2006, Kiehl et al. 2006). However, our results showed that particularly the more common plant species were introduced and that the amount of variation in plant species composition explained by hay treatments was low. After five years, plant species composition of hay amended plots still differed substantially from the donor site. Probably, more attention should be given to the seed presence in the hay (Pywell et al. 1995), or the germination potential of the plant species on the top soil-removed soil. The majority of seeds present in hay comprise of grass species (Smith et al. 1996). This can explain the high abundance in hay amended plots of *Festuca rubra* and *Anthoxanthum odoratum*, which were dominant species at the donor site at the time the hay was collected (Jongejans 2004). Top soil from the donor site could have contained seeds and/or vegetative fragments from species other than those that were present in the hay (Manchester et al. 1999). However, our results suggest that seeds or other plant propagules in the top soil were scarce (Pywell et al. 1995), or that germination and establishment was constrained, as we did not find plant species exclusively in plots where soil was spread.

After land abandonment, a major limitation for establishment of species-rich grassland vegetation is the high nitrogen and phosphorus availability of soils (Marrs 1993). Logically, in absence of top soil removal, soil fertility was high, vegetation was dominated by nitrofilous weedy species and tall forbs, and plant species composition did not appear to succeed towards the species-rich donor site. At the top soil-removed receptor site, where fertility was reduced, plant species composition tended to diverge towards the donor site, but the vegetation remained rather dissimilar to the 'target' *Cirsio-Molinietum* fen meadow. Nevertheless, the plant community at the top soil-removed site was classified as low-productive, species-rich grassland. Plots of all treatments (including the ones without plant and soil introductions) contained several Red List spe-

cies and other species that could be defined as 'target species' (Bal et al. 2001). These species must have been able to disperse from local or regional species pools to the restoration site (Bakker et al. 1996, Soons et al. 2005), or they may have been present in the subsoil and were exposed to soil surface conditions by removing the top soil. This suggests that seed limitation (Bakker and Berendse 1999) was not a major constraint for grassland restoration in our case.

In conclusion, although soil organisms have been shown to play a role in secondary succession (Brown and Gange 1992, De Deyn et al. 2003, Kardol et al. 2006), the establishment of soil organisms on top soil-removed soil is limited. Whereas spreading soil from the fen meadow donor site may have exposed soil organisms to adverse environmental conditions, they even did not survive very well in transplanted turfs. Probably, after land abandonment, introduction of soil or turfs from mid-successional stages may be more effective than soil or turfs from 'target' sites. Future studies should attempt to enhance establishment and survival conditions of soil organisms on ex-arable land in order to make use of their contribution to biodiversity conservation and ecosystem functioning.

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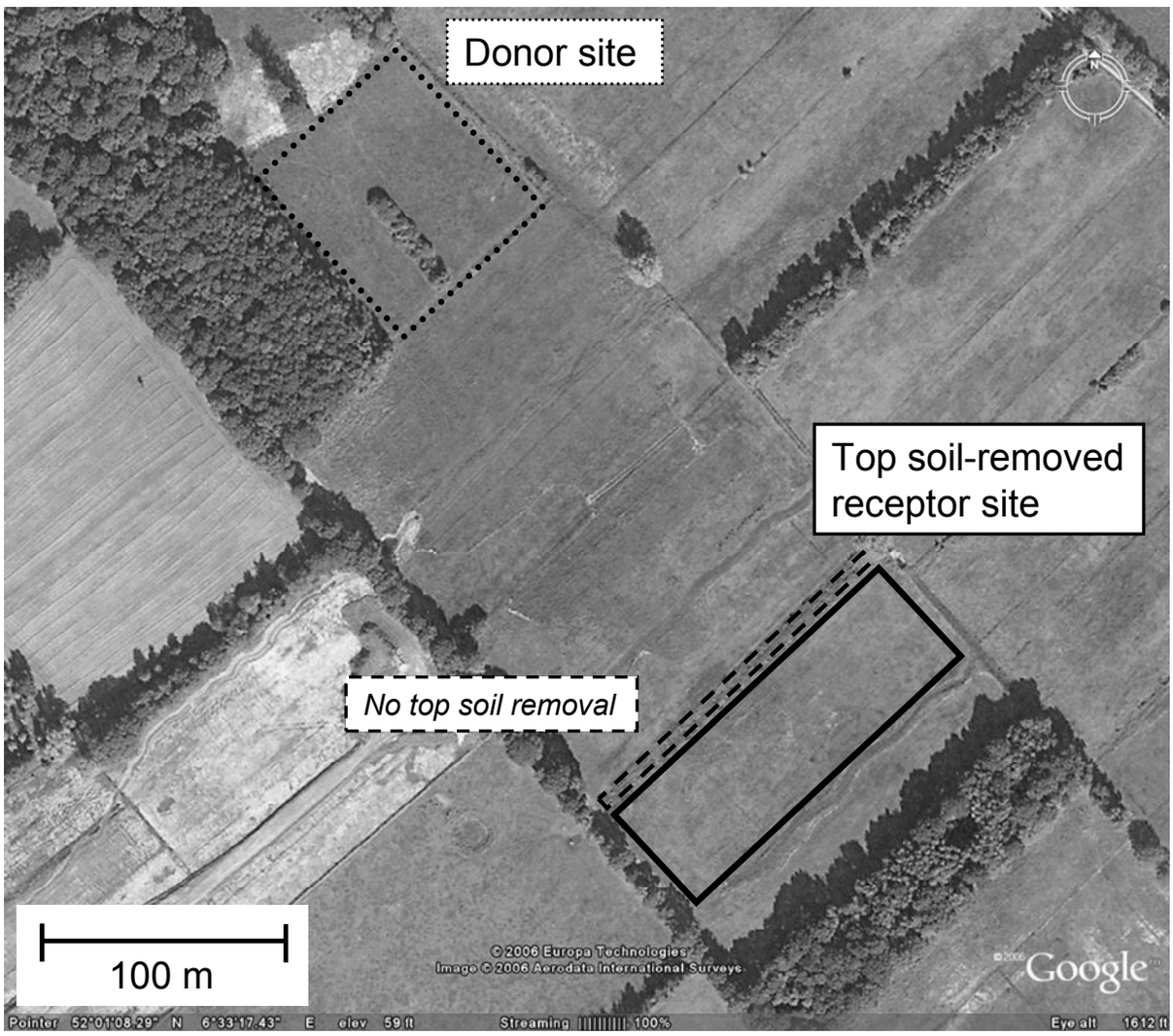
Supplementary material

Appendix 7A Arial picture showing the location of the donor site and the experimental field site.

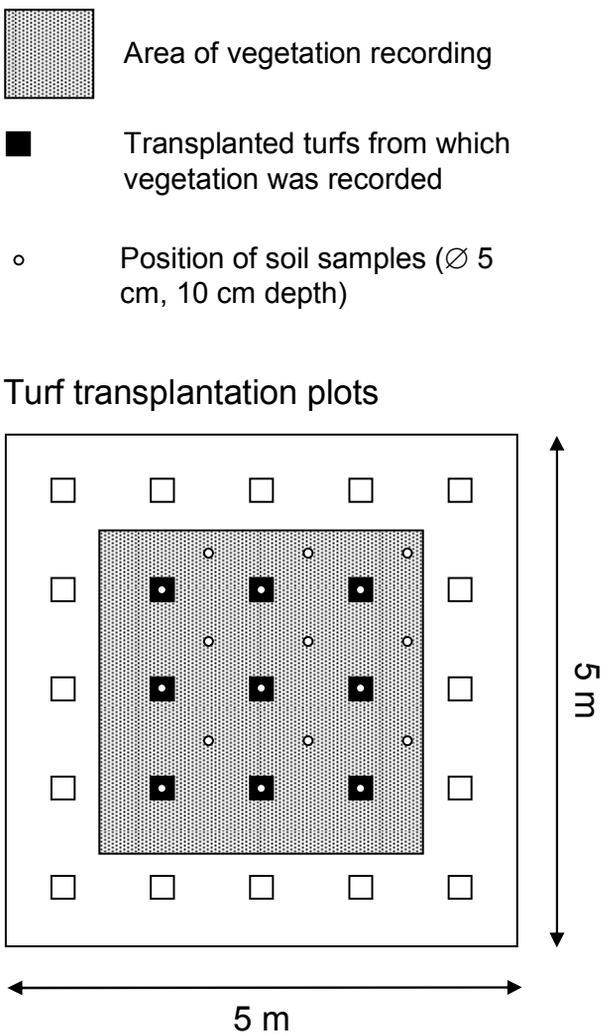
Appendix 7B Diagram showing the positions of vegetation recordings and soil samples in the plots.

Appendix 7C Figure showing densities of nematodes per feeding group in the different treatments.

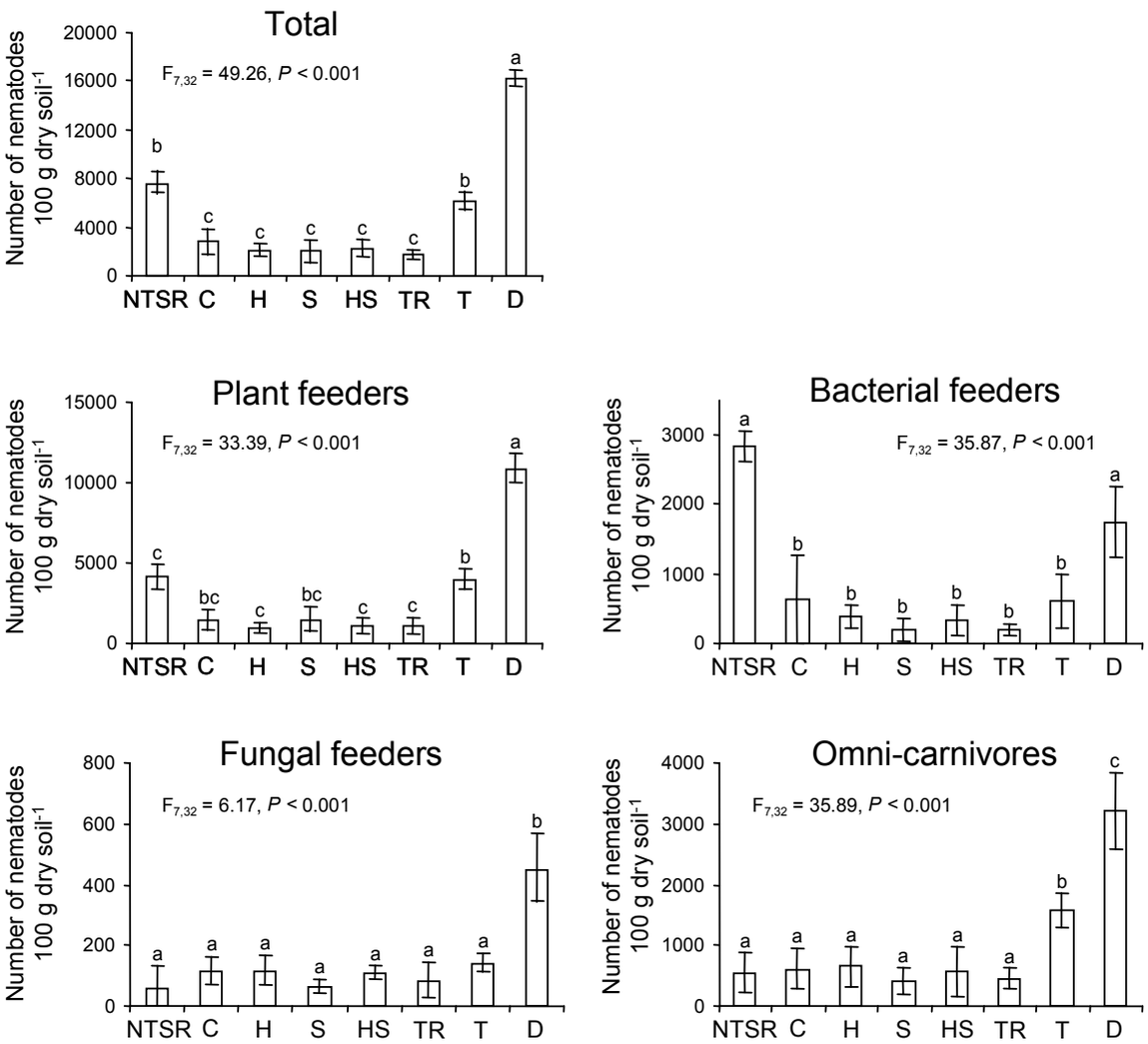
Appendix 7A Arial picture showing the location of the donor site, the top soil-removed receptor site and the field margin where the top soil was not removed. Photograph: Google Earth Beta (v4.0.24XX), release October 2006.



Appendix 7B Diagram showing the positions of vegetation recordings (2003-2006) and soil samples (2003) in turf transplantation plots and in other plots.



Appendix 7C Densities of nematodes per feeding group in 'no top soil removal' (NTSR), control (C), hay (H), soil (S), hay + soil (HS), turf receiving (TR) treatments, and in the transplanted turfs (T) and the donor site (D). Data are mean ± s.e. The statistical results indicate overall treatment effects. Different letters denote significant differences according to Tukey posthoc tests after ANOVA.



Chapter 8

Discussion and synthesis

'Visually stimulating organisms, such as the large, the colourful, the active, and the aggressive, command our attention while the secretive and insidious remain largely ignored' (Price 1980). This certainly relates to the small creatures dwelling belowground. This thesis, in line with other studies (e.g. Van der Putten and Peters 1997, De Deyn et al. 2003, Callaway et al. 2004a, Wardle et al. 2004a,) has shown that soil organisms can affect the outcome of interspecific plant interactions, thereby influencing plant community assembly and temporal changes in plant species composition. Although it has been suggested that a comprehensive recognition of the biotic soil subsystem is necessary for the adequate understanding of plant community patterns and ecosystem processes during land use changes (Wolters et al. 2000, Wardle 2002), most studies in plant community ecology have traditionally been conducted without much explicit consideration of the role of soil organisms. In this thesis I explored plant-soil community interactions and relationships at a range of scales, using descriptive and empirical studies. As a model system, I focused on secondary succession on former agricultural land on sandy soils in the central and eastern part of the Netherlands. In this chapter I will discuss and synthesize the key results in relation to changes in plant and soil community composition and for the practice of ecological restoration.

Successional patterns of soil and plant communities

Above- and belowground sub-ecosystems have been suggested to be closely interlinked (Wardle and Van der Putten 2002, Wardle et al. 2004a). Plant and soil community assembly during secondary succession depends on the initial species composition, colonization from the local species pools, and on the response of the resident and colonizing species to the changing biotic and abiotic environmental conditions. This suggests that differences in response to agricultural disturbance and differences in dispersal abilities between plants and soil organisms could lead to discrepancy in plant-soil organism interactions during initial stages of secondary succession. The positive correlation between similarity in plant community composition and soil nematode community composition, which we observed in the chronosequence of former agricultural fields (Chapter 2), would suggest a parallel plant-soil community development. However, plant and soil nematode communities did not develop in tandem towards the same reference system. The plant community developed towards a mattgrass sward, while the soil nematode community became more similar to that of a heathland community. This indicates, at least partly, autonomous developments in plant and soil communities at the field scale level (Berg and Hemerik 2004). Moreover, there were substantial site-specific differences in the relationship between plant and nematode communities, which could be explained by differential mechanisms operating in initial stages of secondary plant and soil community succession. Plant community development during initial stages following land abandonment is predominantly seed limited (Chapter 6 and Chapter 7), which indicates that community assembly depends on colonization of species from local species pools. In contrast, most soil nematode taxa appear to be initially present following land abandonment (Chapter 2) and changes in soil nematode community composition are mainly due to shifts in dominance patterns in response to altered environmental conditions (Chapter 3).

Agricultural practices differentially affect phylogenetic and trophic groups of soil organisms, which results in a specific bacterial pathway dominated soil food web at the time of abandonment (Brussaard et al. 1990, De Ruiter et al. 1994, Zwart et al. 1994). In this thesis I questioned how the soil community changes during the transition towards species-rich grassland or heathland using soil nematodes as an indicator. After land abandonment, a shift from bacterial to fungal based decomposition pathways (Swift et al. 1979, Klein et al. 1995) and increased soil food complexity (i.e. a higher degree of structure; *sensu* Ferris et al. 2001) was expected. Indeed, the number of fungal-feeding nematodes increased with time since abandonment (Chapter 2). However, even in the oldest fields, numbers of fungal feeding nematodes were substantially lower than in the reference heathlands. Variation within sites of similar age was high and successional increase in fungal feeding nematodes could be largely attributed to a rapid increase in the first years following land abandonment. The data suggest that after land abandonment, initial rapid changes within the soil food web are followed by a phase in which no further changes are observed. This corresponds to the pattern of fungal biomass development, measured in the same chronosequence (Van der Wal et al. 2006b). Also, the abundance of omni-carnivorous nematodes, which have been suggested to be indicative for soil food web complexity (Polis and Strong 1996), did not change after an initial increase (Chapter 2 and Chapter 3). Similarly, the analysis of the mite feeding group composition did not support a successional build-up in soil food web structure or complexity (Chapter 3). This indicates that after a rapid shift in soil food web structure, most likely due to release from physical agricultural disturbances, no substantial changes occur over a period of at least three decades. Indeed, faunal profiles, based on the presence and abundance of functional guilds of nematodes (Ferris et al. 2001), show that the soil food webs of the agricultural sites, two of the three youngest fields and two fields of intermediate age can all be classified as disturbed or maturing, while the soil food webs of all other former agricultural fields were classified as structured (Fig. 8.1). Soil food webs of the reference heathlands were indicated as degraded, implying that these soil systems are nutrient depleted, have high C:N ratios and that decomposition pathways are fungal dominated (Van der Wal et al. 2006b). In accordance with the soil nematode analyses, a comprehensive analysis of soil food web structure, measured by means of trophic levels and decomposition pathways, including all major groups of soil organisms, did also not reveal major changes in soil food web structure during the first three decade following land abandonment (Holtkamp et al., in preparation).

Trophic or feeding-group composition of soil communities could be best explained by the interaction between site and soil properties, which was related to differences in quantity and quality of soil organic matter (Chapter 3; Van der Wal et al. 2006b). Soil organic matter status did not significantly change during the first three decades following land abandonment (Van der Wal et al. 2006b), which may explain why the differences in feeding group composition of soil nematodes and mites were relatively small (Chapter 2 and Chapter 3). During this phase, taxon composition within feeding-groups changed markedly. While taxon composition of soil nematodes could be attributed to shifts in dominance patterns, changes in the taxon composition of oribatid mites were controlled by colonization of new taxa from local species pools. Dispersal constrained succession of mites during early stages of secondary succession, suggesting an individualistic successional trajectory (cf Gleason 1917, 1926). However, on the longer term, mite

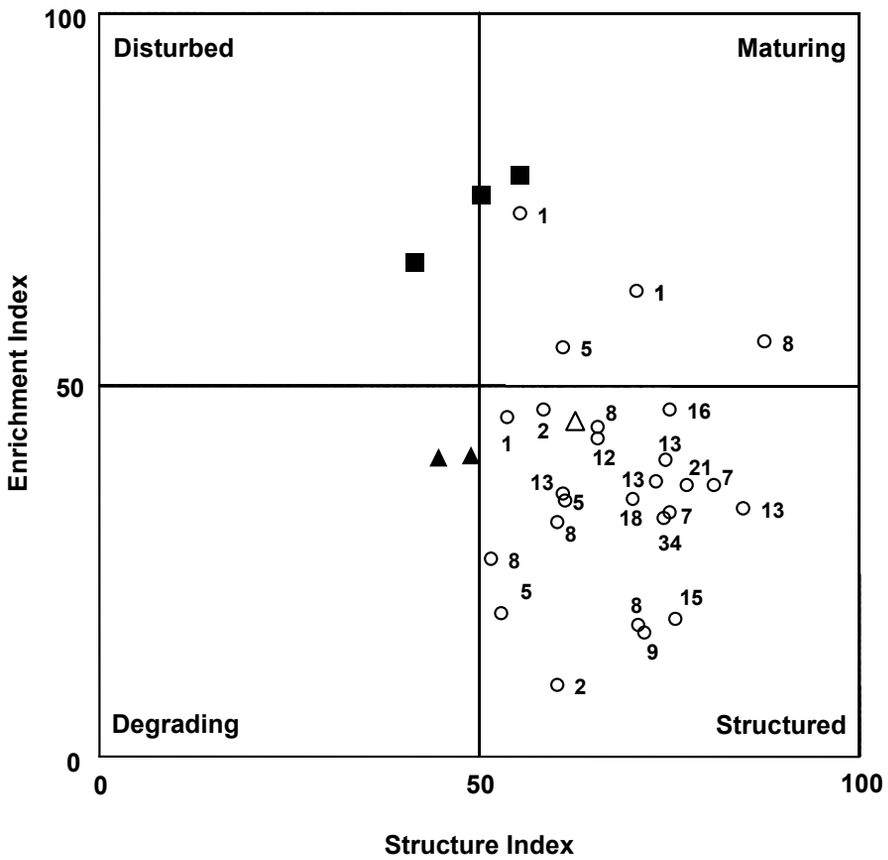


Figure 8.1 Faunal profiles of the chronosequence, representing the structure and enrichment conditions of the soil food web, based on the presence and abundance of functional guilds of nematodes (Ferris et al. 2001). ■ = agricultural field, ○ = former agricultural field, △ = natural site I (matgrass sward), ▲ = natural sites II and III (heathland). Quadrants represent disturbed, maturing, structured and degraded soil food web conditions. Numbers indicate years since abandonment.

community composition may be strongly controlled by abiotic soil conditions and vegetation driven processes (Koehler 1998, Frouz et al. 2001, Zaitsev et al. 2006), suggesting a deterministic and predictable succession trajectory for this group of soil organisms over longer time series (cf Clements 1916, 1928). Successional soil nematode community composition appeared to depend most on the initial composition at the start of land abandonment (cf Egler 1954, Drake 1991). Afterwards, changes in abiotic soil conditions appear to control dominance patterns of nematodes (Chapter 2 and Chapter 3). The low level of replacements in nematode taxa during succession might be explained by the spatial constraints of soil structure, which has been suggested to make competitive exclusion among small soil organisms unlikely to occur (Wanner and Xylander 2005). The differences between mites and nematodes in initial abundance and dispersal abilities, combined with differences in the perception of environmental heterogeneity, resulted in contrasting diversity patterns for these two groups (Chapter 3). In conclusion, in secondary succession, the diversity of one group of organisms may be of low predictive value for the diversity of other groups of organisms (Wolters et al. 2006) and the controlling factors in successional changes in soil community composition may strongly depend on the group of organisms and the community resolution (taxa, feeding groups) considered.

A chronosequence approach can be a useful tool to study long-term successional changes in plant community composition (e.g. Inouye et al. 1987, Coffin et al. 1998, Inouye 1998, Lawson et al. 1999, Bekker et al. 2000, Foster and Tilman, 2000, Witkowski and Wilson 2001, Baer et al. 2002, Wardle et al. 2004b, Keith et al. 2006). Nevertheless, along the chronosequence used in this thesis, soil nematodes and soil mites showed strong site-specific variation in taxon and feeding-group composition which could not be explained by the differences in time since abandonment (Chapter 2 and Chapter 3). Part of this variation may be explained by autogenic factors or by divergent community development resulting from historical differences in former agricultural crop standings and fertilisation regime between the chronosequence sites. Another part of this variation may be attributed to intrinsic differences in soil physical conditions or to differences in the degree of site isolation and the composition of local species pools. Although, in theory, a chronosequence requires all factors other than time to be constant (Luken 1990, Glenn-Lewin and Van der Maarel 1992), differences in historical and local factors are virtually inherent to the approach. Little and fragmentary information was available on the status of the former agricultural fields at the time of abandonment, not allowing to completely control for initial site differences in the selection of sites and in analyses of the results. Therefore, the 'space-for-time-substitution' has introduced some bias, which I could not exactly control.

Plant-soil feedback interactions

In contrast to the descriptive chronosequence study (Chapter 2), results from empirical microcosm studies suggest an important role of soil communities in competitive interactions and successional replacements in secondary plant community succession through biotic plant-soil feedbacks (Chapter 4 and Chapter 5). Changes in the direction of plant-soil feedback (negative or positive) have been suggested to vary across successional gradients as a result of changes in abiotic soil conditions and life history traits of the plant species characteristic for each respective successional stage (Reynolds et al. 2003). Irrespective of abiotic soil conditions, for secondary succession, we showed that plant-soil feedbacks range from negative for early-successional plant species (pioneers, arable weeds), to neutral for mid-successional species, and positive for late-successional plant species (Chapter 4 and Chapter 5). Negative plant-soil feedback reduced the competitive ability of early-successional plants resulting in competitive displacement by mid-successional species (Chapter 5). This suggests that negative plant-soil feedback enhances the rate of succession during early stages of secondary succession and may contribute to the rapid replacement of pioneer species. The positive feedback of the subordinate, late-successional plant species enhanced the evenness of plant communities (Chapter 4) and may contribute to their persistence.

The outcome of individual plant-soil feedbacks (Chapter 5) are the result of the combined effects of plant-specific accumulation of beneficial and harmful soil organisms, which may depend on the present composition of the soil community and on the vulnerability and susceptibility of the plant. We showed that the outcome of plant-soil feedback for late-successional plant species depends on the successional stage of the soil (Chapter 4): the positive feedback was strongest in

late-successional soil. Although in Chapter 4 the soil was merely treated as a 'black box', most likely, this stage-dependent feedback effect was related to a gradual increase of mycorrhizal fungi following land abandonment. Positive plant-soil feedback has been explained by the effects of mycorrhizal fungi on plant growth before (Bever 2002a, Klironomos 2002, Callaway et al. 2003). Therefore, low abundance of mycorrhizal fungi after agricultural disturbance (Smith and Read 1997) may be one of the constraining factors for successful establishment of late-successional plant species. Negative plant-soil feedback for early-successional species was attributable to plant-specific changes in microbial soil communities (Chapter 5). Molecular characterisation of fungal rhizosphere communities revealed a relationship between the biomass production of the early-successional grass *Poa annua* and the composition of the dominant fungal species. Although I did not determine the identity of the organisms involved, microbial root pathogens most likely caused the negative plant-soil feedback (Van der Putten et al. 1993, Bever 1994, Packer and Clay 2000, Klironomos 2002, Callaway et al. 2004b). Negative plant-soil feedbacks, which are reported more often than positive feedback (Bever 2003, Kulmatiski and Kardol, in preparation), did not depend on the successional stage of the soil (Chapter 4). Moreover, biomass production of some early-successional plant species was also reduced by heterospecific microbial inocula. This suggests that negative plant-soil feedbacks are due to plant-specific increase in the density of particular soil pathogens from a common species pool, rather than to host-specific plant-soil organism relationships.

In plant communities, plant-soil feedbacks may affect the outcome of interspecific resource competition (Van der Putten and Peters 1997). In our study, negative plant-soil feedbacks for early-successional species significantly increased when the plants were grown in a competitive environment with heterospecifics (Chapter 5). We observed reduced growth on conspecific soil within a few weeks after planting (Fig. 8.2). Seedlings may be particularly vulnerable to soil pathogens (Augspurger 1990). In absence of heterospecific competition, initial reduction in growth may be overcome when time proceeds. However, in mixed plant communities, competing heterospecifics that do not suffer from pressure of soil pathogens will benefit immediately by taking over resources such as light and nutrients. Interestingly, our results show that effects of feedback between plants and soil biota can have much longer-lasting effects on plant community development than previously thought (Van der Putten et al. 1993). Early-successional plant-soil feedback provided biotic legacies, which influenced dominance patterns of mid-successional plant species. For example, soil legacy effects of the early successional grasses on mid-successional grasses were more pronounced than effects of any of the early-successional species on mid-successional forbs.

In the field, an individual plant will rarely encounter a single-species neighbourhood, but rather a mixture of different neighbouring species. When grown in mixed communities, plants can also affect neighbouring plants via their influences on soil communities, resulting in indirect feedback effects (Bever et al. 1997, Bever 2003). Therefore, net feedback effects for individual plants growing in mixed communities (Chapter 4) can differ from feedback effects when grown in monocultures (Chapter 5). These results highlight the importance of species identity to plant and soil community development (Porazinska et al. 2003, De Deyn et al. 2004b, Bezemer et al.

2006) and imply that in secondary succession, the initial plant species composition, through ecological legacies of plant-soil feedbacks, could lead to long-term, historical contingent effects in successional plant community patterns. Moreover, while the net feedback effects reported in Chapter 4 were separated from resource competition effects, in nature, net feedback effects inherently are affected by resource competition.

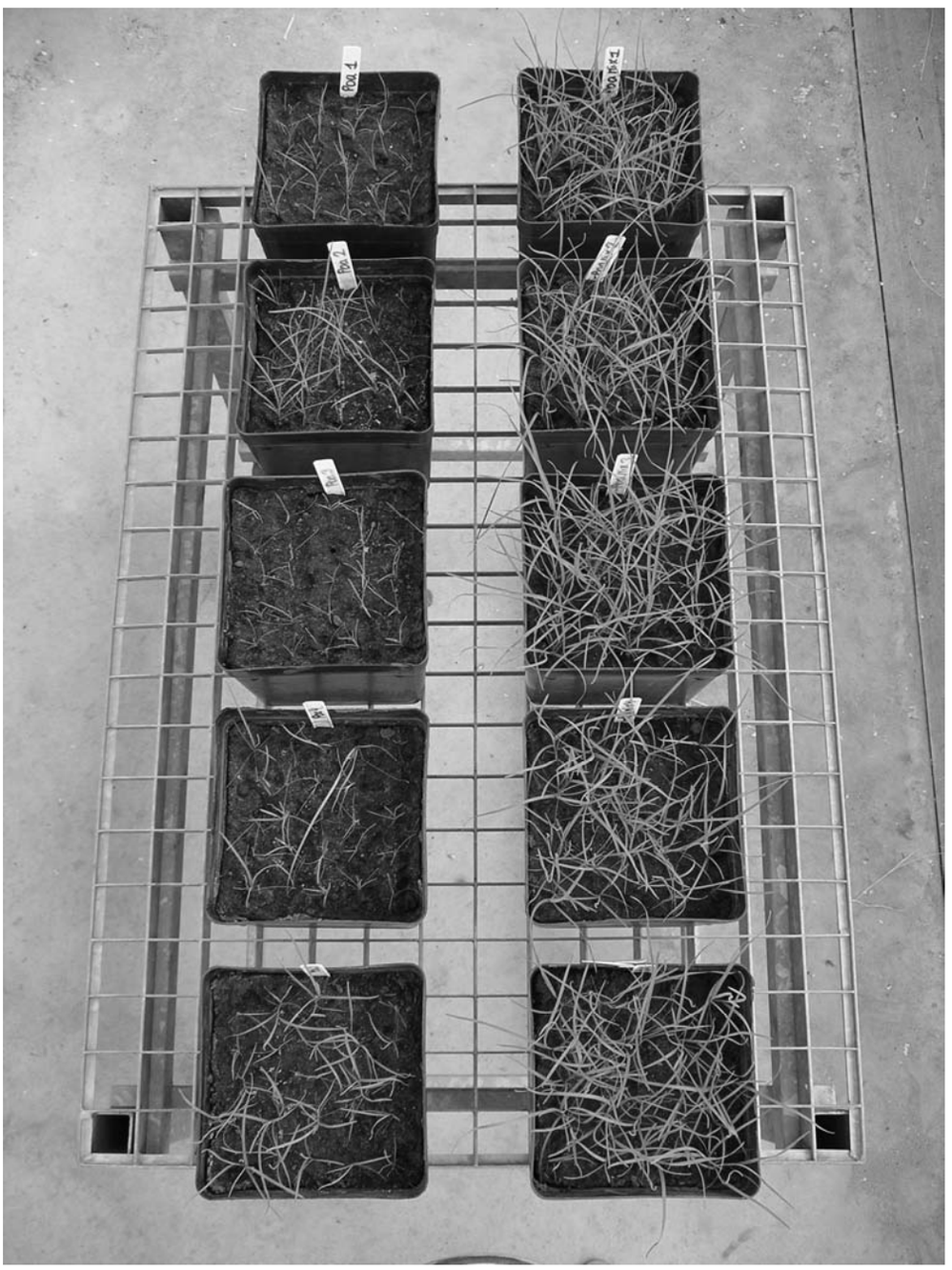


Figure 8.2 Photograph showing the difference in shoot biomass production of the early-successional grass *Poa annua* in five replicate pots after three weeks of growth on own soil (left) and on foreign soil (right).

Restoration of species-rich grasslands and heathlands on former agricultural land

Restoration of species-rich grassland requires a clear understanding of all factors that control plant community assembly, the interactions between these factors, and the relatively importance of these factors over time. After abandonment of agricultural land, high soil fertility (Marrs 1993) and seed limitation (Bakker et al. 1996, Verhagen et al. 2001) have been identified as the major constraining factors for plant community development towards later-successional stages. More recently, soil community development has been suggested to enhance secondary grassland succession (De Deyn et al. 2003). In our study, after agricultural disturbance, soil community development partially depended on dispersal from external species pools, as was shown for oribatid mites (Chapter 3). Such dispersal may be limited (Norton 1994 and references therein), and thereby, potentially constrain plant community succession. Notably, we showed that late-successional plant species display positive feedback particularly with soil organisms from later successional stages (Chapter 4). For restoration of species-rich grasslands this suggests that simultaneous introduction of plant propagules and soil organisms of a species-rich 'target' site will enhance plant community succession and, moreover, will be more effective than introduction of plant propagules or soil organisms alone. However, in a field situation, we could not demonstrate that introduction of later-successional soil organisms, neither by soil spreading nor by turf transplantation, facilitates the establishment of later-successional species (Chapter 7). Soil material originated from *Cirsio-Molinietum* fen meadow and one can question whether soil organisms adapted to the environmental conditions of this mature plant community, would survive and colonize the soil of the abandoned agricultural land (Hedlund and Gormsen 2002). Lack of compatibility in abiotic soil conditions between the organic donor site and the mineral receptor site could be the main reason for failure of establishment of later-successional soil organisms. Soil transfer from intermediate and late-successional stages has been shown effective in establishment of mycorrhizal formations in reforestation on abandoned mine lands (Helm and Carling 1993), but not in mycorrhizal colonization of grassland plants on former agricultural land (Hedlund and Gormsen 2002). The potential of applying soil organism introductions in grassland restoration practices remain tentative and, at least, requires more knowledge about the biotic and abiotic prerequisites for establishment of later-successional soil organisms.

Alternatively, at the field-scale level, effects of plant-soil organism interactions in plant community assembly may be small compared to structuring forces such as abiotic soil conditions and seed dispersal. Particularly, abiotic soil conditions, hence high soil fertility, have received much attention in restoration of species-rich grassland on former agricultural land (e.g. Marrs 1993, Pywell et al. 1994). Top soil removal or microbial immobilisation of plant-available nutrients potentially releases late-successional species from competitive suppression by 'unwanted' early-successional pioneers and weeds (Chapter 6 and Chapter 7). However, plant community development following land abandonment may be much less constrained by high soil fertility than previously thought (Chapter 7). We showed that soil fertility reduction measures applied alone appeared to be ineffective in enhancing establishment of later-successional plant species (Chapter 7). Moreover, sowing mid-successional plant species was highly successful in control treatments where no fertility reduction measures were applied. Apparently the order of arrival

overrules environmental control over plant species composition (Ejrnæs et al. 2006) and once established, priority effects may prevail and prevent replacement of the mid-successional plant communities through invasions by 'unwanted' early-successional weeds (Young et al. 2001, Chase 2003). Seed longevity of most target grassland species is relatively low (Thompson et al. 1997), and, after decades of agricultural land use, most late-successional species will be absent from the seed bank (Bekker et al. 1997). I conclude that during initial stages of secondary succession on abandoned agricultural land, plant community assembly is most strongly constrained by dispersal or recruitment (Poschlod et al. 1998, Bekker et al. 2000, Pywell et al. 2002). Dispersal from local species pools may be rather stochastic, which implies early secondary succession to be a rather unpredictable process, supporting Gleason's individualistic concept of succession (Gleason 1917, 1926). Within this unpredictable process of assembly, soil nutrient status and, potentially, soil community composition function as a filter, removing all species lacking specific combinations of traits (Keddy 1992, Keddy and Weiher 1999, Fattorini and Halle 2004).

Synthesis and conclusions

Community succession and assembly have been suggested to be the core concepts in restoration ecology (Hobbs and Norton, 1996, Lockwood 1997, Young 2000, Young et al. 2001, Young et al. 2005). In community assembly, the Dynamic Environmental Filter Model (Fattorini and Halle 2004) implies a multiple step process which determines whether a new species can join or replace the present community. First, new species must be present in the local species pool (Pärtel et al. 1996, Zobel 1997, Zobel et al. 1998) and be able to disperse to the present community. Second, new species must pass an abiotic filter, which means that they must be able to deal with the chemical and physical conditions. Only if new species pass the abiotic filter they have an opportunity to compete with the established species in the present community. The biotic filter encompasses competitive interactions between species as well as beneficial or harmful interaction with organisms from other groups. To pass this biotic filter the competitive ability of a new species must be sufficient to allow establishment. Importantly, biotic and abiotic filters are dependent on each other and will be affected both by internal and external cues. Moreover, biotic and abiotic filters change over time (Belyea 2004, Fattorini and Halle 2004) and the assembly of one community (i.e. plants) may be affected by the assembly of another community (i.e. soil organisms), through reciprocal alteration of the respective abiotic and biotic filters.

I will synthesize the main results of this thesis using a filter model for plant and soil community assembly during initial stages of secondary succession following abandonment of agricultural land (Fig. 8.3). For plant community development, the pool of pioneer species that is able to colonize from local species pools, or the pool of arable weeds present in the seed bank may strongly control initial stages of secondary plant community succession. Local differences in the composition of these species pool may explain variability in initial plant community composition (Fig. 8.3) [1] (Chapter 2). Successional replacement of early plant communities by later-successional species may be constrained by dispersal limitations [2] (Chapter 6). Active introduction by sowing can reduce dispersal constraints [3] (Chapter 6 and Chapter 7). On the other hand, later-successional species are not necessarily constrained by dispersal limitations [4]

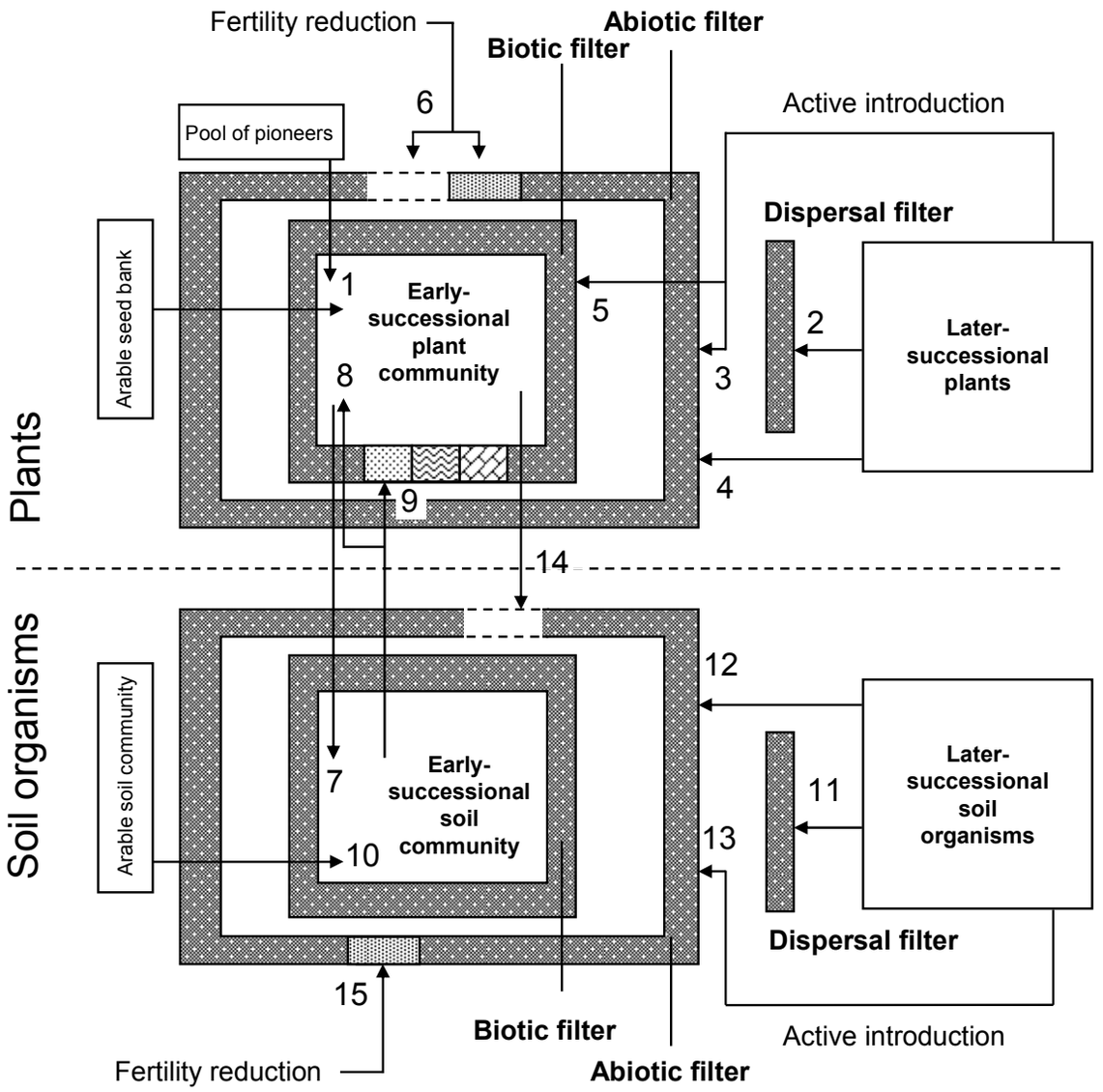


Figure 8.3 Schematic diagram adapted from Fattorini and Halle (2004), illustrating the major controlling factors of initial plant and soil community assembly following abandonment of agricultural land. The numbers are referred to in the text [ ] and point at the main results of this thesis. See text for further explanations.

(Chapter 7) if remnant populations are adjacent (Standish et al. 2007). The abiotic filter (i.e. high soil fertility), often suggested to be the major barrier in grassland restoration (Bakker and Berendse 1999), selects which of the available species are potentially capable to establish and determines their competitive ability relative to other species. The abiotic filter, which can be of minor importance for actively introduced later-successional plant species [5] (Chapter 6), could be reduced or altered by top soil removal (Chapter 6 and Chapter 7) or microbial N-immobilisation [6] (Chapter 7). The final community composition of those species that passed the dispersal constraint and the abiotic filter depend on resource competition with neighbouring species, and on interactions with aboveground and belowground organisms (e.g. Brown 1985, De Deyn 2003): the biotic filter. Direct interactions with soil organisms can strongly affect plant competi-

tive interactions at small spatial and temporal scales (e.g. Van der Heyden et al. 1998, Bradford et al. 2002, De Deyn et al. 2003), but their role in facilitation of establishment of later-successional species at the field scale level could not be demonstrated (Chapter 7). Nevertheless, the other way round, plant species affect the composition of the soil community in a species-specific way [7] (Chapter 4), which in turn affects plant species performance [8] and, hence, competitive interactions (Chapter 4 and Chapter 5). Whether plants alter soil community assembly through alteration of the abiotic or biotic filter cannot be concluded from the experiments described in this thesis. Interestingly, the response to plant-specific changes in soil community composition can vary among later-successional plant species (Chapter 5). Plant community composition during early stages of succession may be strongly influenced by historical (e.g. arable seed bank) and stochastic factors (e.g. Lepš and Rejmanék 1991). This suggests that the plant species that become initially established may cause historical contingency effects in plant community succession through selectively alteration of the permeability of the biotic filter for their successors [9]. Importantly, plant-soil organism interactions can depend on the successional stage of the soil community (not indicated in Fig. 8.3) (Chapter 4). Following land abandonment, the initial soil community may vary from site to site [10] (Chapter 2), depending on historical differences in agricultural management. Successional soil community development may be constrained by dispersal [11] or abiotic and biotic soil conditions [12] (Chapter 2 and Chapter 3). Active introduction of later-successional soil organisms could not be demonstrated as an effective tool to release soil organisms from dispersal constraints (Chapter 6). The abiotic soil condition could have precluded successful establishment [13]. Successional changes in the soil community composition may be primarily controlled by the quantity and quality of plant organic matter input (Chapter 3), i.e. alteration of the abiotic filter [14]. Analogous to plant communities [6], fertility reduction measures such as top soil removal (Chapter 6 and Chapter 7) or addition of carbon substrates [15] (Chapter 7) can alter the abiotic filter for soil organisms. However, it could not be demonstrated that these measures increase the permeability of the abiotic filter for later-successional soil organisms.

In conclusion, both plant and soil communities are not fixed entities, but change constantly over space and time. There is strong potential for interdependence in rate and direction of plant and soil community development in secondary succession following abandonment of agricultural land. So far, most evidence comes from controlled microcosm studies, while in the field plant-soil community relationships appear to be rather idiosyncratic. To improve our understanding of these interactions and the possible applications in restoration of endangered ecosystem, a better understanding of the relative importance of the plant-soil organism interactions, across spatial and temporal scales, is required. Assessment of plant-soil organism interactions in the field is difficult, because the interactions are complex and direct and indirect. A strategic combination of small-scale microcosm experiments and long-term field studies is needed to enhance our ability to explain and predict the effects of plant-soil organism interactions in above- and belowground community assembly.

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Samenvatting

Het omvormen van landbouwgronden tot half-natuurlijke ecosystemen kan het huidige verlies aan soortenrijke graslanden en heidevelden tegen gaan. Pogingen tot herstel van soortenrijke graslanden en heidevelden door landbouwgronden uit productie te nemen, zijn niet altijd succesvol gebleken. Dergelijke omvorming is een vorm van secundaire successie: de opeenvolging van soorten (planten of andere organismen) met als startpunt een 'biologische' nalatenschap na een initiële verstoring. Er is nog steeds veel onbekend omtrent de ontwikkeling van ecosystemen gedurende secundaire successie, met name als het gaat om interacties tussen planten en bodemorganismen. Bodemorganismen kunnen een belangrijke rol spelen in de opbouw en samenstelling van plantengemeenschappen. Dit kan direct door bevorderlijke of schadelijke effecten op de vestiging van zaailingen en op plantengroei en -overleving en indirect door de rol van bodemorganismen in de nutriënten- en koolstofkringloop. Bovendien zijn interacties tussen planten en bodemorganismen afhankelijk van terugkoppelingsmechanismen: planten kunnen de samenstelling van hun bodemgemeenschap beïnvloeden, welke op haar beurt de groei van de plant weer kan beïnvloeden. Tot nu toe is er in secundaire graslandsuccessie na uit-productiename van landbouwgrond weinig aandacht besteed aan de ontwikkeling de bodemgemeenschap, de relatie tussen bodem- en plantengemeenschappen en het potentiële gebruik van bodemorganismen in herstelprogramma's. In dit proefschrift richt ik mij op 1) het analyseren van diversiteitspatronen en veranderingen in de samenstelling van bodem-gemeenschappen in relatie tot secundaire successie van plantengemeenschappen; 2) het bestuderen van de rol van terugkoppelingen tussen planten en bodemorganismen in interacties binnen plantengemeenschappen en in opeenvolging van plantensoorten tijdens secundaire successie; en 3) het testen van effecten van manipulatie van bodemgemeenschappen in het herstel van soortenrijke graslanden.

Nematoden (aaltjes) behoren tot de meest talrijke bodemorganismen in landbouw- en grasland-gronden. Ze vertonen een grote taxonomische diversiteit, bekleden verschillende posities in het bodemvoedselweb en worden gebruikt als indicatoren voor veranderingen in het functioneren van ecosystemen. In een reeks van 26 voormalige landbouwvelden, overeenkomstig in geschiedenis en omgevingsfactoren maar verschillend in de periode van uit-productiename (0 tot 34 jaar; dit noemen we een chronosequentie) hebben we de gemeenschap van nematoden onderzocht en gerelateerd aan de ontwikkeling van de plantengemeenschap. Ter vergelijking hebben we ook half-natuurlijke heiden en een soortenrijk grasland onderzocht. De velden zijn vrijwel allemaal gelegen op de Zuid-Veluwe. Het blijkt dat de nematoden- en plantengemeenschap zich niet noodzakelijkerwijs parallel ontwikkelen in de richting van hetzelfde referentiesysteem. Hieruit concludeer ik dat succesvol herstel van planten-gemeenschappen niet automatisch betekent dat ook de bodemgemeenschap zich over-eenkomstig herstelt. Binnen de chronosequentie selecteerden we drie voormalige land-bouwvelden (vroeg, midden en laat) alsook één van de heidevelden. Op deze vier velden is in samenwerking met Remko Holtkamp (Universiteit Utrecht) en Annemieke van der Wal (NIOO-KNAW) het gehele bodemvoedselweb in kaart gebracht en hebben we op ver-schillende schaalniveaus (qua ruimte en tijd) gekeken naar de biodiversiteits- en gemeenschaps-

ontwikkeling van nematoden en mijten, twee groepen bodemorganismen die op verscheidene plaatsen in het bodemvoedselweb actief zijn. De resultaten geven aan dat het voor de beoordeling van bodemdiversiteit gedurende successie van groot belang is op welke schaal wordt gemeten en welke groep van bodemorganismen als indicator wordt gebruikt. Het blijkt dat de ontwikkeling van de nematodengemeenschap vooral gestuurd wordt door abiotische en biotische veranderingen in de bodem, terwijl de ontwikkeling van de mijtengemeenschap meer bepaald wordt door verspreiding vanuit de omgeving. Wat betreft de ontwikkeling van de trofische structuur van het bodemvoedselweb - een indicatie voor de samenstelling en complexiteit van de voedselketens - blijkt er direct na uit-productiename een grote verandering op te treden, waarna een fase volgt waarin weinig verandert. Deze wordt wellicht veroorzaakt doordat de opbouw van organisch materiaal in de bodem achterblijft bij de veranderingen in de plantengemeenschap.

In kasexperimenten hebben we gekeken naar het effect van terugkoppelingen tussen planten en bodemorganismen op de snelheid en richting van veranderingen in plantengemeenschappen. Voor het eerste experiment construeerden we modelsystemen bestaande uit planten en grond afkomstig uit verschillende successiestadia. De resultaten laten zien dat onafhankelijk van de abiotiek van de bodem, in vroege stadia negatieve terugkoppelingen de plantensuccessie versnellen, terwijl positieve terugkoppelingen de successie stabiliseren door stimulering van langzaamgroeiende plantensoorten uit latere successiestadia. Positieve terugkoppeling met de bodemgemeenschap voor plantensoorten uit latere successiestadia was het sterkst in grond die eveneens afkomstig was uit latere successiestadia. Deze positieve terugkoppeling voor plantensoorten uit latere successiestadia zorgde voor een hogere diversiteit (evenness) in de plantengemeenschap. In een tweede experiment hebben we getest hoe plantensoorten uit vroege successiestadia plant-specifieke veranderingen in de microbiële bodemgemeenschap (bacteriën, schimmels) teweegbrengen en hoe deze veranderingen de competitieve interacties tussen plantensoorten uit vroege en latere successiestadia beïnvloeden. Het blijkt dat terugkoppelingen met de microbiële bodemgemeenschap de vervanging van plantensoorten uit vroege stadia versnelt. Bovendien blijkt dat de terugkoppeling van deze vroege soorten zorgt voor een nalatenschap in de microbiële bodemgemeenschap. Deze nalatenschap werkt door in de dominantiepatronen in plantengemeenschappen uit latere successiestadia.

In veldexperimenten, uitgevoerd in samenwerking met Staatsbosbeheer, hebben we getest of manipulatie van bodemgemeenschappen kan bijdragen aan het herstel van soortenrijke graslanden. Het eerste experiment is uitgevoerd op een voormalig landbouwveld in Assel (Noord-Veluwe) en hier hebben we getest in hoeverre het toedienen van hout en stro (potentieel leidend tot immobilisatie van beschikbare nutriënten) en het afgraven van de nutriëntrijke bovenlaag (de bouwvoor) de ontwikkeling van de planten- en bodemgemeenschap beïnvloedt. In de helft van de behandelingen zaaiden we een mengsel van plantensoorten, karakteristiek voor latere successiestadia, om het relatieve belang van de nutriëntenstatus van de bodem en de mogelijk beperkte verspreidingsmogelijkheden van deze plantensoorten te analyseren. We tonen aan dat het inzaaien meer bepalend is voor de ontwikkeling van de plantengemeenschap dan de maatregelen om de bodemvruchtbaarheid te reduceren. Blijkbaar is het zo dat in het beginstadium

van secundaire successie voor de samenstelling van de plantengemeenschap de volgorde waarin plantensoorten het veld bereiken belangrijker is dan de abiotiek van de bodem. In het tweede veldexperiment, uitgevoerd op een voormalig landbouwveld bij Lievelde (Achterhoek), waarvan na uit-productiename de nutriëntrijke bovenlaag is verwijderd, hebben we getest of gelijktijdige introductie van planten en bodemorganismen afkomstig uit een half-natuurlijk blauwgrasland, de ontwikkeling van de plantengemeenschap richting de 'doelvegetatie' bevordert. We hebben planten en bodemorganismen geïntroduceerd 1) door het uitstrooien van maaisel en grond, onafhankelijk van elkaar of gecombineerd; en 2) door het inbrengen van complete plaggen. We hebben niet kunnen aantonen dat de introductie van bodemorganismen afkomstig uit het blauwgrasland de vestiging van plantensoorten uit het blauwgrasland bevordert. Waarschijnlijk hebben ongunstige bodemomstandigheden (laag gehalte aan organische stof en een sterk fluctuerende waterstand) in het afgegraven landbouwveld de overleving van bodemorganismen in de plaggen beperkt en succesvolle vestiging van de bodemorganismen verhinderd.

Op grond van de kasexperimenten concludeer ik dat er een groot potentieel is voor onderlinge afhankelijkheid van snelheid en richting in ontwikkeling van planten- en bodem-gemeenschappen tijdens secundaire graslandsuccessie op voormalige landbouwgronden. Op kleine ruimtelijke en temporele schaal kunnen terugkoppelingen tussen planten en bodemorganismen de competitieve interacties tussen plantensoorten en de opvolging van plantensoorten uit vroege successiestadia door plantensoorten uit latere stadia sterk beïnvloeden. Als gevolg van ecologische nalatenschap in de bodem kunnen dergelijke terug-koppelingen patronen in plantengemeenschap op grotere temporele schaal beïnvloeden. In een veldsituatie blijken planten- en bodemgemeenschappen zich echter grotendeels onafhankelijk van elkaar te kunnen ontwikkelen en zijn andere factoren, zoals de volgorde waarin plantensoorten arriveren, wellicht meer bepalend voor de plantensuccessie dan het effect van bodemorganismen. We weten nog maar weinig van de omstandigheden waaronder bodem-organismen afkomstig uit latere successiestadia zich het beste kunnen vestigen en vooralsnog hebben we niet kunnen aantonen dat introductie van bodemorganismen een effectieve manier is om soortenrijke graslanden te herstellen.

Dankwoord

Hoewel eenzame werkzaamheden min of meer inherent zijn aan het oio-bestaan, zou dit boekje niets zijn geweest van wat het nu is wanneer het de afgelopen vier jaren bij eenzame werkzaamheden zou zijn gebleven. Het tegendeel was het geval: veel mensen hebben een bijdrage geleverd aan de totstandkoming van dit proefschrift. Of het nu gaat om intensieve samenwerking, een simpel klusje of een losse opmerking, elke bijdrage waardeer ik enorm! Niet iedereen kan ik bij naam noemen (taxonomie blijft een lastig vak), maar toch wil ik een aantal mensen op deze plek persoonlijk bedanken.

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Arnhem, 22 maart 2007
Paul Kardol

The dynamic department of Multitrophic Interactions (summer 2006).
















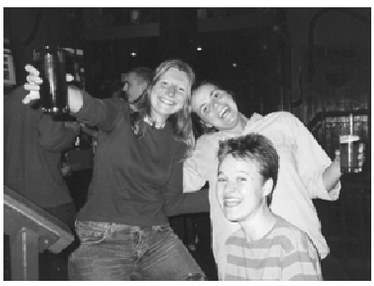






























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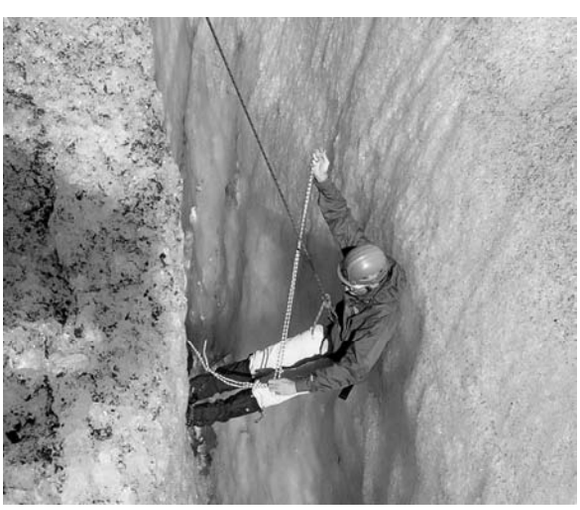
Curriculum vitae

Paul Kardol was born on 30th January 1977 in Oosterbeek, the Netherlands. He attended the Van Lingen College in Arnhem for his secondary education (Atheneum). In 1995, he started the study of Biology at Utrecht University. In 1998, he carried out an undergraduate research project on mesotrophic terrestrialisation in turf ponds, supervised by drs. Tom van den Broek and dr. Boudewijn Beltman at the Landscape Ecology group of the Department of Botanical Ecology and Evolution Biology. In 1999, he carried out a second undergraduate research project entitled 'Breeding in orchards: fitness consequences for Great Tits (*Parus major*)?', supervised by dr. ir. Christel Mols and prof. dr. Marcel Visser in the Department of Animal Population Biology at the Netherlands Institute of Ecology (NIOO-KNAW) in Heteren. After he obtained his MSc degree in 2000, he worked for one year as junior consultant (ecology) at IWACO / Royal Haskoning in 's-Hertogenbosch. In 2002, he was employed for six months at the hydrobiological laboratory of the Waste Water Treatment Authority 'Hollandse Eilanden en Waarden' in Rotterdam, where he was involved in monitoring freshwater macrofauna and vegetation. Hereafter, in November 2002, he started his PhD project in the Department of Multitrophic Interactions at the Netherlands Institute of Ecology (NIOO-KNAW). The study was part of the TRIAS project "Soils in transition: patterns and processes in soil ecosystems during the restoration of natural ecosystems on former agricultural land'. He was supervised by prof. dr. ir. Wim van der Putten and dr. ir. Martijn Bezemer. The research focused on fundamental and applied aspects of plant and soil community assembly in secondary succession on ex-arable lands in the Netherlands and involved a strategic combination of field and glasshouse studies. Within the PhD project he worked in close collaboration with ir. Annemieke van der Wal (microbial ecology), ir. Remko Holtkamp (ecological modelling) and a number of undergraduate students. Most of the results of the PhD project are presented in this thesis. At the end of 2006 he wrote a NWO Rubicon grant proposal, entitled 'Assembly history: biotic plant-soil feedback in spatio-temporal plant community dynamics and invasions', for a two-year postdoctoral study in the Ecosystem Processes Research Group at Landcare Research in Lincoln, New Zealand, in collaboration with dr. Duane Peltzer, and in the Department of Zoology at the University of Hawaii in Honolulu, USA, in collaboration with dr. Tadashi Fukami.



Pitztal glacier, Austria

Ngorongoro crater, Tanzania

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Kardol, P., N.J. Cornips, M.M.L. van Kempen, J.M.T. Bakx-Schotman and W.H. van der Putten. 2007. Microbe-mediated plant-soil feedback in pioneer stages of secondary succession causes long-lasting historical contingency effects in plant community composition. Ecological Monographs 77: xx-xx.

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Van der Wal, A., J.A. van Veen, W. Smant, H.T.S. Boschker, J. Bloem, **P. Kardol**, W.H. van der Putten and W. de Boer. 2006. Fungal biomass development in a chronosequence of land abandonment. Soil Biology and Biochemistry 38: 51-60.

Kardol, P., T.M. Bezemer, A. van der Wal and W.H. van der Putten. 2005. Successional trajectories of soil nematode and plant communities in a chronosequence of ex-arable land. Biological Conservation 126: 317-327.

Manuscripts submitted

Kardol, P., A. van der Wal, A., T.M. Bezemer, W. de Boer, H. Duyts, R. Holtkamp and W.H. van der Putten. Seed addition outweighs soil fertility reduction measures in plant community development during ecosystem restoration on ex-arable land.

Kardol, P. and W.H. van der Putten. Complementary effects of soil organism and plant propagule introductions in restoration of species-rich grassland communities.

Holtkamp, R., **P. Kardol,** A. van der Wal, S.C. Dekker, W.H. van der Putten & P.C. de Ruiter. Soil food web structure during ecosystem development after land abandoment.

Kulmatiski, A. and **P. Kardol**. Getting plant-soil feedback out of the greenhouse: experimental and conceptual approaches.

Manuscripts in preparation

Kardol, **P.**, J.S. Newton, T.M. Bezemer, M. Maraun, A. van der Wal & W.H. van der Putten. Diversity patterns and community development of soil mites and nematodes during secondary grassland succession.

Holtkamp, R., A. van der Wal, **P. Kardol,** S.C. Dekker & P.C. de Ruiter. The effect of soil food web structure on mineralization rates during ecosystem development.

PE&RC PhD Education Statement Form

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 22 credits (= 32 ECTS = 22 weeks of activities)

Review of Literature (5.6 credits)

Succession and assembly theory in ecology restoration (2002-2006)

Post-Graduate Courses (4.8 credits)

Soil ecology: linking theory to practise; FE, SENSE and PE&RC (2003)

Multivariate analysis of ecological data; Faculty of Biological Sciences University of South Bohemia, Czech Republic (2003)

Fragmented habitats and temporal and spatial dynamics of organisms on micro to landscape scale; CONSIDER consortium / Lund University, Sweden (2004)

Deficiency, Refresh, Brush-up and General courses (4.5 credits)

Basic course nematology; Department of Nematology, Wageningen University (2003)

Nematode identification course; Department of Nematology, Wageningen University (2005)

Competence Strengthening / Skills courses (4.2 credits)

Working and planning efficiently; Boertien & Partners (2003)

Techniques for writing and presenting a scientific paper; Wageningen Graduate Schools (2005)

Scientific communication; Biorhiz consortium / Réflexives (2006)

PhD Discussion Groups, local seminars and other scientific meetings (4.5 credits)

'Stimulation Program Biodiversity', Wageningen (2002)

TLinks-meeting, Wageningen (2003)

CONSIDER-meeting, Wageningen (2003)

Symposium 'BodemDiep', SKB-TRIAS, Zeist (poster) (2003)

Heide-workshop, RUN / STW / NWO, Ede (2004)

Soil & Water symposium, TRIAS-SKB, Zeist (poster) (2004)

Current Themes in Ecology, Biological Invasions, Wageningen (2004)

IOBC meeting Multitrophic Interactions, Wageningen (poster) (2005)

Soil & Water symposium, TRIAS-SKB, Zeist (oral presentation) (2005)

KNAW workshop Global Change (oral presentation) (2006)

PE&RC Annual Meetings, Seminars and Introduction Days (3 credits)

Annual meeting Graduate school Functional Ecology; poster (2003); oral presentations (2004 and 2005)

International Symposia, Workshops and Conferences (12.6 credits)

BES symposium 'Soil Biodiversity and Function'; Lancaster, UK (poster) (2003)

XXVIIth International Symposium European Society of Nematology; Rome, Italy (poster) (2004)

Consider Meeting 'Habitat fragmentation'; Lund, Sweden (2004)

XIVth Internationall Colloquium on Soil Zoology and Ecology,; Rouen, France (poster) (2004)

GfÖ Workshop on Restoration Ecology; Giessen, Germany (oral presentation) (2005)

Annual Meeting British Ecological Society (BES); Hertfordshire, UK (oral presentation) (2005)

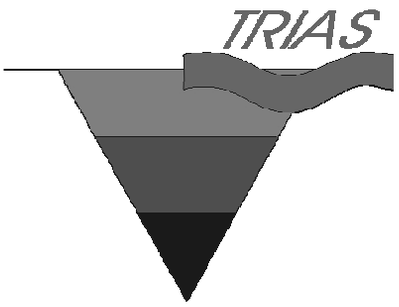
35th Annual Conference GfÖ; Regensburg, Germany (oral presentation) (2005)

XVIIth International Botanical Congress; Vienna, Austria (oral presentation) (2005)

91th Annual Meeting ESA; Memphis, USA (oral presentation) (2006)

Courses in which the PhD candidate has worked as a teacher (0.5 credit)

Functional Biodiversity, Department of Nematology, Wageningen University (2006)



TRIpartite

Approach to

Soil System Processes

The research presented in this thesis was part of the project 'Soils in transition: patterns and processes in soil ecosystems during the restoration of natural ecosystems on former agricultural land (TRIAS-SKB Grant 835.80.011).

Subproject 1 The microbial component of soil food webs and processes.
Ir. Annemieke van der Wal
Department of Terrestrial Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW)

Subproject 2 Soil food web structure, soil ecosystem processes, and vegetation development.
Drs. Paul Kardol
Department of Multitrophic Interactions, Netherlands Institute of Ecology (NIOO-KNAW)

Subproject 3 Food web modelling.
Ir. Remko Holtkamp
Environmental Sciences, Copernicus Institute, Utrecht University

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Stichting
Kennisontwikkeling
Kennisoverdracht
Bodem

Royal Netherlands Academy of Arts and Sciences

NETHERLANDS INSTITUTE OF ECOLOGY

The research presented in this thesis was conducted at the Department of Multi-trophic Interactions, Centre for Terrestrial Ecology of the Netherlands Institute of Ecology (NIOO-KNAW) in Heteren.

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