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Soils in transition: dynamics and functioning of fungi

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Chapter 1

General introduction



General introduction

Restoration of ex-arable land into heathland

Since decades, intensive farming in Europe results in chronic overproduction and the reduction of biological diversity. To counteract these trends, the European Union supports abandonment of agricultural land to restore and increase the area of natural ecosystems and to control agricultural surpluses. In 1990, the Netherlands Ministry of Agriculture, Nature and Food Quality introduced the Ecological Main Structure (EMS), which comprises a coherent network of nature areas and connection zones that should be protected and conserved. The Netherlands has one of the most intensive agricultural systems in Europe, and according to the EMS, policy makers and nature managers aim to (re)create natural ecosystems, in particular *Calluna*-dominated heathlands on sandy ex-arable soils.

Heathlands dominated by *Calluna vulgaris* are characteristic ecosystems in Western Europe. They are highly appreciated because of their cultural historical values and their important role in preservation of flora and fauna (Aerts and Heil, 1993). In the Netherlands, the restoration of heathlands receives much attention as sandy soils were dominated with heathlands before the introduction of artificial fertilizers (De Smidt, 1979). The landscape in these areas is now consisting largely of agricultural land and forests, the latter being planted on former heathlands and sandblowns. The heathlands that have remained have lost much of their original value and diversity because of the expansion of grasses, which has been attributed to increased atmospheric nitrogen input (Heil and Bruggink, 1987).

Several strategies have been performed to create heathland on abandoned agricultural land, which usually has higher soil nutrient levels and a higher soil pH than heathland. The removal of top soil has been applied as one of the management strategies to reduce the levels of nutrients, especially of phosphorus levels (Aerts et al., 1995; Allison and Ausden, 2004). Another restoration technique is the reduction of soil pH by addition of sulphur (Owen and Marrs, 2000; Lawson et al., 2004). Similarly, the addition of heathland propagules either as heather shoots collected from mature heathlands or as translocated heathland turves have been used to restore heathland vegetation (Pywell et al., 1995). However, it has turned out that it is not easy to convert a fertile, arable land into a semi-natural heathland as agricultural management has changed the biological, chemical and physical properties of the soil (Haynes and Naidu, 1998; Six et al., 2006).

The goal of this thesis was to investigate microbially related factors involved in the soil processes during conversion from an arable land into a heathland. The specific focus was on the dynamics and functioning of fungi during the transition process.

The role of saprotrophic fungi in soil ecosystem processes

Saprotrophic fungi play a critical role in many soil processes as they decompose and mineralize soil organic residues, mostly components of plants. This activity of fungi is essential for nutrient cycling in terrestrial ecosystems; carbon dioxide and nutrients are released and again available for photosynthesis and plant growth.

Fungi are better equipped for the degradation of solid plant materials than bacteria are, both through their mode of growth and their enzymatic capabilities. They are filamentous, i.e. they form hyphae, and by doing so they can penetrate solid organic material and translocate nutrients through their cytoplasm (Holland and Coleman, 1987; Klein and Paschke, 2004). The translocation of nutrients enables the fungi to grow on heterogeneously distributed organic substrates and also on substrates that are short in N, P or other essential nutrients (Boddy, 1999). Fungi release extracellular enzymes that hydrolyze complex molecules and polymers into smaller molecules which can be absorbed through cell membranes. Fungi can excrete enzymes for several substrates at the same time which is advantageous in an environment of mixed substrate compounds. For example, plant cell walls contain cellulose, proteins, lignins, hemicelluloses and pectins mixed into a matrix (Adl, 2003). Only a relative few, specialized and widely distributed fungal species belonging to the phyla *Basidiomycota* and *Ascomycota* have the ability to degrade wood, which is composed of cellulose, hemicellulose and lignin (Daniel, 2003). Based on their degradation abilities, these fungi are grouped as white-rot, brown-rot and soft-rot fungi. Of these fungi, only white-rot fungi are able to completely digest lignin, the most recalcitrant part of wood (Shah and Nerud, 2002). Other fungi, sometimes referred to as sugar fungi or molds, are more dependent on soluble sugars or cellulose and can compete with bacteria for these compounds (De Boer et al., 2005). Such fungi are r-selected strategists i.e. they grow quickly when conditions are favourable and form spores when conditions become unfavourable. This enables them to colonize rapidly and avoid competition on virgin resources (Carlile et al., 2001).

During the decomposition of organic material the easily accessible cellulose and hemicellulose are degraded first by r-selected fungi, followed by the colonization of rot fungi that degrade the recalcitrant parts (lignin) of the organic material (De Boer et al., 2005). Soil organic matter decomposition by fungi is largely controlled by environmental conditions, such as moisture content, oxygen concentration and temperature, as well as the composition of the substrate (Nicholas and Crawford, 2003). It is suggested that initial decomposition is limited by nitrogen. However effects of nitrogen fertilization on litter decomposition are contradictory (Fog, 1988; Hobbie, 2005). Long-term additions of nitrogen may decrease the decomposition rate and this has been attributed to a fertilizer-induced increase in decomposer efficiency (production-to-assimilation-ratio) (Franklin et al., 2003). Hyvönen et al., 2005.

suggested that nitrogen deposition could even lead to an increased carbon accumulation in the humus layer. Fungi are involved in the formation of soil aggregates through both mechanical binding by hyphae and chemical binding by fungal-produced polysaccharides (Tisdall, 1991; Bossuyt et al., 2001). This has a positive effect on soil moisture retention and the resistance of soil against erosion (Tisdall and Oades, 1982; Beare et al., 1997). It is generally thought that decomposition processes in soils dominated by fungi result in lower C and N mineralization rates than those dominated by bacteria. This is attributed to the higher C:N ratio of fungi compared to bacteria and the lower turn-over rates of the fungal-feeding fauna (Paul and Clark, 1989; Didden et al., 1994; Zwart et al., 1994). In addition, soil carbon is assumed to be stored more effectively in fungal biomass than in bacterial biomass, since bacteria have shorter generation times and turn-over their biomass at faster rates than do k-selected fungi (Bailey et al., 2002; Hunt et al., 1987). A relatively high amount of fungal biomass would thus give protection against nitrogen leaching and sequester more carbon than bacterial-dominated communities (Bailey et al., 2002; Vinten et al., 2002; Six et al., 2006; De Vries et al., 2006) although this is hardly investigated experimentally. Furthermore, it is assumed that saprotrophic fungi may act as antagonists against plant pathogens (Thornton, 2004). All of these activities of saprotrophic fungi positively contribute to functioning of soils and may be essential for the development of a natural heathland vegetation on ex-arable soil.

Influence of agricultural practices on soil fungi

Many studies reported a reduction of fungal biomass under conventional agricultural management and grassland intensification, whereas bacteria seem to dominate in these systems (e.g. Bloem et al., 1994; Bardgett and McAlister, 1999; Frey et al., 1999; Stahl et al., 1999; De Vries et al., 2006). Also the composition of the fungal community is affected by agricultural management practices (Donnison et al., 2000).

Different explanations have been given to explain the detrimental effects of agricultural management on soil fungi. The removal of organic material as harvestable products in arable ecosystems is preventing the accumulation of a litter layer, which is normally present in natural ecosystems. The absence of a litter layer is associated with a decrease in available substrate for lignocellulolytic fungi (Dick, 1992). This will also influence the quantity and composition of the soil fauna feeding on primary decomposers. The fact that bacteria do not seem to be negatively affected by agricultural management has been ascribed to plowing which brings the substrate into close contact with bacteria, encouraging bacterial growth (Holland and Coleman, 1987). Besides the absence of a litter layer, the application of N fertilizers may inhibit the activity of members of the fungal community

through the suppression of enzyme activity and the build up of recalcitrant and toxic compounds, e.g. amino groups reacting with phenols (Fog, 1988). The negative influence of phosphorus fertilization on arbuscular mycorrhizal colonization of plant roots may also reduce the fungal biomass (Jasper et al., 1979; Kahiluoto et al., 2001). Another explanation for the decrease in fungal biomass is that tillage disrupts hyphal networks, resulting in decreased lengths of fungal hyphae (Lowell and Klein, 2001; Stromberger, 2005). However, an alternative explanation for the observed shorter fungal hyphae in agricultural soil may be the homogeneous presence of resources, allowing fungi to utilize nutrients in their immediate environment without the need for extensive growth (Lowell and Klein, 2001).

Changes of soil biological and chemical properties during land conversion

After abandonment of arable fields, the soil pH is decreasing with time of abandonment (Zeller et al., 2001). Nutrient levels change over time as no fertilization is applied anymore. In addition, nitrate will easily leach into deeper soil layers where it is not taken up by plants or immobilized by micro-organisms. Annual phosphorous (P) gifts have resulted in an enrichment of P of sandy arable fields and this may give rise to leaching of P into deeper soil layers as the sorption capacity of sandy soils for P is generally limited. In addition, it is suggested that a sudden change of land use, such as the abandonment of highly fertilized land, may increase the risk of P leaching (Van Latesteijn, 1998). The quality of organic matter, e.g. the C:N ratio and lignin content, decreases and the amount of organic matter increases as no harvestable product is removed anymore (Knops and Tilman, 2000; Zeller et al., 2001). Hence, the absence of agricultural management gradually results in a more heterogeneous soil environment and the formation of a litter layer.

All the aforementioned changes are expected to favor fungal growth and several studies have already confirmed this. For example, fungal: bacterial biomass ratios were found to increase with successional age in a semi-arid grassland community (Klein et al., 1996) and along an Alaskan forest chronosequence (Ohtonen et al., 1999). As a consequence of increase in fungal biomass, consumers of fungi are also thought to increase in numbers and biomass. Háněl (2003) found increasing populations of fungivorous nematodes in a fallow field. In addition, microarthropod (non-cryptostigmatic mites and omnivorous collembola) biomass was shown to be larger in meadows than in agricultural fields and they were thought to feed on fungi (Vreeken-Buijs et al. 1998). An increase in fungal biomass may thus have an effect on higher trophic levels within the soil foodweb. In addition, interactions between micro-organisms and plants will create positive- (e.g. interaction between a specific plant and beneficial mycorrhizal fungi) or negative- (e.g. microbial pathogens that inhibit plant growth)

feedback loops that influence both plant and microbial community structure (Klironomas, 2003; Van der Putten, 2003). Hence, during land conversion, biological and chemical changes belowground can influence the vegetation aboveground and vice versa.

Constraints on fungal biomass increase in abandoned land

Fungi have a much stronger contribution to decomposition processes in natural ecosystems than in arable land. Therefore, it is expected that fungal biomass will increase during transition from arable land into heathland. Several studies reported increases of fungal biomass in abandoned grassland soils (Klein et al., 1995; Zeller et al. 2000, 2001). However, the constraints on increase of fungal biomass and fungal decomposition processes in ex-arable soil are hardly known.

Several factors may limit the increase of fungal biomass in ex-arable areas. The build-up of organic layers on abandoned arable fields make take several decades and means a slow build-up of habitats and substrates suitable for litter-inhabiting and wood-degrading fungi. Fungi that degrade recalcitrant organic matter have more resistant, long-lasting hyphae than opportunistic fungi that degrade easily available carbon (Cooke and Rayner, 1984). Hence, contribution of such k-selected fungi to an increase in fungal biomass is likely to be more important than that of r-selected fungi that have a high turnover of their hyphae. Therefore, a slow built-up of an organic matter layer may be a constraint for the increase of a more enduringly present fungal biomass. Restriction of fungal growth in former agricultural soil can also be attributed to the presence of anti-fungal compounds that are produced by bacteria, as most agricultural soils are bacterial dominated (Lang et al., 1997; De Boer et al., 1998; Mackie and Wheatly, 1999). Fungal biomass increase may as well be reduced by fungal grazers, such as protozoa, nematodes, and fungivorous mesofauna such as *Collembola* (Old and Darbyshire, 1978; Berg et al., 2004). Protozoa can also indirectly affect fungal populations by stimulating bacterial activity, and enhance antagonistic effects of bacteria on fungi (Levrat et al., 1987). Another constraint for fungal biomass increase in ex-arable land may be the presence of fungicidal residues in soil. Fungicides can inhibit as well as stimulate saprotrophic fungi, i.e. certain groups of fungi can be killed whereas the dead fungal hyphae or the fungicide itself can serve as substrate for other fungi or bacteria (Chen et al., 2001).

An increase in the biomass of arbuscular mycorrhizal fungi is expected to be constrained by high phosphate levels which is common in Dutch arable soils (Jasper et al., 1979; Kahiluoto et al., 2001). The increase of biomass of ericoid mycorrhizal fungi is likely to be limited by the presence of their host plants, such as *Calluna vulgaris* in Dutch dry heathlands.

The role of ericoid mycorrhizal fungi in heathland and in heathland restoration

A characteristic feature of heathland soils is the recalcitrance of organic matter due to a high phenolic content and a low pH, in which mineralization of nutrients is inhibited. In addition, organic acids occur in solution in their most toxic undissociated form (Read, 1991). Together, these factors result in an ecosystem in which the survival and growth of heathland plants is dependent on the presence of an ericoid mycorrhizal infection.

Ericoid mycorrhizas have been regarded as one of the most specific of mycorrhizas because their host range is restricted to a limited number of families of the *Ericales*. In addition, the mycosymbiont in the association belongs to a taxonomic small group of ascomycetes. The ability of the mycosymbiont to mobilize N and P from the organic residues to the plant is important, particularly in the nutrient-poor heathland environment. There is indeed evidence that *Hymenoscyphus ericae*, the most well known ERM fungus, is able to assimilate and translocate nitrate, ammonium, peptides, protein and phosphate to the plant (Pearson and Read, 1975; Bawja et al., 1985; Bawja and Read, 1985, 1986; Leake and Read, 1989a). Substantial quantities of the assimilated products were shown to be transferred to the plant (Bawja and Read, 1985, 1986; Bawja et al., 1985). Metal toxicity and phytotoxic organic acids may hamper the development of heathland plants in the acid heathland soil. However, ERM fungi have been shown to detoxify these compounds or, in the case of phenolic compounds, even use them as carbon source, thereby facilitating seed germination of their host plant (Leake et al., 1989b).

The conversion of a nutrient-rich arable land into a heathland is expected to be difficult since the soil conditions on arable land are completely different from those of heathlands. The fertility, the quality of organic matter and the pH of the soil is much higher in arable land. A high fertility may limit the establishment of *Calluna vulgaris* since fast-growing plant species will have a competitive advantage over slow-growing *Calluna* seedlings. Until now, many studies concerned the ecological role of ERM fungi in mature heathland systems, whereas their role on the natural colonization of *Calluna* in new sites has not been investigated.

Aim, research questions and outline of this thesis

The main aim of this thesis was to analyze the dynamics of biomass and functions of soil fungi during conversion from a high input-output agricultural ecosystem into a low input-output natural ecosystem and to assess the effect of these dynamics on soil ecosystem processes. Until now, most studies on fungal communities in different input-output systems focused either on microbial community structure in different agro-ecosystems, i.e. in soil from conventional agriculture compared to no-till systems (e.g. Frey et al., 1999; Carpenter-Boggs et al., 2003), or they focused on just a few abandoned arable fields that differed many years in field age (Klein et al., 1995; Zeller et al., 2000, 2001). This makes it difficult to assess the relative importance of changes in soil properties or absence of soil management on the gradual development of fungi. Changes in fungal biomass are suggested to be due to changes in soil pH and organic matter quantity and quality, but experimental evidence for this is lacking. It is, therefore, interesting to assess the influences of different quality, composition and size of substrates on the dynamics and decomposition functions of saprotrophic fungi.

The following research questions were formulated to reach the main aim:

1. How do saprotrophic fungi develop during land abandonment and which soil properties influence this development? Is there a difference in the development of r-selected and k-selected fungi in abandoned fields?
2. Which characteristics of organic substrates (recalcitrance, size, accessibility or quality) are influencing the development and functioning of saprotrophic fungi?

In addition to the main aim two other aspects of the transition of ex-arable soils to heathland soils were examined as well. The first aspect concerns the fate of phosphorous in ex-arable, sandy soils. Abandonment of arable soils is suggested to trigger leaching of phosphorous into deeper soil layers. Therefore, it is important to determine this effect as soil nutrient enrichment can threaten groundwater quality. The following research question was addressed to study this:

3. What is the vertical distribution of total phosphorous in relation to time of abandonment of arable fields? Is there a reason for concern about leaching of high amounts of phosphorous into deeper soil layers?

This project was carried out in the context of heathland restoration, and therefore the other aspect considered here concerns our observation of establishment of *Calluna vulgaris* plants

in a recently abandoned arable land, despite high levels of nutrients (P) and the presence of fast-growing forbs and grasses. This led us to question:

4. How can the slow-growing plant species *Calluna vulgaris* establish and maintain itself in a nutrient-rich field dominated by fast growing grasses and forbs?

The first research question is addressed in Chapter 2, in which the results of a study on the development of the fungal and bacterial biomass along a chronosequence of 26 ex-arable fields that differed in the time since abandonment are described. Organic matter quantity and quality, as well as soil acidity and nutrient levels were included in the measurements. It became clear that neither fungal biomass, nor the organic matter quality and quantity reached the level of a natural ecosystem in the time interval of abandoned fields that were studied (about 30 years). A model for the development of fungal biomass in ex-arable soils is proposed. The second question is related to the constraints of saprotrophic fungi to increase in biomass on ex-arable land. In Chapter 4 and 5, the effect of addition of carbon substrate on biomass, activity and composition of soil fungi is shown. Substrates that differed in composition, size and the quality (C:N ratio) were added to ex-arable- and heathland soils. The response of fungi to different accessibility of the substrates, i.e. mixing in the soil versus a separate litter layer, was also studied. The third question is related to the results from Chapter 2, where total phosphorus in the upper 10 cm of ex-arable soils was negatively related to the time of abandonment. In Chapter 3 the results of total P concentrations at different soil depths in four ex-agricultural fields that differed in time since abandonment are described. The last question is addressed in Chapter 6. In this chapter, patches of *Calluna* plants in an abandoned agricultural field are examined for colonization with ericoid mycorrhizal fungi. The soil properties under the patches were compared with those of the rest of the field. A possible mechanism for their establishment on a highly fertilized field with a dominance of fast-growing plant species is discussed.