Soils in transition: dynamics and functioning of fungi

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Chapter 7

General discussion

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In this thesis, results of studies on the dynamics of biomass and functions of saprotrophic fungi during conversion from arable land into semi-natural heathland are described. The main objective of this study was to assess how saprotrophic fungi developed after land abandonment (Chapter 2) and to investigate the principal factors that influenced this development (Chapter 4 and 5). In addition, two other studies related to conversion of ex-arable land into heathland were performed. Firstly, the vertical distribution of phosphorous was measured in different abandoned arable fields to determine the risk of leaching of phosphorous into deeper soil layers after abandonment of highly fertilized arable fields (Chapter 3). Secondly, a study was performed to indicate the possible mechanisms for the establishment and extension of slow-growing *Calluna* plants in a nutrient-rich ex-arable field dominated by fast-growing grasses and forbs (Chapter 6).

Development of fungi during land abandonment

Agricultural management is negatively affecting the biomass of fungi and, in particular in the intensively managed agricultural soils in the Netherlands, bacteria seem to dominate (Bloem et al., 1994; Stahl et al., 1999; De Vries et al., 2006). Natural soils and soils that are less intensively managed have a higher fungal biomass and activity than arable soils (e.g. Bailey et al., 2002; Zhang et al., 2005; Susyan et al., 2005). Therefore, we hypothesized that fungal biomass will increase after abandonment of agricultural fields. In Chapter 2 of this thesis the fungal biomass was quantified along a chronosequence of 26 ex-arable fields in the Netherlands. This chronosequence covered 1-34 years of land abandonment. Three arable fields and three semi-natural heathlands were included, representing the starting point of abandonment and the target for nature development, respectively. Fungal biomass increased only during the first two years after abandonment. Thereafter a leveling off in fungal biomass was observed. Interestingly, soil properties that are known to influence fungal growth, e.g. the quantity and quality of organic matter and the soil pH, did not change over time. It has been suggested that there is a causal relationship between soil disturbance and disruption of the network of mycorrhizal and non-mycorrhizal fungi (McGonigle and Miller, 1996; Kabir et al., 1999; Boddington and Dodd, 2000). Therefore, the conclusion from this study was that the first increase in fungal biomass is due to the absence of soil disturbance brought about by agricultural activities. Fungal biomass is then stabilized and is not increasing any further although it is still far from the level of the heathland. Two possible conceptual models were proposed to predict the development of fungal biomass after land abandonment. The first possibility is that the period of land abandonment that we studied was too short to determine a change in the carrying capacity for fungal biomass. This carrying capacity is likely to be determined by the quantity and quality (e.g. C:N ratio and lignin content) of organic matter and soil pH. Since these soil properties are changing very slowly, the increase of fungal biomass will also be very slowly and possibly not detectable during the first decades after land abandonment. The second model implies that a further increase in fungal biomass is constrained by important soil properties that have to reach a critical threshold before key fungal groups can enter the system. The critical threshold in the ex-arable - heathland development may be the colonization of heather- or woody plants in the ex-arable field. This may trigger the development of ericoid mycorrhizal- and lignocellulose-degrading fungi that can then dominate the decomposition processes. However, in either model, the increase in fungal biomass after land abandonment under these conditions will be slow and will take several decades. A slow change in the biomass of fungi may affect an in increase soil organisms at higher trophic levels in the soil food web. During transition from arable land into a heathland, it was observed that the oldest fields (several decades after land abandonment) were already colonized by later-successional plant species (Kardol et al., 2005). Therefore, it appears that the soil food web structure after land abandonment lags behind the aboveground processes (Holtkamp et al., manuscript in preparation).

The difference in the two proposed models is that the first model aims at a gradual increase in fungal biomass, whereas the second model suggests a stabilization phase after which a rather fast increase in fungal biomass will occur. In ecological theory the idea of a sudden shift to an alternative state has been described before (Scheffer et al., 2001). An alternative state, which can be achieved in both models, may also apply for these soil ecosystems; when fungal biomass is increasing due to the presence of suitable substrates, a change towards a soil with higher resilience may be expected as fungi contribute to soil aggregation and retention of nutrients (Bossuyt et al., 2001 Six et al., 2006; De Vries et al., 2006). In the context of soil ecosystem restoration, the second model may be of interest. According to this model, an increase in fungal biomass is inducible. Possibilities to trigger the increase in fungal biomass in ex-arable land could be the introduction of seeds of woody shrubs such as heather plants or the introduction of organic matter in the form of wood. However, in a 3-year field experiment the addition of wood fragments or straw to a recently abandoned ex-arable soil did not result in an increase in soil fungal biomass (personal observation). Three years after applying the treatments, large pieces of straw and wood were still present in the soil, suggesting that a longer period of time is needed before wood-degrading fungi can degrade the recalcitrant carbon present in wood. Therefore, it should be kept in mind that a fast increase in fungal biomass is not easy to accomplish and it seems that there is a lag phase in fungal biomass increase even after applying carbon substrates for saprotrophic fungal growth.

In Chapter 4 the constraints for an increase in fungal biomass during land abandonment were studied. It was hypothesized that the presence of suitable substrates are limiting the development of slow growing K-selected ligno-cellulolytic fungi. In Chapter 2 it was described that fungal biomass is not further increasing after two years of land abandonment, possibly due to the dominance of opportunistic, r-selected saprotrophic fungi. These fungi grow on the more easily degradable parts of organic matter and have a rapid turn-over of fungal mycelium, resulting in an overall low fungal biomass (Cooke and Rayner, 1984). An increase in the amount and recalcitrance of organic matter was expected to lead to an enduringly increased level of fungal biomass, composed of K-selected fungi. Different carbon substrates (glucose, assumed to stimulate opportunistic fungi and bacteria; cellulose, assumed to stimulate cellulolytic fungi; wood, assumed to stimulate ligno-cellulolytic fungi) were added to one arable, three ex-arable and one heathland soil. Surprisingly, all substrates, even sawdust, induced a quick response in fungal biomass and a subsequent rapid decline within 6 weeks of incubation in the arable and ex-arable soils. Opportunistic fungi were stimulated by the substrates as was confirmed by identification of fungi using sequencing of fungal 18S rDNA gene fragments, enzyme activities and culturing. In the heathland soil, ericoid mycorrhizal fungi appeared to be responsible for the slow decomposition of added carbon. These results show that r-selected fungi are still dominating the decomposition processes in ex-arable soils, even after two decades of land abandonment. Inoculation of sawdust did not successfully induce an increase in K-selected fungi. It is possible that the length of the experiment was too short to measure the decomposition of lignin in the sawdust by ligno-cellulolytic fungi or that sawdust was not a suitable substrate for these fungi. Sawdust has a large surface: volume ratio, maximizing the exposure of easily available cellulose and hemicellulose. This will be most beneficial for opportunistic fungi. Another constraint for a consistent fungal biomass increase may be the absence of sufficient inoculum of K-selected fungi in ex-arable soils. Although it is observed that agricultural soils can contain up to 100 different basidiomycetes species (Lynch and Thorn, 2006), these fungal species may only be present as spores and may need to increase their biomass before they can compete with opportunistic fungi for organic matter.

Fungal succession towards K-selected fungal species is apparently not yet occurring, implying that the stabilization phase of fungal biomass presented in Chapter 2 is composed of opportunistic fungi. The conclusion from this study is that the increase in fungal biomass in ex-arable soils is limited because of the dominance of substrate utilization by r-selected fungi. An additional stimulation to induce an increase in fungal biomass on ex-arable soils may therefore be, together with the addition of suitable substrate and/or host plants, the introduction of an inoculum of K-selected fungi. Perhaps, this inoculum should consist of a certain level of biomass of fungal hyphae that is needed to colonize new substrates in competition with opportunistic fungi. If the basidiomycetes that are found in agricultural soils are present as spores, another constraint to their germination could be the presence of bacterial-produced fungistatic compounds (De Boer et al., 2003). A separate litter layer on the surface of the soil could form a possible niche for spores of saprotrophic K-selected fungi to germinate and develop a biomass of fungal hyphae (Lindahl et al., 2007). This implies that an alternative method to induce an increase in fungal biomass is the addition of substrate on the surface of the soil together with an inoculum of spores of K-selected fungi.

In the study described in Chapter 4 the focus was on the development of saprotrophic fungi. However, in heathland systems the biomass of ericoid mycorrhizal fungi is likely to contribute substantially to the total amount of soil fungal biomass (Read et al., 2004). The introduction of suitable host plants for ericoid mycorrhizal fungi on ex-arable soils could be an alternative experiment to study the constraints for an increase in fungal biomass during soil transition.

Influences of quality, size and accessibility of wood on fungi

In Chapter 4 it was shown that sawdust mixed into the soil induced the growth of opportunistic fungi. Both bacteria and opportunistic fungi were thought to be stimulated by the small size of the substrate and the enhanced contact between wood and soil. Saprotrophic opportunistic fungi can be released from bacterial competition by larger sizes of substrate as was indicated in Chapter 5. A mechanism for this escape can be the growth form of fungi: filamentous fungi are better able to penetrate larger pieces of wood as compared to unicellular bacteria (Holland and Coleman, 1987; Klein and Paschke, 2004). This suggests that the size of the substrate should be integrated in models that study the dynamics of litter chemistry and microbial interactions during litter decay, as substrate size is affecting fungal growth and the rate of decomposition. Unexpectedly, burial of wood also stimulated fungal growth. This may be explained by the exposure of a larger surface of wood for fungal infection. Whereas this exposure will be also beneficial for colonization by bacteria, they are restricted in their ability to grow inside the wood particle and, therefore, fungi have the opportunity to monopolize substrate utilization inside the particle. Another explanation for the positive effect of burial can be the stimulating effect on fungal growth of an increased level of carbon dioxide in soil, which is exceeding the level present in normal air (Stover and Freiberg, 1958). Thus, a combination of a large particle size and burial into the soil seems to be most beneficial for opportunistic fungi. The addition of nitrogen to wood was expected to stimulate initial wood decay as the decay activities of fast-growing fungi and bacteria are usually nitrogen limited (Fog, 1988; Carreiro et al., 2000). Wood decay and the growth yield efficiency of fungi were increased by nitrogen addition although only for the ex-arable soil. The activity of fungi and wood decay in heathland soil was much lower than in the ex-arable soil. Therefore, it was concluded that soil origin and nitrogen availability are also factors that control initial wood decay. The lower activity of fungi in heathland soil may be due to a different composition of the fungal community than in ex-arable soil. Another explanation is the presence of phenolic compounds in heathland soil, inhibiting microbial decomposition (Jalal and Read, 1983). The study lasted for 40 weeks, but even after this period of time no activity of lignin degrading enzymes could be observed. Spores or hyphae of ligno-cellulolytic fungi in ex-arable- and heathland soil were present as checked on a basidiomycetes specific medium (Thorn et al., 1996), so this can not explain the absence of lignin degrading enzyme activity. Possibly the incubation time was too short for these fungi to increase their biomass. Some literature is available on the succession of functional groups of fungi during substrate decomposition (Frankland, 1998; Ruess and Lussenhop, 2005). Each succession is dependent on the composition of the substrate and its environment, but some general patterns are described based on the enzymatic abilities of fungi: substrate is initially colonized by pioneer saprotrophic fungi or sugar fungi, followed by the more specialized polymer degraders. Fungi that are able to breakdown recalcitrant compounds dominate in later successional stages. Basidiomycetes were found to dominate after 6 months of decomposition of oak litter, but in litter of higher quality (lower C:N ratio) the duration of the succession was much shorter (Frankland, 1998). This indicates that the incubation time of the study described in Chapter 5 would probably be long enough for basidiomycetes to proliferate. An alternative explanation for the absence of activity of ligno-cellulolytic fungi may be that, as mentioned above, the biomass of K-selected fungal hyphae was too low to be able to compete with opportunistic fungi and bacteria. Also inhibition of colonization of ligno-cellulolytic fungi by opportunistic fungi might be an additional explanation (Greaves, 1972). Hardly any literature is available on this subject. Studies on the interaction between opportunistic fungi and lignin-degrading fungi in wood may provide insight into the decomposition processes during early and later stages of wood decay.

Vertical distribution of phosphorous in ex-arable soils

During the 1970s the introduction of phosphorous (P) on Dutch arable soils exceeded that of the plant uptake of crops, resulting in substantial accumulation of phosphorous. This contributed to the leaching of high amounts of phosphorous into the groundwater and consequently, to the eutrofication of ground and surface water (Schoumans, 2004). During the 1980s, the maximum threshold value for phosphorous in arable soils was determined (Van der Zee et al., 1990a, b). This threshold value was based on the binding capacity of the soil and the maximum groundwater level under the soil. Based on these criteria, in the 1990s, 70% of the acid, sandy soils in the Netherlands were considered to be P saturated (Breeuwsma et al., 1990; Reijerink and Breeuwsma, 1992). On the longer term, leaching of P from these soils was thought to form a substantial risk for the quality of the groundwater (a source of drinking water). Abandonment of arable soils was suggested to trigger the leaching of P, and has been referred to as the signal for a ‘chemical time bomb’ to explode (Van Latesteijn, 1998).

In Chapter 3 it was shown that the concentration of total P in ex-arable soils decreased with increasing soil depth. The concentration of total P in the deepest soil layer was very low and almost similar to that in the reference heathland soil. Determination of total P includes all phosphorous forms, so also P in soil solution. Therefore, these results indicated that leaching of large amounts of P into deeper soil layers or even to the groundwater should not be a reason for concern in these ex-arable soils. Soils that contain a Bs soil horizon that is characterized by the illuvial accumulation of aluminum and iron may form a storage layer in future when possibly more phosphate leaches out of the surface layers, since inorganic P can react with these irons to form solid compounds (Barrow, 1983; Van der Zee et al., 1987). Phosphorous is then fixed, decreasing the risk of leaching of P and making P unavailable for uptake by plants. However, in future P can again become dissolved and may leach to deeper soil layers (Schoumans, 2004).

Total P in the 0-95 cm soil layer also decreased with increasing years since abandonment, indicating that the observed decrease of total P in the upper soil (10 cm) with years of abandonment in Chapter 2 is probably not due to leaching of large amounts of P in the top soil to lower soil layers. The continuation of accumulation of P for a longer period of time in recently abandoned fields appeared to be the most reasonable explanation for the decrease of P with years of abandonment (Chapter 3).

A high P level may complicate the conversion from arable land into a natural ecosystem. High phosphorous levels on abandoned agricultural field stimulate the growth of undesired plant species and lead to the development of a low-diversity vegetation (Chardon and Sival, 2003). Mining of soil P can be used to decrease the P level: crops are grown without external P addition, followed by the removal of the aboveground plant biomass. Mining of P may be an effective tool to decrease the risk of leaching from P-enriched soil and to increase the speed of restoration of ex-arable soils to a more natural ecosystem (Koopmans, 2004; Sival and Chardon, 2004). In particular in recently abandoned fields mining of soil P could be considered as an option to change the competition between fast- and slow growing plant species, but it seems not necessary to decrease the risk of P leaching.

Establishment of *Calluna vulgaris* on ex-arable soil

The soil conditions on a heavily, fertilized arable soil differ strongly from a heathland ecosystem. Arable soils do not have an organic matter layer and the pH as well as the availability of nutrients are much higher than in heathland soils. After abandonment of arable soil, these conditions will favor the growth of grasses and forbs. Conversion from arable soil into a heathland is therefore complicated, as the slow-growing *Calluna* will be outcompeted by the fast-growing grasses and forbs. Surprisingly, patches of vital *Calluna* (0.7 – 2.0 m) in a 10 year abandoned agricultural field within a dominant vegetation of grasses and forbs were observed in one of the fields of the chronosequence studied in Chapter 2. In Chapter 6, it was described that the nitrogen mineralization and potential nitrogen mineralization (arginine ammonification) was much lower in soil under *Calluna* patches than in the rest of the ex-arable soil. Although nitrogen mineralization under *Calluna* patches was lower, the N:P ratio of *Calluna* shoot material was much higher as compared to those of grasses and forbs, indicating that the latter were more N limited than *Calluna* plants. The lower nitrogen mineralization could not be explained by differences in soil characteristics or in bacterial properties under *Calluna* patches as compared to the rest of the soil. A likely explanation for the reduction of N-mineralizing microbial activity in soil under *Calluna*-patches is the presence of toxic compounds produced by the plant. In N-limited systems with high amounts of allelopathic compounds, it is proposed that the rate-limiting step in the N mineralization process is the decomposition of complex organic N compounds into monomers (e.g. amino acids, amino sugars) by extracellular enzymes (Northup et al. 1995; Schimel and Bennett 2004). This may imply that the toxins produced by *Calluna* prevents the N mineralization process to proceed beyond organic monomers. All *Calluna* roots in the ex-arable soil were colonized by ericoid mycorrhizal fungi. These fungi can take up and transport organic monomers to their hosts (Read, 2004). Thus, ericaceous plants which are colonized by ericoid mycorrhizal fungi have a benefit for organic nitrogen over non-ericaceous plants that are not able to form ericoid mycorrhizal fungi. Interestingly, no *Calluna* seedlings were present in the field, indicating that colonization of new *Calluna* plants was not a continuous process. The relative large size of all patches suggested that they have established soon after abandonment. These observations led us to conclude that rapid successful establishment of *Calluna* plants in ex-arable fields is due to the presence of seedlings soon after abandonment and the

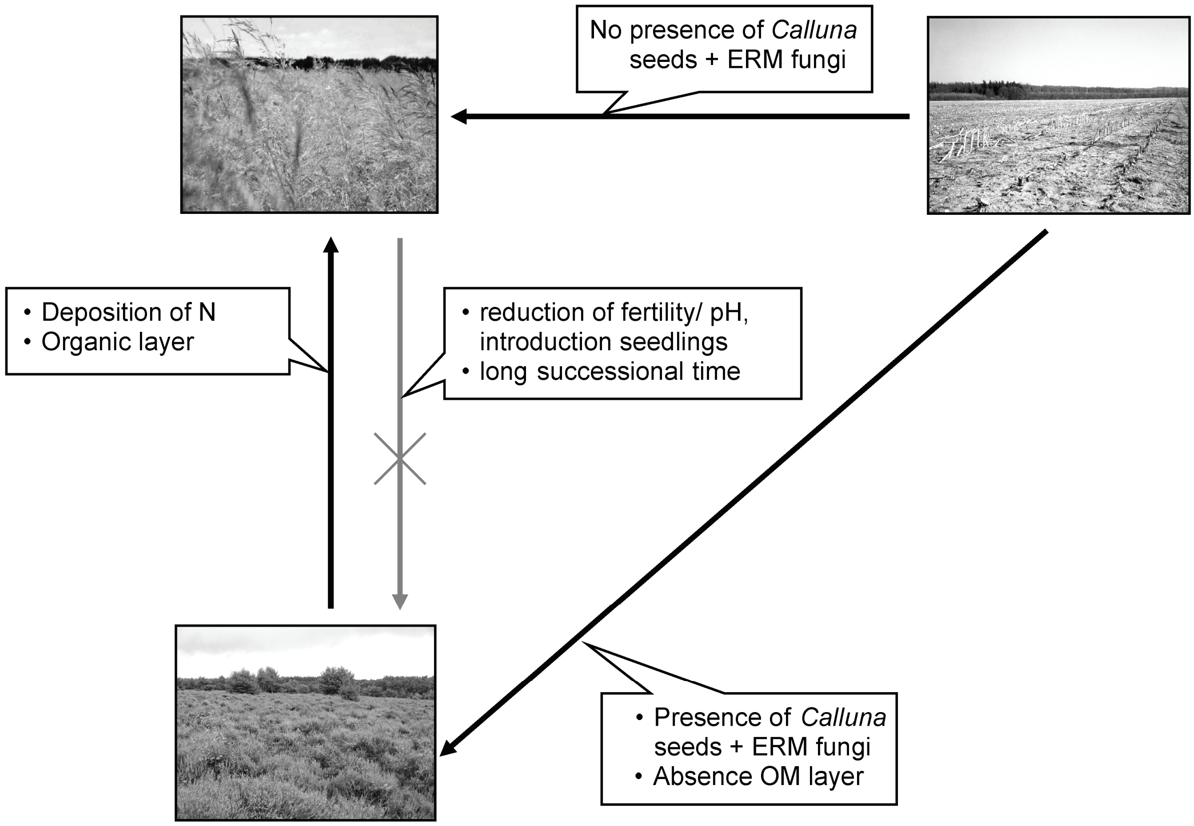

Figure 1: Possible different states during heathland restoration

competitive superiority for organic nitrogen even in a soil with low organic matter content which is probably due to the association with ericoid mycorrhizal fungi.

The conversion from heathland to grassland is a common phenomenon in the Netherlands, and this is generally thought to be due to increased atmospheric deposition of ammonium and lack of management (Berendse and Aerts, 1984; Heil and Bruggink, 1987). In the ex-arable field under study, the opposite situation occurred; heathlands plants were growing within a dominant vegetation of grasses and forbs. After land abandonment, nitrate is rapidly leached into deeper soil layers and net nitrogen mineralization is low as a result of low organic matter content (Marss, 1993; Malý et al., 2000; Chapter 2). In contrast, in mature heathland deposited atmospheric nitrogen will not be oxidized into nitrate and it can, thus, not rapidly leach, resulting in nitrogen enrichment of the top soil layer. Hence, because of the absence of an organic matter layer on ex-arable soils, the conversion to heathland seems to be possible as *Calluna* plants infected with ericoid mycorrhizal fungi are adapted to low accessibility of nitrogen. Interestingly, *Calluna* plants were not limited in their growth by the high phosphorous levels in the ex-arable soil. Therefore, the limited growth of the slow-growing *Calluna* plants on ex-arable soil seems to be rather a matter of competition with fast-growing plant species than the inability of *Calluna* to grow on highly fertilized fields. According to the results of Chapter 6 and the aforementioned hypotheses, different ecosystems can develop after land abandonment (Figure 1). In the presence of *Calluna* seeds and ericoid mycorrhizal fungi immediately after abandonment, a heathland may develop. In the absence of *Calluna* seeds and fungus, the ex-arable soil will be dominated by grasses and forbs. The conversion from grassland into heathland is difficult and can only be achieved after intense restoration management or after a long time of succession. The transition of heathland to grassland has been observed frequently in the Netherlands and is occurring under raised atmospheric nitrogen deposition. This model needs to be confirmed in the field and can be of interest for restoration of heathland.

Management implications

After abandonment of arable fields, the biomass of fungi was found to increase only slowly (Chapter 2) and this increase is limited probably because of the dominance of substrate utilization by r-selected fungi (Chapter 3). An increase in fungal biomass can be of interest for soil ecosystem restoration, as fungi contribute to retention of nutrients and formation of stable soil aggregates. Possibilities to trigger the increase in fungal biomass in ex-arable land could be the introduction of organic matter in the form of recalcitrant organic matter together with sufficient fungal biomass. Hereby, the size of the organic particles is as well important: larger particle sizes are stimulating fungal growth (Chapter 5). Thus, it may be possible to induce the growth of fungal K-selected fungi by applying large woody particles on the surface of the soil together with an inoculum of spores of K-selected fungi, although this is not yet tested in the field. Fungal biomass may as well be increased by the introduction of suitable host plants for ericoid mycorrhizal fungi. Within the context of the risk of phosphorous leaching after abandonment of arable fields, it appears that attempts to decrease the level of phosphorous are not necessary on ex-arable sandy soils in the central part of the Netherlands (Chapter 3). However, mining of soil P could be still of interest to change the competition between fast- and slow growing plant species and to be completely certain that fixed phosphorous will not dissolve again in future. The conversion from an arable field into a heathland may be easily achieved by the introduction of *Calluna* seeds and an inoculum of ericoid mycorrhizal fungi, e.g. soil from a mature heathland, immediately after abandonment of the arable field (Chapter 6).