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The effect of turf cutting on plant and arbuscular mycorrhizal spore recolonisation: Implications for heathland restoration

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ABSTRACT

In two natural heathland vegetations, we analysed the effect of turf cutting on spore numbers of arbuscular mycorrhizal fungi (AMF). Next to this, we performed a controlled factorial experiment to examine the role of AMF for germination and establishment of *Arnica montana* in both turf cut and non-turf cut situations. AMF spore numbers decreased with soil depth, and, along with the topsoil, almost all AMF spores were removed with the removal of the acidified and/or eutrophied organic layer. Recolonisation of AMF spore numbers after turf cutting was slow: spore numbers of approximately 60–95 spores g^{-1} dry soil were found two and a half years after turf cutting, corresponding with 55–70% of AMF spore numbers found in natural field populations of *A. montana*. Since AMF colonisation increased establishment and biomass, and decreased mortality of *A. montana*, it was suggested that lack of AMF after turf removal might complicate the establishment of this herbaceous species. Removal of organic material as a management measure should therefore carefully be applied, taking in consideration the low recolonisation rates of AMF as this can markedly effect the success of restoration.

1. Introduction

Many natural and semi-natural ecosystems in Western Europe, such as heathlands and species-rich grasslands, are threatened by soil acidification and eutrophication mainly resulting from atmospheric nitrogen and sulphur deposition (Roelofs, 1986; Aerts et al., 1991; Uren et al., 1997). These ecosystems have declined considerably in area, and are subject to succession and increased dominance of grasses, with a decrease in diversity (Aerts and Heil, 1993; Bobbink et al., 1998) and a loss of characteristic herbaceous species, such as *Arnica montana* L. (Asteraceae) (Fennema, 1992; Houdijk et al., 1993).

Many attempts to restore nutrient-poor systems have, therefore, focussed on counteracting the detrimental effects of eutrophication and acidification. In Western Europe, measures such as the removal of the eutrophied and/or acidified soil layer (turf cutting) have been shown to restore nutrient poor soil conditions (Aerts and Heil, 1993; Snow and Marrs, 1997) and the restoration of *Calluna vulgaris*-dominated heaths (Helsper et al., 1983; Werger et al., 1985). However,

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De Graaf et al. (1998) found that many herbaceous species did not return after turf removal.

The main objective of turf cutting is to remove the eutrophied and/or acidified soil from the system, but this also removes the majority of the seed pool from the soil. Recolonisation from seed will, therefore, largely depend on seed dispersal or restoration measures such as (re)introduction. However, even if seeds are available, changes in physical and chemical parameters after turf cutting can prevent restoration success. De Graaf et al. (1998) reported an accumulation of ammonium-nitrogen due to an enhanced mineralisation and ongoing atmospheric deposition after turf cutting, and Van den Berg et al. (2003) showed a reduced soil moisture content and a reduced capacity of complexing toxic aluminium after turf cutting due to the removal of organic compounds. Furthermore, the organic layer is an important pool of soil organisms. Many soil organisms, including saprotrophic fungi, collembola and nematodes were found to be affected negatively by turf removal (Klap and Schmidt, 1992, 1995). Organic topsoil is also an important habitat for arbuscular mycorrhizal communities (Rose and Paranka, 1987; Bellgard, 1993). We, therefore, suspect that turf cutting may affect the occurrence and vitality of these communities negatively.

Interactions between arbuscular mycorrhizal fungi (AMF) and plants are expected to be important, especially in nutrient poor systems such as heathlands. AMF colonisation might result in the broadening of the range of habitats in which the species can grow in, due to their beneficial effects (Peat and Fitter, 1993). Colonisation with AMF promotes plant growth by increasing nutrient uptake (Marschner and Dell, 1994; Smith and Read, 1996), and they may interfere with pathogens (Newsham et al., 1995), alter drought resistance (Ruiz-Lozano and Azcon, 1995; Smith and Read, 1996), and increase tolerance to aluminium and soil acidity (Cuenca et al., 2001). AMF have been shown to influence the process of recovery of disturbed ecosystems (Allen, 1991; Johnson et al., 1991; Van der Putten et al., 2001) and help determine the composition of plant communities (Francis and Read, 1994; Van der Heijden et al., 1998; Hedlund and Gormsen, 2002).

The herbaceous species which are currently declining in heathland vegetations, including *Arnica montana*, have AMF interactions (Heijne et al., 1989; Heijne et al., 1996). The presence and/or absence of AMF communities is, therefore, expected to be an important factor for the establishment of these herbs after turf removal.

In this paper, the effect of turf cutting on AMF spore numbers was related to the effect of AMF on the performance of *Arnica montana*, a herbaceous perennial, which, in the Netherlands, is characteristic of nutrient-poor grasslands and dry heathlands. This species is now threatened by extinction due to habitat fragmentation and habitat deterioration, and is, therefore, a bio-indicator species of nutrient poor grasslands and dry heathlands in the Netherlands.

AMF spore numbers in two natural, non-declining *A. montana* populations were analysed at different depths. Next to this, the recolonisation rate of AMF in three field sites was measured before and after turf cutting. We expect that turf cutting will remove most of the AMF and, therefore, hinders the establishment of *A. montana* compared to situations in which AMF were not removed. In order to analyse this, an experiment was performed under controlled conditions in the laboratory, in which seeds of *A. montana* were germinated and seedlings were grown in both undisturbed and turf cut soil cores. The cores were inoculated with a natural AMF culture or remained uninoculated. We hypothesised reduced growth and higher mortalities of *A. montana* in cores without AMF treatment, and that these effects would be more pronounced in the turf cut treatments.

2. Material and methods

2.1. *Spore populations and spore recolonisation in the field*

Spore populations in natural, non-declining populations of *A. montana* were analysed in two nutrient-poor heathland areas, 'Schaopedobbe' (52°57′ N, 6°16′ E) and 'Havelte' (52°48′ N, 6°13′ E), located in the northern of the Netherlands. Both heathlands consists of species-rich heathland vegetation with a high frequency of herbaceous species, including *A. montana*. In both areas, soil samples were collected at three randomly selected sites. The samples were taken with an auger (diameter 3 cm), and divided into 5 depth classes (0–5, 5–10, 10–15, 15–20, 20–25 cm). The first samples were taken in May 2001, and repeated every 4 months one year. Following soil sampling, spores were extracted and counted from 25 ml aliquots by wet-sieving and sucrose centrifugation (Walker, 1991). Spores were then placed in gridded petri dish and counted using a dissecting microscope (×40). Dead spores were distinguished from live ones by careful examination of the morphological characteristics of each spore and their response to punching with a needle in case of doubt. Bulk density was determined by air drying an aliquot of each soil sample (24 h, 105 °C). The total number of spores was expressed per gram dry soil.

The recolonisation rate of AMF after turf cutting was analysed in three experimental fields before and after turf cutting from April 2000 till October 2002, by measuring the increased number of spores per gram dry soil in time. The largest experimental area (400 m^2) was located in the Schaopedobbe nature reserve (**Sdobbe**). The other two areas were located in Havelte nature reserve, and measured 80 m^2 (**Havelte1**) and 200 m^2 (**Havelte2**). The experimental fields were located within 100 m of local *A. montana* populations and were classified as nutrient-poor heathland areas, dominated by grasses. At the start of the experiment, turf was removed to expose the mineral layer (15–20 cm). At each site, three soil samples were randomly taken using an auger (depth 0–5 cm, diameter 3 cm) 1 week before and 1, 3, 7, 13, 18 and 30 months after turf removal. Spores from these soil samples were extracted and counted as mentioned above.

2.2. *Inoculation experiments*

Plants, soil and fungal material used for inoculation experiments originated from the Schaopedobbe heathland. To obtain AMF cultures from the natural *A. montana* population, soil samples were taken with an auger (depth 10 cm, diameter 3 cm) in May 2001. The samples were collected within 30 cm of 15 randomly selected *A. montana* individuals. Spores were extracted by wet-sieving and sucrose