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Effects of mowing on fungal endophytes and arbuscular mycorrhizal fungi in subalpine grasslands

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ABSTRACT

In French subalpine grasslands, cessation of mowing promotes dominance of *Festuca paniculata*, which alters plant diversity and ecosystem functioning. One of the mechanisms underpinning such effects may be linked to simultaneous changes in the abundance of fungal symbionts such as endophytes and arbuscular mycorrhizal fungi. In field conditions, mowing reduced the abundance of the endophyte *Neotyphodium* sp. in leaves of *F. paniculata* by a factor of 6, and increased mycorrhizal densities by a factor of 15 in the soil. In greenhouse experiments, the mycorrhizal colonization of *Trifolium pratense* and *Allium porrum* increased 3- fold and 3.8- fold respectively in mown vs unmown grassland soil. Significantly reduced growth of the two host plants was also observed on soil from the unmown grassland. Such opposite effects of mowing on the two functional groups of fungal symbionts could suggest interactions between these two groups, which in turn could contribute to structuring plant communities in subalpine grasslands.

Introduction

Mutualistic symbionts including arbuscular mycorrhizal fungi (AMF) and endophytic fungi are widespread and play a major role in ecosystem functioning. They can influence plant growth, competitive ability and consequently the structure and the diversity of plant communities (van der Heijden *et al.* 1998; Clay & Holah 1999; van der Heijden *et al.* 2008; De Deyn *et al.* 2011). It is well known that AMF are able to establish a symbiotic relationship with the roots of 70–90 % of land plant species (Smith & Read 2008). They improve plant growth through facilitated uptake of available soil phosphorus and other non-labile mineral nutrients. In addition, they play an essential role in the aggregation and soil structure and reduce plant stress caused by biotic (soil-borne pathogens) and abiotic factors (heavy metals pollution, drought, salinity and mineral nutrient depletion) (Gianinazzi *et al.* 2010).

Similarly, endophytic fungi including grass-endophytes belonging to the genus *Neotyphodium* are known to modify relationships between diversity and ecosystem properties (Rudgers *et al.* 2004; Rudgers *et al.* 2010). They infect 20–30 % of all grass species, forming systemic and asymptomatic infections throughout the aerial parts of the host plant, including the seeds, thereby allowing vertical dispersal of the endophytes from one plant generation to another (Petrini 1991; Stone *et al.* 2000; Arnold 2007; Mouhamadou *et al.* 2011). Endophytes usually inhabit above-ground plant tissues and protect host plants against herbivores and pathogens by

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producing alkaloids that are toxic to insects and grazing mammals (Clay *et al.* 1993; Bush *et al.* 1997; Clay & Schardl 2002; Lemons *et al.* 2005; Schardl *et al.* 2007), and may also negatively affect other organisms, such as soil nematodes (West *et al.* 1990), pathogens causing soil-borne diseases (Gwinn & Gavin 1992), mycorrhizal fungi (Mack & Rudgers 2008) and decomposers (Lemons *et al.* 2005). For example, *Neotyphodium coenophialum*, the tall fescue endophyte, altered the plant community structure by reducing the diversity of the surrounding plant community (Clay & Holah 1999). This effect could be explained by the ability of toxic alkaloids to suppress mycorrhizal fungi essential to the establishment of some other plant species in the community (Chu-Chou *et al.* 1992).

Due to their importance in ecosystem functioning, a number of studies have sought to identify the key factors that regulate fungal symbionts. Management practices seem to be one determining factor. For example, the composition of AMF communities (relative species abundance, species richness) is affected by land management practices such as fertilization, cultivation of non-mycorrhizal crops and tillage (Oehl *et al.* 2003; Kabir 2005; Mathimaran *et al.* 2007; Collins & Foster 2009; Barto *et al.* 2010). Likewise, in temperate grasslands, changes in endophyte communities have been observed in response to mowing (Mouhamadou *et al.* 2011), or between grasslands depending on their management, particularly with effects of overgrazing and liming or sheep grazing (Wilberforce *et al.* 2003). However, such analyses of effects of agricultural management on symbiont communities have mostly focused either on AMF or endophytes. A few studies have, however, considered both groups of fungi simultaneously, but used artificial inoculation. For example, controlled mycorrhizal inoculations have demonstrated interactions between both functional groups such as the suppressive action of AMF by endophytes (Chu-Chou *et al.* 1992; Guo *et al.* 1992; Müller 2003; Mack & Rudgers 2008), or competitive interactions between two endosymbiont species (Mack & Rudgers 2008; Liu *et al.* 2011; Larimer *et al.* 2012). In natural ecosystems, these fungal symbionts can have profound effects on plant ecology, fitness and evolution (Brundrett 2006), and shape plant (Moora & Zobel 1996; Clay & Holah 1999; van der Heijden *et al.* 2008; Gross *et al.* 2010) and soil communities (Omacini *et al.* 2004; van Hecke *et al.* 2005; Casas *et al.* 2011).

The aim of this study was to investigate the impacts of mowing simultaneously on fungal endophytes and AMF, using semi-natural subalpine grasslands as a model. In this ecosystem, plant taxonomic and functional diversity are influenced by the intensity of grassland management (Quétier *et al.* 2007). Grasslands managed by mowing for several centuries harbor a high plant diversity coexisting with dominant graminoids including *Festucapaniculata*. When mowing is stopped and replaced by light grazing, *F. paniculata* becomes highly dominant (50–80 % of total biomass) with a subsequent drastic reduction in plant diversity (Quétier *et al.* 2007). However, no information is available about the impact of mowing on the interactions between fungal endophytes and AMF. We investigated whether mowing management influences infection by endophytes and AMF abundance in field conditions in *F. paniculata* subalpine grasslands and whether there are connections between management practices and the interaction between both functional fungal groups. We focused on the grass-endophytes belonging to the genus *Neotyphodium* which represent a group of plant-associated fungi known to modify relationships between diversity and ecosystem properties (Rudgers *et al.* 2004; Rudgers *et al.* 2010). In addition, we compared across soils from mown vs unmown grasslands AMF effectiveness in root colonization and growth of *Trifolium pratense* and *Allium porrum* used as host plants in a greenhouse experiment.

Materials and methods

Site description

The study was conducted in adjacent mown and unmown permanent grasslands located on the south-facing slopes of the upper valley of the Romanche River of the central French Alps (45.04°N 6.34°E) close to the Lautaret Pass (2057 m a.s.l.). These grasslands have been mown for at least three centuries, but the mowing ceased ca.30 yr ago where slope prevents mechanization, and was replaced by light summer grazing. Plots that are still mown are cut annually in early August and lightly grazed in autumn. As a result the site is split between mown and unmown grasslands, within which we defined 20 m $\times$ 20 m paired plots.

The vegetation of the permanent grasslands is dominated by *F. paniculata* in unmown fields and includes an additional diversity of plants including *F. paniculata*, *Festuca laevigata*, *Bromus erectus*, *Carex sempervirens*, *Meum athamanticum*, *Potentilla* sp., *Trifolium alpestre*, *Trifolium alpinum*, *Trifolium montanum*, *T. pratense*, *Thymus serpyllum* and *Hieracium* sp. in mown fields. Details of the corresponding plant communities and soil properties in mown and unmown fields have been described by Quétier *et al.* (2007) and Robson *et al.* (2007). In particular, cessation of mowing appears to considerably slow down nitrogen cycling and to increase nutrient competition between plants and soil microorganisms (Robson *et al.* 2010).

Experimental approach

In July 2010, we randomly sampled 15 *F. paniculata* tussocks including several tillers from each of mown and unmown grasslands for endophyte characterization and concentration. In parallel, we collected ten bulk soils at a depth of 10–15 cm from three pairs of adjacent plots that were either mown or unmown. The chemical characteristics of the soils of each grassland are presented in Table 1. The soils were used for both the quantification of mycorrhizal densities and for plant growth experiments. For each management type, the ten bulk soils were pooled, passed through 5 mm-sieves and used as a substrate to grow *T. pratense* or *A. porrum* individuals as host plants.

Molecular characterization of leaf endophytes

Three tillers of each of the 15 *F. paniculata* tussocks from mown and unmown grasslands were randomly selected and the leaves were harvested and washed with distilled water. For the extraction of endophyte DNA, 250 mg of leaf of each