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Fungal endophyte diversity in tundra grasses increases by grazing

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ABSTRACT

Fungal endophytes are species rich and ubiquitous, yet, apart from the genus *Epichloë*, their ecology is largely unknown. Here we explore how herbivores affect the diversity of fungal endophytes in tundra grasslands. We assess both hyphal morphological and taxonomic diversity in grass individuals.

By microscopic examination we identified endophytes to be present in all sampled grass individuals whereas identification to taxonomic units were only achieved in a subset of the individuals using laser micro dissection pressure catapulting and culturing for endophyte isolation. Hyphal morphological diversity was significantly higher in grasses exposed to grazing, along with 45 % more taxonomic units achieved.

Our results suggest that grazing is an important mediator of fungal endophyte diversity in tundra grasslands. Furthermore, we suggest laser dissection of stained endophytes as a method for further exploring the ecological role of fungal endophytes.

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Introduction

The development of molecular techniques has facilitated the discovery of an enormous diversity of species of fungal endophytes (Higgins *et al.* 2007; Rodriguez *et al.* 2009; Jensen *et al.* 2011; Higgins *et al.* 2014). The high richness and the omnipresent nature of fungal endophytes indicate they are likely to have important roles in ecosystem processes, be influential to the outcome of species interactions and hence ecosystem functioning (Arnold 2007; Kivlin *et al.* 2013). Fungal

endophytes can produce significant advantages to their host plant by increasing host plant tolerance to different abiotic and biotic stressors (i.e. drought, nutrient deficiency, competition) (Clay 1990; Malinowski & Belesky 1999; Rodriguez *et al.* 2009; Kleczewski *et al.* 2012). In addition, fungal endophytes can benefit plants against herbivores through production of mycotoxins, such as alkaloids, which are physiologically harmful for herbivores (Vicari & Bazely 1993; Schardl *et al.* 2012). Yet, most knowledge on fungal endophyte colonization comes from study systems using the

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endophyte genus *Epichloë* (Leuchtman et al. 2014) from the Clavicipitaceae family and two common agricultural grass species (*Festuca arundinacea* and *Lolium perenne*) (Saikkonen et al. 2006, 2010; Higgins et al. 2011; Sánchez Márquez et al. 2012). Given the diversity of endophytes, *Epichloë* most likely only represent a small fraction of how fungal endophytes are influential to ecosystems, and calls for a broader approach to the study of fungal endophytes.

Plant herbivore interactions are one of the central processes of ecosystems of which fungal endophytes, due to their omnipresent nature, evidently take part. The pronounced ecological effects that species of *Epichloë* can have, and their severe impact to the cattle industry (Strickland et al. 2011), are likely reasons for why research on fungal endophytes in plant herbivore interactions mostly have been limited to this genus also in study systems outside farming systems (e.g. Koh et al. 2006). However, methodologies for detecting these species, such as immunoblot kits or microscope examination, have likely yielded false positives, most probably causing patterns of other fungal endophyte species to be mistaken for that of *Epichloë* (Jensen et al. 2011). For instance, in the search for

Epichloë in tundra grasslands, where their presence causing reindeer grazing avoidance had been hypothesized (Bråthen & Oksanen 2001), false positives were achieved for ten fungal endophyte species, and a total of 59 species were found (Jensen et al. 2011). Moreover, *Epichloë* in the wild is limited to two grass genera (Card et al. 2014), hence the majority of grasses host other fungal endophytes than those belonging to the *Epichloë* genus. The high richness and ubiquitous nature of fungal endophytes strongly suggests fungal endophyte species in general can influence plant herbivore interactions (Jensen et al. 2011), whereas in what ways are still mostly unknown.

The detection of fungal endophyte species is methodologically challenging and hampers our ability to link endophyte species to ecosystem processes. Information about where and when fungal endophyte species appear in vegetation can largely be surveyed through direct PCR and next-generation sequencing approaches (Higgins et al. 2011). Still, more cautious methodology is required to verify that the detected species are actually growing inside the host plant and to what extent they are within the plant tissues. Isolation of

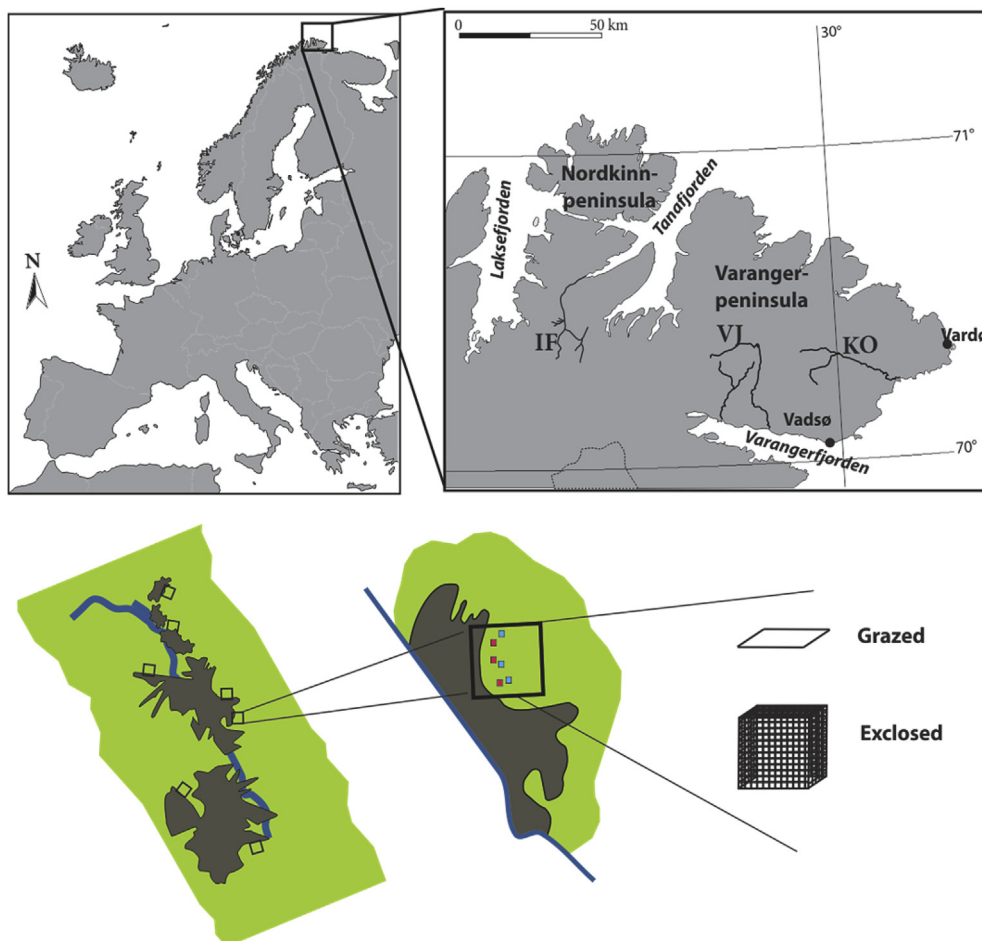


Fig 1 – Geographic location and study design of the herbivore exclusion experiment in tundra grasslands in northern Norway. The three river catchments are denoted as IF = Ifjord, VJ = Vestre Jakobselv and KO = Komag. Within each river catchment, experimental grids were set up along willow thicket edges (IF N = 11, VJ N = 12, KO N = 11). Within each experimental grid, flowering grass individuals of *Anthoxanthum nipponicum*, *Calamagrostis phragmitoides* and *Festuca rubra* were sampled in both grazed and excluded plots.

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