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Role of foliar fungal endophytes in litter decomposition among species and population origins

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ABSTRACT

Litter decomposition is a key ecosystem process which returns nutrients from dead plant material to mineral forms in the soil. We examined whether systemic fungal endophytes modulate recycling of nutrients directly by altering litter decomposition. We studied litter decomposition mediated by *Epichloë* endophytes in litter-bag experiments. We examined direct endophyte effects on litter decomposition in wild populations and cultivars of *Schedonorus phoenix* and *Schedonorus pratensis*. In the first experiment, endophyte presence tended to increase litter decomposition rate in cultivars of the two grass species (*S. phoenix* and *S. pratensis*). However, in the second experiment plant origin had a stronger influence than endophyte symbiosis in *S. phoenix*. Interestingly, the initial level of alkaloids was associated positively with decomposition in *S. phoenix* populations. Characteristics associated with litter quality were not clearly related to either endophytes or decomposition rate. Our results suggest that endophytes can enhance litter breakdown but their role in nutrient cycling is far more complex depending on plant population origin.

1. Introduction

Decomposition is a key process for ecosystem functioning as it releases nutrients from dead organic material into mineral forms, thus controlling nutrient cycling and energy flow (Chapin et al., 2002). Decomposition affects the soil carbon budget, a critical aspect related to global change and climate warming (Wardle et al., 2004). Climate, the quality of litter biomass and the community composition of decomposer organisms are all important factors affecting decomposition (Aerts, 1997). While temperature and humidity explain variation at regional scales, biomass quality and decomposer assemblages can govern decomposition rates at local scales (Zhang et al., 2008; Austin et al., 2014; Karhu et al., 2014). Although microbes are well recognized players in decomposition, the impact of symbiotic organisms, such as plant-associated endophytic fungi, on the process has received little attention (Purahong and Hyde, 2011; Saikkonen et al., 2015). These plant symbionts may affect decomposition through different pathways; they can act as saprobionts in senescencing dead plant materials, but they can also enhance or inhibit the processes by modulating the soil community decomposers through changes in the quality of plant tissues for detritivores.

Virtually all plant leaves are colonized by endophytic fungi, which live internally and asymptomatically within host tissues (Saikkonen et al., 1998; Rodriguez et al., 2009). The majority of these fungi belong to a taxonomically heterogeneous pool of non-systemic endophytes, whose ecological roles are largely unresolved (Saikkonen, 2007; Higgins et al., 2011; Zabalgogeazcoa et al., 2013; Saikkonen et al., 2015). In contrast to non-systemic fungi, some vertically transmitted fungal endophytes form persistent symbioses with their grass hosts and by having strong ecological

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and evolutionary consequences, alter host phenotypic traits (Saikkonen et al., 1998; Clay and Schardl, 2002; Gundel et al., 2010). Studies on function of symbiosis between Pooideae grasses and epichloid fungi (Clavicipitaceae, Hypocreales, Ascomycota) have mostly focused on the bio-protective role of fungal alkaloids against herbivores and pathogens (Clay and Schardl, 2002; Schardl et al., 2007; Saikkonen et al., 2013a). Other ecological effects, including negative effects on decomposers, have been more recently ascribed to these fungal derived compounds (Omacini et al., 2004; Lemons et al., 2005; Siegrist et al., 2010). Besides producing alkaloids, these fungal endophytes may directly affect other plant traits involved in decomposition (Omacini et al., 2004; Gundel et al., 2012; Hamilton et al., 2012; Saikkonen et al., 2015).

Endophytes can modify the phenotype of their host grasses including traits such as the carbon/nitrogen ratio (C:N ratio), phosphorus level, and fiber content of foliage (Lyons et al., 1990; Rasmussen et al., 2008; White and Torres, 2010; Rogers et al., 2011; Hamilton et al., 2012; Vázquez-de-Aldana et al., 2013a) which can directly affect litter decomposition (Berendse et al., 1987; Güsewell and Gessner, 2009; Hall and Silver, 2013). However, past attempts to understand the effects of endophyte symbiosis on these traits and consequently on decomposition, have been inconclusive. Actually, a recent meta-analysis which included all the published papers so far, failed to find an overall significant inhibitory effect of endophytes on the litter decomposition of host grasses (Omacini et al., 2012). In addition to endophyte presence, the plant traits related to litter decomposition depend on host plant genotype, plant ontogenic stage, and grazing history (Burns et al., 2006; Rogers et al., 2011; Jia et al., 2014).

Here we examined the effect of endophyte presence on litter decomposition of two grass species, *Schedonorus phoenix* (formerly *Festuca arundinacea* Schreb., and common name: tall fescue) and *Schedonorus pratensis* (formerly *Festuca pratensis* Huds., and common name: meadow fescue) using litter-bag experiments in a common garden. In accordance with the general tendency of endophytes affecting negatively the decomposition of litter (see review by Omacini et al., 2012), we predicted that endophyte symbiosis would slow down mass loss. As a test of the relative importance of endophyte symbiosis, we manipulated endophyte presence interactively with plant population (tall fescue: three wild origins and one cultivar 'Kentucky-31'; meadow fescue: cultivar 'Kasper'), given the expectation that litter decomposition would vary with plant genotypes (see Madritch and Hunter, 2004).

2. Materials and methods

2.1. Plant material

The endophyte symbiotic (E+) and non-symbiotic (E−) plant material used in the two litter-bag experiments was collected from *S. phoenix* and *S. pratensis* plants grown in a common garden at Ruissalo Botanical Garden (University of Turku, Finland). The *S. phoenix* plant populations were originally collected from three geographic locations around the Baltic Sea, Åland, (Finland), Gotland and Södermanland (Sweden). In addition to these three wild plant populations, one commercial cultivar, 'Kentucky-31', was included in the study. Ten individual plants of each origin and symbiotic status were placed at random in a grid with 1 m^2 for each plant in 2005 (Gundel et al., 2013a,b). In an adjacent plot and following a similar design, ten individual plants of *S. pratensis* (cultivar 'Kasper'), symbiotic and non-symbiotic with endophyte were planted in 2008 (Saikkonen et al., 2013b). In all cases, the endophyte-free plants were obtained by treating symbiotic seeds with heated water before sowing to kill the fungus, and confirming the symbiotic status before planting the plants in the common garden. In the years following plant establishment in the common garden, their symbiotic status was confirmed by checking for the endophyte presence or absence in the seeds produced by symbiotic and non-symbiotic plants, respectively, under light microscope (for details see Gundel et al., 2013a,b; and Saikkonen et al., 2013b).

In autumn 2011, biomass from the 10 plants from each combination of species, population origin, and symbiotic status were harvested and air dried. Dried leaf and pseudostem biomass were enclosed in litter-bags (4 ± 0.05 g/bag; 1 mm^{-2} mesh, 10 × 14 cm size). Two experiments were set up in an enclosure at Ruissalo Botanical Garden, and prior to that, the natural grassland vegetation was removed and soil was superficially homogenized to avoid spatial variation in microbial community.

2.1.1. Experiment 1. Effects of grass species and endophyte symbiosis

The experiment tested the effects of the endophyte symbiosis (E+ and E−) and species identity (*S. phoenix* and *S. pratensis*) on litter decomposition. There were 160 litter-bags from the combination of 2 grass species (*S. phoenix* cultivar 'Kentucky-31' and *S. pratensis* cultivar 'Kasper') and two endophyte statuses (E−, E+). The bags were established in a grid where each place for each bag (experimental unit) was randomly assigned, at a distance of 20 cm from each other, and anchored with 10 cm nails to the ground. The litter-bag experiment corresponded to a full factorial design with 10 replicates and 4 retrieval times. Ten randomly selected bags from each treatment combination were retrieved on four dates: June and September 2012, and May and September 2013. The decomposition rate was determined by weighing the remaining oven dried litter biomass in the bags.

2.1.2. Experiment 2. Effects of plant origin and endophyte symbiosis

The experiment tested the effect of endophyte symbiosis (E+ and E−) and three wild population origins (Åland, Gotland, Södermanland) and one cultivar (Kentucky-31) of *S. phoenix* on litter decomposition. A total of 320 bags from the two endophyte statuses (E+, E−) and four plant origins (three wild origins and one cultivar) were placed on a grid where each litter-bag was 20 cm apart from each other. Similar to experiment 1, the experiment corresponded to a full factorial design with 10 replicates and 4 retrieval times. The four retrieval dates were exactly the same as before. Decomposition was determined by weighing the remaining oven dried litter biomass in each bag.

2.2. Chemical analyses of the litter

Mineral composition (Ca, Cu, Fe, Mg, Mn, N, K, P, S, Zn), carbon content (C), acid detergent fiber (ADF), acid detergent lignin (ADL) and fungal origin alkaloids (ergovaline and peramine) were analyzed at the beginning of the experiments, from three randomly selected litter bags per host species, host origins, and endophyte status.

Mineral composition was determined by the ICP-OES (inductively coupled plasma optical emission spectrometry) method. Samples were digested in concentrated nitric acid (10 ml) and evaporated into about 1–2 ml. The sample was then transferred into a 50 ml volumetric flask, diluted with MILLI-Q purified water, and filtered before the ICP-OES measurement. Mineral and trace elements were measured by high resolution ICP-OES (Perkin Elmer Optina 8300) (Kumpulainen and Paakki, 1987). One blind and 1–3 reference samples were included in each sample batch. Carbon and nitrogen were determined with an automated dry combustion method (Dumas method) by Leco TruMac CN- analyzer, Leco Corporation, USA.

Acid detergent fiber [ADF: cellulose + lignin + ash (minerals and silica)] and acid detergent lignin (ADL: lignin) were determined