

# Direct and indirect effects of the fungal endophyte *Epichloë uncinatum* on litter decomposition of the host grass, *Schedonorus pratensis*

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**Abstract** Microbial plant symbionts have been suggested to mediate plant-soil feedback and affect ecosystem functions. Systemic *Epichloë* fungal endophytes of grasses are found to mediate litter decomposition. These effects are often linked to alkaloids produced by *Epichloë* species, which are hypothesized to negatively affect decomposers. Although endophytes have been found to affect plant community and soil biota, direct (through litter quality) and indirect (through the environment) effects of fungal endophytes on litter decomposition have been scarcely scrutinized. We placed litterbags with endophyte-symbiotic (E+) and non-symbiotic (E–) *Schedonorus pratensis* plant litter in plots dominated by E+ or E– plants of the same species, and followed the dynamics of mass

losses over time. We predicted the endophyte would hinder decomposition through changes in litter quality and that both types of litter would decompose faster in home environments. E+ litter decomposed faster in both environments. The mean difference between decomposition rate of E+ and E– litter tended to be higher in E– plots. Nitrogen and phosphorus, two elements usually associated with high decomposition rates, were significantly lower in E+ litter. We also detected a higher proportion of C in the cellulose form in E+ litter. Contrary to the general assumption, we found that symbiosis with *Epichloë* fungal endophytes can be associated with higher decomposition of plant litter. Since direct effects of *Epichloë* fungi were still stronger than indirect effects, it is suggested that

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besides the alkaloids, other changes in plant biomass would explain in a context-dependent manner, the endophyte effects on the litter decomposition.

**Keywords** *Festuca* · Symbiosis · Plant–microorganism interaction · Plant–soil feedback · Ecosystem processes

## Introduction

Plant–soil feedback affects functional processes of ecosystems such as recycling of nutrients and primary productivity (Zhang et al. 2008; Hobbie 2015). Decomposition that implies the passage of dead plant material to organic matter and minerals can be affected by multiple biotic and abiotic factors (Melillo et al. 1982; Vivanco and Austin 2006; Austin et al. 2014; Cleveland et al. 2014; García-Palacios et al. 2016). Understanding the controls of litter decomposition is crucial for both productivity management in agroecosystems and for estimating carbon budgets in ecosystems (Zhang et al. 2008; Omacini et al. 2012; Austin et al. 2014; Crowther et al. 2015; Hobbie 2015).

Leaf fungal endophytes of the genus *Epichloë* have received certain attention with inconsistent results regarding their effects on host litter decomposition. Asexual *Epichloë* fungal endophytes (Clavicipitaceae) associate with species of cool-season grasses (subfamily Pooideae) growing systemically in the above-ground tissues and passing through generations by vertical transmission (Schardl et al. 2007; Gundel et al. 2017). Considering the 11 independent studies published until 2012, a meta-analysis reported an overall negative, although not significant, effect of endophytes on litter decomposition (Omacini et al. 2012). At the time the meta-analysis was performed, the preponderant hypothesis was that fungal alkaloids produced by endophyte symbionts which have shown deterrent effects on herbivores (see e.g., Clay 1988; Schardl et al. 2007), would have a direct inhibitory effect on litter decomposition. However, as observed for the effects of endophytes on plant–herbivore interactions (Saikkonen et al. 2010, 2013a; Ueno et al. 2016; Shukla et al. 2015), effects on decomposition have proven to be highly variable with negative, neutral, or positive results depending on host species, partners' genotypes, and ecological conditions

(Omacini et al. 2004; Lemons et al. 2005; Siegrist et al. 2010; Gundel et al. 2016; Mikola et al. 2016). Apart from the alkaloids, endophytes have been associated with changes in plant biomass quality that could be also linked to litter decomposition. These effects have been observed at the level of mineral content (e.g., Phosphorus (P), Carbon (C)/Nitrogen (N) ratio), metabolites (e.g., phenolic and antioxidant compounds, and sugar alcohols of fungal origin such as the mannitol), and structural parameters such as content of fibers and lignin (Zabalgoeazcoa et al. 2006; Rasmussen et al. 2008; Rogers et al. 2011; Hamilton et al. 2012; Vázquez-de-Aldana et al. 2013a; Soto-Barajas et al. 2016). However, it has been challenging to establish a direct association between the endophyte symbiosis, the changes in host plant biomass quality, and the decomposition rate of the litter (Siegrist et al. 2010; Gundel et al. 2016; Mikola et al. 2016).

Plant community can have strong effects on abiotic conditions (e.g., humidity and soil temperature), as well as on soil microbial and faunal communities through plant–soil feedbacks, and ultimately affect litter decomposition (Van der Putten et al. 2013; Gong et al. 2015). Some of these changes may be exerted by the endophytes through their impact on the diversity of grassland community (Rudgers and Clay 2008; Saikkonen et al. 2013b; Vázquez-de-Aldana et al. 2013b) and thereby, on the community of decomposers (Lemons et al. 2005; Rudgers and Clay 2008). Thus endophytes may have indirect effects on litter decomposition through effects on plant community and on soil biota. The endophyte *Epichloë coenophiala* of tall fescue (*Schedonorus arundinaceus*) had a negative effect on host litter decomposition through the environment (patches dominated by endophyte-symbiotic plants vs endophyte-free counterparts) although it was of smaller magnitude compared to the direct effect through litter source (Siegrist et al. 2010). With the same grass species, Lemons et al. (2005) found that the exclusion of meso-invertebrates shifted the endophyte effect from decreasing to increasing the rate of litter decomposition. However, endophyte influence on invertebrates (although not necessarily involved in decomposition) associated to host plants has been variable depending on grass species and genotype, and the ecological conditions (see e.g., Rudgers and Clay 2008; Vesterlund et al. 2011; Popay and Jensen

2005; Shukla et al. 2015). Studies have revealed that symbiosis with leaf fungal endophytes affects abundance and structure of the soil microbial community associated with the rhizosphere of tall fescue plants (Buyer et al. 2011; Rojas et al. 2016). Assuming a local adaptation of the soil biota through plant-soil feedback processes, decomposition would be faster if plant litter is similar to the locally produced biomass (home-field advantage hypothesis (HFA); Ayres et al. 2006; Austin et al. 2014; Veen et al. 2015; but see Freschet et al. 2012).

In this paper, we tested direct (through changes in plant litter quality) and indirect (by means of changes in the abiotic and biotic environment) effects of the endophyte *Epichloë uncinatum* on litter decomposition of its host, the perennial grass *Schedonorus pratensis* (common name: meadow fescue). Although the majority of studies have found inhibitory effects of endophytes on litter decomposition (see the meta-analysis by Omacini et al. 2012), we might expect positive or neutral effects of endophytes on litter decomposition based on previous results. A positive effect of the endophyte *E. uncinatum* on litter decomposition of *S. pratensis* was observed in a common garden experiment (Gundel et al. 2016). Using the same symbiotic interaction (*S. pratensis*–*E. uncinatum*), a short-time experiment (it covered 70 days during summer) failed to find either positive or negative effects of the endophyte on litter decomposition (Mikola et al. 2016). Therefore, the relative importance of direct and indirect effects of fungal endophytes on plant litter decomposition is yet uncertain in the long-term. To address this, we used a reciprocal experimental design by crossing litterbags with endophyte-symbiotic (E+) and non-symbiotic (E–) *S. pratensis* plant material in patches dominated by E+ or E– plants of the same species. Since the fungus *E. uncinatum* has been shown to affect plant growth, stand stability, and soil invertebrates (Malinowski et al. 1997; Saikkonen et al. 2013b; Bylin et al. 2014; Shukla et al. 2015), we predicted an interaction between symbiotic status of the plant litter (Litter type, E+ and E–) and symbiont influence through the environment (Environment type, E+ and E–). In accordance with the home-field advantage hypothesis, we also predicted that both types of litter (E+ and E–) will decompose faster in environments dominated by the same plant type (i.e., E+ and E–, respectively).

## Materials and methods

### Origin of plant litter

Endophyte-symbiotic (E+) and non-symbiotic (E–) litter was collected from *S. pratensis* plants growing in a common garden (Ruissalo Botanical Garden, University of Turku, Finland). *Epichloë uncinatum* is the loline-producing endophyte hosted by *S. pratensis* (Craven et al. 2001; Schardl et al. 2007). Individual plants of *S. pratensis* of the Finnish cultivar ‘Kasper’, symbiotic (E+) and non-symbiotic (E–) with endophyte (ten plants each) were placed at random in a grid with 1 m<sup>2</sup> per plant in 2008 (Saikkonen et al. 2013b). Symbiotic status of the established plants was checked and confirmed several times since the establishment of the common garden (see Saikkonen et al. 2013b). At the end of the growing season of the third year (2011), aboveground biomass from the 10 E+ and 10 E– plants was harvested and mixed to E+ and E– batches. The harvest of plant material at the end of the growing season simulates plant senescence before winter in northern latitudes. Air-dried and chopped leaf and pseudostem biomass (E+ or E–) were enclosed in litterbags (100 × 140 mm size, 1 mm<sup>–2</sup> mesh, 4 ± 0.05 g biomass/litterbag).

### Litter decomposition field experiment

In order to examine direct and indirect endophyte effects, we assigned the litterbags into an experimental field established at MTT Agrifood Research Finland (Jokioinen) in May 2006 (Saikkonen et al. 2013b). The experimental field consisted of ten blocks with two paired plots (25 × 39 m) sown with either E+ (79% frequency of endophyte-symbiotic plants) or E– (0% endophyte frequency) seeds of *S. pratensis* cultivar ‘Kasper’ (sowing rate: 20 kg ha<sup>–1</sup>). The symbiotic status remained high (80–90%) and low (0–3%) in E+ and E– plots, respectively (Saikkonen et al. 2013b). Because the cover of *S. pratensis* diminished by approximately 23% in E– plots after four years (Saikkonen et al. 2013b), we identified plant stands within each plot that were dominated by *S. pratensis* in order to avoid the effect of other plant species. After 6 years, total C and N soil contents were not different between E+ and E– plant stands (Mikola et al. 2016).

On October 20, 2011, we placed 12 E+ and 12 E– litterbags on each of the 20 plots (10 E+ and 10 E–

environments). Three E+ and three E− litterbags were randomly removed from each plot at four dates: June and September 2012, and May and September 2013. The decomposition was determined by weighing the remaining mass of litter in each bag, and expressed as percentage of litter mass loss (initial mass – final mass) on a dry weight basis.

### Litter quality parameters

At the beginning of the experiment, three litterbags per symbiotic status were randomly selected and analyzed for mineral and chemical characterization. Mineral concentration (P, K, Ca, Mg, S, Fe, Mn, Zn, and Cu) was analyzed by the ICP-OES method (inductively coupled plasma optical emission spectrometry). For this, samples were digested in concentrated nitric acid (10 ml) and evaporated to 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. Concentration of trace elements was evaluated by high resolution ICP-OES (Perkin Elmer Optima 8300) (Kumpulainen and Paakki 1987). Carbon and nitrogen were determined with an automated dry combustion method (Dumas method) by a 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 using the filter bag technique, with an Ankom Automated Fiber Analyzer A2000, based on the analytical method of Goering and Van Soest (1970).

### Data analysis

For analyzing the direct (symbiotic status of the litter: E+ or E−) and indirect (through the environment: E+ or E−) effects of endophyte symbiosis, time (four levels: days of decomposition), and all their interactions on litter mass loss (g), we estimated a general linear mixed-effects model (lme function, nlme package, Pinheiro et al. 2014). Random effects accounted for the fact that litterbags were nested within 20 plots, and plots were nested within 10 blocks to avoid pseudo-replication (i.e., random intercept models). As a complementary analysis, decomposition rates were estimated by calculating the slope ( $k$ ) of the exponential decay curves for each combination of endophyte-symbiotic status and environment. In the formula: In

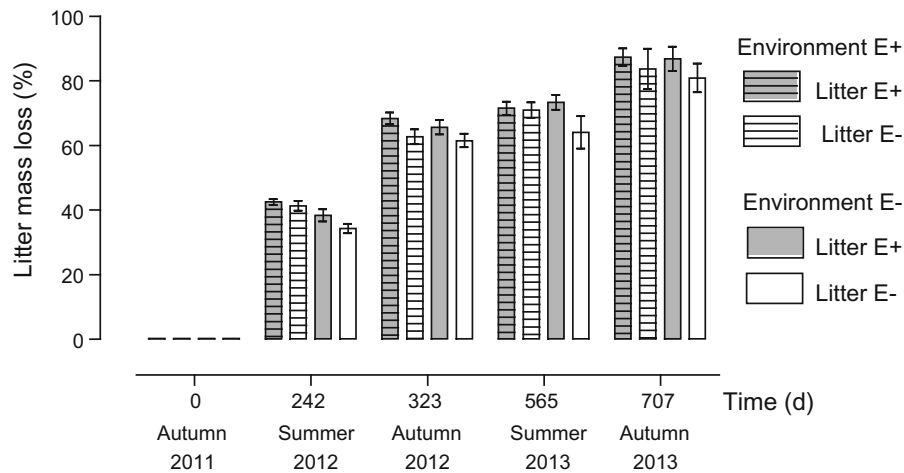
$(M_t/M_0) = y - k_t$ ,  $M_0$  and  $M_t$  represent the initial litter mass and the mass remaining at time  $t$  (in years), respectively,  $y$  is the intercept and  $k$  is the decomposition constant (Wieder and Lang 1982). Least-square regression analyses were used to estimate the  $k$  values considering all the replicates for all treatments (combination of symbiotic status and environment) and decomposition times, including litterbag mass in time 0 (i.e., 4 g). Endophyte effects on each litter quality parameter were analyzed by means of  $t$ -student test. The analyses were performed in R, version 3.0.2 (R Development Core Team 2013).

### Results

The endophyte status of the litter (E+ and E−), the endophyte status of the environment, and decomposition time, interactively affected the litter mass loss of *S. pratensis* ( $F_4 = 2.806$ ,  $P = 0.025$ ; Table 2) (Fig. 1). E+ litter lost more mass than E− litter, and the average difference between E+ and E− litter was significantly greater in environments growing E− grass (Figs. 1, 2). Averaged over the four retrieval times, the mean difference between E+ and E− litter biomass was 0.11 g in environments growing E+ and 0.21 g in environments growing E− grasses. The difference between the environments (E+ and E− plots) was most evident at the first litterbag removal time (summer 2012) when decomposition was 7% higher in endophyte-symbiotic than endophyte-free environments (Fig. 1).

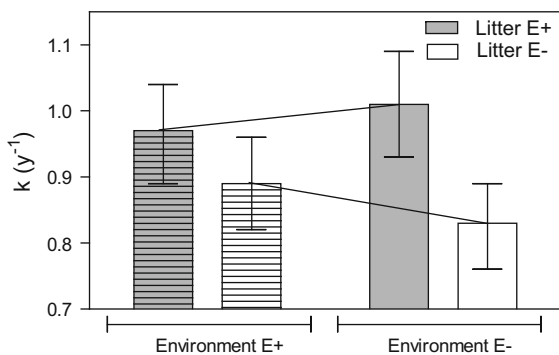
The pattern of biomass loss from the litterbags along experimental time was apparent through decomposition constants for each type of litter in each environment (Fig. 2). The reaction norm shows graphically the interaction between factors in which E+ litter decomposed faster than E− litter but with a tendency to show more decomposition in stands dominated by E− plants than in stands dominated by E+ plants (notice that 95% confidence intervals of E+ and E− litter do not overlap in the E− environment). Thus, E+ litter tended to decompose faster in E− environment than in E+ environment, and E− litter tended to decompose slower in E− environment than in E+ environment (Fig. 2).

Several parameters related to the quality of litter and decomposition rate at the beginning of the experiment differed between E+ and E− litter



**Fig. 1** Temporal dynamics of litter mass loss (%) of *Schedonorus pratensis* cv. Kasper plants symbiotic (E+ dark bars) and non-symbiotic (E- white bars) with fungal endophyte during the experimental time (days). Retrieval times are indicated together with the corresponding season of each year. The

litterbags were placed in an environment where endophyte-symbiotic (E+ patterned bars) or non-symbiotic (E- non-patterned bars) plants of the same grass species were growing. Values are means  $\pm$ 95% CI ( $n = 10$ )



**Fig. 2** Mean values ( $\pm$ 95% CI) of decomposition constants ( $k$ , year<sup>-1</sup>) for endophyte-symbiotic (E+ dark bars) and non-symbiotic (E- white bars) litter from *Schedonorus pratensis* plants (cultivar ‘Kasper’) in environments dominated by endophyte-symbiotic (E+ patterned bars) and non-symbiotic (E- non-patterned bars) plants of the same grass species

**Table 1** Parameters related to quality of endophyte-symbiotic (E+) and non-symbiotic (E-) litter biomass from *Schedonorus pratensis* cv. Kasper plants at the beginning of the decomposition experiment

Parameter	Endophyte-symbiotic status		P values
	E+	E-	
Dry matter (%)	93.38 $\pm$ 0.04	93.40 $\pm$ 0.09	0.843
Ash (%)	7.48 $\pm$ 0.09	7.59 $\pm$ 0.09	0.450
ADF (%)	45.10 $\pm$ 1.81	39.60 $\pm$ 1.25	0.067
ADL (%)	3.72 $\pm$ 0.14	4.15 $\pm$ 0.41	0.374
C (%)	42.66 $\pm$ 0.08	42.85 $\pm$ 0.04	0.316
N (%)	0.69 $\pm$ 0.04	<u>0.94 <math>\pm</math> 0.03</u>	<b>0.006</b>
P (g kg <sup>-1</sup> )	1.78 $\pm$ 0.01	<u>2.12 <math>\pm</math> 0.06</u>	<b>0.004</b>
K (g kg <sup>-1</sup> )	10.11 $\pm$ 0.06	<u>11.08 <math>\pm</math> 0.17</u>	<b>0.005</b>
Ca (g kg <sup>-1</sup> )	5.11 $\pm$ 0.04	5.11 $\pm$ 0.03	0.979
Mg (g kg <sup>-1</sup> )	<u>1.65 <math>\pm</math> 0.01</u>	1.55 $\pm$ 0.01	<b>0.002</b>
S (g kg <sup>-1</sup> )	1.17 $\pm$ 0.01	<u>1.58 <math>\pm</math> 0.01</u>	<b>&lt;0.001</b>
Fe (mg kg <sup>-1</sup> )	116.42 $\pm$ 4.61	<u>190.60 <math>\pm</math> 12.47</u>	<b>0.005</b>
Mn (mg kg <sup>-1</sup> )	<u>53.32 <math>\pm</math> 0.31</u>	43.94 $\pm$ 0.35	<b>&lt;0.001</b>
Zn (mg kg <sup>-1</sup> )	13.98 $\pm$ 0.03	<u>14.83 <math>\pm</math> 0.18</u>	<b>0.009</b>
Cu (mg kg <sup>-1</sup> )	3.50 $\pm$ 0.02	<u>4.34 <math>\pm</math> 0.08</u>	<b>&lt;0.001</b>

Values are mean  $\pm$  SE ( $n = 3$ ). Significant differences ( $t$ -student test) are indicated in bold and the highest value in each significant test is underlined

(Table 1). The endophyte was associated with a significant decrease in litter nitrogen content ( $\approx$ 26% lower) but not in carbon ( $C_{\text{mean}} = 42.75\%$ ). E+ litter was significantly higher in C:N ratio (E+ = 62.20) than E- litter (E- = 45.69) ( $t_4 = 4.244$ ,  $P = 0.013$ ). Mg and Mn contents were higher in E+ litter, while

E– litter was higher in P, Cu, Fe, K, S, and Zn contents (Table 1). There was a marginal positive effect of endophyte on ADF (cellulose + lignin + ash). Together with dry matter, ash, lignin, and Ca contents, the N:P ratio ( $t_4 = 1.726$ ,  $P = 0.159$ ) was not significantly affected by the endophyte symbiosis (N:P  $\approx$  0.41) (Table 1).

## Discussion

In contrast to the hypothesis that *Epichloë* fungal endophytes decelerate litter decomposition of host plants, we found a direct positive effect of the endophyte symbiosis on litter decomposition of the host grass, *S. pratensis*. These results are in accordance with our previous experiment in which the *E. uncinatum* fungal endophyte increased the decomposition rate of the *S. pratensis* litter when incubated in a common garden without vegetation (see Gundel et al. 2016). As we predicted, however, the endophyte modulated decomposition appeared to depend on the frequency of *Epichloë* endophyte-symbiotic grasses in the plant community and its consequences on the biotic and abiotic environment (i.e., indirect effects). Litter from E+ plants tended to decompose faster in both E+ and E– environments compared to litter from E– plants. In contrast to home-field advantage hypothesis (HFA), the decomposition rates of E+ were higher than that of E– litter in the study plots dominated by endophyte-free *S. pratensis* plants. Overall, direct effects of the endophyte status of litter seemed to be more important than indirect effects mediated through the community of decomposers in the soil. Similar direct versus indirect effects were observed for the endophyte *E. coenophiala* on the litter decomposition of its regular host *S. arundinaceus* (Siegrist et al. 2010).

*Epichloë* endophytes can directly promote decomposition by modulating the leaf chemistry or the microbial community, which may act as saprotrophic in abscised plant parts (Zabalgoeazcoa et al. 2013; Saikkonen et al. 2015). The majority of the chemical ecology literature on *Epichloë* endophytes has focused on endophyte-origin alkaloids, which are lolines in the case of *E. uncinatum* (Lehtonen et al. 2005; Schardl et al. 2007; Bylin et al. 2014). Conventionally, *Epichloë* endophytes that produce alkaloids are treated as plant defensive mutualists providing protection to the host plant against herbivores and pathogens

(Saikkonen et al. 2010, 2013a; Huitu et al. 2014; Ueno et al. 2016). Thus, these alkaloids are often hypothesized to also negatively affect decomposer organisms in the soil (Omacini et al. 2012; Saikkonen et al. 2015; Mikola et al. 2016). In contrast to the hypothesis, in our previous study with tall fescue (*S. phoenix*) we detected a positive association between alkaloid level (ergovaline) at the beginning of the trial, and litter decomposition (Gundel et al. 2016). These results do not, however, rule out alkaloid mediated effects on decomposers. Positive effects on decomposition rate might partly be explained by nitrogen burst from quickly degrading alkaloids (Siegrist et al. 2010) or changes in the decomposer community (see Saikkonen et al. 2015; Rojas et al. 2016). Alternatively, *Epichloë* species may differently modify the plant metabolomic profile favoring the stock of labile carbon (e.g., non-structural carbohydrates, phenolic compounds; Rasmussen et al. 2008; Hamilton et al. 2012) and thus differently modulating decomposition processes (García-Palacios et al. 2016).

In order to understand *Epichloë* endophyte-mediated litter decomposition via altered plant quality, we looked at differences in chemistry of leaf litter between E+ and E– plants. Several chemical parameters known to positively affect litter decomposition, such as high N and P contents (Melillo et al. 1982; Vivanco and Austin 2006; Güsewell and Gessner 2009; Gong et al. 2015; Hobbie 2015), were lower in E+ litter compared to those of E– litter. In addition, endophyte presence had no effect on lignin which is known to decelerate decomposition (Melillo et al. 1982; Vivanco and Austin 2006). However, ecological roles of individual chemical parameters may be context dependent. For example, N has sometimes detected to be associated with lower decomposition rates (Hobbie 2015). Contrarian results from studies focusing on individual elements can be partly explained by interactive effects of elements. E+ litter had higher C:N ratio, and lignin:N ratio (Lignin:N<sub>E+</sub> = 5.39, and Lignin:N<sub>E–</sub> = 4.41); acid detergent fiber (ADF) contents tended to be also higher in E+ litter. The latter indicates a greater content of C in the form of cellulose because the proportion of other components of ADF, i.e., ash and lignin, remained the same in E+ and E– litter. Consequently, the cellulose:lignin ratio  $\{[ADF - (ADL + Ash)]/ADL\}$  was 9.11 and 6.70 for E+ and E– litters, respectively.

Differences in litter quality often reflect the relative amount of fungi and bacteria colonizing the litter. A

proper balance of C:N or N:P may be required for the optimal activity of decomposer microorganisms in the litter (Vivanco and Austin 2006; Güsewell and Gessner 2009). Fungi typically dominate microbial communities in nutrient-poor organic matter, as they may have lower nutrient requirements and a lower metabolic activity than bacteria (Güsewell and Gessner 2009). Accordingly, the relative P requirements of fungi should be lower than those of bacteria (Smith 2002), so that fungi are expected to dominate in litter with high N:P ratios. Similarly, fungi dominate and drive decomposition on substrates with high C:N ratios, due to the lower N requirement, while bacteria will dominate on substrates with low C:N ratios. The question to be solved in future studies is how these *Epichloë* endophyte-mediated changes in litter chemistry affect microbial communities that take part in decomposition (Vázquez-de-Aldana et al. 2013c; Zabalgogezcoa et al. 2013; Saikkonen et al. 2015). Our results suggest that cellulose-rich E+ litter with high C:N ratio may favor fungi, especially cellulolytic fungi (Vázquez-de-Aldana et al. 2013a), in decomposing microbial community. Similarly, the greater Mn found in E+ litter, suggests that endophyte symbiosis may promote fungi that use Mn peroxidases to break down lignin and thus decomposition rate (Hobbie 2015). By contrast, N:P ratio remained the same in E+ and E– litters and thus appears to play a less significant role in determining microbial decomposition in this study.

To conclude, *Epichloë* endophytes can affect plant litter decomposition through multiple ecological pathways. In previous studies we have shown that *E. uncinatum* increases plant vigor and thereby promotes

high frequencies of E+ *S. pratensis* individuals in the plant community (Saikkonen et al. 2013b). Thus, endophyte symbiosis can indirectly affect environmental conditions for both degradation of litter by chemical and physical processes or by decomposers via the structure of the plant community and microclimate (e.g., temperature, humidity, etc.). In this study we demonstrated *Epichloë* endophyte-mediated changes in litter quality and that, although influenced by the frequency of infected grasses in the plant community, decomposition rate of E+ litter was higher compared to E– litter, lending no support for home-field advantage hypothesis. Accumulating conflicting literature evidence suggests, however, the effects of endophytes on litter quality and decomposition are context dependent, and are not necessarily translated into changes in nutrient cycling (Mikola et al. 2016).

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

#### Appendix

See Table 2.

**Table 2** Analysis of variance testing for the direct (through plant biomass: symbiotic status of litter) and indirect (through the environment where symbiotic and non-symbiotic grasses

has been growing: Environment type) effects of the fungal endophyte *E. uncinatum* on litter decomposition of the host grass *S. pratensis* along the experimental time (retrieval time)

Source of variation	NumDF	DenDF	F value	P value
Intercept	1	493	22,435.445	<0.001
Symbiotic status of litter (litter type)	1	493	59.732	<0.001
Environment type (environment)	1	9	6.711	0.029
Retrieval time (time)	4	493	4837.596	<0.001
Litter type × environment	1	493	5.424	0.020
Litter type × time	4	493	4.866	0.001
Environment × time	4	493	5.677	<0.001
Litter type × environment × time	4	493	2.806	0.025

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