

# Effects of grassland management, endophytic fungi and predators on aphid abundance in two distinct regions

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## Abstract

### Aims

Fungal endophytes of cool-season grass species produce alkaloids toxic to herbivores, affecting food webs in agricultural and natural ecosystems. Field studies about the effects of endophytes on herbivores are rare and show contradictory results, leading to uncertain conclusions about the nature of endophyte–grass symbiosis. We asked whether the environmental contexts of local and regional scales and predation could modify the effects of endophytes on herbivores.

### Methods

In a full factorial field experiment, we quantified the abundance of the aphid species *Rhopalosiphum padi* on the potted host grass *Lolium perenne*, which was either infected or uninfected with the endophytic fungus *Neotyphodium lolii*. Predators were either excluded or had free access to the pots with the aphids. One hundred and sixty grass pots were located in two regions on altogether 40 grassland sites, half of the sites intensively and half extensively managed. We tested the importance of endophyte infection, study region, management intensity of grasslands, predation and all two-way interactions on aphid abundance.

### Important Findings

Endophyte infection reduced aphid abundance significantly in one study region only. In both regions, we found that the impacts of aphidophagous predators and grassland management intensity on aphid abundance were substantially stronger and more consistent than that of endophytes on aphid abundance. Pots excluding predators and pots placed on extensive grasslands contained higher aphid abundance. The impact of predators and management on aphid abundance were not modified by the endophyte. We conclude that the effect of endophytes on herbivores can be weak in field experiments and depends on environmental context at a regional scale. Hence, more field research efforts are necessary to detect the relative importance of endophytes and the environmental context on biotic interactions in ecosystems.

**Keywords:** bottom-up control, microorganism, multi-trophic interaction, predator exclusion, top-down control

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## INTRODUCTION

Herbivore abundance can be controlled by predators (top down) and by food plant quality and abundance (bottom up) (Hairston *et al.* 1960; Price 2002). Furthermore, microorganisms living within food plants can affect herbivores through their influence on food plant quality (Schardl *et al.* 2004). These microorganisms make up an additional trophic level

that can change trophic cascades and food webs (Hartley and Gange 2009; van der Heijden *et al.* 2008).

Fungal endophytes are examples of these symbiotic microorganisms, which were found in all vascular plant species surveyed for endophytic fungi (Arnold and Lutzone 2007; Stone *et al.* 2000). ‘True’ endophytes of the genus *Neotyphodium* occur only in cool-season grass species; they live hidden within the plant tissue and cause no visible infection (Hartley

and Gange 2009). They have an asexual lifecycle and disperse via the seeds of their host (Schardl et al. 2004). The symbiosis between the endophyte and the grass host is mutualistic, parasitic or a continuum between these two extremes and is a controversially discussed topic in endophyte research (Müller and Krauss 2005; Rudgers et al. 2010; Saikkonen et al. 2010). The host benefits from alkaloids produced by the endophyte–grass association, which are toxic to some herbivores studied (Schardl et al. 2004), e.g. several aphid species populations exhibit slow growth when feeding on grass infected with *Neotyphodium* endophytes, yet grasshoppers are unaffected (Breen 1994; Hartley and Gange 2009; Meister et al. 2006; Saikkonen et al. 2010). The majority of studies on effects of *Neotyphodium* on herbivores and predators have been conducted under laboratory conditions; field studies are less frequent, and the interpretation of their results has been debated (Faeth 2009; Rudgers et al. 2010). Rudgers and Clay (2008) have found that arthropod abundance and diversity decrease in the presence of the endophyte *Neotyphodium coenophialum* in an agronomic grass species. In contrast, Jani et al. (2010) have shown that arthropods not only thrive but also diversify in the presence of an alkaloid-producing endophyte of the genus *Neotyphodium* in a native grass species. An explanation for the contrasting results is the species identity of the host grass and the associated endophyte, which can produce different alkaloids with varying concentrations (Schardl et al. 2004). Another reason might be that in some studies, agronomically important and reared grass species were used, whereas in others, native grass species were examined (Crawford et al. 2010; Faeth and Shochat 2010; Saikkonen et al. 2006; Vesterlund et al. 2011). Several abiotic factors such as drought (Miranda et al. 2011) and nutrient availability (Lehtonen et al. 2005) have been shown to influence herbivore performance on endophyte-infected grass under controlled conditions. But field studies simultaneously executed in different regions are rare and field studies in distinct regions have yielded contradicting results (Jani et al. 2010; Krauss et al. 2007; Omacini et al. 2001; Rudgers and Clay 2008; Saona et al. 2010). We therefore tested whether the environmental contexts of two regions and different management intensity of grasslands affect the influence of endophytes on herbivores under standardized growing conditions. We chose one abundant aphid species (*Rhopalosiphum padi* L.) from one breed as an herbivore and one cultivar of the endophyte–grass association (*Neotyphodium lolii* Glenn, Bacon and Hanlin–*Lolium perenne* L.). These restrictions were necessary, as it has been shown that reactions can vary depending on the genetic background of interacting partners (Bieri et al. 2009; Bultman et al. 2009; Faeth et al. 2002; Hesse et al. 2003; Tintjer and Rudgers 2006).

The toxic effects of endophytes can cascade up the food chain and harm organisms such as predators and parasitoids at higher trophic levels that feed exclusively on herbivores from infected plants (Bultman et al. 2009; de Sassi et al. 2006; Härrri et al. 2008a; Omacini et al. 2001). Some predators might avoid herbivores feeding on endophyte-infected and

alkaloid-producing plants, which could affect interpretations of the nature of the grass–endophyte symbiosis. Host selection for parasitoids was shown under laboratory conditions (Härrri et al. 2008a) but not in a field experiment (Härrri et al. 2008b). Their field experiment was restricted to one field site, whereas here, we tested whether different predator communities at differently managed grasslands and regions alter the effects of endophytes on aphid abundance. In the few field studies conducted on the influence of endophytes relative to other regulatory mechanisms such as nutrient availability or predation, the success of endophytes on aphid control has been determined to be relatively low (Härrri et al. 2008b; Jones et al. 2011; Krauss et al. 2007; Vesterlund et al. 2011).

Independent of endophyte infection, we expect that aphid control in extensively managed grasslands is more effective than in intensively managed grasslands, because extensive land use systems allow better aphid control through increased predator abundance (Roschewitz et al. 2005). Our study design allowed us to test this assumption on a large spatial scale of two distinct regions, in which very extensive and intensive grasslands were selected (Fischer et al. 2010). We studied the effects of endophyte, predation, management intensity, study region and their unknown but interesting interactions on aphid abundance and formulated the following predictions.

The assumed negative effect of the endophyte *N. lolii* on aphid abundance could vary between regions and different management intensities due to different environmental contexts or different predator communities with distinct preferences for aphids from infected plants. We expect that the toxic effect of the endophyte is less important than effects of region, management intensity and predation on aphid abundance. Independent of endophyte infection, we expect a better aphid control by predators in extensively managed grasslands than intensively managed grasslands.

## MATERIALS AND METHODS

### Study species

We selected the agronomically important ‘perennial ryegrass’, *L. perenne* (Poaceae), as study species and host for the endophytic fungus *N. lolii* (Clavicipitaceae). This grass species is native to Europe and Northern Africa, but many grassland populations in Germany are dominated by reared cultivars that are sown with commonly used seed mixtures (e.g. Klapp and Opitz von Boberfeld 2006). All managed seeds in the experiment belonged to the commercial cultivar Grassland Samson, provided by David Hume, AgResearch New Zealand. The grass was either uninfected (E–; identity number A 11104) or infected (E+; identity number A 12038). The *N. lolii* genotype used in our project belong to the ‘wild strain’ or also called ‘standard toxic endophyte strain’, as often used in the Samson Grassland *L. perenne* cultivar (van Zijll de Jong et al. 2008). This endophyte genotype produces the alkaloid components (ergovaline, lolitrem B and peramine) and is neither modified by traditional plant breeding nor by genetic modification. The

infection rate of 100 seeds was 92% for E+ seeds and 0% for E- seeds (D Hume, personal communication). The same cultivar was used in previous experiments, where viability of the endophytes and alkaloid concentrations have been measured (Jones *et al.* 2011; Krauss *et al.* 2007).

We used bird cherry oat aphids *R. padi* (Aphidae) provided by Katz Biotech AG, Germany, as herbivores on *L. perenne*. *Rhopalosiphum padi* is one of the dominant pests in European cereal fields but also feeds on the phloem of various grass species (Blackman and Eastop 2000) including our experimental host plant, *L. perenne* (Jones *et al.* 2011; Krauss *et al.* 2007).

### Study regions and sites

We studied intensively and extensively managed grasslands in two geographically separated regions in Germany to test whether the experimental findings were independent of environmental context. The first study region was in southern Germany in the 'Schwäbische Alb' (hereafter, Alb); the second region was in central Germany, 350 km further north in the 'Hainich-Dün' (hereafter, Hainich). Both regions were dominated by calcareous bedrock. The altitude of the Alb is ~460–860 m above sea level and of the Hainich ~285–550 m above sea level. The Alb (data from the town Ulm) showed average day temperatures of minimum 11.2°C and maximum 21.0°C and had a total precipitation of 327.4 mm during the 3-month study period. The Hainich (data from the town Eisenach) had average day temperatures of minimum 10.7°C and maximum 19.8°C and had total precipitation of 281.1 mm ([www.wetteronline.de](http://www.wetteronline.de)). The Alb is a heterogeneous patchy region comprising intensively managed grasslands interspersed with calcareous grasslands, small agricultural fields and forests. In contrast to the Alb, the Hainich has a larger proportion of bigger and more intensively managed agricultural fields and grasslands. Both regions are part of a large-scale German project called Biodiversity Exploratories (for details, see [www.biodiversity-exploratories.de](http://www.biodiversity-exploratories.de), accessed 17 March 2013; Fischer *et al.* 2010). In each region, we selected 20 grassland study sites separated by a minimum of 2 km to achieve spatial independence. Grasslands were pastures, meadows or a mixture of both. Half the sites in each region were intensively managed and the other half extensively (Table S1, see online supplementary material). Intensively managed grasslands were heavily fertilized and mown two or three times per year and/or grazed by livestock—mainly cattle—for several weeks. Extensively managed grasslands were not fertilized and were mown only once per year and/or grazed for short periods. Most extensively managed study sites were calcareous grasslands with sheep grazing that exhibited a significantly higher diversity of vascular plants (mean  $\pm$  SE,  $44.5 \pm 13.3$ ) than that in intensively managed grasslands ( $21.7 \pm 4.9$ ) (Socher *et al.*, University of Bern, unpublished data).

### Experimental set-up

We cultivated 160 pots (8 l, Ø 22 cm) with 200 seeds per pot of either *N. lolii*-infected or *N. lolii*-uninfected *L. perenne*

grass in commercially available garden compost, which we mixed with soaked water-accumulating granules (Broadleaf P4) to reduce watering efforts in the field. Owing to the high number of seeds, the whole pot was covered with grass even though not all seeds germinated. We placed the plant pots randomly in a climate-controlled greenhouse (16-h light at 19°C, and 8-h darkness at 12°C) and cut the grass twice during its growing period in the greenhouse from mid-March to end of April. At 1 May 2009, we started to transfer the grass pots to the study sites within 6 days. At each of the 40 study sites, we installed four experimental pots (two E+, two E-) spaced 3 m apart. We covered all pots completely with a closed Rantai S48 gauze net (Schachtrupp, Germany) with a mesh width of 0.8 × 0.8 mm to exclude parasitoids and prevent predator colonization before the start of the experiment. We fixed a plastic foil underneath the pots to prevent rainwater from seeping into the ground and the grass roots from growing into the soil (Fig. 1). We watered the plants when necessary after counting the aphids. After 2 weeks of acclimatizing the plants to outdoor conditions, we cut them to a height of 20 cm and exposed 50 *R. padi* aphids to each grass pot. This activity was carried out within 6 days beginning 18 May 2009. The aphids were reared on barley by the supplier (Katz Biotech AG), but before use in the experiment, we fed them for 1 week with uninfected *L. perenne* grass. We used two caged pots at each study site (one E+ and one E-) to exclude predators (see Fig. 1A); two additional pots (one E+ and one E-) on each study site were caged but contained four cut-outs (25 × 15 cm) to provide access for aphid predators while ensuring similar microclimatic conditions for all experimental plants (see Fig. 1B). We counted the number of aphids in each pot 1 week after exposure; subsequent counts took place every second week, always in the same study site sequence. We conducted six counts of each experimental pot between 25 May 2008 and 6 August 2008. One survey of all 160 pots took 6 days because of the large distance between the 40 study sites. We lost two intensively managed study sites in the Alb to vandalism; therefore, 152 experimental grass pots on 38 sites were included in the statistical analyses. A single person conducted all surveys to avoid bias in the searching for and counting of aphids. We counted juvenile, adult and winged aphids separately and also recorded aphidophagous predators: larvae of hoverflies (Syrphidae), lacewing larvae (Chrysopidae), larval and adult ladybirds (Coccinellidae), earwigs (Dermaptera), spiders (Araneae), carabids (Carabidae), rove beetles (Staphylinidae) and mummies (parasitized aphids). We included earwigs because the most abundant species in the region *Forficula auricularia* (Common Earwig) has been shown to be an effective aphid predator in other ecosystems (Piñol *et al.* 2009). We removed predators that had entered predator-exclusion pots. We did not analyse parasitoids separately because the parasitism rate of aphids was very low (0.07% of all counted aphids; mean  $\pm$  SE,  $0.92 \pm 0.14$  individuals per pot) during the study year. We counted aphids and predators in each pot for 7 min based on experience in





**Figure 1:** the two predation treatments. Experimental pots with the host plant *Lolium perenne* in the field with (A) closed gauze net (predator exclusion) and (B) open gauze net (predator access).

previous studies (Härri *et al.* 2008b; Krauss *et al.* 2007). When aphids were very abundant, we counted only half of the pot and extrapolated the number. Occasionally, other aphid species (mostly *Sitobion avenae* F.) colonized the experimental pots in low numbers, so they were not included in the statistical analyses. We harvested the aboveground plant material 1 week after the final aphid count to measure its biomass after 3 days of drying at 80°C.

### Statistical analyses

We conducted all statistical analyses using the software R (R Development Core Team 2012, version 2.15.1). We summed the individual numbers of aphids and predators from all six surveys per grass pot and performed analyses for different life stages of aphids as response variables: number of juvenile aphids, number of adult aphids and winged aphids. Results were similar for juvenile and adult aphids, and the number of winged aphids was too low for adequate statistical analyses. Hence, we present the total number of aphids only. Further response variables are the number of predators and plant biomass. We also tested for the total number of aphids for each count survey separately to assess the temporal dynamics of population growth (shown graphically in Fig. S1, see online supplementary material). We log transformed the data using the equation  $\ln[x + 1]$ , when necessary to meet the assumptions of normality and homoscedasticity in the statistical models.

We used linear mixed-effects models with four binary explanatory variables in the sequence—(i) region (Alb and Hainich), (ii) management intensity (extensive and intensive), (iii) endophyte infection (E+ and E–) and (iv) predation treatment (exclusion and access)—and all two-way interactions between these variables. Furthermore, we analysed aphid abundance separately for the two study regions. We used study site as a random factor, as endophyte and predation treatments were nested within that factor. We calculated Pearson correlations to identify relationships between plant biomass, total number of aphids and total number of predators. For aphid abundance as a response variable, we also tested whether the two covariables total number of predators or plant biomass influenced the effect of the explanatory variables. Arithmetic means and standard errors are given throughout the text and shown in Figs 2–4.

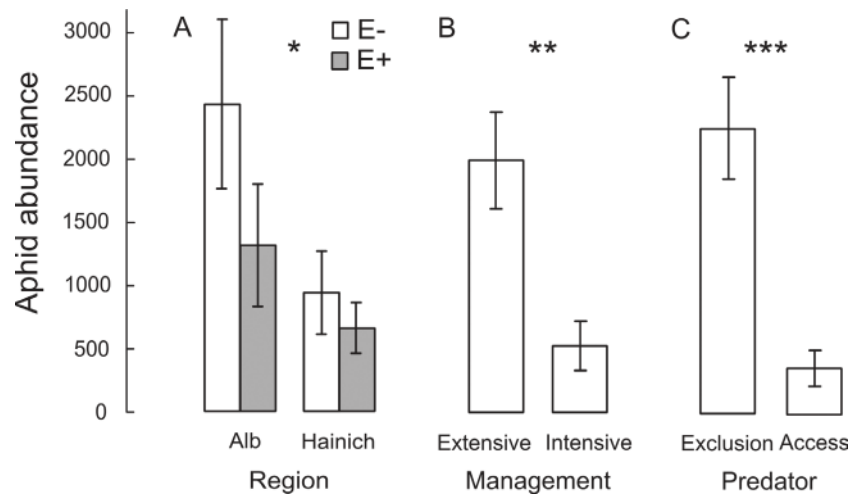
## RESULTS

We counted 198 470 individuals of the aphid species *R. padi* and 1076 aphid predator individuals. The presence of endophyte infection had no consistent negative influence on aphids (Table 1 and Fig. 2A) and significantly reduced aphid abundance in the Alb ( $F_{1,49} = 5.50$ ,  $P = 0.023$ ) but not in the Hainich ( $F_{1,55} = 0.32$ ,  $P = 0.578$ ). The negative effect increased with time and was significant from the third observation onward ( $F_{1,110} = 7.98$ ,  $P = 0.006$ ; Fig. S1, see online supplementary material)—i.e. 5 weeks after aphid exposure on the

**Table 1:** results of mixed-effects models: (A) aphid abundance, (B) predator abundance and (C) biomass as response variables

	(A) Aphid abundance			(B) Predator abundance		(C) Biomass	
	df	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Region	1,34	1.93	0.174	<b>8.96</b>	<b>0.005</b>	0.002	0.965
Management	1,34	<b>11.75</b>	<b>0.002</b>	<b>6.96</b>	<b>0.012</b>	<b>11.51</b>	<b>0.002</b>
Endophyte	1,107	1.64	0.202	2.48	0.117	0.53	0.470
Predation	1,107	<b>69.94</b>	<b>&lt;0.001</b>	<b>13.15</b>	<b>0.0004</b>	0.15	0.696
Region: management	1,34	0.02	0.891	0.05	0.823	2.86	0.100
Region: endophyte	1,107	<b>4.53</b>	<b>0.036</b>	1.23	0.271	0.37	0.543
Region: predation	1,107	1.16	0.284	2.47	0.119	<b>7.04</b>	<b>0.009</b>
Management: endophyte	1,107	1.33	0.252	0.10	0.749	0.02	0.880
Management: predation	1,107	1.04	0.311	1.16	0.285	0.20	0.656
Endophyte: predation	1,107	0.51	0.478	1.34	0.250	1.07	0.303

We show results of the full models with all two-way interactions. We also performed model simplifications as proposed by [Crawley \(2007\)](#) for linear mixed-effects models, but the results did not substantially differ from the full models, and therefore, are not shown. Significant effects are shown in bold and depicted in [Figs 2–4](#).

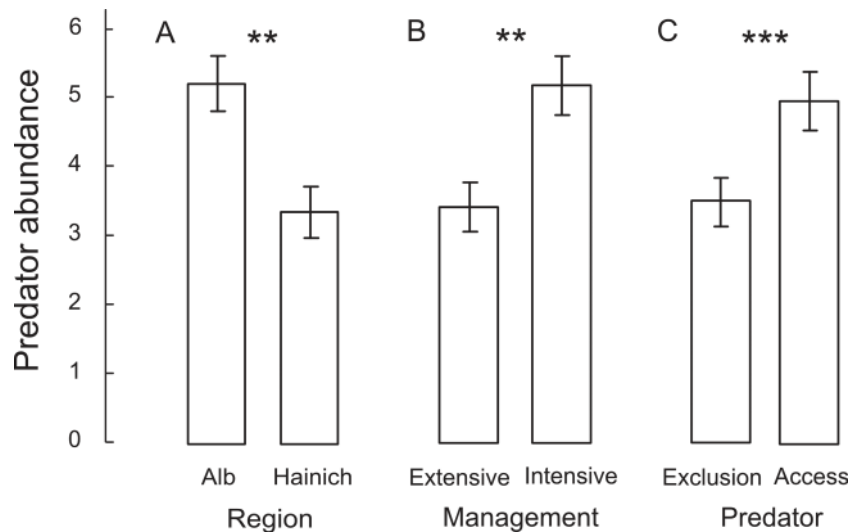


**Figure 2:** effects on aphid abundance. (A) Significant interaction effect of region and endophyte infection on aphid abundance (*Rhopalosiphum padi*) (number of individuals per pot over all six counts). Aphid abundance was significantly lower on grass pots with an infection of *Neotyphodium lolii* (E+) than without an infection (E-) in the study region Alb but not in the study region Hainich, (B) higher on extensively compared to intensively managed grasslands and (C) higher on pots with predator exclusion than on pots with free access for predator. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ; mean  $\pm$  SE is shown.

grass pots. In contrast, the interaction between endophyte infection and the management intensity of the surrounding grasslands was not significant, indicating that management intensity did not significantly influence the effects of endophyte on aphid abundance. The interaction of endophyte infection with the predation treatment was also not significant for aphid abundance, indicating that endophyte infection did not induce a significant predator preference for aphids. The presence of the endophyte did not significantly influence predator abundance ([Table 1](#)).

As intended by the study design, we recorded more aphidophagous predators when predators had free access to the grass pots ([Table 1](#) and [Fig. 3C](#)). Aphid abundance was six

times lower on the predator-accessed pots compared with predator-exclusion pots ([Table 1](#) and [Fig. 2C](#)). Predator abundance was not significantly correlated with aphid abundance ( $r = -0.087$ ,  $P = 0.289$ ). For detailed analyses of predator groups, see [Text S1](#), see online supplementary material. Contrary to expectation, aphid abundance was lower ([Table 1](#) and [Fig. 2B](#)) and predator abundance was higher ([Table 1](#) and [Fig. 3B](#)) on intensively managed grasslands than on extensively managed grasslands, independent of study region. Only 0.02% of all counted aphids ( $0.22 \pm 0.06$  individuals per pot) were winged, indicating that the role of potential aphid dispersal during aphid population growth was of little importance.



**Figure 3:** effects on predator abundance. Predator abundance (number of individuals per pot over all six counts) in the experimental pots was (A) higher in the region Alb than in the region Hainich, (B) lower in extensively managed compared with intensively managed grasslands and (C) lower in pots with predator-exclusion treatment. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ; mean  $\pm$  SE is shown.

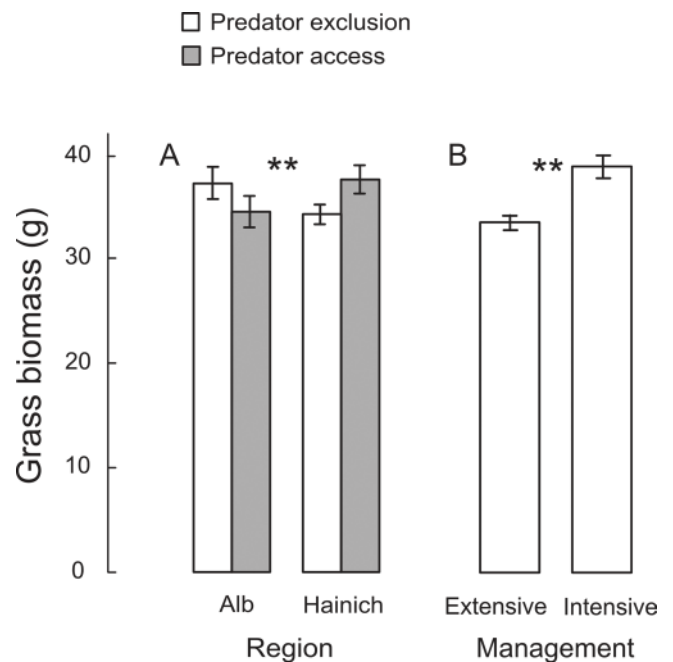
Grass biomass of the experimental pots was not significantly affected by endophyte infection (Table 1). Pots on intensively managed grasslands exhibited a significantly higher plant biomass compared with those on extensively managed grasslands (Table 1 and Fig. 4B). In the Alb, biomass was higher in the closed cages, whereas in the Hainich, biomass was higher in the cages with cut-outs (Table 1 and Fig. 4A). Grass biomass was negatively correlated with aphid abundance ( $r = -0.197$ ,  $P = 0.015$ ).

Analyses including predator abundance ( $F_{1,106} = 1.26$ ,  $P = 0.265$ ) or biomass ( $F_{1,106} = 4.75$ ,  $P = 0.032$ ) as additional cofactors in the full linear mixed-effects models with the response variable aphid abundance did not substantially change the effects of the explanatory variables: region, management intensity, endophyte infection and predator treatment.

## DISCUSSION

In general, our results indicate that endophyte presence in the agronomic grass species *L. perenne* is less important for herbivore suppression than top-down control by predators. Comparing two study regions, we found that endophyte infection with *N. lolii* significantly reduced aphid abundance of *R. padi* in one but not in the other region.

Also previous field studies have shown that endophytes of the genus *Neotyphodium* affect the performance of herbivores inconsistently, raising questions about whether endophyte–grass symbiosis is always a defensive mutualism or parasitism (Rudgers et al. 2010; Saikkonen et al. 2010). In addition to considering the effects of native versus agronomic grass species and the species identity of the interacting partners, it is speculated that the environmental context



might explain the contradicting results in field studies (Faeth and Shochat 2010; Hartley and Gange 2009; Müller et al. 2005). Our study shows evidence that the environmental context of different locations of the study sites can affect the significance and strength of endophyte effects

on herbivores. Studies focussing on plant fitness recorded changes due to an endophyte infection depending on nutrient and water availability (Hesse *et al.* 2003; Malinowski and Belesky 2000; Saona *et al.* 2010), which might have consequences for higher trophic levels. For instance, fertilization of uninfected plants lead to a higher reproduction rate of aphids, explained by better food plant quality, whereas fertilization of infected plants was a disadvantage for aphids probably owing to a higher production of mycotoxins, which require nitrogen (Lehtonen *et al.* 2005; Zhang *et al.* 2009). Our experimental design allowed us to exclude the most frequently studied environmental variable, nutrient availability, from interacting with endophyte infection, because the soil conditions were constant in all of our experimental pots. As we also used interacting species from the same genetic background, the significant endophyte–region interaction must be caused by a large number of unpredictable and uncontrolled environmental conditions, e.g. abiotic conditions like precipitation, temperature and ultraviolet radiation and biotic conditions like species communities of plants, herbivores and predators. Therefore, we conclude that inconsistent results can occur owing to the environmental contexts in the locations of field studies.

Predator abundance was not significantly influenced by the presence of the fungus *N. lolii*. Similarly, the endophyte presence did not modify the effect of predators on aphid abundance or any other variable. Previous field experiments also showed no effects from *N. lolii* on predator abundance (Härri *et al.* 2008b; Jones *et al.* 2011). However, laboratory studies reported significant reduction of fitness and survival rates of aphid predators (de Sassi *et al.* 2006). Also shown in a laboratory experiment, some predators might be able to distinguish between prey from host plants with and without endophyte infection after a learning period and avoid prey from infected plants (Härri *et al.* 2008a). Contradicting other studies hypothesize that volatiles emitted from endophyte-infected plants attacked by herbivores may attract predators of herbivores (McCormick *et al.* 2012; Saikkonen *et al.* 2013). However, in our study, predator abundance on grass plants was independent of endophyte infection and predators neither preferred nor avoided prey from infected plants. In contrast to relatively weak endophyte effects, our results showed strong top-down control of aphids in all grass pots. Such strong impact on herbivore populations has been recorded in natural (Müller and Godfray 1999) and agricultural ecosystems (Schmidt *et al.* 2003). Our result is also in line with another study on the same endophyte–grass association, in which natural enemies are more important and faster acting than endophytes for aphid abundance control (Härri *et al.* 2008b).

Independent of study region and opposite to our expectations, experimental pots on intensively managed grasslands had consistently lower aphid abundance and higher predator abundance compared to those in pots on extensively managed grasslands. We expected better aphid control on extensively

managed grasslands because extensive land use systems have higher flower abundances and plant species richness, which attract aphid predators (Roschewitz *et al.* 2005). However, in our system, extensively managed grasslands were mostly calcareous grasslands with few pasture grasses, tolerable for *R. padi*. Grass species on calcareous grasslands might be less attractive for aphids because they are often dry during the summer months. Intensively managed grasslands with fertilized grass monocultures produce higher grass biomass and contain higher aphid abundances compared to extensive grasslands, allowing also higher predator abundances (Krauss *et al.* 2007). Higher plant biomass, therefore, can enhance herbivore and predator abundance in a bottom-up controlled intensively managed surrounding grassland (Siemann 1998). Weiner *et al.* (2011) have conducted a study on sites in the Alb that overlapped with ours and reported a higher abundance of syrphid flies, one of the main aphid predators, on intensively managed grasslands compared to extensively managed grasslands.

The abundance of herbivores and their functioning can be resource driven by the biomass of the host plants (Price 2002; Pufal and Klein 2013). It is unclear, however, whether our experimental system is mainly top-down or bottom-up controlled. We found no effects of endophyte infection on biomass, which is in contrast to laboratory studies (Hesse *et al.* 2003; Schardl *et al.* 2004). Plant pots with higher biomass showed no increased aphid abundance. In fact, in our study, aphid abundance decreased with increasing grass biomass, which is in contrast to bottom-up control theory (Price 2002). Rather, it implies that high aphid abundance can significantly reduce host plant biomass—a pattern that might occur in our caged pots, because we placed additional aphids at the beginning of the season, but which is less plausible for other experimental designs (e.g. Jones *et al.* 2011) or natural grasslands.

## CONCLUSION

We showed that the impact of the endophytic fungus *N. lolii* on the abundance of aphid *R. padi* is less important than the impact of predators and management intensity. Effects of endophytes on aphid abundance can depend on study region and, therefore, on environmental context, which cannot easily be controlled at the field or regional scale. Conclusions drawn from studies conducted in laboratories, therefore, do not necessarily hold true for field experiments or even for studies conducted in natural ecosystems. Field studies might not be repeatable in other study regions if the impact of an explanatory variable is weak. When the impact of a variable is as strong as in our experiment predator exclusion or management, the results should have a higher probability of holding for many regions. We suggest that abiotic and biotic factors that have been shown to influence the effects of endophytes on herbivores in laboratory experiments must be tested in field studies to ensure their general validity.



## SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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