

Alisha Lemons · Keith Clay · Jennifer A. Rudgers

Connecting plant–microbial interactions above and belowground: a fungal endophyte affects decomposition

Received: 20 January 2005 / Accepted: 12 May 2005 / Published online: 6 July 2005
© Springer-Verlag 2005

Abstract Mutualisms can strongly affect the structure of communities, but their influence on ecosystem processes is not well resolved. Here we show that a plant–microbial mutualism affects the rate of leaf litter decomposition using the widespread interaction between tall fescue grass (*Lolium arundinaceum*) and the fungal endophyte *Neotyphodium coenophialum*. In grasses, fungal endophytes live symbiotically in the aboveground tissues, where the fungi gain protection and nutrients from their host and often protect host plants from biotic and abiotic stress. In a field experiment, decomposition rate depended on a complex interaction between the litter source (collected from endophyte-infected or endophyte-free plots), the decomposition microenvironment (endophyte-infected or endophyte-free plots), and the presence of mesoinvertebrates (manipulated by the mesh size of litter bags). Over all treatments, decomposition was slower for endophyte-infected fescue litter than for endophyte-free litter. When mesoinvertebrates were excluded using fine mesh and litter was placed in a microenvironment with the endophyte, the difference between endophyte-infected and endophyte-free litter was strongest. In the presence of mesoinvertebrates, endophyte-infected litter decomposed faster in microenvironments with the endophyte than in microenvironments lacking the endophyte, suggesting that plots differ in the detritivore assemblage. Indeed, the presence of the endophyte in plots shifted the composition of Collembola, with more Hypogastruridae in the presence of the endophyte and more Isotomidae in endophyte-free plots. In a separate outdoor pot experiment, we did not find strong effects of the litter source or the soil microbial/microinvertebrate community on decomposi-

tion, which may reflect differences between pot and field conditions or other differences in methodology. Our work is among the first to demonstrate an effect of plant–endophyte mutualisms on ecosystem processes under field conditions.

Keywords Collembola · Ecosystem · *Lolium* · Mutualism · *Neotyphodium*

Introduction

Mutualisms and positive interactions between plants and other species can strongly affect the structure of communities (Bever et al. 1997; Bruno et al. 2003; Hartnett and Wilson 1999; Stachowicz 2001). However, whether these interactions influence ecosystem processes is not well resolved (Clay 1994; van der Heijden et al. 1998; Wardle 2002; Rudgers and Clay, in press). In plants, microbial associates feature as some of the most important mutualists (Leuchtman 1992; Smith and Read 1997). For example, a greater species richness of mycorrhizal fungi in the soil increased the diversity of the plant community in an old field in Canada (van der Heijden et al. 1998). Similarly, a bacteria–legume mutualism elevated nitrogen in the soil and enhanced the abundance of non-native species in coastal grasslands in California (Maron and Connors 1996). The presence of a mutualistic fungal endophyte depressed both plant diversity and the biomass of competing plant species in an old field in Indiana (Clay and Holah 1999). These microbially mediated changes in the composition of the plant community may ultimately influence the functioning of ecosystems.

Seed-transmitted fungal endophytes, which can form mutualistic symbioses with grasses, may have an important influence on ecosystem processes. These endophytes grow inside aboveground plant tissues and are asymptomatic for at least part of their life cycle (Clay 1990; Wilson 1995). The endophytes can gain nutrition

Communicated by Alan Knapp

A. Lemons (✉) · K. Clay · J. A. Rudgers
Department of Biology, Indiana University,
Bloomington, IN 47408, USA
E-mail: alemons3@hotmail.com
Tel.: +812-855-1674
Fax: +812-855-6705

and protection from their host plant (Thrower and Lewis 1973). In exchange, these fungi can protect host plants against both biotic and abiotic stresses (Clay 1996; Clay and Schardl 2002; Elmi and West 1995; Hill 1994). These benefits result in part from the production of alkaloids by the fungus, which can render the plant toxic to herbivores and pathogens (reviewed by Clay 1990; Clay and Schardl 2002). Although fungal endophytes have been found in nearly all plants examined (Petrini 1991; Stone et al. 2000), their effects are particularly well understood in association with grasses, where these endophytes occur systemically (rather than locally) in above-ground tissues (Clay 1990). It has been estimated that 20–30% of all grasses form symbioses with systemic endophytes (Leuchtman 1992).

Endophytic fungi may influence ecosystem processes through both direct and indirect pathways. First, senesced leaves from plants with an endophyte may differ in chemical composition (e.g., endophyte alkaloids) from leaves lacking an endophyte (Lyons et al. 1990; Schmidt et al. 1982). This difference may directly affect the rate of decomposition or indirectly affect invertebrate detritivores and microbial decomposers. Similar effects of plant chemistry on decomposition have been documented for other types of anti-herbivore defenses in plants (e.g., Grime et al. 1996; Schadler et al. 2003; Schweitzer et al. 2004; Wardle et al. 2002). Indirect effects are also possible via changes in the plant community associated with endophytes. For example, the presence of an endophyte in a dominant plant host, tall fescue, reduced the diversity of the surrounding plant community (Clay and Holah 1999). Changes in plant diversity and plant composition have been shown to affect ecosystem processes, such as decomposition and soil respiration (Gartner and Cardon 2004; Hector et al. 2000; Knops et al. 2001; Madritch and Hunter 2003). Therefore, the endophyte could reduce the diversity of litter, affecting decomposition, and/or the endophyte could alter the diversity of live plants, affecting the soil detritivore assemblage.

We investigated whether a mutualistic endophyte affects ecosystem processes by examining a widespread symbiosis between the introduced grass, *Lolium arundinaceum* (tall fescue, Poaceae) and the endophyte *Neotyphodium coenophialum* (Clavicipitaceae). *Lolium arundinaceum* is a persistent and invasive exotic grass in the US, occurring across more than 15 million ha of the eastern US alone (Ball et al. 1993; Raloff 2003). *Lolium arundinaceum* continues to be widely planted for turf, forage, and erosion control, has spread into unmanaged habitats as well, and is typically more successful with its endophyte than without (Clay and Schardl 2002). More than 75% of the tall fescue in the US is infected by the endophyte (Ball et al. 1993). Strong effects of the mutualism on decomposition are predicted because of the high levels of nitrogen-rich, anti-fungal and anti-herbivore alkaloids produced by *N. coenophialum* (Bush et al. 1997; Clay and Schardl 2002).

The mechanisms through which invasive species alter native ecosystems (Levine et al. 2003) may depend on

association of the invaders with microbial mutualists. Current research suggests that invasive species can strongly affect ecosystem properties (Mack and D'Antonio 2003; Ogle et al. 2003). For example, invasive exotic species can produce litter that decomposes faster than the litter of co-occurring native species (Ehrenfeld 2003) or alter the environment in which decomposition occurs (Standish et al. 2004). Little is known about the extent to which the effects of invaders depend on their microbial associates. For example, the presence of fungal endophytes in field plots of tall fescue can reduce microbial biomass and soil respiration by more than 80% (Franzluebbers et al. 1999) and can increase soil organic carbon and nitrogen as well as extractable phosphorus compared to plots lacking endophytes (Franzluebbers et al. 1999; Schomberg et al. 2000). Less is known about whether fungal endophytes influence decomposition. In the only other study of which we are aware, the presence of an endophyte (*Neotyphodium* sp.) in the litter of annual ryegrass (*Lolium multiflorum*) significantly reduced the rate of decomposition in indoor and outdoor microcosms when compared with experimentally uninfected plants (Omacini et al. 2004).

Using a combination of field and outdoor pot experiments, we addressed the following three questions: (1) Does the mutualistic endophyte, *N. coenophialum*, alter the rate of decomposition of leaf litter primarily consisting of tall fescue (*L. arundinaceum*)? We used a reciprocal experimental design to test whether effects were due to the presence of the endophyte in the litter or resulted from the unique microenvironment and decomposer assemblage that develops in the presence versus absence of the endophyte in the field. Plots of endophyte-infected or endophyte-free tall fescue were established 3 years prior to our experiment, which allowed for assemblages of both plants and soil invertebrates to develop in association with the endophyte. Thus, our study could encompass potential direct and indirect effects of the microbial mutualist. We then complemented the field experiment with a short-term outdoor pot experiment that aimed to address: (2) to what extent is the soil microbial community responsible for endophyte-mediated differences in decomposition? Finally, we used pitfall traps in the field to survey Collembola, which can increase rates of decomposition (Filsler 2002; Hopkin 1997; Lussenhop 1992; Rusek 1998) and represent one component of a larger detrital food web. We asked, (3) does the endophyte alter the abundance or composition of Collembola?

Materials and methods

Study system

Lolium arundinaceum is a common perennial grass widely planted for pasture and turfgrass and is invasive in some regions of the US (Clay 2001; Hiebert 1990; Raloff 2003). The endophyte (*N. coenophialum*) protects

tall fescue from many consumers by producing alkaloids that render the plant toxic (Breen 1994; Clay 1996). Other benefits of the endophyte include enhanced drought stress tolerance (Elmi and West 1995), increased phosphorous uptake (Malinowski et al. 2000), and greater competitive ability (Clay et al. 1993).

We established field plots at the Indiana University Bayles Road Experimental Field (39°13'9" N, 086°32'29" W). This site is an old agricultural field dominated by *Ambrosia trifida*, *Cerastium vulgatum*, *Cirsium arvense*, *Conyza canadensis*, *L. arundinaceum*, *Poa pratensis*, *Rumex acetosella*, *Solidago* spp., *Sorghum halepense*, and *Trifolium* spp. and previously maintained by mowing. The field was plowed and disked in the fall of 2000 and then seeded with endophyte-infected (E+) or experimentally uninfected (E-) seed at a rate of 45 kg/ha (for details on seed source, germination rates, and infection status see Clay and Holah 1999). Seeds used in the experiment were several generations removed from the original treatment to eliminate the endophyte via long-term seed storage at room temperature. Other plant species were allowed to recruit from the seed bank, vegetative fragments, and nearby vegetation to contribute to the diversity of the plant community in the plots. The experiment consisted of 16 field plots (30 m×30 m) arranged in two rows of eight plots, with endophyte and endophyte-free treatments alternated in a checkerboard design.

Does the endophyte affect the rate of leaf litter decomposition?

We used a time series of litter bag collections to assess litter decomposition. Three pathways through which the endophyte may influence decomposition were considered: (1) the source of the litter (from E+ or E- plots), (2) the invertebrate detritivore assemblage associated with E+ vs. E- fescue, and (3) the microenvironment associated with E+ vs. E- plots. To distinguish among these mechanisms, we used a 2 × 2 × 2 factorial design with a litter source treatment (leaf litter collected from E+ or E- plots), a mesoinvertebrate exclusion treatment [litter bags of large mesh size accessible to micro- and mesoinvertebrates or bags of fine mesh size to exclude macro- and mesoinvertebrates (see also Bradford et al. 2002 and Curry 1969)], and a microenvironment treatment (litter placed into field plots with live E+ or E- tall fescue).

Expectations

If the endophyte altered litter quality in a way that affected decomposition, then we expected a main effect of the litter source. If the presence of live E+ or E- tall fescue in the plots (or associated changes in the community associated with these plants) influenced decomposition, then we expected a main effect of the microenvironment treatment. If mesodetritivores affected decomposition, then we expected a main effect of

the exclusion treatment (i.e., mesh type). Although we could not rule out the possibility that different mesh types changed the microclimate within bags or posed different physical barriers for litter loss in the field (e.g., greater loss is expected with larger mesh size, Bradford et al. 2002), we were primarily interested in whether the effect of litter source or decomposition microenvironment was altered by the mesh type. For example, if differences between E+ and E- litter sources (or decomposition environments) were mainly driven by differences in the associated assemblage of mesodetritivores, then we expected a greater difference in decomposition rate between E+ and E- for large mesh than for small mesh (i.e., significant litter source × exclusion treatment or microenvironment × exclusion treatment interactions).

Litter source

Freshly senesced (standing dead) leaf litter was collected on September 26, 2003, from six randomly chosen 1 m × 1 m subplots in each of the 16 field plots. Litter from the eight plots of each source treatment (E+ or E-) was homogenized, allowed to air dry for 30 days, and fluffed every 2–3 days to facilitate drying. Despite a 25% reduction in the species richness of live plants due to the endophyte (J.A. Rudgers and K. Clay, unpublished data), litter primarily consisted of dead tall fescue leaves: E- litter was 99.2 ± 0.5% tall fescue by weight and E+ litter was 99.0 ± 0.5% tall fescue, with forbs and other grasses present in very small amounts.

Exclusion treatment

Litter bags (10 cm × 10 cm) were constructed from large mesh (1.69 cm² pore size) or small mesh (0.09 mm² pore size) army-grade green mosquito netting. The small mesh size was used to reduce access by mesoinvertebrates, including herbivores, detritivores, and predators (Bradford et al. 2002; Hunter et al. 2003) and including the abundant group of diet and habitat generalists, the Collembola, which range in size from 0.5 mm to 6 mm in length (Hopkin 1997). The bags were sewn along three sides with nylon thread and sealed along the fourth side by doubling over the edge and securing it with three plastic staples from a tag gun (Ningbo MH Industry, Ningbo, Zhejiang, China). Each litter bag was filled with 10 g of air-dried litter.

Decomposition microenvironment

During October 26–31, 2003, 24 bags were placed at randomly chosen locations in each of the 16 plots: six bags of E- small mesh, six E- large mesh, six E+ large mesh, and six E+ small mesh. Flags indicating the location and treatment were placed through the middle of the litter bags, and the bags were secured in two opposite corners by "U"-shaped metal hooks.

Data collection

Bags were collected on three dates: March 11 (135 days), May 12 (197 days), and July 10, 2004 (256 days). On each collection date, eight bags (two bags of each treatment combination) were removed from each of the 16 plots. Upon collection, litter was weighed to determine wet weight, and then thoroughly rinsed through an 18-mm sieve with approximately 2 l of tap water to remove mud and soil. Litter was then dried for a minimum of 3 days at 60°C, and oven-dry weight of the litter was obtained.

Data analysis

Dry weight data were analyzed with repeated measures, mixed model ANOVA (profile analysis, von Ende 2001). The model included the fixed effects of the litter source (E+ or E-), the mesoinvertebrate exclusion treatment (large or small mesh), the microenvironment in which litter was placed (E+ or E-), and the random effect of plot (nested within microenvironment) (Proc MIXED, SAS Institute 2003). All possible interactions were included in the model. Kenward and Roger degrees of freedom calculations were used (ddfm = KR, SAS Institute 2003). Due to the nested design, the variation among plots was used in the denominator of the *F*-test for the effect of microenvironment. Dry weight was log-transformed to meet assumptions of normality (Shapiro-Wilks test) and equality of variances (Levene's test). When interactions in the model were significant, post-hoc Tukey HSD tests were used to determine the significance of differences among the treatment combinations.

To what extent is the soil microbial community responsible for endophyte-mediated differences in decomposition?

We established a pot experiment to determine how the soil microbial community associated with tall fescue may affect leaf litter decomposition. We imposed a litter source treatment (E+ or E- litter) and a soil treatment (sterilized soil, sterilized soil inoculated with live soil collected from E+ plots, or sterilized soil inoculated with live soil from E- plots) in a 2 × 3 factorial design. We also examined initial differences in the nitrogen content of E+ vs. E- litter. Although the pot experiment was only short term (60 days) and used somewhat different methods from the field experiment (bags buried, only small mesh bags used), we include it here for comparison with the field results.

Expectations

If the assemblage of microbes and microinvertebrates associated with the endophyte in the field played an important role in the rate of decomposition, then we expected a significant difference in decomposition be-

tween the E+ and E- soil inoculum treatments. If the presence of the endophyte in litter was important, then we expected a significant effect of litter source. Finally, if microbes from a given soil type interacted differently with E+ vs. E- leaf litter, then we expected a significant litter source × soil treatment interaction.

Litter source

Litter was collected from four randomly chosen locations within each of the E+ or E- field plots on May 14, 2004. Litter was homogenized within a plot, but litter collected from each plot was kept separately. Litter was fluffed every 2–3 days and allowed to air-dry in the lab for 14 days. Litter bags were constructed as described above, but only the small mesh size was used. Each bag was filled with 10 g air-dried litter.

Soil treatment

We used 240 round plastic greenhouse pots (diameter = 19.4 cm, depth = 17.8 cm) filled with soil to 5 cm from the top of the pot. Eighty pots were filled exclusively with sterilized field soil that was collected from a nearby old field in Bloomington, Indiana and autoclaved for 6 h. An additional 80 pots were filled two-third with sterilized field soil and topped with one-third live soil removed from an E+ field plot. Finally, 80 pots were filled two-third with sterilized field soil and topped with one-third live soil from an E- plot. Each pot received inoculum that originated only from a single plot or had sterile soil only. The live soil was removed directly from eight randomly chosen locations within each of the eight E+ and eight E- field plots. Soil was homogenized within a plot, but soil from different plots was kept separately and placed in the pots on the same day. To reduce contamination, every tool used to collect soil was sterilized with antifungal, antiviral, and antibacterial soap (Microcide; National Chemical Laboratories, Philadelphia, PA, USA) and bleach after each exposure to soil.

Treatment combinations

Soil treatments were paired with litter treatments in a reciprocal design. For example, litter collected from plot 1 (E-) was placed in pots with sterilized soil (*n* = 5), pots with E- soil inoculum from plot 1 (*n* = 5), and pots with E+ soil inoculum from plot 2 (*n* = 5); plot 3 (E-) was paired with plot 4 (E+), and so on. This design enhanced the independent replication of both the litter quality and soil inoculum treatments. On May 28, 2004, filled litter bags were added to the pots by burying them by hand ~10 cm below the soil surface. To prevent contamination, a different set of latex gloves were used for each bag that was buried. All 240 pots were randomly assigned locations within a 16 × 15 pot array in the Indiana University Bayles Road Experimental Field.

There were no live plants in the pots, and watering was only from natural rainwater. Litter bags were collected on July 27, 2004 and processed in the same manner as in the field experiment to obtain oven-dry weight.

Data analysis

Dry weight was analyzed with a mixed model ANOVA, including the fixed effects of the litter source and the soil treatment, and the random effect of block (i.e., the pair of plots that served as the source for soil inoculum and litter) (Proc MIXED, SAS Institute 2003). The analysis met assumptions of normality and equality of variances.

Nitrogen content

In addition, we compared the initial nitrogen composition of the E+ vs. E- leaf litter sampled from each plot. Sixteen 10 g samples of litter (one sample per field plot) were oven-dried at 60°C and ground in a 60 mesh Wiley Mill. From each ground sample, a 1-g subsample was removed and sent for analysis by the micro-Kjeldahl digestion procedure using a Lachat Flow Injection Analyzer (J. Dahl, Michigan State University Soil and Plant Nutrient Laboratory). We tested the effect of the endophyte with one-way ANOVA (Proc GLM, SAS Institute 2003). The proportion of nitrogen in the sample was arcsine-square-root transformed to meet assumptions of normality and equality of variances.

Does the endophyte alter the abundance or composition of Collembola?

We assessed whether the endophyte affected the assemblage of Collembola in the *L. arundinaceum* field plots described above. Although Collembola comprise only one part of the litter food web, they are important decomposers that were abundant in the tall fescue system. On June 21, 2004, we established four trap locations in each plot. Each location was flagged at the end of a diagonal transect, 14.1 m from one of the four corners of the 30 m × 30 m plot; the four locations formed a square near the middle of the plot. At each trap location, we placed four pitfall traps ($n = 16$ traps per plot). The traps consisted of 50 ml plastic centrifuge tubes, dug into the ground such that the top of the tube was flush with the soil. The vertical, slippery sides of the plastic tubes ensured that trapped invertebrates did not escape. Using pitfall traps focused sampling efforts on epigeic species (dwelling at the soil surface or on living plants); we did not sample endogeic species (dwelling in the soil) with soil cores because we were most interested in species that would directly interact with litter. The traps were collected on June 24, 2004. Once in the laboratory, tubes were filled with a small amount of 70% ethanol and stored at 4°C. During processing, a 50:50 mixture of sucrose and distilled water was used to float the Collembola to the surface. Specimens were identified

to family following Bland and Jacques (1978), Borrer et al. (1989), and Heyman and Weaver (1997). Morphospecies were classified in part by color following Soto-Adames (2002)

Data analysis

Counts of total Collembola, morphospecies richness, and numbers of individuals in the four most common families (Entomobryidae, Hypogastruridae, Isotomidae, and Sminthuridae) were analyzed with mixed model ANOVA (Proc MIXED, SAS Institute 2003), including the fixed effect of the endophyte treatment, the row, and endophyte × row, and the random effect of plot (nested in endophyte × row). Row was included because Collembola have been shown to be strongly affected by locations within fields in prior work (Salamon et al. 2004). All data were square-root transformed (Sokal and Rohlf 1995) to meet assumptions of normality and equality of variances.

Results

Does the endophyte affect the rate of leaf litter decomposition?

Overall, leaf litter with the endophyte decomposed significantly more slowly than litter lacking the endophyte (Fig. 1; Litter source, Table 1). The endophyte had stronger effects later in the decomposition process, with no difference early on (135 days), 4.4% greater mass than E- litter in May (197 days), and 7.9% greater mass in July (256 days) (Fig. 1).

However, the effect of the endophyte in litter varied with both the microenvironment and the mesoinvertebrate exclusion treatment (i.e., a significant litter

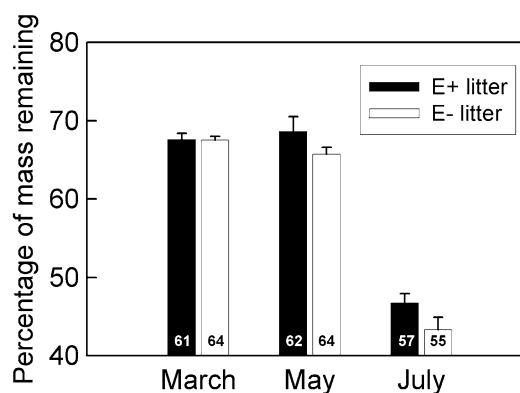


Fig. 1 Effect of the endophyte (*Neotyphodium coenophialum*) treatment on the percentage remaining dry mass of litter from field plots of *Lolium arundinaceum* over three census dates. E+ = litter with the endophyte, E- = litter lacking the endophyte. Bars represent means with S.E. Sample sizes are given on each bar; unequal sample sizes resulted from some bags being lost or damaged in the field

Table 1 Analysis of variance examining the effects of three treatments—(1) litter source (litter collected from plots with or without the endophyte *Neotyphodium coenophialum*), (2) the decomposition microenvironment, and (3) the mesoinvertebrate exclusion treatment (small versus large mesh litter bag) on the dry weight of litter collected from *Lolium arundinaceum* plots across three census dates

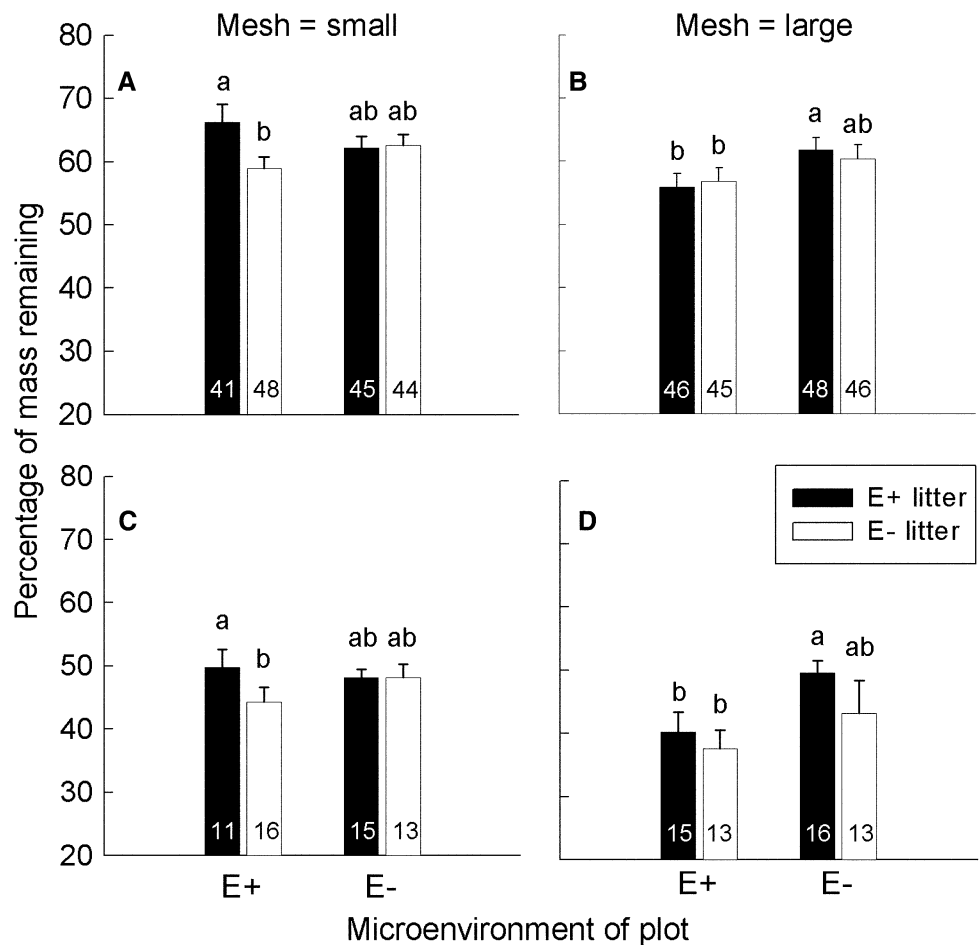
Effect	df	F	p
Litter source	1,325	4.04	0.045
Microenvironment	1,14	4.01	0.065
Litter source × microenvironment	1,325	0.01	0.907
Mesoinvertebrate exclusion	1,192	18.73	< 0.001
Litter source × exclusion	1,230	0.20	0.653
Microenvironment × exclusion	1,192	5.82	0.017
Litter source × microenvironment × exclusion	1,230	4.12	0.044
Census date	2,102	193.16	< 0.001
Census × litter source	2,312	1.65	0.194
Census × microenvironment	2,102	2.14	0.123
Census × litter source × microenvironment	2,312	0.50	0.607
Census × exclusion	2,143	2.59	0.078
Census × litter source × exclusion	2,269	0.41	0.663
Census × microenvironment × exclusion	2,143	1.52	0.222
Census × litter source × microenvironment × exclusion	2,269	0.74	0.480
Planned contrast small mesh: E+ vs. E- microenvironment	1,30	0.08	0.775
Planned contrast large mesh: E+ vs. E- microenvironment	1,30	9.23	0.005

p-values < 0.05 are presented in boldface

source × microenvironment × exclusion interaction, Table 1). The effect of the litter source was strongest in bags of small mesh placed in a microenvironment with the endophyte (Fig. 2a, c), where litter with the endophyte decomposed on average 12% more slowly than litter lacking the endophyte. Litter source differences

were not observed in the large mesh bags that permitted access by mesoinvertebrates, suggesting that mesoinvertebrates may homogenize differences between litter with or without the endophyte (Fig. 2b, d). Furthermore, in large mesh bags, litter with the endophyte decomposed on average 9.5% faster in plots with the

Fig. 2 Effect of the decomposition microenvironment [field plot with (E+) or without (E-) the endophyte *Neotyphodium coenophialum*], the litter source (E+ = litter with the endophyte, E- = litter lacking the endophyte) and the type of mesh litter bag (a, c) small = 0.09 mm² mesh (b, d) large = 1.69 cm² mesh on the percentage remaining dry weight of litter. Graphs a, b show data averaged over the three census dates, and c, d show data from the final census only. Bars represent means with S.E. Sample sizes are given on each bar; unequal sample sizes resulted from some bags being lost or damaged in the field. Different letters indicate significant differences among means within a graph as demonstrated by a Tukey HSD test



endophyte than in plots without the endophyte (Fig. 2b, litter source \times microenvironment \times exclusion, Table 1). In large mesh bags, endophyte-free litter also decomposed faster in plots with the endophyte than in plots lacking the endophyte, although this difference was not significant according to a post-hoc Tukey HSD test ($p=0.11$, Fig. 2b, d). The strong interaction between microenvironment and mesoinvertebrate exclusion suggests that mesoinvertebrates were responsible for the difference in decomposition between endophyte-infected versus endophyte-free microenvironments; differences between microenvironments were not observed in bags of small mesh size (Fig. 2a, c; microenvironment \times exclusion, Table 1).

To what extent is the soil microbial community responsible for endophyte-mediated differences in decomposition?

The soil microbial/microinvertebrate community associated with endophyte versus endophyte-free plots did not affect the rate of decomposition in pots, at least during the short term (60 days) (Fig. 3). Surprisingly, litter decomposed significantly more quickly (12%) in the sterilized soil treatment than in soil inoculated with live soil from either endophyte-infected or endophyte-free field plots (Fig. 3; soil treatment, Table 2).

The presence of the endophyte in litter did not affect the rate of decomposition in the pot experiment (litter source, Table 2). This result contrasts with the finding from the field experiment, where the litter with the endophyte decomposed more slowly than endophyte-free litter. The presence of the endophyte in litter also had no effect on initial total nitrogen content ($F_{1,14}=0.73$, $p=0.41$; mean (S.E.): E+ = 1.04% (0.04), E- = 1.12% (0.08)).

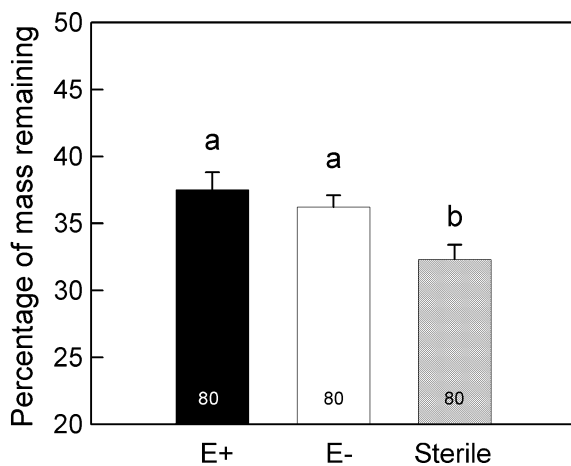


Fig. 3 Pot experiment: effect of the soil inoculation treatment [live soil from plots with the endophyte (E+), live soil from plots without the endophyte (E-) or sterilized soil from a nearby old field (sterile)] on the percentage remaining dry mass of litter collected from field plots of *Lolium arundinaceum*. Bars represent means with S.E.; sample sizes are given on each bar

Does the endophyte alter the abundance or composition of Collembola?

Collembola comprise one component among many in the decomposer food web of old field communities. The total abundance of Collembola did not significantly differ between plots with versus without the endophyte (Fig. 4, Table 3). However, the composition of collembolan families significantly differed between endophyte-infected and endophyte-free plots (Fig. 4, Table 3). Specifically, the abundance of Hypogastruridae was significantly greater in endophyte-infected plots than in endophyte-free plots (Endophyte, Table 3). In contrast, the abundance of Isotomidae was greater in endophyte-free plots than in endophyte-infected plots (Endophyte, Table 3). We found no significant effect of the endophyte on morphospecies richness of Collembola (Endophyte, Table 3, mean (S.E.): E+ = 3.80 (0.42), E- = 3.14 (0.43), $n=8$ plots).

Discussion

Most importantly, the presence of the endophyte in leaf litter slowed decomposition by nearly 8% across all other treatments, demonstrating that the effect of introduced tall fescue on the ecosystem can depend on the presence of a microbial mutualist. A similar effect was found due to a *Neotyphodium* endophyte in *L. multiflorum* in Argentina (Omacini et al. 2004), where litter decomposition was 17% slower with the endophyte. This earlier study used microcosms placed outdoors. Thus, to our knowledge, our work is among the first to show that a fungal endophyte can affect decomposition processes under field conditions.

The reduced rate of decomposition may result from a direct or indirect effect of the endophyte. Direct effects may arise if endophyte alkaloids are toxic to decomposers and detritivores in the same way they are toxic to herbivores and pathogens (Breen 1994; Clay 1996; Clay et al. 1989; Gwinn and Gavin 1992). However, the effects of the endophyte in litter were weakened by mesoinvertebrates, because effects were strongest in the small mesh bags that excluded mesoinvertebrates but

Table 2 Analysis of variance examining the effects of (1) litter source (litter collected from plots with or without the endophyte *Neotyphodium coenophialum*), (2) the soil inoculation treatment (live soil from plots with the endophyte, live soil from plots without the endophyte, or sterilized soil from a nearby old field), and (3) the block on the dry weight of litter in pots

Effect	df	MS	F	p
Litter source	1	0.24	0.06	0.612
Soil treatment	2	5.80	6.22	0.002
Litter source	2	0.06	0.07	0.934
\times soil treatment				
Block	1	25.29	27.12	< 0.001
Error	233	0.93		

p -values < 0.05 are presented in boldface

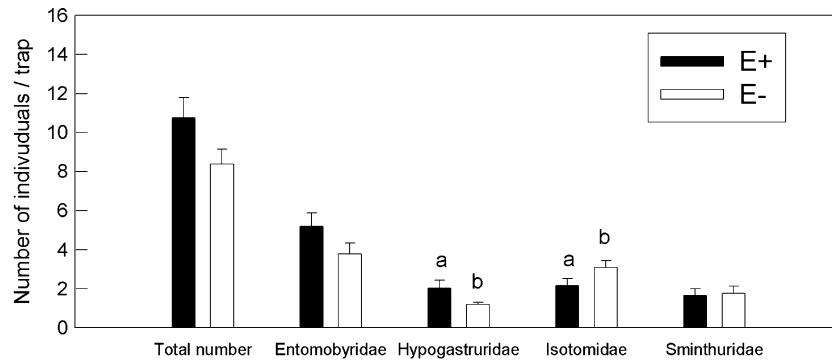


Fig. 4 Effect of the presence of the endophyte (*Neotyphodium coenophialum*) in field plots of *Lolium arundinaceum* on the number of Collembola per pitfall trap, including total number, and the number of the four most commonly observed families (Entomobryidae, Hypogastruridae, Isotomidae, and Sminthuridae). Within

a family, different letters indicate a significant difference between endophyte-infected (E+) vs. endophyte-free (E-) plots (see Table 3). Bars represent means with S.E. Sample size was eight plots per endophyte treatment

allowed microinvertebrates. These results suggest that microbial decomposers and microinvertebrates may be more important than larger invertebrates in driving differences in the rate of decomposition between litter sources. Results are consistent with a previous study that found reduced microbial biomass (>80% lower) in field plots of endophyte-infected versus endophyte-free tall fescue (Franzeluebbbers et al. 1999). In addition, the effects of microbial decomposers may accumulate over time, because litter source did not affect mass loss in the 60 day pot experiment, and source differences grew stronger in the field over 256 days.

A slowing of decomposition may also result as an indirect effect of reduced plant diversity in plots with the endophyte. Effects of litter diversity on the rate of decomposition have been documented in other studies (reviewed by Gartner and Cardon 2004), suggesting a strong potential for indirect effects of the endophyte through changes in litter diversity. For example, increasing the species richness of a plant community resulted in up to 37% more mass loss than predicted from single species treatments (Hector et al. 2000), and a recent review showed that mass loss can be up to 65% greater in mixtures of litter than predicted from single species litters (Gartner and Cardon 2004). Although we

did not record the species richness of the litter, in the same experimental plots, the species richness of living plants was reduced by 25% due to the presence of the endophyte, with no change in total aboveground biomass (J.A. Rudgers and K. Clay, unpublished data; see also Clay and Holah 1999 for a different experiment with a similar result). However, in our study litter was composed of ~99% tall fescue leaves, suggesting that an endophyte-mediated shift in the species composition of litter was unlikely to be driving differences in decomposition. This does not rule out the possibility that changes in the composition of living plants could have strong effects on the soil invertebrate community in ways that alter decomposition.

The presence of the endophyte in leaf litter affected decomposition in the field experiment, but not in the pot experiment. There are several possible explanations for this result. First, in the field we found stronger effects of the presence of the endophyte later in the decomposition process, and the pot experiment was run for only 60 days. We suspect this explanation is unlikely because similar amounts of decomposition occurred in both experiments, regardless of the duration. Second, due to logistical constraints, the experiments were run at different times of the year, which could indicate seasonal

Table 3 Analysis of variance examining the effects of the presence of the endophyte in field plots (endophyte), the block (row), and the plot (nested in the endophyte treatment and row) on the abundance and morphospecies richness of Collembola, including the total

number of Collembola, the morphospecies richness, and the abundance of the four most commonly observed families (Entomobryidae, Hypogastruridae, Isotomidae, and Sminthuridae)

Effect	df	Total number		Morphospecies richness		Number of Entomobryidae		Number of Hypogastruridae		Number of Isotomidae		Number of Sminthuridae	
		F	p	F	p	F	p	F	p	F	p	F	p
Endophyte	1,12	1.34	0.269	1.43	0.255	2.18	0.166	5.85	0.032	5.47	0.037	1.26	0.283
Row	1,12	8.35	0.014	24.48	<0.001	3.41	0.090	9.83	0.009	17.76	0.001	5.14	0.043
Endophyte × row	1,12	0.00	0.959	0.34	0.573	0.10	0.759	3.46	0.088	0.02	0.903	0.57	0.465
Plot (endophyte × row)	12,239	2.33	0.008	0.68	0.774	2.78	0.002	1.61	0.090	1.33	0.201	0.81	0.642

p-values < 0.05 are presented in boldface

variation in effects of the endophyte. Third, the absence of vegetation cover in the pots as well as the burial of bags may have influenced the microbial decomposer and microinvertebrate assemblage differently than in the field. Finally, important decomposers, the mesoinvertebrates, were absent from the pot experiment, although we did not find that mesoinvertebrates caused differences in decomposition of endophyte-infected versus endophyte-free litter in the field.

In addition to differences in litter sources, the decomposition microenvironment in the field (endophyte-infected versus endophyte-free plots) altered the rate of decomposition. Endophyte-infected litter decomposed up to 10% faster when placed in plots with the endophyte than when placed in endophyte-free plots. Importantly, the decomposition microenvironment only affected litter in bags of large mesh size that permitted access by mesoinvertebrates. This result, combined with a lack of a difference in decomposition for endophyte-infected versus endophyte-free soil inoculum from the pot experiment, suggests that mesoinvertebrates may be primarily responsible for the effects of the microenvironment on the rate of decomposition. Why was decomposition faster in endophyte-infected versus endophyte-free plots? One possibility is that plots with the endophyte cultivate an assemblage of mesoinvertebrate detritivores that are particularly effective at decomposing *L. arundinaceum* litter due to the enhanced biomass of tall fescue when it hosts the endophyte (e.g., Clay and Holah 1999). Similar effects are known from other systems, where plants promote assemblages of microinvertebrates that increase the rate of decomposition of their litter (Hansen 1999; Olofsson and Oksanen 2002).

Changes in the composition of Collembola support the hypothesis that endophyte and endophyte-free plots may cultivate different detritivore assemblages, although a broader examination of the decomposer food web beyond just a single census of Collembola is needed. Collembola are known to play an important role in decomposition by redistributing plant material, directly consuming litter, and stimulating microbial activity (Lussenhop 1992; Hopkin 1997; Rusek 1998; Filser 2002). Furthermore, changes in Collembola composition can alter ecosystem processes, such as nitrogen turnover (Mebeles and Filser 1998). In our experiment, the composition, but not total abundance, of Collembola depended on the presence of the endophyte in field plots. Hypogastruridae were more abundant in plots with the endophyte than in plots lacking the endophyte. The opposite was found for the family Isotomidae, with a greater abundance in plots without the endophyte. These results are consistent with other studies that demonstrate Hypogastruridae tolerate toxic environments (e.g., waste dumps and heavy metal sites) better than other families of Collembola (reviewed by Hopkin 1997). In addition, effects on Hypogastruridae may result indirectly from experimental increases in the functional group richness of living plants in the plots, as has been found in Switzerland (Salamon et al. 2004). In contrast, the increase in Isotomidae in the absence of the

endophyte may reflect a sensitivity of this family to toxins, such as alkaloids. For example, the species *Folsomia candida* (Isotomidae) is among the most sensitive species of Collembola to toxins and is commonly used in ecotoxicology studies (Fountain and Hopkin 2005). Clearly, the exact mechanisms underlying the shift in the relative abundance of collembolan families (e.g., competitive interactions, relative tolerance thresholds, plant diversity, etc.) remain unresolved.

This research expands current knowledge on how plant–microbial mutualisms affect ecosystem processes. Here we show that the presence of a mutualistic endophyte affects the rate of leaf litter decomposition and the composition of associated decomposers. Our results on decomposition further demonstrate a role for endophyte–plant mutualisms in ecosystem processes under field conditions.

Acknowledgements Many thanks to Joseph Reznik for assistance identifying Collembola, Jessica Stuaan for sorting Collembola, Jon Dahl for tissue analysis, Keenan Mack and Brette Thompson for help in the field, and two anonymous reviewers for helpful comments on the manuscript. This research was funded by a Howard Hughes Medical Institute Undergraduate Research Capstone Award to A.L., by NSF DBI-0200485 to J.A.R., and by NSF DEB-9727116 to K.C. All experiments complied with the current laws of the USA.

References

- Ball DM, Pedersen JF, Lacefield GD (1993) The tall fescue endophyte. *Am Sci* 81:370–379
- Bever JD, Westover KM, Antonovics J (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *J Ecol* 85:561–573
- Bland RG, Jacques HE (1978) How to know the insects. Wm. C. Brown Company, Dubuque, Iowa
- Borror DJ, Triplehorn CA, Johnson NF (1989) An introduction to the study of insects. Harcourt Brace & Company, Orlando, FL
- Bradford MA, Tordoff GM, Eggers T, Jones TH, Newington JE (2002) Microbiota, fauna, and mesh size interactions in litter decomposition. *Oikos* 99:317–323
- Breen JP (1994) *Acremonium*/endophyte interactions with enhanced plant resistance to insects. *Annu Rev Entomol* 39:402–423
- Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. *Trends Ecol Evolut* 18:119–125
- Bush LP, Wilkinson HH, Schardl CL (1997) Bioprotective alkaloids of grass–fungal endophyte symbioses. *Plant Physiol* 114:1–7
- Clay K (1990) Fungal endophytes of grasses. *Annu Rev Ecol Systemat* 21:275–297
- Clay K (1994) The potential role of endophytes in ecosystems. In: Bacon CW, White JF (eds) *Biotechnology of endophytic fungi of grasses*. CRC Press, Boca Raton, FL, pp 73–86
- Clay K (1996) Interactions among fungal endophytes, grasses and herbivores. *Res Populat Ecol* 38:191–201
- Clay K (2001) Symbiosis and the regulation of communities. *Am Zool* 41:810–824
- Clay K, Holah J (1999) Fungal endophyte symbiosis and plant diversity in successional fields. *Science* 285:1742–1744
- Clay K, Schardl C (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *Am Natural* 160:S99–S127
- Clay K, Cheplick GP, Marks S (1989) Impact of the fungus *Balsania henningsiana* on *Panicum agrostoides*: frequency of infection, plant growth and reproduction, and resistance to pests. *Oecologia* (Berlin) 80:374–380

- Clay K, Marks S, Cheplick GP (1993) Effects of insect herbivory and fungal endophyte infection on competitive interactions among grasses. *Ecology* 74:1767–1777
- Curry JP (1969) The decomposition of organic matter in soil. Part 1: the role of the fauna in decaying grassland herbage. *Soil Biol Biochem* 1:253–258
- Ehrenfeld JG (2003) Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6:503–523
- Elmi AA, West CP (1995) Endophyte infection effects on stomatal conductance, osmotic adjustment and drought recovery of tall fescue. *New Phytol* 131:61–67
- Filser J (2002) The role of Collembola in carbon and nitrogen cycling in soil. *Pedobiologia* 46:234–245
- Fountain MT, Hopkin SP (2005) *Folsomia candida* (Collembola): a “standard” soil arthropod. *Annu Rev Entomol* 50:201–222
- Franzluebbers AJ, Nazih N, Stuedemann JA, Fuhrmann JJ, Schomberg HH, Hartel PG (1999) Soil carbon and nitrogen pools under low- and high-endophyte-infected tall fescue. *Soil Sci Soc Am J* 63:1687–1694
- Gartner TB, Cardon ZG (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos* 104:230–246
- Grime JP, Cornelissen JHC, Thompson K, Hodgson JG (1996) Evidence of a causal connection between anti-herbivore defence and the decomposition rate of leaves. *Oikos* 77:489–494
- Gwinn KD, Gavin AM (1992) Relationship between endophyte infection level of tall fescue seed lots and *Rhizoctonia zeae* seedling disease. *Plant Disease* 76:911–914
- Hansen RA (1999) Red oak litter promotes a microarthropod functional group that accelerates its decomposition. *Plant Soil* 209:37–45
- Hartnett DC, Wilson GWT (1999) Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology* 80:1187–1195
- Hector A, Beale AJ, Minns A, Otway SJ, Lawton JH (2000) Consequences of the reduction of plant diversity for litter decomposition: effects through litter quality and microenvironment. *Oikos* 90:357–371
- van der Heijden MGA et al (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396:69–72
- Heyman S, Weaver J (1997) The Collembola. MOFEP Arthropods Key. <http://www.missouri.edu/~bioscish/coll.html>. In, vol. 2004
- Hiebert RD (1990) An ecological restoration model: application to razed residential sites. *Nat Areas J* 10:181–186
- Hill NS (1994) Ecological relationships of Balansiae-infected graminoids. In: Bacon CW, White JF (eds) *Biotechnology of endophytic fungi of grasses*. CRC Press, Boca Raton, pp 59–71
- Hopkin SP (1997) *Biology of the Springtails (Insecta: Collembola)*. Oxford University Press, Oxford, UK
- Hunter MD, Adl S, Pringle CM, Coleman DC (2003) Relative effects of macro invertebrates and habitat on the chemistry of litter during decomposition. *Pedobiologia* 47:101–115
- Knops JMH, Wedin D, Tilman D (2001) Biodiversity and decomposition in experimental grassland ecosystems. *Oecologia* 126:429–433
- Leuchtman A (1992) Systematics, distribution, and host specificity of grass endophytes. *Nat Toxins* 1:150–162
- Levine JM, Vila M, D’Antonio CM, Dukes JS, Grigulis K, Lavelle S (2003) Mechanisms underlying the impacts of exotic plant invasions. *Proc Roy Soc Lond Ser B-Biol Sci* 270:775–781
- Lussenhop J (1992) Mechanisms of microarthropod–microbial interactions in soil. *Adv Ecol Res* 23:1–33
- Lyons PC, Evans JJ, Bacon CW (1990) Effects of the fungal endophyte *Acremonium coenophialum* on nitrogen accumulation and metabolism in tall fescue. *Plant Physiol (Rockville)* 92:726–732
- Mack MC, D’Antonio CM (2003) The effects of exotic grasses on litter decomposition in a Hawaiian woodland: the importance of indirect effects. *Ecosystems* 6:723–738
- Madritch MD, Hunter MD (2003) Intraspecific litter diversity and nitrogen deposition affect nutrient dynamics and soil respiration. *Oecologia* 136:124–128
- Malinowski DP, Alloush GA, Belesky DP (2000) Leaf endophyte *Neotyphodium coenophialum* modifies mineral uptake in tall fescue. *Plant Soil* 227:115–126
- Maron JL, Connors PG (1996) A native nitrogen-fixing shrub facilitates weed invasion. *Oecologia (Berlin)* 105:302–312
- Mebes KH, Filser J (1998) Does the species composition of Collembola affect nitrogen turnover? *Applied Soil Ecology* 9:241–247
- Ogle SM, Reiners WA, Gerow KG (2003) Impacts of exotic annual brome grasses (*Bromus* spp.) on ecosystem properties of northern mixed grass prairie. *Am Midland Natural* 149:46–58
- Olofsson J, Oksanen L (2002) Role of litter decomposition for the increased primary production in areas heavily grazed by reindeer: a litterbag experiment. *Oikos* 96:507–515
- Omacini M, Chaneton EJ, Ghersa CM, Otero P (2004) Do foliar endophytes affect grass litter decomposition? A microcosm approach using *Lolium multiflorum*. *Oikos* 104:581–590
- Petrini O (1991) Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS (eds) *Microbial ecology of leaves*. Springer-Verlag, New York, New York, pp 179–197
- Raloff J (2003) Cultivating weeds: is your yard a menace to parks and wild lands? *Sci News* 163:232
- Rusek J (1998) Biodiversity of Collembola and their functional role in the ecosystem. *Biodiversity Conserv* 7:1207–1219
- Salamon JA, Schaefer M, Alpei J, Schmid B, Scheu S (2004) Effects of plant diversity on Collembola in an experimental grassland ecosystem. *Oikos* 106:51–60
- SAS Institute, Inc. (2003) SAS version 9.1. The SAS Institute, Cary, North Carolina, USA
- Schadler M, Jung G, Auge H, Brandl R (2003) Palatability, decomposition and insect herbivory: patterns in a successional old-field plant community. *Oikos* 103:121–132
- Schmidt SP et al (1982) Association of an endophytic fungus with fescue toxicity in steers fed Kentucky-31 tall fescue seed or hay. *J Anim Sci* 55:1259–1263
- Schomberg HH, Stuedemann JA, Franzluebbers AJ, Wilkinson SR (2000) Spatial distribution of extractable phosphorus, potassium, and magnesium as influenced by fertilizer and tall fescue endophyte status. *Agron J* 92:981–986
- Schweitzer JA et al (2004) Genetically based trait in a dominant tree affects ecosystem processes. *Ecol Lett* 7:127–134
- Smith SE, Read DJ (1997) *Mycorrhizal symbiosis*, 2nd edn. Academic Press, San Diego
- Sokal RR, Rohlf FJ (1995) *Biometry: the principles and practice of statistics in biological research*, 3rd edn. W.H. Freeman, New York
- Soto-Adames FN (2002) Molecular phylogeny of the Puerto Rican *Lepidocyrtus* and *Pseudosinella* (Hexapoda: Collembola), a validation of Yoshii’s “color pattern species”. *Mol Phylogenet Evolut* 25:27–42
- Stachowicz JJ (2001) Mutualism, facilitation, and the structure of ecological communities. *Bioscience* 51:235–246
- Standish RJ, Williams PA, Robertson AW, Scott NA, Hedderley DI (2004) Invasion by a perennial herb increases decomposition rate and alters nutrient availability in warm temperate lowland forest remnants. *Biol Invasions* 6:71–81
- Stone JK, Bacon CW, White JF (2000) An overview of endophytic microbes: endophytism defined. In: Bacon CW, White JF (eds) *Microbial endophytes*. Marcel Dekker, Inc., New York, New York, pp 3–30
- Thrower LB, Lewis DH (1973) Uptake of sugars by *Epichloe typhina* (Pers. Ex Fr.) Tul. in culture and from its host, *Agrostis stolonifera* L. *New Phytol* 72:501–508
- Wardle DA (2002) *Communities and ecosystems: linking the above-ground and below-ground components*. Princeton University Press
- Wardle DA, Bonner KI, Barker GM (2002) Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. *Funct Ecol* 16:585–595
- Wilson D (1995) Fungal endophytes which invade insect galls: insect pathogens, benign saprophytes, or fungal inquilines? *Oecologia (Berlin)* 103:255–260