Oikos 126: 801–811, 2017
doi: 10.1111/oik.03648

Subject Editor: Matty Berg. Editor-in-Chief: Gerlinde De Deyn. Accepted 26 September 2016

Plant–fungal symbiosis affects litter decomposition during primary succession

Lukas Bell-Dereske, Xiaodong Gao, Caroline A. Masiello, Robert L. Sinsabaugh, Sarah M. Emery and Jennifer A. Rudgers

L. Bell-Dereske (http://orcid.org/0000-0001-9951-2222) (ldereske@unm.edu), R. L. Sinsabaugh and J. A. Rudgers, Dept of Biology, MSC03-2020, Univ. of New Mexico, Albuquerque, NM 87131-0001, USA. – X. Gao and C. A. Masiello, Dept of Earth Science, Rice Univ., Houston, TX 77005, USA. – S. M. Emery, Dept of Biology, Univ. of Louisville, Louisville, KY 40292, USA.

Microbial symbionts of plants can affect decomposition by altering the quality or quantity of host plant tissue (substrate) or the micro-environment where decomposition occurs (conditioning). In C_3 grasses, foliar fungal endophytes (Clavicipitaceae) can increase plant resistance to drought and/or produce alkaloids that reduce herbivory – effects that may also influence host litter composition and subsequent litter decomposition. We studied the effect of the endophyte *Epichloë* sp. on litter decomposition in the Great Lakes dunes (USA) using a reciprocal design altering endophyte presence/absence in both American beachgrass *Ammophila breviligulata* substrate (litter bags) and its conditioning of the decomposition microenvironment. Symbiont treatments were crossed with rain-out shelters that altered growing season precipitation. The first year of decomposition, senesced leaf substrate from *A. breviligulata* with *Epichloë* decomposed 21% faster than endophyte-free substrate. By the third year, conditioning by live symbiotic plants reduced cumulative decomposition by 33% compared to plots planted with endophyte-free plants. Of the traits we examined – litter quantity, C:N ratio, mineral composition, fungal colonization, and carbon chemistry – increased litter quantity via greater tiller production was the primary trait shift associated with endophyte symbiosis. *Epichloë* in *A. breviligulata* litter also altered litter nitrogen decomposition dynamics, as evidenced by lower nitrogen and protein content in decomposed tissue from plants that hosted the endophyte. Differences in initial litter quality and subsequent colonization by saprotrophic fungi were ruled out as key drivers. Altered precipitation had negligible effects on decomposing processes in the dunes. Grass–*Epichloë* symbiosis altered nutrient cycling through increasing the rate of litter decomposition when present in the litter and through reducing litter decomposition by conditioning the decomposition microenvironment. *Epichloë* are widespread symbionts of grasses. Thus, their effects on decomposition could be an important, but often overlooked, driver of nutrient cycling in grass-dominated ecosystems.

Interactions between above- and below-ground communities can be major drivers of the rate of decomposition in terrestrial ecosystems (Wardle et al. 2004, Kardol and Wardle 2010). However, most focus on above-ground interactors has been on herbivores (Wardle et al. 2012), with much less attention to the potential roles of above-ground microbes that live in plants. Separately, both above- and below-ground microbes can influence decomposition (Osono 2006, Purahong and Hyde 2011, Omacini et al. 2012, Nuccio et al. 2013, Yuan and Chen 2014). For example, fungal and bacterial endophytes of leaves can affect decomposition by altering the quantity and composition of host litter (Raghavendra and Newcombe 2013, Rogers et al. 2012, Saikkonen et al. 2015). A predictive framework for understanding the influence of aboveground microbes on litter decomposition requires studies that test for such above–below-ground interactive effects over a broad range of species and ecosystems.

One group of aboveground microbial symbionts, *Epichloë* spp. (Clavicipitaceae, Ascomycota) (Schardl 2010) have been shown to have strong effects on decomposition of host litter, but only has been tested in two non-native host–endophyte systems (Omacini et al. 2012). These fungal endophytes are obligate symbionts of grasses that cannot survive in senesced plant tissue. *Epichloë* can provide a range of benefits to plants, including herbivore deterrence (Crawford et al. 2010), drought tolerance (Oberhofer et al. 2014), and resistance to pathogens (Wäli et al. 2006), in exchange for carbon and shelter within host tissue (Thrower and Lewis 1973, Clay 1990). These benefits, particularly, the production of fungal alkaloids and increases in plant biomass, have potential for cascading effects on decomposition processes. Three prior studies have examined how epichloid endophytes affect decomposition. In all cases, endophyte presence slowed host litter decomposition (reviewed by Omacini et al. 2012). However, the host plants and endophytes in these

studies: *Lolium arundinaceum-Epichloë coenophiala* (Lemons et al. 2005, Siegrist et al. 2010) and *Lolium multiflorum-Epichloë occultans* (Omacini et al. 2004) were non-native to the study ecosytem and occurred in rich agricultural/grassland soils. Thus, prior work may not be broadly representative of *Epichloë* effects in diverse ecosystems, and importantly the effects of native *Epichloë* symbiosis on decomposition remains unknown.

Further work in a greater diversity of systems is also needed in order to uncover the mechanisms through which above-ground endophytes affect decomposition (Fig. 1). Within the two systems tested to date, the authors hypothesized that fungal-produced alkaloids, which can persist in the litter, reduced the rate of host litter decomposition by suppressing microbial activity (Omacini et al. 2004, Lemons et al. 2005, Casas et al. 2011). However, Siegrist et al. (2010) showed that fungal alkaloids were mostly lost from host litter 60 d after leaf senescence, suggesting limited long-term effects of this mechanism. On the other hand, alkaloids could shift the initial saprotrophic community, which may affect long-term decomposition rates (Kivlin and Treseder 2015). Alternative mechanisms have also been proposed. For example, *Epichloë* increase host biomass (Omacini et al. 2006), potentially altering litter production and the microenvironment for decomposition (Omacini et al. 2004). *Epichloë* can increase root exudate production (Omacini et al. 2012), which could also shift the composition of rhizosphere microbial assemblages (Jenkins et al. 2006) (Fig. 1). For example, in *L. arundinaceum* pastures, soil microbial biomass and respiration were 14% lower in fields with *Epichloë* symbiosis than in endophyte-free fields (Franzluebbers et al. 1999). *Epichloë* can also inhibit AMF root colonization on host roots (Chu-Chou et al. 1992) and on the roots of competing plant species (Antunes et al. 2008).

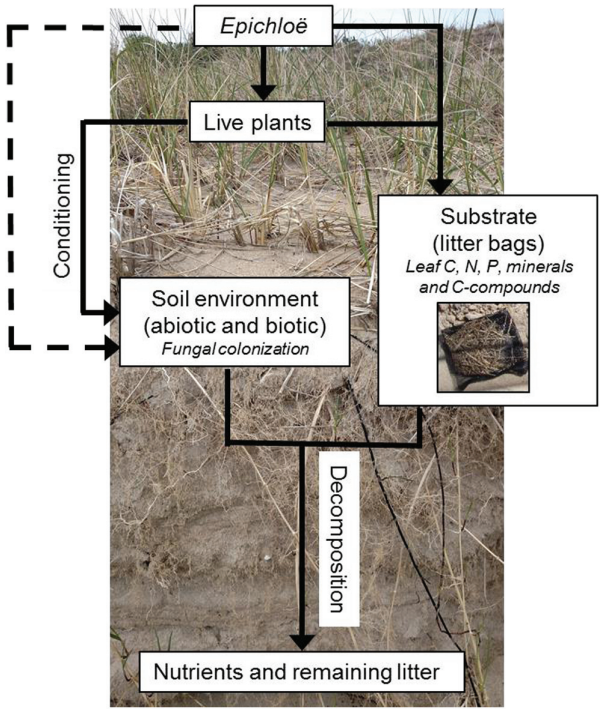

Figure 1. Possible pathways for the effects of *Epichloë* on decomposition of host litter. The fungal endophyte may alter decomposition directly (solid lines) through the amount of litter production (substrate quantity) or the composition of the litter (substrate quality). The endophyte may also alter decomposition indirectly (dashed lines) by shifting the microenvironment in which decomposition occurs (conditioning). At the bottom of each box is the list of mechanisms that we examined for each pathway (in italics).

Additionally, climate may shift the effects of plant symbionts on decomposition (Cheng et al. 2012). This shift in the effects of plant symbionts represents a context dependency in the mutualism which may require altering the abiotic environment to fully understand the effects of the symbiont on the quality and quantity of host litter. However, to our knowledge, no studies have yet examined whether *Epichloë* may alter decomposition under altered climates. Recent research has suggested that altered precipitation may play a larger role than increased temperatures in driving the long term rates of litter decomposition (Suseela et al. 2013). Experiments in temperate grasslands that directly manipulated precipitation showed that drought and reduced total rainfall slowed decomposition (Suseela et al. 2013, Walter et al. 2013), possibly due to reduced microbial activity. Importantly, microbial symbionts can alter host responses to altered environmental conditions (Worchel et al. 2012, Kivlin et al. 2013), which may subsequently affect decomposition rates.

The Great Lakes dune ecosystem may be particularly responsive to climate shifts (Pendleton et al. 2005, 2010). Under the highest CO_2 emission scenarios, general circulation models project that this region will experience a 5°C increase in mean annual temperature by 2070–2099 (Hayhoe et al. 2010). Furthermore, downscaled predictions from the IPCC Fourth Assessment (IPCC 2007) vary from + 19% to –31% change in growing season precipitation (Rudgers et al. 2015) while IPCC Fifth Assesement ensemble model predicts a 10–25% increase in annual preciptiation for the region (IPCC 2014).

We examined *Epichloë* effects on decomposition in the Great Lakes dunes of the USA. *Ammophila breviligulata* is the main dune builder in both the Great Lakes and Atlantic coastal dune ecosystems (Cowles 1899, Lichter 1998a). It hosts an undescribed species of *Epichloë* (Emery et al. 2010). The most commonly used nursery stocks for dune restoration material have 100% endophyte prevalence, whereas the prevalence of *Epichloë* in Great Lakes *A. breviligulata* populations is more variable (~22% of Great Lakes populations were symbiotic, Emery et al. 2010, Emery and Rudgers 2014). To assess the interactive effects of above-ground plant symbionts and climate on decomposition in a native ecosystem, we manipulated *Epichloë* sp. in *Ammophila breviligulata* under alternative precipitation regimes. Specifically, we asked 1) does *Epichloë* affect decomposition directly by altering litter composition (substrate) or indirectly by altering the microenvironment for decomposition (conditioning)? 2) Does the precipitation regime directly affect decomposition or modify how the endophyte affects decomposition? 3) Do the traits that underlie changes in decomposition include shifts in litter substrate quantity, substrate quality, or colonization by saprotrophic fungi?

Methods

Study site

The experiment was located in Leelanau State Park, Leelanau Co., Michigan, USA (45°18′3″N, 85°57′6″W) within a large blowout on the leading edge of a second foredune ~200 m from the Lake Michigan shoreline. In the Great Lakes dunes, the accumulation of an organic layer, soil carbon, and soil nitrogen is slow and only stabilizes ~450 years after succession begins (Lichter 1998b). Thus, small changes in short term litter input and decomposition can have strong effects on long term nutrient accumulation.

Experimental design

In May 2010, we established a 2 × 3 factorial experiment to alter the presence or absence of *Epichloë* sp. symbiosis in *Ammophila breviligulata* populations in the context of a climate manipulation (reduced, ambient or augmented precipitation). Replication consisted of 15 plots (2 m × 2 m) per treatment (90 plots total), each with 25 transplanted *A. breviligulata* individuals, and each randomly assigned to a treatment combination (Emery et al. 2015).

Precipitation manipulation

We constructed modified Sala rain-out shelters to manipulate growing season precipitation (Yahdjian and Sala 2002). Clear, plastic gutters removed ~30% of ambient precipitation from the reduced precipitation plots. We added the collected rain to the augmented water plots to increase the precipitation by ~30%. Both augmented and ambient precipitation plots had mock shelters with gutters oriented upside-down to control for effects on light levels without altering ambient precipitation. A detailed description of the experimental design was presented by Emery et al. (2015). Though *A. breviligulata* is a rhizomatous grass, lateral transfer of water between plots through rhizomes is likely not a problem because each plot is surrounded by 1 m of bare sand and no tillers are growing between plots. Additionally, our precipitation manipulation led to an average of 9% higher moisture in augmented plots compared to reduced plots at both 20 and 40 cm depth (Supplementary material Appendix 1 Table A1, Fig. A1, Rudgers et al. 2015).

Conditioning treatment

To manipulate endophyte presence in *A. breviligulata*, we used endophyte-free seeds collected at a nearby site in Sleeping Bear Dunes National Lakeshore (44°85′8″N, 86°06′3″W) during fall 2006. We used a sterile needle to insert hyphae from *Epichloë* sp. isolates cultured from *A. breviligulata* into the meristem of each seedling (E+ treatment) and sham-inoculated other seedlings (E– treatment) (Leuchtmann and Clay 1988). This inoculation method had an 8% success rate (Emery et al. 2015, Rudgers et al. 2015) similar to *Epichloë* inoculation of other grass species (Chung et al. 1997). Only successfully inoculated genotypes were used for our E+ treatment. Plant responses from the field experiment for 2010–2013 were presented by Emery et al. (2015) and Rudgers et al. (2015). Here, we counted the number of *A. breviligulata* tillers per plot on 27 May 2015 to estimate above-ground plant biomass using allometric equations.

Substrate decomposition bags

Senesced leaves (hereafter, substrate) were collected during April and May 2011 from random individuals of 32 E– genotypes and 21 E+ genotypes of greenhouse-grown *A. breviligulata* planted into a 50:50 mix of sterile sand and Metro Mix 220 (average daily temp 24°C, no supplemental lighting – Houston, TX). The same stock plant genotypes used to establish the field experiment were included in the litter bags. Material was air dried at 25°C and thoroughly mixed within each endophyte status. A total of 540 (270 E– and 270 E+) litter bags (10 × 10 cm) were constructed from fiberglass window screening (3 mm mesh). Each bag contained 4 g of plant material. On 26 May 2011, six randomly chosen litter bags (three E– and three E+) were buried near the center of each 2 × 2 m plot. E+ and E– bags were alternated spatially, placed ~10 cm apart in a circle, and buried ~15 cm deep. We have found that sand accumulation greater than 18 cm can occur over the winter and spring months (Bell-Dereske et al. unpubl.), so a litter burial depth of 15 cm likely happens regularly in the dune ecosystem. Each year, for three years, two bags (one E+ and one E–) were collected from each plot at the end of the growing season (September), allowing assessments of decomposition over ~3 months, ~15 months, and ~27 months of continuous field burial (Table 1).

Table 1. Response variables measured. Material is the substrate analyzed for each response: Fresh litter was collected from greenhouse grown stock plants, year 1 litter was collected on 1 September 2011 (98 d of decomposition), year 2 was collected on 22 September 2012 (485 d), and year 3 was collected on 19 September 2013 (847 d).

Material	Response	Treatments examined	n	Method
Year 1–3 litter	litter decomposition	Substrate × Conditioning × Precipitation × Year	15	litter loss rate (Olson 1963)
Standing dead	litter mass	Conditioning × Precipitation	15	mass balance
Tillers	aboveground biomass	Conditioning × Precipitation	15	field survey
Tillers	tiller turnover	Conditioning × Precipitation	15	monthly census of tiller birth and death
Fresh litter	C:N	Substrate	3	elemental analyzer
Year 3 litter	C:N	Substrate × Conditioning × Precipitation	5	elemental analyzer
Fresh litter	^{13}C NMR	Substrate	3	solid state ^{13}C CP MAS NMR
Year 3 litter	^{13}C NMR	Substrate	3	solid state ^{13}C CP MAS NMR
Fresh litter	mineral composition	Substrate	3	inductively coupled plasma spectroscopy
Year 3 litter	mineral composition	Substrate	3	inductively coupled plasma spectroscopy
Year 3 litter	fungal colonization	Substrate × Conditioning	5	gridline intersect 200X microscopy

Data collection

Bags from 2011 and 2012 were air dried at 25°C and weighed to determine mass loss. The bags collected in 2013 were stored at 4°C for ~1 month, during which time bags were weighed wet and subsampled for microscopy, ^{13}C nuclear magnetic resonance (NMR) spectroscopy, mineral content, and elemental composition (Table 1). The remaining material was air dried to calculate moisture content and mass loss. Due to the limited amount of available substrate and the lack of a strong effect of the precipitation treatment, microscopic and chemical analyses were conducted on subsets of treatment combinations as described in Table 1.

Traits

Aboveground biomass and standing dead litter

Standing dead litter in combination with live biomass links the results from the litter bag experiment to the amount of above-ground decomposition occurring naturally in plots. Biomass of standing dead litter reflects the amount of litter produced by plants minus the loss of litter to decomposition, burial by sand, and mechanical removal by wind. To estimate the effects of *Epichloë* and altered precipitation on birth and death of tillers and litter quantity, we measured the amount of live *A. breviligulata* biomass, tiller turnover rate, and standing dead litter biomass in each plot (Table 1). Near the northeastern corner of each plot we established a permanent 0.25×0.25 m subplot. In 2013, we measured tiller turnover (tiller birth and death rates) in the plots by tagging all tillers the subplot in each plot in May, and then censusing all new and dead tillers in each subsequent month until the end of the season in September. Daily birth (and death) rates of tillers were calculated as the number of new (or dead) tillers at a census divided by tiller number at the previous census and the number of days since the last census. Rates were averaged across all months to give daily birth and death rates for the entire season. In the beginning of June 2014, we removed all of the standing dead litter from each subplot. On 28 June 2015, we surveyed each subplot by counting live tillers and collecting all of the standing dead litter. This litter thus represented ~1 year of accumulation. Litter was oven dried at 80°C for 72 h, then weighed.

We counted the number of live tillers at the whole plot scale on 27 May 2015 to estimate the effects of *Epichloë* on biomass production. At our site, tiller number is related to live biomass by the allometric equation: $y = 1.3519 \times$ tiller number, $r^2 = 0.92$, $p < 0.00001$ (Bell-Dereske and Rudgers unpubl.).

Substrate carbon and nitrogen content

To assess endophyte effects (substrate and conditioning) on litter chemistry, samples from litter bags were oven dried at 60°C for 72 h then ground with a ball mill. Ground samples were dried at 60°C for 72 h then sent to Oklahoma State University Soil, Water, and Forage Laboratory (Stillwater, OK) for percentage carbon (C) and nitrogen (N) analysis on a C:N analyzer.

NMR spectroscopy and litter mineral composition

To examine effects of *Epichloë* on litter substrate carbon chemistry, we subjected both fresh litter (greenhouse-grown litter harvested 24 January 2014 from 15 genotypes of E+ and 15 genotypes of E– (five genotypes per sample)) and decomposed litter (Table 1) to ^{13}C-NMR analysis. To assess the average effect of the endophyte's presence in substrate across our other treatments, we combined subsamples of litter among the conditioning endophyte treatment and plot location for a total of three composite samples of each endophyte substrate treatment. Litter was air dried and ground with liquid nitrogen using a mortar and pestle.

Solid state ^{13}C cross polarization magic angle spinning (CP MAS) NMR spectra were collected on each sample using a solid-state NMR spectrometer. The spectrometer was equipped with 4 mm magic angle spinning (MAS) probe and operated at rotor spinning frequency of 7 kHz. Cross polarization (CP) spectra were acquired by applying a 90 degree ^{1}H pulse, a 1.0 ms ^{13}C contact pulse, composite pulse proton decoupling, and a 5 s recycle delay. The ^{13}C NMR spectra were divided into chemical shift regions corresponding to different functionalities: alkyl C (0–45 ppm), N-alkyl/methoxyl (45–60 ppm), O-alkyl (60–95 ppm), di-O-alkyl (95–110 ppm), aromatic C (110–145 ppm), phenolic (145–165 ppm), and amide/carboxyl (165–215 ppm). The relative allocation of signal was assigned to the seven individual spectral regions. We used a molecular mixing model (MMM) (Baldock et al. 2004) to determine the major biochemical components (e.g. carbohydrate, protein, lignin and lipid) in each sample. The C:N ratio and signal distribution across the seven predefined ^{13}C NMR spectral regions were input into the MMM to model the concentration of each biochemical component. Spinning sideband (SSB) was integrated and corrected during MMM calculation. The MMM additionally provides information on the degree of decomposition of the sample as reported in the alkyl/O-alkyl peak ratios (Baldock et al. 1997). The same fresh and decomposed litter samples described above were analyzed for litter mineral composition (Ca, P, Na, Mg, K, S, Mn, Cu, Fe and Zn) using wet digestion and inductively coupled plasma spectroscopy.

Response

Colonization by saprotrophic fungi

To determine how substrate and conditioning treatments affected the colonization of litter by non-*Epichloë* fungi, we determined the percentage of litter area colonized by fungi using acid fuchsin stain following methods in Brundrett et al. (1996). We used the gridline intersect method to count the presence of distinct fungal structures and measure hyphal length within 30 fields of view at $200 \times$ magnification (Brundrett et al. 1996); each field of view was 1 mm^2 of litter containing 100 ocular grid squares.

Statistical methods

We used a negative exponential model to calculate the litter loss rate: $\ln(x_t/x_0) = -kt$ where x_0 is the initial mass of the litter, x_t is litter mass at year t, and k is the decay constant per year (Olson 1963). Values that showed small increases in mass were treated as a loss of 0 g (9/539 observations). Values with mass loss >100% were treated as a loss of 4 g (6/539 observations). Results did not significantly change when the actual values were analyzed.

We analyzed (k) and C:N litter composition using a general linear mixed effects model containing conditioning endophyte (endophyte presence in the plot), precipitation, and substrate endophyte (endophyte presence in litter) treatments, including all interactions as well as plot as a random, nested factor. As spatial blocking factors, we included categorical variables of plot spatial position indicating column (north–south gradient) and row (east–west gradient) in the analyses of (k). To meet assumptions of Gaussian distributions of errors and homogeneity of variances, we log-transformed year 2 data (2012) and used cube root for year 3 (2013). Years were analyzed separately due to differences in litter mass loss calculation for year 3 litter increasing the variance of the measurement compared to litter from year 1 and 2. Tiller birth and death rates, standing dead litter (g tiller^{-1}), plot level standing dead biomass (y = dead litter per tiller × tiller number), and plot level live biomass were analyzed with general linear models with endophyte treatment, precipitation treatment, spatial position of plots, and all interactions as described above. NMR spectra and mineral composition were statistically analyzed using Welch's two sample t-test in R ver. 3.0.2 (<www.r-project.org>). We analyzed the percentage of litter surface area colonized by fungi with a general linear mixed model including the fixed effects of conditioning endophyte, substrate endophyte, and their interaction, with plot as a random factor. All a general linear mixed effects model and linear models were analyzed using PROC GLMMIX, SAS ver. 9.3 (SAS Inst.).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.11nr8> (Bell-Dereske et al. 2016).

Results

1) Does Epichloë affect decomposition directly by altering litter composition (substrate) or indirectly by altering the microenvironment for decomposition (conditioning)?

Endophyte substrate

The effects of *Epichloë* presence on the rate of litter decomposition varied temporally during the experiment. During the first growing season (May–Sept 2011), substrate produced by E+ plants decomposed 21% faster than E– substrate (Fig. 2a). Percentage mass loss was 20% ± 0.8% SE for E+ substrate and 17% ± 0.8% SE for E– substrate. However, the influence of the endophyte substrate treatment disappeared after the first growing season and had no effect in the second or third year of decomposition (Fig. 2a, Supplementary material Appendix 1 Table A2).

Endophyte conditioning

In contrast, by the third year of ongoing decomposition, litter had decomposed 33% more slowly in E+ *Ammophila breviligulata* conditioned plots compared to endophyte-free (E–) conditioning plots (Fig. 2b). Cumulative litter mass loss over three years was 43% ± 3.2% SE for litter in E+ conditioned plots and 51% ± 3.2% SE for litter in E– conditioned plots. This effect was not observed during the first two years (Fig. 2b), and there was no significant interaction between the endophyte conditioning and endophyte substrate treatments (Supplementary material Appendix 1 Table A2).

2) Does the precipitation regime directly affect decomposition or modify how the endophyte affects decomposition?

For both the litter bags and standing dead litter, precipitation treatments had no significant influence on decomposition. In addition, precipitation did not interact with endophyte substrate or endophyte conditioning treatments to affect either decomposition rates or the amount of standing litter (Supplementary material Appendix 1 Table A2–A3).

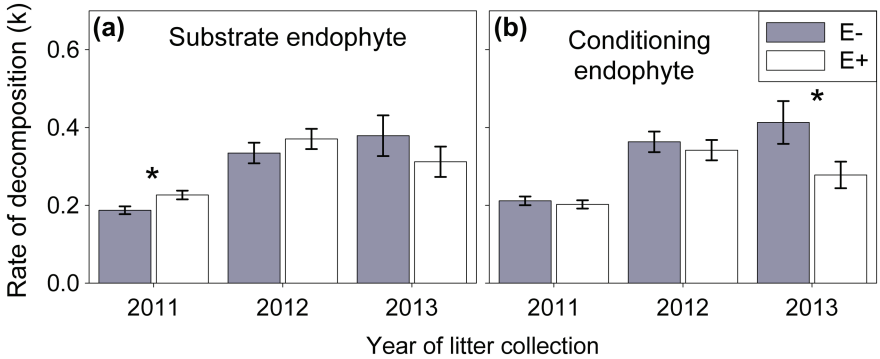

Figure 2. Rates of decomposition of *Ammophila breviligulata* substrate from litter bags deployed in May 2011 and collected in September 2011 (n = 15), 2012 (n = 15), or 2013 (n=14). (a) *Epichloë* (white bars (E+)) substrate decomposed faster than endophyte free substrate (filled bars (E–)) only during the first growing season (2011). (b) Litter bags (of either endophyte status) placed in plots conditioned by *A. breviligulata* with *Epichloë* (white bars (E+)) decomposed more slowly than litter bags placed in endophyte free plots (filled bars (E–)), after three growing seasons (2013). There was no interaction between substrate endophyte status and conditioning endophyte treatment. Error bars are +/– SE. "*" significant pairwise difference at $p \leq 0.05$.

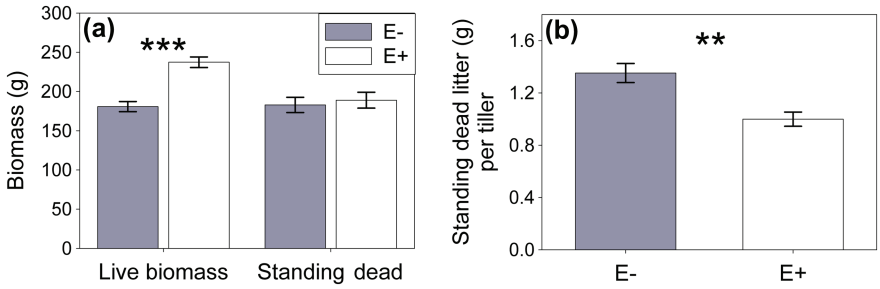

Figure 3. *Ammophila breviligulata* live biomass and standing dead litter per plot and per tiller (n=15). (a) *Epichloë* (white bars (E+)) increased the live biomass per plot for host plant *A. breviligulata* compared to endophyte free plots (filled bars (E–)); however, this increased biomass did not increase the amount of standing dead litter per plot. This was due (b) standing dead litter mass (g) per live tiller of *A. breviligulata* being lower in *Epichloë* plots than in endophyte free plots. Error bars are +/– SE. "**" significant at $p \leq 0.001$ and "***" $p \leq 0.0001$.

3) Do the traits that underlie changes in decomposition include shifts in litter substrate quantity, substrate quality, or colonization by saprotrophic fungi?

Epichloë effects on litter quantity

In a natural field setting, the endophyte could affect the quantity of substrate as well substrate quality and the micro-environmental conditions during decomposition. Endophyte presence in live plants increased the above-ground biomass of *A. breviligulata* by 31% (Fig. 3a). Yet, there was no net change in standing dead litter per plot (Fig. 3a) because *Epichloë* presence in live plants reduced the amount standing dead litter per tiller by 26% compared to endophyte-free plots (Fig. 3b). Furthermore, when we estimated the amount of above-ground litter lost during decomposition as (estimated live biomass – estimated standing dead litter) (Fig. 3a), we found that E+ plots lost, on average, 48.4 g (~20% of live litter biomass) of litter while E– plots showed an insignificant increase in litter of 2.2 g (~1% of live litter biomass). Thus, the presence of *Epichloë* increased the loss of litter by 21% compared to E– plots, exactly the same effect size we reported for *Epichloë* presence in substrate during the first season of decomposition in our litter bags (Fig. 2a). Though, the presence of *Epichloë* increased cumulative sand accumulation in plots during 2010–2013 (Emery et al. 2015), sand accumulation during the period of standing dead tiller accumulation (2014–2015) was generally low (average: 2.08 ± 0.23 SE cm) and did not strongly correlate with the mass of standing dead litter per tiller ($r = 0.17$ $p > 0.1$). Additionally, endophyte presence increased the rate of tiller senescence 84% and had no effect on tiller birth rate (Supplementary material Appendix 1 Fig. A2, Table A3).

Epichloë effects on substrate litter quality

The presence of *Epichloë* affected the downstream composition of litter substrate over the course of decomposition. *Epichloë* did not affect the structural carbon (NMR, Fig. 4a, Supplementary material Appendix 1 Fig. A3), nitrogen composition (Fig. 5a), litter mineral composition (all elements and minerals: Supplementary material Appendix 1 Table A4), or carbon:nitrogen (C:N) ratio (Fig. 5c) of freshly senesced litter. However, after three years of decomposition, E+ substrate had 28% lower amide content than E– substrate (Fig. 4b) and 22% lower N content (Fig. 5b). Despite these shifts in chemistry, there was no significant effect of the substrate *Epichloë* treatment on the percentage of leaf tissue colonized by fungi, over all fungal morphotypes combined (Supplementary material Appendix 1 Fig. A2) or for any individual fungal morphotype (all $p > 0.1$, data not shown). It is unclear if these endophyte-mediated shifts in substrate also affect the C:N ratio because our different methods showed divergent results. *Epichloë* in the substrate increased the C:N ratio of bulked samples of decomposed litter (by 39% compared to endophyte-free litter, n = 3, Fig. 5d). However, during the

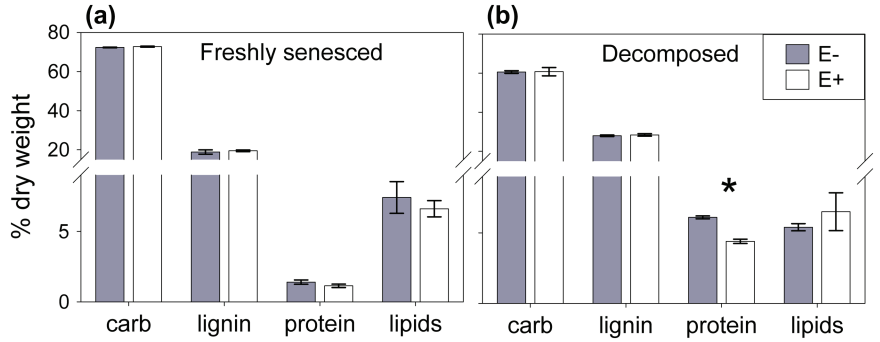

Figure 4. Carbon chemistry of *Ammophila breviligulata* litter, produced by plants with (white bars (E+)) or without (filled bars (E–)) *Epichloë* (n=3). The presence of the endophyte did not affected percentage litter composed of carbohydrates (carb), lignin, protein, and lipids modeled from ^{13}C NMR spectroscopy in (a) freshly senesced litter. However for (b) decomposed litter collected after three years, E+ substrate was depleted in protein compared to E– substrate. Bars are means +/– SE. "*" significant at $p \leq 0.05$.

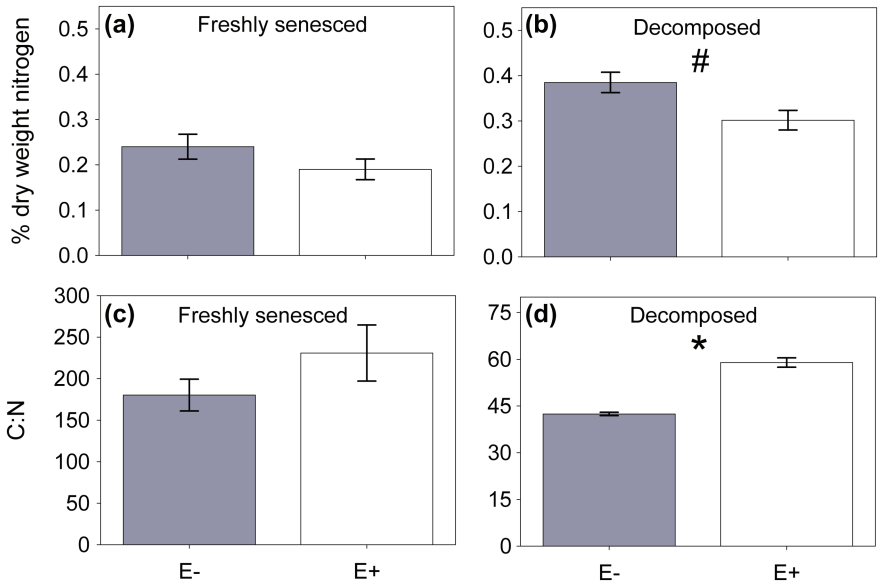

Figure 5. Percentage nitrogen and carbon:nitrogen (C:N) ratio of *Ammophila breviligulata* litter (n = 3). Both (a) fresh and (b) decomposed substrate from *A. breviligulata* with the endophyte (white bars) tended to have lower nitrogen content than substrate from plants without the endophyte (filled bars). (c) There was no significant difference in C:N ratio between freshly senesced *A. breviligulata* substrate with *Epichloë* (E+) and endophyte free (E–) substrate. (d) The carbon:nitrogen ratio was significantly higher in bulked, decomposed substrate from plants with *Epichloë* than without *Epichloë*. "*" signify significant differences between means $p \leq 0.05$. "#" signifies a nearly significant difference between means at $p \leq 0.06$. Bars show means ± SE.

same year of decomposition, the endophyte had no effect on the C:N ratio of samples taken from each plot, where the sample size was larger (5% decrease compare to E–, n = 15, Supplementary material Appendix 1 Table A2).

Epichloë conditioning of the environment for decomposition

After three years of decomposition, endophyte conditioning due to the presence of live plants in field plots had no effect on the C:N ratio of decomposed litter (Supplementary material Appendix 1 Table A2). *Epichloë* conditioning also did not alter the percentage of leaf tissue colonized by fungi for any fungal morphotype (all fungi Supplementary material Appendix 1 Fig. A4; each morphotype: all $p > 0.2$, data not shown).

Discussion

Substrate produced by symbiotic plants had faster initial rates of decomposition than substrate from symbiont-free plants. This increase in initial decomposition rate may have reduced the amount of standing dead litter per tiller in plots where the endophyte was present in live plants. However, the endophyte substrate treatment was not important after the first year of decomposition. Instead, the decomposition microenvironment (i.e. endophyte conditioning) became increasingly important, with endophyte presence in live plants in field plots reducing the rate of decomposition of both E+ and E– substrate types by the third year of decomposition. Mechanisms that may underlie these results include shifts in local nutrient availability, microbial activity, and microbial community composition, but are unlikely to be caused by initial differences in litter quality. These temporally dependent shifts in the importance of *Epichloë* in host plant litter decomposition could have large effects on the nutrient cycling in the nearly sterile dune soil.

Epichloë effects on substrate increased rates of early decomposition

Endophyte symbiosis in *Ammaophila breviligulata* substrate sped up initial rates of litter decomposition. In contrast, all three previous studies found slower decomposition when an *Epichloë* species was present in the substrate (Omacini et al. 2012). One hypothesis for these divergent results is that all prior work examined grass–*Epichloë* symbioses that were non-native introductions to the ecosystem studied and where the interactions between *Epichloë* and below-ground microbes maybe novel. A second hypothesis is that prior studies focused on *Epichloë* species that produce high levels of toxic alkaloids, while the *A. breviligulata* endophyte lacks the genes for alkaloid production, with the exception of the pyrrolopyrazine, peramine (J. A. Rudgers, N. Charlton, C. A. Young, unpubl.). Some possible differences among studies can be ruled out: The length of decomposition in our experiment (98–847 d) overlapped the range of the previous experiments (83 d - Omacini et al. 2004, 170 d - Siegrist et al. 2010, and 256 d - Lemons et al. 2005). Additionally, the percentage of litter lost in sandy soils of the Great Lakes dunes during the first growing season (mean 19% ± 0.6% SE) was comparable to, but on the low end of, litter loss reported in the previous experiments (~15% to 72%, Omacini et al. 2004, Siegrist et al. 2010). After the first growing season, however, the endophyte substrate treatment had no effect on the rate of litter decomposition, suggesting these symbiont

effects were either ephemeral during early succession or show interannual variability. This initial increased decomposition due the endophyte will likely lead to a pulse of nutrients that may lead to ephemeral increases in productivity of the plant community and associated microbial community.

Epichloë altered host leaf traits and litter quantity

Epichloë presence increased aboveground biomass production by 31%, which was comparable to *Epichloë*-driven increases previously reported in this system (3–19% increase in tiller production, Emery et al. 2015). However, the amount of standing dead litter per live tiller was reduced by 26% when *Epichloë* was present. Although *Epichloë* increased the amount of live biomass, the endophyte-driven reduction in dead litter per tiller produced no difference in the amount of standing dead litter at the plot-scale. It is possible that the rapid decomposition of substrate with *Epichloë* could lead to pulses of nutrients and a positive feedback with the host leading to the increased live biomass of *A. breviligulata* we have seen in this system (Emery et al. 2015). Factors such as sand accumulation, tiller turnover, and wind can be ruled out as drivers of the reduction in standing dead litter, as these factors were either minor (e.g. sand accumulation and wind; Emery et al. 2015) or positively related to litter biomass (e.g. tiller turnover). Ruling out sand burial, tiller turnover, and wind removal suggests a direct link between our litter bag experiment and the above-ground decomposition processes: higher initial rates of decomposition of *Epichloë* substrate may cause faster initial leaf litter loss from tillers when *Epichloë* is present in live plants.

The lack of initial differences in substrate chemistry due to *Epichloë* presence suggests that the endophyte's influence on the initial rate of decomposition is not driven by aspects of substrate quality measured in our study. Previous research on *Lolium perenne* and *L. arundinaceum* has found that the presence of *Epichloë* alters the metabolic composition of host plant tissue though many of these effects were dependent on soil nitrogen and CO_2 concentration (Newman et al. 2003, Hunt et al. 2005, Rasmussen et al. 2008, Brosi et al. 2011). *Epichloë* presence reduced the carbon:nitrogen (C:N) ratio of *L. arundinaceum* tissue though this effect was lost during decomposition (Siegrist et al. 2010). It is possible that *Epichloë* alters the surface physical structure or internal architecture of litter in ways that increased access for saprotrophs (particularly microarthropods and bacteria, since fungal colonization was not affected). Dupont et al. (2015) found that *Epichloë* presence increased expression of genes regulating host cell walls and reduced the thickness of the cell walls. Alternatively, the legacy effects of the endophyte could alter utilization/conversion of nutrients from inorganic to organic forms by the soil microbial community (Cornwell et al. 2008, García-Palacios et al. 2016). Other aspects of litter chemistry, such as levels of the endophyte-produced, insect-deterrent alkaloid, peramine (Panaccione et al. 2014), which were not measured here, may also contribute to the effect of the endophyte on substrate decomposition rate.

After three years of decomposition, substrate produced by plants with the endophyte was depleted in protein and nitrogen compared to substrate made by endophyte-free plants. Since we cannot decouple the contributions of plant-derived versus microbe-derived protein, it is difficult to identify specific mechanisms through which the endophyte may be altering nitrogen dynamics in this system. In general, however, higher lignin content slows rates of decomposition (Cornwell et al. 2008). Because we found no effects of *Epichloë* presence on lignin content in fresh litter, effects of *Epichloë* on decomposition rates are not likely occurring through shifts in initial structural carbons, but instead accrue through other (as yet unmeasured) traits, such as leaf toughness and surface structure (García-Palacios et al. 2016), that influence how nutrients are utilized by saprotrophs.

Soil conditioning by *Epichloë* reduced rates of later decomposition

Our results show temporal shifts in the importance of endophyte conditioning on the decomposition microenvironment that may be missed by short-term studies. Notably, plots conditioned by *A. breviligulata–Epichloë* symbiosis significantly reduced the rate of decomposition of both substrate types by the third year of decomposition, with no detectable effects in earlier years. To date, our study is the longest decomposition experiment examining the effects of *Epichloë* symbiosis (847 d, the next longest was 256 d - Lemons et al. 2005). Previous studies have found ephemeral effects of *Epichloë* on short time scales. For example, Siegrist et al. (2010) showed that endophyte conditioning of plots reduced the decomposition of litter only during the earliest stage of decomposition (21 d). In contrast with this result and ours, Lemons et al. (2005) reported that *Epichloë* conditioning increased the decomposition rate compared to endophyte-free plots, but this effect only occurred when a larger mesh (169 mm^2 pore size) was used for litter bag construction, suggesting that effects were driven by invertebrates. Lemons et al. (2005) reported no significant effect of plot endophyte status when a smaller mesh size (0.1 mm^2 pore size, more similar to our mesh) was used. Slower decomposition rates when endophyte-symbiotic plants dominate conditioning suggest that endophyte-mediated alterations in the soil biotic or abiotic microenvironment (e.g. root exudation, carbon priming, nutrient competition) influence later stages of decomposition or ecological succession. Effects of *Epichloë* on the soil micro-environment may become stronger with the length of soil conditioning; however, our experiment fell within the time range of soil conditioning in prior experiments: ~40 months compared to ~10 months (Omacini et al. 2004) to ~75 months (Siegrist et al. 2010). This reduced decomposition over time could lead to increased carbon sequestration similar to what has been recorded in the *Lolium arundinaceum* dominate systems (Franzluebbers et al. 1999).

Mechanisms of *Epichloë* conditioning on the decomposition microenvironment

The mechanism underlying *Epichloë*-altered decomposition that has received the greatest attention has been endophyte-produced alkaloids. Previous screening of four alkaloid gene clusters showed that the *Epichloë* in our *A. breviligulata* has only the genes to produce the pyrrolopyrazine, peramine (J. A. Rudgers, N. Charlton and C. A. Young, unpubl.),

an anti-insect defensive chemical (reviewed by Schardl 1996). Peramine is unique among the *Epichloë* alkaloids in being found throughout host plant tissues (Koulman et al. 2007, Panaccione et al. 2014). Thus, it could affect rhizosphere soils through root exudates or root decomposition. To our knowledge, no previous studies have examined the direct effects of peramine on decomposition or on saprotrophs. Additionally, few studies have examined direct effects of peramine on herbivores; however, it does effectively deter the Argentine stem weevil, a common grass pest (Gerard 2000). We did not measure alkaloid levels in the litter or roots in this experiment, but *Epichloë* has been shown to alter the root exudate profile in *L. arundinaceum*, which also produces peramine (Guo et al. 2015). If endophyte-produced peramine was a major mechanism underlying reduced rates of decomposition in plots with the endophyte, we would expect to see inhibition of saprotrophs. Although we found no effect of the endophyte on the percentage of litter colonized by fungi, endophyte conditioning altered the soil microbial community (i.e. arbuscular mycorrhizal fungi and bacteria, Bell-Dereske et al. unpubl.).

Altered precipitation regime had no direct or indirect effect on the rate of decomposition

Rates of litter decomposition can be accelerated by pulses of precipitation (Chang et al. 2007), but this effect may be most important in ecosystems with high annual precipitation (Manzoni et al. 2010). On the other hand, decomposition rates are typically reduced by drought events (Walter et al. 2013). Surprisingly, we found no effects of either increased or decreased precipitation on rates of litter decomposition in Great Lakes dunes. Given soil moisture levels that are typical for dunes (Baldwin and Maun 1983), our precipitation treatments likely did not reduce the precipitation to drought levels, although they did alter soil moisture in our plots (Emery et al. 2015). Additionally, because dune soils are composed of medium fine sand with no organic layer, water percolates through the soil rapidly (Lichter 1998b). Overall, our data suggest the decomposition processes in Great Lakes dunes are resistant to precipitation variation that falls within 30% of ambient levels, and that symbiotic endophytes trump the effects of precipitation on decomposition rate. Therefore, climate change driven changes in the average precipitation during the growing season may not have strong effects on the nutrient cycling through decomposition, but other aspects of climate change, such as increased temperature (Creamer et al. 2015) and the interactive effect of temperature and precipitation (Suseela et al. 2013), may lead to altered nutrient cycling processes.

Conclusion

This study is the first to report a temporal shift in the relative importance of the pathways through which above-ground fungal symbionts can alter litter decomposition. Initial decomposition of *A. breviligulata* litter was faster if this substrate came from plants hosting the endophyte. This early increase in decomposition rate did not decrease the amount of standing dead biomass per m^2 because the endophyte also increased aboveground plant biomass. Later in the decomposition process, endophyte symbiosis in living host plants slowed the rate of decomposition, perhaps by altering the soil microenvironment. Since previous research has shown that the endophyte is found ~22% of *A. breviligulata* populations and its occurrence is spatially heterogeneous (Emery et al. 2010), both the effects of endophyte in the substrate and conditioning of the soil will likely lead to endophyte-driven spatial heterogeneity in nutrient cycling within the dunes. This spatial heterogeneity may have important implications in successional processes as soil nutrients play an important role in plant succession (Lichter 1998a). Given the widespread occurrence of *Epichloë* within grasses and more generally, of fungal symbionts in plants, temporal shifts in their influence on decomposition processes may have strong effects on nutrient cycling and carbon sequestration during succession in many ecosystems.

Acknowledgements –We thank A. J. Davitt, Isaiah Reynolds, Shelly Gleason, Tamara Milton, Brandon McCormick, Caitlin Rhodes, Catherine Fargen, Emily Moehlman, Alex Bryant, Joy Uwimbabazi, Brad Gottshall, Jeff Masters and Keera Lowe for assistance with field work and experimental set-up.
Funding – This work has been funded by NSF GRFP no. 0940902 and the George Melendez Wright Climate Change Fellowship to LBD, and NSF DEB no. 0918267 and no. 1145588 to JAR and SME.
Conflict of interest – LBD, XG, CAM, RLS, SME and JAR have no conflicts of interest to declare.

References

Antunes, P. M. et al. 2008. Even after death the endophytic fungus of *Schedonorus phoenix* reduces the arbuscular mycorrhizas of other plants. – Funct. Ecol. 22: 912–918.

Baldock, J. A. et al. 1997. Assessing the extent of decomposition of natural organic materials using solid-state 13C NMR spectroscopy. – Soil Res. 35: 1061–1084.

Baldock, J. A. et al. 2004. Cycling and composition of organic matter in terrestrial and marine ecosystems. – Mar. Chem. 92: 39–64.

Baldwin, K. and Maun, M. A. 1983. Microenvironment of Lake Huron sand dunes. – Can. J. Bot. 61: 241–255.

Bell-Dereske, L. et al. 2016. Data from: Plant–fungal symbiosis affects litter decomposition during primary succession. – Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.11nr8>.

Brosi, G. B. et al. 2011. Effects of multiple climate change factors on the tall fescue–fungal endophyte symbiosis: infection frequency and tissue chemistry. – New Phytol. 189: 797–805.

Brundrett, M. et al. 1996. Working with mycorrhizas in forestry and agriculture. – Aust. Centre Int. Agric. Res., Canberra.

Casas, C. et al. 2011. Soil microbial community responses to the fungal endophyte *Neotyphodium* in Italian ryegrass. – Plant Soil 340: 347–355.

Chang, S. C. et al. 2007. Soil fluxes of mineral elements and dissolved organic matter following manipulation of leaf litter input in a Taiwan *Chamaecyparis* forest. – For. Ecol. Manage. 242: 133–141.

Cheng, L. et al. 2012. Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. – Science 337: 1084–1087.

Chu-Chou, M. et al. 1992. Suppression of mycorrhizal fungi in fescue by the *Acremonium coenophialum* endophyte. – Soil Biol. Biochem. 24: 633–637.

Chung, K. R. et al. 1997. Genetics of host specificity in *Epichloë typhina*. – Phytopathology 87: 599–605.
Clay, K. 1990. Fungal endophytes of grasses. – Annu. Rev. Ecol. Syst. 21: 275–297.
Cornwell, W. K. et al. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. – Ecol. Lett. 11: 1065–1071.
Cowles, H. C. 1899. The ecological relations of the vegetation on the sand dunes of Lake Michigan. – Bot. Gaz. 27: 95–117, 167–202, 281–308, 361–391.
Crawford, K. et al. 2010. Fungal endophytes of native grasses decrease insect herbivore preference and performance. – Oecologia 164: 431–444.
Creamer, C. A. et al. 2015. Microbial community structure mediates response of soil C decomposition to litter addition and warming. – Soil Biol. Biochem. 80: 175–188.
Dupont, P. Y. et al. 2015. Fungal endophyte infection of ryegrass reprograms host metabolism and alters development. – New Phytol. 208: 1227–1240.
Emery, S. M. and Rudgers, J. A. 2014. Biotic and abiotic predictors of ecosystem engineering traits of the dune building grass, *Ammophila breviligulata*. – Ecosphere 5: art87.
Emery, S. M. et al. 2010. Variation in endophyte symbiosis, herbivory and drought tolerance of *Ammophila breviligulata* populations in the Great Lakes region. – Am. Midl. Nat. 163: 186–196.
Emery, S. M. et al. 2015. Fungal symbiosis and precipitation alter traits and dune building by the ecosystem engineer, *Ammophila breviligulata*. – Ecology 96: 927–935.
Franzluebbers, A. J. et al. 1999. Soil carbon and nitrogen pools under low- and high-endophyte-infected tall fescue. – Soil Sci. Soc. Am. J. 63: 1687–1694.
García-Palacios, P. et al. 2016. The importance of litter traits and decomposers for litter decomposition: a comparison of aquatic and terrestrial ecosystems within and across biomes. – Funct. Ecol. 30: 819–829.
Gerard, P. J. 2000. Ryegrass endophyte infection affects Argentine stem weevil adult behaviour and susceptibility to parasitism. – N. Z. Plant Protection 53: 406–409.
Guo, J. et al. 2015. Tall fescue cultivar and fungal endophyte combinations influence plant growth and root exudate composition. – Front. Plant Sci. 6.
Hunt, M. G. et al. 2005. Near-term impacts of elevated CO_2, nitrogen and fungal endophyte-infection on *Lolium perenne* L. growth, chemical composition and alkaloid production. – Plant Cell Environ. 28: 1345–1354.
Hayhoe, K. et al. 2010. Regional climate change projections for Chicago and the US Great Lakes. – J. Great Lakes Res. 36: 7–21.
IPCC 2007. Climate Change 2007: synthesis report. Contrib. Working Grps I, II and III to the 4th Assess. Rep. of the Intergovernmental Panel on Climate Change, Geneva, Switzerland.
IPCC 2014. Climate Change 2014: impacts, adaptation and vulnerability. Part B: regional aspects. Contrib. Working Grp II to the 5th Assess. Rep. of the Intergovernmental Panel on Climate Change. – Cambridge Univ. Press.
Jenkins, M. et al. 2006. Assessing short-term responses of prokaryotic communities in bulk and rhizosphere soils to tall fescue endophyte infection. – Plant Soil 289: 309–320.
Kardol, P. and Wardle, D. A. 2010. How understanding aboveground belowground linkages can assist restoration ecology. – Trends Ecol. Evol. 25: 670–679.
Kivlin, S. and Treseder, K. 2015. Initial phylogenetic relatedness of saprotrophic fungal communities affects subsequent litter decomposition rates. – Microbial Ecol. 69: 748–757.
Kivlin, S. N. et al. 2013. Fungal symbionts alter plant responses to global change. – Am. J. Bot. 100: 1445–1457.
Koulman, A. et al. 2007. Peramine and other fungal alkaloids are exuded in the guttation fluid of endophyte-infected grasses. – Phytochemistry 68: 355–360.
Lemons, A. et al. 2005. Connecting plant–microbial interactions above and belowground: a fungal endophyte affects decomposition. – Oecologia 145: 595–604.
Leuchtmann, A. and Clay, K. 1988. Experimental infection of host grasses and sedges with *Atkinsonella hypoxylon* and *Balansia cyperi* (Balansiae, Clavicipitaceae). – Mycologia 80: 291–297.
Lichter, J. 1998a. Primary succession and forest development on coastal Lake Michigan sand dunes. – Ecol. Monogr. 68: 487–510.
Lichter, J. 1998b. Rates of weathering and chemical depletion in soils across a chronosequence of Lake Michigan sand dunes. – Geoderma 85: 255–282.
Manzoni, S. et al. 2010. Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. – Ecol. Monogr. 80: 89–106.
Newman, J. A. et al. 2003. Effects of elevated CO_2, nitrogen and fungal endophyte-infection on tall fescue: growth, photosynthesis, chemical composition and digestibility. – Global Change Biol. 9: 425–437.
Nuccio, E. E. et al. 2013. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. – Environ. Microbiol. 15: 1870–1881.
Oberhofer, M. et al. 2014. Effects of natural hybrid and non-hybrid *Epichloë* endophytes on the response of *Hordelymus europaeus* to drought stress. – New Phytol. 201: 242–253.
Olson, J. S. 1963. Energy storage and the balance of producers and decomposers in ecological systems. – Ecology 44: 322–331.
Omacini, M. et al. 2004. Do foliar endophytes affect grass litter decomposition? A microcosm approach using *Lolium multiflorum*. – Oikos 104: 581–590.
Omacini, M. et al. 2006. Leaf endophytes affect mycorrhizal status and growth of co-infected and neighbouring plants. – Funct. Ecol. 20: 226–232.
Omacini, M. et al. 2012. Grass–endophyte symbiosis: a neglected aboveground interaction with multiple belowground consequences. – Appl. Soil Ecol. 61: 273–279.
Osono, T. 2006. Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. – Can. J. Microbiol. 52: 701–716.
Panaccione, D. G. et al. 2014. Bioactive alkaloids in vertically transmitted fungal endophytes. – Funct. Ecol. 28: 299–314.
Pendleton, E. A. et al. 2005. Coastal change-potential assessment of Sleeping Bear Dunes, Indiana Dunes, and Apostle Islands National Lakeshores to lake-level changes. – US Dept of the Interior.
Pendleton, E. A. et al. 2010. Importance of coastal change variables in determining vulnerability to sea- and lake-level change. – J. Coastal Res. 26: 176–183.
Purahong, W. and Hyde, K. 2011. Effects of fungal endophytes on grass and non-grass litter decomposition rates. – Fungal Divers. 47: 1–7.
Raghavendra, A. K. H. and Newcombe, G. 2013. The contribution of foliar endophytes to quantitative resistance to *Melampsora* rust. – New Phytol. 197: 909–918.
Rasmussen, S. et al. 2008. Metabolic profiles of *Lolium perenne* are differentially affected by nitrogen supply, carbohydrate content and fungal endophyte infection. – Plant Physiol. 146: 1440–1453.
Rogers, A. et al. 2012. Inoculation of hybrid poplar with the endophytic bacterium *Enterobacter* sp. 638 increases biomass but does not impact leaf level physiology. – GCB Bioenergy 4: 364–370.

Rudgers, J. A. et al. 2015. Fungal symbiont effects on dune plant diversity depend on precipitation. – J. Ecol. 103: 219–230.
Saikkonen, K. et al. 2015. Endophytic phyllosphere fungi and nutrient cycling in terrestrial ecosystems. – Curr. Sci. 109: 121–126.
Schardl, C. L. 1996. Epichloe species: fungal symbionts of grasses. – Annu. Rev. Phytopathol. 34: 109–130.
Schardl, C. L. 2010. The epichloae, symbionts of the grass subfamily Poöideae. – Ann. Miss. Bot. Gard. 97: 646–665.
Siegrist, J. A. et al. 2010. Alkaloids may not be responsible for endophyte-associated reductions in tall fescue decomposition rates. – Funct. Ecol. 24: 460–468.
Suseela, V. et al. 2013. Labile compounds in plant litter reduce the sensitivity of decomposition to warming and altered precipitation. – New Phytol. 200: 122–133.
Thrower, L. B. and Lewis, D. H. 1973. Uptake of sugars by *Epichole typhina* (pers. ex fr.) Tul. in culture and from its host, *Agrostis stolonifera* L. – New Phytol. 72: 501–508.

Supplementary material (available online as Appendix oik-03648 at <www.oikosjournal.org/appendix/oik-03648>). Appendix 1.

Wäli, P. R. et al. 2006. Susceptibility of endophyte-infected grasses to winter pathogens (snow molds). – Can. J. Bot. 84: 1043–1051.
Walter, J. et al. 2013. Combined effects of multifactor climate change and land-use on decomposition in temperate grassland. – Soil Biol. Biochem. 60: 10–18.
Wardle, D. A. et al. 2004. Ecological linkages between aboveground and belowground biota. – Science 304: 1629–1633.
Wardle, D. A. et al. 2012. Linking vegetation change, carbon sequestration and biodiversity: insights from island ecosystems in a long-term natural experiment. – J. Ecol. 100: 16–30.
Worchel, E. et al. 2012. Fungal symbionts alter plant drought response. – Microbial Ecol. 65: 671–678.
Yahdjian, L. and Sala, O. 2002. A rainout shelter design for intercepting different amounts of rainfall. – Oecologia 133: 95–101.
Yuan, Z. and Chen, L. 2014. The role of endophytic fungal individuals and communities in the decomposition of *Pinus massonian* needle litter. – PloS ONE 9:e105911.