COMMUNITY ECOLOGY – ORIGINAL RESEARCH

Nitrogen addition and fungal symbiosis alter early dune plant succession

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Abstract
Anthropogenic nitrogen (N) enrichment can have complex effects on plant communities. In low-nutrient, primary successional systems such as sand dunes, N enrichment may alter the trajectory of plant community assembly or the dominance of foundational, ecosystem-engineering plants. Predicting the consequences of N enrichment may be complicated by plant interactions with microbial symbionts because increases in a limiting resource, such as N, could alter the costs and benefits of symbiosis. To evaluate the direct and interactive effects of microbial symbiosis and N addition on plant succession, we established a long-term field experiment in Michigan, USA, manipulating the presence of the symbiotic fungal endophyte *Epichloë amarillans* in *Ammophila breviligulata,* a dominant ecosystem-engineering dune grass species. From 2016 to 2020, we implemented N fertilization treatments (control, low, high) in a subset of the long-term experiment. N addition suppressed the accumulation of plant diversity over time mainly by reducing species richness of colonizing plants. However, this suppression occurred only when the endophyte was present in *Ammophila*. Although *Epichloë* enhanced *Ammophila* tiller density over time, N addition did not strongly interact with *Epichloë* symbiosis to influence vegetative growth of *Ammophila*. Instead, N addition directly altered plant community composition by increasing the abundance of efficient colonizers, especially C_4 grasses. In conclusion, hidden microbial symbionts can alter the consequences of N enrichment on plant primary succession.

Keywords *Ammophila* · *Epichloë* · Fungi · Mutualism · Nitrogen deposition

Introduction

Anthropogenic nitrogen (N) enrichment from agriculture, industry, wastewater, and fossil fuel combustion has more than doubled rates of nitrogen input into the terrestrial nitrogen cycle (Vitousek et al. 1997; Galloway et al. 2008; Fowler et al. 2013), which threatens terrestrial biodiversity and ecosystem functioning (Cleland and Harpole 2010; Su et al. 2022). One component of this enrichment, atmospheric N deposition, has increased nearly threefold in terrestrial systems from 1850 to the present day (Kanakidou et al. 2016), and is predicted to continue to increase during the coming decades (Lamarque et al. 2013; Ciais et al. 2013). Such N enrichment is a complex form of stress for plants because resource addition can have positive or negative effects. For example, N addition can increase plant productivity and fitness (Huberty et al. 1998; Xia and Wan 2008; Guo et al. 2022). Conversely, N inputs can decrease biodiversity due to increased dominance by highly competitive plant species (Stevens et al. 2004; Bobbink et al. 2010; Gross and Mittelbach 2017; Payne et al. 2017).

Reductions in plant community diversity under N enrichment have been well documented in secondary successional systems, such as old fields (Tilman 1987; Huberty et al. 1998; Crawley et al. 2005; Midolo et al. 2019), but much less studied in low-nutrient, successional ecosystems, where N impacts could be strongest (Sparrius et al. 2012). Nutrient inputs could affect foundational ecosystem-engineering plants during early succession, then cascade to alter plant community assembly and ecosystem function (Day et al. 2004, 2018). For example, atmospheric N deposition

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promoted the ecosystem-engineering grass, *Ammophila arenaria*, in dunes of the United Kingdom by increasing grass height and cover, which in turn was associated with decreased plant species richness (Jones et al. 2004).

Predicting how N enrichment affects early succession is more complicated if nutrients alter interactions between microbial symbionts and ecosystem-engineering plants. Increasing a limiting resource, such as N, commonly reduces the benefits of plant–microbe symbioses, particularly those in nutritional mutualisms (reviewed by Kivlin et al. 2013). Symbionts such as mycorrhizal fungi in plant roots can become parasitic with nutrient additions (Johnson et al. 2010; Hoeksema et al. 2010). Increased nutrients can also diminish the biomass of fungal partners by reducing carbon investments from the host plant (Hoeksema et al. 2010; Wipf et al. 2019). Additionally, N enrichment can suppress ecosystem-engineering plants such as nitrogen-fixing lupines, by selecting for less cooperative rhizobia mutualists and reducing plant fitness (Titus 2009; Weese et al. 2015). Alternatively, N addition can cause leaf endophytes, such as *Epichloë* spp., to increasingly benefit host plants as energetic costs of the symbiosis are reduced, and production of N-rich alkaloids that protect plants from herbivory are increased (Lane et al. 1997; Krauss et al. 2007; Hager et al. 2021). In successional ecosystems, such interactions with microbial symbionts could affect the dominance of ecosystem-engineering plants, indirectly changing facilitative or inhibitory interactions that influence rates or trajectories of early succession.

Coastal sand dunes may experience strong impacts of nutrient enrichment on succession through microbial symbioses due to their low nutrient availability and low diversity plant communities dominated by ecosystem engineers. Coastal habitats are threatened by N enrichment because of their proximity to major urban centers and hydrological connections to terrestrial watersheds via river outflows (Crossett et al. 2004; Howarth 2008; Malone and Newton 2020). The U.S. Great Lakes coastal region has high N enrichment due to agricultural, atmospheric, and point-source inputs (Morrice et al. 2008; National Atmospheric Deposition Program 2022). For example, atmospheric N deposition, especially the concentration of NH_4^+, into Great Lakes ecosystems have increased by 400% from historic levels (Du et al. 2014), while dissolved inorganic N in Great Lakes coastal wetlands has risen as a direct result of row-crop agriculture and urbanization in the region (Morrice et al. 2008; Harrison et al. 2020). Understanding potential changes to Great Lakes ecosystems is essential for conservation of these ecosystems in the future.

In the Great Lakes region and North Atlantic Coast, *Ammophila breviligulata*, American beach grass (hereafter *Ammophila*), is the primary dune ecosystem-engineering plant (Garguillo et al. 2004). *Ammophila* can facilitate colonization by other plant species by stabilizing sand and ameliorating harsh physical conditions (Cowles 1899; Zarnetske et al. 2012). However, *Ammophila* can also inhibit succession through plant competition (Cheplick 2005; Hastings et al. 2007), and *Ammophila* die-off is an important stage in dune succession (Maun and Lapierre 1984; Voesenek et al. 1998). Past work demonstrated that nutrient additions increased *Ammophila* growth (Day et al. 2004, 2018), which could alternatively promote abundance and diversity of later colonizers through sand stabilization, or reduce diversity through amplified plant competition. *Ammophila* also hosts the vertically transmitted, systemic fungal endophyte *Epichloë amarillans* (Drake et al. 2018), hereafter *Epichloë*. *Epichloë* is present in approximately one-third of *Ammophila* populations in surveyed Great Lakes sites (Emery and Rudgers 2014) and in nearly all commercially available plants used for restoration (Emery and Rudgers 2010). Our past experimental work showed that *Epichloë* benefited *Ammophila* during the first years of dune succession by increasing aboveground and belowground growth and increasing sand accumulation. Additionally, *Epichloë* presence in *Ammophila* reduced colonizing plant diversity, most likely due to increased *Ammophila* density (Emery et al. 2015; Rudgers et al. 2015; Bell-Dereske et al. 2017). Although *Epichloë* symbiosis has clear ecological impacts on early succession in dunes, the longer-term effects of symbiosis and potential for interactions with nitrogen enrichment remain unknown for this oligotrophic and ecologically important ecosystem.

To evaluate the direct and interactive effects of microbial symbiosis and N addition on dune succession, we used a long-term field experiment to ask: Does N addition alter plant community succession? And if so, is this due to any effects of N addition on growth of *Ammophila*, or through changes to the *Ammophila-Epichloë* symbiosis? We hypothesized that N addition would directly decrease plant community diversity by allowing the best competitors to colonize and dominate the community, as shown in secondary successional ecosystems (Foster and Gross 1998; Crawley et al. 2005). Alternatively, N addition could increase plant diversity by releasing colonizing plants from extreme N limitation and promoting their initial establishment. Indirectly, N addition could increase density of *Ammophila* and slow *Ammophila* die-off (Day et al. 2004), reducing the diversity of later colonizing species and slowing successional change. We expected that the presence of *Epichloë* would interact with N addition to enhance the dominance of *Ammophila* because prior studies on other *Epichloë* symbioses generally report benefits of N to the symbiosis (reviewed by Kivlin et al. 2013). Thus, we hypothesized that the combined effects of N addition and *Epichloë* would slow plant succession and suppress diversity even more than either factor alone.

Methods

Experimental design

In May 2010, we established a factorial field experiment on a bare dune blowout approximately 200 m from the shoreline of Lake Michigan in Leelanau State Park, Michigan, USA (45810.9640 N, 85834.5780 W). This location experiences annual precipitation of 817 mm/year and an average temperature of 7.6 °C (PRISM 2012). Common plants found within this parabolic dune complex are *Ammophila breviligulata, Schizachyrium scoparium, Calamavilfa longifolia, Cirsium pitcheri, Artemesia campestris* (Albert 2000). To manipulate the presence or absence of *Epichloë* in *Ammophila*, we used endophyte-free seeds collected from nearby dunes, germinated seedlings of *Ammophila* in the lab, and either artificially inoculated seedlings with *Epichloë* (E+) or sham inoculated them with sterile water (E-); see details in Emery et al. (2015). Plants were clonally propagated in a greenhouse and then transported to the field. In each of 90 2 m × 2 m plots, we transplanted 25 E+ or 25 E− *Ammophila* plants and monitored plots yearly from 2010–2020.

Because plants can sometimes lose endophyte symbionts over time (Rudgers et al. 2009; David et al. 2019), we used commercial immunoblot kits (Phytoscreen: Agrinostics, Watkinsville, GA) to assess treatment fidelity in two tillers per plot in 2019. Any inconclusive assay results were followed up with microscopy to confirm presence or absence of hyphae within leaf tissue. In 2019, 91% of E+ treatment plot tillers maintained evidence of *Epichloë* infection, while 89% of E− plot tillers still lacked *Epichloë*.

N fertilization treatments

During 2016, we introduced N fertilization treatments to a subset of 60 plots in the long-term experiment (30 E+ and 30 E− plots). One third of plots (10 each E+ and E−) received a low level of N (0.5 g NH_4^+ m^{-2}), corresponding to current atmospheric N deposition rates found near the Chicago, IL urban center (Du et al. 2014), another third received high levels of N (10 g NH_4^+ m^{-2}), comparable to other N addition experiments focused on releasing plants from nutrient stress (e.g., Borer et al. 2014), and the last set of plots received no added N (control). We added N as urea slow-release fertilizer (ESN Urea coated fertilizer: Nutrient Ltd., New Madrid MO), applied twice yearly, once in May at the beginning of the growing season, and again mid-season in July. Due to COVID-19 travel restrictions, we were unable to apply fertilizer in May 2020, but

treatments resumed as planned in July 2020. Every July from 2016 to 2020, we collected data on plant diversity and abundance in all plots. Specifically, we recorded *Ammophila* tiller density (tillers m^{-2}) and the number and identity of all non-*Ammophila* colonizing plant individuals across the entire plot to calculate total colonizer abundance, species richness, Shannon diversity (H'), and Shannon evenness (E') per plot for each year of data collection. Diversity and evenness measurements were density-based, calculated from counts of each individual per treatment plot. Species present in these plots, other than the initially planted *Ammophila*, all colonized naturally. Additionally, all treatment plots were randomized completely to avoid potential effects of biased distribution of seeds or propagules came from heterogeneous seed bank and rain.

Statistical analysis

To evaluate whether N addition and endophyte presence synergistically affected plant successional dynamics, we conducted plot level comparisons of plant community structure over time. First, we used general linear mixed-effects models to examine the responses of plant diversity (H'), species richness, community evenness (E') and *Ammophila* tiller density to the fixed effects of year, nitrogen fertilization treatment, and endophyte presence, with random effects of plot and spatial factors (row and column within the grid of 90 plots) using function lme in the <nlme> package (Pinheiro et al. 2017). Focusing on year × N fertilization × endophyte effects, we applied model selection based on the second-order Akaike information criterion (Burnham and Anderson 1998) to compare a simple model assuming independence of observations across years against models with temporal autocorrelation (either autoregressive 1 or autoregressive 2) (Pinheiro et al. 2017). *AICc* values were obtained with the MuMIn package (Barton and Anderson 2015), and results are reported from the model with the lowest *AICc*. We determined marginal R^2 values of the best models using rsquared in piecewiseSEM (Lefcheck 2016).

Second, we used PERMANOVA to analyze changes in community composition (Clarke and Gorley 2009). PERMANOVA models included fixed effects of year, nitrogen fertilization level, and endophyte presence or absence, and the random effect of plot. PERMANOVA used the Bray–Curtis distance metric with 9999 permutations under a reduced model with Type III SS. We then used pairwise tests to evaluate which treatment combinations differed significantly in plant community composition, with the specific prediction that the combined effects of N fertilization and endophyte presence would be non-additive. We visualized compositional differences in the abundance of plant species using nonlinear multidimensional scaling (NMDS) analysis. The community matrix was constructed with count data,

relating to plant species densities within treatment conditions. Third, heterogeneity of the community in response to the different treatment factors that were indicated as significant in the PERMANOVA was tested using PERMDISP (Anderson 2006), which examines whether treatments differ in the degree of dissimilarity of community composition among replicated plots (e.g., the spread in composition represented by the NMDS graph). Finally, a SIMPER analysis was used to rank plant species by their relative contributions to differences among treatment combinations in community composition in order to identify which species were the strongest drivers of change in the dune plant community (Clarke and Gorley 2009).

Results

Plant community diversity

Overall, nitrogen fertilization and endophyte presence interacted to suppress plant diversity (Table 1). Compared to control plots, plant community diversity (H') was 7% lower in the low nitrogen addition treatment and 17% lower under high nitrogen addition, but only if *Epichloë* was present (Fig. 1a, Online Resource 1). N additions did not significantly affect diversity in the absence of *Epichloë*. The decline in diversity was mainly due to decreased plant species richness, which was reduced by 20% under high nitrogen, but again only if *Epichloë* was present (Table 1, Fig. 1b, Online Resource 1). The response of plant community evenness (E') was more variable than that of species richness, and significantly declined under nitrogen fertilization only when *Epichloë* was absent (opposite to the response of richness) and then only in 2017 (Table 1, Fig. 1c, Online Resource 1).

Plant community composition

N fertilization shifted overall plant community composition, starting 2 years after the N addition treatments began (Table 1, Fig. 2). Low N communities differed from control treatment communities starting in 2018 (pairwise post-hoc tests not shown), and high N communities differed from control communities starting in 2017, although communities treated with high or low N addition did not differ from each other in any year. High N addition also increased heterogeneity (dispersion) in plant species composition among plots by 26% compared to controls, which had more consistent plant composition across the 10 replicate plots (Table 1). The SIMPER analysis indicated that changes in composition were due to grasses increasing and forbs declining in nitrogen addition plots (Online Resource 2). Specifically, *Schizachyrium scoparium* increased by 8%, *Calamovilfa*

Table 1 Statistical results from analysis of plant community composition (PERMANOVA), dispersion (PERMDISP), diversity (H'), evenness (E'), species richness, and *Ammophila* tiller density for the 5-year (2016–2020) sampling period in response to N fertilization and *Epichloë* endophyte presence

Factor	Composition		Dispersion		Diversity		Evenness		Richness		Tillers	
	Pseudo-F	P	Pseudo-F	P	X^2	P	X^2	P	X^2	P	X^2	p
Endophyte	0.78	0.57	NA	NA	3.22	**0.07***	1.85	0.18	1.25	0.263	11.42	**<0.001***
Fertilizer	3.55	**0.003***	12.03	**<0.001***	7.93	**0.02**	4.63	**0.09***	3.04	0.22	3.98	0.14
Year	21.81	**<0.001***	5.27	**0.004***	21.74	**<0.001***	7.72	0.10	53.76	**<0.001***	243.89	**<0.001***
Endophyte × Fertilizer	0.93	0.48	NA	NA	5.75	**0.05***	0.49	0.78	5.46	**0.06***	0.22	0.90
Endophyte × Year	1.53	0.41	NA	NA	8.98	**0.06***	7.84	**0.09***	5.86	0.21	3.13	0.54
Fertilizer × Year	1.07	**0.03***	3.69	**0.002***	4.87	0.77	4.34	0.83	4.27	0.83	16.1	**0.04**
Endophyte × Fertilizer × Year	0.9	0.65	NA	NA	7.75	0.46	16.45	**0.04**	1.78	0.99	9.31	0.32

Bold values indicate the statistical significance

Statistically significant results are indicated by an asterisk (*$p < 0.10$, **$p < 0.05$, ***$p < 0.01$)

Fig. 1 Temporal trends in **a** diversity (Shannon H'), **b** plant species richness, and **c** plant community evenness (E') from 2016 to 2020 for 2 m × 2 m plots in response to nitrogen fertilization treatments when the *Epichloë* endophyte was either absent (top graph) or present (bottom graph). Points are treatment means ± SE
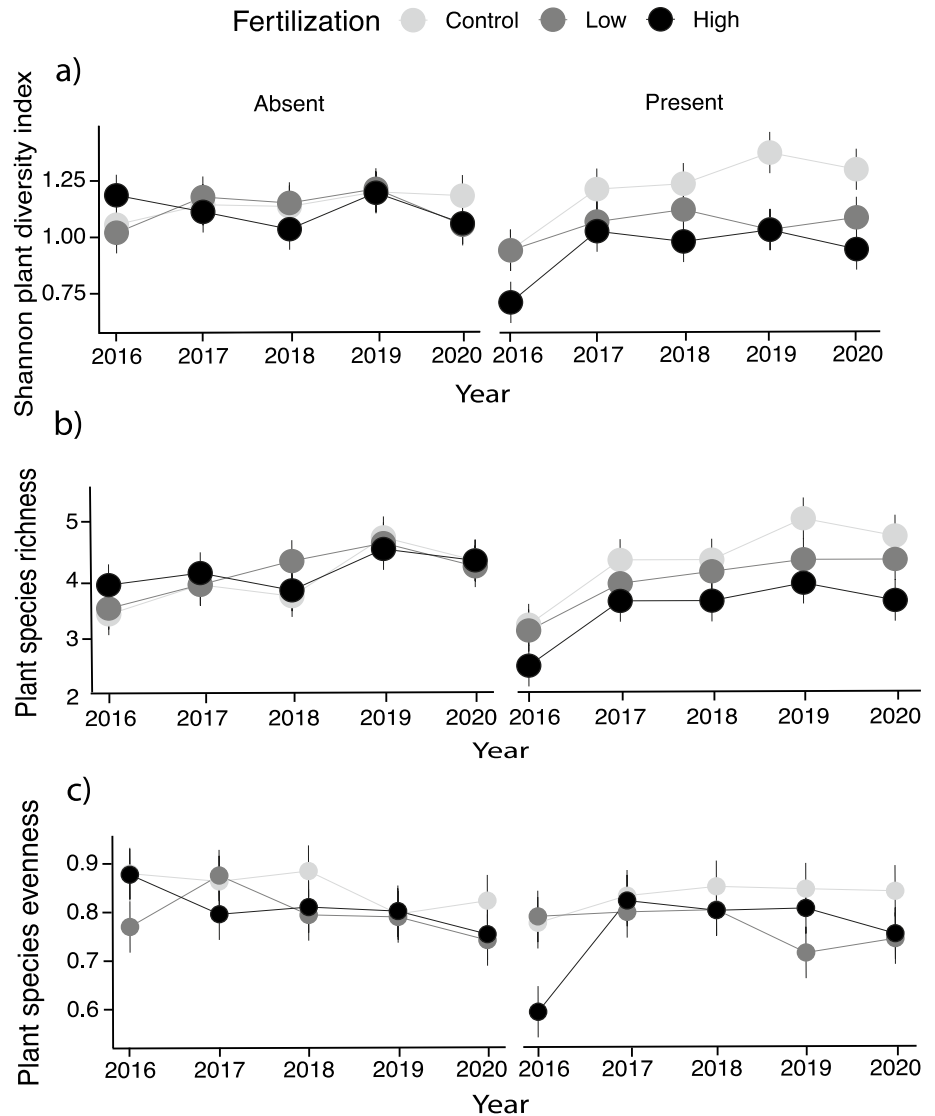
longifolia increased by 81%, and *Agropyron cristatum* increased by 121% with high N addition, while *Artemisia campestris* declined by 76%, and *Cirsium pitcheri* declined by 23%. *Epichloë* presence had no effect on community composition (Table 1).

Ammophila density

Ammophila density declined steadily from 2016–2020 (approximately 19% each year), corresponding to the *Ammophila* die-off that occurs during later stages of dune succession (Fig. 3). Increased resource availability in the form of N addition only altered tiller density in one of the 5 years of the study (Table 1, Fig. 3, Online Resource 1). In 2018, low and high N addition increased tiller numbers by 24–25%. Effects of N addition were non-significant in 2016, 2017, 2019, and 2020 (Online Resource 1). Despite small N fertilization effects, *Ammophila* maintained 19% higher

tiller density on average over time when *Epichloë* was present (Fig. 3). There were no significant interactive effects of endophyte presence and N addition (Table 1).

Discussion

N and *Epichloë* effects on plant community succession

N addition in this freshwater dune system suppressed plant species diversity over time mainly by reducing species richness of the colonizing plant community. However, these effects occurred *only* when the systemic foliar endophyte *Epichloë* was present in *Ammophila*. Our results from this early successional system are consistent with the observation that high levels of N addition that release plants from nutrient stress reduce plant species richness and evenness of later

Fig. 2 Nonmetric multidimensional scaling plot displaying plant community composition means and standard deviations for nitrogen fertilization treatments. 2D stress = 0.19. Ellipses represent standard deviation of point scores for each treatment interaction. Abbreviations represent plant species where AgC is *Agropyron cristatum,* ArC is *Artemisia campestris*, SS is *Schizachyrium scoparium*, CP is *Cirsium pitcher*, and CL is *Calamovilfa longifolia*
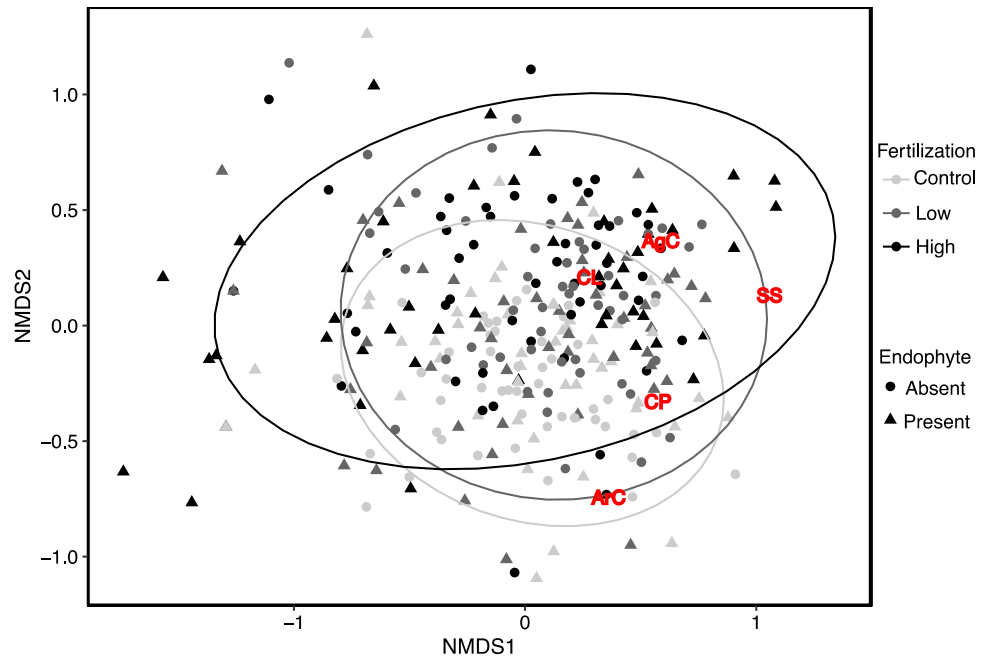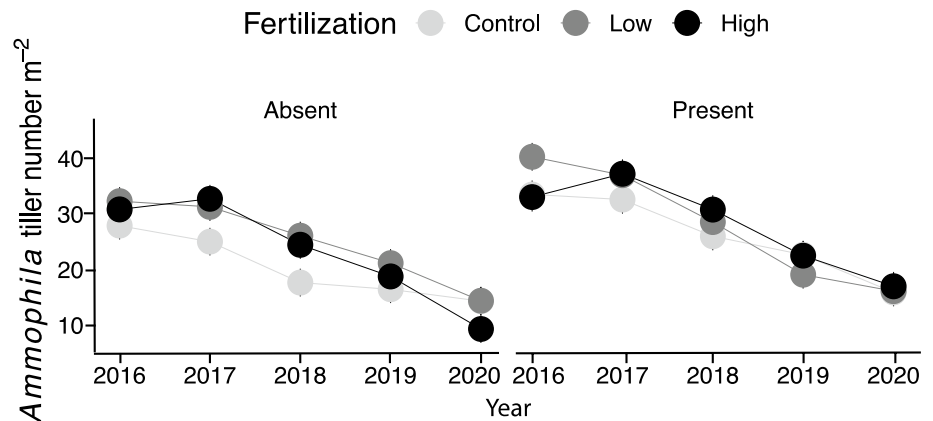

Fig. 3 Temporal trend in tiller density of *Ammophila breviligulata* from 2016–2020 in response to nitrogen fertilization treatments when the *Epichloë* endophyte was either absent (top graph) or present (bottom graph). Points are treatment means ± SE

successional plant communities (Bobbink et al. 2010; Borer et al. 2017; Seabloom et al. 2021). Low levels of N addition, corresponding to more realistic atmospheric N-deposition scenarios, have had mixed effects on plant communities in prior studies (Clark and Tilman 2008; Humbert et al. 2016; Midolo et al. 2019). Therefore, it is notable that even the low N addition treatment in our experiment significantly reduced plant community diversity, indicating that even small increases in anthropogenic N deposition may alter the trajectory of succession in dunes where *Epichloë* is present. This interaction may be due to the suppressive effect that *Epichloë* presence had on colonizing plant diversity in the initial years of this experiment, before N addition treatments started (Rudgers et al. 2015). This is particularly relevant in the context of dune restoration since commercial *Ammophila* plants used in many restorations in both the Great Lakes

region and the Eastern US have *Epichloë* infections, while the majority of natural Great Lakes populations lack symbiosis with *Epichloë* (Emery and Rudgers 2010, 2014). Alternatively, symbioses *Epichloë* are known to enhance secondary metabolite production within plant hosts, specifically alkaloids. Alkaloids are nitrogen-based chemicals, therefore increased nitrogen could increase host production of these secondary metabolites (Lane et al. 1997; Krauss et al. 2007) which could in turn suppress the establishment of later colonizing species.

N fertilization at either high or low levels had direct effects on plant community *composition*, independent of whether *Epichloë* was present, which contrasts against the interaction between N addition and endophyte symbiosis on plant *diversity*. This was in part due to N addition increasing the spatial heterogeneity of plant communities, causing

replicate plots to diverge more in species composition over time. Decreased species richness paired with increased local heterogeneity (dispersion) is a common result of fertilization studies, especially in low-productivity systems (Chalcraft et al. 2008) and this may contribute to temporal stability of these ecosystems (Zhou et al. 2019; Zhang et al. 2019). It is possible for side-effects of fertilization, such as reduced soil pH, to act as environmental filters decreasing plant community heterogeneity in low-productivity systems (Liu et al. 2021). However, changing soil pH is a natural part of early succession in these dunes systems as carbonate minerals dissolve (Lichter 1998). There can be complicated relationships between fertilization, diversity, and time in successional systems (Inouye and Tilman 1995), and more work is needed to understand the consequences of changes in community heterogeneity for successional dynamics.

Additional changes in community composition in response to N addition were due to changes in the abundances of particular species. Two native C4 grass species, *Schizachyrium scoparium* and *Calamovilfa longifolia*, and one non-native C3 grass species, *Agropyron cristatum*, increased under N addition, while two native forbs declined (*Artemisia campestris* and *Cirsium pitcheri*). The increase in grass abundance under N addition may suggest that N reduced the intensity of competition for nutrients with *Ammophila*, especially for the most functionally similar grasses. This phenomenon is well-supported by other studies. In a meta-analysis conducted for grasslands, N addition similarly increased the biomass of grasses and decreased that of forbs (You et al. 2017). Likewise, in secondary successional grasslands, N addition increased growth of C4 grasses and tall clonal species at the expense of C3 grasses and forbs (Suding et al. 2005; Clark et al. 2007; Gross and Mittelbach 2017). N addition was specifically associated with increased abundance of the late-successional grass *Schizachyrium scoparium* in Great Lakes oak-savannah/sand-prairie systems as well (Bird and Choi 2017). In the Great Lakes dunes, both *Schizachyrium scoparium* and *Calamovilfa longifolia* are important successional species, replacing *Ammophila* as dunes stabilize (Olson 1958). However, increased dominance of these grasses in areas with high N enrichment may drive down the abundance of forbs which provide unique biodiversity in this system. In particular, *Cirsium pitcheri* is a federally threatened species that is endemic to Great Lakes dunes and thrives in habitats with moderate levels of sand burial (Pavlovic et al. 1998; Rowland and Maun 2001). This species is already threatened by lakeshore erosion, human disturbance, and climate change (Staehlin and Fant 2015). While this species is known to respond positively to N fertilizer in a greenhouse context (Rowland and Maun 2001), increased dominance by later-successional grass species in response to N deposition appears to inhibit *Cirsium pitcheri* germination and growth in realistic field settings.

N addition is also known to increase the abundances of non-native plant species in communities, which may explain the increase in *A. cristatum* abundance. *Agropyron cristatum* is a non-native grass that is invasive in western US rangelands (Christian and Wilson 1999), and is naturalized in dune habitats in Michigan. It was the only non-native plant species commonly present in our site. Resource availability has been demonstrated to play a critical role in non-native plant invasion in multiple ecosystems (Davis et al. 2000; Borer et al. 2017). For example, cover and dominance of exotic grass species in desert environments increased with increased soil N (Brooks 2003; Barrows et al. 2009). Native species that are adapted to low-nutrient conditions may be outcompeted by more efficient non-native species when soil nutrient levels increase (Brooks 2003; Rickey and Anderson 2004). Such increases in non-native species can overwhelm changes in abiotic conditions (Craig and Henry 2022) and may further alter successional trajectories in the future (Marshall 2021). Managers should take care to prevent the establishment of more noxious non-native dune species such as *Gypsophila paniculata*, *Leymus arenarius*, or *Centaurea stoebe* in this site (Emery and Rudgers 2012; Emery et al. 2013; Reid and Emery 2017).

N and *Epichloë* effects on *Ammophila* growth

N addition had no consistent effect on the tiller density of *Ammophila*. Several studies report that fertilizer addition can increase *Ammophila* tiller number, both in incipient foredune populations and in declining populations (Augustine and Sharp 1969; Seliskar 1995; Boudreau and Houle 2001; Day et al. 2004, 2018), but we found significant N effects in only 1 year of our study (2018). In most study years, N had no significant effect on *Ammophila* density. It may be that *Ammophila* responses to N in our system depend on climate. The year 2018 had intermediate precipitation (904 mm; http://prism.oregonstate.edu). In wet years such as 2019 (1061 mm) and 2020 (973 mm), high rainfall may leach N quickly from plant rooting zones and thereby reduce the influence of N application, similar to what has been shown in some agricultural systems (Peng et al. 2011). In drier years such as 2016 (822 mm) and 2017 (886 mm), plants may be water stressed and unable to use additional N because water limitation dominates (He and Dijkstra 2014). More work is needed to understand how N deposition and climate change might interact to affect plant communities in the future (Komatsu et al. 2019; Kazanski et al. 2021).

Unlike N treatment effects which varied among years, *Epichloë* was associated with consistently increased *Ammophila* tiller density during the last 5 years of our long-term study, most likely as a carry-over of the initial benefits to *Ammophila* growth and survival provided during early years of the experiment (Emery et al. 2015). However, the

rates of decline in tiller abundance during the past 5 years were similar across the two endophyte treatments, indicating that *Epichloë* provided little additional benefit to host plants during *Ammophila* die-off, in contrast to at least one other study of *Epichloë* effects on long-term host plant fitness and decline (Moore et al. 2019). Thus, we conclude that *Epichloë* is most important in early stages of host plant growth in this ecosystem where *Epichloë* plays a crucial role in earlier stages of succession by promoting tillering (Emery et al. 2015) before *Ammophila* die off begins.

Conclusion

To our knowledge, this is the first field study to assess the long-term effects of N addition and grass-endophyte symbiosis on early succession. Although *Epichloë* enhanced *Ammophila* tiller density over time, N addition did not strongly interact with *Epichloë* symbiosis to influence vegetative growth of *Ammophila*, which is notable given the limited number of short-term studies that have found N to increase benefits of fungal symbioses (Kivlin et al. 2013). Instead, N addition directly altered plant community composition by increasing the abundance of efficient colonizers, especially C4 grasses. N addition also suppressed the accumulation of plant diversity over time, though only when the endophyte was present in *Ammophila*. Our results suggest that early growth of ecosystem-engineering species may interact with N addition to affect later community succession. Additionally, high-value endemic species, such as *C. pitcheri*, may be particularly susceptible to N deposition in Great Lakes dune ecosystems.

Declarations

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