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REGULAR ARTICLE

Leaf endophytes mediate fertilizer effects on plant yield and traits in northern oat grass (*Trisetum spicatum*)

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Abstract
Aims Symbiotic fungi commonly increase plant acquisition of soil nutrients. Because prior work has focused on root fungi, we examined how leaf endophytes (*Epichloë*) influenced plant responses to fertilization and altered plant traits that may cascade to food webs and ecosystem processes.

Methods We manipulated endophyte presence/absence in two populations of *Trisetum spicatum*, a wild relative of oat, under a 2 × 2 addition of soil nitrogen (N) and phosphorus (P) in the greenhouse.

Results Endophyte symbiosis altered how plant biomass responded to soil N and how plant traits responded to soil P. Endophytes boosted the biomass gains from N-fertilization in one population. Plants from a second population had weak benefits of symbiosis, but the endophyte altered plant traits, by increasing specific leaf area under P-fertilization, root diameter under low P, and concentration of the fungal alkaloid AcAP under N fertilization. Endophyte presence suppressed the typically observed increase in root hair density in response to soil P limitation. Under low P, symbiotic plants from both populations had improved forage quality relative to symbiont-free plants, although N-fertilization had a larger effect size on forage quality than did symbiosis. Finally, the two populations differed in production of fungal alkaloids, which generally increased in response to fertilization.

Conclusions Predicting how microbial symbionts mediate plant acquisition of nutrients requires understanding how much their effects vary among plant and endophyte genotypes. Here, the magnitude and direction of leaf symbionts' effects on plant yield and traits varied between populations and with soil nutrient availability.

Keywords *Epichloë* · Mutualism · Nitrogen · Phosphorus · *Poaceae* · Mountain ecosystem

Introduction

Symbiosis with fungi is a common plant strategy to increase nutrient uptake (Smith and Read 2008), which could reduce the need for anthropogenic fertilizer inputs. Human additions of nitrogen (N) and phosphorus (P) are major threats to ecosystems worldwide, and reducing these inputs can help mitigate anthropogenic

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disturbances (Gruber and Galloway 2008). While most prior work has focused on root fungi as drivers of plant resource acquisition (e.g., reviewed by Newsham 2011; Smith and Read 2008; van de Wiel et al. 2016), many plants also host fungal endophytes in leaves (Rodriguez et al. 2009). These aboveground symbionts have received less attention than root symbionts for potential benefits to plant nutrition. Studies in wild relatives of crops could be useful for uncovering novel ecological functions of foliar endophytes for agricultural applications.

Prior evidence suggests that foliar fungal endophytes can promote mineral acquisition in grass hosts. First, several previous studies on endophytes in the *Epichloë* genus (family Clavicipitaceae, phylum Ascomycota) have reported that plants with a symbiotic endophyte have greater tissue nutrient content than those without an endophyte. For example, endophyte-symbiotic red fescue (*Festuca rubra*) and wild barley (*Hordeum brevisubulatum*) had greater N and P content than endophyte-free plants (Song et al. 2015; Vazquez-de-Aldana et al. 2013). Similarly, Chinese rye grass (*Leymus chinensis*) with an *Epichloë* sp. had higher photosynthetic N-use efficiency than naturally endophyte-free plants (Ren et al. 2014). Second, endophyte benefits are sometimes stronger under soil nutrient deficiency than when nutrients are less limiting, suggesting endophyte-mediated enhancement of plant nutrient acquisition. For example, an endophyte increased root biomass in tall fescue grass (*Schedonorus arundinaceus*, syn. *F. arundinacea*) under P deficiency (Malinowski and Belesky 1999). In perennial ryegrass (*Lolium perenne*), endophyte presence increased plant biomass more when soil N content was low than when it was high (Lewis 2004). In the same species, endophyte presence increased P-use efficiency, but not P uptake rate, under P deficiency (Ren et al. 2007). However, in some prior studies, the lack of experimental manipulation of endophyte presence has precluded assignment of causality to the endophyte. Reported differences could be caused by plant genotype, rather than by endophyte symbiosis, and other studies have detected no influence of foliar endophytes on plant nutrient acquisition. For example, fertilizer (N, P, K) addition resulted in greater plant biomass regardless of endophyte status in tall fescue *(Schedonorus pratensis* syn. *F. pratensis*) and red fescue, *F. rubra* (Ahlholm et al. 2002b). Also, Ren et al. (2009) reported that symbiotic *L. perenne* in nitrogen-rich soil gained larger shoot biomass and higher levels of soluble sugars than endophyte-free plants in the same rich soil, suggesting nutrient limitation of endophyte benefits, rather than endophyte-mediated nutrient uptake. Experimental manipulation of endophyte presence/absence can help to improve understanding of the causes of observed differences in nutrient acquisition in these complex partnerships.

The mechanisms underlying *Epichloë*-mediated nutrient acquisition are not fully resolved. Thus far, proposed mechanisms for foliar endophyte-mediated nutrient uptake include altered root architecture and changes in root exudate production or composition. For example, under soil P deficiency, endophyte-symbiotic tall fescue grass produced 17% longer root hairs and 11% narrower root diameters than plants with endophytes removed (Malinowski et al. 1999). Similarly, symbiotic perennial ryegrass exposed to soil P-deficiency made longer roots than endophyte-free plants (Ren et al. 2007); changes in root architecture were also confirmed for plants grown in agar, but additionally depended on the source of P (Ding et al. 2015). Endophyte-presence increased total organic carbon and total phenolics in the root exudates of endophyte-symbiotic tall fescue (Guo et al. 2015), although the magnitude of the effect depended on plant genotype. It is possible that endophyte-mediated changes to physiological plant traits additionally affect host interactions with soil microbes in ways that further promote nutrient acquisition (e.g., Bell-Dereske et al. 2017; Chu-chou et al. 1992; Garcia-Parisi et al. 2017). Consideration of the collective genotype of the symbiota (plant + endophyte) could enhance understanding of the context-dependency of nutrient acquisition.

Here, we addressed gaps in understanding of the belowground effects of foliar endophytes using experimental methods in a wild grass-endophyte system. First, we investigated the interactive effects of co-limiting nutrients. Although prior studies have investigated the influence of soil nutrients on endophyte-symbiotic grasses under drought (Ren et al. 2014), herbivory (Krauss et al. 2007), and other environmental factors (Ahlholm et al. 2002a; Vazquez-de-Aldana et al. 2013), studies investigating interactive nutrient combinations have been rare. For example, Li et al. (2012) studied the combined effects of N and P on *Achnatherum sibiricum*. Under nitrogen addition, symbiosis with an *Epichloë* sp. improved acid phosphatase activity enough that plant biomass did not respond to phosphorus deficiency and surpassed that of endophyte-free plants. These results suggested that both N and P may influence the magnitude of endophyte-mediated benefits to plants. Second, understanding whether endophytes alter plant traits is an important step in identifying the mechanisms underpinning foliar endophyte symbioses. Few fertilization studies have examined changes in plant traits other than tissue nutrient content (but see, e.g., Malinowski et al. 1999). For example, detailed analyses of forage quality traits could improve understanding of the consequences for consumers, food web dynamics, and decomposition rates (Lemons et al. 2005). Third, most prior studies have focused on a few agronomic species, with less attention to endophyte-mediation of nutrient acquisition in other taxa (reviewed by Cheplick and Faeth 2009; Omacini et al. 2012).

Here, we assessed yield and plant traits in wild populations of *Trisetum spicatum*, subjected to additions of nitrogen and phosphorus and to experimental removal of two of its natural endophytes. *Trisetum spicatum* is native to the Americas and, to our knowledge, is the closest relative to oat (*Avena sativa*) reported to host foliar endophytes in the *Epichloë* clade. Research on this previously unstudied *Epichloë* host could provide insight into the type of symbiosis that would reduce the need for fertilizer inputs into agroecosystems. We compared plant growth and biomass, physiological traits, and plant nutrient status between two Colorado populations of *T. spicatum* that hosted different *Epichloë* symbionts, in order to address the following questions. *(1) Does foliar endophyte symbiosis ameliorate soil nutrient limitation in plant hosts?* Based on prior research, we predicted that endophyte presence would reduce P limitation, such that biomass differences between E+ and E- plants would be smaller under the addition of inorganic P. Because *Epichloë* spp. produce N-rich alkaloids, we predicted the opposite result for N addition, for which we expected greater biomass differences between E+ and E- plants under N addition than in controls. High N availability may offset the costs of endophyte symbiosis. *(2) Does endophyte symbiosis alter traits associated with nutrient or carbon acquisition?* Based on prior studies, we predicted that endophyte presence would alter root architecture, and that plant investment in nutrient-acquisitive traits (e.g., dense root hairs) would decline with fertilization and endophyte symbiosis. In contrast, we expected investment in carbon-capture traits (e.g., high specific leaf area, anti-herbivore alkaloid production) would increase with fertilization and with endophyte symbiosis due to reduced constraints imposed by nutrient limitation. *(3) Do genotypes of symbiota (plant + endophyte) differ in their responsiveness to soil nutrients?* We tested the degree to which two genetic combinations of plants and endophytes, which differed in their fungal alkaloid chemistry, varied in responsiveness to N and P fertilization.

Materials and methods

Study system

Trisetum spicatum (Poaceae) is a perennial bunchgrass with a wide geographic distribution in mountain ecosystems (Barkworth et al. 2007). We compared the performance of two populations in the Rocky Mountains of western North America: Niwot Ridge in the Colorado Front Range, east of the Continental Divide (40.050278 N, 105.570278 W − 40.060556 N, 105.600556 W, elevation 3297 m–3544 m) and Cottonwood Pass on the western slope of the Continental Divide (38.996143 N, 106.424449 W − 38.84635019 N, 106.4246687 W, elevation 3445 m). Endophyte prevalence at Niwot Ridge was 90% of bulk seed collected. At Cottonwood Pass, prevalence was 17% of field-collected plants (Ranelli et al. 2015), and the endophyte was detected in 86% of seeds collected from nine plants that were endophyte-positive in the field. We used a PCR assay to determine the presence and genotype of the endophyte in each population (Electronic Supplementary Material, Appendix 1).

Endophyte manipulation

To remove the endophyte, we placed field-collected, endophyte-symbiotic seeds into a convection drying oven for 7 d at 60 C. To confirm treatment effectiveness, we scored the endophyte status of each plant microscopically by examining thin sections of the inner leaf sheath from one tiller per plant, staining with aniline blue lactic acid, and scoring at 200× magnification following Bacon and White (1994). At harvest, we again confirmed endophyte status by removing a 1–2 cm section from the basal pseudostem of one tiller from each plant. Each sample was lyophilized, then DNA was extracted using the QIAGEN MagAttract 96 DNA Plant Core Kit. A PCR assay using multiplex 1 (Charlton et al. 2014) that tested for the presence of the genes *tefA*, *perA*, *lolC*, *dmaW* and *idtG* was used to determine the endophyte status of each plant (Electronic Supplementary Material, Appendix 1). The PCR results for endophyte-symbiotic samples at harvest were consistent with the expected endophyte genotype from the Cottonwood Pass and Niwot Ridge samples, the endophyte was detected in all endophyte-present plants, and all endophyte-removed samples lacked amplification.

Experimental design

We imposed a 2 × 2 × 2 × 2 factorial manipulation of the source population (Niwot Ridge vs. Cottonwood Pass), endophyte presence (present vs. removed), nitrogen addition (added vs. not), and phosphorus addition (added vs. not) with $n = 30$ individual plants per treatment combination, with a total of 480 plants. Following experimental endophyte removal, we planted seeds onto the soil surface of 53 cm × 27 cm × 7 cm deep plastic flats filled with potting mix containing vermiculite, Canadian sphagnum peat moss, bark ash, dolomitic limestone, and a proprietary wetting agent (MetroMix 360, Sun Gro Horticulture, Bellevue, WA). In early November 2014, flats were placed on a mist bench and maintained under greenhouse conditions of ~24 °C with 12 h daylight. We transplanted ~5 cm tall seedlings into 23 cm × 10 cm × 10 cm deep plastic pots filled with MetroMix 360. On 26 December 2014, immediately prior to nutrient treatment application, we split each plant into four clones by gently separating subsets of ~6 tillers per clone. Each clone was repotted into 10 cm square plastic pots filled with the same potting mix, and randomly assigned to one of the 2 × 2 nutrient addition treatments, described below. All pots were watered daily with drip emitters.

Nutrient additions

We applied 20 mL of deionized (DI) water per week to control treatment plants. Each week, nitrogen-treated plants received 10 mL of DI water and 10 mL of nitrogen fertilizer (concentration 8.22 g urea per 2.5 L tap water), phosphorus-treated plants received 10 mL deionized water and 10 mL phosphorus fertilizer (4.2 g Triple Super Phosphate per 2.5 L tap water; Hi-Yield, Bonham, Texas, USA), and nitrogen/phosphorus-treated plants received 10 mL nitrogen plus 10 mL phosphorus. Levels of fertilizers were chosen to fall within the range of additions in prior studies with *Epichloë*-symbiotic grasses (Li et al. 2012; Malinowski and Belesky 1999; Malinowski et al. 1998; Newman et al. 2003; Ren et al. 2007, 2009, 2014), and were not intended to mimic rates of N-deposition or agronomic fertilizer application rates. We began fertilizer treatments on 26 Dec 2014 and applied these weekly until harvest on 24–27 Feb 2015. Plants were supplemented with equivalent amounts of additional water as needed between fertilizer applications.

Plant growth and biomass

Every 3 weeks, we counted tiller number and measured the length and width of the longest live leaf to the nearest 0.1 cm to non-destructively estimate plant growth. We took four measurements (26 December, 16 January, 6 February, 20 February); the final measurement occurred immediately prior to harvest. To harvest, we rinsed soil from roots placed over a 1 mm sieve and separated above- and below-ground tissue by clipping with scissors. Plant biomass was dried at 60 C for 2 d in a convection oven, then weighed to the nearest 0.0001 g.

Leaf traits

We assessed three aboveground traits: leaf blade surface area, specific leaf area (SLA), and leaf dry matter content (LDMC), following methods of Perez-Harguindeguy et al. (2013). We collected three fresh, fully expanded non-senesced leaves per plant, placed them into individual 10 mL centrifuge tubes containing DI water and saturated them for 3 h in the dark. Following hydration, we measured leaf area using a flatbed scanner (Canon, Melville, New York). After scanning, we blotted each leaf dry with a paper towel, weighed wet mass to the nearest 0.0001 g, then dried leaves in a convection oven at 60C to obtain leaf dry mass.

Root traits

On 24–27 February 2015, we harvested ~0.36 g wet weight of living roots from each plant. We took digital images using a Leica DFC-480 camera on a Leica MZ12 dissecting microscope with a 50 mm field of view (Leica Microsystems, Wetzlar, Germany). From these images, we measured the diameter (mm) of ten randomly chosen roots using ImageJ analysis software (Schneider et al. 2012). Then, fine roots were permanently mounted onto glass microscope slides with polyvinyl-lactoglycerol. We took digital images at 200× magnification on a Leica DM 2500 compound microscope. The length (mm) of three randomly chosen, intact root hairs, was measured using the measure tool in ImageJ (Schneider et al. 2012) by tracing the root hair tip to its intersection with the main root. Root hair density was evaluated at 200× magnification as the number of root hairs per mm length of root.

Plant nutritional quality

Forage quality analysis was performed on dry leaf biomass collected at harvest. To obtain sufficient biomass for analysis, we combined aboveground biomass from three randomly selected individuals within treatment combinations to reduce the total number of samples from 480 to 160. Dried samples were ground in a Wiley mill (Thomas Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen. Samples were analyzed for nutrient composition using the Foss 6500 near-infrared reflectance spectrophotometer (NIRS) and analyzed with the FOSS ISIScan software (FOSS NIRSystems, Laurel, MD) with prediction equations developed by the NIRS Forage and Feed Testing Consortium (Hillsboro, WI). The forage quality components were crude protein (CP), dry matter content, acid detergent fiber (ADF), neutral detergent fiber (NDF), relative feed value, total digestible nutrients, lignin content, in vitro dry matter digestibility (IVDMD), ash content, and digestible neutral detergent fiber (dNDF). We also collected data on leaf tissue % by dry mass for phosphorus, nitrogen, potassium, calcium, and magnesium using calculations from NIRS data (see Kering et al. 2013; Pittman et al. 2016; Rogers et al. 2014).

Fungal alkaloid chemistry

We quantified endophyte-produced alkaloids on the bulked, ground dry leaf biomass (described above for forage quality analysis). Due to the expense of alkaloid analyses, we randomly chose 5–7 bulked samples per population × fertilizer combination, focused on the treatment with the endophyte present. Samples that were endophyte-free or from a population lacking the genetic capacity to produce the alkaloid (e.g., aminopyrrolizidine in Cottonwood Pass), were run as controls, and alkaloids were below the threshold for detection in all controls. Peramine and chanoclavine were extracted in 50% 2-propanol according to the method of Rasmussen et al. (2011) with the following modifications for detection as reported in Shi et al. (2018). Separation of the alkaloids was performed on an Agilent LC 1290 infinity with a BEH column (Waters, Milford, MA) of 2.1 mm Å~ 150 mm, C18 stationary phase, particle size = 1.7 μm, using 5 mM ammonium acetate (solvent A) and acetonitrile (solvent B) with a linear gradient of (95% A:5% B to 50% A:50% B) at a flow rate of 0.35 mL/min, at 40C in positive mode. The detection of peramine was based on the presence of 248 *m*/*z* MS1 ion and 206 *m*/*z* MS2 ion with a retention time of 5.3 min. The detection of chanoclavine was based on the presence of 257 *m*/*z* MS1 ion and 226 *m*/*z* MS2 ion with a retention time of 7.6 min. Aminopyrrolizidine analyses were performed on 100 mg lyophilized leaf tissue from the Niwot Ridge population using GC (Varian CP-3800, Agilent Technologies) equipped with MS (Varian Saturn 2200, Agilent Technologies), as described previously (Faulkner et al. 2006; Pan et al. 2014). Quinoline was used as an internal standard. The only aminopyrrolizidine detected in any *Trisetum spicatum* plants was *exo*-1-acetamidopyrrolizidine (AcAP), which eluted at 12.2 ± 0.20 min, with parent ion peak (m/z 169) and the characteristic fragment ions at m/z 83 and m/z 108.

Statistical analysis: plant biomass, traits, chemistry

Data were analyzed with mixed effects general linear models implemented in SAS (v. 9.1.3, Cary, NC). Models examined separately the responses of change in tiller number (initial to final), aboveground biomass, and belowground biomass. Each model included the fixed effects of the endophyte treatment, nitrogen treatment, phosphorus treatment, and source population, including all interaction terms as well as the random effect of plant genotype nested in endophyte treatment × source population. We decomposed significant treatment interactions using planned orthogonal contrasts for pairwise contrasts that specifically tested how endophyte presence modified the plant response to nutrient additions. We also used mixed effects models to examine responses of individual plant traits, leaf tissue % N and % P, as well as fungal alkaloid production specific to each population. We also used ANCOVA to explore whether treatments altered relationships between plant traits and biomass production, by testing for trait × treatment interaction effects.

Statistical analysis: plant nutritional quality

Due to the large number of traits measured, we used perMANOVA (Primer v. 6.1, Clarke and Gorley 2009) to test how the endophyte, fertilization, and source population affected the suite of forage quality traits. We normalized traits so that they were analyzed on comparable scales, and ran analyses with a Euclidean distance metric and 9999 iterations. The model included the fixed effects of source population, endophyte presence, nitrogen, and phosphorus, along with all interactions. Following perMANOVA, we visualized treatment effects using non-metric multidimensional scaling analysis (Primer v. 6.1, 9999 iterations, 500 restarts) (Clarke and Gorley 2009). A two-dimensional solution had low stress (=0.14), and stress converged with the number of iterations. Following perMANOVA, we used SIMPER analysis (Primer v. 6.1) to understand which traits contributed most to differences among treatments.

Results

Endophyte variation in *Trisetum spicatum* populations

The endophytes from Cottonwood Pass and Niwot Ridge grouped within the genus *Epichloë* in the maximum likelihood tree generated from *tefA* gene sequences (Supplementary Material, Appendix 1, Fig. S1). However, the two populations of *T. spicatum* were host to different endophyte species, an interspecific hybrid (*E. elymi* × *E. typhina* subsp. *poae*) in Cottonwood Pass and a non-hybrid, *E. amarillans* in Niwot Ridge (Supplementary Material, Appendix 1, Fig. S2). Each endophyte produced a different repertoire of alkaloids as indicated by the presence of genetic markers for alkaloid capability and by direct analysis of alkaloids (Supplementary Material, Appendix 1, Fig. S3). All Cottonwood Pass samples had the same endophyte genetic profile: all *perA* (peramine) markers were detected, as well as markers *dmaW* and *easC* (ergot alkaloid), and *idtQ* (indole diterpene). Based on this genetic profile, we predicted and confirmed the Cottonwood Pass endophytes would produce peramine and the ergot alkaloid chanoclavine. Although *idtQ* was detected, no other *IDT* genes were present, which indicated an inability to produce indole-diterpenes (Supplementary Material, Appendix 1, Fig. S2). The Cottonwood Pass endophyte also had both mating type genes (*mtAC* and *mtBA*), further suppoting that it was a hybrid species (Supplementary Material, Appendix 1, Fig. S2–3). In plants from Niwot Ridge, all *perA* markers were detected as well as *lolC* and *lolA* (lolines), and *easC and cloA* (ergot alkaloids). Although we predicted both peramine and an intermediate within the loline pathway, AcAP would be produced, the only alkaloid detected in endophyte-infected Niwot Ridge samples was AcAP (Supplementary Material, Appendix 1, Fig. S2–3). The Niwot Ridge endophyte contained the ergot alkaloid markers *easC* and *cloA*, but was unable to make ergot alkaloids as the samples lacked *dmaW*, which encodes the first step in ergot alkaloid biosynthesis. The mating type for the Niwot Ridge samples was *MTB* (only the *mtBA* gene was present).

Plant biomass and growth

All plants in the E+ treatment had the endophyte, both at the beginning of the experiment (microscopy) and the end of the experiment (PCR), and all plants in the E- treatment lacked the endophyte. Aboveground plant biomass was generally responsive to additions of nitrogen, but not to phosphorus. However, the two populations of symbiota differed in the sensitivity of aboveground production to endophyte presence and nutrient addition, as indicated by significant population × nitrogen × endophyte interactions for both shoot biomass and tiller production (both $P = 0.04$, Table 1). In the Cottonwood Pass population, nitrogen fertilization increased aboveground biomass of endophyte-bearing plants by 36% and increased tiller production by 32%. Nitrogen addition similarly increased aboveground biomass of endophyte-free plants, by 22% and increased tiller production by 23% (Fig. 1a and b, Table 1). However, the addition of nitrogen to endophyte-bearing plants from Niwot Ridge caused non-significant increases in shoot biomass (12% more) and tiller production (6%), whereas nitrogen fertilization caused endophyte-free Niwot Ridge plants to produce significantly more shoot biomass (23%) and tillers (39%) relative to unfertilized controls (Fig. 1a and b; Table 1). Thus, nitrogen addition increased aboveground biomass in all cases except for in the endophyte-bearing plants from Niwot Ridge. Nitrogen addition decreased root biomass by ~20%, regardless of endophyte status, and patterns of root biomass

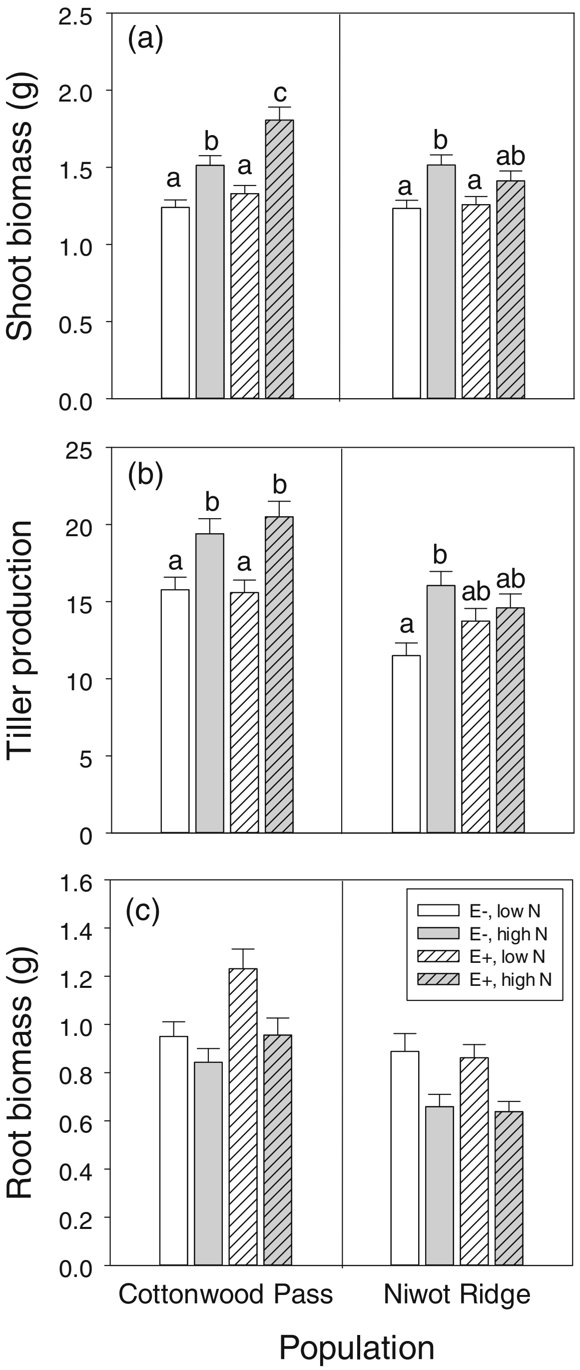


Fig. 1 Means ± s.e. from mixed effects GLMs for the effects of nutrient addition (low/high nitrogen) and endophyte removal (E−/E+) for two source populations (Cottonwood Pass and Niwot Ridge) of *Trisetum spicatum.* **a** Total aboveground shoot biomass, **b** Tiller production (Number of tillers present at end of experiment minus pre-treatment tiller number) and **c** Root biomass. Different lower case letters indicate significant differences among treatments within a population. Full statistical results appear in Table 1

allocation were similar in the two populations (Fig. 1c, $P < 0.0001$, Table 1). Phosphorus treatments did not significantly increase plant biomass, nor did the effect of phosphorus interact with the endophyte or nitrogen treatments (all *n.s.*, Table 1).

Leaf traits

In contrast to plant biomass responses, for which nitrogen effects dominated, plant traits were most sensitive to manipulations of soil phosphorus. Endophyte presence had opposite effects on specific leaf area (SLA) in the two populations, as indicated by a significant population × endophyte interaction ($P = 0.0292$, Table 2). The ability of the endophyte to alter SLA was also contingent on phosphorus availability (a marginal population × endophyte × phosphorus interaction, $P = 0.0562$, Table 2). Specifically, under phosphorus addition, the endophyte increased SLA by 12% for Niwot Ridge plants, but this endophyte effect disappeared under low phosphorus (0% effect size, Fig. 2a). In contrast, for Cottonwood Pass symbiota, endophyte-bearing plants given phosphorus had 9% *lower* SLA than endophyte-free plants under low phosphorus (Fig. 2a). In Niwot Ridge, endophyte presence altered the relationship between SLA and aboveground plant biomass ($F_{1,41} = 7.8$, $P = 0.0078$). With the endophyte present, SLA was not correlated with aboveground biomass (slope = 0.005 ± 0.005 s.e.). With the endophyte removed, aboveground biomass declined with higher SLA (slope = − 0.014 ± 0.007 s.e.). In addition to effects that depended on nutrient treatments, endophyte-bearing plants from both populations had 15% larger leaf blade surface area than endophyte-free plants (endophyte main effect, $P = 0.0238$, Table 2). Mean leaf blade surface area for E+ plants was 2.68 cm^2 ± 0.11 s.e., whereas endophyte-free plants had smaller mean area (2.34 cm^2 ± 0.11 s.e.).

Root traits

As with leaf traits, the ability of the endophyte to alter root traits was contingent on soil resource availability. Soil phosphorus altered the endophyte effect on root diameter, supported by a significant population × endophyte × phosphorus interaction ($P = 0.0227$, Table 2). In Niwot Ridge symbiota under low phosphorus, endophyte presence increased root diameter

Table 1 Analysis of deviance chi-square (X^2) and P-values from mixed effects general linear models evaluating factors affecting the performance of *Trisetum spicatum* plants (shoot biomass, tiller production, and root biomass) including effects of the source population, endophyte removal treatment, additions of soil nitrogen or phosphorus, and all treatment interaction effects

Effect	Shoot biomass		Tiller production		Root biomass	
	X^2	P	X^2	P	X^2	P
Population	5.48	**0.0192**	31.13	**<0.0001**	16.02	**<0.0001**
Endophyte	2.34	0.1264	0.38	0.5354	1.93	0.1650
Population×Endophyte	5.27	**0.0217**	0.00	0.9641	2.56	0.1095
Nitrogen	53.31	**<0.0001**	33.07	**<0.0001**	22.92	**<0.0001**
Population×Nitrogen	3.75	*0.0528*	1.69	0.1933	0.47	0.4949
Endophyte×Nitrogen	0.24	0.6234	0.97	0.3238	0.74	0.3898
Population×Endophyte×Nitrogen	4.17	**0.0411**	4.18	**0.0408**	0.62	0.4326
Phosphorus	0.61	0.4345	0.07	0.7942	0.11	0.7403
Population×Phosphorus	0.21	0.6455	0.05	0.8262	0.01	0.9044
Endophyte×Phosphorus	1.62	0.2025	1.46	0.2273	0.88	0.3472
Population×Endophyte×Phosphorus	0.68	0.4081	0.20	0.6518	0.02	0.8845
Nitrogen×Phosphorus	1.08	0.2995	0.00	0.9885	0.10	0.7546
Population×Nitrogen×Phosphorus	8.76	**0.0031**	0.01	0.9337	0.42	0.5167
Endophyte×Nitrogen×Phosphorus	0.16	0.6906	0.14	0.7130	0.12	0.7250
Population×Endophyte×Nitrogen×Phosphorus	0.14	0.7064	0.15	0.6979	0.07	0.7940

Significant P-values (< 0.05) are shown in bold. Trends ($P \leq 0.06$) are in italic

Table 2 Analysis of deviance chi-square (X^2) and P-values from mixed effects general linear models evaluating factors affecting the traits of *Trisetum spicatum* plants (specific leaf area, leaf blade area, root diameter, and root hair density) including effects of the source population, endophyte removal, additions of soil nitrogen or phosphorus, and all interactions

Effect	Leaf blade surface area		Specific leaf area		Root diameter		Root hair density	
	X^2	P	X^2	P	X^2	P	X^2	P
Population	0.21	0.6446	8.41	**0.0037**	1.03	0.3095	1.08	0.2982
Endophyte	5.11	**0.0238**	0.07	0.7907	0.17	0.6827	0.28	0.5939
Population×Endophyte	1.98	0.1595	4.75	**0.0292**	0.48	0.4887	0.25	0.6160
Nitrogen	1.43	0.2317	1.43	0.2312	3.39	0.0657	2.73	0.0987
Population×Nitrogen	1.08	0.2993	0.08	0.7723	2.71	0.0997	0.01	0.9048
Endophyte×Nitrogen	0.31	0.5783	0.08	0.7738	1.42	0.2336	0.65	0.4218
Population×Endophyte×Nitrogen	0.14	0.7063	0.00	0.9899	0.29	0.5904	0.05	0.8210
Phosphorus	2.26	0.1331	1.20	0.2738	0.00	0.9597	0.89	0.3446
Population×Phosphorus	0.02	0.8905	0.22	0.6378	0.01	0.9202	0.00	0.9859
Endophyte×Phosphorus	1.90	0.1676	0.76	0.3825	0.42	0.5156	3.46	*0.0627*
Population×Endophyte×Phosphorus	0.97	0.3254	3.65	*0.0562*	5.19	**0.0227**	0.01	0.9217
Nitrogen×Phosphorus	1.64	0.1999	0.33	0.5665	1.08	0.2976	2.02	0.1552
Population×Nitrogen×Phosphorus	0.02	0.8780	0.94	0.3331	0.50	0.4789	1.24	0.2651
Endophyte×Nitrogen×Phosphorus	2.78	0.0952	0.74	0.3883	0.80	0.3707	0.21	0.6466
Population×Endophyte×Nitrogen×Phosphorus	3.62	*0.0571*	1.33	0.2486	2.16	0.1418	0.38	0.5392

Significant P-values (< 0.05) are shown in bold. Trends ($P \leq 0.06$) are in italic

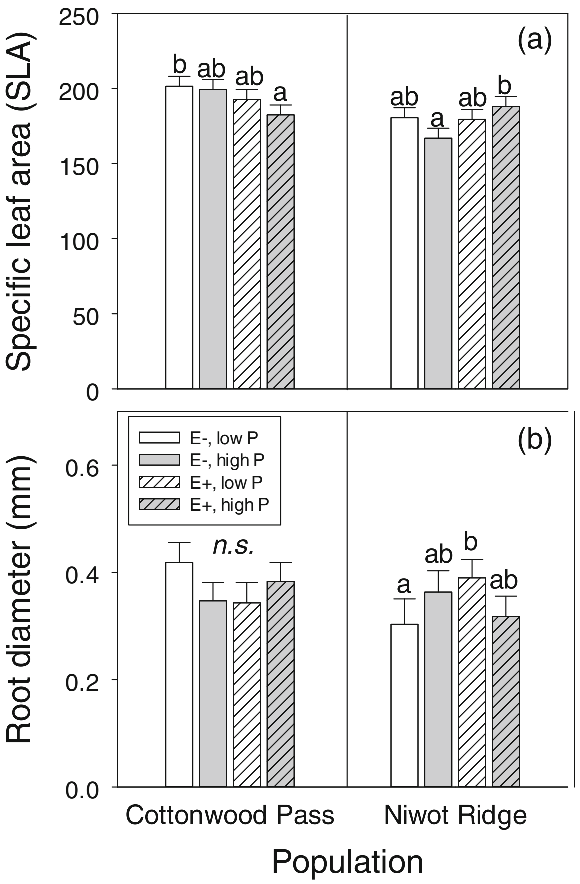


Fig. 2 Means ± s.e. from mixed effects GLMs for the effects of nutrient addition (low/high phosphorus) and endophyte removal (E−/E+) for two source populations (Cottonwood Pass and Niwot Ridge) of *Trisetum spicatum.* **a** Specific leaf area (SLA) and b Root diameter. Different lower case letters indicate significant differences among treatments within a population. Full statistical results appear in Table 2

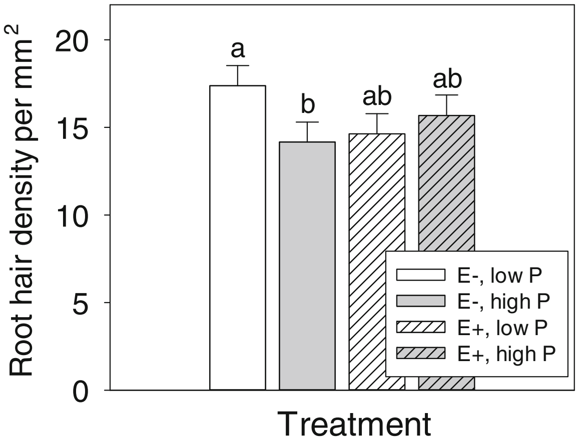


Fig. 3 Means ± s.e. from mixed effects GLM for the effects of nutrient addition (low/high phosphorus) and endophyte removal (E−/E+) on root hair density per mm in *Trisetum spicatum.* Different letters indicate significant differences among treatments. Full statistical results appear in Table 2)

by 28% relative to endophyte-free plants; however, the endophyte did not significantly alter root diameter when phosphorus was supplemented (Fig. 2b). In contrast, for Cottonwood Pass symbiota, phosphorus addition had no significant interactive effect with the endophyte for root diameter (Fig. 2b; $P = 0.52$). Endophyte presence also suppressed the typically observed increase in root hair density in response to soil phosphorus limitation. In both populations, endophyte-free plants produced 22% denser root hairs under low phosphorus than high phosphorus. However, phosphorus limitation had marginal effects on root hair density when the endophyte was present (Fig. 3; endophyte × phosphorus interaction $P = 0.0627$, Table 2).

Plant nutritional quality

The addition of nitrogen fertilizer increased foliar nitrogen content, by up to 48% (Fig. 4a). In the Cottonwood Pass population, this increase in foliar nitrogen content with fertilization was greater when the endophyte was present (48% increase) than when absent (29% increase) (Fig. 4a; endophyte × nitrogen, $F_{1,42} = 8.7$, $P = 0.0053$). However, in the Niwot Ridge population fertilization-mediated increases in leaf nitrogen were ~33%, regardless of endophytes status (Fig. 4a; endophyte × nitrogen, $F_{1,41} = 0.1$, $P = 0.82$).

The addition of phosphorus fertilizer did not alter foliar phosphorus content (Fig. 4b, phosphorus treatment, $F_{1,75} = 0.71$, $P = 0.40$). However, nitrogen fertilization increased foliar phosphorus in the Cottonwood Pass population (population × nitrogen, $F_{1,75} = 4.1$, $P = 0.0461$). In Cottonwood Pass, leaf phosphorus was 0.25% ± 0.003 s.e. under low nitrogen, and 0.27% ± 0.003 s.e. under nitrogen addition (nitrogen, $F_{1,42} = 11.4$, $P = 0.0016$), with no effect of the endophyte treatment on foliar phosphorus (endophyte, $F_{1,42} = 0.001$, $P = 0.98$).

Endophyte presence significantly altered forage quality of *T. spicatum* (perMANOVA, pseudo$F_{1,74} = 2.6$, $P = 0.0392$, Fig. 5). The two populations also differed across the suite of 14 forage quality traits (perMANOVA, pseudo$F_{1,74} = 4.3$, $P = 0.0019$, Fig. 5). Of the 14 traits, a 4% higher calcium content in endophyte-symbiotic plants contributed most to

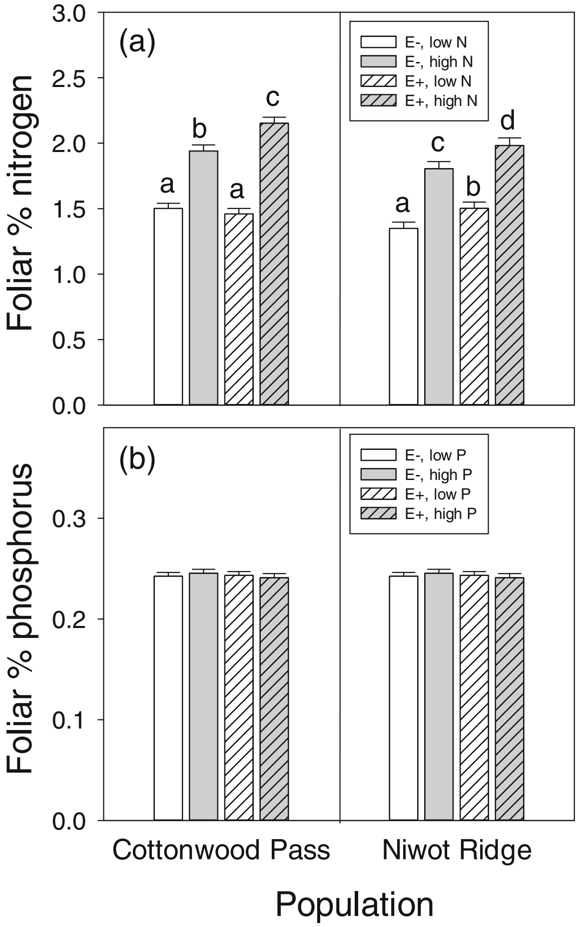


Fig. 4 Means ± s.e. from mixed effects GLM for the effects of nutrient addition and endophyte removal on leaf tissue chemistry of *Trisetum spicatum*. **a** Low/high nitrogen and endophyte removal (E−/E+) effects on percentage leaf nitrogen on a dry mass basis, and **b** Low/High phosphorus and endophyte removal (E−/E+) effects on percentage leaf phosphorus on a dry mass basis. Different lower case letters indicate significant differences among treatments within a population

differences between endophyte treatments according to SIMPER analysis, followed by an 8% increase in crude protein (Appendix 2, Supplementary Table S1). Endophyte presence also increased, in rank order of importance according to SIMPER analysis, relative feed value by ~2% and decreased neutral detergent fiber (NDF) and acid detergent fiber (ADF) by 1–2% relative to endophyte-free plants (Table S1).

Nitrogen addition altered forage quality more than endophyte presence (Fig. 6, perMANOVA, pseudo$F_{1,74}$ = 28.1, P = 0.0001). Of the 14 forage traits, a 36% increase in crude protein under nitrogen fertilization contributed the most to the treatment effect on forage quality (SIMPER analysis; Table S1). Nitrogen addition also increased relative feed value by 6% and total digestible nutrients by 4%. Nitrogen fertilization decreased lignin content by 9% and acid detergent fiber by 5% relative to unfertilized plants (Table S1).

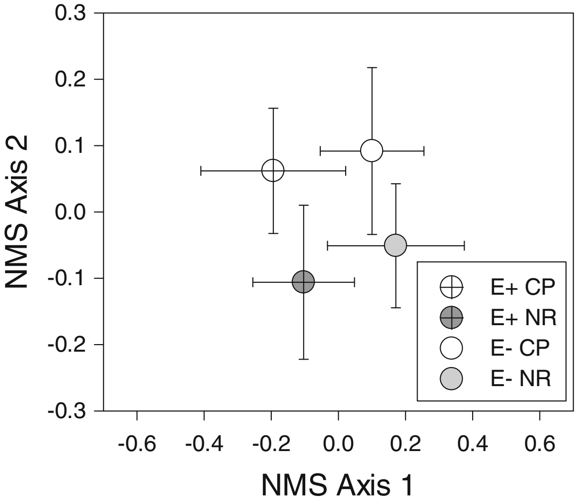


Fig. 5 Non-metric multidimensional scaling (NMS) plot showing differences among centroids ± s.e. across 14 forage quality analysis traits for endophyte (E+/E-) treatments applied to two populations of *Trisetum spicatum* plants, Cottonwood Pass (CP) or Niwot Ridge (NR). NMS 2-dimensional stress = 0.14

Fungal alkaloid chemistry

Both nitrogen and phosphorus additions increased concentrations of endophyte-produced alkaloids, and the two populations differed in types of alkaloids produced. The Cottonwood Pass population produced peramine and chanoclavine. Relative production of peramine was 28% greater under dual additions of nitrogen and phosphorus than in controls that received no supplemental fertilizer (Fig. 7a), suggesting synergistic effects of combined nutrients. The singular effects of fertilization treatments were marginally non-significant (nitrogen $F_{1,21}$ = 3.5, P = 0.0756; phosphorus $F_{1,21}$ = 3.2, P = 0.0893). Phosphorus alone increased the concentration of chanoclavine, an ergot alkaloid, with 15% higher concentration under phosphorus addition relative to water addition (Fig. 7b; $F_{1,21}$ = 5.4, P = 0.0305). The Niwot Ridge population produced only the aminopyrrolizidine, AcAP. Like the other alkaloids, production of AcAP varied with fertilization, although to a much greater degree. Mean AcAP concentration in nitrogen-fertilized plants was 76% greater than in

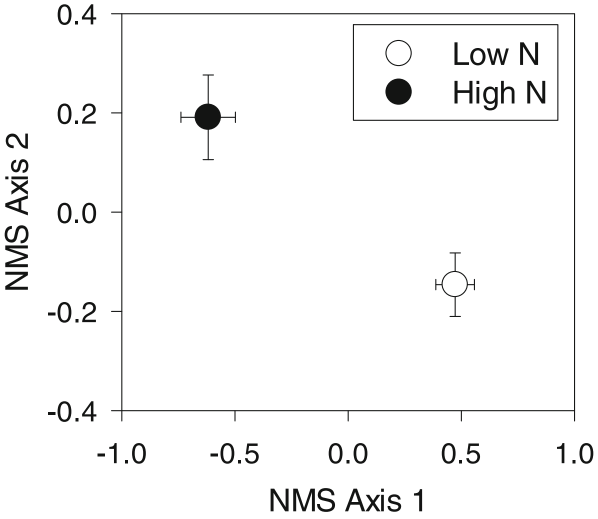


Fig. 6 Non-metric multidimensional scaling (NMS) plot with differences among centroids ± s.e. in 14 forage quality analysis traits for nitrogen additions (low/high) applied to *Trisetum spicatum* plants. NMS 2-dimensional stress = 0.14

controls (Fig. 7c; nitrogen, $F_{1,32} = 6.9$, $P = 0.0132$), and when both N and P were added, AcAP was 140% greater than in controls, although the nitrogen × phosphorus interaction was not significant ($P > 0.9$).

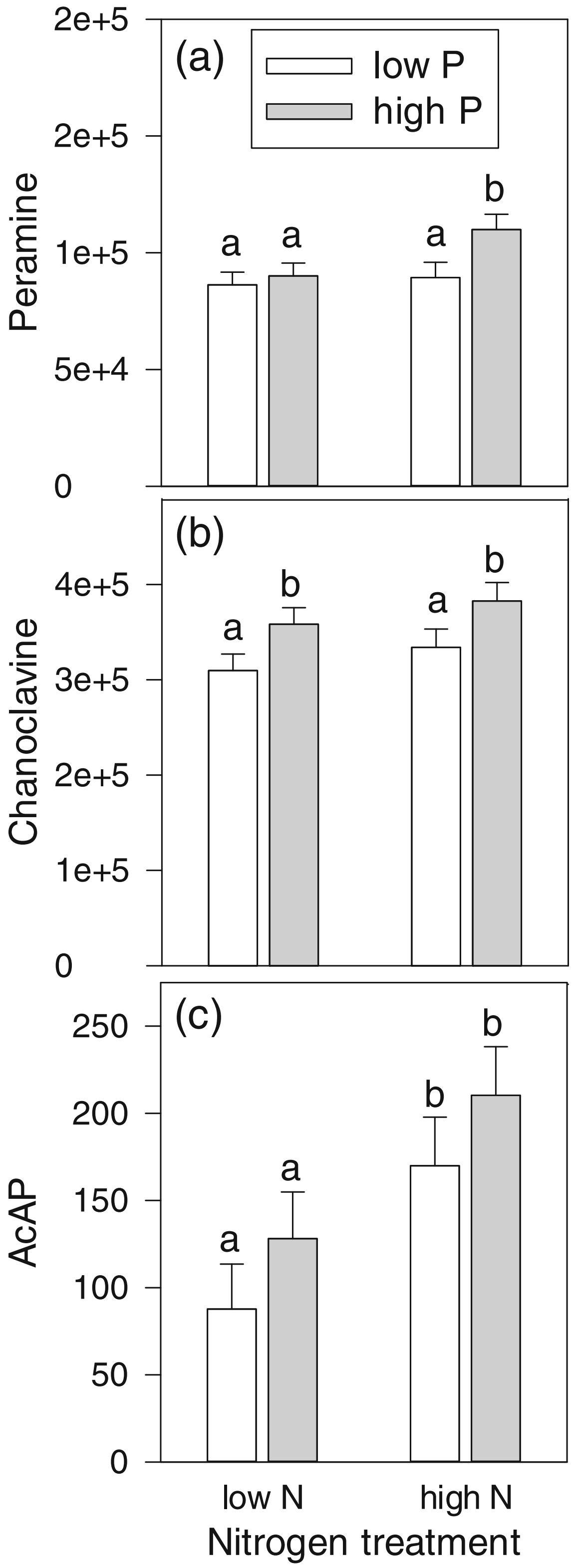
Discussion

Predicting how microbial symbionts mediate plant acquisition of soil nutrients requires understanding the functional differences among plant genotypes and endophyte genotypes. Evaluation of the endophytes present within two populations of *Trisetum spicatum* revealed two new records of endophyte species in this relative of oat: a hybrid of *E. elymi* x *E. typhina* subsp. *poae* (Cottonwood Pass) and a non-hybrid of *E. amarillans* (Niwot Ridge). These endophyte species differed in the alkaloids they produced. Our molecular markers predicted, and direct chemical analysis confirmed, that *E. elymi* x *E. typhina* subsp. *poae* from Cottonwood Pass produced peramine and chanoclavine, whereas *E. amarillans* from Niwot Ridge produced only AcAP. Unexpectedly, we did not see peramine in Niwot Ridge samples even though our markers predicted the capability. Further examination would be required to determine if the *perA* allele was functional, as nonfunctional alleles of this gene have been reported (Berry et al. 2015). Endophyte variation in different host plant populations is not uncommon, but until recently, it was not possible to efficiently evaluate host populations for endophyte diversity (e.g., Charlton et al. 2014; Shymanovich et al. 2017; Takach and Young 2014).

Does endophyte presence ameliorate soil nutrient limitation in plant hosts?

The magnitude and direction of endophyte effects on plant yield varied with soil nutrient levels and differed between populations. In the Cottonwood Pass population, the foliar endophyte boosted the effectiveness of nitrogen fertilizer. Specifically, nitrogen additions caused a 36% increase in aboveground biomass of endophyte symbiotic plants, but only a 22% increase in endophyte-free plants. Further, we detected higher % nitrogen in leaves when the endophyte was present (48% higher than unfertilized) than absent (29% higher than unfertilized). This result was consistent with our initial hypothesis that nitrogen fertilization would increase the benefit of endophyte symbiosis, perhaps by offsetting the nitrogen cost of symbiosis. Ren et al. (2009) showed a similar result, with endophyte-bearing perennial ryegrass gaining greater biomass with nitrogen addition than endophyte-free plants. However, our study included two populations, each with a different endophyte species. Plants from Niwot Ridge showed a weaker response to nitrogen addition when the endophyte was present than when absent, demonstrating that endophyte symbiosis suppressed (rather than boosted) plant responsiveness to fertilizer in this population. This result raised the question, why were symbiota from Niwot Ridge less responsive to fertilization than symbiota from Cottonwood Pass?

Differences between symbiota in production of fungal alkaloids suggests the new hypothesis that some symbiota, such as those from Niwot Ridge, may allocate



◀ **Fig. 7** Means ± s.e. from mixed effects GLM for the effects of soil nitrogen and phosphorus additions on leaf alkaloid chemistry due to endophyte presence in *Trisetum spicatum*. **a** peramine in comparison to an internal standard (relative abundance) Cottonwood Pass population, **b** chanoclavine against an internal standard (relative abundance) Cottonwood Pass population, **c** AcAP against an internal standard (relative abundance) Niwot Ridge population

additional soil nitrogen to the production of alkaloids rather than to plant growth. Indeed, fertilization increased the concentration of AcAP in Niwot Ridge symbiota by up to 140% relative to unfertilized plants. Plants from Cottonwood Pass lacked the genetic capacity to produce aminopyrrolizidines, which have been detected as lolines in other grasses at concentrations up to 2% of plant dry mass (Craven et al. 2001) and are generally considered insect (but not mammalian) deterrents (Schardl et al. 2007). Other work has shown that endophytes can increase production of defensive compounds following fertilization (e.g., Ren et al. 2011). Allocation of resources to defense provides one hypothesis for the weak increase in yield observed for Niwot Ridge plants following fertilization in the greenhouse, where herbivores were absent. Field experiments or insect feeding trials could be useful next steps for testing this ecological significance of these effects. Alternatively, the endophyte in the Niwot Ridge population may restrict N-uptake or N-use efficiency, thereby reducing the biomass gains of fertilization. Further work, such as experimental cross inoculation of each *Epichloë* species into each host, will be required to decouple the effect of different endophyte species from that of differences in plant genotype.

Dual nutrient additions of nitrogen and phosphorus were important for revealing stronger nitrogen-limitation than phosphorus-limitation of *T. spicatum* biomass, as well as differences in the mechanisms by which plants responded to these key soil nutrients. Nitrogen additions generally increased plant biomass, leaf % N content, and fungal alkaloid production. In fact, nitrogen additions also increased leaf % P, consistent with stronger nitrogen than phosphorus limitation in this grass species. In contrast, phosphorus additions had much stronger influences on the plasticity of plants traits, including specific leaf area, root diameter, and root hair density. The interaction between N- and P-additions affected the relative production of peramine, which was 28% greater under dual additions of N and P than in the unfertilized control treatment (Fig. 7a).

However, interactions involving the endophyte, nitrogen, and phosphorus were not statistically significant (Tables 1 and 2). Thus, we found little evidence for dual limitation of *T. spicatum* production by the levels of nutrients in our experiment, and the effects of each soil nutrient were not only largely additive, but also tended to independently influence different types of plant responses.

Does endophyte symbiosis alter traits associated with nutrient or carbon acquisition?

Plants can alter a suite of traits in response to nutrient deficiency, but the degree to which fungal symbionts mediate this trait plasticity is largely unresolved. For example, when phosphorus is limiting, increases in root diameter and root hair density are commonly observed and can increase plant competitiveness under low phosphorus conditions (e.g., Gahoonia et al. 1997; Ma et al. 2001; Richardson et al. 2009). Similarly, grasses from infertile habitats have been shown to increase root hair length and density under low nitrogen conditions (e.g., Boot and Mensink 1990). Our results suggest a wide range of endophyte-mediated effects on trait plasticity. For leaf traits, soil nutrient additions increased the degree to which the endophyte affected trait expression. Under phosphorus addition, symbiotic plants had improved carbon capture, with 28% greater SLA than endophyte-free plants, whereas these differences were nil under low phosphorus (Niwot Ridge population). In contrast, for root traits, such as diameter and root hair density, endophyte presence suppressed the plant's adjustment of traits to the soil resource environment. For instance, root hair density in both populations showed the typical increase under low phosphorus in the absence of the endophyte, but remained low following fertilization when the endophyte was present, suggesting that the endophyte may alleviate some phosphorus limitation through mechanisms other than changes in root architecture. These results are supported by previous literature documenting altered investment in root traits (including root hair length, architecture, exudate composition, or diameter) in other species of foliar endophyte-bearing grasses (Malinowski et al. 1998, 1999; Ren et al. 2007; Ding et al. 2015; Guo et al. 2015). A working hypothesis, then, is that symbiotic plants do not require as large an investment in nutrient-obtaining root structures because of the endophyte's ability to increase nutrient acquisition via other mechanisms, such as alterations in the chemistry of root exudates or interactions with root symbiota, such as mycorrhizal fungi (Mack and Rudgers 2008).

While leaf and root traits yield important insight into potential mechanisms of endophyte-mediated nutrient acquisition, analysis of forage quality translates the net trait changes into their value for consumers. To our knowledge, our study is among the first to test for endophyte-mediation of forage quality in a wild grass species, and the only study thus far on an oat relative. We discovered that the influence of endophyte symbiosis on plant nutritional quality was largely independent of soil nutrient availability. Endophyte symbiosis increased crude protein, relative feed value, and calcium content, and reduced acid and neutral detergent fiber, regardless of the level of fertilization. However, in the Cottonwood Pass population, nitrogen addition caused larger increases in leaf nitrogen content if plants had the endophyte than when the endophyte was removed. Our results suggest a potential paradox: that endophytes increase forage quality for consumers, while at the same time producing defensive alkaloids, such as insect-deterring peramine and aminopyrrolizidine. These dual effects could be harnessed for forage improvements by cultivating symbiota that simultaneously deter insect herbivores and increase forage quality and yield, as may occur in Cottonwood Pass symbiota. In addition, such benefits may have relevance for oat production because of the phylogenetic relatedness of *Trisetum* to *Avena*. However, the magnitude of endophyte-mediated increases in forage quality was smaller than the direct effects of N fertilization, which increased leaf % N, crude protein, and relative feed value 3–4 times more than did endophyte presence.

Do genotypes of symbiota differ in their responsiveness to soil nutrients?

Across the board, we detected divergent responses in two populations of symbiota (plant genotype + fungal species) in the *T. spicatum-Epichloë* system. Generally, plants from Cottonwood Pass showed stronger benefits of symbiosis than those from Niwot Ridge, which overall had lower production. The benefits of symbiosis in the Cottonwood Pass population were associated with fewer changes in the traits we measured and were magnified with nitrogen, but not phosphorus, fertilization. In contrast, Niwot Ridge plants experienced relatively weak benefits of endophyte symbiosis, and these benefits were stronger under nitrogen limitation. However, Niwot Ridge symbiota were more likely than those from Cottonwood Pass to have endophyte-mediated changes in plant and fungal traits, including SLA, root diameter, and fungal alkaloids. Due to this high variability between just two populations of plants associated with two *Epichloë* species, we suggest that studies of single host-endophyte genotype combinations will not be useful for predicting responses at the scale of species or landscapes.

Conclusion

Our results demonstrate a direct role of foliar fungal endophytes in mediating soil nutrient acquisition in a native grass. We showed that the direction and magnitude of endophyte effects depended strongly on the identity of host-endophyte symbiota, and that symbiosis can alter a range of traits that mediate resource acquisition. Importantly, we also revealed that endophyte presence can improve plant forage quality, although such improvements were weaker than the gains from fertilizer addition.

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References

Ahlholm JU, Helander M, Henriksson J, Metzler M, Saikkonen K (2002a) Environmental conditions and host genotype direct genetic diversity of *Venturia ditricha*, a fungal endophyte of birch trees. Evolution 56:1566–1573

Ahlholm JU, Helander M, Lehtimaki S, Wali P, Saikkonen K (2002b) Vertically transmitted fungal endophytes: different responses of host-parasite systems to environmental conditions. Oikos 99:173–183

Bacon CW, White JF Jr (1994) Stains, media, and procedures for analyzing endophytes. In: Bacon CW, White JF Jr (eds) Biotechnology of endophytic fungi of grasses. CRC Press, Boca Raton

Barkworth ME, Capels KM, Long S, Anderton LK, Piep MB (eds) (2007) Flora of North America volume 24: North of Mexico: Magnoliophyta: Commelinidae (in part): Poaceae, part 1. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico, New York.

Bell-Dereske L, Takacs-Vesbach C, Kivlin SN, Emery SM, Rudgers JA (2017) Leaf endophytic fungus interacts with precipitation to alter belowground microbial communities in primary successional dunes. FEMS Microbiology Ecology 93(6). https://doi.org/10.1093/femsec/fix036

Berry D, Takach JE, Schardl CL, Charlton ND, Scott B, Young CA (2015) Disparate independent genetic events disrupt the secondary metabolism gene *perA* in certain symbiotic *Epichloë* species. Appl Environ Microbiol 81:2797–2807

Boot RGA, Mensink M (1990) Size and morphology of root systems of perennial grasses from contrasting habitats as affected by nitrogen supply. Plant Soil 129:291–299

Charlton ND, Craven KD, Afkhami ME, Hall BA, Ghimire SR, Young CA (2014) Interspecific hybridization and bioactive alkaloid variation increases diversity in endophytic *Epichloë* species of *Bromus laevipes*. FEMS Microbiol Ecol 90:276–289. https://doi.org/10.1111/1574-6941.12393

Cheplick GP, Faeth SH (2009) Ecology and evolution of grass-endophyte symbiosis. Oxford University Press, Oxford

Chu-chou M, Guo G, An ZQ, Hendrix JW, Ferris RS, Siegel MR, Dougherty CT, Burrus PB (1992) Suppression of mycorrhizal fungi in fescue by the *Acremonium coenophialum* endophyte. Soil Biol Biochem 24:633–637

Clarke KR, Gorley RN (2009) Primer version 6.1.10 user manual and tutorial. Primer-E, Plymouth

Craven KD, Blankenship JD, Leuchtmann A, Hignight K, Schardl CL (2001) Hybrid fungal endophytes symbiotic with the grass *Lolium pratense*. Sydowia 53:44–73

Ding N, Kupper JV, DH MN Jr (2015) Phosphate source interacts with endophyte strain to influence biomass and root system architecture in tall fescue. Agron J 107:662–670. https://doi.org/10.2134/agronj14.0135

Faulkner JR, Hussaini SR, Blankenship JD, Pal S, Branan BM, Grossman RB, Schardl CL (2006) On the sequence of bond formation in loline alkaloid biosynthesis. Chembiochem 7:1078–1088

Gahoonia TS, Care D, Nielsen NE (1997) Root hairs and phosphorus acquisition of wheat and barley cultivars. Plant Soil 191:181–188

Garcia-Parisi PA, Lattanzi FA, Grimoldi AA, Druille M, Omacini M (2017) Three symbionts involved in interspecific plant-soil feedback: epichloid endophytes and mycorrhizal fungi affect the performance of rhizobia-legume symbiosis. Plant Soil 412:151–162. https://doi.org/10.1007/s11104-016-3054-3

Gruber N, Galloway JN (2008) An earth-system perspective of the global nitrogen cycle. Nature 45:293–296. https://doi.org/10.1038/nature06592

Guo J, McCulley RL, DH MN Jr (2015) Tall fescue cultivar and fungal endophyte combinations influence plant growth and root exudate composition. Front Plant Sci 6:183. https://doi.org/10.3389/fpls.2015.00183

Kering MK, Butler TJ, Biermacher JT, Mosali J, Guretzky JA (2013) Effect of potassium and nitrogen fertilizer on switchgrass productivity and nutrient removal rates under two harvest systems on a low potassium soil. Bioenergy Res 6: 329–335

Krauss J, Harri SA, Bush L, Husi R, Bigler L, Power SA, Muller CB (2007) Effects of fertilizer, fungal endophytes and plant cultivar on the performance of insect herbivores and their natural enemies. Funct Ecol 21:107–116

Lemons A, Clay K, Rudgers JA (2005) Connecting plant-microbial interactions above and belowground: a fungal endophyte affects decomposition. Oecologia 145:595–604

Lewis GC (2004) Effects of biotic and abiotic stress on the growth of three genotypes of *Lolium perenne* with and without infection by the fungal endophyte *Neotyphodium lolii*. Ann Appl Biol 144:53–63. https://doi.org/10.1111/j.1744-7348.2004.tb00316.x

Li X, Ren A, Han R, Yin L, Wei M, Gao Y (2012) Endophyte-mediated effects on the growth and physiology of *Achnatherum sibiricum* are conditional on both N and P availability. PLoS One 7:e48010. https://doi.org/10.1371/journal.pone.0048010

Ma Z, Bielenberg DG, Brown KM, Lynch JP (2001) Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. Plant Cell Environ 24:459–467. https://doi.org/10.1046/j.1365-3040.2001.00695.x

Mack KML, Rudgers JA (2008) Balancing multiple mutualists: asymmetric interactions among plants, arbuscular mycorrhizal fungi, and fungal endophytes. Oikos 117:310–320. https://doi.org/10.1111/j.2007.0030-1299.15973.x

Malinowski DP, Belesky DP (1999) *Neotyphodium coenophialum*-endophyte infection affects the ability of tall fescue to use sparingly available phosphorus. J Plant Nutr 22: 835–853

Malinowski DP, Belesky DP, Hill NS, Baligar VC, Fedders JM (1998) Influence of phosphorus on the growth and ergot alkaloid content of Neotyphodium coenophialum-infected tall fescue (*Festuca arundinacea* Schreb.). Plant Soil 198: 53–61

Malinowski DP, Brauer DK, Belesky DP (1999) The endophyte *Neotyphodium coenophialum* affects root morphology of tall fescue grown under phosphorus deficiency. J Agron Crop Sci 183:53–60

Newman JA, Abner ML, Dado RG, Gibson DJ, Brookings A, Parsons AJ (2003) Effects of elevated CO_2, nitrogen and fungal endophyte-infection on tall fescue: growth, photosynthesis, chemical composition and digestibility. Glob Chang Biol 9:425–437

Newsham KK (2011) A meta-analysis of plant responses to dark septate root endophytes. New Phytol 190:783–793

Omacini M, Semmartin M, Perez LI, Gundel PE (2012) Grass-endophyte symbiosis: a neglected aboveground interaction with multiple belowground consequences. Appl Soil Ecol 61: 273–279. https://doi.org/10.1016/j.apsoil.2011.10.012

Pan J, Bhardwaj M, Faulkner JR, Nagabhyru P, Charlton ND, Higashi RM, Miller AF, Young CA, Grossman RB, Schardl CL (2014) Ether bridge formation in loline alkaloid biosynthesis. Phytochemistry 98:60–68

Perez-Harguindeguy N, Diaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L, Wright IJ, Ray P, Enrico L, Pausas JG, de Vos AC, Buchmann N, Funes G, Quetier F, Hodgson JG, Thompson K, Morgan HD, ter Steege H, van der Heijden MGA, Sack L, Blonder B, Poschlod P, Vaieretti MV, Conti G, Staver AC, Aquino S, Cornelissen JHC (2013) New handbook for standardised measurement of plant functional traits worldwide. Aust J Bot 61:167–234. https://doi.org/10.1071/bt12225

Pittman JJ, Arnall DB, Interrante SM, Wang N, Raun WR, Butler TJ (2016) Bermudagrass, wheat, and tall fescue crude protein forage estimation using mobile-platform active-spectral and canopy-height data. Crop Sci 56:870–881. https://doi.org/10.2135/cropsci2015.05.0274

Ranelli LB, Hendricks WQ, Lynn JS, Kivlin SN, Rudgers JA (2015) Biotic and abiotic predictors of fungal colonization in grasses of the Colorado Rockies. Divers Distrib 21:962–976. https://doi.org/10.1111/ddi.12310

Rasmussen S, Lane GA, Mace W, Parsons AJ, Fraser K, Xue H (2011) The use of genomics and metabolomics methods to quantify fungal endosymbionts and alkaloids in grasses. In: Hardy N, Hall R (eds) Plant Metabolomics. Methods in Molecular Biology. Methods and Protocols, vol 860. Humana Press.

Ren AZ, Gao YB, Zhou F (2007) Response of *Neotyphodium lolii*-infected perennial ryegrass to phosphorus deficiency. Plant Soil Environ 53:113–119

Ren AZ, Gao YB, Wang W, Wang JL, Zhao NX (2009) Influence of nitrogen fertilizer and endophyte infection on ecophysiological parameters and mineral element content of perennial ryegrass. J Integr Plant Biol 51:75–83. https://doi.org/10.1111/j.1744-7909.2008.00721.x

Ren AZ, Li X, Han R, Yin LJ, Wei MY, Gao YB (2011) Benefits of a symbiotic association with endophytic fungi are subject to water and nutrient availability in *Achnatherum sibiricum*. Plant Soil 346:363–373. https://doi.org/10.1007/s11104-011-0824-9

Ren A, Wei M, Yin L, Wu L, Zhou Y, Li X, Gao Y (2014) Benefits of a fungal endophyte in Leymus chinensis depend more on water than on nutrient availability. Environ Exp Bot 108:71–78. https://doi.org/10.1016/j.envexpbot.2013.11.019

Richardson AE, Hocking PJ, Simpson RJ, George TS (2009) Plant mechanisms to optimise access to soil phosphorus. Crop Pasture Sci 60:124–143. https://doi.org/10.1071/cp07125

Rodriguez RJ, White JF, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182: 314–330. https://doi.org/10.1111/j.1469-8137.2009.02773.x

Rogers JK, Young CA, Mosali J, Norton SL, Hopkins AA (2014) Stockpiled forage yield and nutritive value of summer-dormant and summer-active tall fescue in a marginal environment. Forage and Grazinglands 12:1–9. https://doi.org/10.2134/FG-2014-0065-RS

Schardl CL, Grossman RB, Nagabhyru P, Faulkner JR, Mallik UP (2007) Loline alkaloids: currencies of mutualism. Phytochemistry 68:980–996

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675

Shi C, An S, Yao Z, Young CA, Panaccione DG, Lee ST, Schardl CL, Li C (2018) Toxin-producing *Epichloë bromicola* strains symbiotic with the forage grass, *Elymus dahuricus*, in China. Mycologia 109:847–859

Shymanovich T, Charlton ND, Musso AM, Scheerer J, Cech NB, Faeth SH, Young CA (2017) Interspecific and intraspecific hybrid Epichloë species symbiotic with the north American native grass *Poa alsodes*. Mycologia 109:1–16. https://doi.org/10.1080/00275514.2017.1340779

Smith SE, Read DJ (2008) Mycorrhizal Symbiosis, 3rd Edition. Academic Press, p 800.

Song M, Chai Q, Li X, Yao X, Li C, Christensen MJ, Nan Z (2015) An asexual *Epichloe* endophyte modifies the nutrient stoichiometry of wild barley (*Hordeum brevisubulatum*) under salt stress. Plant Soil 387:153–165. https://doi.org/10.1007/s11104-014-2289-0

Takach JE, Young CA (2014) Alkaloid genotype diversity of tall fescue endophytes. Crop Sci 54:667–678. https://doi.org/10.2135/cropsci2013.06.0423

van de Wiel CCM, van der Linden CG, Scholten OE (2016) Improving phosphorus use efficiency in agriculture: opportunities for breeding. Euphytica 207:1–22. https://doi.org/10.1007/s10681-015-1572-3.

Vazquez-de-Aldana BR, Garcia-Ciudad A, Garcia-Criado B, Vicente-Tavera S, Zabalgogeazcoa I (2013) Fungal endophyte (*Epichloe festucae*) alters the nutrient content of *Festuca rubra* regardless of water availability. PLoS One 8: e84539. https://doi.org/10.1371/journal.pone.0084539