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ARTICLE
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Nonlinear responses of ericaceous and ectomycorrhizal Arctic shrubs across a long-term experimental nutrient gradient

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Abstract

In the Arctic tundra, warming is anticipated to stimulate nutrient release and potentially alleviate plant nutrient limitations. Typically simulated by fertilization experiments that saturate plant nutrient demand, future increases in soil fertility are thought to favor ectomycorrhizal (EcM) over ericaceous shrubs and have often been identified as a key driver of Arctic shrub expansion. However, the projected increases in fertility will likely vary in their alleviation of nutrient limitations. The resulting responses of shrubs and their mycorrhizae across the gradient of nutrient limitation may be nonlinear and could contradict the current predictions of tundra vegetation shifts. We compared the functional responses of two dominant shrubs, EcM dwarf birch (*Betula nana*) and ericaceous Labrador tea (*Rhododendron tomentosum*), across a long-term nitrogen and phosphorus fertilization gradient experiment in Arctic Alaska. Using linear mixed-effects modeling, we tested the responses of shrub cover, height, and root enzyme activities to soil fertility. We found that *B. nana* cover and height linearly increased with soil fertility. In contrast, *R. tomentosum* cover initially increased, but decreased after surpassing the intermediate levels of increased soil fertility. Its height did not change. Enzyme activity did not respond to soil fertility on EcM-colonized *B. nana* roots, but sharply declined on *R. tomentosum* roots. Overall, the nonlinear responses of shrubs to our fertility gradient demonstrate the importance of experiments grounded in replicated regression design. Our results indicate that under moderate increases in soil fertility, Arctic shrub expansion may not only include deciduous EcM shrubs but also ericaceous shrubs. Regardless of shifts aboveground, changes in root enzyme activity belowground point to some EcM shrub species playing a more influential role in tundra soils; as EcM roots remained steady in their liberation of soil organic nutrients with heightened soil fertility, degradative root enzyme activity on the dominant ericaceous shrub dropped—in some instances with even the slightest increase in fertility.

KEYWORDS
Arctic tundra, *Betula nana*, Ericaceae, fertilization, long-term experiment, root enzyme, soil fertility

INTRODUCTION

Almost a century of research has determined that Arctic tundra plants are nutrient limited (Bliss, 1971; Chapin et al., 1995; Haag, 1974; Mack et al., 2004; Russell et al., 1940; Shaver & Chapin, 1980). To test the hypotheses of nutrient limitation, researchers implemented experimental fertilization at high doses that were intended to saturate plant nutrient demand (Chapin et al., 1986). These experiments were designed to answer a binary question: Is tundra vegetation nutrient limited or not? Decades of measuring changes in these studies have not only found ample support for the presence of plant nutrient limitation but have also molded our understanding of tundra ecosystem processes.

From these experiments, we have learned that (1) not all plants or tundra ecosystem types equally benefit from added nutrients (Chapin et al., 1995; Shaver et al., 2001; Van Wijk et al., 2003), (2) nutrient addition interacts with other environmental factors to determine the responses of ecosystem pools and fluxes (Bret-Harte et al., 2008; Gough et al., 2012; Hicks et al., 2020; Mack et al., 2004; McLaren & Buckeridge, 2019; Stark & Grellmann, 2002), and (3) short-term responses often differ from long-term trends. Yet, as current research attempts to apply the knowledge gained from these studies to predict future Arctic vegetation change—specifically woody shrub expansion—the binary experimental design is proving to be insufficient. This design is unable to capture the potentially nuanced, nonlinear plant responses that occur across a gradient of nutrient limitation (Cottingham et al., 2005). Given that warming-induced nutrient release will likely be spatially and temporally heterogeneous (Keuper et al., 2012; Salmon et al., 2016, 2018), identifying the functional shape of plant response across a soil fertility gradient is increasingly important as we consider the pace and implications of Arctic vegetation change. In light of this, a new question has emerged: How do tundra shrubs respond when nutrient limitations are alleviated but not fully relieved? (Hobbie et al., 2002).

Most studies of relationships between nutrient availability and Arctic shrub expansion have focused on deciduous ectomycorrhizal (EcM) shrubs rather than evergreen ericaceous shrubs (Myers-Smith et al., 2011; Vowles & Björk, 2019). In high-dose fertilization experiments, deciduous EcM shrubs, such as *Betula* spp., frequently become dominant canopy species and outcompete evergreen ericaceous shrubs (DeMarco, Mack, Bret-Harte, Burton, et al., 2014; Shaver et al., 2001; Zamin et al., 2014). This has been hypothesized to result from enhanced nutrient acquisition (Bret-Harte et al., 2008; Clemmensen et al., 2006; Deslippe et al., 2011) and plastic growth strategies (Bret-Harte et al., 2001; Heskel et al., 2013; Prager et al., 2020; Zamin & Grogan, 2012). However, when increases in soil fertility do not fully lift nutrient limitations, ericaceous shrubs may remain competitive with EcM shrubs (Chapin, 1980; Grime, 1977). Evergreen ericaceous shrubs respond positively to warming and subtler increases in fertility (Elmendorf et al., 2012; Vowles & Björk, 2019; Weijers et al., 2018; Zamin et al., 2014). Their growth, therefore, may have a quadratic, or hump-shaped, relationship with soil fertility, which would imply ericaceous shrubs could co-expand with EcM shrubs at intermediate stages of the nutrient limitation gradient. Co-expansion of these shrubs would broaden the pool of species we consider to be important when considering the impacts of expansion. Despite the differing effects these contrasting shrub functional types may have on important ecosystem properties and functions, such as litter input (Christiansen et al., 2018; DeMarco et al., 2011; DeMarco, Mack, & Bret-Harte, 2014; Hobbie, 1996; Hobbie & Gough, 2004), albedo (Chapin et al., 2005; Loranty et al., 2011; Sturm et al., 2005), or mycorrhizal activity (Andresen et al., 2022; Read & Perez-Moreno, 2003; Vowles & Björk, 2019), the response of ericaceous shrubs across a soil fertility gradient and the level of fertility at which EcM shrubs dominate remain largely unexplored topics. Experimental soil fertility gradients that capture the responses of both these contrasting shrub functional types can provide insight on the potential for co-expansion and help to incorporate nonlinear responses into ecosystem models.

Shifts in belowground mycorrhizal function are likely to occur as EcM and ericaceous shrubs change in abundance. Ecto- and ericoid mycorrhizae (ErM) exert a strong influence over nutrient cycling in Arctic tundra through their exudation of degradative enzymes (Hobbie & Hobbie, 2006; Read & Perez-Moreno, 2003). These enzymes target organically bound nutrients in soil and allow for plant nutrient acquisition in an environment with limited nutrient mobilization. As shrubs travel the gradient from nutrient limited to not, their mycorrhizal-associated enzyme activity may have a quadratic response. Sharp increases in soil fertility can reduce activity by shifting mycorrhizal fungal communities toward nitrophilic species with low degradative capacity (Lilleskov et al., 2002). Additionally, heightened soil fertility could reduce host plant C allocation to their fungal partners, and thus enzyme production, by (1) decreasing dependence on mycorrhizal nutrient acquisition or (2) decreasing host plant success in interspecific competition. Subtle increases in fertility, by contrast, may stimulate activity. Changes in the activity of degradative root enzymes, concurrent with the aboveground expansion of shrubs, will alter tundra soil processes and could affect C dynamics in a globally important C-rich ecosystem (Clemmensen et al., 2021; Parker et al., 2021; Schuur et al., 2015). Therefore, pairing aboveground shrub responses with those of root enzyme activity across a fertility gradient is a necessary step to understanding the nuanced consequences of shrub expansion.

Direct measurements of shrub-associated mycorrhizal enzyme activity are limited. In Arctic tundra, EcM fungal communities and abundance change with naturally and experimentally increased soil nutrient availability (Clemmensen et al., 2006; Deslippe et al., 2011; Timling et al., 2012; Urcelay et al., 2003), though sometimes in opposing ways. Our previous work indirectly points to a quadratic relationship between soil fertility and EcM-associated root enzyme activity: long-term high-dose fertilization reduced enzyme activity while warming moderately increased soil fertility and stimulated the activity of some enzymes (Dunleavy & Mack, 2021a). The decreases with fertilization occurred even as host shrub cover became monodominant. The enzymatic response of ericaceous roots to increased tundra soil fertility is unknown. Some evidence suggests the preferential acquisition of organic forms of nitrogen (N) by ericaceous plants even in the presence of inorganic N (Michelsen et al., 1996; Read, 1991). Genetically, ErM often have a higher enzymatic capacity than EcM and at times can act saprophytically (Martino et al., 2018; Perotto et al., 2018; Ward et al., 2022). Given this, ericaceous root enzymes may be less sensitive to heightened soil fertility than the enzymes of obligately partnered EcM roots.

In this study, we compared the functional responses of two dominant tundra shrubs, EcM *Betula nana* and ericaceous *Rhododendron tomentosum*, and their root enzymes across a long-term nutrient addition gradient experiment in Arctic Alaska. To capture aboveground shrub response, we measured both shrub cover and height. We also assayed potential root activities across a suite of five enzymes varying in targeted substrate and degradative mechanism. We hypothesized cover, height, and root enzyme activities of both shrub species would initially rise as soil fertility increased and nutrient limitations were alleviated (Figure 1). Once nutrient limitations were fully relieved, *B. nana* biomass would continue to linearly increase, yet *R. tomentosum* would decrease as informed by prior high fertilization studies (Bret-Harte et al., 2001; Shaver et al., 2001; Zamin et al., 2014). At this point, both shrubs would decrease in their root activity. In *B. nana*, this decrease would be in response to a weakening of its reliance on mycorrhizal nutrient acquisition; in *R. tomentosum*, it would be in parallel to its aboveground decline. Additionally, we hypothesized the ericaceous *R. tomentosum* root enzymes would be less sensitive to changes in soil fertility than those of the

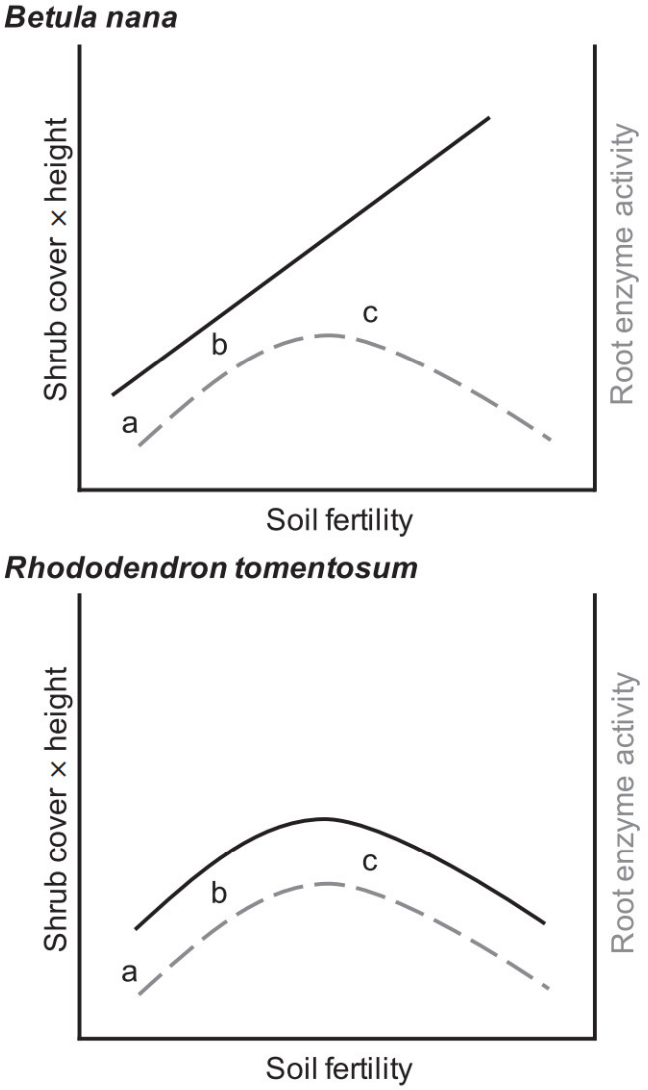

FIGURE 1 Hypothesized relationship of *Betula nana* and *Rhododendron tomentosum* cover × height (solid black line), and root enzyme activity (dashed gray line) with soil fertility. Low fertility levels at (a) result in low shrub growth and root activity. As fertility rises at (b), cover and activities increase. Nutrient limitations are relieved at (c): *B. nana* continues to increase in aboveground mass, but *R. tomentosum* decreases. Root activities decrease on both species. Hypothesized relationships are not drawn to scale.

EcM-associated *B. nana* because of their putative preference for organic N.

MATERIALS AND METHODS

Study site

We conducted this study near Toolik Lake at the Arctic Long-Term Ecological Research site (68°38′ N, 149°34′ W, 780 m above sea level) on the North Slope of Alaska. The site is located on a gradual east-facing slope in moist acidic tussock tundra. Vegetation is dominated by roughly equal biomasses of graminoids (*Eriophorum vaginatum*, *Carex bigelowii*) and dwarf deciduous and evergreen shrubs (*B. nana*, *Salix pulchra*, *R. tomentosum*, *Vaccinium vitis-idaea*), with a nearly continuous surface cover of mosses. Soils are characterized as Gelisols and consist of a moist organic horizon with a mean thickness of 30 cm over a silty mineral horizon underlain by continuous permafrost. The site is located on Itkilik I glacial drift, ~50–120 kyr old (Hamilton, 2003). Atmospheric N deposition is minimal, ranging from 8 to 56 mg N m^{-2} $year^{-1}$ (Hobara et al., 2006; National Atmospheric Deposition Program, 2020).

A nutrient addition gradient experiment was established in 2006 (Appendix S1: Figure S1). This experiment consists of four replicate blocks that contain six 5 × 20 m plots. Each plot receives one of six treatments. Treatments include control, 0.5, 1, 2, 5, and 10 g N m^{-2} $year^{-1}$ fertilization with half the amount of P on a mass basis (0.25, 0.5, 1, 2.5, and 5 g P m^{-2} $year^{-1}$, respectively). Plots were fertilized with ammonium nitrate (NH_4NO_3) and triple superphosphate (P_2O_5) every spring following snowmelt until 2012. Since 2012, plots have been fertilized with ammonium chloride and sodium nitrate (NH_4Cl and $NaNO_3$) due to a change in US policy of obtaining NH_4NO_3; P fertilization has remained the same. Previous studies report on plant community, ecosystem C fluxes, and leaf traits (Heskel et al., 2012; Prager et al., 2017, 2020).

Sample collection

We sampled organic soils from 5 cm below the surface of the green moss to 15 cm deep using a 4.2-cm-diameter metal corer between 2 and 13 July 2018, when vegetation is near peak biomass. Nine cores were taken from each of the six plots for EcM-colonized *B. nana* root tip collection across a 3 × 3 grid in a 3 × 3 m area (Appendix S1: Figure S1). In control plots and fertilizer plots that received 2 g and 10 g N m^{-2} $year^{-1}$, we also sampled *R. tomentosum* rhizomes and roots for ericaceous root tip collection at the same points we cored. These were verified to be *R. tomentosum* by connecting belowground material to aboveground growth and were sampled between 5 and 15 cm deep. We took an additional three cores within each sampling area per plot for soil chemical analyses. Cores and plants were stored intact at 4°C for no more than 72 h before excising root tips. Soil analyses were conducted on the day of core collection.

B. nana and *R. tomentosum* cover was visually estimated in five permanent 1-m^2 quadrats per plot in late July 2020 by the Arctic LTER and Toolik Field Station Spatial and Environmental Data Center, using the methods described by Gough (2019). Shrub height was measured as the stem length from the base of each stem to the apical tip, taken from four random shrubs per quadrat per species.

Soil analysis

We measured soil moisture, bulk %C and %N, and total dissolved organic carbon (TOC), total dissolved nitrogen (TN), salt-extractable inorganic N (dissolved inorganic N [DIN]; NH_4-N and NO_3-N), and orthophosphate (PO_4-P) concentrations. On the day of collection, coarse material (>2 mm) was removed from cores and the remaining soil was homogenized by hand. A subsample was dried at 60°C for 48 h for gravimetric soil moisture and bulk C:N analysis. The dried sample was ground and bulk C:N content was measured via combustion mass spectrometry on an Elemental Analyzer (Costech Analytical Technologies, USA). Total organic carbon and TN were extracted from 10 g of field moist soil in 50 mL 0.5 M K_2SO_4. Extracts were shaken for 2 h on a shaker table, placed overnight at 4°C to settle, and vacuum-filtered through a Whatman GF/A filter. The filtrate was frozen and stored at −20°C until further analysis. A subsample of filtrate was processed for TOC and TN via combustion on a Total Organic Carbon Analyzer TOC-L (Shimadzu Corporation, Japan) and a separate subsample was processed for DIN on a SmartChem 200 Discrete Chemistry Analyzer (Unity Scientific, USA) via colorimetry using the ammonium salicylate method (Unity Scientific Method 210-203D) for NH_4-N detection and the nitrate method (Unity Scientific Method NO3-001-A) for NO_3-N detection. Dissolved organic nitrogen (DON) was calculated as TN minus DIN. Orthophosphate was extracted from 2.5 g of air-dry soil in 10 mL of 0.025 N H_2SO_4 and 0.05 N HCl double acid solution. Extracts were shaken for 5 min on a shaker table and vacuum-filtered through a Whatman No. 5 filter. We measured PO_4-P concentrations of filtrate using the ascorbate method (Murphy & Riley, 1962).

Absorbances were read at 880 nm on a Powerwave XS microplate reader (Biotek, USA).

Root enzyme activity

To characterize root enzyme activity and allocation to organic nutrient acquisition, we conducted extracellular enzyme assays on individual EcM and ericaceous root tips, corresponding to *B. nana* and *R. tomentosum*, respectively. On the same day that we conducted assays, we excised a maximum of six healthy root tips from each core and plant, verified by turgidity and a white inner stele under a stereomicroscope, for a maximum of 40 EcM root tips and 40 ericaceous fine root ends (hereafter "root tips") per plot. The total number of root tips sampled per plot ranged between 16 and 40 tips due to a lack of healthy roots in some plots. To capture the heterogeneity among roots within a plot, we took no more than two root tips from each fine root segment within a core or from each plant. Excised root tips were stored in 100 μL DI water in 96-well plates at 4°C for no more than 9 h and on average 5 h before starting enzyme assays.

Following the root tip enzyme assay protocol described in Pritsch et al. (2011) and the peroxidase assay in Johnsen and Jacobsen (2008), we measured the potential activities of five enzymes—two associated with labile N acquisition (leucine amino peptidase [EC 3.4.11.1] and *N*-acetyl glucosaminidase [hereafter chitinase; EC 3.2.1.14]), one with P acquisition (phosphomonoesteratase [hereafter phosphatase; EC 3.1.3.2]), and two with nutrient acquisition via oxidative breakdown of complex carbon compounds (laccase [hereafter phenol oxidase; EC 1.10.3.2] and peroxidase [EC 1.11.1.7])—on root tips in a series of fluorometric and colorimetric assays. The activities of three hydrolytic enzymes were measured using fluorogenic substrates (4-methylumbelliferone [MUB] *N*-acetyl glucosaminide for chitinase and 4-MUB-phosphate for phosphatase; 7-amino-4-methylcoumarin labeled leucine for leucine aminopeptidase); activities of two oxidative enzymes were measured using colorimetric substrates (2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid), hereafter ABTS, for phenol oxidase and 3,3′,5,5′-tetramethylbenzidine, hereafter TMB, for peroxidase).

During assays, we sequentially incubated EcM and ericaceous root tips in concentrated substrate solutions in buffers at the appropriate pH associated with each enzyme. Root tips were incubated in the dark at room temperature on a shaker table. At the end of leucine aminopeptidase, chitinase, and phosphatase assays, we transferred the incubated solution to clean, black microplates filled with 150 μL 1 M Tris stop solution and measured the fluorescence at 360 ± 20 nm excitation and 460 ± 20 nm emission. At the end of phenol oxidase assays, incubation solutions were transferred to clean, clear plates and absorbance was measured at 420 nm. Incubation solutions from peroxidase assays were transferred to clean, clear plates filled with 30 μL 1 M H_2SO_4 and absorbance was measured at 450 nm. All fluorescence and absorbance measurements were taken on a Synergy HTX microplate reader (Biotek, USA). Root tips were rinsed between incubations with diluted incubation buffer before beginning the assay for the next enzyme. After completing the final assay, root tips were scanned in 60 μL tap water in a 96-well plate and the projected surface area was measured in WinRHIZO software program (Regent Instruments, Québec, Canada). Potential enzyme activity was calculated as picomoles of substrate converted per square millimeter of root tip surface area per minute (hereafter pmol $\text{mm}^{-2}\ \text{min}^{-1}$) using standard curves for hydrolase substrates and the Beer–Lambert law for ABTS ($\varepsilon_{420} = 3.6 \times 10^4\ \text{M}^{-1}\ \text{cm}^{-1}$; Pritsch et al., 2011) and TMB ($\varepsilon_{450} = 5.9 \times 10^4\ \text{M}^{-1}\ \text{cm}^{-1}$; Josephy et al., 1982).

Statistical analysis

We analyzed the effects of nutrient addition on aboveground and belowground shrub traits using linear mixed-effects models. All analyses were performed in R version 4.0.2 (R Core Team, 2019). General linear mixed-effects models were fit using the function "glmmTMB" in the package *glmmTMB* (Magnusson et al., 2017). To ensure fertilizer treatments produced the intended increases in nutrient availability, we tested the effect of nutrient addition on soil DIN and PO_4-P using log-gamma linear mixed models. Models included fixed effects of amount of fertilizer added, with both first- and second-order polynomial terms, and random effects of plot within block. We fit subsequently reduced models and chose the best fitting model based on Akaike information criterion (AIC) values.

To account for the collinearity of relationships among soil chemical variables, we created soil fertility indices from the axes of a nonmetric multidimensional scaling (NMDS) ordination to use in subsequent analyses. We fit a two-dimensional ordination using the Bray–Curtis dissimilarity matrix of relativized DIN, PO_4, DON, TOC, and bulk soil C:N ratio, %C, and %N using the function metaMDS in the package *vegan* (Oksanen et al., 2019). We then extracted the axis values as soil fertility indices that we used as fixed effects in linear mixed-effects models described below. We tested Pearson's correlation between each NMDS axis and each soil chemical variable to characterize the changes in soil chemistry represented by each index. Axis 1 and Axis 2 correlated with inorganic and organic soil chemical variables, respectively, and thus hereafter referred to as the inorganic and organic indices.

We used linear mixed-effects models to assess the effect of soil fertility indices and species on shrub cover, shrub height, and root enzyme activity. Shrub cover and height models were fit using a normal distribution; enzyme activity models were fit using a log-gamma distribution. Models were comprised of the interactive fixed effects of species with inorganic and organic indices. Because we hypothesized a quadratic relationship between soil fertility and some of our dependent variables, we included first- and second-order polynomial terms for both indices in our full models. To account for the spatial dependencies in our nested sampling design, we included nested random effects of plot within block for shrub cover models; quadrat within plot within block for shrub height models; and core within plot within block for enzyme activity models. We used backward model selection to find the best fitting model for each of our dependent variables based on AIC values. Afterward, residuals for each best fitting model were visually inspected for homoscedasticity. We then calculated the marginal and conditional R^2 for the best fit model via the trigamma method using the function "r.squaredGLMM" in the package *MuMIn* (Barton & Barton, 2012).

To reconcile zeros within our enzyme activity data with a log-gamma distribution, we added a value on the same order of magnitude as the minimum activity of each enzyme. For leucine aminopeptidase, phosphatase, and peroxidase, zero values made up 0.5%, 0.1%, and 0.9%, respectively, of the total sample size. For phenol oxidase, zero values made up 35% of the sample size. To ensure adding a minimum value did not alter the model outcome, we ran preliminary parallel models excluding zeros for leucine aminopeptidase, phosphatase, and peroxidase activities, and a zero-adjusted gamma model for phenol oxidase activity. We did not find that adding a minimum value affected model residuals or coefficient estimates. To verify the relationships found between enzyme activities and the fertility indices were not an artifact, we looked for similar relationships between enzyme activities and the amount of fertilizer added, and present the results in Appendix S1.

RESULTS

Soil fertility

In general, nutrient addition increased nutrient availability in the intended manner. Fertilization was linearly related to DIN concentration and quadratically related to PO_4-P concentration (Appendix S1: Table S1). DIN increased with increasing amount of fertilizer added; concentrations increased gradually until 5 g N m^{-2} $year^{-1}$, after which concentrations increased rapidly ($R_m^2 = 0.36$, $R_c^2 = 0.40$; Figure 2a). Orthophosphate concentrations increased until a similar inflection point as DIN, after which they decreased ($R_m^2 = 0.31$, $R_c^2 = 0.31$; Figure 2b). TOC, DON, and bulk soil %C, %N, and C:N ratio did not correlate with fertilization.

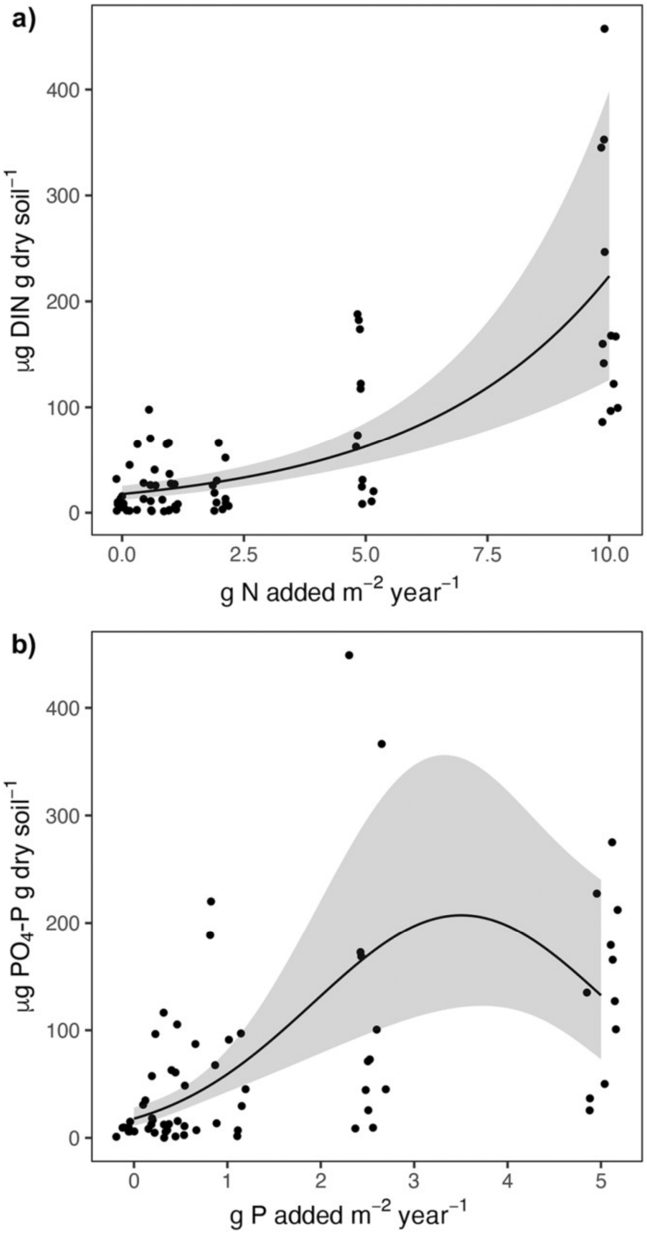

FIGURE 2 Responses of soil extractable (a) dissolved inorganic N (DIN) ($n = 71$ cores) and (b) PO_4-P ($n = 66$ cores) to 12 years of experimental fertilization at Arctic LTER, Toolik Lake, Alaska. Lines and shading represent linear mixed-effects model results with 95% CLs.

To capture the overall change in soil chemical environment with nutrient addition, we created a two-dimensional NMDS ordination (stress = 0.14) and extracted two indices of soil fertility from its two axes (Figure 3). The first axis, hereafter referred to as the inorganic index, positively correlated with DIN, DON, and PO_4-P (Table 1). The second axis, hereafter the organic index, positively correlated with bulk soil %C, %N, and PO_4-P. This axis also negatively correlated with TOC, DON, and bulk soil C:N ratio. The organic axis did not present strong patterns relevant to our hypothesis, and we have thus limited its discussion in the presented analysis. A full exploration of the effects of the organic axis on vegetation and root enzyme activity can be found in Appendix S1.

Aboveground plant cover

We modeled shrub cover and height as a function of the quadratic fixed effects of the inorganic index and its interaction with species to test our hypothesized relationship of cover and height with soil fertility (Appendix S1: Table S2). Shrub cover was strongly explained by the inorganic index, though the nature of the relationship depended on species ($R_m^2 = 0.40$, $R_c^2 = 0.50$; Figure 4a). In support of our hypothesis, *B. nana* cover linearly increased with the inorganic index, while *R. tomentosum* cover displayed a negative quadratic relationship. *R. tomentosum* cover increased until intermediate nutrient levels, after which it sharply decreased. *B. nana* stem heights increased with increasing inorganic index ($R_m^2 = 0.49$, $R_c^2 = 0.57$; Figure 4b), while those of *R. tomentosum* did not change.

Root enzyme activity

We modeled root activities of each enzyme as a function of the quadratic fixed effects of the inorganic and organic indices and their interaction with species to test for the hypothesized quadratic relationship to soil fertility and for differences among species (Appendix S1: Table S3). Contrary to our hypothesis, the relationship between enzyme activities and the inorganic index did not form the predicted negative quadratic shape and differed greatly between EcM *B. nana* and ericaceous *R. tomentosum* roots. In three of the five enzymes, we found a significant interaction between inorganic index and species (Table 2). Generally, activities on EcM roots did not significantly respond to the inorganic index (Table 2). In contrast, we found that activities on ericaceous roots responded nonlinearly to the inorganic index, as shown by significant second-order polynomial model parameters (Table 2). However, they did not form the quadratic shape we predicted (Figures 5–7). Additionally, intercept estimates of ericaceous root activities were significantly

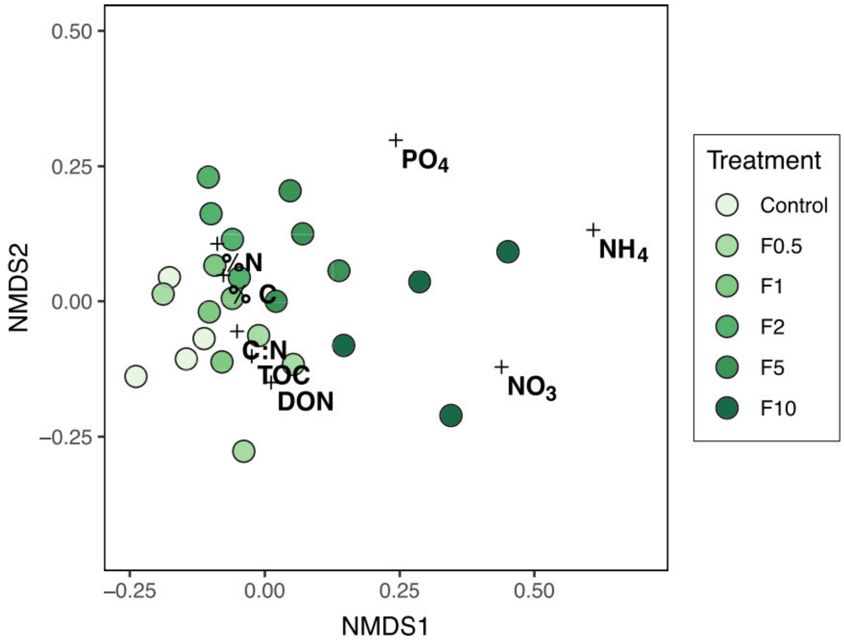

FIGURE 3 Nonmetric multidimensional scaling (NMDS) of the Bray–Curtis distances between relativized soil chemical variables among experimental plots. Circles represent plots ($n = 24$) and are colored by fertilization level. Plus signs represent the loadings of each soil chemical variable on the ordination and show the influence of each variable on axes values.

lower than those of EcM roots for all enzymes (Table 2). The activities of all enzymes but chitinase were related to the inorganic rather than the organic index. The best fitting models for four of the five enzymes contained the fixed effects of the inorganic index while those for only two of the five enzymes contained the fixed effects of the organic index (Table 2; Appendix S1: Figure S3). However, the shape of the relationship to the inorganic index varied among enzymes, even within their broad classes (Figures 5–7).

TABLE 1 Pearson's correlation test results of soil chemical variables with nonmetric multidimensional scaling (NMDS) Axes 1 and 2.

Axis	Soil variable	r	p
Inorganic index (NMDS1)	NH$_4$-N	0.84	<0.001***
	NO$_3$-N	0.89	<0.001***
	PO$_4$-P	0.70	<0.001***
	Dissolved organic N	0.35	0.089†
	Bulk soil C:N	0.04	0.829
	Bulk soil %C	−0.30	0.152
	Bulk soil %N	−0.30	0.156
	Total organic C	0.25	0.248
Organic index (NMDS2)	NH$_4$-N	0.14	0.522
	NO$_3$-N	−0.15	0.479
	PO$_4$-P	0.55	0.005*
	Dissolved organic N	−0.57	0.004*
	Bulk soil C:N	−0.51	0.011*
	Bulk soil %C	0.56	0.005*
	Bulk soil %N	0.74	<0.001***
	Total organic C	−0.55	0.005*

*$p < 0.05$; ***$p < 0.001$; $^\dagger p < 0.1$.

Responses of hydrolytic enzymes to the inorganic index were mixed. Leucine aminopeptidase activities did not support our quadratic hypothesis and instead linearly decreased with the inorganic index on both ericaceous and EcM roots. Model results showed that increased inorganic fertility resulted in decreased leucine aminopeptidase activity (Figure 5a). Chitinase activities on either species were not related to the inorganic index (Figure 5b). Phosphatase activities on ericaceous roots were quadratically related to the inorganic index, but again, not in the predicted shape. Activities sharply decreased initially and then reached a plateau, showing that even low increases in nutrient availability negatively affected ericaceous phosphatase activity (Figure 6). EcM phosphatase activities, though, were not significantly related to the inorganic index.

Responses of oxidative enzyme activities, similar to those of hydrolytic enzymes, did not support our hypothesized quadratic relationship. Oxidative enzymes correlated with the inorganic index. Phenol oxidase activities were quadratically related to the inorganic

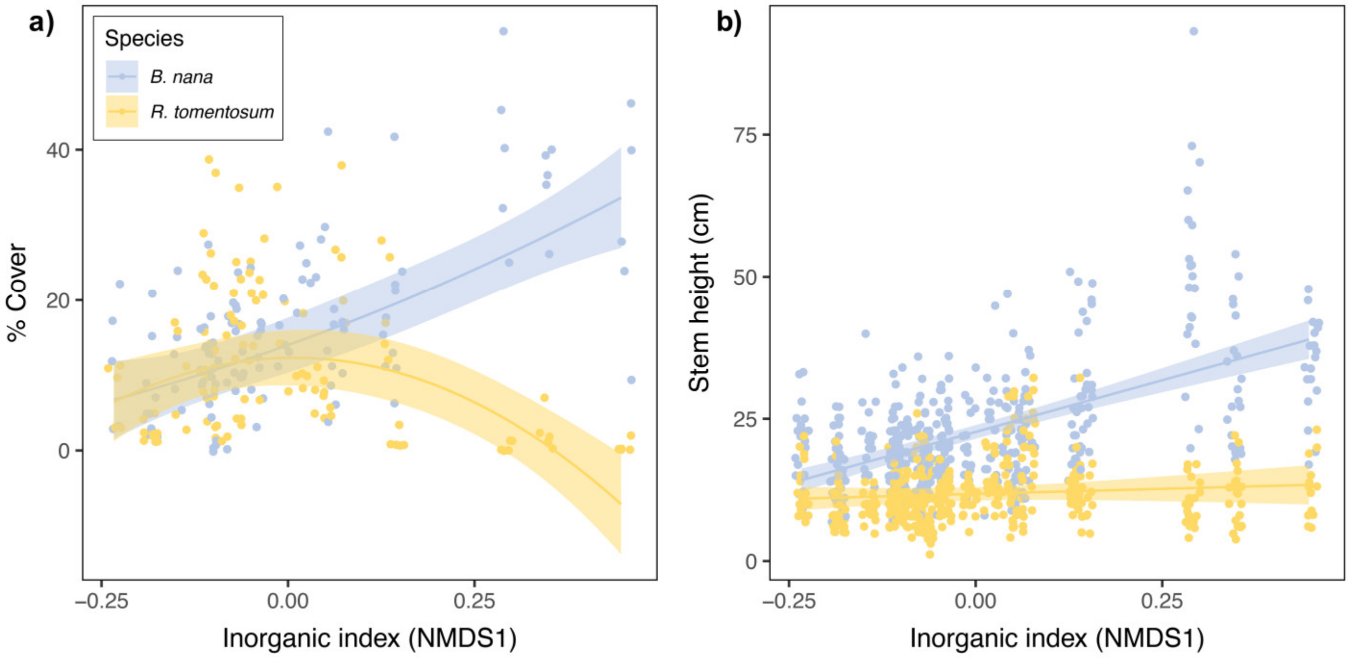

FIGURE 4 Responses of *Betula nana* and *Rhododendron tomentosum* (a) cover and (b) stem height to the inorganic soil fertility index. Lines and shading represent linear mixed-effects model results with 95% CLs. Cover: $n = 120$ *B. nana* and *R. tomentosum* quadrats each. Stem height: $n = 458$ *B. nana* stems and $n = 465$ *R. tomentosum* stems. NMDS, nonmetric multidimensional scaling.

TABLE 2 Parameter estimates, SE, *z* values, *p* values, and marginal and conditional R^2 from the best fitting model for enzyme activities on individual ectomycorrhizal (EcM) *Betula nana* and ericaceous *Rhododendron tomentosum* root tips.

Final model variables by enzyme	Coefficient	SE	*z*	*p*	R^2_m	R^2_c	*N* (roots)
Leucine aminopeptidase					0.07	0.34	*n* = 757 EcM; *n* = 430 erica.
(Intercept)	4.0	0.1	36.6	<0.001***			
Inorganic index	−1.0	0.3	−3.1	0.002*			
R. tomentosum (intercept)	−0.6	0.1	−8.9	<0.001***			
Chitinase					0.51	0.61	*n* = 760 EcM; *n* = 430 erica.
(Intercept)	6.3	0.1	84.8	<0.001***			
Organic index	2.6	1.9	1.4	0.171			
Organic index^2	−2.5	1.7	−1.5	0.141			
R. tomentosum (intercept)	−1.7	0.1	−36.0	<0.001***			
R. tomentosum:organic index	2.1	1.6	1.3	0.192			
R. tomentosum:organic index^2	5.7	1.9	3.0	0.003*			
Phosphatase					0.38	0.51	*n* = 739 EcM; *n* = 382 erica.
(Intercept)	7.1	0.1	88.2	<0.001***			
Inorganic index	2.7	1.6	1.7	0.098†			
Inorganic index^2	1.3	1.5	0.9	0.373			
Organic index	0.1	0.4	0.3	0.746			
R. tomentosum (intercept)	−1.3	0.1	−23.4	<0.001***			
R. tomentosum:inorganic index	−11.4	1.6	−7.0	<0.001***			
R. tomentosum:inorganic index^2	6.6	1.8	3.6	<0.001***			
R. tomentosum:organic index	−1.0	0.4	−2.4	0.018*			
Phenol oxidase					0.11	0.23	*n* = 760 EcM; *n* = 433 erica.
(Intercept)	3.8	0.3	14.3	<0.001***			
Inorganic index	−0.9	10.2	−0.1	0.926			
Inorganic index^2	−0.1	9.2	−0.02	0.988			
R. tomentosum (intercept)	−3.2	0.2	−14.4	<0.001***			
R. tomentosum:inorganic index	−20.6	5.8	−3.6	<0.001***			
R. tomentosum:inorganic index^2	22.6	7.0	3.2	0.001*			
Peroxidase					0.37	0.60	*n* = 750 EcM; *n* = 425 erica.
(Intercept)	6.1	0.1	63.3	<0.001***			
Inorganic index	6.9	3.8	1.8	0.069†			
Inorganic index^2	2.8	3.4	0.8	0.418			
R. tomentosum (intercept)	−1.6	0.1	−27.3	<0.001***			
R. tomentosum:inorganic index	−7.9	1.6	−5.0	<0.001***			
R. tomentosum:inorganic index^2	4.3	2.0	2.2	0.027*			

Note: Parameters without species name represent EcM root tips; those with *R. tomentosum* describe the adjustment for ericaceous (erica.) root tips.
$^{*}p < 0.05$; $^{***}p < 0.001$; $^{\dagger}p < 0.1$.

index on ericaceous roots, sharply decreasing initially and then reaching a plateau (Figure 7a). EcM phenol oxidase activities, though, did not change with either index. Peroxidase activities on EcM roots linearly increased with increasing inorganic index, suggesting increased nutrient availability steadily increased EcM activities (Figure 7b). In contrast, ericaceous peroxidase activities were quadratically related to the inorganic index. Initially, these activities slightly decreased with increasing index values but then returned to similar levels of activity at the highest index values as were at lowest values.

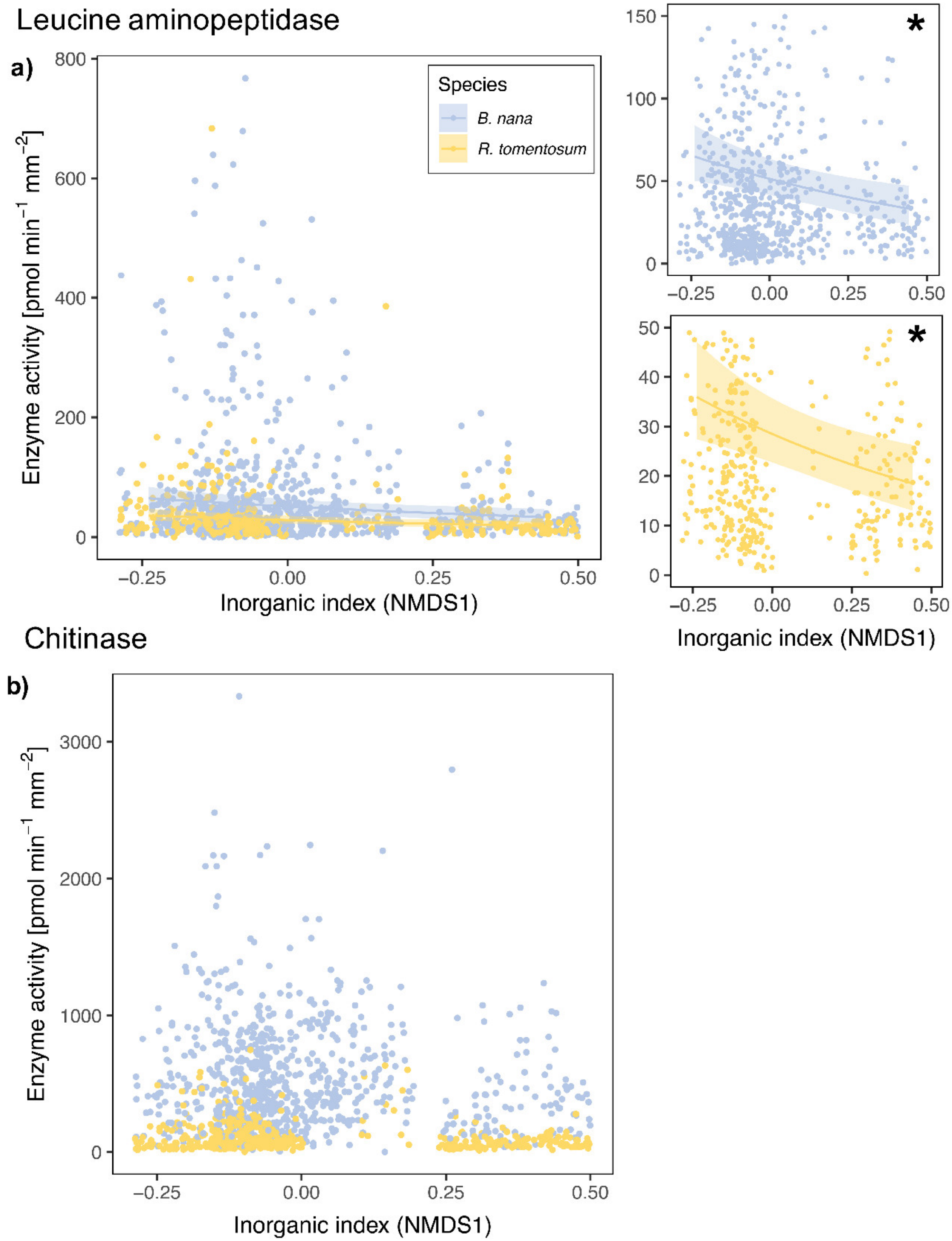

FIGURE 5 Responses of (a) leucine aminopeptidase and (b) chitinase activities to inorganic soil fertility index. Because of the large differences in the ranges of enzyme activities between ectomycorrhizal *Betula nana* and ericaceous *Rhododendron tomentosum* roots, we present model results on the full scale and on a scale proportional to the interquartile range of each root type's activity. Colors represent root species. Lines and shading represent linear mixed-effects model results with 95% CLs. An asterisk indicates parameter estimates that are significantly different from zero. NMDS, nonmetric multidimensional scaling.

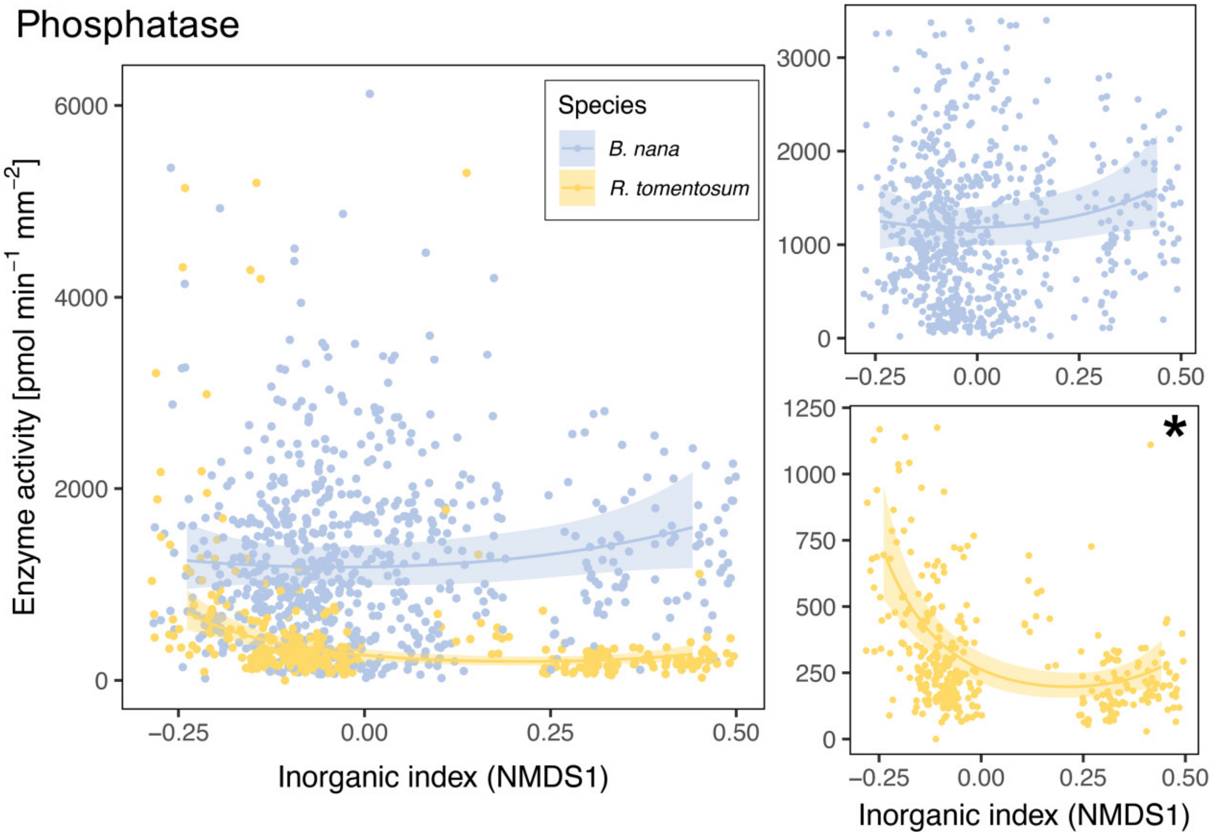

FIGURE 6 Responses of phosphatase activities to the inorganic soil fertility index. Because of the large differences in the ranges of enzyme activities between ectomycorrhizal *Betula nana* and ericaceous *Rhododendron tomentosum* roots, we present model results on the full scale and on a scale proportional to the interquartile range of each root type's activity. Colors represent root species. Lines and shading represent linear mixed-effects model results with 95% CLs. An asterisk indicates parameter estimates that are significantly different from zero. NMDS, nonmetric multidimensional scaling.

DISCUSSION

We tested the functional responses of cover, height, and root enzyme activity of two contrasting dominant tundra shrubs to an experimental soil fertility gradient. We hypothesized that responses would (1) differ between shrub species and (2) be nonlinear. Our results supported our hypotheses on shrub cover and height, which were heavily informed by prior knowledge, but provided mixed support for our hypotheses on root enzymes. While root enzyme activities responded differently between shrub species and were generally nonlinear, they did not form the quadratic shape that we hypothesized. *B. nana* cover and height increased linearly throughout the soil fertility gradient while its root enzyme activities remained relatively unchanged. Conversely, *R. tomentosum* cover initially increased, but decreased after surpassing the intermediate levels of soil fertility. Its root enzyme activity decreased throughout the gradient, and in some enzymes, sharp decreases occurred with slight increases in soil fertility. We propose that interspecific competition and shifts in mycorrhizal allocation acted as underlying drivers of the observed shrub responses to increasing soil fertility.

Co-expansion of ericaceous and EcM shrubs

Our results showed that ericaceous shrubs benefited from moderate increases in soil fertility. This suggests ericaceous shrubs, often overlooked in North American studies relating to nutrient availability and shrub expansion, are likely co-expanding with EcM shrubs in response to heightened soil fertility. The increasing abundance of ericaceous shrubs like *R. tomentosum* is not a new finding. Support for ericaceous shrub expansion has been reported by both observational and experimental studies in Europe and North America (Elmendorf et al., 2012; Vuorinen et al., 2017; Zamin et al., 2014). The building evidence for ericaceous shrub expansion in connection with moderate increases in nutrient availability demands further study, especially given the potential ecosystem consequences of

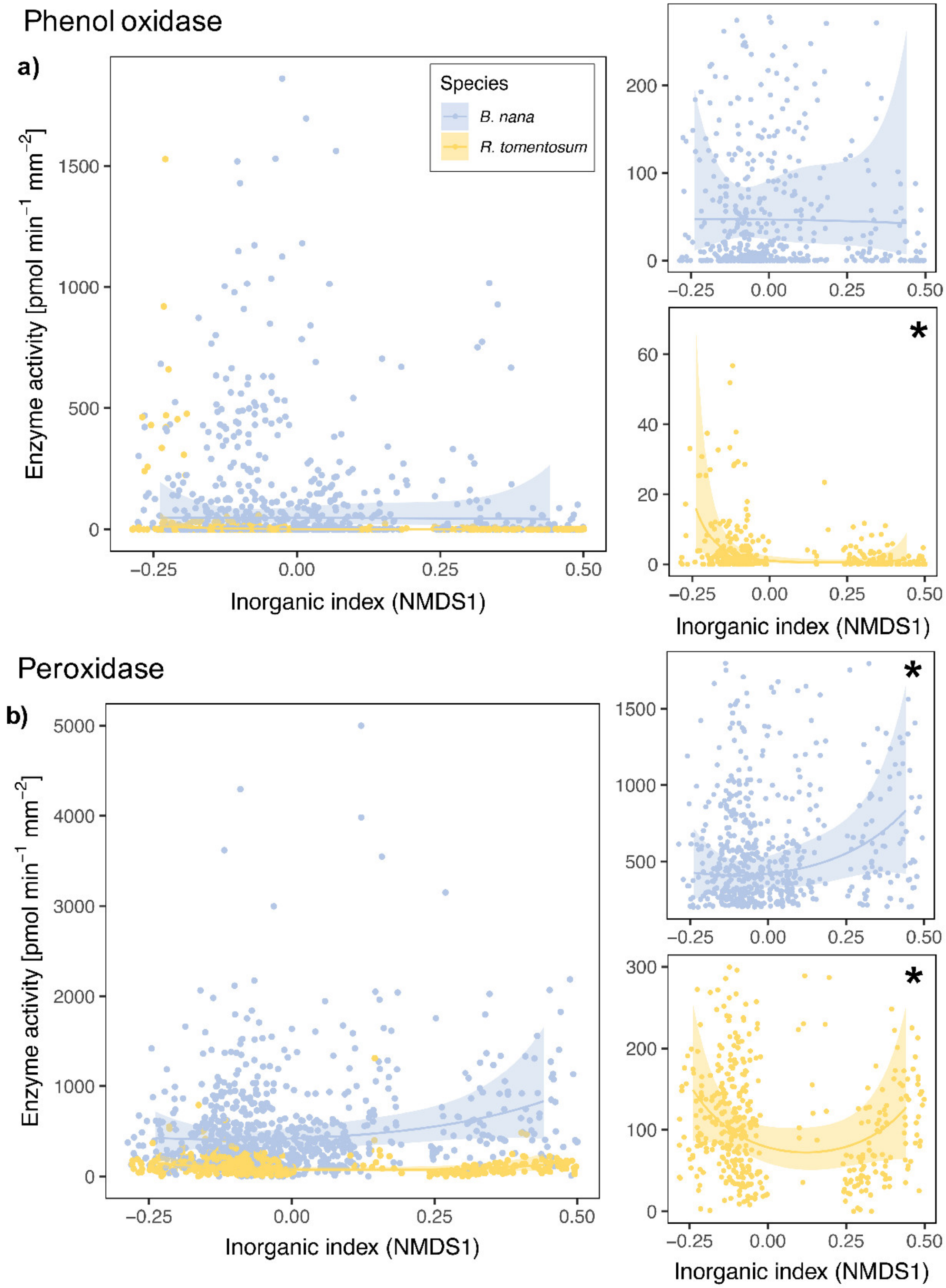

FIGURE 7 Responses of (a) phenol oxidase and (b) peroxidase activities to the inorganic soil fertility index. Because of the large differences in the ranges of enzyme activities between ectomycorrhizal *Betula nana* and ericaceous *Rhododendron tomentosum* roots, we present model results on the full scale and on a scale proportional to the interquartile range of each root type's activity. Colors represent root species. Lines and shading represent linear mixed-effects model results with 95% CLs. An asterisk indicates parameter estimates that are significantly different from zero. NMDS, nonmetric multidimensional scaling.

this vegetation shift (Vowles & Björk, 2019). Ericaceous shrubs have lower litter quality and quantity than EcM shrubs (Hobbie, 1996), associate with different mycorrhizal fungi, interact differently with herbivores (Vowles et al., 2017), and likely will not increase snow cover in the same manner that taller EcM shrubs do. Additionally, like their EcM counterparts, ericaceous shrubs are active at the thaw front and acquire recently released N from thawing permafrost via their mycorrhizal fungi (Hewitt et al., 2020). These attributes could positively affect ecosystem C storage by either boosting plant growth or moderating decomposition rates in a warmer environment. Incorporating the quadratic response of ericaceous shrubs found in this study into models of vegetation change will improve our ability to predict future co-expansion and its implications for ecosystem processes.

Though *R. tomentosum* benefited from small to moderate increases in nutrient availability, the cover begins to decline when *B. nana* height significantly exceeds the maximum height of *R. tomentosum* (Figure 4b). Interspecific competition with *B. nana* has previously been hypothesized to be a driver of the decrease in ericaceous shrubs at high levels of soil fertility (Bret-Harte et al., 2001). Experimentally increased nutrient availability initiates a plastic growth response in *B. nana*, where it grows long shoots and shades out competing ericaceous shrub and graminoid species. Throughout our soil fertility gradient, *B. nana* height steadily increased while *R. tomentosum* did not change. Though this is not direct evidence that *B. nana* outcompeted *R. tomentosum* for light in its journey toward dominance across the gradient, it provides further support for this hypothesis. Additionally, these results offer insights for instances when *B. nana* does not initiate shoot elongation. In the Scandinavian Arctic, *B. nana* was relatively unresponsive aboveground to long-term high-dose fertilization (Van Wijk et al., 2003). Instead, plant communities remained similar to unfertilized plots as the biomass of all vascular species increased. Varying mechanisms have been proposed to explain the observed regional differences in whether or not *B. nana* grows long shoots, including localized soil characteristics (Jonasson et al., 1999) and investment in defensive phenolic compounds (Graglia et al., 2001). Our results in combination with those of past studies suggest that *R. tomentosum* abundance may not decrease in dominance with higher soil fertility if *B. nana* does not grow long shoots.

Root enzyme responses

Enzyme activities on ericaceous roots were more sensitive to changes in soil fertility than those on EcM roots (Appendix S1: Figure S4). In contrast to unchanging activity on EcM roots, ericaceous root activity declined even as ericaceous shrub cover increased. We offer two possible explanations. On one hand, greater sensitivity could reflect that *R. tomentosum* readily reduced enzyme production—a metabolically expensive process—to account for changes in soil fertility. In tundra and other ecosystems, increased soil fertility can increase plant allocation to aboveground material (Iversen et al., 2015; Poorter et al., 2012). For *R. tomentosum*, this shift may be initially beneficial, but lose its effectiveness once interspecific competition for light with *B. nana* becomes a larger factor. On the other hand, the greater sensitivity of ericaceous roots may indicate that these shrubs respond poorly to inorganic nutrient addition, perhaps physiologically. In this case, the observed drop in root activity served as an early sign of the detrimental effect of added nutrients. Zamin et al. (2014) found negative responses of ericaceous shrubs to high-dose fertilization regardless of EcM shrub presence. While the decrease in ericaceous shrubs was speculated to result from trade-offs associated with higher leaf nutrient concentrations, our results suggest it could relate to belowground processes. Overall, the relative insensitivity of *B. nana* root enzyme activity compared with the sharp sensitivity of *R. tomentosum* could convey a competitive advantage to *B. nana* for nutrient acquisition. Regardless of the drivers behind the greater sensitivity of ericaceous enzyme activity, its implications point to a decreasing influence of ericaceous shrubs in tundra soil nutrient cycling as climate warms and nutrient availability increases.

We were surprised to find the activities of most enzymes on EcM roots did not change, even at the highest levels of soil fertility. We hypothesized that higher soil fertility levels would reduce EcM-associated root activities based on the ecological market theory involved in mycorrhizal relationships (Franklin et al., 2014). This predicts that when nutrients are readily available, plants will lower their C allocation to mycorrhizae. Furthermore, our prior research in a nearby 28-year experiment found sharp declines in root activity with high-dose fertilization. While we expected to find similar results, the difference in the length of these two experiments—more than a decade and a half—could explain the conflicting results. Similar to the lack of EcM response in our study, the initial 15 years of fertilization in the longer running experiment did not affect EcM biomass and colonization (Clemmensen et al., 2006; Deslippe et al., 2011). Long-term studies in Arctic tundra demonstrate temporal dependencies in a variety of responses to a changing climate, including plant communities (Chapin et al., 1995; Shaver et al., 2001), soil microbial communities (Koyama et al., 2014; Rinnan et al., 2007), and ecosystem C and nutrient cycling (Christiansen et al., 2018; Mack et al., 2004). Mycorrhizal root communities and function may be no different than these other variables in their temporal response. Although EcM root enzyme activity generally did not respond to increasing soil fertility, the ratio of root activity to aboveground cover and height decreased. This points to a drop in allocation toward enzymatic acquisition of nutrients and is consistent with other studies that identify a proportional decrease in belowground allocation in EcM shrubs (DeMarco, Mack, Bret-Harte, Burton, et al., 2014; Zamin et al., 2014), backing our hypothesized weakening of host shrub reliance on mycorrhizae.

One enzyme—peroxidase—served as an exception to the general insensitivity found in EcM root activities. EcM-associated peroxidase activities rose with soil fertility and were more sensitive than those on ericaceous roots. We posit that increased peroxidase activities on EcM roots may be in response to increases in woody litter with greater shrub cover. Heightened peroxidase activity is a potential mechanism that may allow EcM shrubs to maintain dominance by supporting fungal partners that can readily degrade their senesced material. Other studies have found fertilization stimulated the activities of other C-degrading enzymes, similar to peroxidase, on EcM roots (Jones et al., 2012) and in bulk tundra soils (Koyama et al., 2013). Nutrient-related enzymes in bulk tundra soil, however, either did not respond or decreased with fertilization (Koyama et al., 2013; McLaren & Buckeridge, 2019). Furthermore, high-dose fertilization near these sites accelerates decomposition and reduces ecosystem C storage (Mack et al., 2004). Together, these results indicate that faster decomposition rates occurring with experimentally increased soil fertility likely arose from stimulated activity of free-living soil microbes and EcM-associated C-degrading enzymes rather than that of ericaceous root enzymes.

N and P interactions with increased soil fertility

Similar to previous studies, we found inorganic nutrient concentration was an important driver of shrub dynamics in our experiment. However, the question remains of whether N or P contributes more to plant community shifts.

Phosphorus has the potential to be an important element in deciduous shrub expansion. Across several tundra ecosystems, N and P co-limit plant growth (Chapin et al., 1995). At the highest levels of fertilization, we found that DIN concentrations steeply rose, while PO_4-P concentrations began to decrease. This nonlinear relationship might suggest a potential shift in the limiting element from N to P at these higher levels of DIN concentration. Recent evidence for co-limitation is variable. In a long-term experiment in Arctic Canada, plants in plots fertilized with only N experienced a weaker positive response than those in plots fertilized with both N and P (Zamin & Grogan, 2012). In contrast, McLaren and Buckeridge (2019) found evidence supporting a primary limitation by N in a factorial N and P addition experiment near our study site. Given that N availability may increase through warming and thawing soils as well as future anthropogenic N deposition in the North American Arctic (Galloway et al., 2008; Gong et al., 2018; Linder et al., 2013), P may become a more important nutrient in vegetation and C dynamics (Jiang et al., 2016; Street et al., 2018; Wieder et al., 2015). As permafrost thaw deepens, weathering materials will become more accessible, likely opening a new source of P into tundra ecosystem cycling. The extent to which potential P inputs will alleviate plant P demand, however, is understudied. Further research should be conducted to understand the constraint future P limitation may have on shrub expansion and how increases in plant-accessible P through permafrost thaw may alter these constraints.

Conclusions

This study adds to our understanding of the mechanisms and consequences of Arctic shrub expansion by measuring the responses of two contrasting shrub functional types across a long-term experimental soil fertility gradient. Because future nutrient releases in tundra soils are likely to be spatially and temporally heterogeneous, field-based regression analyses over a continuous gradient, such as those conducted in this study, are increasingly important in developing predictive models of shrub expansion. Given that the effects of heightened soil fertility can differ across tundra ecosystem types and that EcM and ericaceous shrub responses will likely vary across species, further research should compare shrub responses to environmental gradients among various tundra types. While caution should be taken in interpreting these results in areas with different dominant EcM and ericaceous shrub species, the results presented here suggest that as soil fertility increases in Arctic tundra, the resulting vegetation shift heavily depends on the amount of nutrients released. In areas with weaker increases in soil fertility, both deciduous EcM and evergreen ericaceous shrub cover could increase. In areas with stronger increases in fertility, however, deciduous EcM shrubs like *B. nana* will likely dominate while evergreen ericaceous shrubs like *R. tomentosum* decrease.

Additionally, we predict that as we move to a greener tundra with higher nutrient availability, EcM fungi on *B. nana* will play a larger role in liberating soil organic nutrients than they have in the past as their host plant expands and as the roots of competing ericaceous shrubs decrease in degradative enzyme activity.

AUTHOR CONTRIBUTIONS

Michelle C. Mack and Haley R. Dunleavy conceived the ideas and designed the methodology. Haley R. Dunleavy collected and analyzed the data and led the writing of the manuscript. Michelle C. Mack acquired the funding, supervised the project, and contributed critically to manuscript drafts. Both authors provided final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Dunleavy & Mack, 2021b) are available from the Arctic Data Center: https://doi.org/10.18739/A2125QB07.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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