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Long-term low-level factorial nitrogen and phosphorus additions significantly impact a Low Arctic mesic tundra plant community, but species responses differ from high-level fertilization: Implications for predicting climate warming effects

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Abstract

Arctic climate warming is expected to enhance plant growth-limiting nutrients in tundra soils, thereby affecting community composition. Much of our understanding of nutrient influences on tundra plants is derived from very high level fertilization experiments. Here, we report effects of 11–19 years of unusually low-level factorial annual nitrogen (N) and phosphorus (P) additions (at rates 1/10th of most previous studies) to mesic birch hummock tundra vegetation. We measured aboveground biomass of all species and birch shoot lengths (total and current year's extension) in 1 and 9 m^2 sampling areas respectively. Only the low N treatment had significant biomass effects, increasing the moss group and some infrequent vascular species, reducing others, and having no effects on the dominant shrubs (including birch). Hence, overall community composition was altered, but in markedly different ways to classic high-level fertilization responses. However, birch new shoot extension in the 9 times larger sampling areas was strongly stimulated by each of the separate low-level N and P additions, and even more so by their combination, indicating that its stem apical primary growth was NP co-limited. Overall, our results demonstrate that this tundra plant community is sensitive to very low-level (but climatically-realistic) soil nutrient enhancements, and suggest that changes will be slow (multiple decades), and likely to favour species whose growth is primarily N-limited.

Key words: plant community, soil nutrients, spatial scale, multi-decadal temporal dynamics

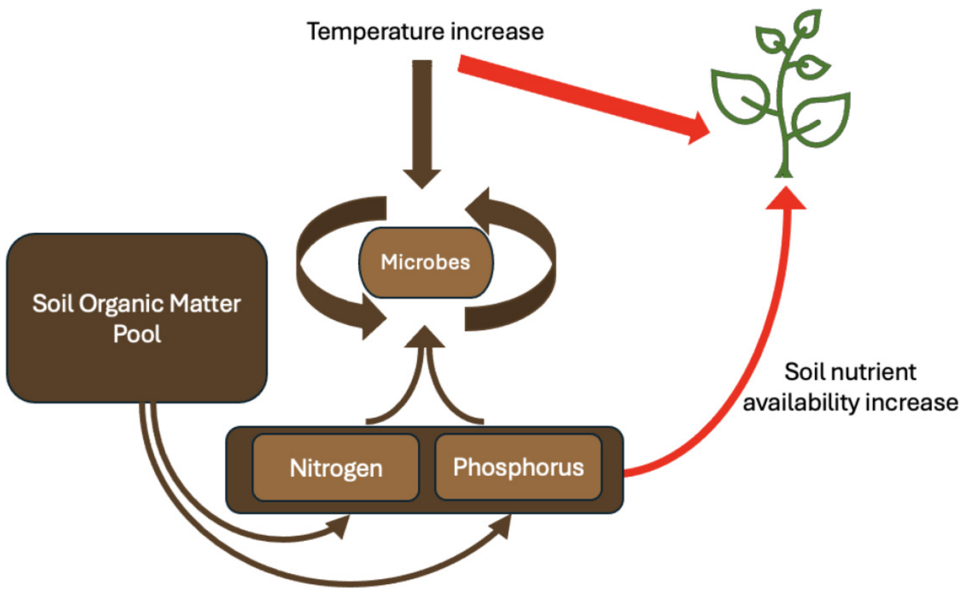
1. Introduction

1.1. Recent climate warming in the Arctic and corresponding vegetation change

Climate change is occurring particularly rapidly in the Arctic, where mean annual surface air temperature has increased nearly four times as fast as the global average (Rantanen et al. 2022). Warming is expected to stimulate microbial decomposition of tundra soil organic matter (SOM), increasing soil nutrient availabilities (Haag 1974). The consequent changes in plant species' niche space will drive changes in tundra vegetation community composition (i.e., the identity and relative abundances of species) (McKane et al. 2002; Holt 2009).

Recent repeat photography, long-term ecological monitoring, and tree dendrochronology studies of Arctic tundra ecosystems have already chronicled significant vegetation shifts in the past few decades (Myers-Smith et al. 2011; Bjorkman et al. 2018; Mekonnen et al. 2021). For example, mesic birch hummock tundra is a widespread vegetation-type across the Low Arctic, and its principal deciduous shrub Birch (*Betula glandulosa* Michaux) has been undergoing range expansion in many places (Mekonnen et al. 2021). This pan-Arctic phenomenon may be attributed to direct effects of climate warming on plant growth as well as indirect effects associated with soil fertility increases due to warming-enhanced microbial decomposition of SOM (Chapin et al. 1992) (Fig. 1). However, the relative importance of these two effects has not been determined in the context of realistic climate warming in the near future. Furthermore, in contrast to the widespread observations of enhanced deciduous shrub cover across the Arctic, evergreen shrub increases in response to warming have not been frequently reported except for some locations in Scandinavia (e.g., Maliniemi et al. 2018), and at our Daring Lake, NWT, Canada research site (Zamin et al. 2014). Evergreen responses could be particularly important since their relatively slow growth rates and production of recalcitrant litter may act as a negative feedback that would slow warming-induced increases in soil nutrient availability for other species (Vowles and Bjork 2018). In summary, given the

Fig. 1. Conceptual diagram illustrating the direct and indirect (i.e. soil mediated) effects of climate warming that may influence tundra plant community growth, and structural composition. For each plant species, photosynthesis and growth may be stimulated directly by warmer air temperatures, and/or by indirecly by the enhanced soil nutrients associated with warming-induced soil microbial decomposition of soil organic matter that increases availabilities of soil soluble (nitrogen) N and (phosphorus) P. The extent to which some plant species respond more than others to these direct and indirect effects, the relative magnitudes of warming-enhanced availabilities of these two nutrients, and the relative growth limitation by N as compared to P for each species, will together ultimately determine tundra plant community structure under a warmer climate.



potential for such interactions, understanding the impacts of increased availabilities of all plant growth-limiting nutrients on each of the individual species within tundra communities will be necessary to accurately predict Low Arctic vegetation responses to a changing climate.

1.2. Long-term nitrogen and phosphorus fertilization experiments to investigate tundra plant community growth limitation

Combined nitrogen (N) and phosphorus (P) fertilizer addition experiments have been used at several Low Arctic tundra research sites to investigate nutrient limitation of plant growth (i.e., plant growth responses when the potential for limitation by specific nutrients is eliminated by adding them at rates that greatly exceed annual plant uptake) (Chapin et al. 1995; Jonasson et al. 1999; Mack et al. 2004; Van Wijk et al. 2004). Generally, N + P fertilization causes growth to increase dramatically in systems dominated by deciduous vegetation (Shaver and Jonasson 1999; Graglia et al. 2001). This growth response is attributed to the naturally faster tissue/nutrient turnover rates (i.e., movement of elements through biogeochemical cycles) associated with deciduous shrub species due to their annual shedding of leaves (Van Wijk et al. 2004). By contrast, evergreen species have less developmental plasticity and have tended to exhibit a negligible response to fertilization, perhaps due to their slower turnover rates (Van Wijk et al. 2004).

High amounts of N and P have been added not just in combination, but also separately in at least two low Arctic sites to investigate the individual influences of these two elements (Zamin et al. 2014; McLaren and Buckeridge 2019). At Toolik Lake, proportional cover (based on visual estimates) of *Betula nana* (a closely related shrub species to *B. glandulosa*) was stimulated in the N and N + P addition plots, but inhibited by P addition alone, suggesting its growth was primarily N-limited (McLaren and Buckeridge 2019). To our surprise, the corresponding experiment at Daring Lake demonstrated that birch apical shoot extension (current year's primary growth) was stimulated by each of the separate N and P additions, and even more so by their combination (Zamin and Grogan 2012). A subsequent follow-up harvest study confirmed this NP co-limitation pattern for shoot biomass of birch and also for the widespread and very common Arctic sedge *Eriophorum vaginatum* (Zamin et al. 2014)). Furthermore, the forb *Rubus chamaemorus* was primarily P-limited (Zamin and Grogan 2014). Together, these results fundamentally question the widespread dogma that growth of all Arctic mesic tundra plant species is primarily N-limited (Shaver and Chapin 1980; McKane et al. 2002; Mack et al. 2004), and they therefore challenge the assumption that climate-warming enhancement of soil N availability will be the primary driver of shifts in future tundra plant community composition.

In contrast to Birch, the long-term additions of high amounts of N and P (hereafter "high N" and "high P") at Daring Lake significantly decreased evergreen shrub biomass including the originally dominant evergreen shrub *Rhododendron tomentosum* (Zamin et al. 2014). These declines were attributed to possible nutrient toxicity (i.e., exposure to nutrient levels beyond their natural soil fertility range), nutrient-altered competitive interactions, or delayed pre-winter shoot hardening (Zamin et al. 2014). Negative responses in such experiments cannot be used to infer that those nutrients do not in fact limit growth: All that can be concluded is that the negative impacts of the nutrient addition were more substantial than any potential positive growth enhancements. Therefore, when plants experience moderately enhanced soil fertility (i.e., with low-level nutrient additions that avoid the potential for negative feedbacks associated with over-fertilization), certain species' growth may respond positively, and thus reveal possible nutrient limitations that could not be detected in high-level nutrient addition studies. In that context, it is noteworthy that a separate 8 year summer greenhouse treatment (i.e., with no direct fertilization but probable warming-induced indirect increases in soil nutrient availability) increased shoot biomass of all vascular species—including the evergreens (Zamin et al. 2014). Finally, although several species responded positively to the high-level N additions, the overall lack of species responses to the low-level N additions (Zamin et al. 2014) is intriguing, and suggests that either the two nitrogen treatment levels have resulted in fundamentally different trajectories of community change, or that the low-level N addition simply had not reached a sufficient fertility level to drive change after 8 years—but might do so over a longer period such as the 19 years of treatments investigated in this study below.

Overall, the results of these long-term fertilization experiments demonstrate that (a) nutrient availability is an important limitation on plant growth and driver of plant community composition in Low Arctic tundra; (b) some species are much more responsive than others to enhanced nutrient availability; (c) some species are much more responsive than others to supplements of nitrogen as compared to phosphorus; and (d) responses to low-level N additions do not mimic those of high-level N addition—at least after 8 years of nutrient supplementation.

1.3. Why might it be important to investigate species' growth limitation responses to N and P separately?

P mobilization into plant-available pools may be less responsive than N to soil warming for several reasons: First, N is mobilized into its various plant-available soil solution pools by the microbial decomposition of SOM (Brady and Weil 2008). Soil temperature therefore is a primary control on soil N supply into its plant-available pools (ammonium, nitrate, and dissolved organic N). By contrast, although tundra plant-available P supply from microbial decomposition of SOM is also controlled by soil temperature, the primary P product (phosphate) is much less soluble than the N products, and tends to precipitate out of solution very rapidly (Brady and Weil 2008)—especially at the low pH typical of tundra soils (Murrmann and Peech 1969). Second, soil microbes have relatively low N:P ratios in comparison to plants (and SOM) and so they require larger amounts of P relative to N from the soil solution (Jonasson et al. 1999). Accordingly, soil microbes are expected to internally accumulate more of the additional P compared to the N that is released as a result of warming-enhanced decomposition of SOM, thus altering the relative proportions of each element in the plant-available nutrient pool. In summary, these biogeochemical differences between N and P cycling in tundra soils suggest that N availability to plants may be enhanced proportionally more than P under future Arctic warming. Consequently, those plant species that are primarily growth-limited by N may be more favoured than species that are either NP co-limited or P-only limited, resulting in altered species niche space and hence shifts in plant community composition.

1.4. The thorny issue of spatial and temporal sampling scales that permeates interpretation of observed recent ambient, and experimental warming-induced, vegetation changes

Temporal changes in individual plant species abundances within mesic birch hummock tundra have been tracked at our Daring lake site using 1 m^2 long-term vegetation point-frame monitoring sub-plots that were established in control and summer greenhouse warming treatment plots in 2004, and measured in 2005, 2011, and 2017. The greenhouse warming treatment significantly enhanced birch shoot biomass after 8 years (i.e., in 2011) as expected from other studies (Chapin et al. 1995), but to our surprise, growth of evergreens was enhanced substantially more than birch (Zamin et al. 2014). However, sampling within the same greenhouse experimental plots (but different sub-plots) in 2017 revealed a significant warming effect only for *Vaccinium vitis-idaea* (Gu and Grogan 2020). In other words, these two measurements of shoot biomass in the same experiment (based on 1 m^2 point-framing of different sub-plots, each with an associated biomass harvest of different 0.16 m^2 areas) yielded conflicting growth results between the two different sampling years (Zamin et al. 2014; Gu and Grogan 2020). We conclude that high spatial variability in plant species distributions in mesic birch hummock tundra vegetation (Figs. E2 and E4) may explain such inconsistencies, and therefore that larger sampling areas are likely to yield more robust conclusions.

Species growth responses to nutrient addition treatments may be determined non-destructively by point-framing (Gu and Grogan 2020) and by measuring plant dimensions (e.g., new shoot length) (e.g., Zamin and Grogan 2012), or destructively by harvests of shoots and/or their component parts (e.g., Bret-Harte et al. 2002; Zamin et al. 2014; Berner et al. 2018; Gu and Grogan 2020). For species' whose total aboveground biomass is accumulated over multiple years (i.e., all the deciduous and evergreen shrubs in birch hummock tundra vegetation), total shoot biomass may not be a very sensitive indicator of responses to treatments. This is especially true if the aboveground biomass has been accumulated over a similar or longer timeframe than the full course of the experimental manipulation. Accordingly, for such species, measurements of apical shoot extension (i.e., current year's shoot primary growth) can be particularly effective for detecting changes in growth rate in response to recent changes (Zamin et al. 2014). Therefore, to optimize the sensitivity of our determination of *B. glandulosa*'s response to low-level enhancements of soil fertility, we included an additional unusually large spatial scale (9 m^{-2}) non-destructive sampling of birch shoot length extension in our study below.

1.5. What is the likely impact on tundra plant community growth of more climatically realistic (i.e. low magnitude) increases in soil nutrient availability due to warming?

Historically, tundra fertilization experiments with combined N plus P additions at very high levels (10 g N m^{-2} and 5 g P m^{-2} $year^{-1}$—which are typical application rates in temperate zone agriculture), have yielded fast dramatic growth responses (Chapin et al. 1995; Jonasson et al. 1999; Mack et al. 2004). While these experiments are extremely useful for identifying which soil nutrients limit growth of particular species, they are undoubtedly excessive in terms of the enhanced soil nutrient levels expected with climate warming. For example, the largest estimates we are aware of suggest N mineralization would be increased by 7 g N m^{-2} $year^{-1}$ for a 3 °C soil temperature rise (Mack et al. 2004). However, other researchers contest that the initial soil fertility responses to warming are unlikely to be of that magnitude (Hobbie et al. 2002). Furthermore, when other ecosystem processes such as nitrogen losses via leaching and denitrification as well as soil microbial nutrient accumulation are accounted for, much lower levels of increased soil fertility are likely (Hobbie et al. 2002; Zamin and Grogan 2012). In conclusion, the high-level annual addition rates in almost all N and P fertilization experiments to date are unlikely to reflect the near-future enhanced soil nutrient availabilities expected with climate warming.

Accordingly, we established a long-term factorial low-level N × P experiment (with addition rates 10% of the classic high-level additions) to test the responses of each species/growth form within a birch mesic hummock tundra plant community to the moderately enhanced soil fertility that might reasonably be expected with Arctic climate warming over the next few decades. Our overall goals were to determine whether (1) plant species differ in their responses to either low-level N or P enhancement; (2) species whose growth has been demonstrated as NP-colimited (using the high N × P additions) display this same colimitation at a much lower but more climatically-realistic levels of N and P enhancement; (3) those species that responded negatively to high N, P, or N + P, respond positively to lower (more climatically-realistic) level enhancements of those same nutrients; and (4) overall plant community composition is significantly altered by low-level N, P, or N + P additions.

In summary, after at least 11 years of the combined low-level factorial N + P fertilization treatments, this novel—and as far as we know unique—experiment separates N and P responses to realistic soil fertility increases with climate warming so as to improve our capacity to predict future Low Arctic mesic tundra vegetation changes. The following hypotheses were tested using a combination of robust community shoot biomass harvests (1 m^2 replicate sampling areas) and birch shoot length measurements (9 m^2 replicate sampling areas), both of which were made at substantially larger areal sampling scales than most previous investigations to better account for the high species spatial variability previously noted (Zamin et al. 2014; Gu and Grogan 2020):

1. Species with previously demonstrated positive growth responses to high-level factorial inputs of either N or P (*Rubus chamaemorus*) will respond positively to low-level inputs of those same nutrients.
2. Species with previously demonstrated negative growth responses to high-level factorial inputs of either N and/or P (*Rhododendron tomentosum*, *V. vitis-idaea*, *Vaccinium uliginosum*, *Andromeda polifolia*, bulk mosses, and bulk lichens) will respond positively to low-level inputs of those same nutrients.
3. Species whose growth has previously been demonstrated to be NP-colimited (i.e., *B. glandulosa* and *E. vaginatum* - Zamin et al. 2014) will be significantly more responsive to the combined low-level N + P treatment than to additions of either low N or P alone.
4. Plant community composition will be significantly altered in response to 19 years of low-level N inputs.

2. Methods

2.1. Site description and plot selection

The study was conducted in the central Canadian Low Arctic at the Tundra Ecological Research Station (TERS) near Daring Lake, Northwest Territories, Canada (64°52′N, 111°33′W). The research area occupies a gently sloped valley and is enclosed to the North by the Thelon esker, an 800 km long ridge of sub-glacial riverine deposits (Dredge et al. 1999). It contains several tundra vegetation-types extending a sloped hydrological gradient from the dry heath esker near the top, to wind-sheltered taller shrub slopes, mesic birch hummock tundra, and then wet sedge vegetation in the watercourses and wetlands in the lowest elevations of the valley (Fig. E1) (Nobrega and Grogan 2007). The region is underlain by continuous permafrost (>160 m depth), and maximum depth of the overlying summer thawed soil active layer varies by soil-type and vegetation-type from 0.3 to 1.2 m depth (Dredge et al. 1999; Lafleur and Humphreys 2008). Weather monitoring at TERS (1996–2014) indicated a mean annual air temperature of −8.9 °C, mean July temperature of 13.4 °C, and mean growing season rainfall of 260 mm (Humphreys and Lafleur 2011; Campeau et al. 2014).

Our experiment was focused entirely on mesic birch hummock tundra vegetation, which fits within the erect dwarf-shrub, moss tundra category of arctic vegetation-types (Raynolds et al. 2024), and is categorized by maximum birch shrub height of 40 cm. This vegetation-type features the evergreen shrubs labrador tea (*Rhododendron tomentosum* (Harmaja)—for which *Rhododendron subarcticum* (Harmaja) is a synonym), lingonberry (*Vaccinium vitis-idaea* L.), and bog rosemary (*Andromeda polifolia* L.), the deciduous shrubs dwarf birch (*Betula glandulosa* Michx.) and blueberry (*Vaccinium uliginosum* L.), the herb sedge cottongrass (*Eriophorum vaginatum* L.), and the forb cloudberry (*Rubus chamaemorus* L.), as well as a substantial diversity of mosses and lichens.

2.2. Annual factorial nitrogen and phosphorus addition treatments

During the establishment of the experiment in 2004, 10 similar plots (5 m × 7 m each) of birch hummock tundra vegetation were selected in the research valley and then were randomly allocated to one of the following two treatments: control, and low-level N (LN; 1 g N m^{-2} $year^{-1}$). In summer 2012, as a consequence of the high-level factorial N and P fertilization experiment results reported in the Zamin and Grogan 2012 study, all plots were halved to expand the initial low-level N experiment to a full factorial N and P experiment consisting of 20 plots (5 m × 3.5 m each; $n = 5$) and four treatments: control and low-level N as described above, and low-level P (LP; 0.5 g P m^{-2} $year^{-1}$), and low-level N + low-level P (LNLP; (1 g N + 0.5 g P) m^{-2} $year^{-1}$). Since it had been running for over a decade, we assume that the more recently established P treatment had taken full effect by the time of the study reported here. This assumption will be supported if we observe P growth responses in the longest-living species in particular (since all the other shorter-lived species will have undergone the treatment for greater proportions of their lifespans). All nutrient additions were applied in July or August of each growing season (Fig. E2), with the N as granular ammonium nitrate (NH_4NO_3) from 2004 to 2015 and then as urea (CH_4N_2O) from 2016 onwards, and the P as 45% phosphorus pentoxide (P_2O_5).

2.3. Birch shoot length measurements

At the end of the 2022 growing season, we measured total shoot length, and current year's new shoot extension, of all individual birch stems in 3 m × 3 m sampling areas that were located so that their centres corresponded with the exact centres of the low N and low P factorial experimental plots (i.e., the birch sampling areas were located using a structured random approach). This relatively large sampling area was chosen because despite birch's visual prominence in birch hummock tundra vegetation, it is a relatively sparsely distributed species compared to most other species in this community (~2 ramets per m^2—Zamin et al. 2014), and tends to cluster into ramets given its clonal growth (Weis and Hermanutz 1988). For each birch individual ramet within the sampling area, the lengths of all stems arising from the soil surface and their associated branches were measured. Concurrently, any new shoot apical extension (i.e., current year's primary growth) for each individual stem was identified by the distinct colour change from white dried resin gland-covered stem tissue to fresh brown and relatively supple resin gland-covered apical stem tissue (Fig. E3), and measured separately. New shoot extension was expected to serve as a more sensitive measure of birch responses to the treatments since this primary growth measure is the product of the current year only. By contrast, total shoot length is the product of many years because individual birch shoots can live for over 30 years, and so this measure may include growth that occurred under the ambient conditions prior to initiation of the experimental treatment. Since very considerable time was required to identify all *B. glandulosa* individual ramets within each of the 9 m^2 plots, and then to measure all total and new shoot growth of each individual main stem and its branches, we were logistically restricted to measuring only three randomly selected plots from the five experimental replicates in each treatment.

2.4. Biomass sample collection

Aboveground plant biomass was harvested in 1 m^2 sampling areas of all replicate plots from the factorial low N × P experiment in late summer of 2023. Harvest areas were designated using a structured randomisation approach by placing the North-East corner of the 1 m^2 sampling area 50 cm inwards from the Northern and Eastern edges of each full (5 × 3.5 m) treatment plot. However, sampling at that location would sometimes impinge on the 1 m^2 permanent point-frame monitoring zone within each treatment plot, and so in those instances the harvest area was instead located 50 cm inwards from the Southern and Western edges of the treatment plot. Shoots along the boundary edges of each sampling area were teased apart to ensure that only individuals rooted within that area were included in the harvest. The 1 m^2 sampling area was then completely harvested by cutting out small turves of soil and associated plants with a serrated knife (~10–20 cm × 10–20 cm × ~10 cm depth) and placing them all in a large plastic bag for transport to the field lab (Fig. E4).

For sorting to the species level, each live shoot of all vascular plants was followed down to the first adventitious root or junction with the underlying rhizome—whichever occurred first—at which point the shoot was clipped and sorted by species. Lichen pieces >1 cm width were plucked from the top soil/moss surface and all species were grouped together for biomass. For mosses, the green-brown boundary was used to differentiate above- from belowground growth, and all green moss tissue for all species was clipped and grouped together. Standing dead biomass of vascular species, leaf litter, and dead moss were discarded (i.e., not included in the sorted sample components). For birch, live leaves were separated from stems to separately quantify each component of aboveground biomass. Finally, all sorted biomass components were dried at 65 °C in a fan-assisted oven for ~7 days and weighed immediately afterwards.

We returned to the site to measure multiple soil variables in the treatment plots a year later (25 August 2024) at points directly adjacent to each plot's harvested area that were covered by intact vegetation. The active layer (i.e., late August soil thaw depth) was measured 15 cm away from each of the four corners of the harvested area and averaged. To determine soil bulk density and late August gravimetric soil moisture content, a serrated breadknife was used to cut down the edges of a 20 cm × 20 cm area. To prevent disturbance of the internal soil structure/volume, the enclosed soil block was then gently pulled out by grasping the two lateral sides, and placed on a cutting board. Subsamples (~5 × ~5 cm area × ~10 cm deep) were cut from the outermost side of the main block (furthest from the harvested sample area) and their dimensions carefully measured and recorded. The subsamples were then sealed in plastic bags for fresh weight determination at the field lab, followed by transport back to Queen's for ovendrying at 65 °C over at least 5 days and then dry weight measurement. Finally, the organic layer depth was determined in the 20 cm × 20 cm hole by first identifying the transition from organic to mineral soil, and then measuring its depth from the soil top surface (i.e., the green-brown transition in the moss layer).

2.5. Data analysis

All data were first visually and statistically inspected to ensure that the response variables and associated predictors met the assumptions of normality and homogeneity of variance using the *lmtest* and *car* packages in R (v.0.9-40, Zeileis and Hothorn 2002; v. 3.1-3, Fox and Weisberg 2019, R Core Team 2016). All data satisfied the assumptions, and the subsequent analyses were conducted without any data transformations.

Betula glandulosa new and total shoot length measurements (late summer 2022) were analysed using two-way analyses of variance (ANOVAs) with N addition and P addition as main effects, as well as their interaction. Afterwards, Tukey post hoc tests were performed to analyse multiple comparisons among the treatments.

For the 2023 harvested plant biomass data of each species or growth form, two-way analyses of co-variance (ANCOVAs) were used to test the effects of N addition and P addition and their interaction as main effects, and the soil variables as covariates. In the case of *B. glandulosa* where leaves were separated from stems, these individual biomass components were analysed separately, and then together as total aboveground biomass. The full model included each of the four measured soil variables (active layer depth, bulk density, gravmetric moisture %, and organic layer depth), and all other possible models were created by stepwise removal of these variables for a total of 16 possible models. To select the best-fitting model, the *model.sel* function from the *MuMIn* package (v.1.48.4; Bartoń 2024) was used to calculate the Akaike information criterion (AICc) and estimate the relative goodness of fit and quality of the various models. If multiple competing models were estimated to have AICcs occurring within delta ≥2, the top model was selected by carefully comparing AICc weights, and the relative importance of each of the variables, to choose the best-fitting model. All assumptions of ANCOVA were checked by visual inspection of residual plots in addition to Global Validation of Linear Models Assumptions using the *gvlma* package (v. 1.0.0.3; Pena and Slate 2006). While some models did not pass stringent tests of assumptions (which are ill-suited for small sample sizes, like the Durbin–Watson test for autocorrelation of disturbances), they all passed visual inspections of residuals and normality. *P* values and *F*-statistics for each linear model were obtained using the “Anova” function in the *car* package. Statistically significant individual treatment effects were determined using multiple comparisons, specifically Tukey’s HSD post hoc tests using the *multcomp* package (v. 1.4-26; Zeileis and Hothorn 2002) while the direction of the correlation with environmental covariates was inferred from the coefficients in the species’ linear model.

Impacts of the nutrient addition treatments on overall community structure were tested by calculating Pielou’s evenness and Shannon’s Diversity indices for the shoot biomass of vascular plant species, and performing separate ANOVAs and subsequent Tukey’s post hoc tests.

We used a principal components analysis (PCA) to further investigate the impacts of the factorial nutrient additions on overall community composition and the biomass of each species and growth form, as well as to visually interpret correlations with environmental variables. The PCA ordination was performed with the *prcomp* function in R using the aboveground biomass data for each species and growth form as active constraints, whereas qualitative supplemental factors N and P, and qualitative environmental variables were overlain passively. Despite all biomass having the same unit, species biomass was scaled to unit variance given the disparity between the most abundant and least abundant species and growth forms. To further interpret the PCA visual trends and to test for significance of the N and P treatment effects and their interaction, as well as correlations with the soil environmental variables, we conducted a PERMANOVA with the *vegan* package in R (Oksanen et al. 2024) using Bray–Curtis dissimilarity indices generated from the aboveground biomass data for all plant species and growth forms.

3. Results

3.1. Long-term effects of the factorial low nitrogen and phosphorus additions on aboveground biomass

Of the seven principal vascular plant species in this mesic birch tundra vegetation community, only aboveground biomass of the evergreen shrub *A. polifolia* was significantly enhanced by the long-term low-level N additions (Tables 1, 2, and A1, Fig. B1), while the sedge *E. vaginatum* also tended to increase (but not quite significantly $P < 0.06$) (Tables 1, 2, and A2, Fig. B2). Conversely, both *V. vitis-idaea* and *Rubus chamaemorus* aboveground biomass were significantly reduced by the low N addition treatment (Tables 1, 2, A3, and A4, Figs. B3 and B4). The long-term low-level P additions had no statistically significant effects on any of the vascular species, and there no significant N × P interactions. In summary (Table 2), although there were some significant individual species responses to N as outlined above, neither the dominant *Rhododendron tomentosum* (nor *B. glandulosa*, or *V. uliginosum*) were altered (Tables 1 and A5–A9, Figs. B5–B7), and so total vascular plant shoot biomass of this low arctic mesic tundra community was not significantly affected by any of the fertilization treatments (Tables 1 and A10).

By contrast, the non-vascular components of the plant community were relatively sensitive to the P addition treatments. Moss total aboveground biomass (i.e., bulked across species) was significantly enhanced by the combination of the low N plus low P addition, but not by either addition on its own (Tables 1, 2, and A11, Fig. B8). Furthermore, the bulked lichens—which generally are the largest biomass component of the total community (Table 1)—were the only measured plant category that tended to respond negatively

Table 1. Plant total aboveground biomass (g m^{-2}) for all vascular species and non-vascular growth forms in the factorial low-level N × P experiment in late summer 2023 after 19 years of N addition and 11 years of P addition.

	Control	Low N	Low P	Low N + P
Betula glandulosa				
Leaves	4.0 (1.3)	7.3 (4.6)	4.4 (2.9)	6.1 (2.3)
Stems	23 (7.4)	46 (29)	36 (22)	31 (11)
Total aboveground	27 (8.5)	53 (34)	40 (25)	37 (13)
Rhododendron tomentosum				
Total aboveground	120 (10)	150 (21)	130 (28)	190 (57)
Vaccinium vitis-idaea				
Total aboveground	85 (5.7)	61 (6.6)	100 (22)	41 (13)
Andromeda polifolia				
Total aboveground	19 (11)	45 (3.9)	11 (6.7)	35 (11)
Vaccinium uliginosum				
Total aboveground	5.6 (4.7)	10 (5.1)	13 (10)	24 (7.0)
Eriophorum vaginatum				
Total aboveground	22 (7.0)	37 (12)	23 (5.4)	26 (12)
Rubus chamaemorus				
Total aboveground	3.3 (2.2)	1.1 (0.80)	3.8 (1.5)	0.42 (0.23)
Total vascular	**281 (14)**	**354 (47)**	**328 (63)**	**354 (50)**
Mosses (bulked)				
Total aboveground	81 (40)	78 (18)	58 (20)	140 (21)
Lichens (bulked)				
Total	200 (39)	140 (25)	120 (20)	120 (29)
Total vasc. + non-vascular	**560 (40)**	**570 (40)**	**500 (72)**	**616 (57)**

Note: Data are means with standard errors in parentheses ($n = 5$).

to the low-level P addition, though not quite significantly ($P < 0.06$) (Tables 1, 2, and A12, Fig. B9). Overall, as a result of these small magnitude and counteracting effects, the entire plant community (i.e., vascular plus non-vascular) total aboveground biomass was not significantly affected by either the low N or low P addition, nor their combination (Tables 1, 2, A13).

3.2. Divergent species and growth form responses to environmental soil measures

Although none of the measured soil characteristics differed significantly among the treatments (Table C1), the ANCOVA analyses indicated that variation in certain species' and non-vascular growth form's biomass across the plots was correlated with some of these soil characteristics. For example, aboveground biomass of the dominant evergreen shrub *Rhododendron tomentosum* was positively correlated with soil active layer depth, and negatively correlated with both late August plot soil moisture and soil bulk density (the latter not quite significantly $P < 0.06$) (Tables 2, A5, and C1). The other major evergreen shrub *V. vitis-idaea* was also correlated negatively with late August soil moisture (not quite significantly $P < 0.06$) (Tables 2, A3, and C1), while the minor evergreen *A. polifolia* tended to be negatively correlated with soil bulk density (Tables 2, A1, and C1). Abundance of the deciduous shrub *V. uliginosum* was negatively correlated with soil organic layer depth, while biomass of its counterpart *B. glandulosa* (either separately as leaves, stems, or as total shoot) was not significantly related to any of the measured soil characteristics (Tables 2, A6–A9, and C1). As might be expected from its typical distribution across low Arctic landscapes, biomass of the principal sedge *E. vaginatum* was strongly positively correlated with late August soil moisture, and negatively correlated with soil active layer depth (Tables 2, A2, and C1). This pattern along with the reverse result for *Rhododendron tomentosum* described above is consistent in that a shallower soil active layer can often correspond with relatively moist surface soil, and therefore suggests that while the sedge grows best in wet inundated conditions, the evergreen prefers relatively dry microsites. Finally, shoot biomass of the forb *Rubus chamaemorus* was negatively correlated with soil bulk density (Tables 2, A4, and C1). Overall, the ANCOVA analysis of total vascular plant biomass indicated no significant correlations with any of the measured soil characteristics (Tables A10 and C1).

For the non-vascular components of this mesic birch hummock community, the total aboveground moss biomass was not significantly correlated with any of the soil characteristics (Tables 2, A11, and C1). However, consistent with the inverse soil active layer/surface soil moisture pattern outlined above, and their general preference for dry surface soils, total lichen biomass was positively correlated with soil active layer depth (Tables 2, A12, and C1). Consequently, given that lichens are generally the largest biomass component of this vegetation-type (Table 1), it is not surprising that total above-

Table 2. Summary of factorial low N × low P treatment effects and significant environmental correlates in the statistical analyses of aboveground biomass for the seven major vascular plant species and mosses and lichens from the 2023 1 m^2 harvest of mesic birch hummock tundra vegetation.

	Low N addition	Low P addition	Low N + P addition	Soil active layer depth	Soil moisture	Soil bulk density	Soil organic layer depth
B. glandulosa							
Leaves				NA		NA	NA
Stems				NA		NA	NA
Total aboveground				NA		NA	NA
R. tomentosum							
Total aboveground				↑ **	↓ *	↓ †	NA
V. vitis-idaea							
Total aboveground	↓ **			NA	↓ †	NA	NA
A. polifolia							
Total aboveground	↑ **			NA	NA	↓ †	NA
V. uliginosum							
Total aboveground				NA	NA		↓ *
E. vaginatum							
Total aboveground	↑ †			↓ **	↑ *		NA
R. chamaemorus							
Total aboveground	↓ *			NA		↓ *	NA
Mosses (bulked)							
Total aboveground			↑ *	NA	NA		NA
Lichens (bulked)		↓ †		↑ *	NA	NA	NA
Total vascular plus non-vascular biomass				↑ †	NA	NA	NA

Note: Model effects and correlations that are significant are indicated as: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$; ↑ = increase in biomass; ↓ = decrease in biomass; NA = not included in species' linear model. In the case of the factorial Low N and P additions, effects are based on Tukey results, whilst for the environmental variables, correlations are based on the coefficients of the ANCOVA models (see Tables A1–A11 for details).

ground biomass of the community (i.e., vascular species plus non-vascular growth forms) across the plots tended (P < 0.07) to be positively correlated with soil active layer depth (Tables 2, A13, and C1).

3.3. Long-term effects of the factorial low nitrogen and phosphorus additions on community structure

The low-level N addition significantly altered community composition (i.e., species' presence and their relative abundances) according to a permutational Analysis of variance (PERMANOVA) ($F = 3.2$, $P = 0.005$, Table 3). By contrast, neither the P addition, nor the N × P interaction, significantly affected community composition (Table 3). These results are supported by Pa CA of the plant community biomass data which explained 27% of the variance in the first axis, and 23% in the second (Fig. 2). Visually, the ellipse clusters of the two treatments with N addition (i.e low N, and low N + P) overlap substantially, and both generally align similarly along PC1 (Fig. 2). Furthermore, the positions of those species whose biomass responded positively to low N addition (i.e., *A. polifolia,* and *E. vaginatum*) loaded positively on PC1, while those that responded negatively (i.e., *V. vitis-idaea, Rubus chamaemorus*) loaded in the opposite direction (Fig. 2). Of all measured soil characteristics, only soil active layer depth tended to be a significant (negative) covariate in the PCA ($P < 0.07$; Table 3), and it was associated closely with the second PC axis representing 23% of the variance (Fig. 2).

Finally, in terms of overall structure of the full vascular plant community, neither richness nor evenness were significantly affected by any of the treatments and therefore Shannon diversity was unaltered (Tables A14 and D1).

3.4. Long-term effects of the factorial low nitrogen and phosphorus additions on *B. glandulosa* total shoot length and new shoot extension

Birch growth was determined within the same experimental plots in the late summer of 2022 at a nine-fold larger spatial scale compared to the 2023 harvest year data described above (i.e., the shoot length measurements were made on all birch plants within a 9 m^2 sampling area, while the 2023 shoot biomass harvests were of a 1 m^2 sampling area). In contrast to the harvest results indicating no treatment effects on any component of *B. glandulosa* shoot biomass (Tables 1, 2, and A7–A9, Fig. B7), total shoot length of birch plants was significantly enhanced by each of the separate low-level N and P additions, with no significant interaction (Table 4, Fig. 3). Even more surprisingly, birch new shoot extension (representing primary stem growth during the year of sampling) was significantly enhanced not just by the separate N and P additions,

Table 3. PERMANOVA results of the factorial low N × low P treatment effects on aboveground biomass based on Bray–Curtis dissimilarities of 999 permutations of the late summer 2023 harvest data.

	Df	Sum Sq	R^2	*F* value	*P* value
Nitrogen	1	0.1	0.2	4	0.004 **
Phosphorus	1	0.03	0.03	0.8	0.6
Nitrogen × Phosphorus	1	0.04	0.04	1	0.4
Soil active layer depth	1	0.07	0.08	2	0.07 †
Soil organic layer depth	1	0.03	0.03	0.7	0.6
Soil bulk density	1	0.04	0.06	1	0.3
Soil moisture	1	0.05	0.07	2	0.2
Residual	12	0.5	0.5		
Total	19	0.8	1.0		

Note: The analysis tested the effects of the factorial fertilization treatments and the environmental variables on plant community composition, and significance levels are denoted with asterisks: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table 4. Statistical analysis of the effects of the factorial low-level N and P additions on birch total shoot length (i.e. full shoot length extension that includes growth from the current year plus all previous years), and birch new shoot length (i.e., apical shoot extension (primary growth) in the current growing season only), for all birch shrub ramets per randomly located 9 m^2 sampling area within the mesic birch hummock tundra N x P plots ($n = 3$ replicates) measured in late summer 2022.

	Df	Sum Sq	Mean Sq	*F* value	Pr (>*F*)
Birch total shoot length (per 9 m^2)					
Nitrogen	1	25 290 937	25 290 937	6	0.04*
Phosphorus	1	49 898 408	49 898 408	11	<0.01**
Nitrogen × Phosphorus	1	1395 372	1395 372	0.3	0.6
Residuals	8	34 993 585	4374 198		
Birch total new shoot length (per 9 m^2)					
Nitrogen	1	197 762	25 290 937	52	<0.001***
Phosphorus	1	86 106	49 898 408	23	<0.01**
Nitrogen × phosphorus	1	35 154	1395 372	9	0.02*
Residuals	8	3821	4374 198		

Note: Results of a two-way mixed-model factorial ANOVA are presented with significance levels denoted as follows: *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

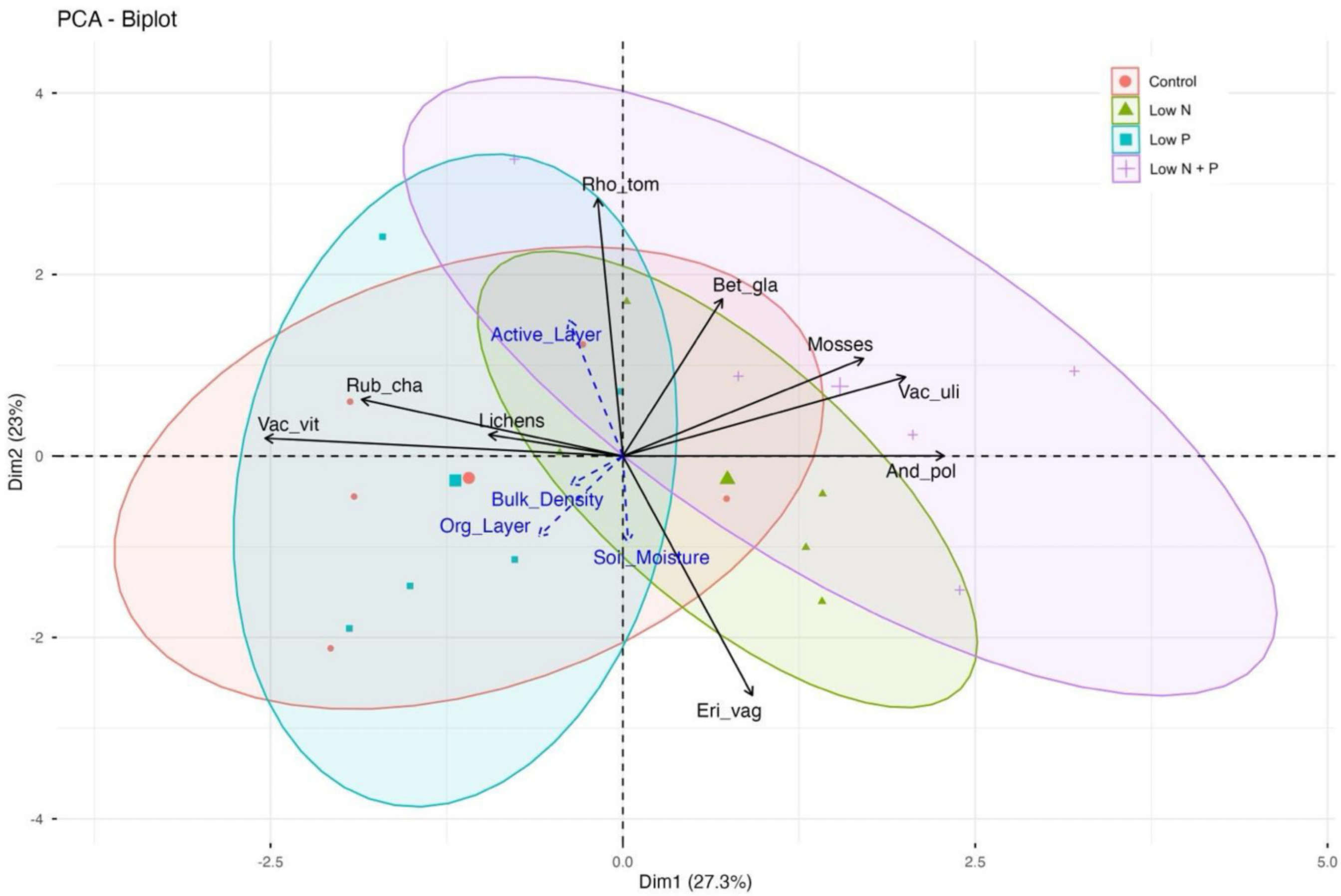
but also by their combination (Table 4, Fig. 3). This significant positive interaction between the treatments resulted in a ten-fold growth increase in the N + P plots relative to the controls (Fig. 3), and demonstrates conclusively that birch shoot primary growth in the year of sampling was clearly co-limited by the availability of N and P.

4. Discussion

4.1. Overview

Our results are surprising in at least two ways. First, our unusually low-level N and P annual additions for 19 and 11 years, respectively, did significantly change this mesic Low Arctic tundra plant community. Although it is well established that high-level nutrient additions greatly alter tundra plant communities (Chapin et al. 1995; Jonasson et al. 1999; Mack et al. 2004), the potential impacts of lower level nutrient additions have only rarely been investigated. For example, no significant impacts on % cover of two common tundra shrubs were observed at addition rates less than or equal to 2 g N plus 1 g P m^{-2} per year in recent investigations across an experimental gradient with multiple levels of N + P additions that had been running for 14 years (Dunleavy and Mack 2024; Williamson et al. 2024). Hence, whether the particularly low-level additions used in our study (1 g N and 0.5 g P m^{-2} per year) would have any significant effects on the plant community was not anticipated from these two other potentially comparable studies (which notably were based on % cover visual estimates rather than actual plant harvest data). Second, the changes we observed were not in directions that would have been predicted from high-level fertilization or experimental warming studies—where responses are generally greatest in the dominant deciduous and evergreen shrubs. In our case, the changes were primarily in the non-vascular component of the community since the mosses were enhanced ∼×1.7 by N + P and the lichens declined ∼×0.6 in response to P (Table 1). By contrast, the two vascu-

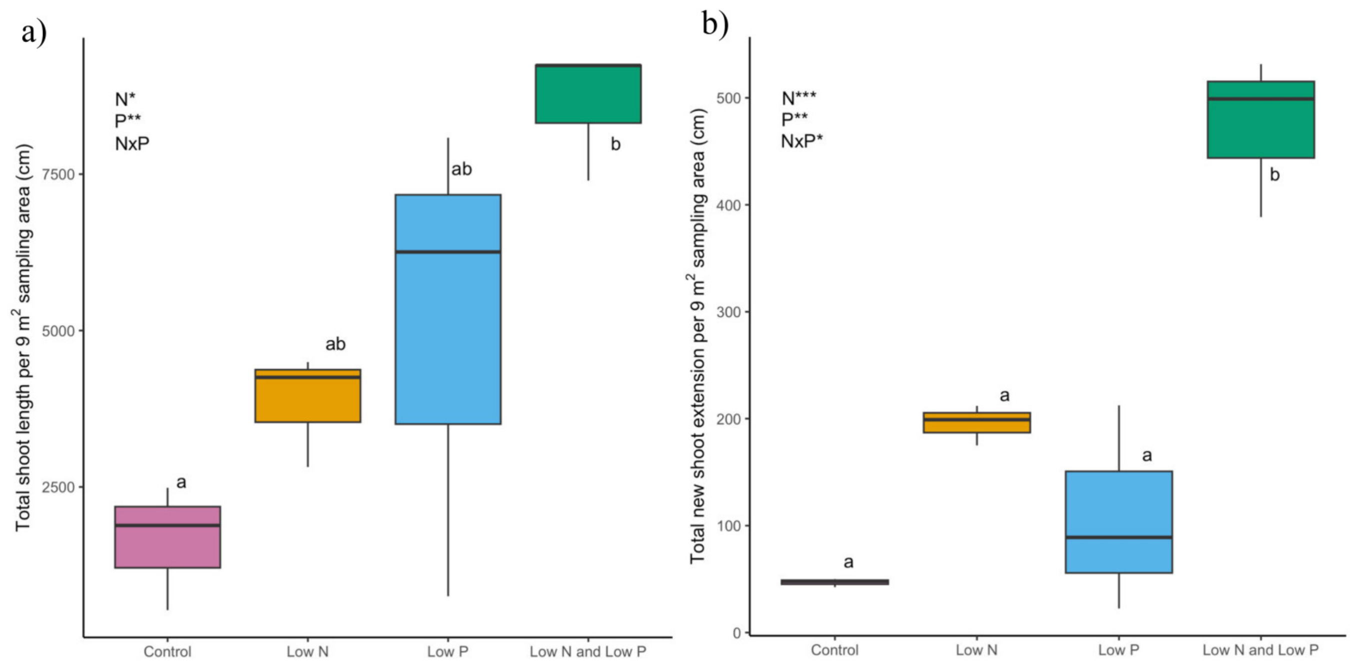
Fig. 2. Principal components analysis (PCA) of plant species aboveground biomass and community composition determined by the late summer 2023 harvest in response to the factorial low-level N and P additions. Plot community composition in the different treatments is categorised with legend key shapes (Control—circles; Low N—triangles; Low P—squares; Low N plus Low P—crosshairs), solid black vectors represent species or growth forms' contributions to the variance (active constraints on the analysis), while dashed blue vectors represent the passive supplemental soil variables measured at the time of the harvest (see Table 2 for details). Ellipses in the PCA represent the 75% confidence intervals for each treatment. Species abbreviations: And pol = *Andromeda polifolia*; Bet gla = *Betula glandulosa*; Rho tom = *Rhododendron tomentosum*; Rub cha = *Rubus chamaemorus*; Vac uli = *Vaccinium uliginosum*; Vac vit = *Vaccinium vitis-idaea*; and Eri vag = *Eriophorum vaginatum*.



lar species that were stimulated by N (*A. polifolia* and *E. vaginatum*) are very small components of the total community shoot biomass, and only *V. vitis-idaea* is a significant biomass component of the two species that were inhibited by N (Tables 1 and 2). Thus, our results in comparison to previous high-level fertilization studies suggest that the trajectories of individual plant species abundances and overall tundra vegetation change in response to enhanced nutrient availabilities may greatly depend on the magnitude of soil fertility enhancement.

In the context of climate change impacts, we and many other research groups across the Arctic have recorded significant changes in tundra plant communities that have been exposed to experimental warming (Elmendorf et al. 2012*a*; Zamin et al. 2014; Bjorkman et al. 2018), or that have been experiencing ambient climate warming over the past few decades (Chapin et al. 1995, Elemendorf et al. 2012*b*; Bjorkman et al. 2018; Gu and Grogan 2020). Conceptually, growth and composition within tundra plant communities might be altered by climate change due to direct temperature effects on above-ground processes such as photosynthesis, as well as indirect effects due to warming-induced SOM decomposition that enhances soil nutrient availability belowground (Fig. 1). Our study here specifically investigated the latter indirect effect by experimentally enhancing soil nutrients levels to a modest amount (1 g N and 0.5 g P m^{-2} per year) that might be realistic of near-future climate-warming impacts. For comparison, total vascular plant growing season N accumulation in the new apical shoot growth and fine root pools just prior to senescence is ~6 g N m^{-2} for this birch hummock tundra community (Vankoughnett and Grogan 2016). Therefore, assuming that at least half the N in those pools is lost in leaf litter and root turnover, net total vascular plant N uptake from the soil each year may range from 1 up to 3 g N m^{-2} per year. In summary, if plant-available N and P levels in our experiment had indeed been elevated to levels that are realistic of climate-warming impacts, we conclude that increases in soil nutrient availability - ei-

Fig. 3. Total birch shrub shoot length (*a*), and new apical shoot extension (i.e. current year's primary shoot growth) (*b*) summed per 9 m^2 sampling area under control, and factorial low N and low P supplementation in mesic birch hummock tundra. These non-destructive growth data were collected in late summer 2022. Boxplots show the median (line), interquartile range (box), with the whiskers extending to the most extreme data points ($n = 3$ replicates). Two-way mixed-model factorial ANOVAs were used for statistical analyses. Boxes that do not share superscript letters in common are statistically significantly different ($p < 0.05$) based on Tukey post hoc tests.



ther alone, or in combination with direct effects of increased aboveground temperature - will be a primary driver of climate warming impacts on this tundra vegetation-type (Fig. 3). Our study is therefore a rare demonstration of the fundamental importance of even very modest nutrient availability enhancements as critical determinants of tundra vegetation change.

Furthermore, our results suggest that climate warming-induced enhancements to soil N availability are likely to be more critical than enhancements to soil P availability in driving initial plant species' and community composition responses in mesic birch hummock tundra. In this study, the only such long-term low-level factorial N and P addition experiment in low Arctic vegetation that we are aware of, our results clearly demonstrate that species' responses to high-level factorial N and P addition experiments do not mirror what can be expected with lower, more climatically-realistic, soil fertility enhancements. Consequently, projections of tundra vegetation change that have been made based on such high-level fertilization experiments (e.g., Chapin et al. 1995; Jonasson et al. 1999; Mack et al. 2004) probably do not accurately model the impacts of anticipated climatic changes (Fig. 1).

Finally, our results emphasize the critical importance of scale in interpretation of ecological data -something that has been highlighted as a "fundamental issue in all ecology" for a long time (e.g., Levin 1992) but nevertheless often overlooked. Here, we sampled birch growth at two different spatial scales and two different temporal scales, and our results yielded opposing conclusions. Only by sampling at a sufficient spatial scale to account for the high spatial variability of individual birch plants within birch hummock tundra vegetation did we detect that growth of this species was in fact stimulated by the separate low-level N and P additions (Fig. 3). And only by measuring its new stem extension in the year of sampling (i.e., current year's primary growth) did we detect the significant and very strong interaction indicating that birch growth was co-limited by N and P availability (at least in the year of sampling—2022). This latter result supports our previous studies based on separate data from 2010 and 2011 (Zamin and Grogan 2012; Zamin et al. 2014) demonstrating independently once again that birch growth in this vegetation-type at Daring Lake at least, is *consistently* co-limited by the soil's supply of N and P. However, we suggest that the co-limitation observed here in this study is particularly novel and important because it is a clear demonstration that even at the low levels of nutrient enhancement that can be realistically anticipated with climate warming over the next few decades, birch growth will be strongly co-limited by N and P. Given the fundamental biogeochemical differences between tundra soil N and P cycling that suggest N availability to plants is likely to be enhanced proportionally more than P under future Arctic warming (see Section 1.3 above), our demonstration here of birch NP co-limitation suggests that this species will be increasingly constrained by a shortage of P relative to N, whereas those other species whose growth are primarily limited by N availability alone will tend to be more favoured.

4.2. Effects of long-term factorial low-level nitrogen and phosphorus additions on plant species' aboveground biomass did not mirror effects from a previous experiment with high-level additions at the same site

4.2.1. Did those species that had previously responded positively to the high-level factorial N and P inputs respond in the same way to the low-level additions?

Previous experiments using high-level factorial inputs of N and P have not only guided our understanding of tundra plant growth limitation by nutrient availability, but have also been used as a basis to infer likely shifts in plant community as a result of climate change-induced increases in soil nutrients. In an earlier study at Daring Lake, 8 years of factorial high-level N and P additions increased *B. glandulosa* new stem apical production (i.e., primary stem growth) by ∼×10 with N addition, and by ∼×4 with P addition (Zamin et al. 2014). Similarly, total shoot biomass for *B. glandulosa* was increased ∼×2 in response to high N addition, and ∼×2.5 in response to high P addition. Only two other species in this birch hummock tundra community responded positively to any of the high-level factorial inputs of N and P after eight years of treatment. Shoot biomass of the forb *Rubus chamaemorus* was increased ∼×5 in response to high-level P additions, while *E. vaginatum* shoot biomass was increased ∼×1.5 under the N treatment, ∼×2 in response to the P treatment, and increased ∼×6 by the combined high N + P treatment (Zamin et al. 2014). Our Hypothesis 1 that these species would respond similarly to additions of the same nutrients at much lower levels (i.e., 10% of the high-level rate) was generally not supported. *B. glandulosa* total aboveground biomass in the 2023 1 m^2 sampling area harvest (i.e., at the same spatial scale as the high-level study) was not significantly enhanced by either N or P, or their combination (Tables 1 and 2). Together, these contradictory biomass results from the previous high-level N × P studies at our site versus this low-level N × P experiment really challenge the widespread assumption that high-level fertilizer addition experiments across the Arctic are capable of providing useful insights into species' responses to climate warming-induced enhancements that elevate soil nutrient availability.

4.2.2. Did those species that had previously responded negatively to the high-level factorial N and P inputs respond positively to the low-level additions?

For the common low-lying evergreen shrubs *Rhododendron tomentosum* and *V. vitis-idaea*, the previous high-level factorial inputs of N and P resulted in negative total shoot biomass responses to additions of either N or P alone (ranging from ∼×0.1 to ∼×0.5) as well as to their combination (Zamin et al. 2014). By contrast, the long-term low-level factorial N and P additions reported here had no inhibitory effect on *Rhododendron tomentosum* (Tables 1 and 2). However, there were no significant positive growth responses either, refuting our Hypothesis 2. Likewise, *V. vitis-idaea* shoot biomass was not significantly enhanced by any of the additions (Table 1). In fact, just as in the 2010 harvest where its shoot biomass tended to be reduced by the low-level N addition treatment (Zamin et al. 2014), this inhibitory growth response to low-level N addition became statistically significant in the 2023 harvest data (Table 2). By contrast, shoot biomass of the infrequent evergreen shrub *A. polifolia*, which had been inhibited by high P addition after 8 years (Zamin et al. 2014), was enhanced ∼×2 by the low-level N addition—making it the only species' result supporting Hypothesis 2. Finally, the infrequent deciduous shrub *V. uliginosum* which had been significantly reduced by the high-level N additions after eight years (Zamin et al. 2014) was unaffected by the low-level N or P treatments, again refuting Hypothesis 2. Together, these results are surprising because we expected that the relatively low levels of nutrient addition in our study would fertilize below any posited thresholds for these species' theorised adverse physiological effects due to nutrient toxicity or delayed winter hardening. Hence, we had predicted that these species would respond positively to the low-level nutrient enhancements, but Hypothesis 2 was clearly generally refuted, suggesting that either shoot growth of a significant number and variety of species within mesic birch hummock tundra vegetation (i.e., *Rhododendron tomentosum*, *V. vitis-idaea*, and *V. uliginosum*) may be more limited by factors other than soil N or P availability, or that the ongoing low-level nutrient additions had been largely sequestered by the soil microbial biomass (see Section 4.3 ahead), or that the additions altered plant compensatory allocation to belowground biomass (which was not measured), or that these species were outcompeted for the added nutrients by the positively responding components of the plant community.

4.2.3. Did the large biomass components—the lichens and mosses—respond to the low-level factorial N and P inputs?

Critically, mosses and lichens together constituted about half of the total community aboveground biomass (Table 1). Total lichen biomass was not significantly affected by the 19 years of low-level N additions, but was greatly diminished (∼×0.6) by the low-level P addition ($P < 0.06$) (Table 1). Correspondingly, the low-level N addition had no effect after 8 years but total lichen community biomass declined substantially in each of the high-level N and P additions and especially in response to combined N and P (Zamin et al. 2014).

Total aboveground biomass of mosses as a group did not respond to either the low-level N or P additions, but was significantly stimulated (∼×1.7) by the N + P combination, suggesting co-limitation (Table 2). These responses contrast with those after 8 years, where total moss community aboveground biomass tended to be enhanced in the low-level N addition treatment but was significantly reduced in the high-level N addition plots (Zamin et al. 2014).

Together, these marked shifts in non-vascular growth form response patterns between the two different biomass harvests indicate that temporal scale is a critical determinant for their responses to enhancements in soil N and P availability. These patterns of results could be attributed to the same mechanisms postulated immediately above to explain the results for Hypothesis 2.

4.2.4. Did those species that had been demonstrated as NP-colimited in the previous high-level factorial N and P experiment respond in the same way to the low-level additions?

In the Daring Lake factorial high-level N and P addition experiment that was harvested after eight years (Zamin et al. 2014), not only did the separate nutrient additions stimulate growth of *B. glandulosa* and *E. vaginatum* (as described in detail in Section 4.2.1 above), but the N + P combination resulted in even larger growth responses (indicating that their growth was co-limited by N and P). For example, *B. glandulosa* new stem apical production (i.e., primary stem growth) was increased ∼×10 and ∼×4, respectively, by the separate N and P additions, and ∼×18 with the N + P addition (Zamin et al. 2014). Similarly, total *B. glandulosa* shoot biomass was increased ∼×2 and ∼×2.5, respectively, by the separate N and P additions, and ∼×3 by the N + P combination (Zamin et al. 2014). Shoot biomass of the Graminoid *E. vaginatum* was increased ∼×1.5 and ∼×2, respectively, by the separate N and P additions, and ∼×6 with the N + P addition (Zamin et al. 2014). By contrast, we found here that although *E. vaginatum* total aboveground biomass in the 2023 1 m^2 sampling area harvest tended to be enhanced by the low-level N additions ($P < 0.06$), it was not enhanced by P, nor by the combined addition of N and P, refuting Hypothesis 3 (Table 1). Similarly, *B. glandulosa* total aboveground biomass was not significantly enhanced by either N or P or their combination in the 2023 harvest, also refuting Hypothesis 3 (Table 1). In summary, despite the much longer duration of the treatments and the larger harvested sampling area in our current study compared to the earlier one (Zamin et al. 2014), neither *E. vaginatum* nor *B. glandulosa* displayed the same NP co-limitation growth responses to the same nutrients when they had been added at a 10× lower addition rate. This contradiction in species' responses between the high-level and low-level factorial N × P addition experiments raises issues about deducing the presence and ecological significance of NP co-limited growth as compared to single nutrient limitation, and therefore may have fundamental implications for our understanding of the influence of nutrient availabilities on tundra plant community structure.

4.2.5. *Betula glandulosa* growth responses differed for the 1 m^2 compared to the 9 m^2 sampling areas: Are significant responses of certain relatively sparsely distributed species going undetected because they are not sampled at an appropriate scale?

Betula glandulosa responses to low levels of enhanced soil fertility are particularly important because of the significance of this deciduous shrub in the context of overall greening and shrubification across the Arctic over the past few decades (Myers-Smith et al. 2011; Mekonnen et al. 2021). Hence, during the late summer of 2022—the year prior to the aboveground harvest—we focussed only on *B. glandulosa* and made several non-destructive measures of its shoot growth in the same factorial low-level N and P treatments described above. In contrast to the 2023 results from the 1 m^2 sampling area harvest, and despite having fewer replicate sample plots (five replicates for the 1 m^2 biomass harvest compared to three replicates for the 9 m^2 shoot length measurements), total birch shoot length measurements in 2022 at the 9 m^2 spatial sampling scale indicated that birch shoot growth was, in fact, enhanced by the separate low-level N and P additions (∼×2 and ∼×3, respectively) but there was no significant interaction (Table 4, Fig. 3). Even more interestingly, birch new shoot extension (representing primary stem growth during the year of sampling) was enhanced not just by the separate N and P additions (∼×4 and ∼×2, respectively), but there was also a significant positive interaction between these treatments resulting in a remarkable ten-fold growth increase in the N + P plots relative to the controls (Table 4, Fig. 3).

Of these three birch shoot growth metrics (i.e., total shoot biomass, total shoot length, and total new shoot apical extension), only the most temporally sensitive and spatially extensive one supported the co-limitation hypothesis (#3). Note that new shoot extension length is very closely correlated with biomass of the current year's shoot tip growth (Zamin and Grogan 2012), and therefore it is very unlikely that these comparisons across scales are confounded because different measurement variables were used for the two scales. To explain the contrasting results, it is first important to note that vegetative reproduction is the dominant growth mode for *B. glandulosa* and leads to clustering of individuals resulting in sparse and clustered distributions (Hermanutz et al. 1989; Myers-Smith et al. 2011) (∼2 ramets per m^2 at our site (Zamin and Grogan 2012)), whereas seedling recruitment is generally infrequent. Hence in summary, our results from the two different spatial scales demonstrate that for sparsely distributed species like birch, unconventionally large spatial sampling scales are clearly critical for providing sufficient sensitivity to detect responses to relatively "benign" but probably climatically-realistic treatments.

Likewise, only the measure of new shoot apical extension revealed the hypothesized NP co-limitation effect, presumably because it captures growth responses over the current growing season only, as opposed to the total shoot length which captures accumulated growth over many growing seasons that often span longer than the age of the experiment.

In our case, birch shoots were estimated to be ∼36 years old in this vegetation-type at our site (Vankoughnett and Grogan 2016), whereas the experimental N and P manipulations were started 19 and 11 years respectively before the harvest. Overall, these results suggest that important birch responses to low arctic ecosystem experimental manipulations may have been undetected in studies that were based on intense but small-scale harvests or point-frame data. Note that all other species in our study community are considerably smaller in stature and are present at substantially higher densities, and therefore we assume that the 1 m^2 harvest area we used is appropriate for those data (although we acknowledge that this assumption warrants testing). We would however caution other researchers to be very wary of using harvest or point-frame sampling areas <1 m^2 for tundra vegetation analyses. Finally, the shortcomings of measuring total shoot biomass as comparared to the much more temporally sensitive current year's stem extension are likely to be particularly critical for understanding responses of other woody shrubs—perhaps especially—the long-lived evergreen shrub *Rhododendron tomentosum* which also primarily reproduces vegetatively (Racine et al. 1987), and is the most dominant vascular shrub in our community (Table 1). More generally, we conclude that our contrasting Birch results reported here are an important reminder that the choice of measurement variable in any science investigation is critically important. Being acutely aware of the temporal and spatial scales of whatever experimental processes and underlying "natural" changes are affecting the phenomena under study (Levin 1992) provides fundamental context for that choice, and for interpreting the resulting data.

4.3. The low-level nitrogen addition significantly shifted overall plant community composition

The PERMANOVA analysis associated with the PCA indicated that community composition was significantly altered by the low-level N additions (Fig. 2), supporting Hypothesis 4. These changes in mesic birch hummock tundra plant community composition align with the widespread dogma that tundra plant species' growth is primarily limited by soil N availability (Shaver and Chapin 1980; McKane et al. 2002; Mack et al. 2004). Furthermore, this conclusion that N availability drives community composition (Fig. 2) aligns with the separate statistically significant positive aboveground biomass responses of the infrequent evergreen *A. polifolia* (and *E. vaginatum*—$P < 0.06$), as well as the negative responses of the abundant evergreen shrub *V. vitis-idaea* and the forb *Rubus chamaemorus* (Table 2). However, these results offer a snapshot of tundra plant community responses to an experiment where the N addition treatment was initiated substantially before the P addition (start years 2004 and 2012, respectively). Despite the P additions having been relatively recent (11 years), their significant or almost significant effects on two of the plant components (lichens and mosses—Tables 2, A11, and A12) strongly suggest that the P addition had had sufficient time to impact not just soil nutrient availability, but also the plant community. However, even if so, it is unknown at what rate and magnitude the soil N and P availabilities may have been enhanced, and the timeline of when these enhancements into the plant-available soil solution may have occurred. For example, the tundra soil microbial biomass has a remarkable ability to take up large amounts of added N and retain it almost completely for at least three subsequent years (Churchland et al. 2010). Hence, we anticipate that the soil microbes in our experiment may have accumulated and stored these annual nutrient additions (especially early on), thereby moderating the effect of experimental nutrient addition in terms of actually enhancing soil nutrient availability to plants. To test the potential end result of this hypothesis in terms of interpreting the patterns of plant biomass data reported here, we are currently quantifying soil microbial N and P pools from all harvested plots in this experiment as a follow-up study.

Furthermore, differences in N and P cycling are expected under future climate because soil microbial N:P ratios are considerably lower than those of plants (Murrmann and Peech 1969;), and so microbes are expected to accumulate and sequester proportionally more of the P than N that is released by warming-induced SOM decomposition. Consequently, those plant species in tundra communities that are primarily N rather than P or NP co-limited are likely to become more favoured with climate warming. Overall, in summary, we conclude that the diverse species and growth-form responses to our long-term low-level factorial N and P additions reported here strongly suggest that the magnitudes and relative rates of change in soil N and P availabilities to plants with climate warming will be critical determinants of future mesic tundra plant community structure (Fig. 1).

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is dedicated to the memory of Queen's University Department of Biology botanist Dr. Adele Crowder.

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The authors declare there are no competing interests.

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Appendix A. Species and growth form and community richness/diversity statistical analyses results

Table A1. *Andromeda polifolia* total aboveground biomass two-way factorial Analysis of covariance (ANCOVA) results.

	Sum of squares	df	*F* value	Pr (>*F*)
Nitrogen	**2797.3**	**1**	**9**	**0.009****
Phosphorus	346.0	1	1	0.3
Nitrogen × phosphorus	235.8	1	1	0.4
Bulk density	1244.7	1	4	0.06†
Residuals	4640.1	15		

Note: Type I sum of squares, degrees of freedom, *F*-values, and *P* values for each factor and interaction are presented with significance levels denoted with asterisks: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table A2. *Eriophorum vaginatum* total aboveground biomass two-way factorial ANCOVA results.

	Sum of squares	df	*F* value	Pr (>*F*)
Nitrogen	1,087.8	1	4	0.06†
Phosphorus	233.7	1	1	0.4
Nitrogen × phosphorus	344.2	1	1	0.3
Active layer	**2,973.8**	**1**	**11**	**0.005****
Soil moisture	**1,627.2**	**1**	**6**	**0.03***
Bulk density	728.7	1	3	0.1
Residuals	3,446.6	13		

Note: Type I sum of squares, degrees of freedom, *F* values, and *P* values for each factor and interaction are presented with significance levels denoted with asterisks: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table A3. *Vaccinium vitis-idaea* total aboveground biomass two-way factorial ANCOVA results.

	Sum of squares	df	*F* value	Pr (>*F*)
Nitrogen	**11 372.1**	**1**	**15.**	**0.001****
Phosphorus	0.02	1	0	1
Nitrogen × phosphorus	2291.2	1	3	0.1
Soil moisture	3235.6	1	4	0.05†
Residuals	11 291.5	15		

Note: Type I sum of squares, degrees of freedom, *F* values, and *P* values for each factor and interaction are presented with significance levels denoted with asterisks: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table A4. *Rubus chamaemorus* total aboveground biomass two-way factorial ANCOVA results.

	Sum of squares	df	*F* value	Pr (>*F*)
Nitrogen	**46.9**	**1**	**6**	**0.03***
Phosphorus	0.07	1	0.01	0.9
Soil moisture	6.3	1	0.8	0.4
Bulk density	**45.1**	**1**	**6**	**0.03***
Nitrogen × phosphorus	5.0	1	0.6	0.4
Residuals	107.4	14		

Note: Type I sum of squares, degrees of freedom, *F* values, and *P* values for each factor and interaction are presented with significance levels denoted with asterisks: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table A5. *Rhododendron tomentosum* total aboveground biomass two-way factorial ANCOVA results.

	Sum of squares	df	*F* value	Pr (>*F*)
Nitrogen	1314.7	1	0.4	0.5
Phosphorus	6436.3	1	2	0.2
Nitrogen × phosphorus	3562.9	1	1	0.3
Active layer	**35 382.0**	**1**	**11**	**0.005****
Soil moisture	**25 015.4**	**1**	**8**	**0.02***
Bulk density	12 658.7	1	4	0.07†
Residuals	41 508.6	13		

Note: Type I sum of squares, degrees of freedom, *F* values, and *P* values for each factor and interaction are presented with significance levels denoted with asterisks: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table A6. *Vaccinium uliginosum* total aboveground biomass two-way factorial ANCOVA results.

	Sum of squares	df	F value	Pr (>F)
Nitrogen	178.4	1	1	0.3
Phosphorus	433.0	1	2	0.1
Nitrogen × phosphorus	3562.9	1	1	0.3
Organic layer	**1258.8**	**1**	**7**	**0.02***
Bulk density	538.9	1	3	0.1
Residuals	2560.0	14		

Note: Type I sum of squares, degrees of freedom, F values, and P values for each factor and interaction are presented with significance levels denoted with asterisks: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table A7. *Betula glandulosa* total stem biomass two-way factorial ANCOVA results.

	Sum of squares	df	F value	Pr (>F)
Nitrogen	58.1	1	0.03	0.9
Phosphorus	0.4	1	0	1
Nitrogen × phosphorus	1343.2	1	0.7	0.4
Soil moisture	3350.2	1	2	0.2
Residuals	27 320.7	15		

Note: Type I sum of squares, degrees of freedom, F values, and P values for each factor and interaction are presented with significance levels denoted with asterisks: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table A8. *Betula glandulosa* total leaf biomass two-way factorial ANCOVA results.

	Sum of Squares	df	F value	Pr (>F)
Nitrogen	12.1	1	0.3	0.6
Phosphorus	0.4	1	0.01	0.9
Nitrogen × phosphorus	7.2	1	0.2	0.7
Soil moisture	76.1	1	2	0.2
Residuals	658.9	15		

Note: Type I sum of squares, degrees of freedom, F values, and P values for each factor and interaction are presented with significance levels denoted with asterisks: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table A9. *Betula glandulosa* total aboveground biomass two-way factorial ANCOVA results.

	Sum of squares	df	F value	Pr (>F)
Nitrogen	123.2	1	0.05	0.8
Phosphorus	1.6	1	0.001	1
Nitrogen × phosphorus	1,547.7	1	0.6	0.4
Soil moisture	4,436.5	1	2	0.2
Residuals	36,189.2	15		

Note: Type I sum of squares, degrees of freedom, F values, and P values for each factor and interaction are presented with significance levels denoted with asterisks: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table A10. Total aboveground biomass of all vascular species two-way factorial ANCOVA results.

	Sum of squares	df	F value	Pr (>F)
Nitrogen	12 208	1	1.1	0.3
Phosphorus	2630	1	0.2	0.6
Nitrogen × phosphorus	**2795**	1	0.3	0.6
Residuals	177 639	15		

Note: Type I sum of squares, degrees of freedom, F values, and P values for each factor and interaction are presented with significance levels denoted with asterisks: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table A11. Mosses (bulked) total aboveground biomass two-way factorial ANCOVA results.

	Sum of squares	df	*F* value	Pr (>*F*)
Nitrogen	7929.5	1	3	0.1
Phosphorus	2407.1	1	0.8	0.4
Nitrogen × phosphorus	**17 197.4**	**1**	**5**	**0.03***
Bulk density	8374.8	1	3	0.1
Residuals	47 373.5	15		

Note: Type I sum of squares, degrees of freedom, *F* values, and *P* values for each factor and interaction are presented with significance levels denoted with asterisks: †$P < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table A12. Lichens (bulked) total biomass two-way factorial ANCOVA results.

	Sum of squares	df	*F* value	Pr (>*F*)
Nitrogen	4520.0	1	1	0.2
Phosphorus	12 442.6	1	4	0.06†
Nitrogen × phosphorus	2745.4	1	0.9	0.4
Active layer	**21 991.5**	**1**	**7**	**0.02***
Residuals	46 034.8	15		

Note: Type I sum of squares, degrees of freedom, *F* values, and *P* values for each factor and interaction are presented with significance levels denoted with asterisks: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table A13. Total aboveground biomass of all vascular species plus non-vascular growth forms two-way factorial ANCOVA results.

	Sum of squares	df	*F* value	Pr (>*F*)
Nitrogen	16 729	1	1.4	0.3
Phosphorus	155	1	0.01	0.9
Nitrogen × phosphorus	8402	1	0.7	0.4
Soil moisture	45 985	1	3.7	0.07†
Residuals	187 735	15		

Note: Type I sum of squares, degrees of freedom, *F* values, and *P* values for each factor and interaction are presented with significance levels denoted with asterisks: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table A14. Summary of the statistical analyses of the factorial low N × low P treatment effects on species richness, evenness, and Shannon diversity from the late summer 2023 1 m^2 aboveground biomass harvest for the seven major vascular plant species in mesic birch hummock tundra.

Richness	Df	Sum sq	Mean sq	*F* value	Pr(>*F*)
Nitrogen	1	0.05	0.05	0.2	0.7
Phosphorus	1	0.05	0.05	0.2	0.7
Nitrogen × phosphorus	1	0.05	0.05	0.2	0.7
Residuals	16	5	0.30		
Evenness	Df	Sum sq	Mean sq	*F* value	Pr(>*F*)
Nitrogen	1	0.007	0.007	2	0.4
Phosphorus	1	0.00007	0.00007	0.02	0.5
Nitrogen × phosphorus	1	0.005	0.005	1	0.7
Residuals	16	0.06	0.003		
Shannon diversity	Df	Sum sq	Mean sq	*F* value	Pr(>*F*)
Nitrogen	1	0.04	0.01	0.7	0.3
Phosphorus	1	0.006	0.006	0.5	0.9
Nitrogen × phosphorus	1	0.002	0.002	0.2	0.2
Residuals	16	0.2	0.01		

Appendix B. Species and growth form biomass responses

Fig. B1. Andromeda polifolia total aboveground biomass per 1 m^2 sampling area in response to long-term factorial low N and low P supplementation of mesic birch hummock tundra ($n = 5$). Bolded lines indicate the median while boxes and whiskers represent the 25th and 75th, and 0th and 100th percentiles, respectively. All points are displayed, either as points or as the median or box percentiles, while whiskers are omitted if outliers are significantly different from the other points. Any statistically significant treatment effects from Table A1 are indicated with corresponding symbols.

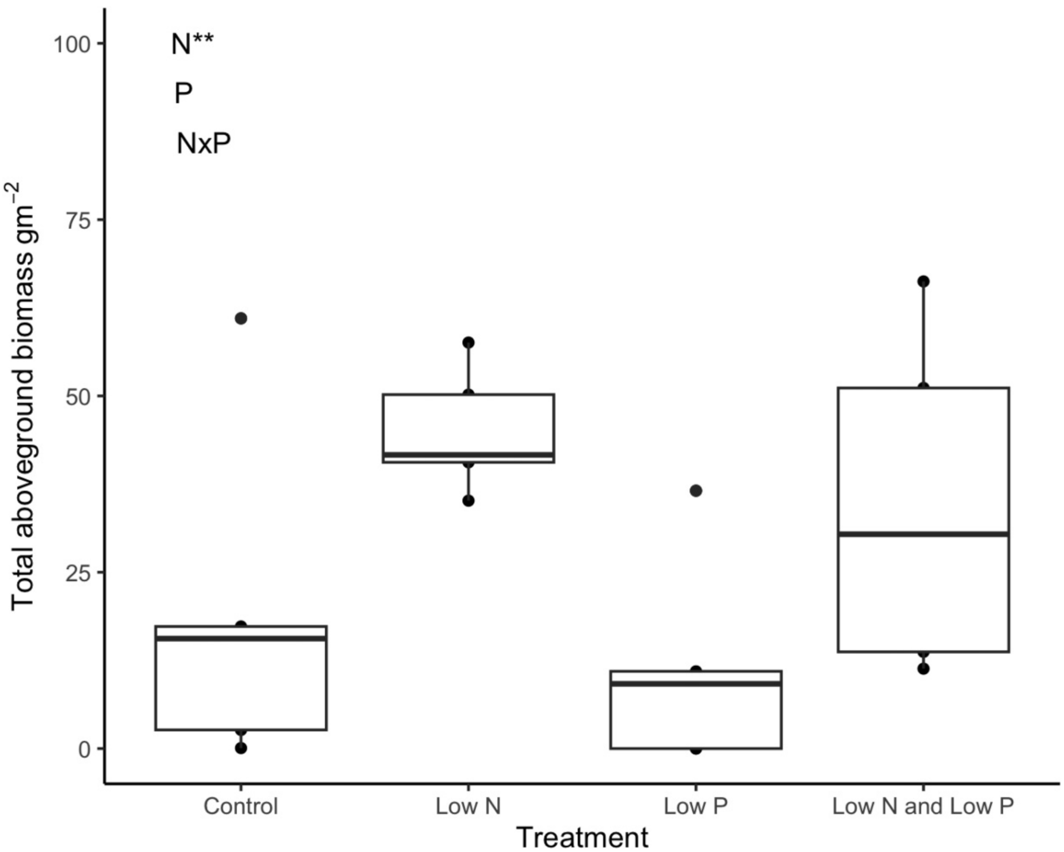

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Fig. B2. *Eriophorum vaginatum* total aboveground biomass per 1 m^2 sampling area in response to long-term factorial low N and low P supplementation of mesic birch hummock tundra ($n = 5$). Bolded lines indicate the median while boxes and whiskers represent the 25th and 75th, and 0th and 100th percentiles, respectively. All points are displayed, either as points or as the median or box percentiles, while whiskers are omitted if outliers are significantly different from the other points. Any statistically significant treatment effects from Table A2 are indicated with corresponding symbols.

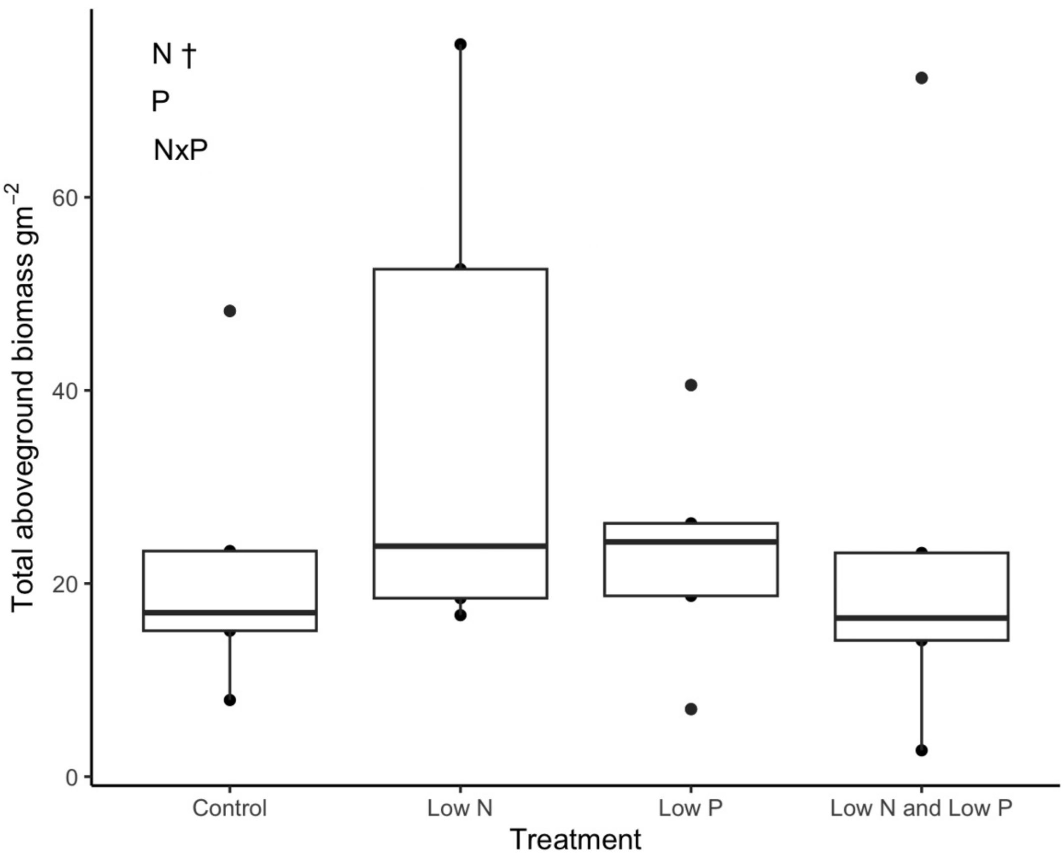


Fig. B3. *Vaccinium vitis-idaea* total aboveground biomass per 1 m^2 sampling area in response to long-term factorial low N and low P supplementation of mesic birch hummock tundra ($n = 5$). Bolded lines indicate the median while boxes and whiskers represent the 25th and 75th, and 0th and 100th percentiles, respectively. All points are displayed, either as points or as the median or box percentiles, while whiskers are omitted if outliers are significantly different from the other points. Any statistically significant treatment effects from Table A3 are indicated with corresponding symbols.

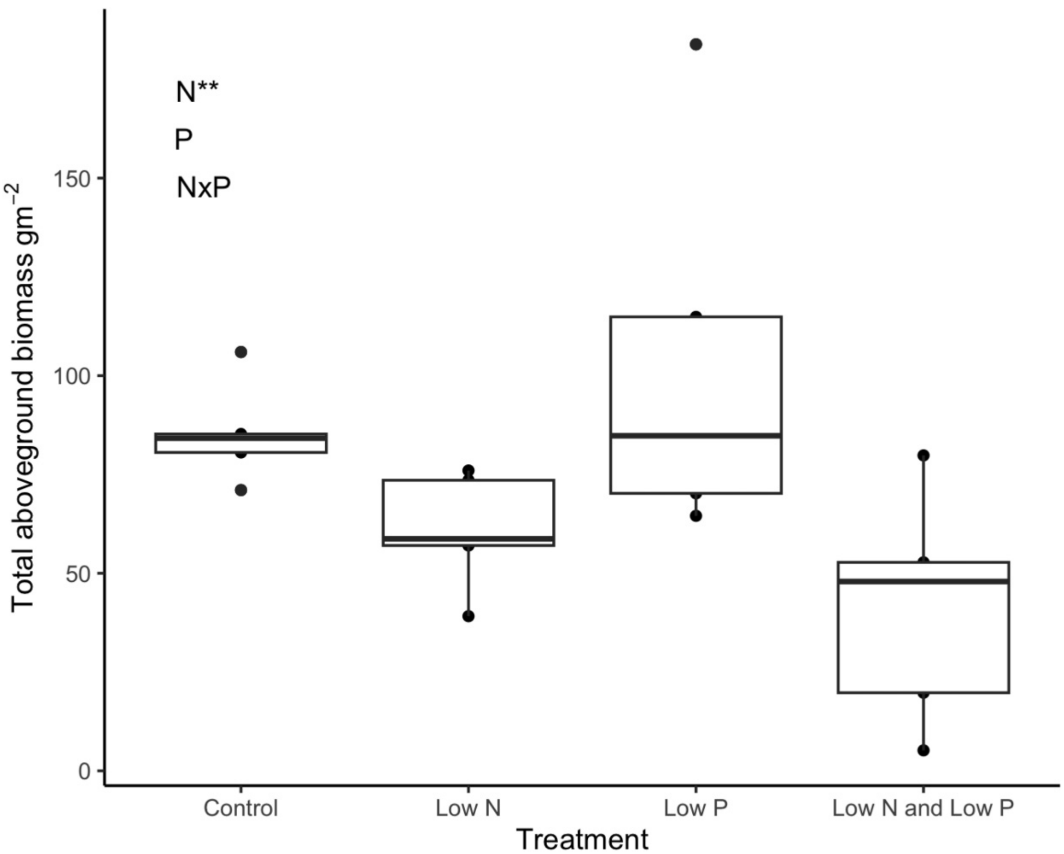


Fig. B4. *Rubus chamaemorus* total aboveground biomass per 1 m^2 sampling area in response to long-term factorial low N and low P supplementation of mesic birch hummock tundra ($n = 5$). Bolded lines indicate the median while boxes and whiskers represent the 25th and 75th, and 0th and 100th percentiles, respectively. All points are displayed, either as points or as the median or box percentiles, while whiskers are omitted if outliers are significantly different from the other points. Any statistically significant treatment effects from Table A4 are indicated with corresponding symbols.

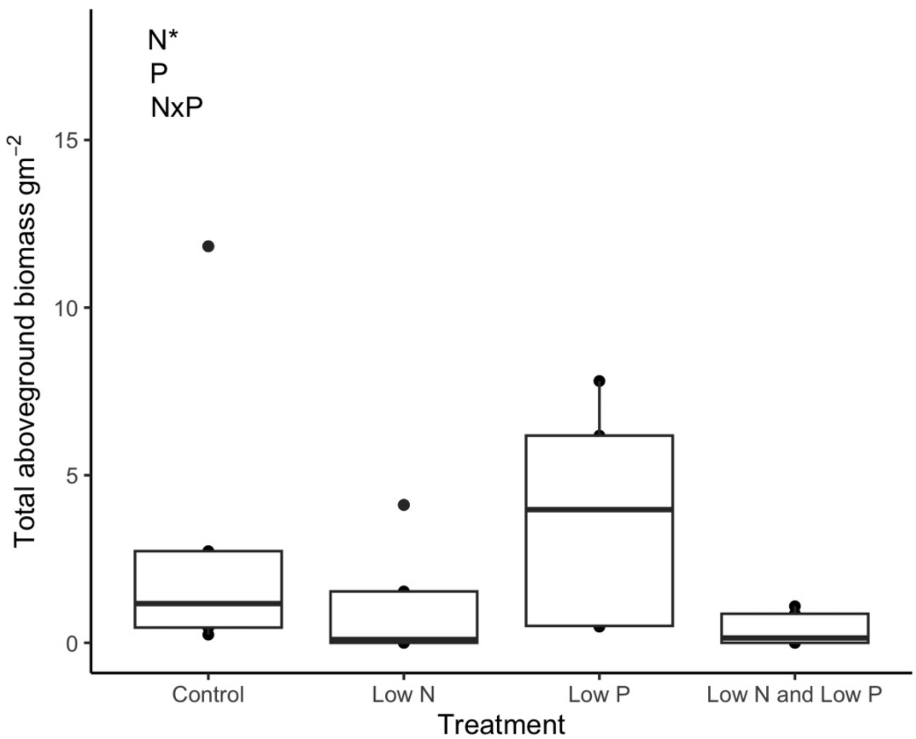


Fig. B5. *Rhododendron tomentosum* total aboveground biomass per 1 m^2 sampling area in response to long-term factorial low N and low P supplementation of mesic birch hummock tundra ($n = 5$). Bolded lines indicate the median while boxes and whiskers represent the 25th and 75th, and 0th and 100th percentiles, respectively. All points are displayed, either as points or as the median or box percentiles, while whiskers are omitted if outliers are significantly different from the other points. Any statistically significant treatment effects from Table A5 are indicated with corresponding symbols.

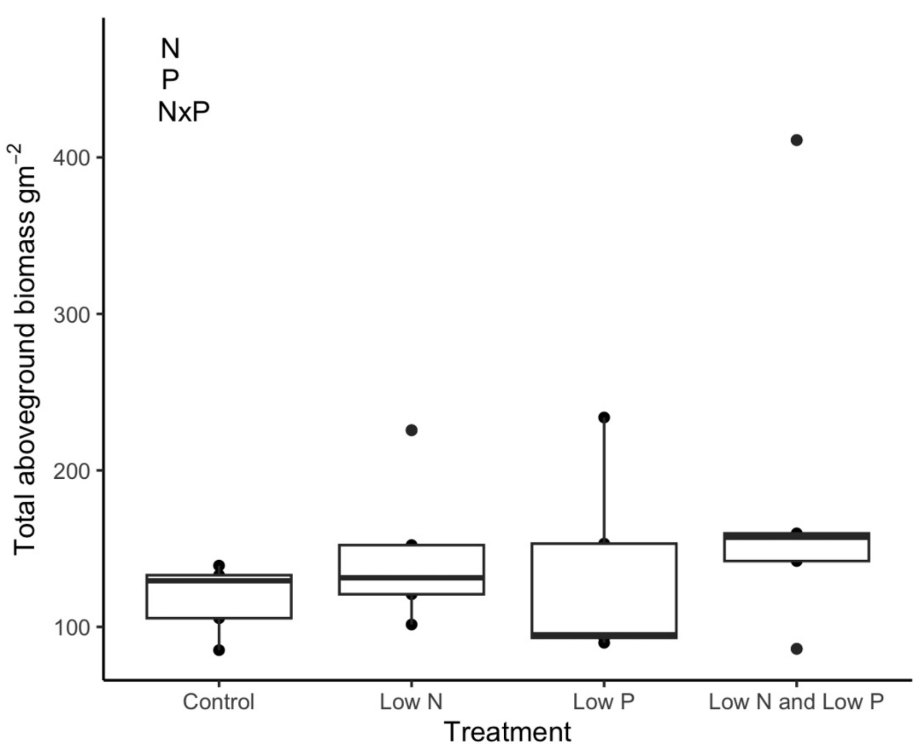


Fig. B6. *Vaccinium uliginosum* total aboveground biomass boxplot of per 1 m^2 sampling area in response to long-term factorial low N and low P supplementation of mesic birch hummock tundra ($n = 5$). Bolded lines indicate the median while boxes and whiskers represent the 25th and 75th, and 0th and 100th percentiles, respectively. All points are displayed, either as points or as the median or box percentiles, while whiskers are omitted if outliers are significantly different from the other points. Any statistically significant treatment effects from Table A6 are indicated with corresponding symbols.

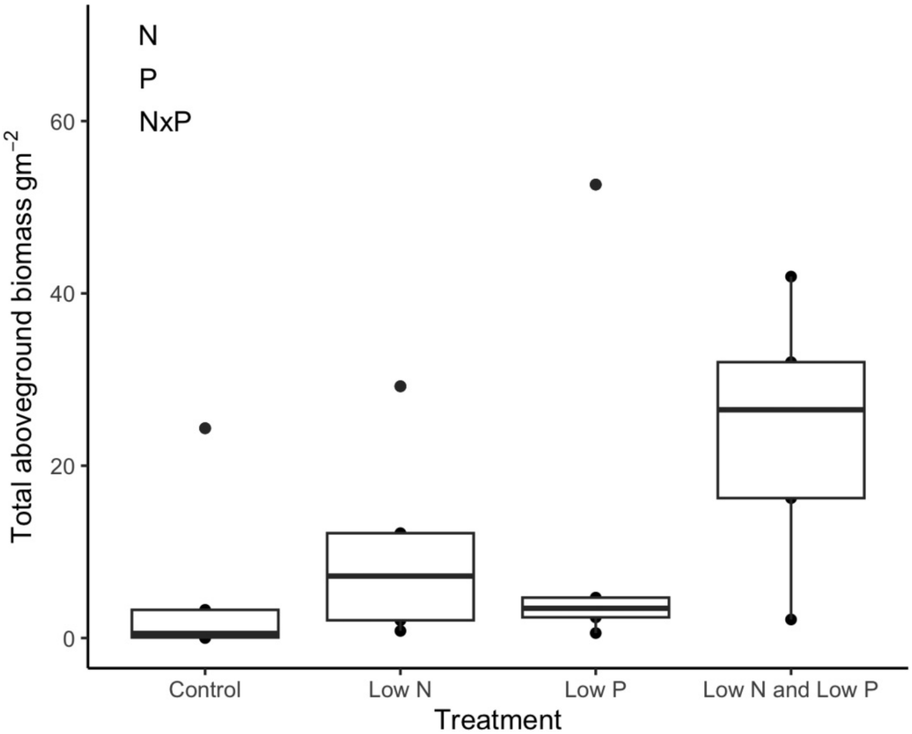


Fig. B7. *Betula glandulosa* total aboveground biomass boxplot of per 1 m^2 sampling area in response to long-term factorial low N and low P supplementation of mesic birch hummock tundra ($n = 5$). Bolded lines indicate the median while boxes and whiskers represent the 25th and 75th, and 0th and 100th percentiles, respectively. All points are displayed, either as points or as the median or box percentiles, while whiskers are omitted if outliers are significantly different from the other points. Any statistically significant treatment effects from Table A7 are indicated with corresponding symbols.

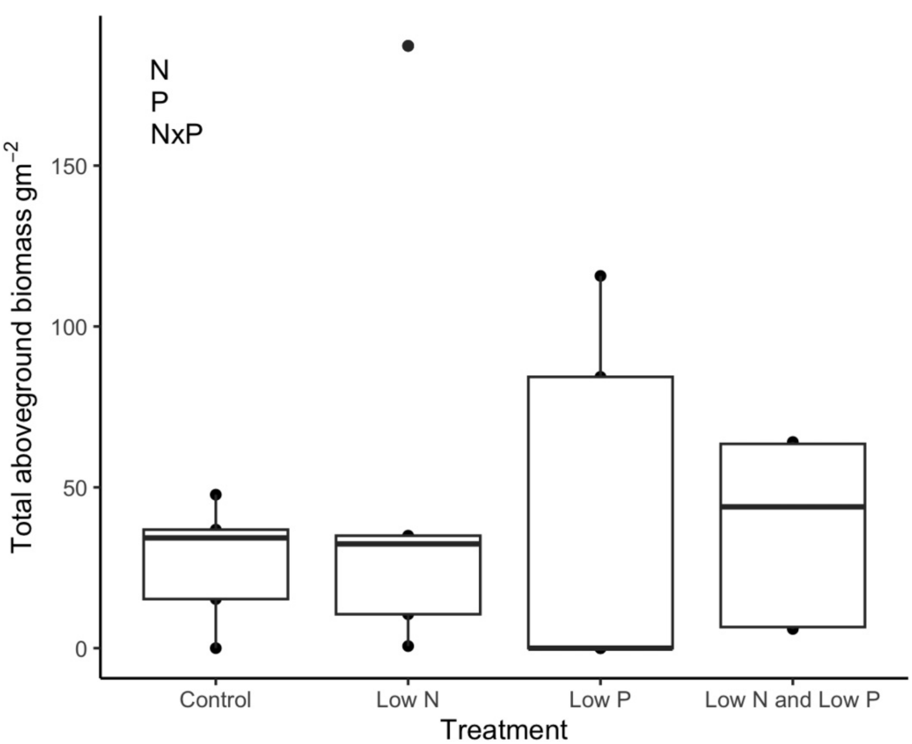


Fig. B8. Mosses (bulked) total aboveground biomass boxplot per 1 m^2 sampling area in response to long-term factorial low N and low P supplementation of mesic birch hummock tundra ($n = 5$). Bolded lines indicate the median while boxes and whiskers represent the 25th and 75th, and 0th and 100th percentiles, respectively. All points are displayed, either as points or as the median or box percentiles, while whiskers are omitted if outliers are significantly different from the other points. Any statistically significant treatment effects from Table A11 are indicated with corresponding symbols.

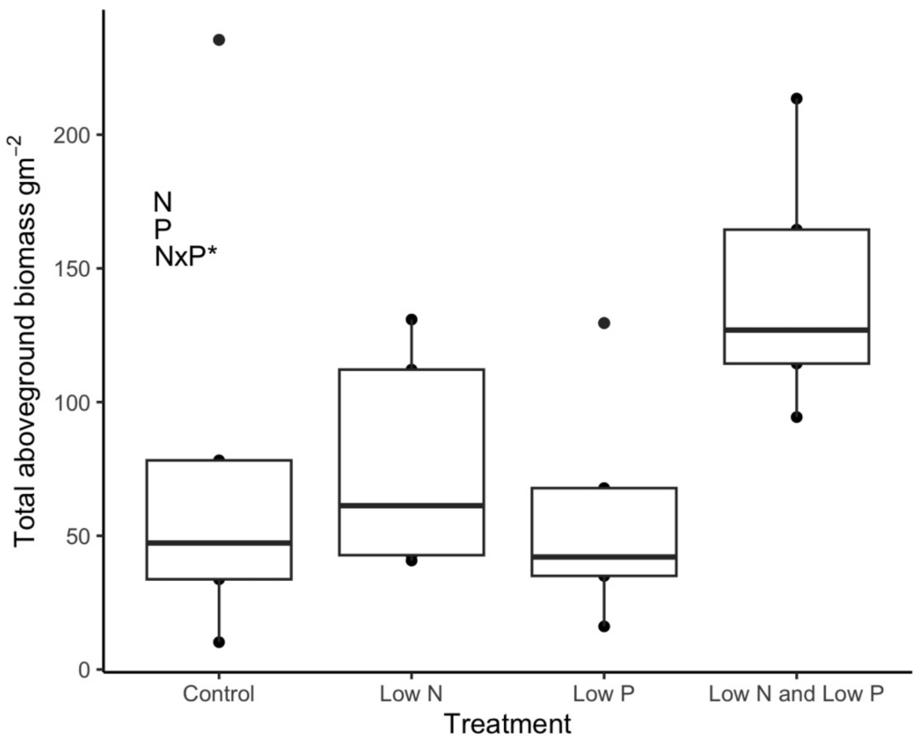
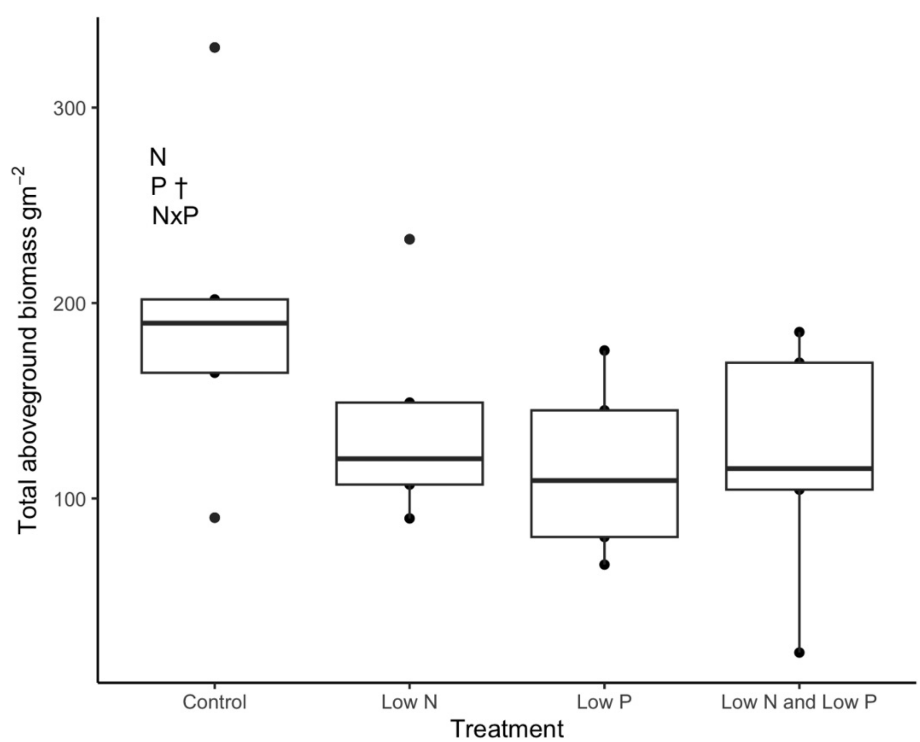


Fig. B9. Lichens (bulked) total biomass per 1 m^2 sampling area in response to long-term factorial low N and low P supplementation of mesic birch hummock tundra ($n = 5$). Bolded lines indicate the median while boxes and whiskers represent the 25th and 75th, and 0th and 100th percentiles, respectively. All points are displayed, either as points or as the median or box percentiles, while whiskers are omitted if outliers are significantly different from the other points. Any statistically significant treatment effects from Table A12 are indicated with corresponding symbols.



Appendix C. Soil characteristics across plots

Table C1. Soil characteristics presented across experimental plots.

Plot	Soil active layer depth (cm)	Soil moisture (g cm^{-3})	Soil bulk density (g cm^{-3})	Soil organic layer depth (cm)
C1	36 (0.4)	388	0.17	30
C2	46 (0.2)	221	0.18	25
C3	52 (0.3)	423	0.12	27
C4	43 (0.1)	244	0.15	19
C5	66 (0.1)	375	0.16	16
LN1	49 (0.1)	497	0.10	22
LN2	43 (0.5)	265	0.13	27
LN3	54 (0.1)	434	0.14	16
LN4	39 (0.1)	187	0.10	32
LN5	54 (0.2)	94	0.15	26
LP1	37 (0.2)	117	0.17	18
LP2	54 (0.3)	436	0.13	13
LP3	44 (0.1)	204	0.14	21
LP4	40 (0.2)	415	0.08	45
LP5	58 (0.2)	654	0.13	25
LNLP1	40 (0)	301	0.12	20
LNLP2	42 (0.3)	384	0.16	27
LNLP3	50 (0.1)	211	0.17	13
LNLP4	46 (0)	257	0.18	27
LNLP5	69 (0.1)	243	0.14	18

Note: Mean values are presented for soil active layer depth ($n = 4$), with standard error in parentheses. Soil moisture data were gravimetrically determined on samples removed in late August 2022.

Appendix D. Plant community structure across the plots

Table D1. Species richness, Pielou's evenness, and the Shannon diversity indices for the plant communities in each of the factorial N × P experiment plots based on all seven vascular species.

Plot	Species richness	Species evenness	Shannon diversity
C1	6	0.6	1.1
C2	6	0.7	1.3
C3	7	0.8	1.5
C4	7	0.9	1.7
C5	7	0.6	1.2
LN1	7	0.7	1.4
LN2	6	0.9	1.7
LN3	6	0.8	1.5
LN4	7	0.7	1.3
LN5	7	0.7	1.4
LP1	7	0.7	1.5
LP2	6	0.8	1.4
LP3	6	0.6	1.4
LP4	7	0.8	1.5
LP5	7	0.6r	0.8
LNLP1	6	0.8	1.2
LNLP2	7	0.8	1.6
LNLP3	6	0.8	1.1
LNLP4	6	0.8	1.4
LNLP5	7	0.4	1.1

Appendix E. Supplementary images

Fig. E1. Photo from the top of the Thelon esker viewing the Daring Lake research valley ~ 300 km north of Yellowknife, NWT, Canada. All plots of the factorial low N × P addition experiment (and experimental warming plots, factorial high N × P addition experiment, and other manipulations) are located in the mesic birch hummock tundra downslope to the right of the esker, and upslope of the wetland watercourse at the base of the valley.

Fig. E2. Photo of annual fertilization addition in the factorial low-level N × P experiment. The appropriate granulated nutrient aliquots for each plot were weighed and split into five vials to be sprinkle-dispersed over five 1 m wide lanes of the plot to improve evenness of nutrient application. In this particular instance, the low N addition is being applied to a LN and to a directly adjacent LNLP plot, and each row is a 1 m wide temporary lane across the full 5 m × 7 m area (i.e., the full area for the two plots combined).

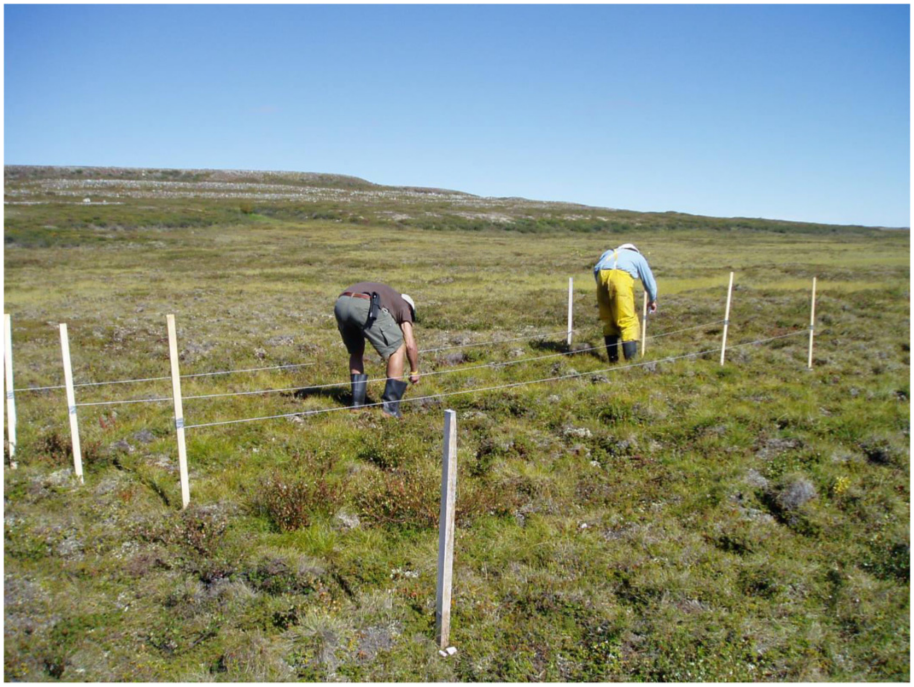


Fig. E3. Image depicting the measurement of B. glandulosa new apical shoot extension from one plot of the factorial low-level N × P experiment in the late summer of 2022. The transition from previous years' growth to current year's growth is seen at the base of the ruler.

Fig. E4. (*a*) Delineation of a 1 m^2 section of one plot from the factorial low-level N × P addition experiment before harvesting; (*b*) harvest of turves of soil and associated plants in the late summer of 2023; and (*c*) sample turf before transport to the field lab for careful extraction of all aboveground plant biomass and subsequent sorting to species-level.

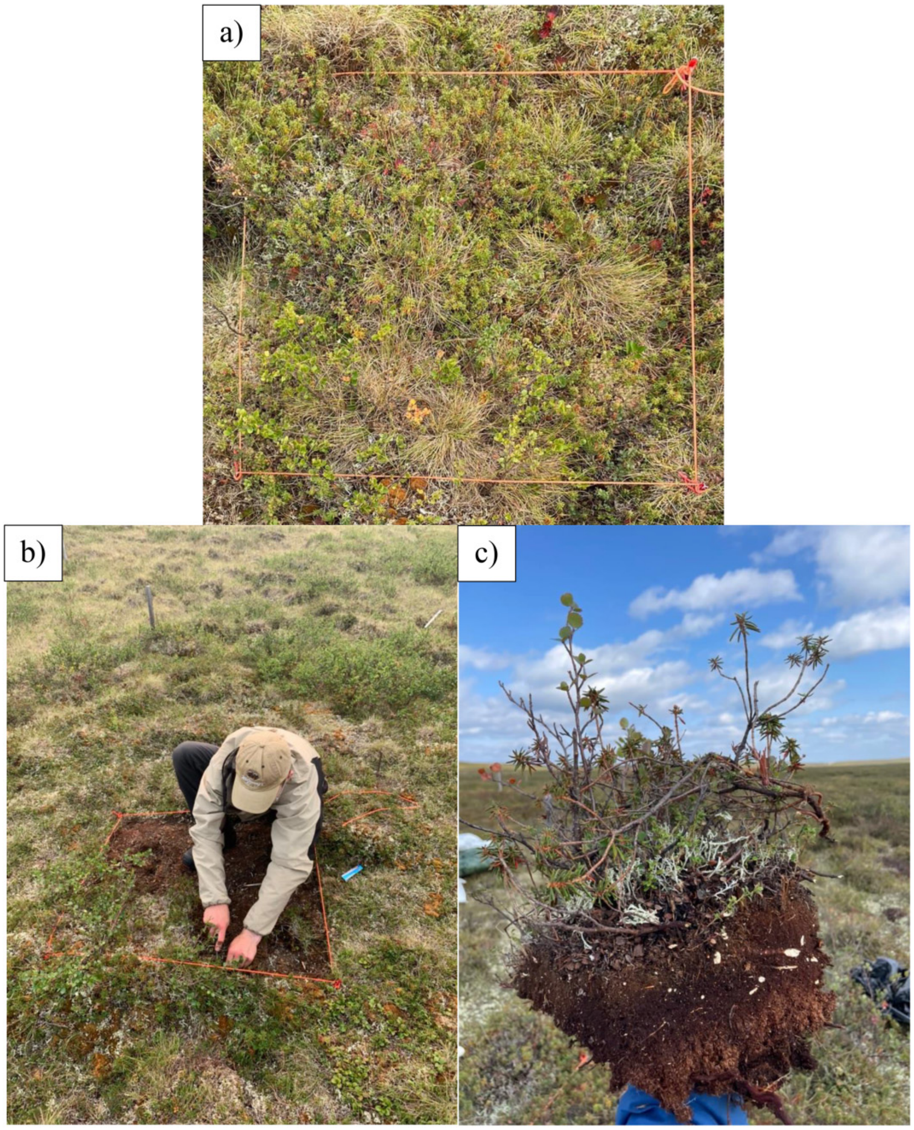


Fig. E5. Plant aboveground biomass from the factorial low N × P addition experiment sorted to the species and non-vascular growth form level at the Daring lake research tent in late summer of 2023.

Appendix F. Environmental impacts

The rationale for including this table is that every scientific lab and field activity involves environmentally-damaging impacts. As we become increasingly knowledgeable about the impacts that humanity's activities are having on the biosphere, we all—and scientists/researchers in particular—have a growing responsibility to acknowledge our impacts, and respond accordingly. Awareness is the fundamentally necessary pre-requisite to doing something about the problem. Inclusion of this table identifying and explicitly articulating specific measures that were deliberately taken in our science/research studies to minimize their environmental impacts is a first step toward advancing awareness. Note that the two measures included were probably the most environmentally significant, but even if not, the focus of this initiative at this stage is on the "big picture"—awareness—not on the detail of comparing different measures or evaluating if the chosen measure was actually most effective in mitigating the impact.

Table F1. Specific measures taken by the authors to reduce the environmental impacts of the scientific activities reported in this study.

Category of science-associated activity	Details of the science-associated activity	Ecological impacts of the activity that may be avoidable	Measure taken to reduce those ecological impacts
Travel	Chartered air travel from Yellowknife to the remote tundra research field-site at Daring Lake for three summers	Greenhouse gas emissions associated with each charter flight	Colin's coordination of travel plans among the different research groups maximised the number of passengers and amount of gear per flight, and minimised the total number of flights
Project materials	Use of garbage bags and ziploc bags for sample transport and storage	Increased inputs of plastic wastes into landfills	Heavy duty garbage bags were reused as long as possible to transport harvested material for sorting. Heavy duty ziploc bags were washed and reused for future lab activities.
Sample transport	Mailing biomass samples and transporting un-sorted soil and plant materials	Greenhouse gas emissions associated with each charter, and commercial flight, and ground transport of samples	Sorted biomass samples were compressed into the fewest and smallest boxes possible. Un-sorted harvest material was reduced to only the critical layer of soil for each turf to reduce the weight of the packages for transport.