Decoupled above- and belowground responses to multi-decadal nitrogen and phosphorus amendments in two tundra ecosystems

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Abstract

Global change in the Arctic promotes deeper soil thaw and enhanced soil microbial activity, increasing nitrogen (N) and phosphorus (P) availability to plants and microbes in strongly nutrient-limited ecosystems. This critical, positive climate feedback has been examined through fertilization experiments that describe short-term (<10y) above- or belowground responses to combined NP additions, with evidence of enhanced shrub growth, nutrient availability, and soil organic matter decomposition. There has been less opportunity for long-term comparisons of both above- and belowground responses with factorial N and P additions in different systems, despite broad awareness that ecosystem response can shift with time, and the potential for decoupled above- vs. belowground or N vs. P responses, currently and with further predicted global change. We examined the response of the plants, soil microbes and soil nutrients, to factorial N and P additions in the moist acidic tundra (MAT; 26 y of nutrient additions) and moist non-acidic tundra (MNT; 16 y). Aboveground, the MAT plant community continues to change as predicted by earlier studies: functional groups responded independently to N and P, but NDVI-biomass, especially of *Betula nana,* only increased with N-addition. Unlike shorter-term MNT studies, the MNT vegetation, which does not include *B. nana*, shows few new fertilization responses. Belowground responses were not predicted by aboveground responses in either MAT or MNT. In contrast to the N-response aboveground, MAT microbial biomass responded positively and microbial phosphatase activity negatively to P additions, implying possible release from microbial P-limitation. Critically, earlier published results of declines in soil total carbon (C) with combined NP addition in the MAT are not present in the long-term. We make two conclusions: 1) arctic ecosystems are not universally N-limited but also exhibit complex responses to P alone or in combination with N.; and 2) the presence or absence of key

vegetation species can cascade from aboveground to belowground and restrict the extrapolation of responses of nutrient addition in a single Arctic ecosystem to other Arctic ecosystems, the short-term to the long-term, or aboveground to belowground.

Key words: Extracellular enzyme activity; Fertilization; Long-term; Microbial biomass; Moist non-acidic tundra; Nutrient limitation; Soil carbon; Toolik LTER

Introduction

Arctic regions are warming more rapidly than other parts of the globe (Huang et al. 2017) resulting in rapid ecological changes in arctic ecosystems (Post et al. 2009). Organisms respond both directly to warmer temperatures, but also to changes in ecosystem properties resulting from the warming. For example, deeper soil thaw is expected to enhance nutrient availability in the Arctic through increased rates of decomposition and nutrient mineralization (Nadelhoffer et al. 1991, Hobbie et al. 1999, Hinzman et al. 2005, Wang et al. 2017). Interest in how plant and soil communities will respond to increases in nutrient availability has resulted in a number of long-term fertilization experiments in a variety of arctic ecosystems across North America (Shaver et al. 2014, Gough et al. 2016) and Scandinavia (Jonasson et al. 1999, Van Wijk et al. 2004). Although the long-term levels of nutrient additions are often unrealistic in terms of nutrient release with warming, these experiments have been extremely fruitful for characterizing responses to changing ecosystem nutrient limitations, and the effects of fertilization aboveground (e.g. Chapin et al. 1995, Bret-Harte et al. 2001, Campioli et al. 2012), belowground (Jonasson et al. 1999, Rinnan et al. 2007, Deslippe et al. 2011, Koyama et al. 2013), and to a limited extent,

59 above-and belowground combined (Chapin et al. 1995, Gough et al. 2002, Mack et al. 2004,
60 Rinnan et al. 2007, Haugwitz et al. 2011), are well described. Resampling of these experiments,
61 however, has highlighted the importance of time, as the longer-term responses rarely reflect
62 responses of the shorter term (<10 y). For instance, responses of particular plant groups to
63 nutrient additions may be positive in the short term but negative in the longer term (Campioli et
64 al. 2012, Shaver et al. 2014), and the soil microbial community may require more than 10 years
65 of fertilization to show effects (Rinnan et al. 2007). Long-term fertilization experiments that
66 more extensively explore above- and belowground responses are especially useful for helping us
67 understand the whole ecosystem response to changes in nutrient availability.
68 The type of nutrient addition also limits our knowledge of tundra responses to long-term
69 increased nutrient availability: historically, researchers have favored combined N and P ("NP")
70 fertilization responses, whereas the independent (factorial) addition of N and P are less common.
71 NP fertilization experiments (control and NP combined treatments only) have provided good
72 evidence in arctic ecosystems that nutrient addition alters vegetation structure (e.g. increasing
73 dominance by deciduous shrubs (Shaver et al. 2001, Mack et al. 2004), enhances soil nutrient
74 availability (Chapin et al., 1995), and enhances soil organic matter (SOM) decomposition (Mack
75 et al. 2004, Koyama et al. 2013)). However, soil N and P cycles are not always coupled
76 (Rastetter et al. 2013, Sundqvist et al. 2014), and may decouple further with global change. For
77 instance, arctic mineral soils are often frozen, such that the short-term source of P for most
78 tundra plants and microbes may be via recycled organic matter P (Chapin et al. 1978, Giblin et
79 al. 1991, Jonasson et al. 1999), and thus N and P may respond differently to enhanced soil thaw
80 over longer time periods. In addition, early experiments in Alaskan ecosystems that examined
81 vegetation responses to factorial N and P additions found a consistent increase in growth with N-

addition (albeit with a strong interaction between N and P) (Shaver and Chapin 1980), and faster accumulation of N than P in tussock sedges when both nutrients were added (Shaver and Chapin 1986, Shaver and Chapin 1995), promoting the paradigm that arctic ecosystems are strongly N-limited. However, theoretical P limitation or NP co-limitation proposed by these same authors lead to the initiation of Alaskan tundra factorial N and P experiments. This theory has been supported in short-term N and P factorial fertilization experiments in Alaskan, Canadian and Swedish tundra ecosystems that report potential N and P co-limitation of numerous arctic ecosystems (e.g. heath tundra - Gough et al. 2002, Gordon et al. 2011, Sundqvist et al. 2014, Street et al. 2018; mesic meadow - Giesler et al. 2012; birch hummock - Zamin and Grogan 2012), as well as evidence that some carbon (C) rich ecosystems, such as wet sedge tundra, may be primarily P-limited (Chapin et al. 1975, Shaver and Chapin 1995, Nadelhoffer et al. 2002). Therefore, an assessment of the long-term effects of factorial N and P addition on both above- and below-ground properties is necessary for understanding potential ecosystem responses to shifting nutrient supplies with a changing climate, even in systems that are historically described as N-limited.

The objectives of this study were fourfold: 1) to characterize the long-term response of nutrient amendments (N, P and NP) to arctic tundra in comparison to earlier studies; 2) to investigate similarities in response above and belowground; 3) to compare the response in two common arctic tundra ecosystems; and 4) to understand if these systems are responsive to P in addition to N. We examined these objectives using multi-decadal, factorial N and P fertilization experiments in both moist acidic tundra (MAT) and the geologically younger moist non-acidic tundra (MNT) at Toolik LTER, Alaska. MAT is the most common arctic vegetation type in northern Alaska and MNT is the second most abundant vegetation type in the region (Raynolds

et al. 2006). At these sites, we measured the vegetation community structure, aboveground biomass based on normalized difference vegetation index (NDVI), soil nutrient stocks and extractable pools, the microbial biomass, and the activity of three extracellular enzymes that are representative of organic matter decomposition. Although there have been several studies that have examined aspects of these control and NP plots, the effects of the full factorial fertilization experiment on plant and soil properties have not been published for the 26-year history of the experiment (MAT), or since year 4 in the experiment's 16-year duration (MNT). In response to our objectives, are hypotheses are also fourfold: 1) long-term responses will differ from earlier published NP results, as feedbacks between above- and belowground adjust over time; 2) above- and belowground responses will be decoupled, in response to the competition between plants and soil microbes; 3) there will be ecosystem-specific responses that reflect the unique plant and microbial communities; and 4) we will find responses to N and P alone and interactively, reflecting the potential for arctic ecosystems, or components of arctic ecosystems, to be limited by both nutrients.

Materials and Methods

STUDY SITE

The study was conducted at the Toolik LTER in the northern foothills of the Brooks Range, Alaska (68° 38' N and 149° 43' W, elevation 760 m). Annual average temperature is -7 ^{o}C, and temperatures are generally above freezing between June-August, with an average temperature of 10 °C in July (Shaver et al. 2006). The area is underlain by continuous permafrost ca. 200m thick, so there is no deep drainage of soil water and little or no connection with

128 groundwater (Hinzman et al. 1991), with the depth of the active layer ranging from 30-50 cm and
129 they may not thaw each year to the mineral layer. Maximum thaw occurs in late-August to early-
130 September. Soils are gelisols (formed over permafrost), with high organic content (Shaver et al.
131 2014).

132 We conducted this study in two contrasting tussock tundra ecosystems: MAT and MNT
133 (Table 1). Tussock tundra is the most widespread vegetation type in Alaskan upland tundra – the
134 two ecosystems described here differ based on acidity, and correspondingly, on vegetation
135 composition. The MAT site is located on the older Itkillik II glacial surface where the substrate
136 is 50 k – 120 k years old, whereas the MNT is located on the younger Itkillik I glacial surface
137 where the substrate is 11.5-25 k years old (Hamilton 2003). MAT soils have a pH = 3-4, whereas
138 the MNT soil pH is neutral (pH= 6-7). Characteristics of the soils from the two ecosystems,
139 including bulk density and depth of the organic layer, as well as bulk nutrient concentrations, are
140 detailed in Table 1. The two ecosystems have a similar plant functional type composition but
141 plant biomass in the MNT is less than the MAT, and species diversity higher (Gough et al.
142 2000).
143
144 EXPERIMENTAL DESIGN

145 Fertilization treatments in both experiments are maintained by the Toolik LTER with a
146 full-factorial addition of N (10 g N m^{-2} y^{-1} as NH_4NO_3) and P (5 g P m^{-2} y^{-1} as P_2O_5), annually in
147 pellet form following snowmelt (early June). At the time of our study, the MAT site had been
148 fertilized annually for 26 years (established in 1989) and the MNT for 16 years (established in
149 1997). This study used a 5 x 20 m plot from each factorial N and P treatment, from three (MNT)
150 or four (MAT) replicate blocks that were separated by a minimum of 20 m.

151

152 VEGETATION SAMPLING

153 Aerial percent cover was estimated in each plot in mid-July 2013. The percent cover of
154 mosses, lichens and all vascular plant species was visually estimated within eight -1 m^2 adjacent
155 quadrats, each with 20 x 20 cm sub-quadrats, placed along the edge of each plot (0.25 m inside
156 to avoid edge effects).

157 NDVI was used as a measure of the abundance of photosynthetically active vegetation
158 (Rouse et al. 1974) and is highly sensitive to variation in aboveground biomass of vegetation in
159 the MAT at this site (Boelman et al. 2005); hereafter, we refer to NDVI estimates as 'NDVI-
160 biomass'. We calculated the normalized difference vegetation index (NDVI) as (NIR – R) / (NIR
161 + R), where NIR indicates mean reflectance at near-infrared wavelengths (841 - 876 nm) and R
162 mean reflectance at visible red wavelengths (620 - 670 nm). Spectral radiance measurements
163 were collected by the Toolik LTER using a hand-held dual channel spectrophotometer (Unispec
164 DC, PP Systems, Amesbury, MA, USA) (Shaver and Gough 2015). Radiance measurements
165 were taken throughout the summer of 2014 on multiple dates for each plot (2013 measurements
166 were not available for both ecosystems). On each date, five replicate scans were taken 1 m apart
167 along a 5 m transect located ~ 0.5 m from the edge of each plot. For this study, we selected the
168 dates that occurred nearest to peak NDVI measurements (10 July, 28 July, 6 Aug and 9 Aug). All
169 spectral measurements were converted to reflectance values and were interpolated to 1 nm
170 intervals. Vegetation indices were calculated for replicated scans, and then averaged per block
171 and across all 4 collection days before analysis.

172 Vegetation data was analyzed as relative percent cover for each species and for functional
173 groups (calculated as the sum of all component species). Relative percent cover was calculated

174 by dividing the cover of the individual species or functional group in each plot by the total plant
175 cover recorded for that 1m^2 plot.
176
177 SOIL SAMPLING AND PREPARATION
178 Organic and mineral soil horizons were sampled from MAT and MNT in early July 2013.
179 When present, tussocks of *Eriophorum vaginatum* were avoided when sampling so sampling
180 occurred only in intertussock areas (moss dominated areas between tussocks), because of distinct
181 micro-topographic differences between tussock and intertussock areas. In the MAT, *E.*
182 *vaginatum* grows in dense tussocks which cover ca. 20 % of the ground surface area, whereas the
183 remainder of the surface area, and dominant ground cover type, is classified as 'intertussock' and
184 is composed of moss that is well colonized by evergreen and deciduous shrubs at both sites. In
185 the MNT, both *E. vaginatum* and tussocks are less abundant, and tussocks cover <10% of the
186 ground surface area. A single ca. 10 cm x 10 cm column of soil was cut from each plot to the
187 depth of the permafrost using a serrated knife. All organic horizons were < 20 cm deep, and were
188 separated into the upper organic (0-5 cm depth) and lower organic (5-15 cm depth) layers, to
189 allow comparison with previous studies that separated by depth (Mack et al. 2004, Sistla and
190 Schimel 2013) and because ecosystem nutrient pools and microbial biomass can vary strongly by
191 depth in the organic horizon. The mineral layer was sampled either to permafrost, or the upper 10
192 cm only, whichever was less. Mineral soils were sampled for MAT only, as the permafrost
193 extended into the organic horizon for all MNT plots, preventing mineral soil sampling.
194 Soils were separated into layers in the field, bulk density subsamples were collected using
195 a sharp knife from the center of each layer and measured, and then all samples were returned to
196 the field lab. Bulk density samples were weighed, and oven dried for calculating gravimetric

197 water content and CN analysis. The rest of the samples were homogenized by hand and all large
198 roots (>1 mm diameter) removed. The homogenized soil was frozen at -20 ^{o}C and shipped to
199 University of California Santa Barbara for the analyses below.
200
201 SOIL AND MICROBIAL EXTRACTION AND ANALYSIS
202 Soil samples (10 g) were extracted from frozen with 40 ml of deionized water by shaking
203 for 3 h. Duplicate samples for estimates of the microbial biomass flush ('fumigated') were
204 extracted in the same manner but with the addition of 1 ml $CHCl_3$ (Fierer et al. 2003). All
205 extracts were vacuum filtered through 1 μm pore size glass fiber filter paper and sparged for 30
206 min with compressed air (to remove residual C from the $CHCl_3$), then frozen at -20 ^{o}C until
207 analysis.
208 Water-extractable organic C (EOC) and total N (ETN) contents in the fumigated and non-
209 fumigated extracts were determined by oxidative combustion and infrared (EOC; Nelson &
210 Sommers, 1982) or chemiluminesence (ETN) analysis (TOC-TN autoanalyzer, Shimadzu,
211 Kyoto, Japan). Water-extractable NH_4^+-N, NO_3^--N and PO_4-P in non-fumigated extracts and
212 PO_4-P in the fumigated extracts were determined colorimetrically, using automated flow analysis
213 (Lachat autoanalyser) and the salicylate (NH_4^+-N), sulphanilamide (NO_3^--N) and molybdate blue
214 (PO_4-P) methods (Mulvaney 1996).
215 Microbial biomass C, N and P flushes were calculated as the difference between EOC,
216 ETN or PO_4-P in fumigated and non-fumigated extracts. No correction factor was applied for
217 incomplete $CHCl_3$-release, or sorption of P because these values are not known for water-
218 extraction for these two ecosystems. The final nutrient concentration of all extracts was
219 corrected for dilution by the water content of the sample.

220

221 SOIL MICROBIAL EXTRACELLULAR ENZYME ANALYSIS

222 We assayed for the activity of three hydrolytic enzymes that acquire carbon, nitrogen and
223 phosphorous at the terminal stages of organic matter decomposition: cellulose-degrading β-
224 glucosidase, chitin-degrading N-acetyl-glucosaminidase (NAG) and phosphatase (McLaren et al.
225 2017). Soil was thawed and blended with 0.05M acetate buffer (MAT: pH 5; MNT: pH 6). Soil
226 slurries were pipetted onto 96 well plates to which fluorescing 4-methylum-belliferone (MUB)
227 tagged substrate (β-D-glucoside, N-acetyl-α-D-glucosaminide and phosphate) was added, with 8
228 analytical replicates per soil. The assays were incubated at 22 ^{o}C for ~2-4.5 h (previously
229 determined, by substrate, for these soils, to be during the phase of linear increase in activity) and
230 then the reaction was stopped by adding 20 µL of 0.5 M NaOH. Sample fluorescence was read
231 with a TECAN Infinite Pro 200 plate reader (Tecan Group Ltd., Männedorf, Switzerland) at 365
232 nm excitation, 450 nm emission. For each substrate, we measured the background fluorescence
233 of soils and substrate and the quenching of MUB by soils, and used standard curves of MUB to
234 calculate nmol of substrate hydrolyzed per hour per g of soil. Soil pools and rates were converted
235 from µg g^{-1} to g m^{-2} and nmol g^{-1} h^{-1} to mmol m^{-2} h^{-1} by correcting for the depth and bulk density
236 of each replicate sample.

237

238 TOTAL SOIL CNP ANALYSIS

239 Soil CN was analyzed on dried, ground soil for each soil layer using a dry combustion
240 total CN analyzer (Perkin Elmer 2400 at NC State University Environmental Testing Service).
241 Soil total P was analyzed using a strong-acid soluble digest (EPA method 3050B digested at NC
242 State University Environmental and Agricultural Testing Service Laboratory). The digestates

were analyzed using an Inductively-coupled plasma-optical emission spectrometer (ICP-OES; Perkin Elmer Model 8000, Waltham MA USA) with a cross- flow nebulizer.

STATISTICAL MODELS

We analyzed for differences in vegetation functional groups between ecosystems and treatments using a fully factorial MANOVA with site, N and P as the main factors. As the MANOVA for functional group was significant (Roy's Max Root $P<0.05$), and include significant interactions between sites and nutrient additions, each functional group was tested independently for each site using a fully factorial mixed model with N and P as the main factors and block as a random effect (REML), for each ecosystem. As the dominant species were different between sites we did not run the full ecosystem and treatment model on species specific cover. Instead, dominant species at each site (>5 % cover) were each analyzed independently using a fully factorial model as above. Our models for NDVI, soil pools and rates were similar, with block as a random effect, and N, P and depth (the latter not for NDVI) as fixed effects, for each ecosystem. The factors N and P were treated as binary dummy variables, such that Control plots = (0, 0), N-addition plots = (1, 0), P-addition plots = (0, 1), and N + P addition plots = (1, 1), for the factors (N, P), respectively. For MAT soil we analyzed the organic and mineral soil separately, in order to allow a qualitative comparison between ecosystems (mineral soil results are presented in Appendix S2: Tables S1 and S2). Because of the different time since experimental inception, we did not include both ecosystems in the same model, although we do discuss qualitative differences between the two systems. To enable this comparison, all data are presented on a per m^2 basis (scaled by depth and bulk density, see Table 1). Results on a per gram basis are available in the online data archive (see Data Accessibility Statement). All data

266 were assessed for normality (Shapiro-Wilks) and heteroscedasticity and in most cases, log
267 transformed (or square root arcsine transformed for vegetation data) before analysis before
268 analysis with JMP 10.0 (2010, Cary NC USA).
269
270 **RESULTS**
271
272 *Vegetation responses to long-term nutrient addition*
273 Overall, the vegetation responses to both N and P additions were stronger in the MAT
274 than the MNT (Fig. 1, Table 2, Appendix S1: Table S1 & S2). In the MAT, NDVI-biomass
275 increased with N addition, but did not respond to P (Fig. 1, Appendix S1: Table S1). Two
276 functional groups responded interactively to N x P: the decrease in evergreen shrubs with N was
277 moderated by P, and the increase in deciduous shrubs with N overwhelmed the generally
278 negative effect of P. Moss and graminoids decreased with the addition of N, and graminoids also
279 decreased with the addition of P. Forbs increased with the addition of N and P, independently.
280 The MAT response was dominated by *Betula nana* (deciduous shrub) and *Rubus chamaemorus*
281 (forb), two species that were in low abundance in the MNT (Figure 1c, f, Appendix S1: Table
282 S2).
283 In the MNT, NDVI-biomass did not change with either N or P additions, and there was
284 no interactive response. Graminoids responded positively to N, and moss responded negatively
285 to P. There were no N x P interactive responses in the MNT.
286
287 *Soil Microbial Biomass C, N and P responses to long-term nutrient addition*

In the MAT organic soils, both N and P additions, as well as depth, altered microbial biomass (Table 2, Appendix S1: Table S3). MBC in MAT shallow organic soils decreased with P addition but increased with P addition in deeper organic soils (P x Depth), and did not respond to N at either depth (Fig. 2a). MAT MBN decreased with N addition, especially in deeper organic soils (N x Depth, Fig. 2b). Similar to MBC, MAT MBN decreased with P addition in shallow organic soils, but increased in deeper organic soils (P x Depth, Fig. 2b). MAT MBP increased with P addition, but only in deeper organic soils (P x Depth, Fig. 2c).

Control plot MBC in the MNT was double that in the MAT (Fig. 2a, d) and there were fewer effects of N and P addition on MBC in the MNT compared with the MAT. In the MNT, MBC and MBN did not change with N or P addition (Table 2, Appendix S1: Table S4, Fig. 2d, e), whereas MBP increased with P addition but only in deeper organic soils (Table 2, P x Depth, Appendix S1: Table S4, Fig. 2f).

Potential extracellular enzyme activity responses to long-term nutrient addition

In the MAT organic soils, there was no effect of any fertilization treatment, or depth, on the activity of β-glucosidase (Table2, Appendix S1: Table S3, Fig. 3a). In contrast, the activity of NAG showed an N x P interaction, where the decrease with N addition was moderated by the simultaneous addition of P (Fig. 3b). Similarly, the N x P interaction for phosphatase activity in the MAT organic soils was driven by a decrease with the addition of P, and this decrease was moderated when N was added simultaneously (N x P, Fig. 3c).

Control plot β-glucosidase and NAG activity was ~ten times larger in MNT than in MAT organic soils, although control plot phosphatase activity was a similar magnitude in the two systems (Fig. 3). Unlike the generally negative effect of fertilizers on enzyme activity in the

311 MAT, the effect of nutrient additions on MNT enzyme activity was positive (Table 2, Appendix
312 S1: Table S4). The activity of β-glucosidase increased with the addition of N, and with organic
313 soil depth (Fig. 3d). NAG activity in the MNT also increased with N, more so with P addition (N
314 x P, Fig. 3e). Phosphatase activity in the MNT increased with N addition (Fig. 3f).
315
316 *Soil Extractable C, N and P responses to long-term nutrient addition*
317 Extractable organic carbon in the MAT organic soils did not respond to fertilization
318 (Table2, Appendix S1: Table S3, Fig. 4a), and extractable total nitrogen increased with N
319 addition (Fig. 4b). The two inorganic components of total N (ammonium and nitrate) responded
320 to both N and P addition in MAT organic soils: Extractable NH_4^+-N increased with N addition,
321 and independently decreased with P addition (Fig. 5a). Similarly, extractable NO_3^--N increased
322 with N addition, but this increase was moderated by the simultaneous addition of P (N x P, Fig.
323 5b). Effects of N or P additions on soil phosphate in the MAT organic soils were complex with
324 interactions between N x P x Depth: extractable PO_4-P increased with P addition, an effect that
325 was decreased with the simultaneous addition of N (N x P), especially in deeper organic soils (N
326 x P x Depth, Fig. 4c).
327 MNT control plot extractable total C and N pools (ETN and NO_3^--N) were generally
328 similar between MAT and MNT (Table 2, Appendix S1: Table S4, Fig. 4), although control plot
329 extractable PO_4-P and NH_4^+-N in MNT were ~double of that in the MAT (Fig. 4c, f). Extractable
330 organic C in the MNT increased with depth (Fig. 4d). Total extractable N in the MNT increased
331 with N addition (Fig. 4e). Extractable NO_3^--N in the MNT increased with N addition (Fig. 5d),
332 and extractable phosphate increased with P addition (Fig. 4f).
333

Soil Total C, N and P responses to long-term nutrient addition

In MAT organic soils, soil total C and total soil N both increased with depth, but did not respond to either N or P addition (Table 2, Appendix S1: Table S3, Fig. 6a, b). Soil total P increased with both P addition and with depth (Fig. 6c). As with other MNT control plot variables, total soil pools of C, N and P in the MNT were ~ double the MAT (Fig. 6). Soil total C in the MNT increased at depth but did not respond to fertilizer (Table 2, Appendix S1: Table S4, Fig. 6d). Soil total N in the MNT was higher in the deeper organic soils, and increased when N and P were added together (N x P, Fig. 6e). Soil total P in the MNT increased when both nutrients were added in combination (N x P) and this interaction was enhanced in the surface organic soils (N x P x Depth, Fig. 6f).

Discussion

We had a four-part objective in this study: 1) to characterize the long-term response of factorial nutrient (N and P) amendments to arctic tundra in comparison to earlier studies; 2) to investigate similarities in response above and belowground; 3) to compare the response in two common arctic tundra ecosystems; and 4) to understand if these systems are responsive to P in addition to N. The two ecosystems that we studied differed in vegetation, soil and microbial response to similar long-term N and P additions, with predictably complex interactions. Nonetheless, in both ecosystems we found that (1) the early, strong ecosystem response to NP additions above and belowground has been maintained, although in support of our first hypothesis, the components of these responses have changed through time. (2) In this first study to examine a complementary suite of above- and belowground responses to factorial nutrient additions in Alaskan tundra, our second hypothesis was supported, and we found a decoupling of

the above- and belowground responses, highlighting that aboveground responses cannot necessarily be used to predict those belowground. (3) Our third hypothesis regarding ecosystem-specific responses was also supported in that we found a higher number of responses aboveground in the MAT as compared to the MNT and many more interactive belowground responses in the MNT and thus conclude that Arctic tundra ecosystems have different sensitivities to long-term nutrient amendments. Finally, (4) our fourth hypothesis about N and P responses was also supported as we found multiple indications of ecosystem response to P, in addition to N and their interactions, in vegetation and in soil, implying a need to expand our investigation of P in tundra ecosystems. We expand upon these phenomena below.

Aboveground community and NDVI-biomass response to multi-decadal nutrient amendments

There were strong responses of P addition on the cover of multiple vegetation functional groups in the MAT. We found decreases in the proportion of graminoids and increases in forbs with P additions. The response of these functional groups mirror their response to nitrogen addition, indicating an independent co-limitation (i.e., they responded independently to N and P with no interaction between the two nutrients). In contrast, NDVI-biomass increased only with N, rather than P addition. This indicates that either both overall productivity and vegetation composition are controlled by different nutrients, or that a few functional groups, which are regulated primarily by N, determine NDVI-biomass. We suggest the latter explanation given the strong response of deciduous shrubs, and *Betula nana* in particular, to fertilization treatments.

Although *B. nana* responded interactively to N and P addition (Appendix S1: Table S1), the overall response was dominated by the effect of N; when N and P were added simultaneously the plots resembled an exaggerated version of the N-alone plots with almost complete dominance

of deciduous shrubs. Increases in *B. nana* with NP fertilization in this ecosystem have been attributed to high plasticity of *B. nana* growth, with *B. nana* changing biomass allocation and increasing the rate of new meristem production with fertilization (Bret-Harte et al. 2001). In contrast to our findings that N determines the *B. nana* response, in a Canadian mesic birch tundra, Zamin et al. (Zamin and Grogan 2012, Zamin et al. 2014) found that after six to eight years of fertilization both N and P additions independently increased *B. glandulosa* apical stem growth and P alone resulted in an increase in leaf growth. Regardless, this response may still be driven by N, as Zamin & Grogan (2012) attributed the increases in growth with P to an increase in N availability which resulted from P addition. Also in contrast to our findings, in heath tundra in Sweden, Jonasson et al. (1999) found that although *B. nana* was present in the heath plant community, NP fertilization did not lead to *B. nana* dominance and rather increased biomass across all functional groups, possibly because the fertilization treatment was relatively short (5 years) in comparison with the experiments we describe here.

The plant functional group abundance we describe in the NP plots after 26 years of fertilization is largely predicted by shorter-term results from this and other near-by fertilization experiments, yet the plant community appears to be continuing to change. Relatively early in the experiment (four years of fertilization) a decline in moss, graminoids and forbs and an increase in the abundance of *B. nana* were reported (Chapin et al. 1995, Hobbie et al. 2005). After nine years, moss, graminoids and evergreen shrubs continued to decline (although were still present in the community) and there were continued large increases in dominance by *B. nana* (Chapin et al. 1995, Shaver et al. 2001). After 15 years of fertilization there was strong dominance by *B. nana* (90% of total biomass), with the next most abundant plant (*Rhododendron palustre,* an evergreen shrub) making up only 4% of the biomass, followed by a low growing perennial forb *Rubus*

403 *chamaemorus* at <2% biomass (Shaver et al. 2001). Currently, after 26 years of fertilization,
404 while *B. nana* remains dominant, we also report an increase in the abundance of *R. chamaemorus*
405 with cover reaching nearly 40% in NP plots. Although our characterization of species change is
406 by proportional cover and not biomass, which is more commonly reported from these
407 experiments, there are strong correlations in this ecosystem between cover and biomass (Gough
408 and Hobbie 2003). This increase in *R. chamaemorus* may be facilitated by the recent die-back of
409 *B. nana* in fertilized plots where standing dead *B. nana* makes up 10% of the canopy, removing
410 potential light limitation for the *R. chamaemorus* growing in the understory.
411 In contrast with the MAT ecosystem, there were very few effects of 16 years of
412 fertilization by P on vegetation cover in the MNT, supporting our prediction of stronger
413 responses to P addition in the MAT. Further, in contrast with the MAT where responses have
414 become stronger over time, long-term effects on the MNT resemble those on the shorter term.
415 After 4 years of fertilization Gough and Hobbie (2003) reported an increase in above-ground
416 biomass with NP addition, resulting primarily from increases in graminoids, and a smaller
417 decrease in biomass with P addition. After 16 years of treatment, however, we found no effects
418 of fertilization by either nutrient on NDVI-biomass. Gough and Hobbie (2003) also reported
419 increases in graminoid and forb cover and decreases in evergreen shrubs within plots that had
420 been fertilized by both nutrients (but not in plots with single nutrient additions). After 12 more
421 years of fertilization we found no evidence of this earlier reported co-limitation and the only
422 changes in plant cover we detect is an increase in graminoids. Earlier responses, similar to those
423 we report, were primarily driven by graminoids, and *Carex bigelowii* in particular (Gough and
424 Hobbie 2003). In the MAT any positive effect of fertilization on graminoids is overwhelmed by
425 the large increases in *B. nana* cover, which quickly outcompetes the other species. *B. nana* is

absent from the MNT and, as predicted by Gough and Hobbie (2003), long term community changes in this community were subtle because of a lack of species which respond dramatically to fertilization treatments. Further, as suggested by Jonasson et al. (1999), the successional pathway when nutrient availability increases in different arctic ecosystems is going to depend on both the plant community and soil and microbial properties in that ecosystem and thus we must be careful in extrapolating the results to other arctic ecosystems.

Belowground biomass and activity response to multi-decadal nutrient amendments

A release of microbial nutrient limitation may be indicated by an increase in MBC and a decrease in the microbial acquisition effort for that nutrient (i.e., activity of the enzyme(s) that increases the availability of that nutrient), according to threshold element ratio (TER) theory (Sterner and Elser 2002, Sinsabaugh and Follstad Shah 2012). In our study, MBC only responded positively to P addition, and only in the deep organic soil of the MAT (Fig. 2). True to TER theory, this MBC increase with P addition was accompanied by lower phosphatase activity (Fig 3c), which typically indicates a decrease in microbial attempts at P-acquisition (although some of this decrease may be from the phosphate ion competing for the enzyme active site). This match with theory for P amendments has been found in other organic soil systems (Pinsonneault et al. 2016), including these same soils with NP addition in an earlier study (Koyama et al. 2013). In fact, this P-acquisition response to P amendments is more common than C- or N-acquisition responses in the literature (reviewed in Burns et al., 2013), perhaps because the P-acquisition enzyme is more specific (N-acquisition via NAG also acquires C), or because phosphatase production is more universal in microbial communities, as it is a relatively simple trait to acquire (encoded by one gene (Martiny et al. 2015)). Combined, the increased biomass

449 and decreased acquisition effort we found provides support for microbial P limitation in these
450 MAT organic soils. In contrast, MNT organic soil and MAT mineral soil microbes appear to be
451 opportunistic about P storage since P addition increased MBP in deep organic soils of the MNT
452 without also increasing MBC (Appendix S1: Table S4). Microbial P storage in tundra soils has
453 been reported before (Chapin et al. 1978, Buckeridge et al. 2016) and slow turnover of P from
454 microbial biomass may provide a mechanism for tundra microbes to overcome permafrost-
455 locked mineral P stores (Jonasson et al. 1999) – the mineral soils in the MNT were not ice-free in
456 the year that we sampled, for instance. Previous studies have indicated that the MNT system may
457 be less limited by P, despite higher available Ca and Mg concentrations (Whittinghill and Hobbie
458 2012) which could bind and reduce P availability. For instance, the MNT has higher soil P
459 availability and foliar P concentrations, compared to the MAT (Hobbie and Gough 2002). Our
460 biomass and enzyme results from these long-term nutrient amendments support these earlier
461 conclusions that the MNT is less P-limited than the MAT.
462 Based on the paradigm that these tundra ecosystems are strongly N-limited, we found
463 surprisingly few effects of independent N additions on soil microbial biomass or activity in either
464 ecosystem. An earlier short-term laboratory incubation of control MAT soil with N-amendments
465 concluded microbial N-limitation for the MAT (Sistla et al. 2012). However, our long-term *in*
466 *situ* results found no MBC response to N additions in either ecosystem. These contradictory
467 laboratory and field responses may be due to competition or community differences in lab and
468 field. Field microbes may not be accessing the available N, and we believe the most likely reason
469 is the very strong and competitive biomass response in the MAT by *B. nana* (Chapin et al. 1995,
470 Jonasson et al. 1999, Shaver et al. 2001). Alternatively, or in addition, the *in situ* microbial
471 community may have changed structure (Deslippe et al. 2005) and/or physiology in response to a

long-term high N supply. Interestingly, independent additions of N strongly decreased MBN in the MAT deep organic soil (Fig. 2). MBN declines could be a result of several mechanisms, including direct N (or P) toxicity and associated declines in base cation or C availability (reviewed in Treseder 2008) or declines of Acidobacteria with N (Ramirez et al. 2012), a dominant tundra bacterial phylum (Chu et al. 2010). The lack of decline in MBC with N or P additions implies that long-term fertilizer amendment did not depress total microbial population sizes, but that some other mechanism, such as community restructuring, alters ecosystem N pools by reducing the typically substantial portion of (extractable) soil N that is stored in tundra microbial biomass.

Unlike the microbial biomass response to either N or P independently, most enzymes responded interactively to N and P. An exception to this was the positive response of the cellulose-degrading β-glucosidase to N additions in the MNT. Based on an earlier study in the MAT NP plots, we expected a stimulation of C-degrading enzymes in the MAT system (Koyama et al. 2013). However, our results raise an important caveat of enzyme activity assays: we measured two C-acquiring enzymes (β-glucosidase and NAG) and found that only NAG was stimulated in the shallow MAT NP soils, whereas Koyama et al. (2013) found that NP stimulated most C-degrading enzymes (although, as with this study, not β-glucosidase). Therefore, we suggest caution when using single enzymes as ecosystem indicators; multiple enzymes within each nutrient acquisition group provide a more robust response. Nonetheless, in both ecosystems, we found interactive effects of N and P on N-acquiring and P-acquiring enzyme activity: in the MAT, the reduction in P- or N-acquisition activity with P or N, respectively, is lessened with the addition of the other nutrient (Fig. 3b, c). In the MNT, P and N additions additively increase P-

494 or N-acquisition activity (Fig. 3e, f). These interactive responses in the MNT are likely indicative
495 of the high microbial N and P costs for enzyme fabrication.
496 Extractable pools provide mixed messages in long-term nutrient addition experiments, as
497 they represent the short-term net sum of nutrients not taken up by plants and microbes (i.e. not
498 limiting) plus the result of any priming of native soil nutrient pools (i.e. limiting), in a temporally
499 unstable pool. Nonetheless, broad conclusions can be drawn from these data. Not surprisingly, in
500 both systems available N pools typically increased with N amendments and available P with P
501 amendments. However, available N and P pools were generally lower when added together in the
502 MAT, implying short-term co-limitation of N and P in that system, in particular with regards to
503 nitrate availability. Similar interactions were found in a 7-year nutrient amendment study in the
504 Canadian low arctic tundra, although in that case ammonium availability was particularly
505 sensitive to NP interactions and declined when P was added simultaneously (Zamin et al. 2014).
506
507 *Belowground total soil C, N and P response to multi-decadal nutrient amendments*
508 Soil total C stocks provide a long-term signal of the effects of N and P on SOM
509 decomposition, and SOM stabilization processes, and thus summarize above and belowground
510 long-term ecosystem response to N and P fertilization. Surprisingly, we found a lack of effects of
511 N or P on soil total C in the MAT, unlike reported C-losses in the same system, after shorter
512 periods of nutrient amendment (5 years - Koyama et al. 2013, 6 years - Nadelhoffer et al. 2002
513 and 19 years - Mack et al. 2004). However, our results parallel other long-term nutrient
514 amendment results in the same system (22 years of amendments - Koyama et al. 2013), implying
515 that short-term ecosystem C-loss with the removal of plant-nutrient limitations do not persist
516 beyond two decades. This is possibly a response to aboveground community shifts over time, as

summarized above: earlier soil total C-loss corresponded with increases in deciduous shrub biomass (Mack et al. 2004), which has now started to decline.

Soil total P accumulates with P additions in the MAT. In contrast to this independent effect of P in the MAT, in the MNT system we found interactive effects of N and P (and depth). We saw an increase in N and P storage with NP amendment in the organic soils in addition to much higher enzyme activity in this ecosystem (compare ecosystem axes in Fig. 3) and in the NP plots in particular. This higher enzymatic activity is in contrast with lower microbial respiration in the MNT ecosystem (Whittinghill and Hobbie 2012) and may imply higher carbon use efficiency, at least when nutrient limitations are removed.

We suggest that the strong interactive effects belowground in the MNT reflect the low fertilization response aboveground in this system. In the MAT, belowground responses may be pre-empted by strong competition by *B. nana* in particular for both N and P and as a result we see repressed enzyme activity and low microbial biomass. In contrast, the MNT vegetation community contains fewer long-term responsive plant species and is associated with belowground enhanced enzyme activity and available nutrients. It is possible that this has promoted much larger microbial biomass and total C, N and P stores in the MNT (compare ecosystem y-axes in Figs. 2 & 6), especially in the NP plots.

In summary, whereas the MAT ecosystem was dominated by an aboveground response, especially *B. nana*, to NP, the MNT ecosystem was dominated by the belowground response (Table 2). Effects of fertilization on ecosystems are often characterized based on the response aboveground, even though the belowground response may differ or may even respond in the absence of an aboveground effect (e.g. Jonasson et al. 1999). Two clear conclusions from these very different above-and belowground and ecosystem trajectories are: 1) arctic ecosystems are

not universally N-limited but also exhibit complex responses to P alone or in combination with N.; and 2) the presence or absence of key vegetation species (*B. nana* and *R. chamaemorus*) can cascade from aboveground to belowground and restrict the extrapolation of responses of nutrient addition in a single Arctic ecosystem to other Arctic ecosystems, the short-term to the long-term, or aboveground to belowground. Therefore, we cannot generalize the response of Arctic tundra ecosystems to long-term nutrient amendment. An extension of this conclusion is that we should use caution before assuming that Arctic ecosystems will respond similarly to a changing world, and should broaden our long-term manipulations to multiple different tundra ecosystems, with different vegetation and microbial communities, different C stocks, and potentially different sensitivities to global change.

Data Accessibility Statement

- Species cover: Arctic Data Center Entry

http://dx.doi.org/10.6073/pasta/8a2999c9ed297a184aaca7057e1ae177

- Soil microbial biomass C, N and P; extracellular enzyme activity, soil extractable C, N and P; soil total C, N and P, in g m^{-2} and μg g^{-1}: Arctic Data Center Entry

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Table 1. Characteristics of the two arctic ecosystems in this study (Mean (SE)). Moist acidic tundra (MAT) was most recently glaciated 50,000 – 100,000 years ago and moist non-acidic tundra (MNT) 11,500 - 25,000 years ago (Hamilton 2002). Experimental fertilizer additions were at the rate of 10 g N m^{-2} y^{-1}; 5 g P m^{-2} y^{-1} in both ecosystems, starting in 1989 (MAT) or 1997 (MNT). % Ca, Mg, K, and Al are from Hobbie and Gough 2002, %C, N and P measured in the control plots during this study, and pH from LTER measurements in 2013.

	MAT			MNT		
	Organic (0-5cm)	Organic (>5cm)	Mineral	Organic (0-5cm)	Organic (>5cm)	Mineral
pH	4.3 (0.1)		4.5 (0.1)	7.0 (0.1)		6.9 (0.2)
Depth of Organic	12.0 (1.2)			15.7 (2.2)		
Bulk Density (g cm^{-3})	0.10 (0.03)	0.13 (0.01)	0.78 (0.30)	0.19 (0.07)	0.37 (0.23)	
Soil moisture (%)	686.3 (104.4)	595.0 (51.0)	78.9 (17.4)	426.0 (49.6)	355.7 (19.3)	
%C	43.45 (0.46)	39.19 (1.86)	9.2 (3.48)	39.75 (1.56)	35.50 (1.69)	
%N	1.24 (0.07)	1.22 (0.06)	0.46 (0.18)	1.59 (0.28)	1.89 (0.22)	
%P	0.08 (0.01)	0.09 (0.01)	0.05 (0.02)	0.09 (0.01)	0.09 (0.01)	
%Ca	0.31 (0.06)		0.05 (0.01)	2.53 (0.18)		0.42 (0.04)
%Mg	0.12 (0.01)		0.19 (0.02)	0.17 (0.02)		0.33 (0.01)
%K	0.16 (0.05)		0.17 (0.03)	0.11 (0.02)		0.15 (0.02)
%Al	0.88 (0.22)		1.48 (0.08)	0.66 (0.10)		1.41 (0.10)

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Table 2. Effects of long-term N and P addition on NDVI-biomass and abundance, soil microbial and nutrient pools and process rates in moist acidic tundra ("MAT", experiment established 1989) and moist non-acidic tundra ("MNT", experiment established 1997). Summary data for each ecosystem are based on a two-way factorial ANOVA (N x P) for vegetation responses and a three-way ANOVA (N x P x Depth) for soil responses Cells are filled for significant (α=0.05) individual responses to N or P addition or N x P interactions (light gray = positive or dark gray = negative), and "D" an interaction between N, P, or NxP and soil depth. Non-signficant responses are not displayed. Two- and three-way interactions have complex results that are described in more detail in the text and figures. In general, a positive (light gray) interaction indicates that one factor enhanced the response of another, whereas a negative (dark gray) interaction indicates that one factor inhibits another.

Response Category	Response Variable	MAT			MNT		
		N response	P response	Net N x P response	N response	P response	Net N x P response
Vegetation biomass	NDVI	+					
Vegetation functional group relative abundance	Moss	-				-	
	Lichen						
	Evergreen	-	+	-			
	Forb	+	+				
	Graminoid	-	-		+		
	Deciduous	+		+			
Microbial biomass pools	MBC		+D				
	MBN	-D	+D				
	MBP		+D			+D	
Extracellular enzyme rates	BG				+		
	NAG			+	-		+
	Phosphatase		-	+	+		
Soil extractable pools	Organic C						
	Total N	+			+		
	NH_4^+-N	+	-				
	NO_3^--N	+	-	-	+		
	PO_4-P		+D	-D		+	
Soil total pools	C						
	N				+		+
	P		+		+	+	+D

+ (Positive effect)
- (Negative effect)
D = N or P interaction with depth

Figure Captions.

Figure 1. The effects of long-term nutrient additions on Normalized Difference Vegetation Index (NDVI) (a,d), proportional cover of vegetation functional groups (b,e) and dominant species (c,f), in moist acidic tundra (MAT: a,b) and moist non-acidic tundra (MNT: c,d). The treatment "Control" has received no nutrient additions, "N" has received 10 g N y^{-1} (as NH_4NO_3), "P" has received 5 g P y^{-1} (as P_2O_5), and "NP" has received 10 g N + 5 g P y^{-1}, since 1988 in the MAT and since 1997 in the MNT. Refer to text for further experimental details. Bars are means (MAT, n=4; MNT, n=3) + one standard error. Functional groups in (b) and (e) are represented by color as indicated in the legend, and also are arranged top-to-bottom as in the legend. "Bet nan" = *Betula nana*; "Eri vag" = *Eriophorum vaginatum*; "Rho pal" = *Rhododendron palustre*; "Rub cha" = *Rubus chamaemorus*.

Figure 2. The effects of long-term nutrient additions on soil microbial biomass carbon (MBC; a,d), nitrogen (MBN; b,e) and phosphate (MBP; c,f), in moist acidic tundra (MAT: a,b,c) and moist non-acidic tundra (MNT: d,e,f), each in the shallow (0-5 cm) and deep organic (>5 cm) soil. The treatment "Control" has received no nutrient additions, "N" has received 10 g N y^{-1} (as NH_4NO_3), "P" has received 5 g P y^{-1} (as P_2O_5), and "NP" has received 10 g N + 5 g P y^{-1}, since 1988 in the MAT and since 1997 in the MNT. The microbial biomass is the uncorrected flush of nutrients released upon extraction with water and chloroform. Refer to text for further experimental and method details. Bars are means (MAT, n=4; MNT, n=3) + one standard error. Asterisks next to text in plots represents significant ($\alpha = 0.05$) main effects and interactions from a 3-way factorial ANOVA (N x P x Depth) within each ecosystem (degrees of freedom: MAT: 1,21; MNT: 1,14). P-value significance codes: <0.001 = ***, <0.01 = **, <0.05 = *.

Figure 3. The effects of long-term nutrient additions on the potential soil enzyme activity of β-glucosidase (a,d), N-acetyl-glucosiminidase (b,e) and phosphatase (c,f), in moist acidic tundra (MAT: a,b,c) and moist non-acidic tundra (MNT: d,e,f), each in the shallow (0-5 cm) and deep organic (>5 cm) soil. The treatment "Control" has received no nutrient additions, "N" has received 10 g N y^{-1} (as NH_4NO_3), "P" has received 5 g P y^{-1} (as P_2O_5), and "NP" has received 10 g N + 5 g P y^{-1}, since 1988 in the MAT and since 1997 in the MNT. Refer to text for further experimental details. Bars are means (MAT, n=4; MNT, n=3) + one standard error. Asterisks next to text in plots represents significant ($\alpha = 0.05$) main effects and interactions from a 3-way factorial ANOVA (N x P x Depth) within each ecosystem (degrees of freedom: MAT: 1,21; MNT: 1,14). P-value significance codes: <0.001 = ***, <0.01 = **, <0.05 = *.

Figure 4. The effects of long-term nutrient additions on water-extractable soil organic carbon (a,d), total nitrogen (b,e) and phosphate (c,f), in moist acidic tundra (MAT: a,b,c) and moist non-acidic tundra (MNT: d,e,f), each in the shallow (0-5 cm) and deep organic (>5 cm) soil. The treatment "Control" has received no nutrient additions, "N" has received 10 g N y^{-1} (as NH_4NO_3), "P" has received 5 g P y^{-1} (as P_2O_5), and "NP" has received 10 g N + 5 g P y^{-1}, since 1988 in the MAT and since 1997 in the MNT. Refer to text for further experimental details. Bars are means (MAT, n=4; MNT, n=3) + one standard error. Asterisks next to text in plots represents significant ($\alpha = 0.05$) main effects and interactions from a 3-way factorial ANOVA (N x P x

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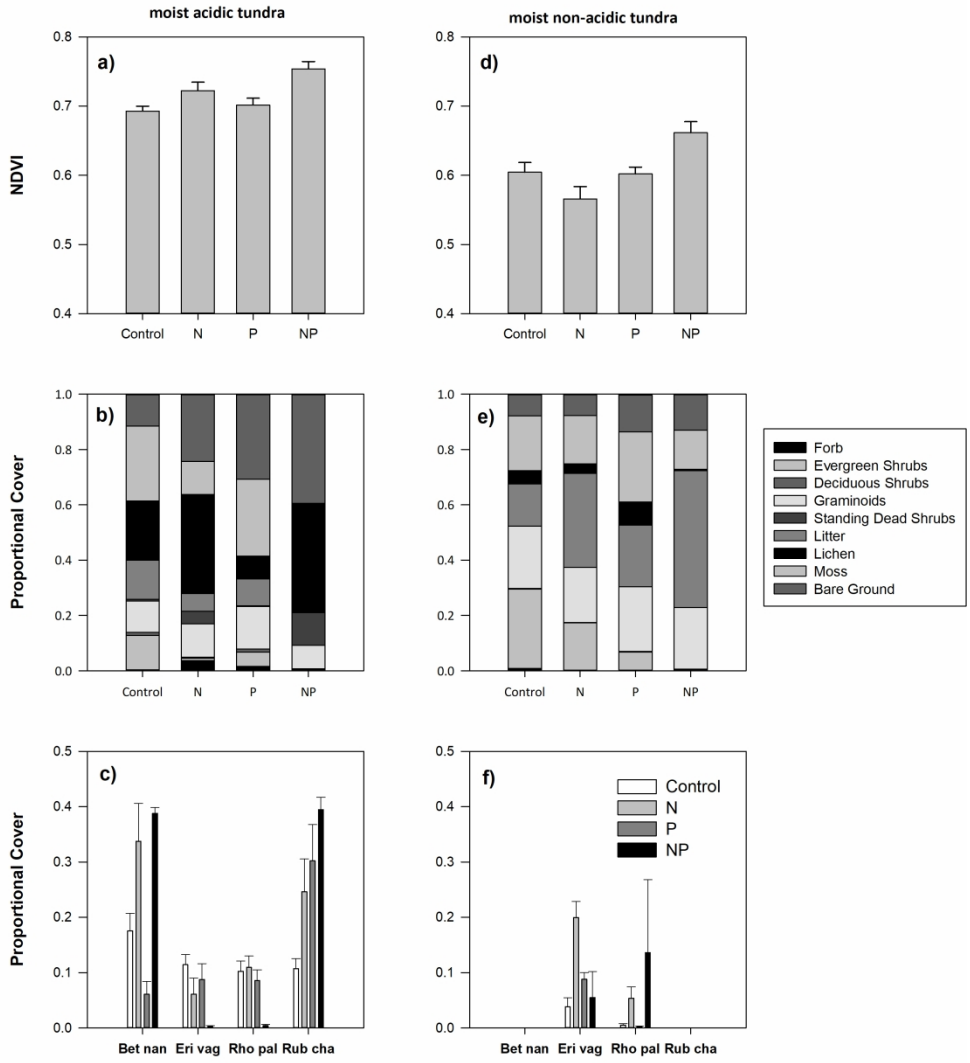
Figure 5. The effects of long-term nutrient additions on water-extractable soil ammonium-N (a,c), and nitrate-N (b,d), in moist acidic tundra (MAT: a,b,c) and moist non-acidic tundra (MNT: d,e,f), each in the shallow (0-5 cm) and deep organic (>5 cm) soil. The treatment "Control" has received no nutrient additions, "N" has received 10 g N y^{-1} (as NH_4NO_3), "P" has received 5 g P y^{-1} (as P_2O_5), and "NP" has received 10 g N + 5 g P y^{-1}, since 1988 in the MAT and since 1997 in the MNT. Refer to text for further experimental details. Bars are means (MAT, n=4; MNT, n=3) + one standard error. Asterisks next to text in plots represents significant (α = 0.05) main effects and interactions from a 3-way factorial ANOVA (N x P x Depth) within each ecosystem (degrees of freedom: MAT: 1,21; MNT: 1,14). P-value significance codes: <0.001 = ***, <0.01 = **, <0.05 = *.

Figure 6. The effects of long-term nutrient additions on total soil carbon (a,d), nitrogen (b,e) and phosphorus (c,f), in moist acidic tundra (MAT: a,b,c) and moist non-acidic tundra (MNT: d,e,f), each in the shallow (0-5 cm) and deep organic (>5 cm) soil. The treatment "Control" has received no nutrient additions, "N" has received 10 g N y^{-1} (as NH_4NO_3), "P" has received 5 g P y^{-1} (as P_2O_5), and "NP" has received 10 g N + 5 g P y^{-1}, since 1988 in the MAT and since 1997 in the MNT. Refer to text for further experimental details. Bars are means (MAT, n=4; MNT, n=3) + one standard error. Asterisks next to text in plots represents significant (α = 0.05) main effects and interactions from a 3-way factorial ANOVA (N x P x Depth) within each ecosystem (degrees of freedom: MAT: 1,21; MNT: 1,14). P-value significance codes: <0.001 = ***, <0.01 = **, <0.05 = *.



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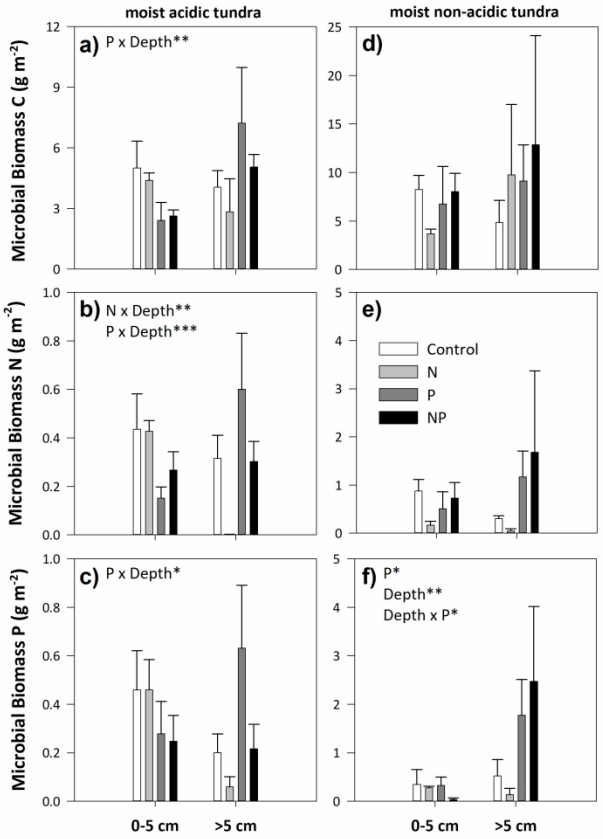

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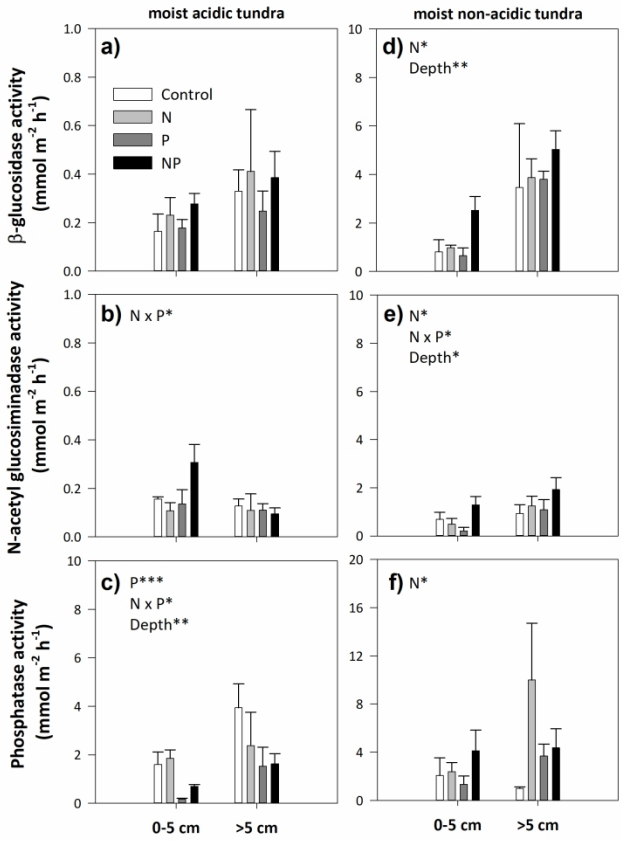

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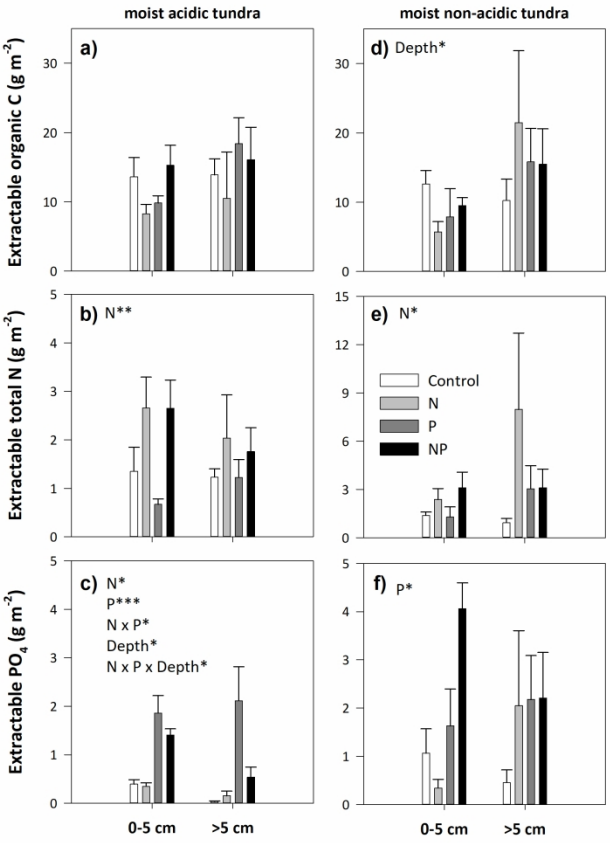

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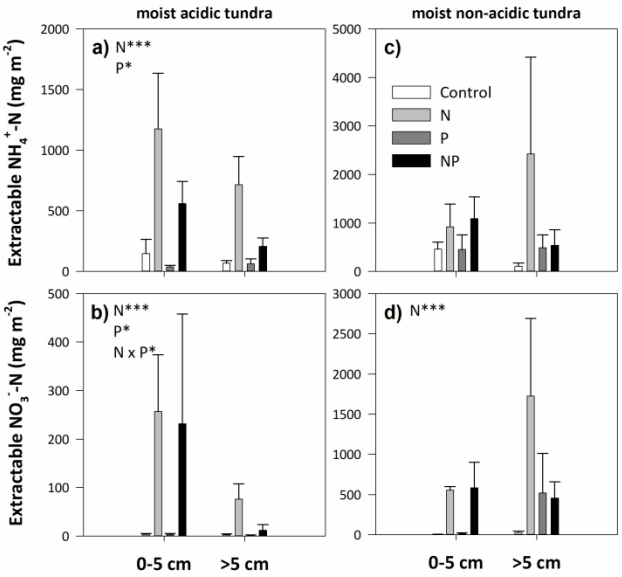

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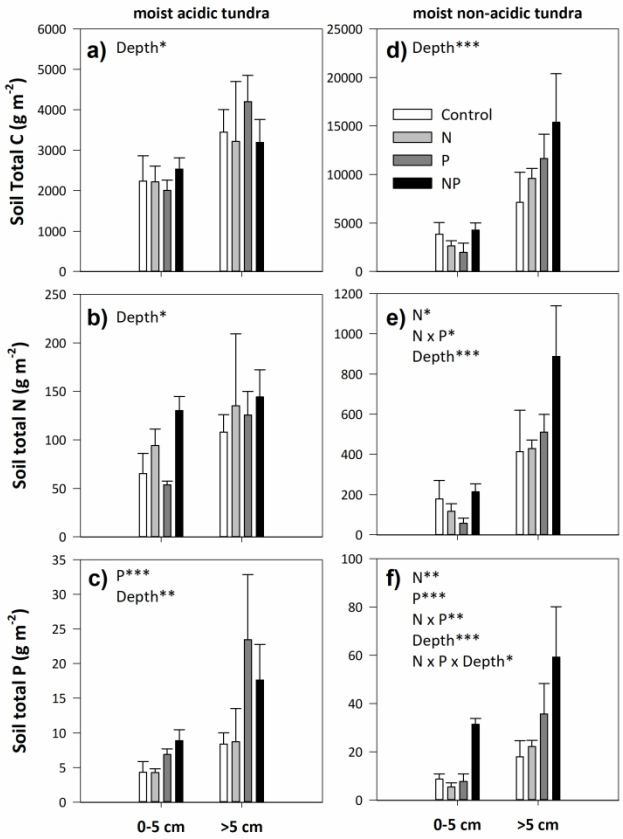

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