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Plant and microbial responses to nitrogen and phosphorus addition across an elevational gradient in subarctic tundra

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Abstract: Temperature and nutrients are major limiting factors in subarctic tundra. Experimental manipulation of nutrient availability along elevational gradients (and thus temperature) can improve our understanding of ecological responses to climate change. However, no study to date has explored impacts of nutrient addition along a tundra elevational gradient, or across contrasting vegetation types along any elevational gradient. We set up a full factorial nitrogen (N) and phosphorus (P) fertilization experiment in each of two vegetation types (heath and meadow) at 500 m, 800 m and 1000 m elevation in northern Swedish tundra. We predicted that plant and microbial communities in heath or at lower elevations would be more responsive to N addition while communities in meadow or at higher elevations would be more responsive to P addition, and that fertilizer effects would vary more with elevation for the heath than for the meadow. Although our results provided little support for these predictions, the relationship between nutrient limitation and elevation differed between vegetation types; most plant and microbial properties were responsive to N and/or P fertilization but responses often varied with elevation and/or vegetation type. For instance, vegetation density significantly increased with N + P fertilization relative to the other fertilizer treatments, and this increase was greatest at the lowest elevation for the heath but at the highest elevation for the meadow. Arbuscular mycorrhizae decreased with P fertilization at 500 m for the meadow, but with all fertilizer treatments at in both vegetation types at 800 m. Fungal to bacterial ratios were enhanced by N + P fertilization for the two highest elevations in the meadow only. Additionally, microbial responses to fertilization were primarily direct rather than indirect via plant responses, pointing to a decoupled response of plant and microbial communities to nutrient addition and elevation. Because our study shows how two community types differ in their responses to fertilization and elevation, and because the temperature range across this gradient is ~3°C, our study informs on how

nutrient limitation in tundra may be influenced by temperature shifts that are comparable to those expected under climate change during this century.

Keywords: fertilization experiment, global warming, plant-soil linkages, plant functional groups, fungal-to-bacterial ratios, above- and belowground communities.

INTRODUCTION

Temperature is a major limiting factor for high latitude communities and ecosystems such as subarctic tundra, and these systems are therefore predicted to be particularly responsive to global warming (IPCC 2007). Since organisms and communities regulate ecosystem processes, understanding how they will respond to long-term warming is essential for predicting the effect of global climate change on tundra ecosystem functioning (Wookey et al. 2009). Increasing elevation is associated with a decline in temperature, so elevational gradients represent powerful natural experiments which may inform about community responses to variation in temperature at the landscape-scale when other abiotic factors remain relatively constant (Körner 2007, Sundqvist et al. 2013). As such, several observations have shown that organisms and communities, both above- and belowground, are highly responsive to elevation (and associated changes in climatic factors) in many locations around the world (e.g. Whittaker 1956, Hodkinson 2005, Bahram et al. 2012).

Declining temperatures associated with increasing elevation can directly influence community properties through limiting metabolism and process rates for both plants (Hoch and Körner 2012) and soil microbes (Schinner 1982, Margesin et al. 2008). However, reduced

66 temperatures associated with increasing elevation can also exert indirect effects on organisms
67 and communities (e.g. Hodkinson 2005, Sundqvist et al. 2013). One important indirect effect
68 involves impairment of fluxes and biological availability of soil nutrients resulting from reduced
69 temperatures associated with increasing elevation (Vitousek et al. 1988, Vitousek et al. 1994,
70 Sveinbjörnsson et al. 1995). This can occur for instance through lower temperatures at high
71 elevations influencing species composition, or impairing microbial activity, nutrient inputs,
72 organic matter quality, decomposition and nutrient mineralization rates (Vitousek et al. 1988,
73 Vitousek et al. 1994, Sveinbjörnsson et al. 1995, Sundqvist et al. 2011a). We currently have a
74 limited understanding of the mechanistic basis through which this indirect effect of elevation (i.e.
75 via soil nutrient availability) may influence communities in ecosystems that are likely to be
76 heavily impacted by global warming such as tundra. Since temperature limits soil nutrient
77 mineralization rates, global warming is also expected to significantly enhance nutrient
78 availability in arctic tundra (ACIA 2005). An increased understanding of community responses
79 to the indirect effect of elevation on nutrient availability should therefore inform on responses of
80 tundra ecosystems to future climate change.

81 Tundra ecosystems are widely recognized as highly nutrient limited. This has been
82 confirmed through numerous experiments in both alpine and arctic tundra that have revealed
83 strong effects of fertilization on aboveground properties and processes such as plant biomass, net
84 primary productivity and plant community composition (Gough et al. 2000, Bret-Harte et al.
85 2008, Haugwitz and Michelsen 2011). With regard to belowground responses, fertilizer additions
86 in tundra have also been shown to reduce soil carbon (C) storage (Mack et al. 2004), and drive
87 shifts in the relative proportion of bacterial and fungal biomass in variable directions (Rinnan et
88 al. 2013, Wardle et al. 2013). Studies in other ecosystems have also revealed consistent shifts in

soil bacterial community structure in response to nitrogen (N) addition (Ramirez et al. 2010) and a greater responsiveness of soil microbes to phosphorus (P) addition under low soil P availability (Craine et al. 2007) or high N deposition levels (Liu et al. 2012). Nitrogen is often considered the main limiting nutrient in high-altitude and high-latitude ecosystems (Tamm 1991, Aerts and Chapin 2000), but a growing number of studies suggest that plant productivity for some tundra species and vegetation types is limited by P or co-limited by N and P (e.g. Seastedt and Vaccaro 2001, Weintraub 2011, Giesler et al. 2012, Zamin and Grogan 2012). Some studies have also provided evidence for a change in the relative importance of P versus N limitation with elevation (van de Weg et al. 2009). Specifically, results from the Swedish subarctic tundra suggest that P relative to N limitation increases with elevation-associated declines in temperature (Sundqvist et al. 2011b). However, no study has tested this experimentally through the use of fertilizer addition in tundra ecosystems. Indeed, to our knowledge the only study to have explicitly tested this across any elevational gradient is Fisher et al. (2012) who applied N and P fertilizers across a tropical forest gradient in the Peruvian Andes and showed that N limitation increased with elevation.

Here, we use a N and P fertilization experiment designed to alleviate N and/or P limitation across an established elevational gradient in Swedish subarctic tundra (Sundqvist et al. 2011a,b, 2012, Milbau et al. 2013), to explore how nutrient limitation impacts plant and soil microbial communities across ecosystems that experience contrasting temperature regimes. The Swedish subarctic tundra is a mosaic of different vegetation types, and two functionally contrasting vegetation types, heath and meadow, co-occur at all elevations along our gradient. Heath vegetation is dominated by evergreen and deciduous dwarf-shrubs, while the meadow vegetation is dominated by faster growing plants such as forbs, graminoids and sedges. The

heath vegetation has been shown to have lower soil pH and N availability, but higher P availability and soil fungal to bacterial ratios compared to meadow vegetation (Björk et al. 2007, Eskelinen et al. 2009, Sundqvist et al. 2011a, Giesler et al. 2012). Plant and microbial communities for these two vegetation types also display vastly different responses to elevation (Sundqvist et al. 2011a). Previous work in our study system has also shown that foliage and leaf litter N:P ratios increase with elevation for both vegetation types, indicating an overall increase in the relative importance of P versus N limitation with increasing elevation (Sundqvist et al. 2011b). Further, while soil fungal to bacterial ratios increase and available concentrations of N and P decline with increasing elevation for the heath, these properties show idiosyncratic responses to elevation for the meadow (Sundqvist et al. 2011a). Because responses of soil abiotic, plant, and microbial properties to elevation have been described for both the heath and the meadow (Sundqvist et al. 2011a,b), this system provides strong opportunities for studying the response of highly contrasting plant and microbial communities to the alleviation of N and P limitation across the same elevational gradient.

It is widely recognized that the plant and soil microbial subsystems are often interlinked (Wardle 2002, Bardgett and Wardle 2010). For example, plant species adapted to more fertile soils which produce high quality litter are generally associated with a more bacterial-based soil microbial community compared to plants growing on less fertile soils which often have a relatively higher dominance of fungi (e.g. Coleman et al. 1983, Eskelinen et al. 2009). However, some recent studies have pointed to a decoupled response of plant and microbial communities to fertilization (Wardle et al. 2013) while others have not (Suding et al.2008); we still know very little about the generality of this decoupled response. Additionally, to our knowledge, no study has explored the effect of fertilization for both plant and microbial communities at multiple

points across the same elevational gradient. We used this study system to test the following three hypotheses: 1) Plant and soil microbial communities at lower elevations will be more responsive to N addition and higher elevational communities will be more responsive to P addition. We base this on previous measures of plant tissue N:P ratios across the study system which suggest increasing P relative to N limitation with increasing elevation (Sundqvist et al. 2011b). 2) Phosphorus fertilization will have a greater effect on plant and microbial properties in the meadow and N fertilization will have a greater impact in the heath. This is based on previous measures of that higher concentrations of mineral N occur in the meadow soils and higher mineral P concentrations occur in the heath soils (Sundqvist et al. 2011a). 3) The influence of addition of N and P (either singly or in combination) on plant and microbial properties will be determined by interactive effects between elevation and vegetation type (heath vs. meadow) because fertilizer effects will change more with elevation for the heath than for the meadow. This third hypothesis is based on previous findings that concentrations of soil available nutrients decline unidirectionally with elevation for the heath but not for the meadow (Sundqvist et al. 2011a). We tested these hypotheses in the Swedish subarctic tundra which contain large gradients of elevation and a mosaic of contrasting vegetation types and which is therefore highly regulated by local-scale variation in temperature and nutrient availability. Using this study system we aim to advance our mechanistic understanding of how community properties and aboveground-belowground linkages in these ecosystems may respond to global change.

MATERIALS AND METHODS

Study site

158

159 This study was conducted on the north-east facing slope of Mt. Suorooaivi (1193 m a.s.l.)
160 situated 20 km south east from Abisko, northern Sweden (68°21'N,18°49'E), previously
161 described by Sundqvist et al. (2011a). The climate in this area is subarctic with a growing season
162 of approximately three months. Previous measurements of air temperature during the summer
163 months have shown that temperature declines by ~3°C from 400 m to 1000 m across the study
164 site (Sundqvist et al. 2011a,b, Appendix A). The mean annual precipitation measured in the
165 proximity of the study area (Abisko Scientific Research Station) was 310 mm for the period
166 1913 – 2000, with the highest mean monthly precipitation in July (51 mm) and the lowest in
167 April (12 mm) (Kohler et al. 2006). Summer precipitation at Mt Suorooaivi ranges between 230
168 and 290 mm and has been shown to vary little across elevations in the proximity of the study
169 area (Karlsson et al. 2005) as well as across two contrasting elevations in the study site itself
170 (Sundqvist 2011). At the study site, the bedrock consists of salic igneous rocks and quartic and
171 phyllitic hard schists. The forest line, formed by *Betula pubescens* ssp. *czerepanovii* (mountain
172 birch), is situated at an elevation of 500-600 m at the study site. Two co-dominant vegetation
173 types occur in a mosaic at all elevations; meadow (dominated by forbs, graminoids and sedges)
174 and heath (dominated by deciduous and evergreen dwarf-shrubs). For more details on the study
175 system see Sundqvist et al. (2011a,b, 2012).

176

177 *Experimental setup*

178

179 In July 2008, a total of 96 plots (each 1 × 1 m, with the outer 10 cm as a buffer) were established,
180 i.e., 16 plots in heath vegetation and 16 plots in meadow vegetation at each of three elevations

(500, 800 and 1000 m), in the proximity of plots used in previous studies in this system (Sundqvist et al. 2011a,b, 2012). The size of each plot is characteristic of the minimum size of patches of each vegetation type across the study site, and is of a sufficient size for assessing vegetation responses in both vegetation types (Sundqvist et al. 2011a). Within each elevation, the mean distance of each plot to the nearest plot for the same treatment is approximately 10 m (and the mean distance between the two most distant plots is approximately 100 m). Due to high spatial heterogeneity over very short distances (often on the order of a few meters) in microtopography and soil fertility characteristic of these communities (Björk et al. 2007), it is expected that the 10 m distance among plots is sufficient to ensure adequate independence among them (Sundqvist et al. 2011a, 2012). Plots at the 500 m elevation were situated in open birch forest immediately below the forest line, and plots at 800 m and 1000 m were devoid of trees. Gradients of this type are powerful for exploring the role of temperature and associated changes in climate in influencing ecosystem properties when other abiotic factors do not co-vary with elevation (Körner 2007, Sundqvist et al. 2013). In this study system, all plots have approximately the same aspect (north east facing slope), and parent material is independent of elevation, so that climate is the principal abiotic factor that varies with elevation (Sundqvist et al. 2011a).

At each elevation, in each vegetation type, four replicates of the 16 plots were each randomly assigned to one of four treatments, i.e., Control (unamended), N-addition, P-addition and N+P addition. To alleviate N and P limitation for each plot which received N and P addition, fertilizers were added annually from 2008 to 2010 in the amount of 10 g N m^{-2} yr^{-1} as NH_4NO_3, and 5 g P m^{-2} yr^{-1} as superphosphate ($Ca(H_2PO_4)_2 \cdot H_2O$) which is in line with rates used in previous studies on nutrient limitation in arctic tundra (e.g., Jonasson 1992, Chapin et al. 1995,

Mack et al. 2004, Rinnan et al. 2007) as well as other ecosystems (Vitousek 2004). Fertilizers were added for the first time in July 26, 2008. For the two following years half of the total annual amount was added when all plots were snow free (June 25, 2009 and June 8, 2010), and half was added three weeks later (i.e. July 16, 2009 and June 29, 2010).

Vegetation survey

Tundra vegetation is well known to be responsive to nutrient additions within a two-year period (e.g. Chapin and Shaver 1985, Jonasson 1992). In order to assess the effect of fertilizer addition after two years on vegetation properties across our study site, total cover of each species was measured by point quadrat analysis (Goodall 1952), in each plot, over 8-20 July, 2010. This was performed by recording the total number of times the vegetation of each species was intercepted from a total of 100 downward projecting points in each plot (Wardle et al. 2003) using the inner 80 × 80 cm of each plot, with the outer 10 cm serving as a buffer zone. This cover data was also used for calculating Shannon's diversity index (hereafter 'Shannon's diversity') and total vegetation density (Sundqvist et al. 2011a), as well as the cover of each plant functional group, i.e., forbs, graminoids, sedges, deciduous and evergreen dwarf-shrubs, pteridophytes. Further, total species richness was also recorded by noting all plant species present within this 80 × 80 cm area, including those not intercepted by any points.

Soil sampling and analysis

Fresh soil was sampled from each plot after two years, over August 23-27, 2010. In each plot, at least 5 samples were taken to ensure a minimum of 0.2 L of humus. The whole humus layer was sampled using a 45-mm diameter soil core, and the depth of the humus layer for each core was recorded. All cores collected in each plot were bulked into one composite sample and brought to the laboratory on the same day as sampling, where they were kept cold (+2°C) for a maximum of 24 h before further analysis. For each bulked sample from each plot, the humus soil was homogenized using a 4-mm sieve. Soil pH was measured in a soil:water suspension (50 mL de-ionized water and 6 g fresh weight soil shaken overnight and sedimentation for 1 h). Gravimetric soil moisture was determined after drying (105 °C, 24 h) and soil organic matter (OM) content was determined by loss on ignition (550 °C, 4 h). To measure soil available concentrations of N (NH_4-N and NO_3-N) and P (PO_4-P) for each plot, a subsample of 5 g fresh weight soil was extracted in 80 mL 1 M KCl and concentrations were determined by colorimetry on an AutoAnalyser III (SEAL Analytical, Kontram OmniProcess AB, Sweden). All mineral nutrient concentrations are expressed as mg g^{-1} OM. A subsample of dried (70 °C, 3 days) and ground (ball mill; Retsch MM 301) soil from each plot was analyzed for total % carbon (C), N (Leco TruSpec CN Furnace) and P (nitric acid:perchloric acid digestion followed by inductively coupled plasma analysis) (Thompson and Wood 1982).

Soil microbial community composition was characterized for a subsample of each bulked soil sample by using phospholipid fatty acid (PLFA) analysis as described by Frostegård et al. (1991) with minor modifications. Briefly, 0.5 g fresh soil samples were extracted in chloroform-methanol-phosphate buffer (1:2:0.8 v/v/v), and the extracted lipids were fractionated into neutral lipids, glycolipids and polar lipids on silic acid columns by successive eluation with chloroform, acetone and methanol. The methanol fraction (containing phospholipids) was subjected to mild

alkaline methanolysis to transform the fatty acids into free methyl esters and analyzed on a gas chromatograph (GC), equipped with a flame ionization detector. The PLFAs i15:0, a15:0, i16:0, 16:1ω9, 16:1ω7t, 16:1ω7c, i17:0, a17:0, cy17:0, 18:1ω7 and cy19:0 were used to indicate relative bacterial biomass, the PLFA 18:2ω6 was used as an indicator of relative fungal biomass (Frostegård and Bååth 1996) and the PLFA 16:1ω5 was used as an indicator of relative arbuscular mycorrhizal (AM) biomass (Olsson 1999). The PLFAs 10Me17:0 and 10Me18:0 were used to indicate relative actinomycete biomass (Lechevalier 1977, Kroppenstedt 1985). The PLFAs chosen to represent fungi and the sum of the PLFA markers chosen to represent bacteria were used to calculate the fungal to bacterial ratio (Frostegård and Bååth 1996) for each plot. All PLFAs were calculated as nmol g^{-1} OM.

Statistical analysis

The effects of vegetation type, elevation, N addition, P addition, and all possible interactions among these factors, on plant, soil microbial and soil abiotic variables were analyzed using four-way ANOVA. Whenever ANOVA yielded significant effects we used Duncan's test at $P = 0.05$ to assess differences among means. To further evaluate the response of the plant and microbial communities to these factors as well as their relationships with each other and with soil abiotic properties, multivariate approaches were used. First, detrended correspondence analysis (DCA) was applied to the plant species data set, which revealed a gradient length of the first axis > 5 SD, indicating a unimodal response (ter Braak and Šmilauer 2002). Thus, canonical correspondence analysis (CCA) was applied to the plant species data set using soil properties as explanatory environmental variables. Each explanatory variable that explained an additional amount of

variation at $P < 0.05$ was retained by manual forward selection using Monte Carlo permutations (999 unrestricted permutations) (ter Braak and Šmilauer 2002). The sample scores for the first and the second axis for all plots derived from the CCA were further analyzed using four-way ANOVAs as described above to test the effects of vegetation type, elevation, N addition and P addition on plant community composition. The microbial data set (PLFAs) was firstly subjected to DCA which revealed a gradient length of the first axis < 1 SD, indicating a linear response (ter Braak and Šmilauer 2002). As such, the microbial data set was further subjected to a partial redundancy analysis (RDA) using soil abiotic properties and plant functional group cover as explanatory environmental variables, with species standardized by error variance (Borcard et al. 1992, ter Braak and Šmilauer 2002). The most discriminating combination of environmental variables was selected by the 'forward selection' Monte-Carlo procedure, exactly as described above. The sample scores derived from the RDA were further analyzed by four-way ANOVAs as described above to test the effects of vegetation type, elevation and fertilization (N, P addition) on soil microbial community composition. All univariate analyses were performed in SPSS Statistics 20.0 and all multivariate analyses were performed using CANOCO for Windows 4.5.

RESULTS

Plant community properties

Both species richness and Shannon's diversity were highest for the meadow where they peaked at 800 m, while for the heath they were lowest at 800 m (Figures 1A-D, Appendix B). Fertilizer treatments by themselves had no overall effect on either variable, but there was a significant N ×

vegetation type × elevation interaction for Shannon's diversity because at 500 m for the heath and 800 m for the meadow, Shannon's diversity was significantly reduced by N+P fertilization relative to the treatments without N addition (Figure 1A-D, Appendix B). Vegetation density did not differ between vegetation types but did differ among elevations; overall, density was highest at the 500 m elevation for the heath, and at the 500 and 1000 m for the meadow (Figure 1E,F, Appendix B). Vegetation density was significantly increased by N + P fertilization relative to the other treatments, and this increase was greatest at the lowest elevation for the heath and at the highest elevation for the meadow (Figure 1E-F; N × P × vegetation type × elevation interaction: $F = 5.2$, $P = 0.008$).

Deciduous and evergreen shrub cover was highest on heath while cover for all other plant functional groups was highest on the meadow (Figure 2, Appendix B). There was also an interactive effect of elevation × vegetation type on all plant functional groups (Appendix B). For the heath, pteridophytes, deciduous shrubs, graminoids and forbs declined, while evergreen shrubs and sedges increased with increasing elevation. Conversely, for the meadow, deciduous and evergreen shrubs, graminoids and sedges increased while pteridophytes and forbs declined with increasing elevation (Figure 2, Appendix B). All plant functional groups were responsive to N and/or P fertilization (except for pteridophytes) and responses often differed significantly amongst fertilizer treatments, vegetation types and elevations (Figure 2, Appendix B,C). For the heath at the 500 m elevation only, deciduous and evergreen shrubs were significantly reduced by N+P addition, and evergreen shrubs were also reduced by N added alone. Meanwhile, graminoid cover in the heath was increased by N and N+P fertilization at 500 m and by N +P fertilization at 1000 m (Figure 2, Appendix B,C). For the meadow, deciduous shrubs were least in the N+P fertilization treatment at 800 m, and in the N and N+P fertilization treatments at 1000 m, while

evergreen shrubs were least in the N and N+P fertilization at 800 m. Further, in the meadow, graminoids were greatest in the N+P fertilization treatment at all elevations, while sedges were least in the N+P fertilization treatment at 800 m and 1000 m (Figure 2, Appendix B,C). Overall, forbs were reduced by N fertilization and this reduction was greatest for the meadow (significant N × vegetation type interaction; Figure 2, Appendix B).

Soil microbial community composition

Overall, actinomycete, AM, and bacterial biomasses were highest for the meadow, while fungal biomass and the fungal to bacterial ratio were highest for the heath (Figure 3, Appendix D). For both vegetation types, fungal biomass was highest at 800 m, the fungal to bacterial ratio was least at 500 m and the bacterial biomass was unresponsive to elevation (Figure 3). While there was a significant effect of elevation on AM biomass according to ANOVA (Appendix D), post-hoc analyses revealed no significant differences amongst elevations (data not shown). There was a significant overall effect of N and/or P fertilization on most microbial properties, and the effect of fertilization was often dependent on elevation and/or vegetation type (Figure 3, Appendix D). Overall, N fertilization slightly increased fungal biomass and P addition decreased bacterial biomass but this was not statistically significant for either variable. There was also an interactive effect of P fertilization and vegetation type on fungal biomass (Figure 3, Appendix D). Specifically for the heath, at the 800 m elevation bacterial biomass was reduced by all fertilizer additions (Figure 3), and at 1000 m, actinomycete biomass was increased by N fertilization (data not shown, Appendix D). Meanwhile for the meadow, at 800 and 1000 m the fungal to bacterial ratio was increased by N+P fertilization while the bacterial and fungal biomass were both

unresponsive to fertilization within each elevation (Figure 3). Further for the meadow, AM biomass was reduced by P fertilization at 500 m, and for both vegetation types AM biomass was reduced by all fertilizer treatments at the 800 m elevation (E × P interaction: $F = 3.2$, $P = 0.048$, N × P interaction: $F = 5.9$, $P = 0.017$; data not shown).

Soil abiotic properties

Overall, total soil C (%), N to P ratios and PO_4-P concentrations were highest for the heath, and pH, P and NH_4-N concentrations were highest for the meadow (Figure 4, Appendix E,F). For the heath, N was highest at 500 and 800 m, NH_4-N concentrations were highest at 800 m and lowest at 500 m and PO_4-P concentrations declined with increasing elevation, and no other abiotic soil variables revealed any simple relationships with elevation for either vegetation type (Figure 4, Appendix E,F). Phosphorus fertilization generally increased soil P and PO_4-P concentrations and reduced the N to P ratio (Figure 4, Appendix E,F). There was also a significant interactive effect of P addition and elevation on PO_4-P concentrations because the increase in PO_4-P was greatest at the lowest elevation (Figure 4, Appendix E). Meanwhile, N fertilization added alone significantly increased NH_4-N concentrations especially at mid-elevations (significant N × elevation interaction), but this increase was mostly not significant when P was added together with N (significant N × P interaction, Figure 4, Appendix E). There was a significant interactive effect of P addition and vegetation type on PO_4-P because responses of PO_4-P to fertilization were overall greater for the heath than for the meadow (Figure 4, Appendix E). Further, relative to the control, pH was reduced by N+P fertilization at 500 m and increased by N fertilization at 800 m for the heath, while it was reduced by N+P fertilization relative to the control and N

fertilization plots at 800 m for the meadow (significant N × P × elevation interaction; Appendix E, Appendix F). Concentrations of NO_3-N were only detected in 56 % of the samples (data not shown). For the heath, the lowest detectable NO_3-N concentrations were found in control plots at 1000 m (0.07 ± SE 0.001 μg g^{-1} OM, n = 2) and the highest in N+P addition plots at 800 m (0.6 ± SE 0.1 μg g^{-1} OM, n = 3). For the meadow, the lowest detectable NO_3-N concentrations were found in control plots at 1000 m (0.8 ± SE 0.5 μg g^{-1} OM, n = 3) and the highest in N addition plots at 1000 m (2.4 ± SE 0.8 μg g^{-1} OM, n = 4). No NO_3-N was detected in any P addition plots at 500 m for either vegetation type, in any control plots in meadow at 500 m, or in any P addition plots in meadow at 800 m.

Linking vegetation and soil microbial data to soil properties

The CCA for the plant community data revealed that axis 1 was clearly separated by vegetation type, with pH and bacterial and AM biomass associated with meadow plots and the fungal to bacterial ratio, soil C and moisture content associated with heath plots (Figure 5A-B). Plots at different elevations were separated along axis 2 by soil N (associated with the 500 m elevation for both vegetation types), P (associated with the meadow at 500 m elevation), C and PO_4-P (associated with the heath at 500 m elevation), and NH_4-N (associated with the meadow at 800 and 1000 m) (Figure 5A). The CCA also showed that certain plant species were more associated with specific elevations and this effect was stronger for the meadow than for the heath (Figure 5B); specifically for the meadow plots, species composition below the tree line at 500 m differed greatly from that of the other two elevations. The ANOVAs revealed that there were no main effects of N and P addition on axis 1 and 2 scores derived from CCA (Appendix B). However,

there was a significant interactive effect of P addition and vegetation type on axis 1 scores (F = 4.1, P = 0.046) because P fertilization increased these scores for the heath but reduced them for the meadow (although this was not significant within vegetation types). According to the post hoc tests, the 500 m elevation differed significantly from the 800 and 1000 m elevations along axis 1 and 2 for the meadow, and all elevations differed significantly from each other along both axes for the heath (Figure 5A).

Partial RDA for the soil microbial data showed that axis 1 was mainly separated by forbs and pH (which were greater in meadow plots), and by C (which was greater in heath plots) (Figure 6A-B). Meanwhile, plots at different elevations were separated along axis 2 by soil N (greatest in 500 m plots), sedges (greatest in 1000 m meadow plots) and soil moisture (greatest in 1000 m heath plots). Some PLFA markers were also associated with particular vegetation types and elevations (Figure 6B). For instance, bacterial markers were mainly associated with the meadow, the fungal marker (18:2ω6) was associated with higher elevations for the heath, and the AM and actinomycete markers with high elevations for the meadow. The ANOVAs revealed that axis 1 scores from the RDA were significantly affected by P and vegetation type, as well as interactive effects of N × elevation, and N × vegetation type × elevation (Appendix D). As such, axis 1 scores differed significantly between heath and meadow and increased overall with P fertilization. Duncan's post hoc test (P = 0.05) also revealed that axis 1 scores significantly increased with fertilization at the 800 m elevation for the heath only. Meanwhile, axis 2 scores were significantly influenced by the interactive effect of N × vegetation type (F = 7.9, P = 0.010) because N fertilization tended to decrease axis 2 scores for the meadow but not for the heath. Axis 2 scores were also responsive to the effect of elevation (Appendix D) and the vegetation type × elevation interaction (F = 15.4, P < 0.001). Duncan's post hoc test at P = 0.05 revealed

that this was because all elevations differed significantly from each other for the heath, and the 500 m differed significantly from the 800 and 1000 m for the meadow.

DISCUSSION

Responses to fertilization across contrasting elevations and vegetation types

Nutrient limitation often increases with increasing elevation and associated declines in temperature (Sveinbjörnsson 1995, Kitayama et al. 1998, Johnson et al. 2000), but some recent observational studies suggest that the relative importance of different limiting nutrients changes with elevation (van de Weg et al. 2009, Fisher et al. 2012). In addition, plant and soil microbial community composition can be closely associated (Bardgett and Wardle 2010, Eisenhauer et al. 2010), and vegetation responses to elevation can thus influence microbial responses (Löffler et al. 2008, Sundqvist et al. 2011a). Previous work in our study system has shown increasing limitation of plants by P relative to N with increasing elevation (Sundqvist et al. 2011b), and we therefore predicted that community properties at low and high elevations would be more responsive to N and P addition respectively. However, we did not find this for either plant or microbial communities, leaving our first hypothesis unsupported. While no comparable fertilization study has been performed across contrasting elevational gradients in tundra, our findings are also inconsistent with a recent fertilization study in Peru which revealed declining foliar N:P ratios and increasing responsiveness of plant growth to N addition as elevation increased from lowland tropical forest to montane cloud forest (Fisher et al. 2012). However, we found that different plant functional groups showed contrasting responses to fertilization, and to

433 the interactive effects of fertilization with elevation and vegetation type. These varying responses
434 of different floristic components are likely to have contributed to preventing community-level
435 properties, such as total cover, species richness and Shannon's diversity, from showing simplistic
436 responses to fertilization across the gradient. These findings are at least partly consistent with
437 experimental manipulation studies in tundra systems spanning between five and 21 years that
438 have shown complex interactions of fertilization and plant removal among coexisting plant
439 functional groups (Suding et al. 2008, Bret-Harte et al. 2008, Wardle et al. 2013). Similarly,
440 fertilization influenced the soil microbial community but not in any consistent way across the
441 gradient, which may be explained by different components of the microflora showing contrasting
442 responses to fertilization and elevation.

443 We further predicted a greater effect of P fertilization on plant and microbial properties in
444 meadow vegetation than in heath and a greater effect of N fertilization in heath compared to
445 meadow. Although soil nutrient concentrations in the unfertilized plots suggested higher soil N
446 and lower soil P availability in the meadow than the heath (Figure 4, Sundqvist et al. 2011a,
447 Giesler et al. 2012), we found little support for our second hypothesis. Instead, most plant and
448 microbial variables that we measured were only weakly responsive, or unresponsive, to the
449 interactive effect of either N or P addition with vegetation type, meaning that heath and meadow
450 did not differ much in their responsiveness to either fertilizer. However, vegetation density
451 consistently showed a strong positive response to simultaneous N and P addition for both heath
452 and meadow. This result after two years of fertilization is consistent with other experiments that
453 have been conducted over both the same (Madan et al. 2007) and longer (4-9 years) time-span
454 (Shaver et al. 1998, Gough and Hobbie 2003, Zamin and Grogan 2012) in showing tundra

vegetation to be most responsive to the combined effect of N and P. This means that N and P can often co-limit tundra plants across contrasting vegetation types (Onipchenko et al. 2012).

Overall, N and P addition also had an overall positive effect on the fungal to bacterial ratios for both vegetation types, although this was statistically significant for only two elevations in the meadow. While the method used here (PLFA analysis) may not detect all potential changes in the microbial community, our results point to N and P fertilization favoring soil fungi over bacteria in at least some of our plots. In these cases, they therefore suggest that alleviation of N and P addition could be associated with a change in the competitive strength among major functional groups of soil microbes (Fierer et al. 2007). Since fungal based food-webs can be associated with a more conservative nutrient cycling in comparison to bacterial based food webs, this observed change could potentially also influence nutrient turnover rates (Wardle et al. 2004), although this requires further study. While our results may also be indicative of at least some plant and microbial properties being co-limited by N and P in our study system, they provide little evidence that the extent of this co-limitation differs strongly among vegetation types.

Interactive effects of fertilization, elevation and vegetation type

In previous work on this system, concentrations of available soil nutrients declined with elevation for the heath but showed idiosyncratic responses for the meadow (Sundqvist et al. 2011a); we therefore predicted that plant and microbial variables would be most responsive to fertilization at higher elevations for the heath. A three way interactive effect of fertilization, elevation and vegetation type is needed for this hypothesis to hold. However, with regard to the plant community, we only found such an interactive effect for some variables and never in a

direction consistent with our third hypothesis. Instead, fertilization sometimes had greater effects on plant properties at the higher (and colder) elevations for the meadow but at lower (and warmer) elevations for the heath (Figure 1, 2); this was especially apparent for the positive fertilization effects on vegetation density and graminoids, and negative effects on dwarf-shrub cover. These results emphasize that the role of temperature in controlling tundra plant community responses to improved nutrient availability varies among vegetation types. As such, they suggest that with increasing temperature, N and P becomes less limiting factors for plants dominating more fertile tundra soils, but more limiting for plants dominating poorer soils. Our results for the meadow are consistent with several studies in other ecosystems suggesting that increasing elevation (and thus declining temperature) is associated with reduced soil fertility and thus increased nutrient limitation (Vitousek et al. 1988, 1994, Sveinbjörnsson et al. 1995, Johnson et al. 2000). In contrast, for the heath, our results indicate that low temperatures constrain plant responses to improved nutrient availability at high elevations, consistent with work showing high elevation tundra communities dominated by slow growing dwarf-shrubs to be relatively resistant to fertilization (Haugwitz and Michelsen 2011). This highlights how opposing patterns of plant nutrient limitation can occur even across the same elevational gradient when different vegetation types are considered.

We also often found differences among heath and meadow vegetation in terms of how fertilizer effects on soil microbial properties changed with elevation, but again not in the direction predicted by our third hypothesis. However, most microbial variables were responsive to the interactive effects of fertilization with elevation and/or vegetation type, which highlights the importance of environmental context in determining when and how microbial communities respond to increased supply of N and P in the tundra landscape. Our data also showed that N+P

addition caused an increase in the ratio of fungal to bacterial biomass at the mid- and high elevation for the meadow. This is inconsistent with predictions that greater nutrient availability supports a more bacterial-based energy channel (Coleman et al. 1983, Bardgett and Wardle 2010), and with the results of some previous fertilizer experiments in tundra (Schmidt et al. 2000, Wardle et al. 2013). However, fungal to bacterial ratios were also least at the lowest (and thus warmest) elevation for both vegetation types (Figure 3). Therefore, our results from two years of fertilization corroborate findings from a long-term (10 yr) climate change experiment in subarctic tundra heath showing a positive effect of fertilization (NPK), but not warming, on fungal to bacterial ratios (Rinnan et al. 2007). As such, the effect of alleviation of nutrient limitation by fertilization on soil microbial properties may not necessarily mirror the effect of long-term changes in temperature on microbes through influencing soil nutrient availability (Rinnan et al 2007). Our results also at least partly contrast with those from an 18 year warming experiment in the Alaskan arctic suggesting that increasing dwarf shrub (*Betula nana*) growth in response to warming has an important role in enhancing soil fungal biomass (Deslippe et al. 2012). Taken together, our findings instead suggests that future warmer temperatures may be associated with an increased role of the bacterial-based energy channel in tundra soils regardless of nutrient availability or vegetation type.

Decoupled responses of plants and microbes to fertilization

Changes in both plant and soil abiotic properties along elevational gradients are known to influence soil microbial community responses to elevation (Wagai et al. 2011, Sundqvist et al. 2011a, Bahram et al. 2012) and our data is consistent with this (Figure 6). Further, while addition

of N and P to our plots always raised soil available levels of N and P at specific elevations, they also influenced other properties that could potentially impact on soil microbial communities, such as plant community composition (Grayston et al. 1998, Orwin et al. 2010) and soil pH (Lauber et al. 2009, Rousk et al. 2010). However, we found little evidence that plant community changes mediated the response of the microbial community composition to fertilization across contrasting elevations in the manner recently shown for the response of aboveground consumers (de Sassi et al. 2012). This was because those plant and soil abiotic properties that were most strongly altered by fertilization did not have any great impact on microbial community composition (Figure 6). As such, within elevations microbial community composition changed significantly in response to fertilization only at 800 m for the heath (as revealed by Duncan's post hoc test at $P = 0.05$; see results text), but this did not occur in association with any plant or abiotic soil variable that was responsive to fertilization (Figure 6A). Generally, our results are broadly in agreement with experimental evidence from studies spanning five to 11 years that have shown that the dominant effects of fertilization on soil microbial communities operate directly (Marshall et al. 2011, Wardle et al. 2013) rather than indirectly via shifts in vegetation composition and thereby plant-soil feedbacks (Suding et al. 2008). They further highlight how these direct effects can differ greatly among communities, and depend on factors such as type of nutrient addition (i.e. N and/or P), elevation and vegetation type.

Conclusions

Our results show that plant and microbial properties in tundra can be responsive to elevation and fertilization as well as their interaction, but that these responses can vary among vegetation types,

and with plant and soil microbial communities sometimes responding in markedly different ways. These results have several implications. First, they show that the response to nutrient addition and increasing elevation for both plant and microbial communities is not constant among differing vegetation types. Instead, we found that significant plant responses to N and P added in combination (i.e. Shannon's diversity and vegetation density) was greatest at lower elevations for heath but at higher elevations for meadow vegetation, and that while microbial responses to these nutrients were weaker these also differed among vegetation types and elevations. Second, they suggest that plant community responses to nutrient addition may not necessarily be an important determinant of microbial community responses irrespective of elevation or vegetation type, indicative of a decoupled response of plant and microbial communities to changes in temperature and soil fertility. These results further suggest that the relative importance of temperature versus nutrient limitation differs for tundra plant and microbial communities across tundra landscapes. However, we stress that further work is needed to understand the mechanistic basis for these decoupled responses. Since our results are also broadly in line with more long-term manipulations of plant functional groups and nutrients in tundra (Wardle et al. 2013) plant community responses to climate change may be poor predictors of microbial community responses in these ecosystems. Finally, they highlight how manipulating soil fertility for contrasting vegetation types across an elevational gradient may help us better understand the mechanistic basis through which changes in temperature influence plant and soil communities, and the linkages between them, at the landscape-scale (Sundqvist et al. 2013). Since elevational gradients allow us to determine how communities and ecosystems respond to changes in temperature that are of similar magnitude to those expected to occur through global climate change (Fukami and Wardle 2005, Malhi et al. 2010, Sundqvist et al. 2013), this type of

information is essential for predicting tundra responses to climate change across vegetation types and levels of soil fertility (Wookey et al. 2009).

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ECOLOGICAL ARCHIVES MATERIAL

Appendix A. Daily summer mean temperature across the study site.

Appendix B. ANOVA results testing for effects of N and P fertilization, vegetation type, elevation, and their interactions on vegetation properties.

Appendix C. Plant functional group cover in control and fertilizer plots across the study site.

Appendix D. ANOVA results testing for effects of N and P fertilization, vegetation type, elevation, and their interactions on soil microbial properties.

Appendix E. ANOVA results testing for the effects of N and P fertilization, vegetation type, elevation, and their interactions on soil abiotic properties.

Appendix F. Soil abiotic properties (means ± standard errors) in control and fertilizer plots across the study site.

FIGURE CAPTIONS

Figure 1. Shannon's diversity index (A, B), species richness (C, D) and vegetation density (E, F) in plots without fertilizer addition (Control), or amended with N, P, or N+P, for subarctic heath and meadow vegetation across an elevational gradient. Error bars = standard errors (N = 4). Within each panel, groups of 4 bars topped by the same capital letters are not significantly different at $P < 0.05$ (Duncan's test). Within each group of 4 bars, bars topped by the same lower case letters are not significantly different at $P < 0.05$; letters are not presented when none of the 4 bars differ significantly from any of the others (Duncan's test). ANOVA results are given in Appendix B.

Figure 2. The mean (±SE) cover (%) of plant functional groups in plots without fertilizer addition (Control) or amended with N, P or N+P for subarctic heath (A) and meadow (B) along an elevational gradient. ANOVA results given in Appendix B, and actual values with SEs are given in Appendix C.

Figure 3. Fungal biomass (A, B), bacterial biomass (C, D) and fungal:bacterial ratio (E, F) in plots without fertilizer addition (Control) or amended with N, P or N+P for subarctic heath and meadow vegetation across an elevational gradient. Error bars = SE (N = 4). Within each panel, groups of 4 bars topped by the same capital letters are not significantly different at $P < 0.05$ (Duncan's test). Within each group of 4 bars, bars topped by the same lower case letters are not significantly different at $P < 0.05$; letters are not presented when none of the 4 bars differ significantly from any of the others (Duncan's test). ANOVA results are given in Appendix D.

832

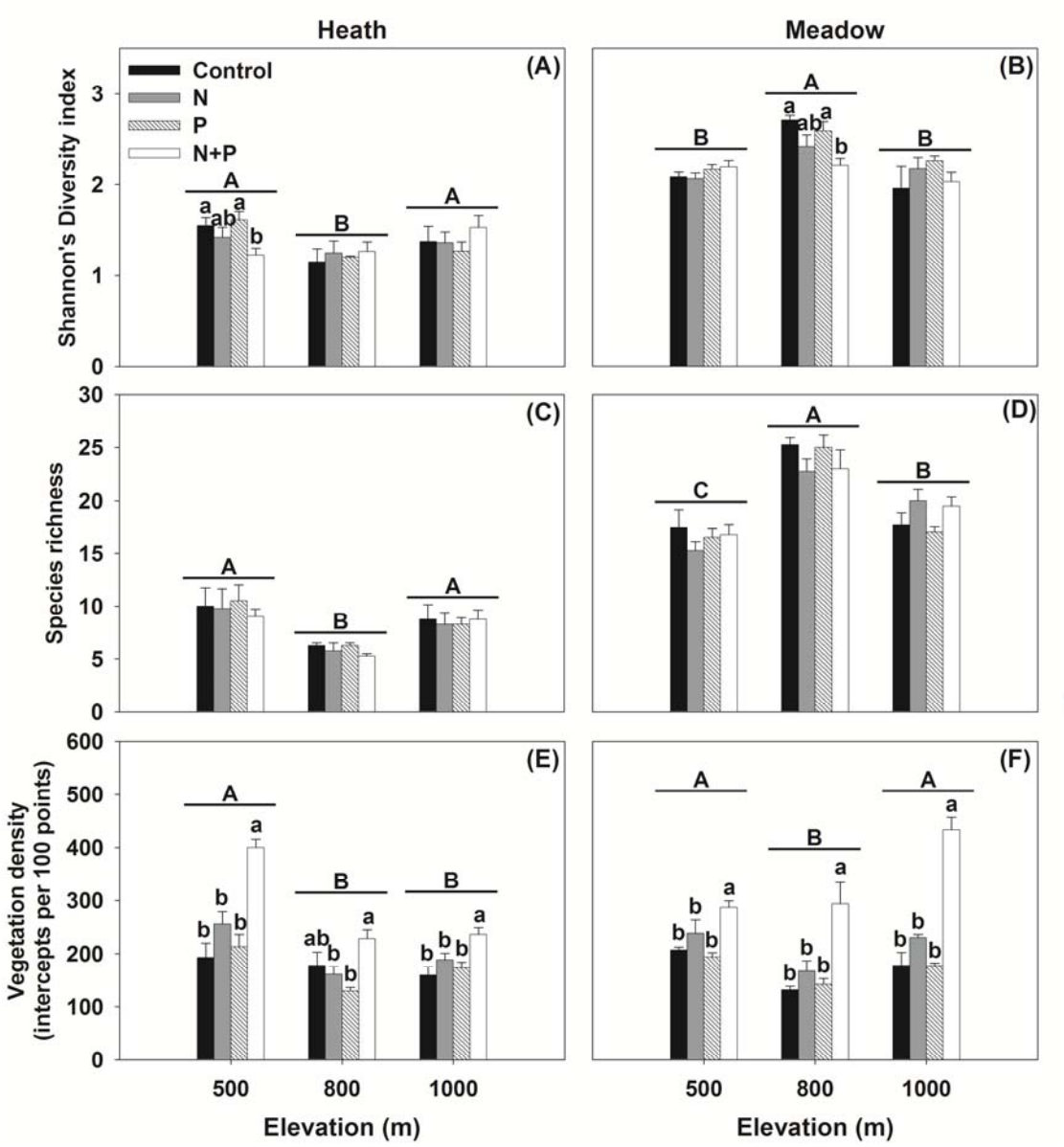
833 Figure 4. Concentrations of soil NH_4-N (A, B) and PO_4-P (C, D) in plots without fertilizer
834 additions (Control) or amended with N, P or N+P in subarctic heath and meadow vegetation
835 along an elevational gradient. Error bars = standard errors (N = 4). Within each panel, groups of
836 4 bars topped by the same capital letters are not significantly different at $P < 0.05$ (Duncan's test).
837 Within each group of 4 bars, bars topped by the same lower case letters are not significantly
838 different at $P < 0.05$; letters are not presented when none of the 4 bars differ significantly from
839 any of the others (Duncan's test). ANOVA results are given in Appendix D.

840

841 Figure 5. Canonical correspondence analysis of plant community composition constrained by
842 soil properties. (A) Biplot displaying means of the axis scores for each fertilizer treatment at each
843 elevation within each vegetation type, and environmental (soil abiotic and biotic) variables.
844 Square symbols are meadow plots and circles are heath plots. Whenever Duncan's post hoc test
845 at $P = 0.05$ revealed significant differences among elevations, and among plots within elevations
846 and vegetation types these are presented in the results text. (B) Biplot of species and
847 environmental (soil abiotic and biotic) variables. Species with ≥ 20% cover in one or more plots
848 are shown: *A. odo = Anthoxanthum odoratum, B. nan = Betula nana, C. alp = Cicerbita alpina,*
849 *C. big = Carex bigelowii, C. lap = Calamagrostis lapponica, C. tet = Cassiope tetragona, D. fle*
850 *= Deschampsia flexuosa, E. her = Empetrum hermaphroditum, F. ovi = Festuca ovina, F. viv =*
851 *Festuca vivipara, G. dry = Gymnocarpium dryopteris, G. syl = Geranium sylvaticum, M. eff =*
852 *Milium effusum, P. cae = Phyllodoce caerulea, R. ace = Rumex acetosa, V. bif = Viola biflora,*
853 *V. myr = Vaccinium myrtillus, V. uli = Vaccinium uliginosum, V. vit = Vaccinium vitis-idaea.* C
854 = total soil carbon, N = total soil nitrogen, P = total soil phosphorous, Moisture = Soil moisture

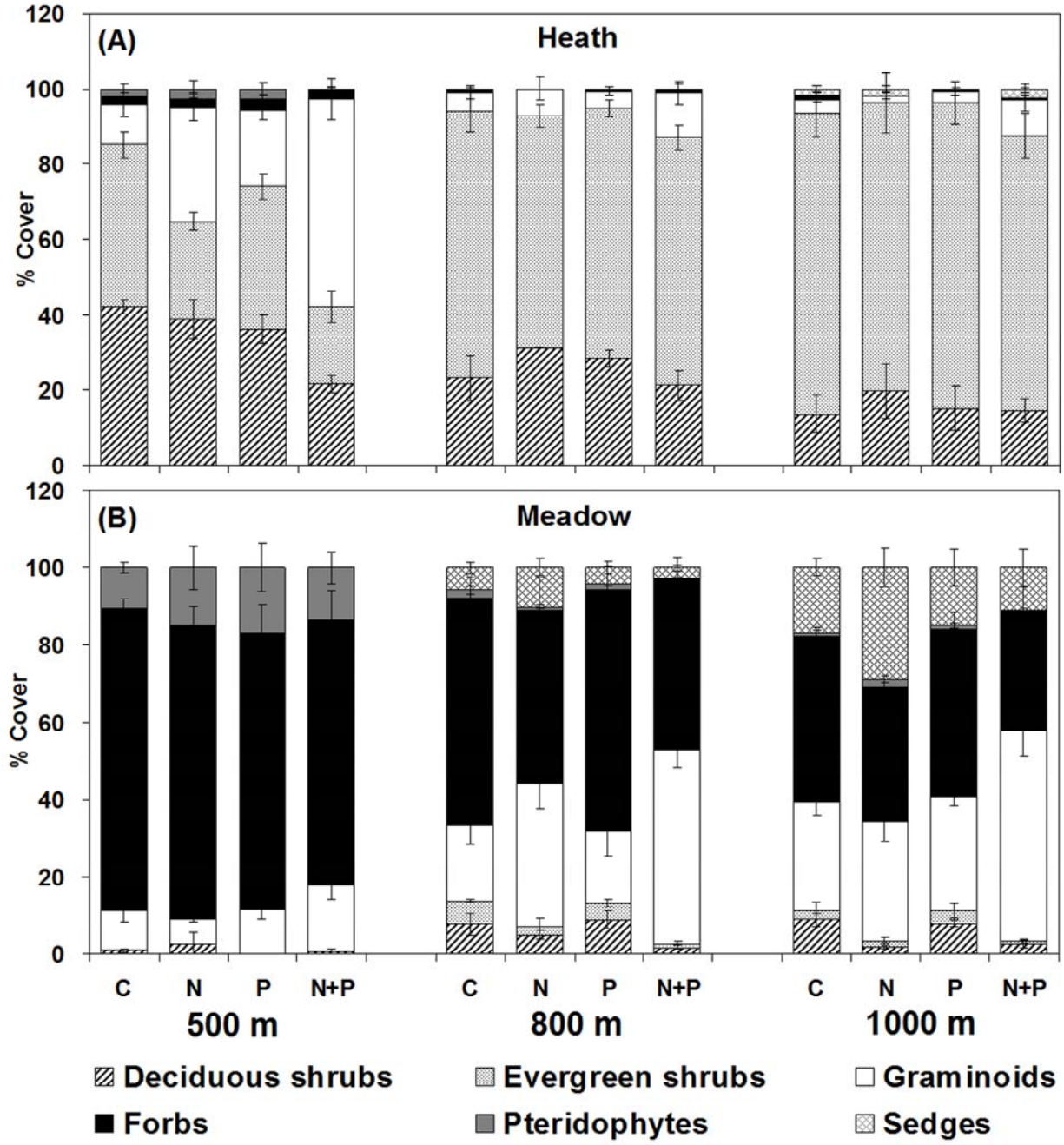
content, AM = Arbuscular mycorrhizae, F:B ratio = Fungal-to-Bacterial ratio. The biplot displays 37.1% of the variance explained by soil properties. The sum of all canonical eigenvalues is 2.122.

Figure 6. Partial RDA of soil microbial community composition constrained by plant species and abiotic soil properties. (A) Biplot displaying means of the axis scores for each fertilizer treatment at each elevation within each vegetation type, and environmental (i.e., plant and abiotic soil) variables. Square symbols are meadow plots and circles are heath plots. Whenever Duncan's post hoc test at $P = 0.05$ revealed significant differences among elevations, and among plots within elevations and vegetation types these are presented in the results text. (B) Biplot of microbial phospholipid fatty acids and environmental (i.e., plant and abiotic soil) variables. The sum of all canonical eigenvalues is 0.571 and the biplot displays 57.1% of the variance explained by plant and soil properties. The relative amount of variance explained by plant and soil properties is 2.2% and 7.9%, respectively with a shared variance of 47.0%. For the PLFAs, triangles = bacteria, the square = fungi, the star = arbuscular mycorrhizal fungi and circles = actinomycetes.



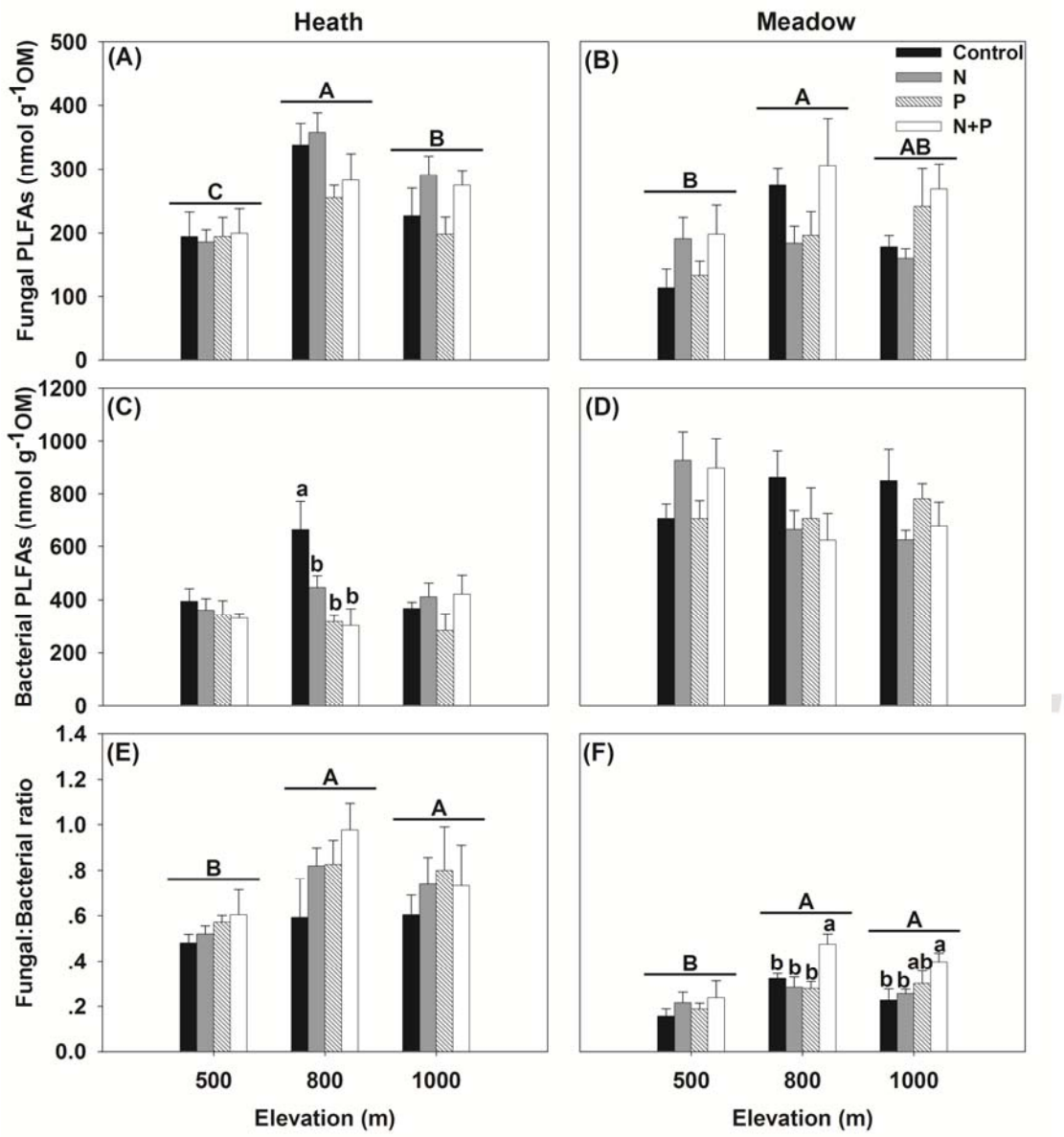
872

873 Figure 1



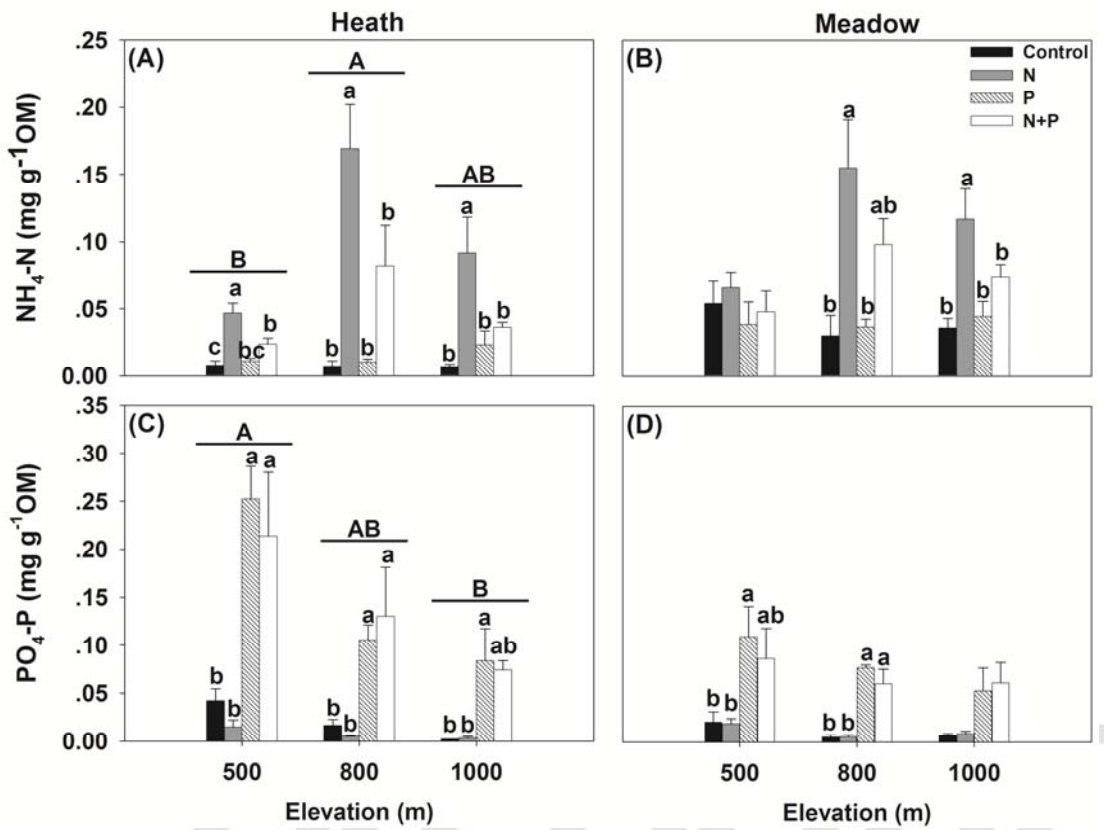
874

875 Figure 2



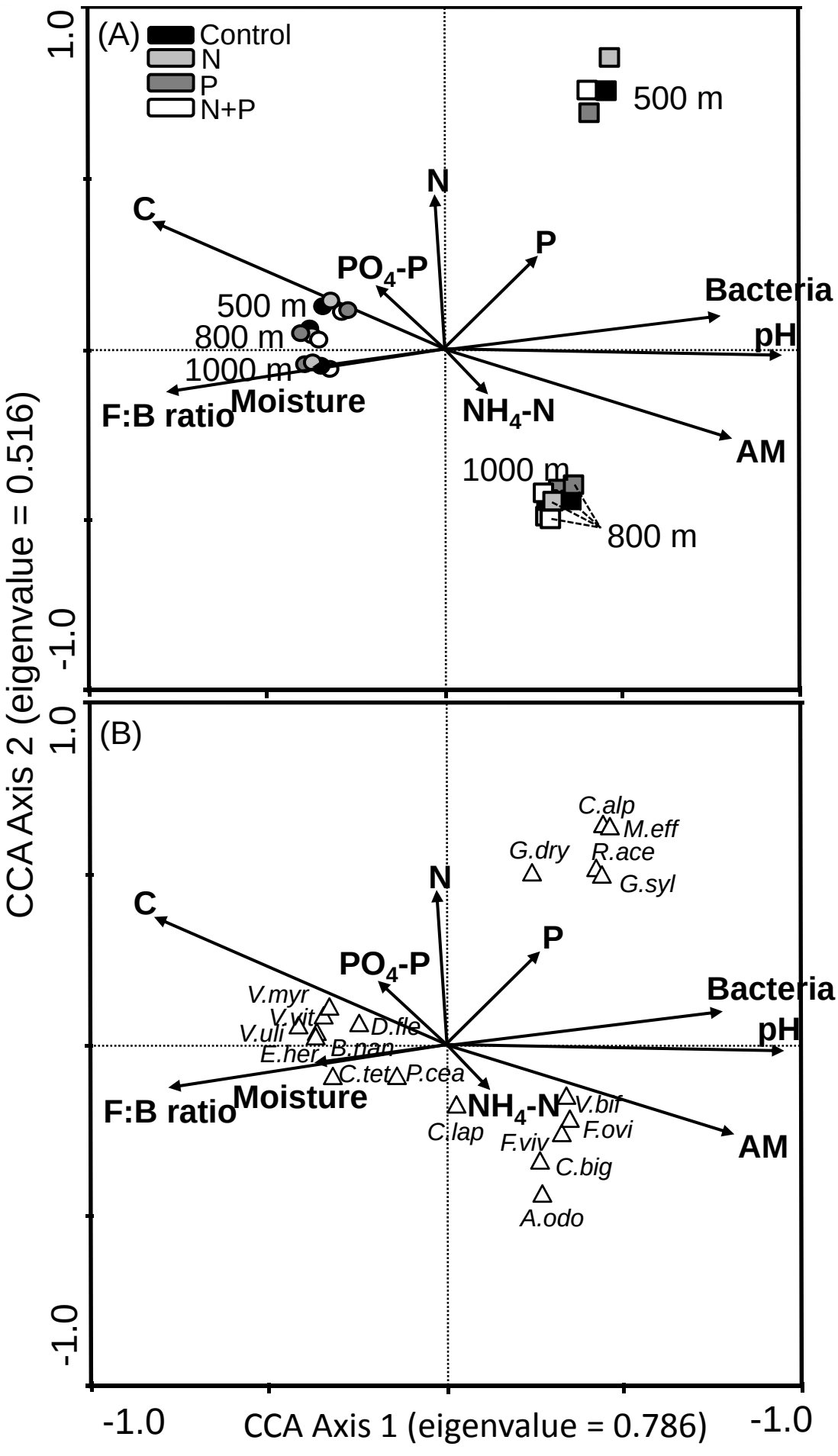
876

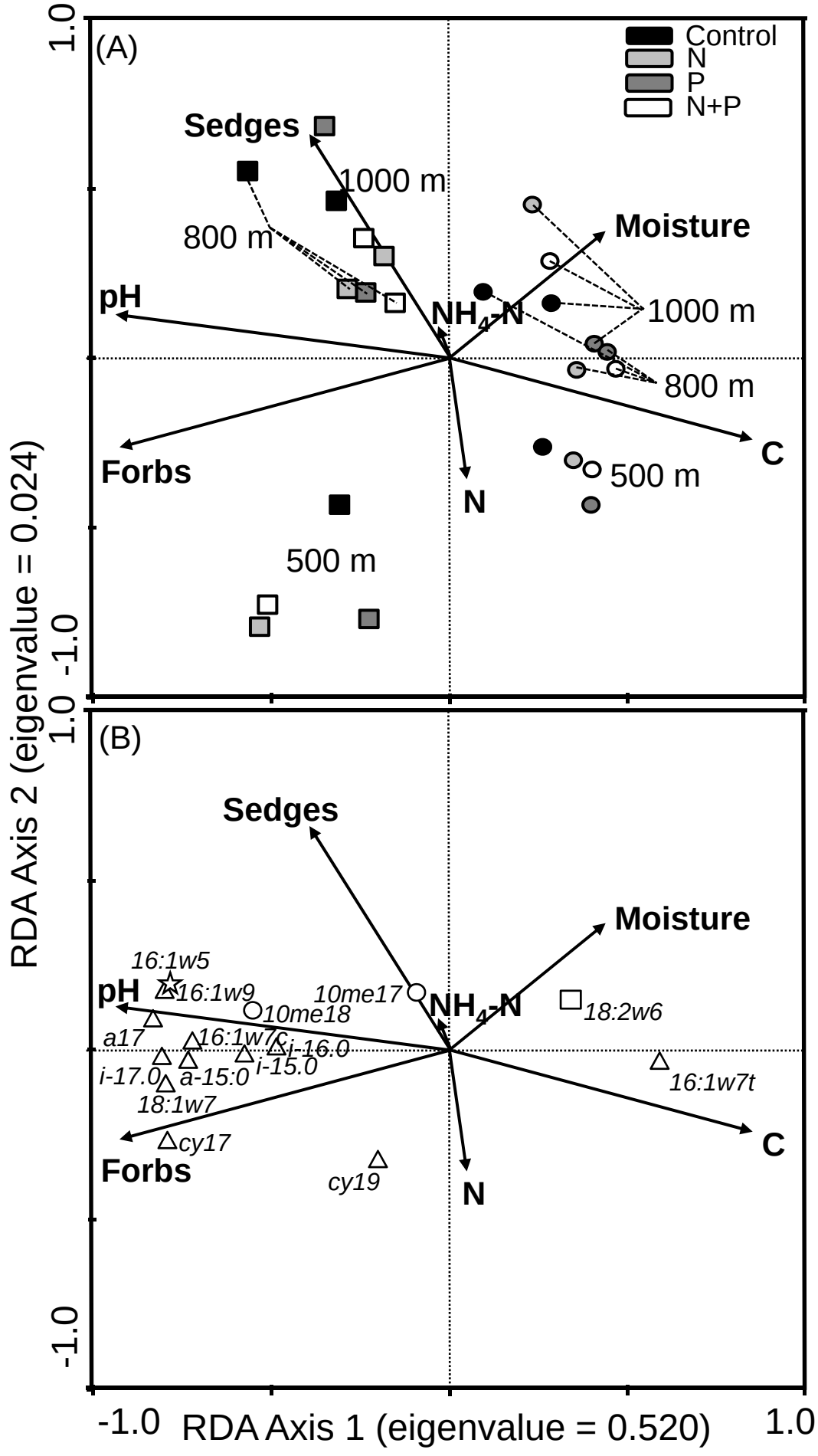
877 Figure 3



878

879 Figure 4


(A)
Control
N
P
N+P
500 m
C
N
P
PO4-P
Bacteria
pH
500 m
800 m
1000 m
F:B ratio
Moisture
NH4-N
AM
1000 m
800 m
1.0
-1.0
(B)
C.alp
M.eff
G.dry
R.ace
G.syl
C
N
P
PO4-P
Bacteria
pH
V.myr
V.vit
V.uli
D.fle
E.her
B.nan
C.tet
P.cea
F:B ratio
Moisture
NH4-N
V.bif
C.lap
F.ovi
F.viv
AM
C.big
A.odo
1.0
-1.0
-1.0
CCA Axis 1 (eigenvalue = 0.786)
-1.0
CCA Axis 2 (eigenvalue = 0.516)


(A)
Control
N
P
N+P
Sedges
1000 m
800 m
Moisture
pH
NH4-N
1000 m
800 m
C
Forbs
N
500 m
500 m
(B)
Sedges
Moisture
16:1w5
pH
16:1w9
10me17
NH4-N
18:2w6
10me18
a17
16:1w7c
i-16.0
i-17.0
a-15:0
i-15.0
16:1w7t
18:1w7
cy17
C
Forbs
cy19
N
1.0
-1.0
1.0
-1.0
RDA Axis 2 (eigenvalue = 0.024)
-1.0
RDA Axis 1 (eigenvalue = 0.520)
1.0