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Covering Letter

Dear Editors of Soil Biology & Biochemistry,

Please find attached a revised version of the manuscript SBB10882 “Do shifts in life strategies explain microbial community responses to increasing nitrogen in tundra soil?” submitted for publication in Soil Biology & Biochemistry

The comments of the reviewers were again very constructive and helpful. We have carefully considered all comments and revised the manuscript accordingly. We hope that the manuscript is now suitable for publication.

On behalf of all authors,

Minna Männistö

*Revision Notes

Responses to reviewers comments

Reviewer #1: I have two concerns in the revised manuscript, but believe they can be corrected.

I believe that all the measurements (except respiration in Fig. 3) were made only at the end of six-week incubation. Therefore, the authors cannot explicitly say that "N addition significantly increased/decreased XX (e.g. L367)" by comparing results of N-amended and control soils, because the authors did not actually measure changes over time. A correct description should be "XX was significantly higher/lower (or not significantly different) in N-amended soils than the controls (e.g. L392-393)." These results MAY INDICATE (or IMPLY) that the N addition "changed" XX over time assuming that the control soils maintained their properties relatively unaffected by the 6-week incubation. Such descriptions are all over the manuscript, and I want the authors to thoroughly correct them. I point some of the examples below (I bet I miss a few).

L335 "reduced"
L367 "Increased" copy number
L374 "increased"
L390 "decreased"
L480 "shift"
L493 "Decreased"
L511 "increased"
L513 "increased"
L550 "decreasing"
Some "Highlights" as well.

RESPONSE:
We agree to this point and have made the following corrections:
L 339: PLFAs were lower
L351: LAP acticity was lower
L355: NAG activity was higher
L370: gene copies were higher
L372: Higher copy numbers
L379-383: abundance was higher/lower
L402: diversity and species richness were lower, dominance higher
L485: higher copy numbers
L491: abundance was higher
L493: abundance was lower
L519: detected at high abundance
L555: lower microbial biomass

My second concern is in Discussion, 4.2. It looks like the authors cherry-picked references that are consistent with the results of this study, and ignored those inconsistent. For instance, Fiefer et al. (2007 Ecology) categorized Bacteroidetes as copiotrophic, but the authors found that N-amended soils had less Bacteroidetes than the controls. Fierer et al. (2007) also reported that beta-proteobacteria (Class, not Phylum, by the way) was copiotrophic - how about in this study? Table 4 shows a significant N effect for Betaproteobacteria. Is it consistent with Fierer et al. 2007? Maybe not based on Fig. 5. If so, do not ignore the inconsistency, but discuss it (e.g. L506-508).

Response:
We did in fact detect a negative response of Betaproteobacteria and Bacteroidetes to N addition. As

earlier noted (by reviewer 1 in the first round), copiotrophy was tested by adding labile carbon by Fierer et al. (2007) and it is likely that members of Bacteroidetes and Betaproteobacteria correlate positively with the fresh labile carbon and are not directly influenced by N.

We have added the following sentence in the discussion (Lines 498-504):
Contrary to what was observed in this study, field experiments have shown increased abundances of Betaproteobacteria and Bacteroidetes in N amended plots (Ramirez et al., 2010; Koyama et al., 2014). Both taxa responded positively to increased labile C and were categorized as copiotrophs byFierer et al. (2007). Increased N availability may thus have little direct effect on these taxa but the observed positive responses in the field were likely mediated through increased primary production by plants as suggested by Ramirez et al. (2010).

I also have minor comments below.

Highlights
The fourth highlight is confusing. It should be gene copies per unit DNA, not just gene copies.
R: Corrected

L99-102. Awkward. Change it like "…to more copiotrophic species which have higher growth rates and carbon use efficiencies, and utilize simpler…"
R: Corrected

L109. Remover "-" between "animal" and "interactions".
R: Corrected

L115-116. Awkward. Rewrite it like ", grazing also leaves significant temporal variation in soil nitrogen"
R: Corrected

L121. Replace "show" with "is associated with"
R: Corrected

L126. Change "nutrient" to "nitrogen"
R: changed to “N”

L138-139. "yearly" and "every year" are redundant.
R: We have revised the sentence: *In a heavily grazed area, reindeer migration causes a drastic nitrogen pulse every year… (L136)*

L143-151. I don't like the way that the authors set up the specific hypotheses. It looks like there are two hypotheses [with 1), and 2)], but the two predictions are no more than expected results from one hypothesis. Remove 1) and 2).
R: Corrected (L 140-148).

L 214. I believe these analyses were conducted for soils after six-week incubation. If so, it's better to mention to it here.
R: Corrected in line 213

L308. I want to make sure that the authors have the accession numbers by the time it's accepted.
R: Accession numbers have been added to lines 307-309.

L314. "Phyla" is still used throughout the manuscript, despite the authors are using some Classes in comparison (e.g. alpha-Proteobacteria, gamma-Proteobacteria). Taxa should be the correct term. I want the authors to correct them throughout the manuscript (e.g. L325, L386, L432, L484).

R: Throughout the manuscript, we have replaced “phyla” with “taxa” or in some cases (lines 327, 840) with “phyla or class” to avoid confusion to OTU-level data.

L333. Replace "proxies" with "indices"
R: Corrected (L 337)

L347. Confusing. The subject of the sentence should be "LAP calculated per SOM", NOT "Nitrogen addition"
R: Corrected to “When calculated per SOM LAP activity was lower and NAG activity higher in N amended microcosms compared to the controls.” (L351-352)

L356. Are "microbial metabolic potential" and "microbial metabolic quotient" the same? If so, be consistent. What is the unit of qCO2? Please provide the result of "microbial metabolic potential" either in figure/table in the text or that in appendix. ANOVA table is not good enough to see what was observed.

Response: Yes they are the same. We have corrected these for consistency and made the following changes:

- We added description of the calculation to the Methods section (lines 194-196)
- We added the qCO2 values in the Results section (lines 360-363)
- We changed the term “metabolic acticity” to microbial metabolic quotient in Discussion (line 554)

L362. Replace "changes" with "differences"
R: Corrected
L379. I believe the authors should provide figures of UniFrac results as supplemental materials. The results of statistical analyses are not enough for readers to tell what N amendment/grazing did on bacterial community structure.

Response:
We used CAP (constrained canonical analyses of principle coordinates) analysis of weighted and unweighted UniFrac distances to visualize the effects of N amendment and grazing history on the community structure. We have added a description of the analysis in the methods section (L 329-332), provided the ordination as a supplementary figure and added text in the Results section (L 395-400).

L383-385, and L392-393. This is a correct description - "indicated significant differences", NOT "N amendment
R: Corrected

L384. "N" is used instead of "nitrogen". Be consistent throughout the manuscript.
R: For consistency we have now used N for nitrogen and C for carbon throughout the manuscript as suggested also by reviewer 2.

L409. Add "dominated by" between "be" and "more"
R: Corrected

L434-345. Delete "the N amended microcosms and" - the previous sentence was all about difference between grazed and non-grazed soils. The following sentence should focus on the topic, otherwise readers will be confused.
R: Corrected

L434. "N" is used here again.
R: We have now used “N” throughout the revised MS

L453. The authors are not supposed to use "&" for two authors but "and" - See page 11 in Author Information Pack (https://www.elsevier.com/wps/find/journaldescription.cws_home/332?generatepdf=true).
R: Corrected

L468-469. These fungal operon numbers need citations.
R: We have added the reference to Baldrian et a., 2013 and references therein

L549. Define "metabolic activity" and how it was calculated.
R: Done, see the earlier response for L356

L574-575. "in to" should be "into"
R: Corrected

L579. "C" is used, instead of "carbon". Be consistent throughout the manuscript.
R:All nitrogen and carbon abbreviated to N and C

L595. Add "respectively" after "grazing"
R: Corrected

Table 1.

Be consistent in units - for instance, ug/g SOM is used for microbial N in Table 1, but mg/g SOM is used for Fig. 1.

R: We have changed the unit for Fig 1.

In Table 1, OM is used, but SOM is used in Fig. 1 - are they the same? If so, be consistent.

R: yes they are the same, we have corrected the OM to SOM in Table 1

Microbial N was around 0.5 mg/g OM (i.e. 500 ug/g SOM) when soils were collected (Table 1), but 50 mg/g SOM at the end of experiment (Fig. 1). They are different in two orders of magnitude. Am I correct? If so, can you discuss it?

R: thank you for pointing this out. We had miscalculated values for Fig 1. These have now been corrected, microbial N was around 500 ug/g SOM

Reviewer #2: The revised manuscript focuses more directly on the N-amendments and does a much more convincing job of linking the results presented to existing knowledge of N and C cycling. This version has clarified the theoretical approach used and assumptions of the copiotroph vs. oligotroph dichotomy, and even provides a plausible explanation to reconcile previous conflicting results obtained for microbial activity in response to N amendments. I find this manuscript to be an interesting contribution and would like to see it published in SBB. I have only minor suggestions to improve the clarity and presentation of the manuscript.

Please indicate significant differences among treatments in Fig. 1.
R: Done

My feeling is that for simplicity sake Table 3 could be relegated to supplementary materials if significant effects were noted in the upper right hand corner of Fig 3 (move legend to left hand panel).
R: we agree and have added the significant effects in Fig 3 and moved Table 3 to supplementary materials.

Remove results for rRNA gene copy numbers from Table 2 and instead indicate significant effects in Figure 4.
R: Done

Indicate significant differences among treatments in Fig. 6.
R: Done

After first use please abbreviate nitrogen as N and carbon as C consistently.
R: Corrected throughout the MS

L30 "…nutrient pulses in the soils located along migratory routes."
R: Corrected

L110 "…high temporal and spatial variation…"
R: Corrected (L 109)

Add accession numbers to L308.
R: Done

The first and second paragraphs of the discussion are quite repetitive. As the first paragraph provides only a brief summary, which is explored in greater detail in subsequent subsections, I think it could simply be deleted. However if retained please revise the first sentence for clarity. It is far too long and convoluted. I suggest:

L408 "We predicted that microorganisms from soils under heavy grazing, experiencing associated pulses in nitrogen, would be more copiotrophic…"

L411"…grazing history had little impact on microbial activities…"

L413 "…depending on grazing history. Instead N addition…"

R: We agree to the comment and have removed the first paragraph.

L505 "…SOM under N limitation…"

R: Corrected

L564 "variation in N availability." (punctuation missing)
R: Corrected

Highlights

Similar bacterial community structure in soils under different grazing intensities.

N amendment decreased respiration in tundra soil.

N amendment decreased biomass but increased rRNA copy numbers per unit DNA.

Copiotrophic taxa were more abundant in N amended soils.

Do shifts in life strategies explain microbial community responses to increasing nitrogen in tundra soil?

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Key words: tundra, nitrogen, bacterial communities, PLFA, enzyme activities, qPCR, next generation sequencing

ABSTRACT

Subarctic tundra soils store large quantities of the global organic carbon (C) pool as the decomposition of plant litter and soil organic matter is limited by low temperatures and limiting nutrients. Mechanisms that drive organic matter decomposition are still poorly understood due to our limited knowledge of microbial communities and their responses to changing conditions. In subarctic tundra large grazers, in particular reindeer, exert a strong effect on vegetation and nutrient availability causing drastic nutrient pulses in the soils located along the migratory routes. Here we studied the effect of increased nitrogen (N) availability on microbial community structure and activities by laboratory incubations of soil collected from two sites with contrasting grazing intensities. We hypothesized that heavily grazed soil experiencing nutrient pulses harbor more copiotrophic taxa that are able to respond positively to increases in available N leading to increased enzyme activities and respiration. Contrary to our hypothesis, there were only minor differences in the microbial community composition between the lightly and heavily grazed soils. N amendment shifted the bacterial community composition drastically, but the changes were similar at both grazing intensities. The relative abundance of diverse *Actinobacteria* and *Rhodanobacter*-affiliated *Gammaproteobacteria* increased in the N amended microcosms, while the abundance of *Acidobacteria, Alphaproteobacteria, Deltaproteobacteria, Verrucomicrobia* and *Bacteroidetes* decreased. Contrary to our hypotheses, increased N availability decreased respiration and microbial biomass at both grazing intensities, while increased N availability had little influence on the extracellular enzyme activities. We propose that similar to what has been reported in other systems, elevated N availability suppressed microbial respiration and biomass by favoring copiotrophic species with faster growth rates and with limited capabilities to decompose recalcitrant organic matter. Similar responses in soils from contrasting vegetation types, soil organic matter (SOM) quality and N availabilities in response to grazing intensity indicate that nutrient pulses may have a strong direct impact on the microbial communities. Responses detected using laboratory incubations

49 are likely amplified in the field where the direct effect of increased N availability is combined with
50 increase in labile C through changes in plant production and species composition.

51

52 ## 1. Introduction

53 As subarctic tundra soils store a significant proportion of the global soil C stock, there is a
54 considerable interest in understanding how the C stored in these systems will respond to changes in
55 environmental conditions. In addition to low temperatures as a factor that limits primary production
56 and soil microbial activity, the role of N availability for soil C decomposition is considered to be a
57 key factor in the responses of soil C stocks to climate change (Hobbie et al., 2002; Robinson, 2002;
58 Mack et al., 2004). Accelerated SOM decomposition and nutrient mineralization in response to
59 climate warming may lead to increased nutrient availability, which can in turn further increase
60 decomposition rates. However, the role of increased N availability on soil microbial biomass and
61 activities remains under dispute due to the fact that there are two contrasting lines of evidence
62 regarding the direction of the N effect on soil microbial biomass and activities. The first line of
63 evidence suggests a negative effect of increased N, as fertilization experiments across biomes have
64 indicated that increased N availability reduces microbial biomass and activities (Treseder, 2008;
65 Ramirez et al., 2010, 2012). The negative effect of N enrichment on respiration has been attributed to
66 inhibitory effects of added N on ligninolytic enzymes (e.g. Sinsabaugh 2010), reduced need of the
67 microorganisms to decompose organic matter for N acquisition (Craine et al., 2007), and switching
68 of the microbial community from overflow metabolism to utilization of C for growth (Schimel &
69 Weintraub, 2003). In overflow metabolism microorganism respire the excess C that cannot be
70 utilized for growth due to nutrient limitations. Thus adding N to nitrogen limited systems may reduce
71 respiration as it allows the microbes to utilize the excess C for growth (Schimel & Weintraub, 2003).
72 The “nitrogen mining hypothesis” (Craine et al., 2007) suggests that SOM decomposition may be

driven by N limitation whereby microbes utilize complex organic matter as a N source rather than a C source.

Especially in Arctic tundra, another line of evidence suggests a positive effect of increased N. In these extremely N-limited tundra soils, low N availability may limit the production of extracellular enzymes and, hence, increased N availability may accelerate SOM decomposition by enhancing microbial enzyme production (Schimel & Weintraub, 2003; Wallenstein et al., 2009; Sistla et al., 2012), especially enzymes that hydrolyze C-rich compounds (Koyama et al., 2013; Stark et al., 2014). Long-term fertilization experiments in Alaskan tundra have shown increased C losses due to increased N availability in the soil organic layer (Mack et al., 2004), suggesting that increased N availability subjects centuries old SOM to microbial decomposition (Nowinski et al., 2008).

The divergent results on the effects of N on microbial biomass and activities reflect the close and complex inter-linkages between soil N and C cycles. The mechanisms by which microbial communities influence the nutrient cycles are still poorly understood, but the community structure is considered to be a key determinant of the functions (Fontaine et al. 2003; Fierer et al., 2012; Chen et al., 2014; Leff et al., 2015). Both field and laboratory experiments have identified major shifts in microbial community structure after fertilization treatment (Nemergut et al., 2008; Ramirez et al., 2010; 2012; Fierer et al., 2012; Koyama et al., 2014; Leff et al., 2015) which are considered to reflect concurrent changes in microbial biomass (Fierer et al., 2012), enzymatic activities (Koyama et al., 2014) or soil respiration (Ramirez et al., 2010; 2012). It has been suggested that N availability is an important determinant for the dominant life strategy of the soil microbial community (Fontaine et al., 2003; Fierer et al., 2012; Chen et al., 2014; Leff et al., 2015). The priming effect theory predicts that under N limitations, microbial communities are dominated by slow growing K-strategists (oligotrophic species) that are able to mine nutrients in SOM whereas under abundant N concentrations, microbial communities are dominated by fast-growing r-strategists (copiotrophic species) that utilize mineral N and more labile C (Fontaine et al., 2003; Chen et al, 2014). A decrease

98 in respiration and enzyme activities through increased N availability has been linked to a shift in the
99 microbial community to more copiotrophic species which have higher growth rates and carbon use
100 efficiencies, and utilize simpler C sources with reduced need for extracellular enzyme production
101 (Fierer et al., 2012; Ramirez et al., 2012; Leff et al., 2015). The abundance of *Actinobacteria,*
102 *Proteobacteria* and *Bacteroidetes* has been shown to increase with increased N availability and these
103 are suggested to represent copiotrophic taxa while members of the *Acidobacteria* and
104 *Verrucomicrobia* have been considered to represent oligotrophic taxa (Fierer et al., 2007, 2012;
105 Ramirez et al., 2012; Leff et al., 2015).
106 Investigations on the effects of increased N availability on soil microbial activity and
107 community composition have considered mainly steady increases in soil N availability due to long-
108 term fertilization, while little attention has been paid to plant-animal interactions, which may result
109 in high temporal and spatial variation in soil N availability. Large migratory grazers have a
110 substantial effect on vegetation across the world's biomes, with important effects on soil C storage as
111 well as N availability (Tanentzap and Coomes 2012). In subarctic tundra, grazing by reindeer
112 (*Rangifer tarandus* L., same species as caribou in the northern parts of the American continent)
113 causes important changes in vegetation (Zamin and Grogan 2012; Bernes et al., 2015), N availability
114 (Olofsson et al., 2004), and soil microclimate (Stark et al., 2015), but grazing also leaves significant
115 temporal variation in soil N. Reindeer migrate annually between summer and winter ranges
116 depending on the availability of suitable forage, and in sites located along the migration routes
117 reindeer herds cause substantial N pulses with drastically increasing N concentrations repeated each
118 year (Stark and Väisänen 2014). Migratory grazing is often associated with a shift in the dominant
119 vegetation from evergreen and deciduous dwarf shrubs to a grassland that is associated with
120 enhanced plant productivity, soil N availability, and microbial respiration (Olofsson et al., 2004).
121 Tundra systems along migration routes thus differ from the surrounding systems in several ways;
122 while lightly grazed systems experience continuously low N concentrations and are dominated by

123 slow-growing dwarf shrub plant species, systems along the migration route experience drastic N
124 pulses each year during the reindeer migration and are dominated by graminoids. This difference in
125 the natural patterns of soil N concentrations could provide interesting opportunities for investigating
126 the soil microbial responses to increasing N, and offer novel insights into the capacity of microbial
127 communities for adapting to different patterns of soil nutrient availability.
128 Here, we hypothesized that – resulting from community-level adaptations to prevailing N
129 levels – soil microbial responses to increasing N availability depend on natural patterns in soil N
130 concentrations. Understanding this relationship was expected to improve our understanding on the
131 basic mechanisms by which microbial communities influence tundra soil C and N cycles. We tested
132 the hypothesis through laboratory incubations with experimental N additions to soils from two sites
133 with contrasting reindeer grazing intensities. In a lightly grazed area, the access of reindeer during
134 migration is prevented by a fence built in the 1960's, the vegetation is dominated by dwarf shrubs
135 and the soil contains low N concentrations with little temporal variation (Stark and Väisänen 2014).
136 In a heavily grazed area, reindeer migration causes a drastic N pulse every year, the vegetation is
137 dominated by graminoids and the soil shows higher N concentrations as well as extreme temporal
138 variation during the growing season. We hypothesized that parallel to shifts in vegetation and soil N,
139 grazing has induced shifts in the microbial community composition towards higher functional
140 adaptation of the soil microbial community to sudden increases in N. More specifically, we predicted
141 that bacterial communities and decomposition under lightly grazed N-poor soils are driven by more
142 oligotrophic, slow growing K-strategist bacteria that utilize organic N by decomposing the
143 recalcitrant C pools (a.k.a nitrogen mining). Consequently increased N availability triggers only
144 weak responses in the microbial activities. On the other hand, we predicted that bacterial
145 communities in the heavily grazed soils with higher N availability are dominated by more
146 copiotrophic r-strategist species that respond more strongly to increased nutrient availability which in

147 turn is detected as increased respiration, enzyme activities for C acquisition and significant shifts in
148 microbial community structure reflecting growth of the faster growing r-strategist taxa.
149

150 ## 2. Materials and methods

151

152 ### 2.1 Study site, sampling and laboratory incubation

153

154 Soil for the laboratory incubation was obtained from a mesic tundra heath (Raisduoddar, Norway
155 [69°39'N, 27°30'E]) located in the suboceanic section of northernmost Fennoscandia (Oksanen and
156 Virtanen, 1995). Owing to a pasture rotation fence built in the 1960s, one sub-section in Raisduoddar
157 is used by the reindeer only briefly for passage (hereafter referred to as light grazing). Vegetation
158 under light grazing is dominated by evergreen and deciduous dwarf shrubs (*Empetrum nigrum* ssp.
159 *hermaphroditum*, *Betula nana*, *Vaccinium vitis-idaea*), and the soil is N poor (Table 1; Stark et al.
160 2002; Olofsson et al. 2004). Another sub-section has been subjected to intensive grazing for the past
161 50 years during the annual reindeer migration period during the first half of August (hereafter
162 referred to as heavy grazing). Owing to the repeated disturbance, vegetation under heavy grazing is
163 dominated by graminoids (*Carex* spp.), plant productivity is high, and the soil is N rich (Table 1;
164 Stark et al., 2002; Olofsson et al., 2004). Soil N concentrations are at highest during the reindeer
165 migration, when soil NH_4-N concentration under heavy grazing are approx. 270 µg g^{-1} SOM relative
166 to values of approx. 24 µg g^{-1} SOM under light grazing (Stark and Väisänen 2014).
167 Five blocks were established along the reindeer fence that separates lightly and heavily
168 grazed sub-sections (distance between blocks > 20 m). Within each block, we selected plots of about
169 5 × 5 m at both lightly and heavily grazed sides of the reindeer fence (distance between plots with
170 differing grazing intensity < 20 m). Soil material was collected by coring approx. 2 kg of fresh soil,
171 which corresponded to 10-15 soil cores (diameter 7.5 cm) to approx. 5 cm depth in the soil organic

172 layer (Stark et al., 2015). Prior to the experiment, soils were pre-incubated for two months at 4°C in
173 order to deplete soils of plant-derived labile C substances with rapid turnover rate (e.g. root
174 exudates). After the pre-incubation, soils were sieved (mesh 2 mm) and soil moisture (drying at
175 105ºC, 12 h), organic matter content (loss on ignition at 475ºC, 4 h), and water-holding capacity
176 (WHC) determined. Sub-samples were also taken for analysis of soil N concentrations.
177 Soil microcosms were established by weighing 30 g of soil obtained from each of the five
178 blocks per grazing treatment in 500 ml glass bottles capped with rubber stoppers. Soil moisture was
179 adjusted to 30% of the WHC. N was added as NH_4NO_3 to the N amended (+N) microcosms from a
180 100 g^{-1} stock solution to a final concentration of 12.5 mg NH_4NO_3/g SOM, corresponding to 8.75 mg
181 of N per gram of soil C (Hartley et al. 2010). Controls were amended with the same volume of milli-
182 Q water. We used the same dosage of NH_4NO_3 as in Hartley et al. (2010), because these soils
183 constituted the most similar soils in the literature compared with our site and thus created an
184 interesting comparison. Soils were incubated for six weeks at 9°C after which soil was sampled for
185 microbial activity, community composition and soil chemical analyses. The effect of NH_4NO_3 on
186 soil pH was tested in a separate experiment by adding NH_4NO_3 and water to 10 g of soil in the same
187 ratio. Soil pH was measured immediately and after 4 week incubation in 3:5 v/v soil:water
188 suspensions (Denver Instrument Model 220).
189
190 2.2 Microbial respiration and extracellular enzyme activities
191
192 Microbial respiration (total CO_2-C release) was analyzed at selected time points from the headspace
193 of the incubation bottles using an Agilent 6890N GC equipped with a ShinCarbon ST micropacked
194 column (Restek) and thermal conductivity detector. Microbial metabolic quotient (qCO_2, respiration
195 per biomass) was calculated after 6 weeks of incubation by dividing the respiration rate (mg CO_2 h^{-1}
196 g^{-1} SOM) by total PLFA concentration (μmol PLFA g^{-1} SOM).

197 The effect of N addition on potential extracellular enzyme activities (EEAs) was tested
198 after the 6 week incubation as described earlier (Stark et al., 2015). Briefly, betaglucosidase (BG),
199 β-N-acetylglucosaminidase (NAG), acid-phosphatase (AP) and leucine aminopeptidase (LAP)
200 activities were analyzed using para-nitrophenyl(pNP)-β-glucopyranoside (5 mM), pNP-β-N-
201 acetylglucosaminide (3 mM), pNP-phosphate (5 mM) and leucine p-nitroanilide (5 mM) as
202 substrates, respectively. Three g sub-samples of soil were suspended in 60 ml sodium acetate buffer
203 (50 mM, pH 5.0), after which 100 µl of the sample was mixed with 200 µl of enzyme substrate in a
204 96-well plate. Blanks with 100 µl of the sample mixed with 200 µl of sodium acetate were prepared
205 to account for the color in the soil slurry and the background absorbance of the enzyme substrate was
206 analyzed using blanks with 100 µl of sodium acetate mixed with 200 µl of enzyme substrate.
207 Following incubations at room temperature, samples were centrifuged and 100 µl of the supernatant
208 transferred into another 96-well plate, 5 µl of 1.0 M NaOH added, and the absorbance was measured
209 at 410 nm using a Multiscan FC microplate reader (Thermo Scientific).
210
211 2.3 Soil N, microbial biomass and community composition
212
213 Soil and microbial biomass N were analyzed after the 6 week incubation. A ~3 g subsample of soil
214 was extracted with 50 mL of 0.5 M K_2SO_4 while another subsample was extracted using the same
215 method after chloroform fumigation for 18 h (Brookes et al., 1985). The concentration of NH_4-N was
216 determined from soil extracts according to the standard protocol (SFS 3032) using a Shimadzu UV-
217 1700 spectrophotometer. NO_3-N was determined from soil extracts via flow analysis (FIA Perstorp).
218 The total extractable N in both soil and fumigated extracts was oxidized to NO_3 (Williams et al.,
219 1995) and then analyzed as NO_3-N (FIA, Perstorp). Microbial N was calculated by subtracting the
220 total extractable N of the soil extracts from that of the fumigated extracts.

Phospholipid fatty acid (PLFA) and lipid phosphate (PO_4) concentrations were used as proxies for soil microbial biomass (Findlay et al., 1989; Frostegård et al., 1991; Frostegård and Bååth, 1996). Lipids for both analyses were extracted from 1 g (wet weight) of freeze-dried soil using 10 ml of a one-phase mixture (1:2:0.8 v/v/v) of chloroform, methanol and 0.15 M citrate buffer (pH 4.0) for 2 hours after which the tubes were centrifuged at 1500 x g for 10 min. The supernatant was transferred to a new tube and the soil was re-extracted with 5 ml extraction solvent for 1 h, centrifuged and supernatants from the first and second extraction combined. The phases were separated by adding 4 ml of chloroform and 4 ml of citrate buffer. After overnight separation, the lipids were separated into neutral lipids, glycolipids and phospholipids in silicic acid columns as described by Frostegård et al. (1991). The phospholipid fraction was then subjected to mild alkaline methanolysis (White et al. 1979) after which the fatty acid methyl esters were analyzed as described by Männistö and Häggblom (2006). PLFA 18:2ω6c was used to indicate fungal biomass [including saprotrophic, ectomycorrhizal and ericoid mycorrhizal fungi (Olsson 1999; Ruess et al. 2002)], while the sum of PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω9c, i17:0, a17:0, 17:0, cyclo-17:0, 18:1ω7c and cyclo-19:0 was used to indicate bacterial biomass (Frostegård and Bååth 1996). For the lipid-PO_4 analysis, 0.2 ml of the lipid phase (containing all lipid fractions) was pipetted to a new test tube and dried under a stream of N. Lipids were digested with 1.8 ml saturated potassium persulfate by incubating the samples at 95°C for 50 h. PO_4 was measured by the malachite green method as described by Findlay et al. (1989) using a Shimadzu UV-1700 spectrophotometer.

Total genomic DNA was extracted from 0.25 g of soil using a modified phenol-chloroform-isoamylalcohol (PCI) protocol after Griffiths et al. (2000). In brief, 0.5 g of acid-washed and baked glass beads (0.1 mm) together with 500 µl PCI (ratio 25:24:1) and 500 µl cetrimonium bromide buffer (CTAB) were added to the soil sample followed by bead beating on a Precellys 24 Dual homogenizer for 30 s at 5500 rpm. Samples were centrifuged (16000 x g, 5 min) and the extraction repeated with PCI followed by a final extraction with chloroform-isoamyl alcohol (CI;

246 ratio 24:1). DNA was precipitated with 30% PEG solution for 1 h on ice, washed with ice-cold 70%
247 ethanol and the DNA pellet was re-suspended in 1x TE buffer and stored at -80°C for further
248 processing.
249 Amplification of the V1-V3 region of the 16S rRNA gene was done using the primer pair
250 27F (5'-AGAGAGTTTGATCMTGGCTCAG- 3', Lane, 1991) and 518R (5'-
251 ATTACCGCGGCTGCTGG- 3; Muyzer et al., 1993). A 25 µl PCR reaction contained 5 µl 5x
252 Phusion HF buffer (Thermo Scientific), 0.5 µl dNTPs (10 mM), 0.5 µl bovine serum albumin (20
253 mg/ml), 1.25 µl of each primer (10 µM), 1 U Phusion High Fidelity polymerase (Thermo Scientific)
254 and ~50 ng of the DNA extract, filled up with PCR grade water. PCR amplification was carried out
255 on a Biometra TProfessional Basic cycler (Biometra, Germany) with 98°C for 30 s, followed by 30
256 cycles of 98°C, 10 s; 52°C, 30 s; and 72 °C for 180 seconds, and a final elongation step of 5 min at
257 72°C. Successful PCR products were re-amplified for 6 cycles with the same primer pair, but
258 including adaptor A (5'-CCATCTCATCCCTGCGTGTCTCCGAC- 3') and unique 10-12 bp long
259 barcodes in the beginning of the forward primer to allow Ion Torrent sequencing and assignment to
260 specific samples. PCR products were cleaned using the Agencourt AMPure XP magnetic beads
261 purification system (Beckman Coulter) and quantified using the Qubit dsDNA HS Assay Kit
262 (Invitrogen). Amplicons were then combined in equimolar concentrations for sequencing. Next,
263 pooled samples were re-amplified using the PGM-specific sequencing adaptor A (5'-
264 CCATCTCATCCCTGCGTGTCTCCGAC 3') and P1_338r (5'–
265 CCTCTCTATGGGCAGTCGGTGAT TGCTGCCTCCCGTAGGAGT–3') for 6 cycles using the
266 Platinum PCR SuperMix (Life Technologies) to reduce the size of the template suitable for Ion
267 Torrent sequencing (region V1–V2) and to add adapter P1 to the end of the product. The product was
268 then purified using the Agencourt AMPure XP beads and seeded into an Ion PGM Template OT2
269 reaction following manufacturer's instructions (Life Technologies). Templated beads were enriched

using the Ion OneTouch ES system and sequencing libraries were loaded on Ion 316 Chips and sequenced using the Ion PGM Sequencing 400 Kit.

Quantitative PCR (qPCR) was performed in triplicate to evaluate bacterial 16S rRNA and fungal 18S rRNA gene copy numbers using the CFX96 Real-time PCR detection system (Bio Rad). qPCR for bacteria was done with the SsoFast Kit (BioRad) and the primer pair Eub341f (3'-CCT ACG GGA GGC AGC AG-5') and Eub534r (3'-ATT ACC GCG GCT GCT GG-5') (Muyzer et al., 1993). Each 15 µl reaction mixture contained 7.5 µl 2x SsoFast EvaGreen qPCR Supermix (Biorad), 0.375 µl of each primer (10 µM), 4.25 µl PCR grade water and 2.5 µl template in a 1000-fold dilution. PCR conditions were 98°C for 2 min followed by 40 cycles of 98°C, 5 s; 56°C, 20 s (following a plate read). Genomic DNA from *Granulicella tundricola* MPACTX9 was used as a standard. qPCR for fungi was done with the KAPA Sybr Fast qPCR Kit (KAPA Biosystems) and the primer pair FF390 (3'-CGA TAA CGA ACG AGA CCT-5') and FR1 (3'-AIC CAT TCA ATC GGT AIT-5') (Vainio and Hantula, 2000). Each 15 µl reaction mixture contained 7.5 µl KAPA Sybr Fast qPCR Mix (KAPA Biosystems), 0.375 µl of each primer (10 µM), 4.25 µl PCR grade water and 2.5 µl template in a 1000-fold dilution. PCR conditions were 97°C for 2 min followed by 40 cycles of 95°C, 20 s; 50°C, 30 s and 72°C, 30 s (following a plate read). A plasmid containing the target sequence amplified from *Phialocephala fortinii* was used as a standard for the fungal qPCR. A melt curve read for each qPCR run was done after the last cycle from 65°C to 95°C in 0.5°C increments for 5 s. In addition, qPCR products were checked on a 1% agarose gel for the correct size or the appearance of additional bands.

Sequences were analyzed using Mothur (Schloss et al., 2009) and QIIME (Caporaso et al., 2010a) software packages. Sequences shorter than 250 bp or which contained ambiguities and homopolymer stretches of more than 8 bases were removed. Chimera check was performed using UCHIME (Edgar et al., 2011) and singleton sequences were removed. After quality filtering 78403 reads were obtained from all samples, with an average of 3920 reads per sample (min = 2206, max =

6202). For downstream analyses all samples were rarefied to an equal sequence number to avoid heterogeneity in sequencing depth which would affect calculations for α- and β-diversity. Sequences were then clustered into operational taxonomic units (OTU) using UCLUST (Edgar, 2010) with a sequence similarity value of 97%, and representative sequences were randomly chosen from each cluster. Taxonomy assignment of the OTUs was based on the naïve bayesian RDP classifier (Wang et al., 2007) using a SILVA-based reference database (www.mothur.org/wiki/Silva_reference_files) with a confidence threshold of 60%. Alpha diversity indices (Faith's phylogenetic diversity, Chao1, dominance and observed OTUs) were calculated using a script available in QIIME. Dominance is here Dominance=1-Simpson index. For β-diversity analyses, representative OTU sequences were aligned to the SILVA reference database using PyNAST (Caporaso et al. 2010b) and a phylogenetic tree was build using FastTree (Price et al., 2009), followed by calculation of weighted and unweighted UniFrac distance metrics (Lozupone et al., 2006; 2011).

Sequences were deposited in the Sequence Read Archive of NCBI under accession number SRP069050 (runs SRR3133443, SRR3133444, SRR3133446 - SRR3133455, SRR3133457 - SRR3133464).

2.4 Statistical analyses

The effects of N and grazing intensity on EEAs, soil and microbial N, lipid-PO_4, total-PLFA, bacterial and fungal copy numbers, bacterial diversity indices and the relative abundances of dominant bacterial taxa were tested using the linear mixed effects model (PASW 12.0 Statistical software) with N addition (control, N+) and grazing intensity (light grazing, heavy grazing) as fixed factors and block as a random factor nested within grazing intensity. The effects of N and grazing intensity on microbial respiration were tested with the same model but incubation time was added to

319 the model as a repeated factor. Logarithmic transformations were used to meet the assumptions of
320 the mixed model when necessary.
321 The effect of N and grazing intensity on bacterial community structure was tested using
322 PERMANOVA (Anderson, 2001) with N and grazing intensity as fixed factors and block as a
323 random factor nested within grazing intensity. Weighted and unweighted UniFrac distances of 16S
324 rRNA gene sequences were calculated using Qiime (as described above) and used as the distance
325 measure for PERMANOVA procedure using 999 permutations for the probability tests. To test shifts
326 within the dominant bacterial taxa, the abundance (number of sequences) in OTUs classified to
327 specific phylum or class were used. The OTU data was square root normalized, Bray-Curtis
328 similarities were calculated using PRIMER 6 software (Clarke and Gorley, 2006) and
329 PERMANOVA tests performed as described for the UniFrac distances. Canonical analysis of
330 principal coordinates (CAP, Anderson and Willis 2003) was used to visualize shifts in the bacterial
331 community composition. Weighted and unweighted UniFrac distances were used as the metric and N
332 as the grouping factor for CAP.
333
334 **3. Results**
335
336 **3.1 Effect of grazing intensity and N addition on microbial biomass and soil nitrogen**
337 After 6 weeks of incubation all indices for microbial biomass were significantly lower in N-amended
338 microcosms compared to the controls (Fig 1, Table 2). PLFA analysis indicated that especially
339 bacteria responded negatively to the N addition, as bacterial PLFAs were lower in the N amended
340 microcosms. There were no statistically significant differences in the fungal PLFAs between N
341 amended and control soils. Grazing intensity of the site did not influence the total biomass indicators
342 (total PLFAs, lipid-PO_4 or microbial-N) or bacterial PLFAs, but the abundance of fungal PLFAs was
343 significantly higher in the lightly compared to heavily grazed soils (Fig. 1, Table 2). The

concentrations of total extractable N was significantly increased by N addition, being 0.11 mg g^{-1} SOM and 2.77 mg g^{-1} SOM in control and N-amended soils under light grazing, respectively, and 0.24 mg g^{-1} SOM and 3.29 mg g^{-1} in control and N-amended soils under heavy grazing, respectively (Table 2). Soil pH at the beginning of the experiment was between 5.0 and 5.2, NH_4NO_3 addition decreased pH on average by 0.67 units.

3.2 Effect of grazing intensity and N addition on microbial activities

When calculated per SOM LAP activity was lower and NAG activity higher in N amended microcosms compared to the controls. When the enzyme activities were calculated per total PLFA concentration (as a proxy for total biomass), there were no statistically significant differences in LAP, BG or AP activities between the N amended and control microcosms or in lightly and heavily grazed soils, but NAG activity was significantly higher in the N amended microcosms. Grazing intensity affected only NAG activity (calculated per organic matter) which was lower in the heavily grazed soil (Table 2; Fig. 2).

Microbial respiration declined over the 6 week incubation in both N amended and control microcosms regardless of previous grazing intensity (Fig. 3, Table S1). However, respiration declined more rapidly in the N amended microcosms. N addition resulted in a lower microbial metabolic quotient (respiration per biomass) when calculated per total PLFA concentrations. In the lightly grazed soils qCO_2 was 5.79 vs. 4.23 CO_2 h^{-1} $mmol^{-1}$ PLFA for control vs. N amended microcosms, respectively, compared to 5.78 vs. 4.25 mg CO_2 h^{-1} $mmol^{-1}$ PLFA, in the heavily grazed soils, indicating that decreased respiration was not solely caused by the decline in microbial biomass.

3.3 Effect of N addition on microbial community structure in soils of different grazing intensity

Quantitative PCR (qPCR) analysis was used to detect changes in the bacterial and fungal ribosomal RNA gene copy numbers. Previous grazing intensity had no significant effect on bacterial copy

numbers, but fungal copy numbers were higher in the heavily than in the lightly grazed soil (Table 2, Fig. 4). Both bacterial 16S rRNA and fungal 18S rRNA gene copies were higher in the DNA extracted from the N amended microcosms compared to the control microcosms (Table 2, Fig. 4). Higher copy numbers per ng DNA indicate that the composition of the bacterial and fungal communities shifted in the N amended microcosms to species with a higher number of 16S/18S rRNA gene copies. On the other hand, in soils under intensive grazing the fungal community appeared to consist of species with higher 18S rRNA gene copy numbers while there was no significant grazing effect on bacterial rRNA gene copy numbers (Fig. 4).

Bacterial communities were further compared by 16S rRNA gene sequence analysis. Classification of sequences indicated minor differences in the abundance of different taxa in the lightly and heavily grazed soils. However, the abundance of *Actinobacteria* was higher in N amended soils of both grazing intensities and the abundance of *Gammaproteobacteria* was higher especially in the N amended heavily grazed soils, while the abundance of members of *Acidobacteria, Bacteroidetes*, *Alpha-, Beta-* and *Deltaproteobacteria, Planctomycetes* and *Verrucomicrobia* were lower in N amended soils of both grazing intensities (Table 3, Fig. 5).

PERMANOVA analysis of the weighted UniFrac distances further indicated that after 6 week incubation the soil bacterial community structures were not significantly different in the heavily and lightly grazed soils, but N addition shifted the communities under both grazing intensities (Table 4). Shifts in the bacterial communities tended to differ in soils with different grazing history (grazing x N interaction, $p=0.062$). PERMANOVA analysis of the unweighted UniFrac distances indicated significant differences in the N amended vs. control microcosms but no interaction with grazing intensity. To detect shifts in the community structure within the dominant taxa, we tested the OTU data (abundance of sequences in different OTUs) of each phylum and in addition different *Proteobacteria* and *Acidobacteria* classes separately. PERMANOVA analysis indicated significant shifts within all major taxa in the N amended microcosms, while grazing

intensity affected only *Gammaproteobacteria* and no grazing x N interactions were detected (Table 4). Canonical analysis of principal coordinates (CAP, Anderson and Willis, 2003) was used to visualize the grouping of samples based on the weighted and unweighted UniFrac distances. Similarly as the other analyses, CAP indicated that the bacterial community structure was controlled by N addition more than grazing history. Moreover, Spearman correlation of the CAP axes with dominant bacterial taxa supported the strong correlation of *Gammaproteobacteria* and *Actinobacteria* with the N amended soils (Fig. S1 in the supplementary materials). Comparison of α-diversity indices indicated that the diversity (Faith's phylogenetic diversity) and species richness (Chao1, observed species) were lower, but the dominance was higher in N amended microcosms of soils from both grazing intensities (Fig. 6, Table S2 in the supplementary materials). There were no significant differences in the species richness between the heavily and lightly grazed soils, but dominance was significantly higher in the heavily grazed soils. Moreover, N amendment decreased species richness more in the heavily than lightly grazed soil. These results indicate that while grazing intensity had no effect on the bacterial diversity, there were more dominant groups in the heavily grazed soils. N amendment increased the dominance in soils of both grazing intensities which decreased the diversity and species richness especially in the heavily grazed soils.

Comparison of the relative abundance of the 50 most abundant OTUs in the N amended microcosms identified several dominant OTUs that responded to the N amendment. The most abundant OTUs that increased in the N amended microcosms were generally members of *Actinobacteria* (orders *Actinomycetales, Acidimicrobiales* and *Solirubrobacterales*) and *Gammaproteobacteria* (*Rhodanobacter* spp.) while those that responded negatively to N amendment included members of *Alphaproteobacteria* (*Rhizobiales*), *Acidobacteria* and *Gammaproteobacteria* (*Sinobacteraceae*) (Table S3 in the supplementary materials).

4. Discussion

419

420 4.1 Differences in microbial community composition in response to grazing intensity

421

422 We predicted that the bacterial community composition should be more copiotrophic in microcosms
423 with soil from the heavily grazed site with higher mineral N concentrations and stronger seasonal
424 fluctuation. Higher N concentrations in the soil solutions have been linked to a higher dominance of
425 r-selected microorganisms which utilize simpler organic compounds and are not able to mine N by
426 degrading recalcitrant SOM (Fontaine et al., 2003; Fierer et al., 2012; Chen et al., 2014; Leff et al.,
427 2015). However, there were only minor differences in the bacterial community structures between
428 the grazing intensities as evidenced by both qPCR analysis which indicated no shifts in the bacterial
429 copy numbers and sequence analysis which indicated no significant differences in the bacterial
430 community structure (weighted or unweighted UniFrac distances), diversity or abundance of most of
431 the dominating taxa. Of the dominating taxa, only the abundance and diversity of
432 *Gammaproteobacteria* differed between the lightly and heavily grazed soils. These differences
433 resulted mainly from the higher abundance of *Rhodanobacter* affiliated OTUs in the soil from heavy
434 grazing pressure. The minimal effects of grazing on bacterial community composition were
435 surprising given the large differences in the plant community structures and N availability between
436 soils under light and heavy grazing. In other soil biomes, members of *Acidobacteria* and
437 *Verrucomicrobia* have been reported to correlate negatively, while *Actinobacteria, Bacteroidetes,*
438 *Alpha-, Beta-* and *Gammaproteobacteria* have been found to correlate mostly positively with
439 increased N availability in field studies (Nemergut et al., 2008; Cambell et al., 2010; Ramirez et al.,
440 2010; Fierer et al., 2012; Koyama et al., 2014; Leff et al., 2015). Vegetation type and shifts in plant
441 community composition after fertilization have been reported to influence bacterial community
442 structure in the Alaskan tundra (Chu et al., 2011) and grasslands across the globe (Leff et al., 2015).
443 Besides soil N availability, the 50-year difference in grazing intensity had also significantly altered

444 the chemical quality of accumulated soil organic matter in our study site. Characterization of soil C
445 using ^{13}C-NMR spectroscopy showed higher proportion of carbohydrates under light grazing and
446 higher proportion of aliphatic-not-O-substituted C under heavy grazing (Väisänen et al., 2015). We
447 suggest that the similarity of bacterial community composition in soils from different grazing
448 intensities may partially result from the fact that we used pre-incubated soils that are depleted in
449 labile C by e.g. plant root exudates. We found no effects of grazing on respiration and EEAs in the
450 present laboratory incubation, but under field conditions and fresh soil samples, respiration, BG and
451 LAP activities are found to be consistently higher under heavy than light grazing (Stark and
452 Väisänen, 2014). In pre-incubated soils, bacteria associated with plant exudates may decrease and
453 consequently, bacterial community composition to a large extent depicts the influence of the
454 chemical quality of accumulated soil organic matter. By contrast, under field conditions the supply of
455 plant-derived labile C may be a strong driver of the bacterial communities as well as microbial
456 respiration rates. This notion is supported by our findings that the effect of grazing on bacterial
457 community composition was slightly stronger in fresh soils collected from the same study site in the
458 following year (manuscript in preparation).
459 Total PLFA, lipid phosphate and microbial-N concentrations were similar at both
460 grazing intensities indicating no clear differences in microbial biomass. However, the
461 fungal:bacterial ratio and the concentration of the fungal fatty acid 18:2ω6,8 indicated higher fungal
462 abundance in soils under light grazing, which likely reflects the dominance of ecto- and ericoid
463 mycorrhizal vegetation (*B. nana, Empetrum* and *Vaccinium* species) in the lightly grazed area as
464 oppose to the graminoid-dominated vegetation in the heavily grazed area. Moreover, contrary to
465 fungal PLFA concentrations, fungal 18S rRNA gene copy numbers were higher in soils under heavy
466 than light grazing indicating that the fungal community structures differed between the grazing
467 intensities. The ribosomal RNA gene copy numbers of fungi vary widely and numbers between 20
468 and 200 copies per genome have been reported (Baldrian et al., 2013 and references therein), while

469 forest soil fungal isolates were reported to contain 5 x 10^5 to more than 1 x 10^7 copies ng^{-1} DNA
470 (Baldrian et al., 2013). The high variability in copy numbers between different species, or even
471 strains within the same species, limits the applicability of qPCR as a marker for fungal biomass
472 while the fungal PLFA 18:2ω6 has been proposed as a more reliable estimate for fungal biomass
473 (Frostegård and Bååth, 1996; Baldrian et al., 2013). Taken together, fungal PLFA concentrations
474 indicated that a long history of heavy grazing has reduced the fungal biomass while simultaneously
475 shifting the community composition to species with considerable higher ribosomal RNA gene copy
476 numbers.
477
478 4.2 Effects of N addition on bacterial community composition
479
480 Experimental manipulation of soil N resulted in significant differences in the soil bacterial
481 community composition between N amended and control soils. Nearly all measures, i.e. 16S rRNA
482 gene copy numbers, UniFrac distances, and abundance and composition of different taxa indicated
483 significantly different bacterial community structures in the N amended and control soils after 6
484 week incubations. We hypothesized that N amendment would increase the proportion of
485 copiotrophic to oligotrophic bacterial taxa as suggested by Fierer et al. (2012). Higher copy numbers
486 in the N amended microcosms, as indicated by the qPCR analysis, was in line with this hypothesis as
487 the ribosomal RNA gene copy numbers have been linked to bacterial life strategy. Low copy
488 numbers are attributed to oligotrophic life style (K-strategists) while high copy numbers reflect
489 ability to respond dynamically to favorable growth conditions (Klappenbach et al., 2000). Sequence
490 analysis indicated that the abundance of *Actinobacteria* and *Gammaproteobacteria* members was
491 higher in N amended soils especially of the high grazing intensity, while the relative abundance of
492 e.g. members of *Acidobacteria*, *Alpha-*, *Beta-* and *Deltaproteobacteria, Bacteroidetes*,
493 *Planctomycetes* and *Verrucomicrobia* were lower in N amended microcosms. The negative response

494 to N in the relative abundance of *Acidobacteria, Planctomycetes* and *Verrucomicrobia* is in line with
495 a N induced shift from oligotrophic to copiotrophic taxa as these are considered to represent
496 oligotrophic bacteria (Fierer et al., 2007) and have been shown to respond negatively to increased
497 nutrient availability earlier both in field (Fierer et al., 2012; Leff et al., 2015) and laboratory
498 incubation studies (Ramirez et al., 2012). Contrary to what was observed in this study, field
499 experiments have shown increased abundances of *Betaproteobacteria* and *Bacteroidetes* in N
500 amended plots (Ramirez et al., 2010; Koyama et al., 2014). Both taxa responded positively to
501 increased labile C and were categorized as copiotrophs by Fierer et al. (2007). Increased N
502 availability may thus have little direct effect on these taxa but the observed positive responses in the
503 field were likely mediated through increased primary production by plants as suggested by Ramirez
504 et al. (2010). Similar to this study, *Actinobacteria* and *Gammaproteobacteria* have been reported to
505 respond positively to increased N availability in field (Ramirez et al., 2010; Campbell et al., 2010;
506 Leff et al., 2015) and laboratory studies (Ramirez et al., 2012). *Actinobacteria* and *Proteobacteria*
507 are considered to include copiotrophic taxa that may benefit from increased N availability either
508 directly or indirectly as an increase in labile C through increased plant primary production (Fierer et
509 al., 2012; Eilers et al., 2010). Moreover, copiotrophic r-strategist taxa are considered to out-compete
510 oligotrophic K-strategists at higher N availability because of their faster growth compared to the K-
511 strategists that benefit from their ability to mine N from SOM under N limitation (Fontaine et al.,
512 2003; Chen et al., 2014). However, studies indicate that within both phyla, there are members with
513 different life –strategies and categorizing taxa in the different life history strategies remains
514 challenging.

515 In this study we detected two distinctly different groups among the order
516 *Xanthomonadales* (*Gammaproteobacteria*). *Rhodanobacter* related sequences were more abundant
517 in the heavily grazed soil and N amended soils, while members of the family *Sinobacteraceae* were
518 more abundant in the control soils and responded negatively to N amendment. *Rhodanobacter*

519 related sequences were detected at high abundance in fertilized plots of a field study at the same site
520 (Männistö, unpublished data) and in a long-term fertilized tundra soil in Alaska (Campbell et al.,
521 2010) indicating that N availability has a strong and direct effect on members of this taxon. OTUs
522 related to the *Sinobacteraceae* have been abundantly detected in other, generally N-poor tundra soils
523 where the abundance shifts similarly as the oligotrophic *Acidobacteria* (Männistö et al., 2013;
524 Männistö, unpublished). Opposite shifts to nutrient and substrate concentrations are thus detected
525 within taxa of the same order, highlighting the importance of analyzing the communities within fine
526 taxonomic resolution and questioning the use of phylum level identification to classify the sequences
527 into K- and r-strategists.
528 Similarly, while diverse members of *Actinomycetales* were shown to respond to labile
529 C and were linked to copiotrophic, fast growing bacteria (Goldfarb et al., 2011), also other
530 mechanisms could underlie the effects of N amendment on the abundance and community
531 composition *Actinobacteria*. Some *Actinobacteria* have been linked to utilization of recalcitrant
532 substrates such as lignocellulolytic C (Goodfellow & Williams, 1983; 1987; Ball et al., 1989;
533 Větrovský et al., 2014) and known to possess a wide diversity of enzyme activities for
534 decomposition of lignin- derived substrates (Bugg et al., 2011; le Roes-Hill et al., 2011).
535 *Actinobacteria* may upregulate the lignocellulolytic activity under increased N availability (Barder &
536 Crawford, 1981) and increased N availability has been shown to affect the structure of
537 *Actinobacteria* community (Eisenlord & Zak, 2010) and richness of genes involved in the
538 depolymerization of lignin and other plant polysaccharides (Eisenlord et al., 2013). Increase in
539 *Actinobacteria* in the N amended microcosms may thus be linked to their ability to degrade
540 recalcitrant C in addition or instead of preference to labile C. Several *Actinobacteria* phylotypes,
541 including those related to the orders *Actinomycetales* and *Acidimicrobineae*, were reported to
542 increase in warmed plots of a field experiment in Alaska. Increase of these *Actinobacteria* was linked
543 to a decrease in labile C availability in warmed plots and these phylotypes were considered K-

544 strategists capable of utilizing the more recalcitrant C pools (Deslippe et al., 2012). N induced
545 increases in a wide diversity of *Actinobacteria* likely reflects different life-strategies within this
546 phylum with a wide diversity of functions related to C cycling.
547
548 4.3 Effect of increased N availability on microbial activity – a potential link to changes in bacterial
549 life strategy?
550
551 Contrasting with our hypothesis, N addition did not increase microbial activities for C acquisition,
552 but instead respiration and microbial biomass declined with N addition irrespective of grazing
553 intensity. Microbial respiration was lower in N amended microcosms both when calculated per SOM
554 and per microbial biomass, indicating decreased microbial metabolic quotient (qCO2) which in
555 combination with lower microbial biomass led to a considerable decrease in microbial release of
556 CO_2. A decrease in respiration and microbial biomass after an increase in N availability has been
557 reported in many field and laboratory studies (Treseder, 2008). Importantly, decreased qCO2 would
558 be consistent with the copiotrophy theory stating that enhanced N availability increases rapidly
559 growing copiotrophic microbial species with higher carbon use efficiencies and faster turnover rates
560 (Fierer et al. 2012). The more copiotrophic community is less likely to decompose more recalcitrant
561 SOM which further reduces respiration and EEAs (Fierer et al., 2012; Ramirez et al., 2012). Reduced
562 respiration in the N amended microcosms is in line also with the priming effect theory predicting that
563 under higher N availability, r-strategist members of the decomposers utilize the less recalcitrant C
564 without the need of priming the decomposition of the recalcitrant SOM for N acquisition (Fontaine et
565 al., 2003; Chen et al., 2014). Changes in the community structure together with increased ribosomal
566 RNA copy numbers indicate that the decrease in microbial respiration in the N amended microcosms
567 is linked to an increase in r-strategist taxa that compete better at higher N concentrations.

568 Unexpectedly, this mechanism functioned similarly at both systems with stable N concentrations and
569 systems experiencing high seasonal variation in N availability.
570 Some field experiments in tundra ecosystems have shown increased microbial biomass,
571 respiration and enzyme activities after N fertilization (Koyama et al., 2013, Stark et al., 2014), while
572 other experiments have shown decreased microbial activity in response to N addition (Stark and
573 Grellmann, 2002). Furthermore, using the exactly same dosage of N addition in laboratory
574 incubations in sub-arctic soils, Hartley et al. (2010) found no effects of N addition on microbial
575 respiration. The reason why N addition leads to such differing consequences in different experiments
576 remains unknown. It is possible that soil microbial responses in field experiments are largely
577 mediated by changes in the C availability due to increasing plant biomass and shifts in plant species
578 composition, which affects litter and root exudate quality and quantity, rather than directly from
579 increased N availability. Moreover, laboratory incubations with sieved soil do not take into account
580 the plant-mycorrhiza interactions that may be important in determining the effects of increased N
581 availability (Leff et al., 2015). The effect of N fertilization on microbial activities and community
582 structure may also depend on the duration of the fertilization treatment (Koyama et al., 2013; 2014;
583 Campbell et al., 2010). High N concentrations may inhibit soil microorganisms directly by increasing
584 osmotic potential in soil solution or indirectly by decreasing pH or altering C availability (Treseder
585 2008). Similar to what was detected by Hartley et al. (2010), addition of NH_4NO_3 decreased soil pH,
586 which could be a possible explanation for the reduced respiration. However, the shift in the bacterial
587 community composition does not support this mechanism since the taxa that increased in abundance
588 in the N amended microcosms (*Actinobacteria, Gammaproteobacteria*) were ones that have been
589 reported to respond negatively to reduced pH, while *Acidobacteria* that decreased in abundance in
590 the N amended microcosms, have been correlated with lower pH (Lauber et al., 2009, Männistö et
591 al., 2007). Moreover, earlier studies have indicated that N induced decline in respiration is neither
592 linked to decrease in pH nor to the form of N utilized in the experiment (Ramirez et al., 2010).

5. Conclusions

This study showed drastic shifts in the tundra soil microbial community structure after N addition. However, contrary to our hypothesis, the responses of the bacterial communities to increased N availability were highly similar in N-poor and N-rich soils collected from habitats under light and heavy grazing, respectively. Increased abundance of members of *Actinobacteria* and *Gammaproteobacteria* in response to N addition with a concurrent decrease in *Acidobacteria* and respiration would support the theory of the increase of copiotrophic taxa, introduced by Fierer et al. (2012). However, another possible mechanism explaining the decrease in respiration and increase of the abundance of *Actinobacteria* would be the depletion of the labile C in the pre-incubated soils. Thus, while the community shift to a higher share of copiotrophic species may partially explain changes in the microbial activities, other mechanisms, such as the capacity to degrade recalcitrant C substances, are also likely. Changes in N availability likely affects the communities by multiple mechanisms, which shift the community structure to maintain the ecosystem functioning.

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References

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32-46.

617 Anderson, M.J., Willis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of
618 constrained ordination for ecology. Ecology 84, 511e525.

619 Baldrian, P., Větrovský, T., Cajthaml, T., Dobiášová, P., Petránková, M., Šnajdr, J., Eichlerová, I.,
620 2013. Estimation of fungal biomass in forest litter and soil. Fungal Ecology 6, 1-11.

621 Ball, A.S., Betts, W.B., McCarthy, A.J., 1989. Degradation of lignin-related compounds by
622 actinomycetes. Applied and Environmental Microbiology 55, 1642-1644.

623 Barder, M.J., Crawford, D.L., 1981. Effects of carbon and nitrogen supplementation on lignin and
624 cellulose decomposition by a streptomyces. Canadian Journal of Microbiology 27, 859-863.

625 Bernes, C., Bråthen, K., Forbes, B., Speed, J.M., Moen, J., 2015. What are the impacts of
626 reindeer/caribou (rangifer tarandus L.) on arctic and alpine vegetation? A systematic review.
627 Environmental Evidence 4, 1-26.

628 Brookes, P.C., Kragt, J.F., Powlson, D.S., Jenkinson, D.S., 1985. Chloroform fumigation and the
629 release of soil nitrogen: The effects of fumigation time and temperature. Soil Biology and
630 Biochemistry 17, 831-835.

631 Bugg, T.D., Ahmad, M., Hardiman, E.M., Singh, R., 2011. The emerging role for bacteria in lignin
632 degradation and bio-product formation. Current Opinion in Biotechnology 22, 394-400.

633 Campbell, B.J., Polson, S.W., Hanson, T.E., Mack, M.C., Schuur, E.A., 2010. The effect of nutrient
634 deposition on bacterial communities in arctic tundra soil. Environmental Microbiology 12, 1842-
635 1854.

636 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer,
637 N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E.,
638 Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R.,

639 Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010a.
640 QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335-336.

641 Caporaso, J.G., Bittinger, K., Bushman, F.D., DeSantis, T.Z., Andersen, G.L., Knight, R., 2010b.
642 PyNAST: A flexible tool for aligning sequences to a template alignment. Bioinformatics (Oxford,
643 England) 26, 266-267.

644 Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E.,
645 Kuzyakov, Y., 2014. Soil C and N availability determine the priming effect: Microbial N mining and
646 stoichiometric decomposition theories. Global Change Biology 20, 2356-2367.

647 Chu, H., Neufeld, J.D., Walker, V.K., Grogan, P., 2011. The influence of vegetation type on the
648 dominant soil bacteria, archaea, and fungi in a low arctic tundra landscape. 75, 1756-1765.

649 Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth, 192pp.

650 Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition.
651 Ecology 88, 2105-2113.

652 Deslippe, J.R., Hartmann, M., Simard, S.W., Mohn, W.W., 2012. Long-term warming alters the
653 composition of arctic soil microbial communities. FEMS Microbiology Ecology 82, 303-315.

654 Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics
655 (Oxford, England) 26, 2460-2461.

656 Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves
657 sensitivity and speed of chimera detection. Bioinformatics 27,
658 2194-2200.

659 Eilers, K.G., Lauber, C.L., Knight, R., Fierer, N., 2010. Shifts in bacterial community structure
660 associated with inputs of low molecular weight carbon compounds to soil. Soil Biology and
661 Biochemistry 42, 896-903.

662 Eisenlord, S.D., Freedman, Z., Zak, D.R., Xue, K., He, Z., Zhouc, J., 2013. Microbial mechanisms
663 mediating increased soil C storage under elevated atmospheric N deposition. Applied and
664 Environmental Microbiology 79, 1191–1199.

665 Eisenlord, S.D., Zak, D.R., 2010. Simulated atmospheric nitrogen deposition alters actinobacterial
666 community composition in forest soils. Soil Biology and Biochemistry **74**:1157–1166

667 Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria.
668 Ecology 88, 1354-1364.

669 Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative
670 metagenomic, phylogenetic and physiological analyses of soil microbial communities across
671 nitrogen gradients. The ISME journal 6, 1007-1017.

672 Findlay, R.H., King, G.M., Watling, L., 1989. Efficacy of phospholipid analysis in determining
673 microbial biomass in sediments. Applied and Environmental Microbiology 55, 2888-2893.

674 Fontaine S., Mariotti A., Abbadie L., 2003. The priming effect of organic matter: a question
675 of microbial competition? Soil Biology & Biochemistry 35, 837–843.

676 Frostegård, Å., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid

677 phosphate in soils of different organic content. Journal of Microbiological Methods 14, 151-163.

678 Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and
679 fungal biomass in soil. Biology and Fertility of Soils 22, 59-65.

680 Goldfarb, K.C., Karaoz, U., Hanson, C.A., Santee, C.A., Bradford, M.A., Treseder, K.K.,
681 Wallenstein, M.D., Brodie, E.L., 2011. Differential growth responses of soil bacterial taxa to carbon
682 substrates of varying chemical recalcitrance. Frontiers in microbiology 2, 94.

683 Goodfellow, M., Williams, S.T., 1983. Ecology of actinomycetes. Annual Review of Microbiology
684 37, 189-216.

685 Griffiths, R.I., Whiteley, A.S., O'Donnell, A.,G., Bailey, M.J., 2000. Rapid method for coextraction
686 of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based
687 microbial community composition. Applied and Environmental Microbiology 66, 5488-5491.

688 Hartley, I.P., Hopkins, D.W., Sommerkorn, M., Wookey, P.A. 2010. The response of organic matter
689 mineralisation to nutrient and substrate additions in sub-arctic soils. Soil Biology & Biochemistry 42,
690 92–100.

691 Hobbie, S., Nadelhoffer, K., Högberg, P., 2002. A synthesis: The role of nutrients as constraints on
692 carbon balances in boreal and arctic regions. Plant and Soil 242, 163-170.

693 Klappenbach, J.A., Dunbar, J.M., Schmidt, T.M., 2000. rRNA operon copy number reflects
694 ecological strategies of bacteria. Applied and Environmental Microbiology 66, 1328-1333.

695 Koyama, A., Wallenstein, M.D., Simpson, R.T., Moore, J.C., 2014. Soil bacterial community
696 composition altered by increased nutrient availability in arctic tundra soils. Frontiers in microbiology
697 5, 516.

698 Koyama, A., Wallenstein, M.D., Simpson, R.T., Moore, J.C., 2013. Carbon-degrading enzyme
699 activities stimulated by increased nutrient availability in arctic tundra soils. PloS one 8, e77212.

700 Lane, D.J., 1991. 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M.
701 (Eds.), Nucleic Acid Techniques in Bacterial Systematics. John Wiley and Sons,

New York, NY, pp. 115-175.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75, 5111-5120.

Leff, J. W., Jones, S.E., Prober, S.M., Barberán, A., Borer, E. T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M.H., McCulley, R.L., La Pierre, K., Risch, A.C., Seabloom, E.W., Schütz, M., Steenbock, C., Stevens,C.J., Fierer, N.,2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences 112, 10967-10972.

le Roes-Hill, M., Khan, N., Burton, S.G., 2011. Actinobacterial peroxidases: An unexplored resource for biocatalysis. Applied Biochemistry and Biotechnology 164, 681-713.

Lozupone, C., Hamady, M., Knight, R., 2006. UniFrac--an online tool for comparing microbial community diversity in a phylogenetic context. BMC bioinformatics 7, 371.

Lozupone, C., Lladser, M.E., Knights, D., Stombaugh, J., Knight, R., 2011. UniFrac: An effective distance metric for microbial community comparison. The ISME journal 5, 169-172.

Mack, M.C., Schuur, E.A.G., Bret-Harte, M., Shaver, G.R., Chapin, F.S., 2004. Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. Nature 431, 440-443.

Männistö, M.K., Häggblom, M.M., 2006. Characterization of psychrotolerant heterotrophic bacteria from finnish lapland. Systematic and Applied Microbiology 29, 229-243.

Männistö, M.K., Tiirola, M., Häggblom, M.M., 2007. Bacterial communities in arctic fjelds of finnish lapland are stable but highly pH-dependent. FEMS Microbiology Ecology 59, 452-465.

723 Männistö, M.K., Kurhela, E., Tiirola, M., Häggblom, M.M., 2013. *Acidobacteria* dominate the active
724 bacterial communities of Arctic tundra with widely divergent winter-time snow accumulation and
725 soil temperatures. FEMS Microbiology Ecology 84, 47-59.

726 Muyzer, G., de Waal, ,E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by
727 denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding
728 for 16S rRNA. Applied and Environmental Microbiology 59, 695-700.

729 Nemergut, D.R., Townsend, A.R., Sattin, S.R., Freeman, K.R., Fierer, N., Neff, J.C., Bowman,
730 W.D., Schadt, C.W., Weintraub, M.N., Schmidt, S.K., 2008. The effects of chronic nitrogen
731 fertilization on alpine tundra soil microbial communities: Implications for carbon and nitrogen
732 cycling. Environmental Microbiology 10, 3093-3105.

733 Nowinski, N., Trumbore, S., Schuur, E.G., Mack, M., Shaver, G., 2008. Nutrient addition prompts
734 rapid destabilization of organic matter in an arctic tundra ecosystem. Ecosystems 11, 16-25.

735 Oksanen, L., Virtanen, R., 1995. Topographic, altitudinal and regional patterns in continental and
736 suboceanic heath vegetation of northern Fennoscandia. Acta Botanica Fennica 153, 1-80.

737 Olofsson, J., Stark, S., Oksanen, L., 2004. Reindeer influence on ecosystem processes in the tundra.
738 Oikos 105, 386-396.

739 Olsson, P.A., 1999. Signature fatty acids provide tools for determination of the distribution and
740 interactions of mycorrhizal fungi in soil. FEMS microbiology ecology 29, 303-310.

741 Price, M.N., Dehal, P.S., Arkin, A.P., 2009. FastTree: Computing large minimum evolution trees
742 with profiles instead of a distance matrix. Molecular biology and evolution 26, 1641-1650.

743 Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M.A., Fierer, N., 2010. Consistent effects of
744 nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology 91, 3463-3470.

745 Ramirez, K.S., Craine, J.M., Fierer, N., 2012. Consistent effects of nitrogen amendments on soil
746 microbial communities and processes across biomes. Global Change Biology 18, 1918-1927.

747 Robinson, C., 2002. Controls on decomposition and soil nitrogen availability at high latitudes. Plant
748 and Soil 242, 65-81.

749 Ruess, L., Häggblom, M.M., García Zapata, E.J., Dighton, J. 2002. Fatty acids of fungi and
750 nematodesâ€"possible biomarkers in the soil food chain? Soil Biology and Biochemistry 34, 745-
751 756.

752 Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon
753 and nitrogen limitation in soil: A theoretical model. Soil Biology and Biochemistry 35, 549-563.

754 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski,
755 R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn,
756 D.J., Weber, C.F., 2009. Introducing mothur: Open-source, platform-independent, community-
757 supported software for describing and comparing microbial communities. Applied and
758 Environmental Microbiology 75, 7537-7541.

759 Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil
760 Biology and Biochemistry 42, 391-404.

761 Sistla, S.A., Asao, S., Schimel, J.P., 2012. Detecting microbial N-limitation in tussock tundra soil:
762 Implications for arctic soil organic carbon cycling. Soil Biology and Biochemistry 55, 78-84.

763 Stark, S. and Grellmann, D. 2002. Soil microbial responses to herbivory in an arctic tundra heath at
764 two levels of nutrient availability. *Ecology* 83, 2736-2744.

765 Stark, S., Männistö, M.K., Ganzert, L., Tiirola, M., Häggblom, M.M., 2015. Grazing intensity in
766 subarctic tundra affects the temperature adaptation of soil microbial communities. Soil Biology and
767 Biochemistry 84, 147-157.

768 Stark, S., Männistö, M., Eskelinen, A., 2014. Nutrient availability and pH jointly constrain microbial
769 extracellular enzyme activities in nutrient-poor tundra soils. Plant and Soil 383, 373-385.

770 Stark, S., Strömmer, R., Tuomi, J., 2002. Reindeer grazing and soil microbial processes in two
771 suboceanic and two subcontinental tundra heaths. Oikos 97, 69-78.

772 Stark, S., Väisänen, M., 2014. Insensitivity of soil microbial activity to temporal variation in soil N
773 in subarctic tundra: Evidence from responses to large migratory grazers. Ecosystems 17, 906-917.

774 Tanentzap, A.J., Coomes, D.A., 2012. Carbon storage in terrestrial ecosystems: Do browsing and
775 grazing herbivores matter? Biological Reviews 87, 72-94.

776 Treseder, K.K., 2008. Nitrogen additions and microbial biomass: A meta-analysis of ecosystem
777 studies. Ecology Letters 11, 1111-1120.

778 Vainio, E.J., Hantula, J., 2000. Direct analysis of wood-inhabiting fungi using denaturing gradient
779 gel electrophoresis of amplified ribosomal DNA. Mycological Research 104, 927-936.

780 Väisänen, M., Sjögersten, S., Large, D., Drage, T., Stark, S. 2015. Long-term reindeer grazing limits
781 warming-induced increase in C release: potential role of soil C quality. *Environmental Research*
782 *Letters* 10 094020. doi:10.1088/1748-9326/10/9/094020

783 Větrovský, T., Baldrian, P., 2013. The variability of the 16S rRNA gene in bacterial genomes and its
784 consequences for bacterial community analyses. PloS one 8, e57923.

785 Větrovský, T., Steffen, K.T., Baldrian, P., 2014. Potential of cometabolic transformation of
786 polysaccharides and lignin in lignocellulose by soil actinobacteria. PloS one 9, e89108.

787 Wallenstein, M.D., McMahon, S.K., Schimel, J.P., 2009. Seasonal variation in enzyme activities and
788 temperature sensitivities in arctic tundra soils. Global Change Biology 15, 1631-1639.

789 Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive bayesian classifier for rapid
790 assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental
791 Microbiology 73, 5261-5267.

792 White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie, R.J., 1979. Determination of the
793 sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40, 51-62.

794 Williams, B.L., Shand, C.A., Hill, M., O'Hara, C., Smith, S., Young, M.E., 1995. A procedure for the
795 simultaneous oxidation of total soluble nitrogen and phosphorus in extracts of fresh and fumigated
796 soils and litters. Communications in Soil Science and Plant Analysis 26, 91-106.

797 Zamin, T.J., Grogan, P., 2013. Caribou exclusion during a population low increases deciduous and
798 evergreen shrub species biomass and nitrogen pools in low arctic tundra. Journal of Ecology 101,
799 671-683.

800

801 Figure legends

802 Fig. 1. The effect of N amendment on the microbial biomass measures lipid phosphate, microbial N,
803 total PLFA, bacterial PLFA and fungal PLFA concentrations after 6 week incubation in soils
804 collected from lightly (LG) and heavily grazed (HG) tundra. Values are means ± S.E., N=5. Effect of
805 N amendment and grazing intensity are indicated by significance levels *, $p<0.05$ and **, $p<0.01$.

806 Fig. 2. Potential N-acetyl-glucosaminidase (NAG) and leucine aminopeptidase (LAP) activities
807 after 6 week incubation in N amended and control soils from lightly and heavily grazed tundra.
808 Effect of N amendment and grazing intensity are indicated by significance levels *, $p<0.05$ and **,
809 $p<0.01$.

810 Fig. 3. Effect of N amendment on the microbial respiration during the 6-week incubation in soils
811 collected from lightly and heavily grazed tundra. Effect of N amendment, grazing intensity and
812 incubation time are indicated by significance levels *, $p<0.05$ and **, $p<0.01$.

813 Fig. 4. Effect of N amendment on bacterial 16S rRNA gene and fungal 18S rRNA gene copies in the
814 DNA of lightly and heavily grazed soils after 6 week incubation. Effect of N amendment and grazing
815 intensity are indicated by significance levels *, $p<0.05$ and **, $p<0.01$.

816 Fig. 5. The relative abundance of bacterial sequences related to dominant taxa after 6 week
817 incubation of N amended and control soils from lightly and heavily grazed tundra. Values are means
818 ± S.E., N=5

819 Fig. 6. Faith's phylogenetic diversity (PD), species richness (Chao 1), dominance and observed
820 OTUs in N amended and control soils from lightly and heavily grazed tundra after 6 week
821 incubation. Effect of N amendment and grazing intensity are indicated by significance levels *,
822 $p<0.05$ and **, $p<0.01$.

823

824 Supplementary information

825 Table S1. The effects of nitrogen addition, grazing, incubation time, and their interactions on the
826 rates of microbial respiration of CO_2 during a 6-week laboratory incubation. F- and *P*-values are
827 obtained by the Linear Mixed Effects Model (LME). Significance levels $P < 0.05$ are indicated in
828 bold.

829 Table S2. The effects of N addition and grazing and their interactions on bacterial diversity indices.
830 F- and *P*-values are obtained by the Linear Mixed Effects Model (LME). Logarithmic (*)
831 transformations were used when necessary to meet the assumptions of LME. Significance levels $P <$
832 0.05 are indicated in bold.

833 Table S3. Average number of 50 most abundant OTUs in the lightly and heavily grazed soils with or
834 without N amendment. Significant effects of grazing intensity and N amendment were tested using
835 Linear Mixed Effects Model (LME). Logarithmic transformations (*) were used when necessary to
836 meet the assumptions of LME. Significance levels *, $p < 0.1$; **, $p < 0.05$; ***, $p < 0.01$

837 Figure S1. Canonical analysis of principal coordinates (CAP) of weighted and unweighted UniFrac distances
838 before (T0) and after 6-week laboratory incubation with (N) or without (C) nitrogen amendment. LG and HG
839 soils were collected from lightly and heavily grazed tundra, respectively. Vectors show Spearman correlations
840 of the dominant phyla or class with the CAP axes (only those with lengths >0.3 are shown).

841

Table 1. Soil properties under light and heavy grazing in Raisduoddar study site. Values are mean and S.E. in parentheses, N = 5.

Soil properties	Light grazing	Heavy grazing
SOM (%)	75.6 (2.3)	68.4 (8.0)
pH	5.1 (0.1)	5.2 (0.1)
NH_4-N (µg g^{-1} SOM)	15.0 (5.6)	61.7 (29.1)
NO_3-N (µg g^{-1} SOM)	3.1 (0.2)	9.8 (6.3)
Extractable organic N (µg g^{-1} SOM)	102.9 (13.4)	105.6 (17.2)
Microbial N (µg g^{-1} SOM)	495.5 (64.6)	504.8 (41.5)

Table 2. The effects of nitrogen addition and grazing and their interactions on microbial metabolic quotient (qCO_2), the potential activities of β-glucosidase (BG), N-acetyl-glucosamidase (NAG), acid-phosphatase (AP), and leucine-aminopeptidase (LAP), the concentration of lipid phosphate (lipid-PO_4), total, bacterial, and fungal PLFAs, microbial and inorganic N, and bacterial and fungal rRNA gene copy numbers after a 6-week laboratory incubation. F- and *P*-values are obtained by the Linear Mixed Effects Model (LME). Logarithmic (*) and square root (†) transformations were used when necessary to meet the assumptions of LME. Significance levels $P < 0.10$ are indicated by underline and $P < 0.05$ in bold.

		Nitrogen		Grazing		Nitrogen × Grazing	
		F	*P*	F	*P*	F	*P*
qCO_2		25.1	**< 0.001**	0.1	0.726	2.0	0.192
BG	OM	0.5	0.498	0.1	0.724	0.1	0.786
	$PLFA_{tot}$	1.2	0.316	0.1	0.826	0.0	0.991
NAG	OM*	4.0	<u>0.079</u>	4.3	<u>0.073</u>	1.1	0.330
	$PLFA_{tot}$	17.4	**0.004**	0.6	0.453	2.4	0.168
AP	OM	0.4	0.524	0.0	0.991	3.2	0.110
	$PLFA_{tot}$	2.0	0.202	0.2	0.683	2.9	0.131
LAP	OM	3.5	<u>0.079</u>	1.5	0.240	0.8	0.389
	$PLFA_{tot}$	0.1	0.711	0.0	0.892	0.3	0.622
NH_4-N $_{(log)}$		290.2	**< 0.001**	6.8	**0.031**	2.2	0.176
NO_3-N $_{(log)}$		329.6	**< 0.001**	2.5	0.156	1.4	0.276
Microbial N $_{(log)}$		9.8	**0.014**	0.2	0.704	1.1	0.334
Lipid-PO_4		11.3	**0.011**	1.1	0.409	0.8	0.409

Total PLFAs	23.5	**0.002**	2.5	0.154	0.3	0.576
Bacterial PLFAs	18.2	**0.004**	0.2	0.698	0.1	0.728
Fungal PLFAs	0.6	0.475	8.4	**0.020**	0.1	0.725
F:B ratio	4.8	0.065	5.7	**0.044**	0.3	0.632

Table 3. The effects of nitrogen addition and grazing and their interactions on the abundance of the main bacterial phyla after a 6-week laboratory incubation. F- and *P*-values are obtained by the Linear Mixed Effects Model (LME). Logarithmic (*) and square root (†) transformations were used when necessary to meet the assumptions of LME. Significance levels $P < 0.10$ are indicated by underline and $P < 0.05$ in bold.

	Nitrogen		Grazing		Nitrogen × Grazing	
Phylum	F	*P*	F	*P*	F	*P*
Acidobacteria all	17.4	**< 0.001**	2.2	0.157	2.3	0.149
Acidobacteria SD1	5.54	**0.032**	2.498	0.134	0.354	0.560
Acidobacteria SD2	15.36	**0.004**	1.41	0.269	3.912	0.083
Acidobacteria SD3	27.83	**<0.001**	7.144	**0.017**	4.754	**0.044**
Actinobacteria	17.5	**< 0.001**	0.0	0.983	0.0	0.983
Alphaproteobacteria	42.7	**< 0.001**	0.05	0.826	13.3	**0.006**
Betaproteobacteria	16.1	**0.004**	2.3	0.165	0.04	0.842
Deltaproteobacteria	86.3	**< 0.001**	0.17	0.688	1.2	0.311
Gammaproteobacteria	5.1	0.054	5.1	0.053	4.9	0.059
Bacteroidetes	16.6	**< 0.001**	0.48	0.51	19.0	**0.002**
Planctomycetes	117.3	**< 0.001**	1.5	0.240	0.8	0.389
Verrucomicrobia	59.4	**< 0.001**	3.1	0.098	1.5	0.235
Gemmatimonadetes	15.0	0.055	2.0	0.199	0.4	0.527
Chloroflexi	0.03	0.879	5.9	**0.041**	9.9	**0.014**

Table 4. The effects of nitrogen addition and grazing and their interactions on bacterial community composition after a 6-week laboratory incubation. Pseudo-F- and *P*-values were obtained by Permanova analyses of weighted and unweighted UniFrac distances or OTUs within the dominant phyla. Significance levels $P < 0.10$ are indicated by underline and $P < 0.05$ in bold.

	Nitrogen		Grazing		Nitrogen × Grazing	
	Pseudo-F	*P*	Pseudo-F	*P*	Pseudo-F	*P*
Whole community						
Weighted UniFrac	8.439	**0.004**	1.334	0.207	2.783	<u>0.062</u>
Unweighted UniFrac	1.652	<u>0.074</u>	1.061	0.285	1.147	0.364
OTUs	4.326	**0.002**	1.693	0.124	1.184	0.306
Phyla						
Acidobacteria	3.111	**0.018**	1.652	0.114	1.084	0.385
Actinobacteria	4.050	**0.003**	1.674	0.144	1.179	0.313
Bacteroidetes	5.500	**0.004**	1.491	0.150	0.786	0.661
Alphaproteobacteria	1.724	<u>0.076</u>	1.669	0.122	0.954	0.526
Betaproteobacteria	4.742	**0.005**	1.569	0.177	1.324	0.269
Deltaproteobacteria	7.283	**0.001**	1.049	0.344	1.480	0.212
Gammaproteobacteria	2.989	**0.015**	1.740	**0.041**	1.624	0.139
Planctomycetes	3.834	**0.007**	1.520	0.105	1.369	0.222
Verrucomicrobia	3.237	**0.017**	1.172	0.337	1.202	0.319

Figure 1

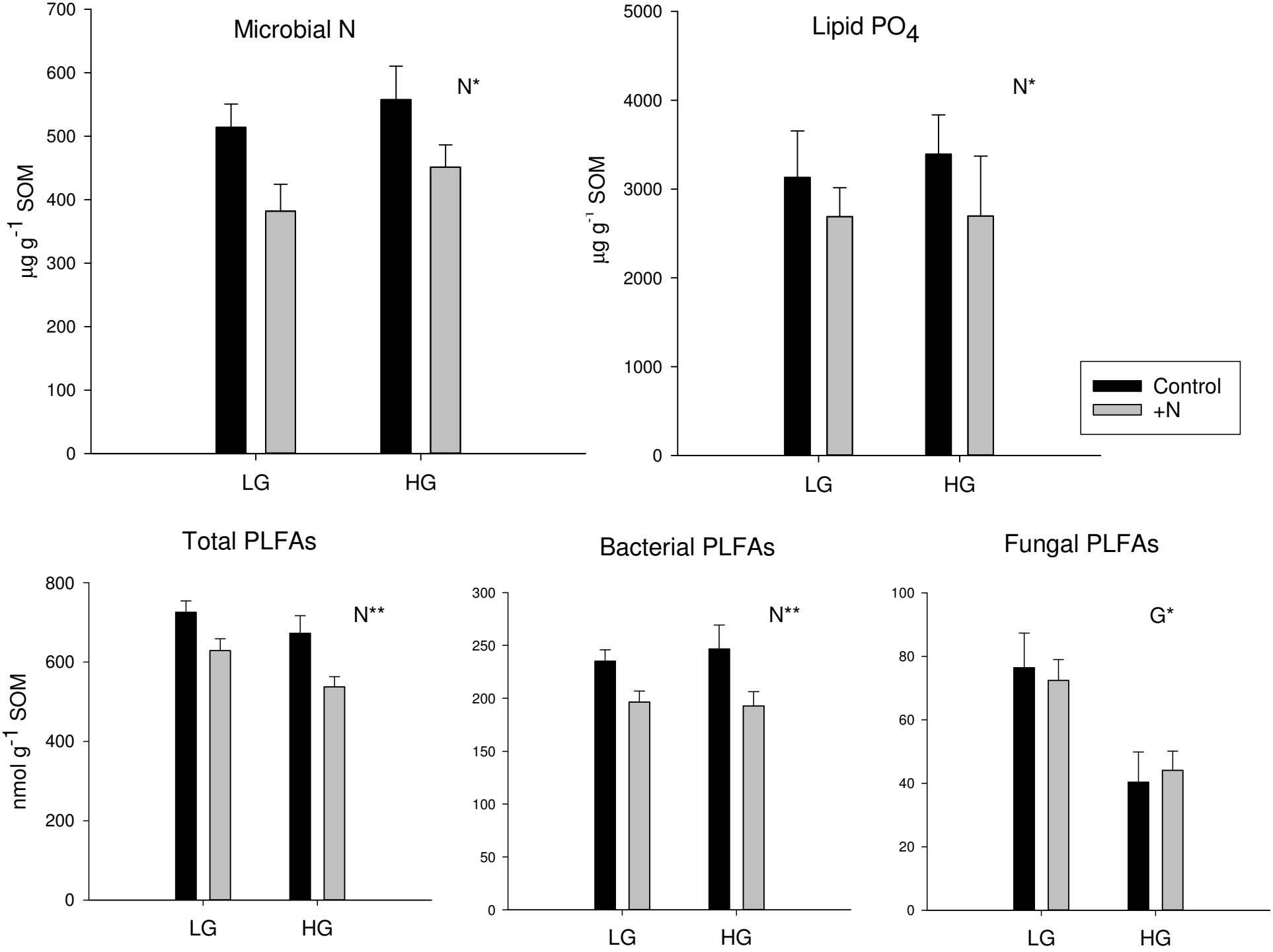

Microbial N
N*
µg g-1 SOM
700
600
500
400
300
200
100
0
LG
HG
Lipid PO4
N*
µg g-1 SOM
5000
4000
3000
2000
1000
0
LG
HG
Control
+N
Total PLFAs
N**
nmol g-1 SOM
800
600
400
200
0
LG
HG
Bacterial PLFAs
N**
300
250
200
150
100
50
0
LG
HG
Fungal PLFAs
G*
100
80
60
40
20
0
LG
HG

Figure 2

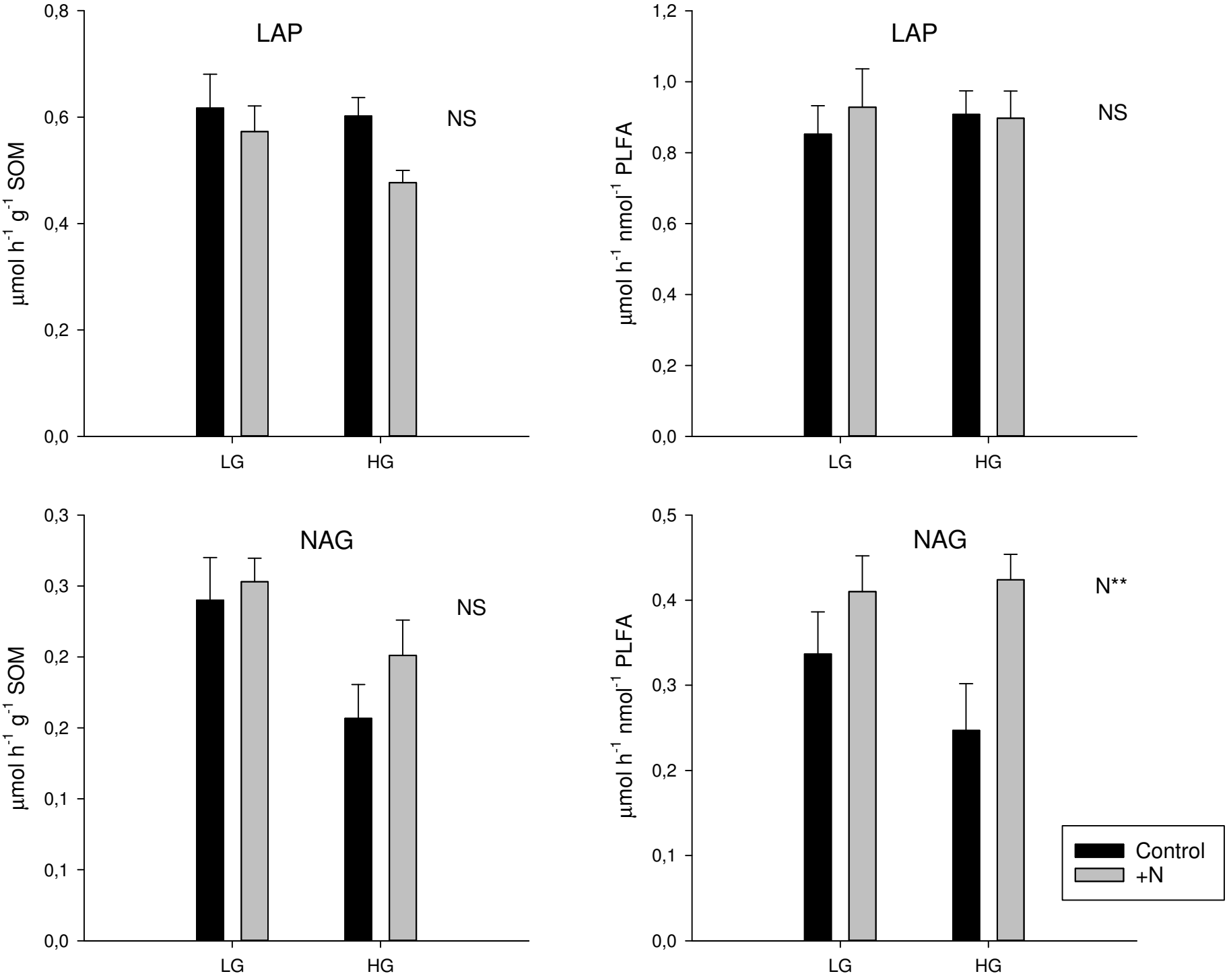

LAP
NS
µmol h-1 g-1 SOM
0,8
0,6
0,4
0,2
0,0
LG
HG
LAP
NS
µmol h-1 nmol-1 PLFA
1,2
1,0
0,8
0,6
0,4
0,2
0,0
LG
HG
NAG
NS
µmol h-1 g-1 SOM
0,3
0,2
0,1
0,0
LG
HG
NAG
N**
µmol h-1 nmol-1 PLFA
0,5
0,4
0,3
0,2
0,1
0,0
LG
HG
Control
+N

Figure 3

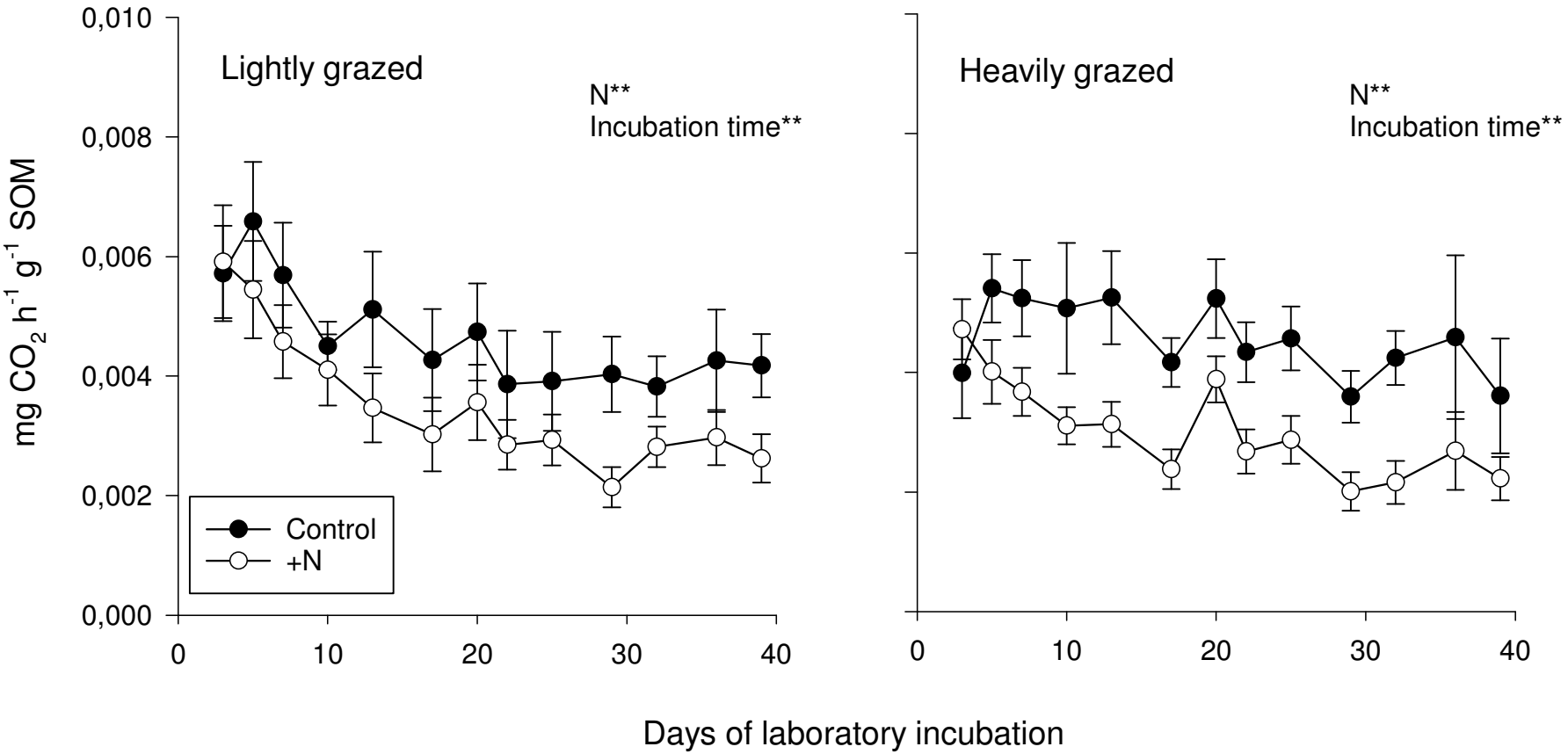
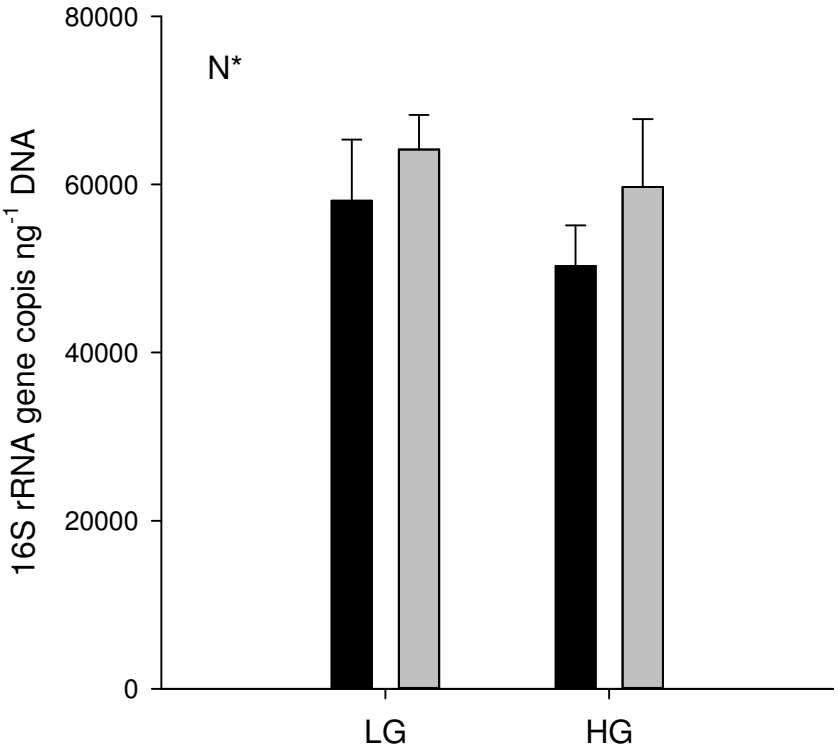

Lightly grazed
N**
Incubation time**
Heavily grazed
N**
Incubation time**
mg CO_2 h^{-1} g^{-1} SOM
0,010
0,008
0,006
0,004
0,002
0,000
Control
+N
0
10
20
30
40
Days of laboratory incubation

Figure 4


80000
60000
40000
20000
0
N*
16S rRNA gene copis ng-1 DNA
LG
HG

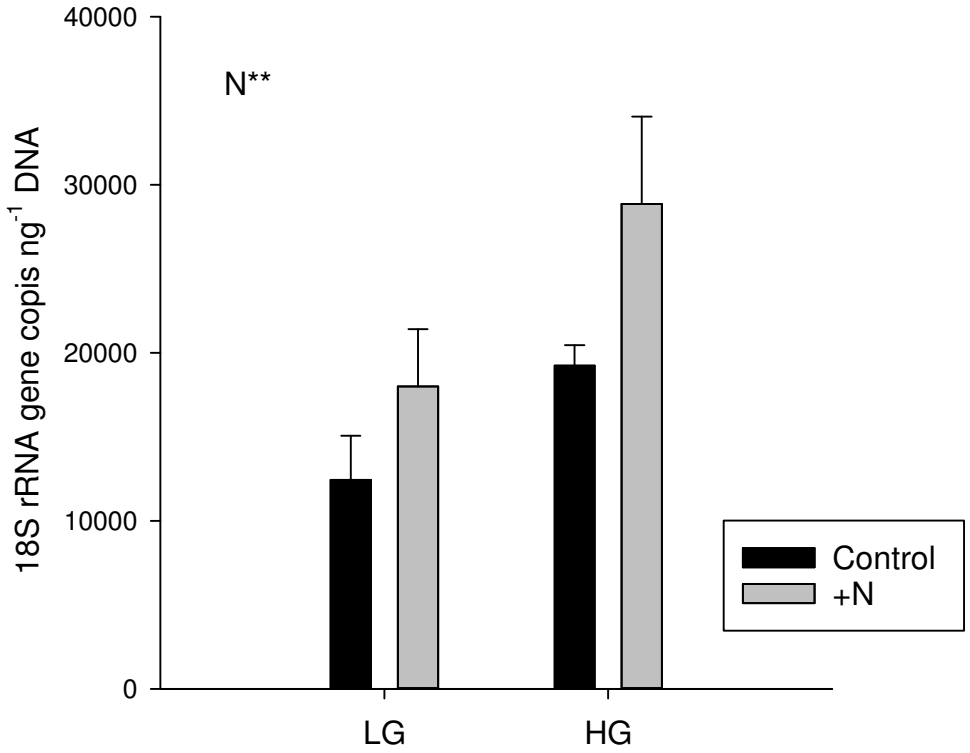

40000
30000
20000
10000
0
N**
18S rRNA gene copis ng-1 DNA
LG
HG
Control
+N

Figure 5

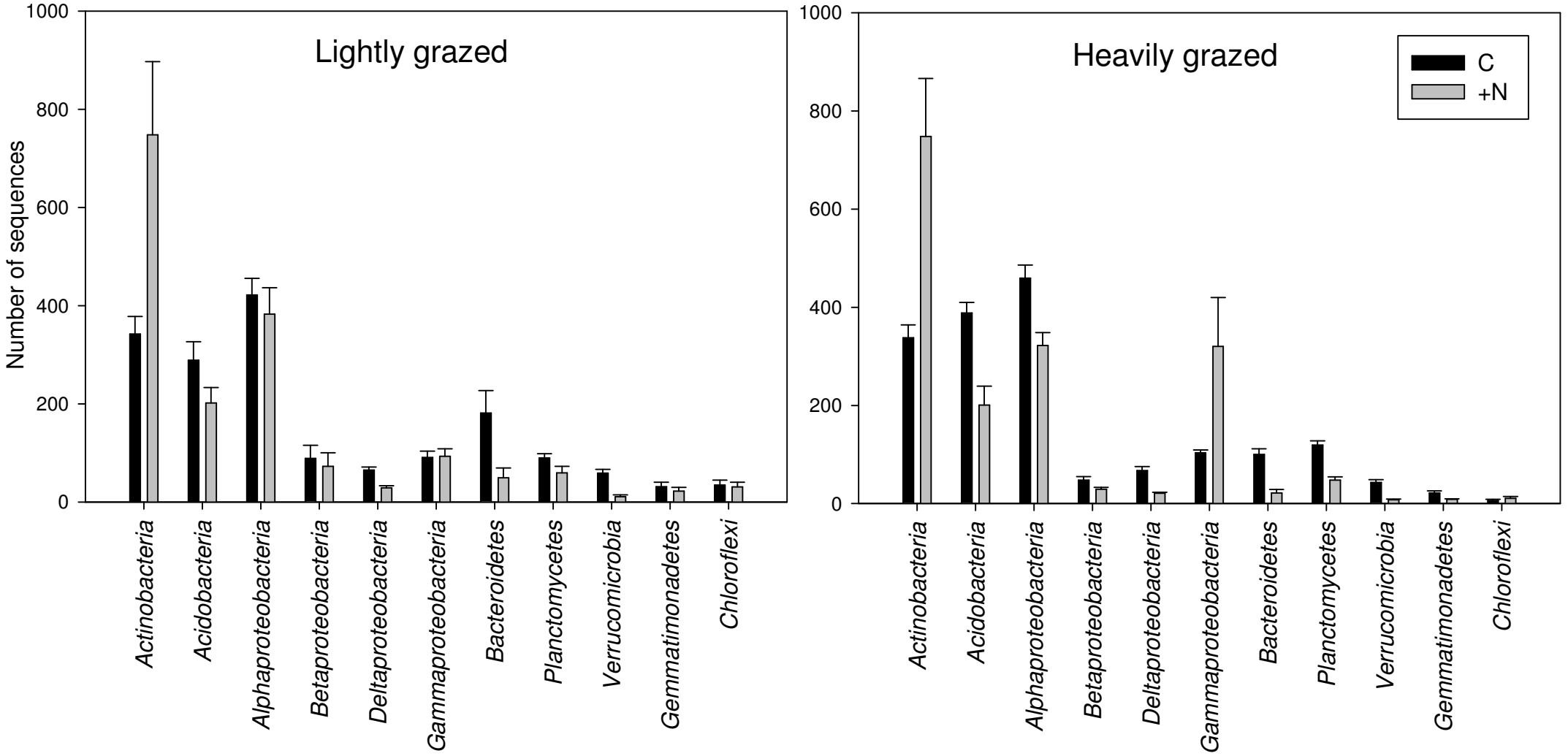

Lightly grazed
Heavily grazed
C
+N
Number of sequences
0
200
400
600
800
1000
Actinobacteria
Acidobacteria
Alphaproteobacteria
Betaproteobacteria
Deltaproteobacteria
Gammaproteobacteria
Bacteroidetes
Planctomycetes
Verrucomicrobia
Gemmatimonadetes
Chloroflexi

Figure 6

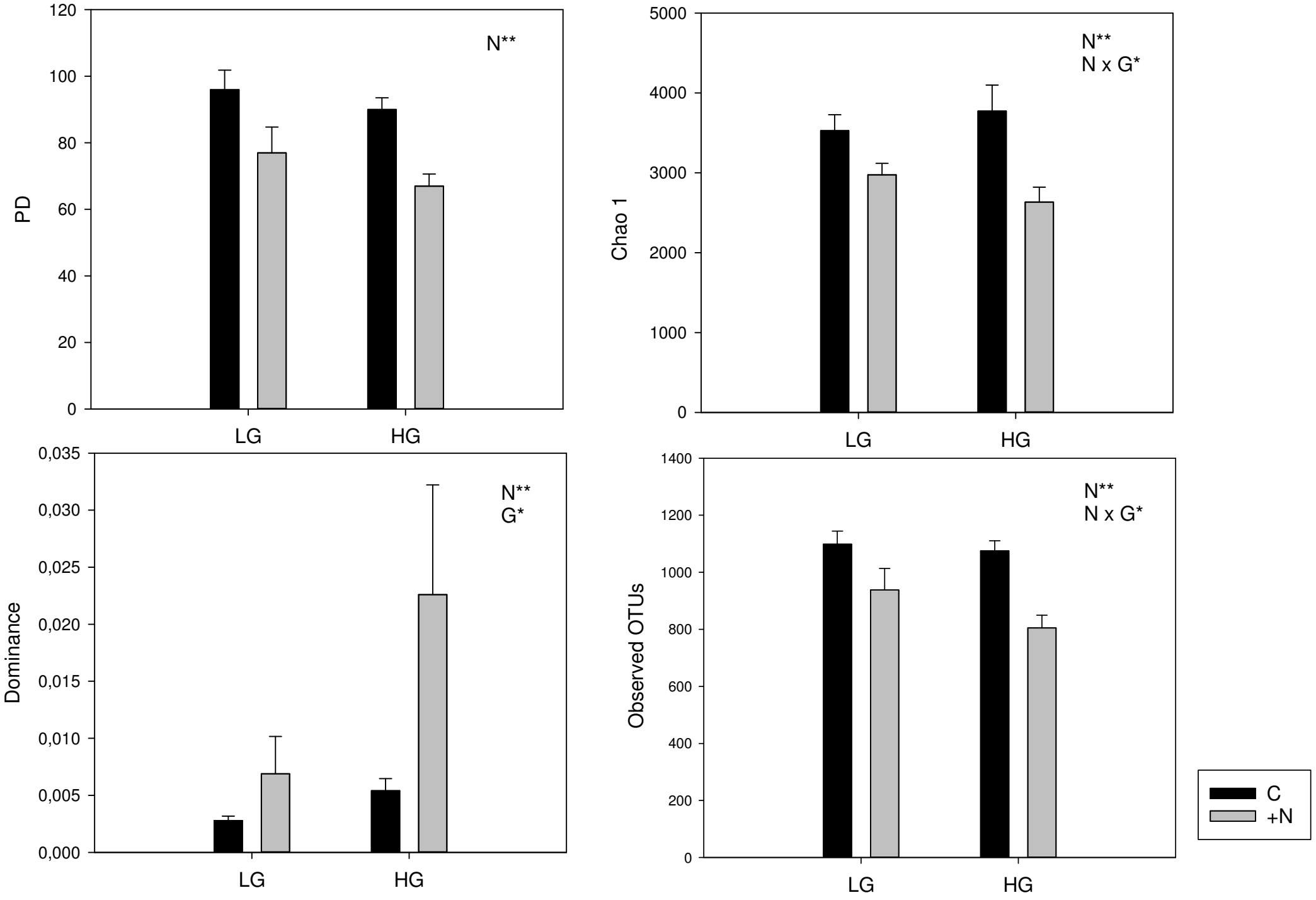

PD
120
100
80
60
40
20
0
N**
LG
HG
Chao 1
5000
4000
3000
2000
1000
0
N**
N x G*
LG
HG
Dominance
0,035
0,030
0,025
0,020
0,015
0,010
0,005
0,000
N**
G*
LG
HG
Observed OTUs
1400
1200
1000
800
600
400
200
0
N**
N x G*
LG
HG
C
+N

Fig S1

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Table S1

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Table S2

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Table S3

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