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

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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

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 <u>lower</u>
L351: LAP acticity was <u>lower</u>
L355: NAG activity was <u>higher</u>
L370: gene copies were <u>higher</u>
L372: <u>Higher</u> copy numbers
L379-383: abundance was <u>higher/lower</u>
L402: diversity and species richness were <u>lower,</u> dominance <u>higher</u>
L485: <u>higher</u> copy numbers
L491: abundance was <u>higher</u>
L493: abundance was <u>lower</u>
L519: detected at <u>high</u> abundance
L555: <u>lower</u> 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.

1

2

3 Do shifts in life strategies explain microbial community responses to increasing nitrogen

4 in tundra soil?

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21

24   ABSTRACT

25   Subarctic tundra soils store large quantities of the global organic carbon (C) pool as the

26   decomposition of plant litter and soil organic matter is limited by low temperatures and limiting

27   nutrients. Mechanisms that drive organic matter decomposition are still poorly understood due to our

28   limited knowledge of microbial communities and their responses to changing conditions. In subarctic

29   tundra large grazers, in particular reindeer, exert a strong effect on vegetation and nutrient

30   availability causing drastic nutrient pulses in the soils located along the migratory routes. Here we

31   studied the effect of increased nitrogen (N) availability on microbial community structure and

32   activities by laboratory incubations of soil collected from two sites with contrasting grazing

33   intensities. We hypothesized that heavily grazed soil experiencing nutrient pulses harbor more

34   copiotrophic taxa that are able to respond positively to increases in available N leading to increased

35   enzyme activities and respiration. Contrary to our hypothesis, there were only minor differences in

36   the microbial community composition between the lightly and heavily grazed soils. N amendment

37   shifted the bacterial community composition drastically, but the changes were similar at both grazing

38   intensities. The relative abundance of diverse *Actinobacteria* and *Rhodanobacter*-affiliated

39   *Gammaproteobacteria* increased in the N amended microcosms, while the abundance of

40   *Acidobacteria, Alphaproteobacteria, Deltaproteobacteria, Verrucomicrobia* and *Bacteroidetes*

41   decreased. Contrary to our hypotheses, increased N availability decreased respiration and microbial

42   biomass at both grazing intensities, while increased N availability had little influence on the

43   extracellular enzyme activities. We propose that similar to what has been reported in other systems,

44   elevated N availability suppressed microbial respiration and biomass by favoring copiotrophic

45   species with faster growth rates and with limited capabilities to decompose recalcitrant organic

46   matter. Similar responses in soils from contrasting vegetation types, soil organic matter (SOM)

47   quality and N availabilities in response to grazing intensity indicate that nutrient pulses may have a

48   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

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

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

74   C source.

75   Especially in Arctic tundra, another line of evidence suggests a positive effect of

76   increased N. In these extremely N-limited tundra soils, low N availability may limit the production

77   of extracellular enzymes and, hence, increased N availability may accelerate SOM decomposition by

78   enhancing microbial enzyme production (Schimel & Weintraub, 2003; Wallenstein et al., 2009;

79   Sistla et al., 2012), especially enzymes that hydrolyze C-rich compounds (Koyama et al., 2013; Stark

80   et al., 2014). Long-term fertilization experiments in Alaskan tundra have shown increased C losses

81   due to increased N availability in the soil organic layer (Mack et al., 2004), suggesting that increased

82   N availability subjects centuries old SOM to microbial decomposition (Nowinski et al., 2008).

83   The divergent results on the effects of N on microbial biomass and activities reflect the

84   close and complex inter-linkages between soil N and C cycles. The mechanisms by which microbial

85   communities influence the nutrient cycles are still poorly understood, but the community structure is

86   considered to be a key determinant of the functions (Fontaine et al. 2003; Fierer et al., 2012; Chen et

87   al., 2014; Leff et al., 2015). Both field and laboratory experiments have identified major shifts in

88   microbial community structure after fertilization treatment (Nemergut et al., 2008; Ramirez et al.,

89   2010; 2012; Fierer et al., 2012; Koyama et al., 2014; Leff et al., 2015) which are considered to reflect

90   concurrent changes in microbial biomass (Fierer et al., 2012), enzymatic activities (Koyama et al.,

91   2014) or soil respiration (Ramirez et al., 2010; 2012). It has been suggested that N availability is an

92   important determinant for the dominant life strategy of the soil microbial community (Fontaine et al.,

93   2003; Fierer et al., 2012; Chen et al., 2014; Leff et al., 2015). The priming effect theory predicts that

94   under N limitations, microbial communities are dominated by slow growing K-strategists

95   (oligotrophic species) that are able to mine nutrients in SOM whereas under abundant N

96   concentrations, microbial communities are dominated by fast-growing r-strategists (copiotrophic

97   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

layer (Stark et al., 2015). Prior to the experiment, soils were pre-incubated for two months at 4°C in order to deplete soils of plant-derived labile C substances with rapid turnover rate (e.g. root exudates). After the pre-incubation, soils were sieved (mesh 2 mm) and soil moisture (drying at 105ºC, 12 h), organic matter content (loss on ignition at 475ºC, 4 h), and water-holding capacity (WHC) determined. Sub-samples were also taken for analysis of soil N concentrations.

Soil microcosms were established by weighing 30 g of soil obtained from each of the five blocks per grazing treatment in 500 ml glass bottles capped with rubber stoppers. Soil moisture was adjusted to 30% of the WHC. N was added as NH_4NO_3 to the N amended (+N) microcosms from a 100 g^{-1} stock solution to a final concentration of 12.5 mg NH_4NO_3/g SOM, corresponding to 8.75 mg of N per gram of soil C (Hartley et al. 2010). Controls were amended with the same volume of milli-Q water. We used the same dosage of NH_4NO_3 as in Hartley et al. (2010), because these soils constituted the most similar soils in the literature compared with our site and thus created an interesting comparison. Soils were incubated for six weeks at 9°C after which soil was sampled for microbial activity, community composition and soil chemical analyses. The effect of NH_4NO_3 on soil pH was tested in a separate experiment by adding NH_4NO_3 and water to 10 g of soil in the same ratio. Soil pH was measured immediately and after 4 week incubation in 3:5 v/v soil:water suspensions (Denver Instrument Model 220).

2.2 Microbial respiration and extracellular enzyme activities

Microbial respiration (total CO_2-C release) was analyzed at selected time points from the headspace of the incubation bottles using an Agilent 6890N GC equipped with a ShinCarbon ST micropacked column (Restek) and thermal conductivity detector. Microbial metabolic quotient (qCO_2, respiration per biomass) was calculated after 6 weeks of incubation by dividing the respiration rate (mg CO_2 h^{-1} g^{-1} SOM) by total PLFA concentration (μmol PLFA g^{-1} SOM).

The effect of N addition on potential extracellular enzyme activities (EEAs) was tested after the 6 week incubation as described earlier (Stark et al., 2015). Briefly, betaglucosidase (BG), β-N-acetylglucosaminidase (NAG), acid-phosphatase (AP) and leucine aminopeptidase (LAP) activities were analyzed using para-nitrophenyl(pNP)-β-glucopyranoside (5 mM), pNP-β-N-acetylglucosaminide (3 mM), pNP-phosphate (5 mM) and leucine p-nitroanilide (5 mM) as substrates, respectively. Three g sub-samples of soil were suspended in 60 ml sodium acetate buffer (50 mM, pH 5.0), after which 100 µl of the sample was mixed with 200 µl of enzyme substrate in a 96-well plate. Blanks with 100 µl of the sample mixed with 200 µl of sodium acetate were prepared to account for the color in the soil slurry and the background absorbance of the enzyme substrate was analyzed using blanks with 100 µl of sodium acetate mixed with 200 µl of enzyme substrate. Following incubations at room temperature, samples were centrifuged and 100 µl of the supernatant transferred into another 96-well plate, 5 µl of 1.0 M NaOH added, and the absorbance was measured at 410 nm using a Multiscan FC microplate reader (Thermo Scientific).

2.3 Soil N, microbial biomass and community composition

Soil and microbial biomass N were analyzed after the 6 week incubation. A ~3 g subsample of soil was extracted with 50 mL of 0.5 M K_2SO_4 while another subsample was extracted using the same method after chloroform fumigation for 18 h (Brookes et al., 1985). The concentration of NH_4-N was determined from soil extracts according to the standard protocol (SFS 3032) using a Shimadzu UV-1700 spectrophotometer. NO_3-N was determined from soil extracts via flow analysis (FIA Perstorp). The total extractable N in both soil and fumigated extracts was oxidized to NO_3 (Williams et al., 1995) and then analyzed as NO_3-N (FIA, Perstorp). Microbial N was calculated by subtracting the total extractable N of the soil extracts from that of the fumigated extracts.

221   Phospholipid fatty acid (PLFA) and lipid phosphate (PO_4) concentrations were used as

222 proxies for soil microbial biomass (Findlay et al., 1989; Frostegård et al., 1991; Frostegård and

223 Bååth, 1996). Lipids for both analyses were extracted from 1 g (wet weight) of freeze-dried soil

224 using 10 ml of a one-phase mixture (1:2:0.8 v/v/v) of chloroform, methanol and 0.15 M citrate buffer

225 (pH 4.0) for 2 hours after which the tubes were centrifuged at 1500 x g for 10 min. The supernatant

226 was transferred to a new tube and the soil was re-extracted with 5 ml extraction solvent for 1 h,

227 centrifuged and supernatants from the first and second extraction combined. The phases were

228 separated by adding 4 ml of chloroform and 4 ml of citrate buffer. After overnight separation, the

229 lipids were separated into neutral lipids, glycolipids and phospholipids in silicic acid columns as

230 described by Frostegård et al. (1991). The phospholipid fraction was then subjected to mild alkaline

231 methanolysis (White et al. 1979) after which the fatty acid methyl esters were analyzed as described

232 by Männistö and Häggblom (2006). PLFA 18:2ω6c was used to indicate fungal biomass [including

233 saprotrophic, ectomycorrhizal and ericoid mycorrhizal fungi (Olsson 1999; Ruess et al. 2002)], while

234 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

235 cyclo-19:0 was used to indicate bacterial biomass (Frostegård and Bååth 1996). For the lipid-PO_4

236 analysis, 0.2 ml of the lipid phase (containing all lipid fractions) was pipetted to a new test tube and

237 dried under a stream of N. Lipids were digested with 1.8 ml saturated potassium persulfate by

238 incubating the samples at 95°C for 50 h. PO_4 was measured by the malachite green method as

239 described by Findlay et al. (1989) using a Shimadzu UV-1700 spectrophotometer.

240   Total genomic DNA was extracted from 0.25 g of soil using a modified phenol-

241 chloroform-isoamylalcohol (PCI) protocol after Griffiths et al. (2000). In brief, 0.5 g of acid-washed

242 and baked glass beads (0.1 mm) together with 500 µl PCI (ratio 25:24:1) and 500 µl cetrimonium

243 bromide buffer (CTAB) were added to the soil sample followed by bead beating on a Precellys 24

244 Dual homogenizer for 30 s at 5500 rpm. Samples were centrifuged (16000 x g, 5 min) and the

245 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

270   using the Ion OneTouch ES system and sequencing libraries were loaded on Ion 316 Chips and

271   sequenced using the Ion PGM Sequencing 400 Kit.

272   Quantitative PCR (qPCR) was performed in triplicate to evaluate bacterial 16S rRNA and

273   fungal 18S rRNA gene copy numbers using the CFX96 Real-time PCR detection system (Bio Rad).

274   qPCR for bacteria was done with the SsoFast Kit (BioRad) and the primer pair Eub341f (3'-CCT

275   ACG GGA GGC AGC AG-5') and Eub534r (3'-ATT ACC GCG GCT GCT GG-5') (Muyzer et al.,

276   1993). Each 15 µl reaction mixture contained 7.5 µl 2x SsoFast EvaGreen qPCR Supermix (Biorad),

277   0.375 µl of each primer (10 µM), 4.25 µl PCR grade water and 2.5 µl template in a 1000-fold

278   dilution. PCR conditions were 98°C for 2 min followed by 40 cycles of 98°C, 5 s; 56°C, 20 s

279   (following a plate read). Genomic DNA from *Granulicella tundricola* MPACTX9 was used as a

280   standard. qPCR for fungi was done with the KAPA Sybr Fast qPCR Kit (KAPA Biosystems) and

281   the primer pair FF390 (3'-CGA TAA CGA ACG AGA CCT-5') and FR1 (3'-AIC CAT TCA ATC

282   GGT AIT-5') (Vainio and Hantula, 2000). Each 15 µl reaction mixture contained 7.5 µl KAPA Sybr

283   Fast qPCR Mix (KAPA Biosystems), 0.375 µl of each primer (10 µM), 4.25 µl PCR grade water and

284   2.5 µl template in a 1000-fold dilution. PCR conditions were 97°C for 2 min followed by 40 cycles

285   of 95°C, 20 s; 50°C, 30 s and 72°C, 30 s (following a plate read). A plasmid containing the target

286   sequence amplified from *Phialocephala fortinii* was used as a standard for the fungal qPCR. A melt

287   curve read for each qPCR run was done after the last cycle from 65°C to 95°C in 0.5°C increments

288   for 5 s. In addition, qPCR products were checked on a 1% agarose gel for the correct size or the

289   appearance of additional bands.

290   Sequences were analyzed using Mothur (Schloss et al., 2009) and QIIME (Caporaso et al.,

291   2010a) software packages. Sequences shorter than 250 bp or which contained ambiguities and

292   homopolymer stretches of more than 8 bases were removed. Chimera check was performed using

293   UCHIME (Edgar et al., 2011) and singleton sequences were removed. After quality filtering 78403

294   reads were obtained from all samples, with an average of 3920 reads per sample (min = 2206, max =

295 6202). For downstream analyses all samples were rarefied to an equal sequence number to avoid

296 heterogeneity in sequencing depth which would affect calculations for α- and β-diversity. Sequences

297 were then clustered into operational taxonomic units (OTU) using UCLUST (Edgar, 2010) with a

298 sequence similarity value of 97%, and representative sequences were randomly chosen from each

299 cluster. Taxonomy assignment of the OTUs was based on the naïve bayesian RDP classifier (Wang

300 et al., 2007) using a SILVA-based reference database (www.mothur.org/wiki/Silva_reference_files)

301 with a confidence threshold of 60%. Alpha diversity indices (Faith's phylogenetic diversity, Chao1,

302 dominance and observed OTUs) were calculated using a script available in QIIME. Dominance is

303 here Dominance=1-Simpson index. For β-diversity analyses, representative OTU sequences were

304 aligned to the SILVA reference database using PyNAST (Caporaso et al. 2010b) and a phylogenetic

305 tree was build using FastTree (Price et al., 2009), followed by calculation of weighted and

306 unweighted UniFrac distance metrics (Lozupone et al., 2006; 2011).

307 Sequences were deposited in the Sequence Read Archive of NCBI under accession

308 number SRP069050 (runs SRR3133443, SRR3133444, SRR3133446 - SRR3133455, SRR3133457 -

309 SRR3133464).

310

311 2.4 Statistical analyses

312

313 The effects of N and grazing intensity on EEAs, soil and microbial N, lipid-PO_4, total-PLFA,

314 bacterial and fungal copy numbers, bacterial diversity indices and the relative abundances of

315 dominant bacterial taxa were tested using the linear mixed effects model (PASW 12.0 Statistical

316 software) with N addition (control, N+) and grazing intensity (light grazing, heavy grazing) as fixed

317 factors and block as a random factor nested within grazing intensity. The effects of N and grazing

318 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

344   concentrations of total extractable N was significantly increased by N addition, being 0.11 mg g^{-1}

345   SOM and 2.77 mg g^{-1} SOM in control and N-amended soils under light grazing, respectively, and

346   0.24 mg g^{-1} SOM and 3.29 mg g^{-1} in control and N-amended soils under heavy grazing, respectively

347   (Table 2). Soil pH at the beginning of the experiment was between 5.0 and 5.2, NH_4NO_3 addition

348   decreased pH on average by 0.67 units.

349

350   **3.2 Effect of grazing intensity and N addition on microbial activities**

351   When calculated per SOM LAP activity was lower and NAG activity higher in N amended

352   microcosms compared to the controls. When the enzyme activities were calculated per total PLFA

353   concentration (as a proxy for total biomass), there were no statistically significant differences in

354   LAP, BG or AP activities between the N amended and control microcosms or in lightly and heavily

355   grazed soils, but NAG activity was significantly higher in the N amended microcosms. Grazing

356   intensity affected only NAG activity (calculated per organic matter) which was lower in the heavily

357   grazed soil (Table 2; Fig. 2).

358   Microbial respiration declined over the 6 week incubation in both N amended and control

359   microcosms regardless of previous grazing intensity (Fig. 3, Table S1). However, respiration

360   declined more rapidly in the N amended microcosms. N addition resulted in a lower microbial

361   metabolic quotient (respiration per biomass) when calculated per total PLFA concentrations. In the

362   lightly grazed soils qCO_2 was 5.79 vs. 4.23 CO_2 h^{-1} mmol^{-1} PLFA for control vs. N amended

363   microcosms, respectively, compared to 5.78 vs. 4.25 mg CO_2 h^{-1} mmol^{-1} PLFA, in the heavily grazed

364   soils, indicating that decreased respiration was not solely caused by the decline in microbial biomass.

365

366   **3.3 Effect of N addition on microbial community structure in soils of different grazing intensity**

367   Quantitative PCR (qPCR) analysis was used to detect changes in the bacterial and fungal ribosomal

368   RNA gene copy numbers. Previous grazing intensity had no significant effect on bacterial copy

369   numbers, but fungal copy numbers were higher in the heavily than in the lightly grazed soil (Table 2,

370   Fig. 4). Both bacterial 16S rRNA and fungal 18S rRNA gene copies were higher in the DNA

371   extracted from the N amended microcosms compared to the control microcosms (Table 2, Fig. 4).

372   Higher copy numbers per ng DNA indicate that the composition of the bacterial and fungal

373   communities shifted in the N amended microcosms to species with a higher number of 16S/18S

374   rRNA gene copies. On the other hand, in soils under intensive grazing the fungal community

375   appeared to consist of species with higher 18S rRNA gene copy numbers while there was no

376   significant grazing effect on bacterial rRNA gene copy numbers (Fig. 4).

377   Bacterial communities were further compared by 16S rRNA gene sequence analysis.

378   Classification of sequences indicated minor differences in the abundance of different taxa in the

379   lightly and heavily grazed soils. However, the abundance of *Actinobacteria* was higher in N

380   amended soils of both grazing intensities and the abundance of *Gammaproteobacteria* was higher

381   especially in the N amended heavily grazed soils, while the abundance of members of *Acidobacteria,*

382   *Bacteroidetes*, *Alpha-, Beta-* and *Deltaproteobacteria, Planctomycetes* and *Verrucomicrobia* were

383   lower in N amended soils of both grazing intensities (Table 3, Fig. 5).

384   PERMANOVA analysis of the weighted UniFrac distances further indicated that after

385   6 week incubation the soil bacterial community structures were not significantly different in the

386   heavily and lightly grazed soils, but N addition shifted the communities under both grazing

387   intensities (Table 4). Shifts in the bacterial communities tended to differ in soils with different

388   grazing history (grazing x N interaction, p=0.062). PERMANOVA analysis of the unweighted

389   UniFrac distances indicated significant differences in the N amended vs. control microcosms but no

390   interaction with grazing intensity. To detect shifts in the community structure within the dominant

391   taxa, we tested the OTU data (abundance of sequences in different OTUs) of each phylum and in

392   addition different *Proteobacteria* and *Acidobacteria* classes separately. PERMANOVA analysis

393   indicated significant shifts within all major taxa in the N amended microcosms, while grazing

394 intensity affected only *Gammaproteobacteria* and no grazing x N interactions were detected (Table

395 4). Canonical analysis of principal coordinates (CAP, Anderson and Willis, 2003) was used to

396 visualize the grouping of samples based on the weighted and unweighted UniFrac distances.

397 Similarly as the other analyses, CAP indicated that the bacterial community structure was controlled

398 by N addition more than grazing history. Moreover, Spearman correlation of the CAP axes with

399 dominant bacterial taxa supported the strong correlation of *Gammaproteobacteria* and

400 *Actinobacteria* with the N amended soils (Fig. S1 in the supplementary materials). Comparison of α-

401 diversity indices indicated that the diversity (Faith's phylogenetic diversity) and species richness

402 (Chao1, observed species) were lower, but the dominance was higher in N amended microcosms of

403 soils from both grazing intensities (Fig. 6, Table S2 in the supplementary materials). There were no

404 significant differences in the species richness between the heavily and lightly grazed soils, but

405 dominance was significantly higher in the heavily grazed soils. Moreover, N amendment decreased

406 species richness more in the heavily than lightly grazed soil. These results indicate that while grazing

407 intensity had no effect on the bacterial diversity, there were more dominant groups in the heavily

408 grazed soils. N amendment increased the dominance in soils of both grazing intensities which

409 decreased the diversity and species richness especially in the heavily grazed soils.

410 Comparison of the relative abundance of the 50 most abundant OTUs in the N amended

411 microcosms identified several dominant OTUs that responded to the N amendment. The most

412 abundant OTUs that increased in the N amended microcosms were generally members of

413 *Actinobacteria* (orders *Actinomycetales, Acidimicrobiales* and *Solirubrobacterales*) and

414 *Gammaproteobacteria* (*Rhodanobacter* spp.) while those that responded negatively to N amendment

415 included members of *Alphaproteobacteria* (*Rhizobiales*), *Acidobacteria* and *Gammaproteobacteria*

416 (*Sinobacteraceae*) (Table S3 in the supplementary materials).

417

418 **4. Discussion**

419

4.1 Differences in microbial community composition in response to grazing intensity

421

We predicted that the bacterial community composition should be more copiotrophic in microcosms with soil from the heavily grazed site with higher mineral N concentrations and stronger seasonal fluctuation. Higher N concentrations in the soil solutions have been linked to a higher dominance of r-selected microorganisms which utilize simpler organic compounds and are not able to mine N by degrading recalcitrant SOM (Fontaine et al., 2003; Fierer et al., 2012; Chen et al., 2014; Leff et al., 2015). However, there were only minor differences in the bacterial community structures between the grazing intensities as evidenced by both qPCR analysis which indicated no shifts in the bacterial copy numbers and sequence analysis which indicated no significant differences in the bacterial community structure (weighted or unweighted UniFrac distances), diversity or abundance of most of the dominating taxa. Of the dominating taxa, only the abundance and diversity of *Gammaproteobacteria* differed between the lightly and heavily grazed soils. These differences resulted mainly from the higher abundance of *Rhodanobacter* affiliated OTUs in the soil from heavy grazing pressure. The minimal effects of grazing on bacterial community composition were surprising given the large differences in the plant community structures and N availability between soils under light and heavy grazing. In other soil biomes, members of *Acidobacteria* and *Verrucomicrobia* have been reported to correlate negatively, while *Actinobacteria, Bacteroidetes, Alpha-, Beta-* and *Gammaproteobacteria* have been found to correlate mostly positively with increased N availability in field studies (Nemergut et al., 2008; Cambell et al., 2010; Ramirez et al., 2010; Fierer et al., 2012; Koyama et al., 2014; Leff et al., 2015). Vegetation type and shifts in plant community composition after fertilization have been reported to influence bacterial community structure in the Alaskan tundra (Chu et al., 2011) and grasslands across the globe (Leff et al., 2015). 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 5×10^5 to more than 1×10^7 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).

593

5. Conclusions

595

596 This study showed drastic shifts in the tundra soil microbial community structure after N addition.

597 However, contrary to our hypothesis, the responses of the bacterial communities to increased N

598 availability were highly similar in N-poor and N-rich soils collected from habitats under light and

599 heavy grazing, respectively. Increased abundance of members of *Actinobacteria* and

600 *Gammaproteobacteria* in response to N addition with a concurrent decrease in *Acidobacteria* and

601 respiration would support the theory of the increase of copiotrophic taxa, introduced by Fierer et al.

602 (2012). However, another possible mechanism explaining the decrease in respiration and increase of

603 the abundance of *Actinobacteria* would be the depletion of the labile C in the pre-incubated soils.

604 Thus, while the community shift to a higher share of copiotrophic species may partially explain

605 changes in the microbial activities, other mechanisms, such as the capacity to degrade recalcitrant C

606 substances, are also likely. Changes in N availability likely affects the communities by multiple

607 mechanisms, which shift the community structure to maintain the ecosystem functioning.

608

613

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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

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

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	<u>0.065</u>	5.7	**0.044**	0.3	0.632

Table 3

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.

Phylum	Nitrogen		Grazing		Nitrogen × Grazing	
	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

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
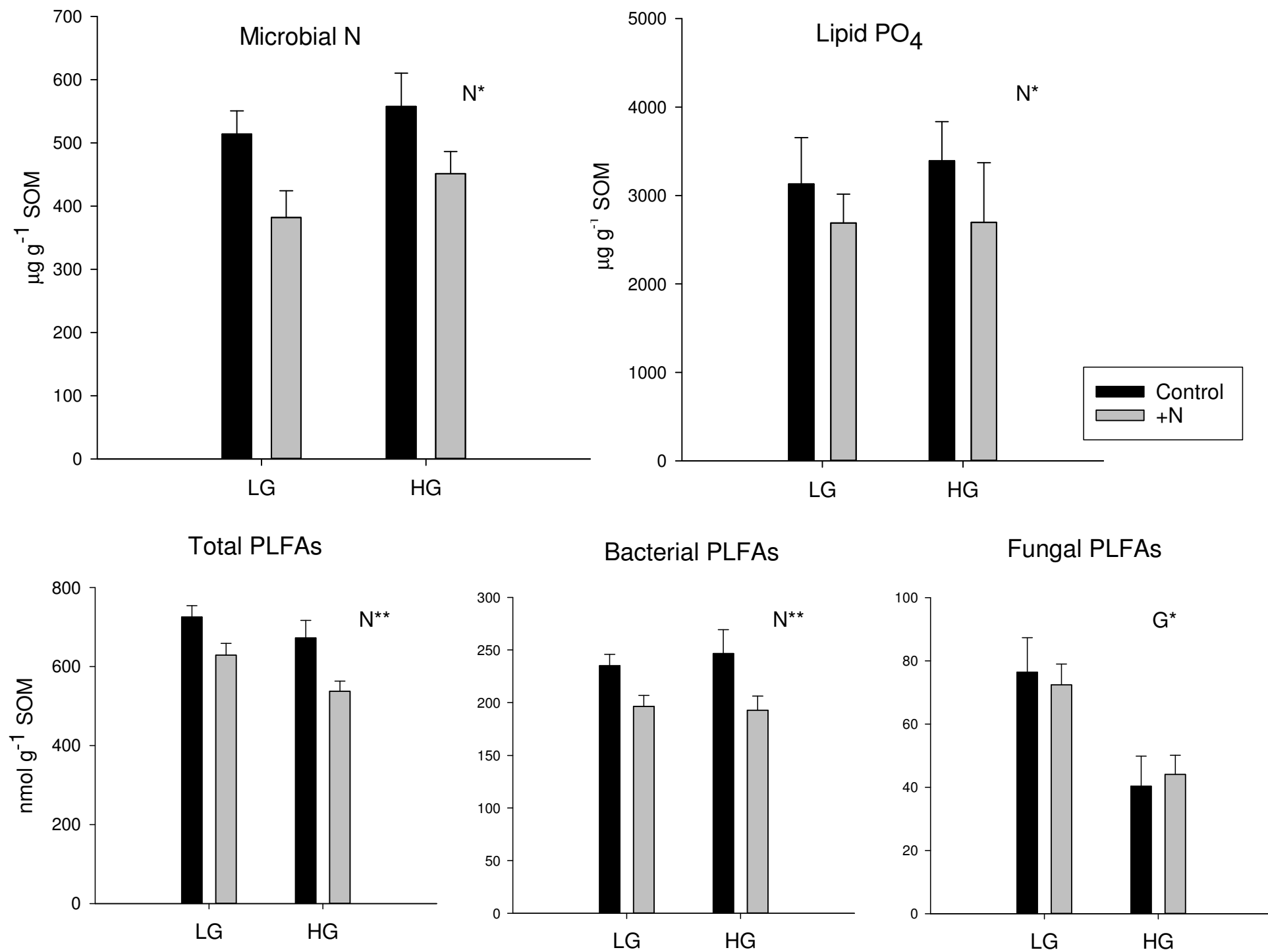
Figure 2
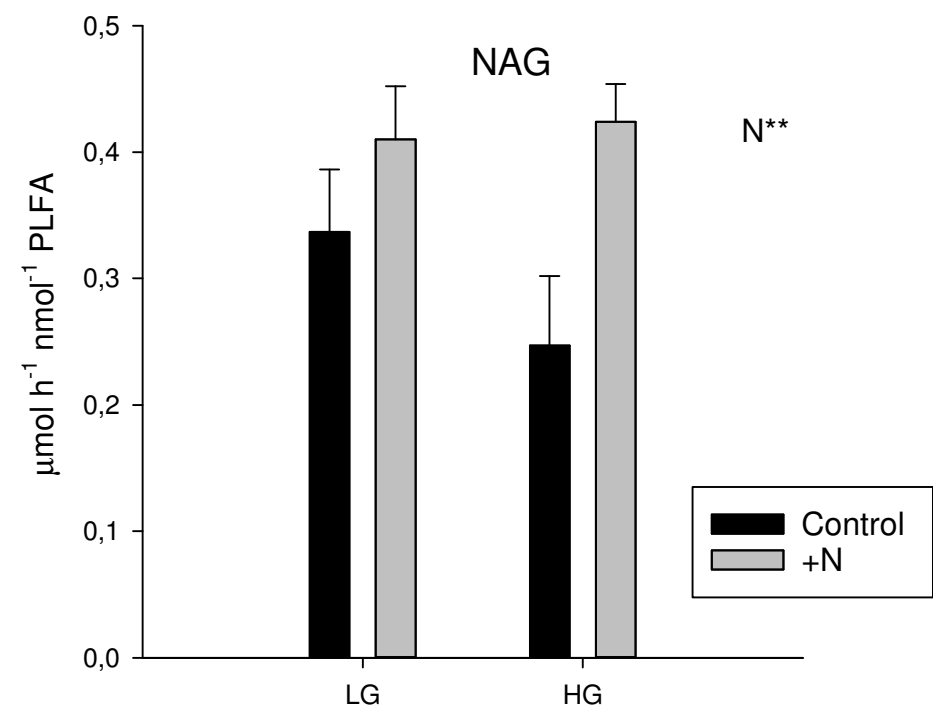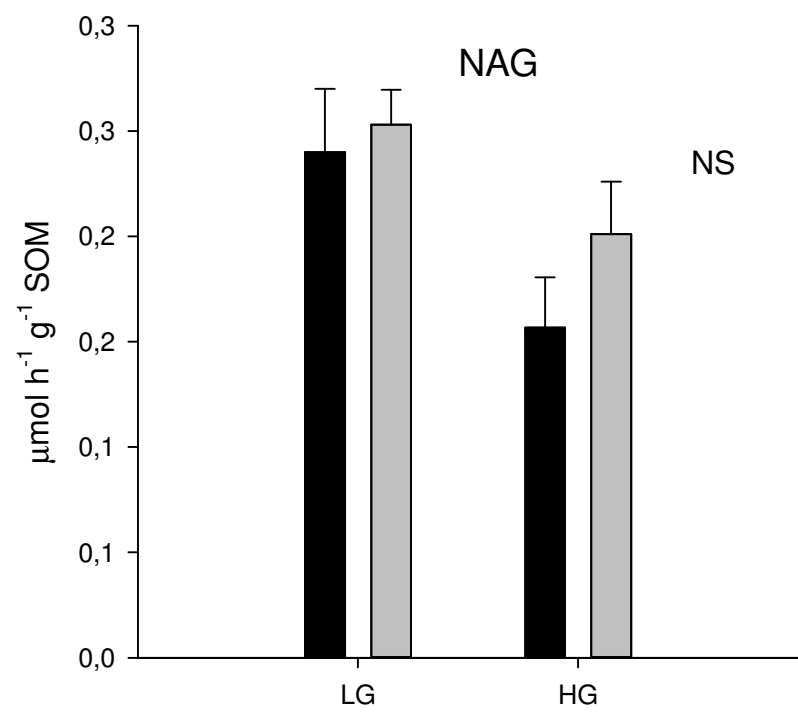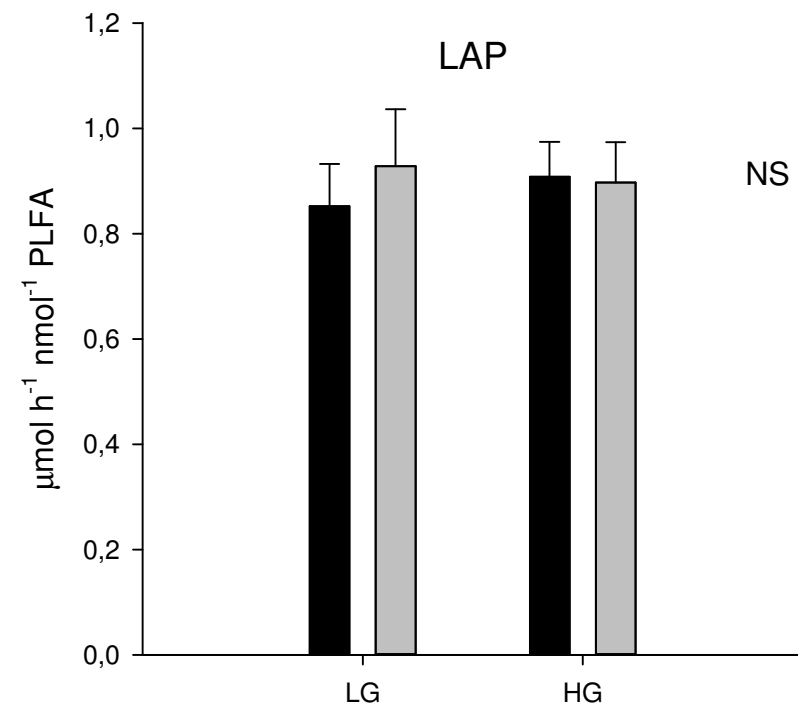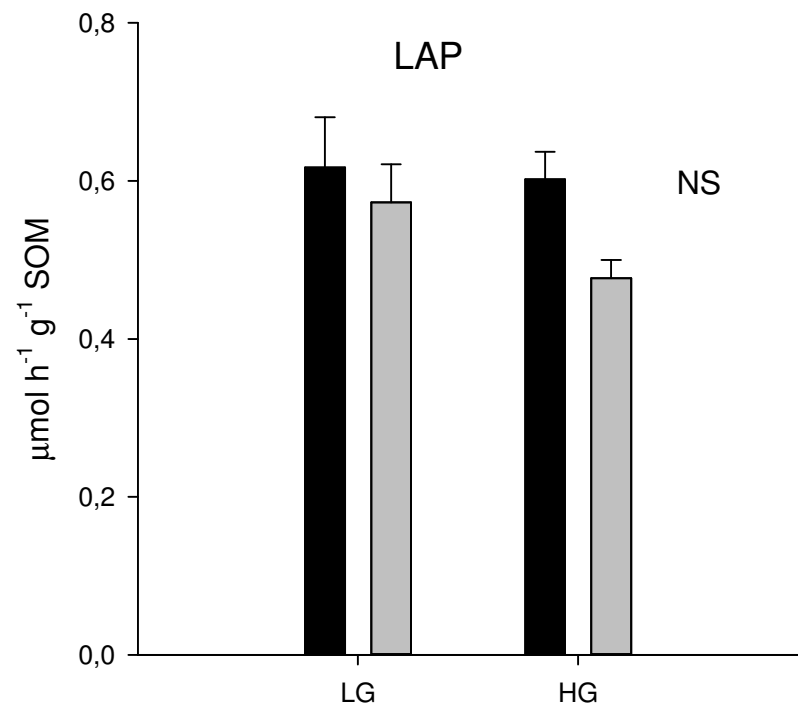
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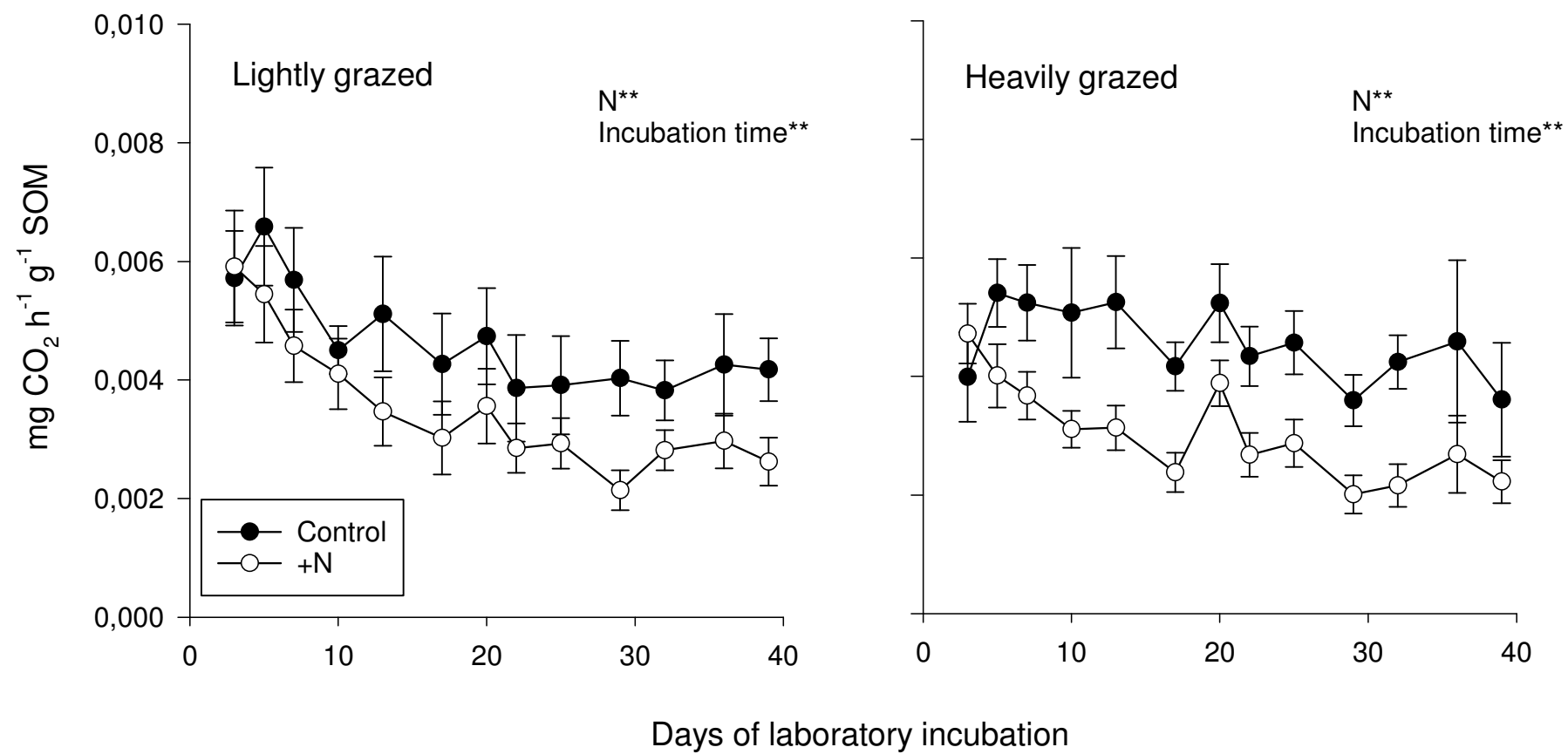
Figure 4

Figure 5

Figure 6

Fig S1

Table S1

Table S2

Table S3