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1 **Coupled carbon and nitrogen losses in response to seven years of chronic warming**  
2 **in subarctic soils**

3

4 Running title: **Coupled losses of C and N from subarctic soils**

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26

27 Research Article

28 **Abstract**

29 Increasing temperatures may alter the stoichiometric demands of soil microbes and  
30 impair their capacity to stabilize carbon (C) and retain nitrogen (N), with critical  
31 consequences for the soil C and N storage at high latitude soils. Geothermally active  
32 areas in Iceland provided wide, continuous and stable gradients of soil temperatures to  
33 test this hypothesis. In order to characterize the stoichiometric demands of microbes  
34 from these subarctic soils, we incubated soils from ambient temperatures after the  
35 factorial addition of C, N and P substrates separately and in combination. In a second  
36 experiment, soils that had been exposed to different *in situ* warming intensities (+0,  
37 +0.5, +1.8, +3.4, +8.7, +15.9 °C above ambient) for seven years were incubated after  
38 the combined addition of C, N and P to evaluate the capacity of soil microbes to store  
39 and immobilize C and N at the different warming scenarios. The seven years of chronic  
40 soil warming triggered large and proportional soil C and N losses ( $4.1 \pm 0.5 \% \text{ } ^\circ\text{C}^{-1}$  of  
41 the stocks in unwarmed soils) from the upper 10 cm of soil, with a predominant  
42 depletion of the physically accessible organic substrates that were weakly sorbed in soil  
43 minerals up to 8.7 °C warming. Soil microbes met the increasing respiratory demands  
44 under conditions of low C accessibility at the expenses of a reduction of the standing  
45 biomass in warmer soils. This together with the strict microbial C:N stoichiometric  
46 demands also constrained their capacity of N retention, and increased the vulnerability  
47 of soil to N losses. Our findings suggest a strong control of microbial physiology and  
48 C:N stoichiometric needs on the retention of soil N and on the resilience of soil C stocks  
49 from high-latitudes to warming, particularly during periods of vegetation dormancy and  
50 low C inputs.

51

52

53 **Keywords:** Substrate induced respiration, microbial biomass, temperature increase,  
54 nitrogen immobilization, microbial carbon and nutrients limitation, nitrogen loss

## 55 **1. Introduction**

56 Global warming is expected to accelerate the decomposition of soil organic matter  
57 (SOM) more than its production, causing large releases of CO<sub>2</sub> to the atmosphere and  
58 positive feedbacks to the climatic system (Davidson and Janssens et al. 2006, Jenkinson  
59 et al. 1991). Soils at northern latitudes store more than half of the surface-soil carbon  
60 (C) (Tarnocai et al. 2009). As their SOM decomposition has been strongly limited by  
61 low temperatures and they are warming more rapidly, they are particularly vulnerable to  
62 temperature driven C losses (Smith et al. 2015, Crowther et al. 2016). As such, warming  
63 of northern soils may potentially increase global concentrations of atmospheric CO<sub>2</sub>  
64 (McGuire et al. 2009). Model predictions for future CO<sub>2</sub> emissions and climate change  
65 projections by the Intergovernmental Panel on Climate Change (IPCC) remain,  
66 nonetheless, largely uncertain (Friedlingstein et al. 2006, Todd-Brown et al. 2013),  
67 partly due to the lack of accurate representation of vegetation and soil microbial  
68 feedbacks (Bardgett et al. 2013, Friedlingstein et al. 2006) and interactions between C  
69 and nutrient cycles (Bardford et al. 2016, Friedlingstein et al. 2006).

70

71 The coupling between C and nitrogen (N) biogeochemical cycles is especially tight in  
72 northern ecosystems. Low temperatures constrain the depolymerization and  
73 mineralization rates of soil organic N and the release of N-monomers and mineral N,  
74 thus limiting plant productivity (Hobbie et al. 2002, Schimel and Bennett 2004, Todd-  
75 Brown et al. 2013). Rising temperatures are expected to accelerate the rates of microbial  
76 N transformations and alleviate the plant N limitations in these ecosystems, thus  
77 increasing plant productivity and C inputs to the soil (Dormann and Woodin 2002,  
78 Natali et al. 2012, Wu et al. 2011). Increases in vegetation productivity at warmer  
79 temperatures can even offset the soil C losses associated with the accelerated SOM  
80 mineralization rates from soil microbes (Melillo et al. 2002, Sistla et al. 2013, IPCC  
81 2013). The vulnerability of soil C stocks to warming will therefore depend on the  
82 capacity of soils to retain nutrients and ultimately on the ability of plants to profit from  
83 the enhanced nutrient availability.

84

85 Soil microbial biomass plays a fundamental role in the stabilization of soil C (Liang et al.  
86 2017, Miltner et al. 2012) and as a short- and long-term N reservoir in soils at high  
87 latitudes (Bardgett et al. 2003, Zogg et al. 2000). A large fraction of the N pool in these  
88 cold ecosystems is contained in microbial biomass (Jonasson et al. 1996, Xu et al.

89 2013). This large N storage potential and the low N mineralization rates imply that  
90 microbes successfully compete with plants for the limiting N pools during the growing  
91 season (Dunn et al. 2006, Skouw Haugwitz et al. 2011), but also that microbial turnover  
92 and N release may represent a major pathway for plant N uptake during periods of  
93 declining microbial populations (Bardgett et al. 2003). Microbial N retention becomes  
94 even more crucial in ecosystems with a period of vegetation dormancy or senescence,  
95 such as at high latitudes, when the short photoperiod and low temperatures prevent  
96 vegetation productivity and N uptake (Bardgett et al. 2005). Microbial immobilization  
97 then becomes a crucial mechanism to minimize potential N losses from the system  
98 during relatively long winter periods (Groffman et al. 2011, Jonasson et al. 1996, Kaiser  
99 et al. 2011). Warming can, however, desynchronize the intimate seasonal coupling  
100 between microbial N immobilization and vegetation uptake in these ecosystems  
101 (Bardgett et al. 2005, Jaeger et al. 1999, Lipson et al. 1999), leading to potential soil N  
102 and C losses.

103

104 The physiological response of soil microbes to warmer temperatures may elicit shifts in  
105 their resource demands, and cause disequilibria on plant-microbial interactions.  
106 Although vegetation growth is generally N limited at high latitude ecosystems, C has  
107 been found to limit soil microbial growth and biomass even at these high latitudes (Wild  
108 et al. 2015). Warmer temperatures may cause persistent increases in microbial  
109 respiratory demands and the depletion of the most physically accessible organic  
110 substrates in soil (Marañón-Jiménez et al. 2018), thus compromising the C available to  
111 maintain constant levels of standing biomass. According to the ecological  
112 stoichiometric theory, soil microbes regulate their elemental composition by retaining  
113 elements in which they are limited and releasing those in excess (Serner and Elser  
114 2002). This implies a predominance of microbial N mineralization to N immobilization  
115 in strongly C-limited microbes. Warming-induced increases in N mineralization during  
116 periods of inactive plant N uptake and accessible C inputs may consequently lead to  
117 potential losses of soil N by dissimilatory pathways, either by nitrate leaching or  
118 gaseous N fluxes (Turner and Henry 2010). Temperature-driven N losses may account  
119 for the smaller increase in plant productivity compared to net N mineralization and soil  
120 respiration rates frequently observed in experimental warming experiments (Bai et al.  
121 2013, Lu et al. 2013, Rustad et al. 2001), causing divergences between observed and  
122 predicted soil C losses for high latitudes (Todd-Brown et al. 2013, McGuire et al. 2018).

123 The potential changes in the capacity of subarctic soils to retain N have not been  
124 explored mechanistically yet, even though this information is fundamental to constrain  
125 the climate change projections of productivity and soil organic C (SOC) of northern  
126 ecosystems.

127

128 Geothermally active areas in Iceland provide stable, continuous and wide gradients of  
129 soil temperature (Sigurdsson et al. 2016) that encompass the full range of warming  
130 scenarios projected by the IPCC for the northern region (IPCC, 2013). This allow  
131 testing for non-linear responses to soil warming and the inference of realistic  
132 predictions of soil biogeochemical processes. Previous studies at the same experimental  
133 plots from these soil temperature gradients found a linear reduction of  $1.28 \pm 0.16$  ton  
134 SOC ha<sup>-1</sup> per °C degree of warming from the upper 10 cm of soil (Leblans et al. 2016).  
135 Warming increased C losses by accelerating the mass-specific C mineralization rates of  
136 soil microorganisms (Marañón-Jiménez et al. 2018, Walker et al. 2018). Surprisingly,  
137 enhanced N mineralization in these N-limited soils did not lead to higher vegetation  
138 productivity according to the predictions of most ecosystem models (Todd-Brown et al.  
139 2013). On the contrary, aboveground and belowground plant biomass did not change.  
140 Vegetation apparently did not benefit from the N released at higher temperatures,  
141 probably due to ecosystem N losses. Despite the large and rapid loss of soil C, soil C:N  
142 stoichiometry indeed remained unaltered (Leblans et al. 2016), implying a proportional  
143 loss of N.

144 In order to assess the mechanisms underlying this coupled soil C and N loss, we  
145 incubated soils that had been exposed for seven years to a range of warming intensities  
146 in the field due to geothermal activity (0 - 15.9 °C above ambient, hereafter “*in situ*  
147 temperatures”). In a first set of soil incubations, the factorial addition of C, N and P  
148 substrates separately and in combination to soils from ambient temperatures allowed us  
149 to characterize the stoichiometric demands of the microbes from these subarctic soils  
150 (hereafter “experiment of stoichiometric demands characterization”). In a second set of  
151 soil incubations, the combined addition of C, N and P to the warmed soils along the  
152 geothermal gradient allowed us to evaluate the capacity of soil microbes to store and  
153 immobilize C and N as affected by different warming scenarios, both at ambient  
154 nutrient conditions and when C, N and P are plentiful (hereafter “experiment of  
155 warming impacts on soil C and N retention”). Regarding the microbial stoichiometric

156 demands from these subarctic soils, we hypothesized that soil microbes have strong C  
157 limitation due to the short growing period for vegetation (low C inputs) and the high  
158 clay content of these soils (high physical protection). We also hypothesized that this C  
159 limitation and a restricted C:N stoichiometric plasticity of soil microbes limit the  
160 immobilization of mineralized N. Regarding the warming impacts on soil C and N  
161 retention, the total losses of C from these (Leblans et al. 2016, Poeplau et al. 2016) and  
162 many other soils (Hicks Pries et al. 2017, Crowther et al. 2016, Melillo et al. 2017)  
163 exposed to warmer temperatures, and the increasing mass-specific respiration rates of  
164 soil microbes (Marañón-Jiménez et al. 2018), led us to hypothesize a depletion of the  
165 most physically accessible substrates in soil. We also hypothesized that these C scarcity  
166 conditions in warmer soils impair the C retention by microbial biomass and the  
167 immobilization of the mineralized N that is released from SOM at warmer temperatures.  
168 These two complementary experiments will therefore contribute to elucidate the causes  
169 of the divergences on the soil C losses between field warming experiments and model  
170 predictions at high latitude ecosystems.

171

## 172 **2. Methods**

### 173 *2.1. Study site*

174 Soils were collected at the ForHot research site in the Hengil geothermal area, 40 km  
175 east of Reykjavik, Iceland (64°00'01"N, 21°11'09"W; 83-168 m a.s.l.), which has been  
176 described in detail by Sigurdsson et al. (2016). Mean annual air temperature, annual  
177 precipitation and wind speed were 5.2 °C, 1460 mm and 6.6 m s<sup>-1</sup>, respectively  
178 (Synoptic Station, 9 km south of Hveragerdi, Icelandic Meteorological Office, 2016).  
179 The mean temperatures of the warmest and coldest months, July and December, were  
180 12.2 and -0.1 °C, respectively. The growing season normally starts in late May and ends  
181 in late August. Snow cover is not permanent during winter due to the mild oceanic  
182 climate, but the soil typically freezes for at least two months during mid-winter. The  
183 main vegetation type is unmanaged grassland, dominated by *Agrostis capillaris*,  
184 *Ranunculus acris* and *Equisetum pratense*, all perennial species with short-lived  
185 aboveground parts that regrow each year from underground stems or rhizomes. Sites  
186 had been grazed by sheep for centuries (low-intensity grazing), but this practice was  
187 ceased in the 1970's (Sigurdsson et al. 2016).

188



189 The soil in the area has been subjected to warming since May 2008 due to geothermal  
190 activity, when an earthquake shifted geothermal systems to previously unwarmed soils.  
191 Hot groundwater warmed the underlying bedrock and surfaced along faults in the soil  
192 crust. Soil temperatures were highest near these faults and declined perpendicular to  
193 them. No signs of soil contamination by geothermal byproducts, such as exchangeable  
194 sulfur, were found (Sigurdsson et al. 2016). The soils are Andosols with a silty-loamy  
195 texture.

196

### 197 *2.2. Experimental design and soil sampling*

198 Five replicate transects were established in 2012, each covering six levels of *in situ* soil  
199 warming: 0, 0.5, 1.8, 3.4, 8.7 and 15.9 °C above ambient (mean annual temperatures in  
200 the upper 10 cm of soil). A 0.5 × 0.5 m plot was established for each warming level for  
201 soil sampling (n = 6 *in situ* temperatures × 5 replicate transects = 30 plots). Soil  
202 temperature was monitored hourly at 10 cm soil depth using TidbiT v2 HOBO Data  
203 Loggers (Onset Computer Corporation, Bourne, USA). Despite the seasonal and daily  
204 oscillations of soil temperatures, the temperature increases above ambient were rather  
205 constant along the year and vertically down to ca. 20-25 cm depth (Sigurdsson et al.  
206 2016). The mean annual soil temperatures and main soil parameters are indicated in  
207 Table 1. Plant community composition showed no changes in dominant plant species up  
208 to +8.7 °C warming (Gudmundsdóttir et al. 2014, Michielsen 2014). At the most  
209 extreme warming level (15.9 °C above ambient) the vegetation community shifted  
210 towards a higher dominance of non-vascular plants (mosses) (Leblans, personal  
211 communication).

212

213 After seven years of soil warming (August 2015), samples from the upper 10 cm of  
214 mineral soil were collected from all plots. The mean soil temperature in unwarmed plots  
215 two weeks prior to sampling was 11.9±0.3 °C. Soils from each warming level were  
216 sieved to 2 mm, mixed and homogenized to constitute a composite sample. The samples  
217 were then stored at 5 °C, which is approximately the mean annual temperature of the  
218 ambient unwarmed soil, until the analyses and incubations.

219

### 220 *2.3. Initial soil parameters*

221 Three subsamples of 15, 7.5 and 7 g of fresh soil were extracted with 2 M KCl, 0.5 M  
222 NaHCO<sub>3</sub> and 0.5 M K<sub>2</sub>SO<sub>4</sub>, respectively, within 24 h of sampling. Ammonium (NH<sub>4</sub><sup>+</sup>)

223 and nitrate ( $\text{NO}_3^-$ ) were determined from the KCl extracts (Bremner and Keeney 1965).  
224 Half of the  $\text{NaHCO}_3$  and  $\text{K}_2\text{SO}_4$  extract volume was digested at 400 °C with  $\text{H}_2\text{SO}_4$  with  
225 selenium as a catalyst. Total phosphorus (P) and total extractable N ( $\text{TN}_{\text{K}_2\text{SO}_4}$ ) were  
226 determined from the digested  $\text{NaHCO}_3$  and  $\text{K}_2\text{SO}_4$  extracts, respectively. Available  
227 inorganic P ( $\text{P}_{\text{inorg}}$ ) was determined from the undigested  $\text{NaHCO}_3$  extracts (Olsen et al.  
228 1954) and dissolved organic C ( $\text{DOC}_{\text{K}_2\text{SO}_4}$ ) and  $\text{NH}_4^+$  from the undigested  $\text{K}_2\text{SO}_4$   
229 extracts. Organic P ( $\text{P}_{\text{org}}$ ) and dissolved organic N ( $\text{DON}_{\text{K}_2\text{SO}_4}$ ) were determined as the  
230 difference between digested and undigested  $\text{NaHCO}_3$  and  $\text{K}_2\text{SO}_4$  extracts, respectively  
231 (Jones and Willett 2006). Two other pools of soluble organic C were quantified using  
232 extractants of different ionic strengths. For this, two subsamples of 10 g of fresh soil  
233 were extracted with deionized water ( $\text{DOC}_{\text{water}}$ ), which is a common measure of readily-  
234 soluble C, and a weak phosphate buffer at 10 mM (0.33 mM  $\text{KH}_2\text{PO}_4$  and 6.67 mM  
235  $\text{Na}_2\text{HPO}_4$ ) adjusted to pH 7.0 ( $\text{DOC}_{\text{buffer}}$ ), which extracts both the readily-soluble C and  
236 weakly adsorbed C in clay minerals (Nelson et al. 1994, Kaiser and Zech 1999). The  
237 lower ionic strength and pH of the buffer solution compared to the 0.5 M  $\text{K}_2\text{SO}_4$   
238 solution reduces the flocculation of organic colloids and the re-adsorption of the  
239 solubilized C onto the diffuse double layer surrounding clay particles (Haney et al.  
240 2001). The relative accessibility of extractable soil C pools ( $\text{DOC}_{\text{K}_2\text{SO}_4}$ ,  $\text{DOC}_{\text{water}}$ ,  
241  $\text{DOC}_{\text{buffer}}$ ) was calculated as the ratio of DOC to SOC pools.

242

243 Another set of subsamples of the same mass of fresh soil were also extracted as  
244 described above for determining microbial biomass C and total microbial N and P by  
245 fumigation-extraction (Jenkinson and Powlson 1976). Microbial biomass C ( $\text{C}_{\text{micro}}$ ),  
246 total microbial N ( $\text{N}_{\text{micro}}$ ) and total microbial P ( $\text{P}_{\text{micro}}$ ) were determined as the  
247 difference in  $\text{DOC}_{\text{K}_2\text{SO}_4}$ ,  $\text{TN}_{\text{K}_2\text{SO}_4}$  and total P between the fumigated and unfumigated  
248 subsamples, respectively. All analyses were performed by colorimetric detection with a  
249  $\text{San}^{++}$  Continuous Flow Analyzer (Skalar Analytical B.V., Breda, The Netherlands).  
250  $\text{NO}_3^-$  was determined after reduction to  $\text{NO}_2^-$  and formation of the diazo complex at 540  
251 nm wavelength (EN-ISO 13395).  $\text{NH}_4^+$  was determined after reaction with salicylate, a  
252 catalyst and active chlorite solution to form a green colored complex at 660 nm  
253 wavelength (ISO 11732).  $\text{TN}_{\text{K}_2\text{SO}_4}$  and  $\text{NH}_4^+$  in digested and undigested  $\text{K}_2\text{SO}_4$  extracts  
254 respectively, were determined colorimetrically at 660 nm wavelength.  $\text{DOC}_{\text{K}_2\text{SO}_4}$  was  
255 determined after reaction with phenolphthalein at 550 nm wavelength (ISO 5667-3).  
256  $\text{P}_{\text{inorg}}$  was determined colorimetrically as phospho-molybdc complex at 880 nm

257 wavelength in both digested and undigested extracts (ISO 15681-2). Total soil organic  
258 C and total soil N (SOC and TN, respectively) were determined from dry soils by dry  
259 combustion at 850 °C with a Thermo Flash 2000 NC Analyser  
260 (Thermo Fisher Scientific, Delft, The Netherlands). Inorganic C is not detectible in  
261 these volcanic soils (Arnalds 2015), so total C can be considered as organic C. Soil pH  
262 was determined by stirring and settling in deionized water in a ratio 1:5 (Pansu and  
263 Gautheyrou 2006).

264

265 We calculated the stoichiometric C:N imbalance between soil organic pools and  
266 microbial biomass following Mooshammer et al. 2014a, as the ratio of C:N in the SOM  
267 pools (SOC:TN and  $\text{DOC}_{\text{K}_2\text{SO}_4}:\text{TN}_{\text{K}_2\text{SO}_4}$ ) over microbial biomass C:N ( $\text{C}_{\text{micro}}:\text{N}_{\text{micro}}$ ).  
268 The C:N imbalance is then a measure of the divergence between the C:N stoichiometry  
269 of soil microbes and soil organic substrates, where C:N imbalance < 1 thus reflects a  
270 lack of C in SOM pools for soil microbes.

271

#### 272 *2.4. Substrate addition and soil incubation*

273 Subsamples of 40 g (dry equivalent) of fresh soil from the unwarmed ambient plots  
274 (hereafter “incubation flasks”) were distributed into flasks within 72 h after sampling. In  
275 order to determine the stoichiometric demands of soil microorganisms and their  
276 capacity of C storage and N immobilization (experiment of stoichiometric demands  
277 characterization), a 1-ml of deionized water solution with a source of C, N, P or their  
278 combinations (hereafter “addition”) was added to each flask. We hypothesized that  
279 losses of soil N were associated with a restricted capacity of microbial N  
280 immobilization, so we tested the effect of two levels of N addition instead of the CP  
281 combination. C was added as glucose (1.73 mg of glucose  $\text{g}^{-1}$  dry soil, that is, 0.69 mg  
282  $\text{C g}^{-1}$ ), N was added as  $\text{NH}_4\text{NO}_3$  (0.1 mg of  $\text{NH}_4\text{NO}_3 \text{ g}^{-1}$ , that is, 34  $\mu\text{g N g}^{-1}$  for the N  
283 addition level and 0.05 mg of  $\text{NH}_4\text{NO}_3 \text{ g}^{-1}$ , 17  $\mu\text{g N g}^{-1}$  for the “half-N” addition level)  
284 and P was added as  $\text{KH}_2\text{PO}_4$  (0.101 mg  $\text{KH}_2\text{PO}_4 \text{ g}^{-1}$ , 23  $\mu\text{g P g}^{-1}$ ). The amount of  
285 substrates added accounted for ca. 1 % of the initial soil C content and 0.7 and 0.35 %  
286 of the initial soil N content for N and “half-N”, respectively (Table 1). Phosphorous  
287 retention is generally >90 % for Icelandic Andosols (Arnalds et al. 1995), so that the P  
288 added was ca. ten times the initial available inorganic P soil content to ensure that  
289 enough P was accessible to soil microbes. These amounts of substrates were chosen to  
290 ensure the alleviation of potential C and nutrient limitations of soil microbes while

291 avoiding potential changes in soil pH. The corresponding combination of the above C,  
292 N and P concentrations were used for the CN, NP and CNP addition levels, equivalent  
293 to a weight ratio of 20:1:0.67 for the CNP addition level. A set of incubation flasks was  
294 also incubated after the addition of 1 ml of deionized water without substrate (hereafter  
295 “water-only”).

296

297 The response of microbial biomass to soil warming and the capacity of the warmed soils  
298 to retain N in presence of available nutrients (experiment of warming impacts on soil C  
299 and N retention) was determined by incubating the samples from each *in situ* warming  
300 level with “water-only” and with added C, N and P in combination (CNP) as a single  
301 addition level, using the same soil mass and substrate concentrations as above (see  
302 Marañón-Jiménez et al. 2018 for further details). Soil moisture was adjusted to 60 %  
303 water-holding capacity in all incubation flasks, and the soil was mixed to ensure an even  
304 distribution of the solution.

305

306 The soils were then incubated at the mean annual soil temperature in the field (5 °C)  
307 and allowed to equilibrate for 12 h. This time lapse was determined in a preliminary  
308 assay using the same soils based on the time needed to obtain acceptable coefficients of  
309 variability (<20 %) of microbial respiration. Microbial respiration (i.e. substrate induced  
310 respiration) was then measured in all samples using an infrared gas analyzer (EGM-  
311 4/SRC-1, PP-Systems, Hitchin, UK) coupled to a custom-made chamber with a fan and  
312 vent. Incubation flasks were partially closed during the incubation to prevent drying but  
313 allow the gas exchange. The flasks were ventilated with a fan for ca. 2 minutes prior  
314 each respiration measurement to release the accumulated CO<sub>2</sub> in soil pores and in the air  
315 layer closed to the soil surface. Flasks were immersed in a water bath at a constant  
316 temperature of 5 °C to maintain the targeted temperature during the respiration  
317 measurements. Temperature was continuously monitored during the measurements and  
318 incubation using TidbiT v2 HOB0 Data Loggers (Onset Computer Corporation,  
319 Bourne, USA). Gravimetric soil moisture stayed constant at 60 % water-holding  
320 capacity throughout the experiment.

321

322 The incubation temperature of the soil samples was then increased progressively to 30  
323 °C over 6 days (4.6 °C per day) in an incubator with adjustable temperature, allowing us  
324 to discard any potential limitation of low incubation temperatures on the microbial

325 substrate uptake and growth (Nedwell 1999).  $C_{\text{micro}}$ ,  $N_{\text{micro}}$  and the remaining  $\text{DOC}_{\text{K}_2\text{SO}_4}$ ,  
326  $\text{NH}_4^+$  and  $\text{DON}_{\text{K}_2\text{SO}_4}$  in the soil were determined for all incubated samples as described  
327 above six days after the C and nutrient additions to allow soil microbes to take up the  
328 substrates. We were only interested in relative differences among treatments, so the  
329 concentrations in the microbial fraction presented here were not corrected for extraction  
330 efficiency. All fractions are presented relative to soil dry mass.

331

### 332 2.5. Data analyses

333 The effect of *in situ* soil warming on initial soil and microbial C and nutrient contents  
334 and ratios prior to the incubations was tested using one-way ANOVAs, and differences  
335 among warming levels were further tested by post hoc tests with Tukey correction for  
336 multiple testing. The effects of C, N and P substrate additions on microbial respiration,  
337  $C_{\text{micro}}$ ,  $N_{\text{micro}}$ , microbial C:N ratios; the remaining  $\text{DOC}_{\text{K}_2\text{SO}_4}$ ,  $\text{NH}_4^+$  and  $\text{DON}_{\text{K}_2\text{SO}_4}$  and  
338 the  $\text{DOC}_{\text{K}_2\text{SO}_4}:\text{TN}_{\text{K}_2\text{SO}_4}$  ratio in unwarmed soils (experiment of stoichiometric demands  
339 characterization) after the incubation were tested using one-way ANOVAs, and  
340 differences among addition levels were further tested by post hoc tests with Tukey  
341 correction for multiple testing. The differences from soils without any addition were  
342 also tested using post hoc Dunnett's tests, using the "water-only" unamended soils as  
343 control. The effect of soil warming, substrate addition (C, N and P combined) and their  
344 interaction on microbial respiration,  $C_{\text{micro}}$ ,  $N_{\text{micro}}$ , the microbial C:N ratio (experiment  
345 of warming impacts on soil C and N retention) were tested using two-ways ANOVAs,  
346 with "addition" and "*in situ* soil warming" as fixed factors. Differences among *in situ*  
347 warming levels were further tested by post hoc tests with Tukey correction for multiple  
348 testing. The effect of substrate addition on the above variables was also tested for each  
349 warming level separately by one-way ANOVAs. Data were transformed when required  
350 to improve normality and homoscedasticity (Quinn and Keough, 2009). Stoichiometric  
351 ratios were calculated on a mass basis. Statistical analyses and model construction were  
352 performed using JMP 13.0 (SAS Institute). All results are presented as means  $\pm$   
353 standard errors.

354

## 355 3. Results

356 3.1. Response of microbial biomass C and respiration of ambient soils to the addition of  
357 C, N and P

358 Microbial biomass C (non-corrected for extraction efficiency) constituted only 0.63 %  
359 of the SOC in this subarctic soil but contained four times more C than the  $\text{DOC}_{\text{K}_2\text{SO}_4}$   
360 pool (Table 1). Microbial respiration increased ca. 12 h after the C addition ( $P < 0.001$ ),  
361 but N addition and P addition did not cause any significant changes in the rate of  
362 microbial respiration (Fig. 1a), either alone or in combination with C.

363

364 Microbial biomass C responded to the additions very similarly to microbial respiration.  
365 It increased 29-47 % approximately six days after the addition of a labile C substrate  
366 (glucose) (Fig. 1b,  $P < 0.001$ ), while it even decreased in response to the N and P  
367 additions alone. Microbial biomass C, however, increased after the combined addition  
368 of N and P either alone or in combination with C.

369

### 370 *3.2. Response of microbial N of ambient soils to the addition of C, N and P*

371 The microbial N pool represented ca. three times the total extractable N in the soil  
372 (Table 1). Most of this extractable soil N (79 %) was in an organic form, while  $\text{NH}_4^+$   
373 and  $\text{NO}_3^-$  represented only 17 % and 3 % of this pool, respectively (Table 1). Total  
374 microbial N only increased significantly in response to the combined addition of C and  
375 N (Fig. 2a,  $P = 0.02$ ), although values also increased, but not significantly, in all the rest  
376 of the addition levels. Consequently, the C addition also caused a depletion of the  $\text{NH}_4^+$   
377 in soil (Fig. 2b,  $P < 0.001$ ). Circa 82 and 72 % of the  $\text{NH}_4^+$  initially available was  
378 depleted from the soil when C and N were added in combination in the CN and CNP  
379 addition levels, respectively (Fig. 2b), while a large proportion (86, 81 and 111 % for  
380 “half-N”, N and NP, respectively) still remained in the soil otherwise. In contrast, soil  
381  $\text{DON}_{\text{K}_2\text{SO}_4}$  decreased in response to N-only addition (Fig. 2c,  $P = 0.007$ ).

382

### 383 *3.3. Response of microbial C:N ratios of ambient soils to the addition of C, N and P*

384 The C:N ratios of  $\text{K}_2\text{SO}_4$ -extractable soil organic substrates decreased to lower values  
385 than in microbial biomass after six days of incubation (C:N imbalance  $< 1$ , Fig. 3).  
386 Microbial C:N ratios increased significantly in response to the CNP addition and  
387 decreased after the addition of N and P only ( $P < 0.001$ ).

388

### 389 *3.4. Response of easily accessible soil C pools and C:N ratios to warming*

390 Seven years of continuous warming provoked a substantial depletion of the pools of  
391 DOC extracted with  $\text{K}_2\text{SO}_4$  and with phosphate buffer ( $\text{DOC}_{\text{K}_2\text{SO}_4}$  and  $\text{DOC}_{\text{buffer}}$ ,

392 respectively, Fig. 4a), while the most readily-available DOC pool ( $\text{DOC}_{\text{water}}$ ) did not  
393 show a consistent decreasing pattern with soil temperatures *in situ*. Moreover, the  
394 relative accessibility of the  $\text{DOC}_{\text{buffer}}$  pool, calculated as the ratio of  $\text{DOC}_{\text{buffer}}$  to SOC  
395 pools, decreased with the intensity of soil warming up to 8.7 °C above ambient  
396 ( $P < 0.001$ , Fig. 4b), while the relative accessibility of the  $\text{DOC}_{\text{K}_2\text{SO}_4}$  pool was not  
397 substantially affected below this soil warming intensity. Nonetheless, the non-  
398 extractable C pools (SOC) were depleted in a higher proportion at the highest warming  
399 level (15.9 °C above ambient, Table 1), contributing to increase the relative accessibility  
400 of both the  $\text{DOC}_{\text{K}_2\text{SO}_4}$  and  $\text{DOC}_{\text{buffer}}$  pools. The relative accessibility of the  $\text{DOC}_{\text{water}}$   
401 pool remained however unaffected by *in situ* soil warming.

402

403 Soil warming also decreased the pools of soil  $\text{DOC}_{\text{K}_2\text{SO}_4}$  and  $\text{TN}_{\text{K}_2\text{SO}_4}$  proportionally,  
404 without any significant shifts in  $\text{DOC}_{\text{K}_2\text{SO}_4}:\text{TN}_{\text{K}_2\text{SO}_4}$  ratios along the *in situ* temperature  
405 gradient (Fig. 4c). Even though the C:N ratios of soil organic matter (SOC:TN) were  
406 2.3 times higher than the C:N ratios of microbial biomass, the imbalance from the C:N  
407 of the extractable fraction of organic substrates ( $\text{DOC}_{\text{K}_2\text{SO}_4}:\text{TN}_{\text{K}_2\text{SO}_4}$ ) was initially close  
408 to one (Fig. 4c), since the C:N ratios of the extractable organic pools were much lower  
409 than the ratios of the total organic matter pools. Warming did not cause shifts in the  
410 stoichiometric imbalance between the extractable organic substrates and microbial  
411 biomass, given the coupled and proportional losses of C and N from both biomass and  
412 soil (Fig. 4c).

413

### 414 3.5. Response of soil microbes to warming and to the addition of C, N and P

415 Despite the depletion of the easily accessible soil C pools, microbial respiration only  
416 decreased slightly with *in situ* warming ( $P = 0.04$ , Fig. 5a), and this decrease was only  
417 significant at unamended samples (“water-only”,  $P = 0.03$ ). *In situ* soil warming however  
418 decreased substantially both microbial biomass C and N ( $P < 0.001$  for both variables),  
419 with the largest changes between 1.8 and 3.4 °C above ambient (Fig. 5b, c). Microbial  
420 C:N ratios thus did not change significantly with *in situ* soil warming, although variance  
421 increased at the warmest soils (Fig. 5d,  $P = 0.13$ ).

422

423 The addition of a substrate containing a labile source of C, N and P (CNP) increased  
424 microbial respiration in a similar magnitude across all *in situ* warming levels ( $P < 0.001$

425 for “addition” effect,  $P=0.87$  for “addition” and “*in situ* soil warming” interactions, Fig.  
426 5a). In contrast, the substrate added increased microbial biomass C only in soils from  
427 moderate warming levels  $<3.4$  °C ( $P<0.001$ , Fig. 5b), but it did not increase at higher  
428 warming levels ( $P<0.001$  for “addition” and “*in situ* soil warming” interactions), even  
429 though the amount of remaining DOC was still higher than in unamended soils  
430 ( $P<0.01$ ). Microbial N showed very similar response ( $P<0.001$  for “addition” effects,  
431 Fig. 5c), but the interaction between “addition” and “*in situ* soil warming” was not  
432 significant in this case ( $P=0.18$ ). Microbial C:N ratios, however, did not change  
433 substantially in response to the added CNP substrate ( $P=0.10$ , Fig. 5d), although they  
434 tended to increase in response to the addition at *in situ* warming levels  $\leq 3.4$  °C ( $P=0.05$   
435 for “addition” and “*in situ* soil warming” interactions), indicating a proportionally  
436 higher retention of C than N.

437

#### 438 **4. Discussion**

439 Nitrogen was lost in the same proportion as C in these subarctic soils (Table 1, Fig. 4c),  
440 so that the C:N ratios did not change substantially along the *in situ* soil temperature  
441 gradient. This is in contrast to the increase in the availability of soil mineral N and  
442 vegetation productivity generally observed in field warming experiments (Dieleman et  
443 al. 2012, Dormann and Woodin 2002, Wu et al. 2011). The proportional loss of both  
444 elements points to the tight C:N stoichiometric coupling as a mechanism. Soil C losses  
445 in response to warmer temperatures have frequently been observed, but experimental  
446 results do not always match model predictions for high-latitude ecosystems (Todd-  
447 Brown et al. 2013, McGuire et al. 2018). Overlooking the relevance of the C and N  
448 stoichiometric needs of soil microbes for soils to retain these elements can be a potential  
449 cause of these divergences. Soil warming provoked the depletion of a large fraction of  
450 the easily accessible C pools in these soils (Fig. 4), where microbial C limitation was  
451 already strong (Fig. 1), leading to substantial reductions in microbial biomass and in the  
452 capacity of N retention of soil microbes. The strict C and N stoichiometric needs of soil  
453 microbes may have determined the coupled losses of C and N from warmed soils,  
454 accounting for the constant soil C:N ratios.

455

##### 456 *4.1. C, N and P limitation of microbes in high-latitude soils*

457 Nutrient immobilization by soil microbes can strongly control biogeochemical cycling  
458 in ecosystems where temperatures limit the release of nutrients from SOM (Skouw



459 Haugwitz et al. 2011). In these subarctic soils, most of the soil N was in organic form  
460 and the microbial N pool represented ca. three times the total extractable N, pointing to  
461 the high sensitivity of N biogeochemical fluxes and soil N storage capacity to changes  
462 in microbial biomass N. The soils in our incubations have been exposed *in situ* to  
463 constant temperature increases relative to ambient temperatures (Sigurdsson et al.  
464 2016), so an increase in mineralization rates and N release to the soil are expected  
465 throughout the year. Litter decomposition and mass-specific mineralization rates of the  
466 microbes from the same study site were accordingly higher in warmer soils (Leblans et  
467 al. 2016, Marañón-Jiménez et al. 2018). The short photoperiod and low temperatures,  
468 however, limited vegetation productivity and nutrient uptake during winter dormancy  
469 (Leblans et al. 2017). The role of soil microbes in nutrient immobilization for  
470 preventing nutrient leaching is therefore crucial during this period, and particularly  
471 during winter thaws (Yano et al. 2015).

472

473 Soil microorganisms in these subarctic soils were strongly C limited even at ambient  
474 temperatures, indicated by a large and equivalent increase in respiration and biomass in  
475 response to C addition (Fig. 1). By contrast, microbial respiration was not altered by the  
476 N or P additions, and microbial biomass even decreased after the addition of these  
477 nutrients alone (Fig 1b). Besides the low vegetation inputs during prolonged winter  
478 periods, the strong C limitation can be also partly associated with the low accessibility  
479 of most organic substrates, which are sorbed by soil minerals of high specific surface  
480 area in these volcanic-ash soils. The large differences between SOC and DOC pools  
481 points to a high proportion of non-extractable C strongly occluded (Poeplau et al. 2016).  
482 More than ten times organic C was extracted by phosphate buffer than by water in the  
483 ambient soils, which also indicates a high proportion of soil C weakly adsorbed to  
484 colloidal surfaces (Hayes, 1985). The high adsorption capacity of the fine-textured soils  
485 may promote a long-lasting microbial C limitation that, most likely, aggravate in winter,  
486 when plant C inputs decrease.

487

488 The relationship between the C:N stoichiometry of soil microorganisms and SOM  
489 substrates governs the predominant biogeochemical pathways by which microbes meet  
490 their stoichiometric needs using available resources (Mooshammer et al. 2014b).  
491 Accordingly, soil microorganisms retain limited elements and release those in excess

492 (Sterner and Elser 2002). The microbial C:N ratios in the soils at ambient temperatures  
493 (C:N=5.41±0.15, Fig. 4c) were slightly lower than those reported for grassland soils  
494 (C:N=6.6) and global averages (C:N=7.6) (Xu et al. 2013). The SOC:TN ratios of SOM  
495 (C:N=11.97±0.07) were also lower than for grasslands (C:N=13.3) and globally  
496 (C:N=16.4), and the ratios were even lower in the pool of extractable SOM  
497 (C:N=6.02±0.72, Fig. 4c). The relatively low microbial C:N ratios in these subarctic  
498 soils and a C:N imbalance in relation to the extractable organic pools close to one (Fig.  
499 4c) indicate that N immobilization was not required in large amounts to meet their  
500 stoichiometric needs. On the contrary, a net mineralization occurred during the soil  
501 incubation in non-amended soils (Fig. 2b), while the immobilization of mineral N was  
502 conditioned by the supply of an accessible C pool and the production of new microbial  
503 biomass (Figs. 1b and 2).

504

505 Carbon limitation and the strict C:N stoichiometric needs of soil microbes  
506 (Zeichmeister-Boltenstern et al. 2015) actually constrained microbial N immobilization.  
507 Only the C addition provoked a significant increase in microbial N (Fig. 2a), and N  
508 immobilization was highest when C and N were added in combination, although the  
509 addition of inorganic N alone also stimulated microbial N immobilization slightly. A  
510 86, 81 and 111 % of the total  $\text{NH}_4^+$  initially available still remained in the soil six days  
511 after addition for the “half-N”, N and NP addition levels, respectively (Fig. 2b), while  
512 only 18 to 28 % remained when C was also added for the CN and CNP additions. The  
513 decrease of microbial biomass (Fig. 1b) and the predominant use of DON as C source  
514 when only N was added (Fig. 2c) are further evidences of C limitation for microbial  
515 growth and N immobilization (Farrell et al. 2014). Similar C constraints of microbial N  
516 demands have been observed in Siberia (Wild et al. 2015), reminding the need to frame  
517 the concept of C or nutrient limitation to specific ecosystem components or processes  
518 rather than generalizing to entire ecosystems. Sub-surface soils (>5 cm depth) also  
519 showed no capacity for net retention of increased N inputs after 20 years of fertilization  
520 experiment in Alaska, leading to a net C loss (Mack et al. 2004). Soils with relatively  
521 low C:N ratios may also present a secondary microbial P limitation. The addition of P in  
522 these soils may fuel the synthesis of P-rich mRNA for protein transcription (Elser et al.  
523 1996), enhancing immobilization of soil DON for protein synthesis up to certain level,  
524 where the N immobilization is again saturated and limited by C availability (Hessen et  
525 al. 2007). This limitation was evidenced by the decrease in microbial biomass in

526 response to the P addition (Fig. 1b). In contrast, the simultaneous supply of N and P  
527 needed for protein synthesis may have promoted the allocation of soil organic substrates  
528 for microbial growth, resulting in increases in microbial biomass (Fig. 1b). Soils with  
529 low C:N ratios where the N storage function of soil microbes is not supported by a  
530 continuous supply of easily accessible C will be therefore vulnerable to N losses.

531

#### 532 *4.2. Response of microbial cycling to soil warming*

533 Seven years of continuous soil warming led to a substantial loss of total soil C and N  
534 from the upper 10 cm (Table 1), but not all pools of SOC were depleted equally. Both  
535  $\text{DOC}_{\text{K}_2\text{SO}_4}$  and  $\text{DOC}_{\text{buffer}}$  pools decreased significantly with *in situ* soil warming, while  
536 the  $\text{DOC}_{\text{water}}$  pool did not show a consistent decreasing pattern (Fig. 4a). In relative  
537 terms, soil warming provoked a predominant depletion of the  $\text{DOC}_{\text{buffer}}$  pool in relation  
538 to the total SOC up to +8.7 °C warming (Fig. 4b), indicating a proportional decrease of  
539 the soil organic C adsorbed within the soil minerals. Water-extractable C is known as  
540 the most readily-available C pool for soil microbes, but it has also shown a lower  
541 biodegradability compared to the buffer-extractable C pool when both pools are fully  
542 accessible to soil microbes (Nelson et al. 1994, Wagai and Sollings 2002). Soil  
543 microbes may have resorted on the weakly-adsorbed C fraction, the largest DOC pool in  
544 these soils, as a predominant C source as the water-extractable C pool was depleted at  
545 increasing soil temperatures. Increasing rates of depolymerization and solubilization  
546 from the weakly-adsorbed SOM fraction may have also contributed to increase the  
547 water-extractable C inputs, compensating the microbial consumption of this pool.  
548 Nonetheless, the non-extractable C pools (SOC) also experienced a predominant  
549 depletion at the most extreme warming level (15.9 °C above ambient), probably causing  
550 a decrease in the surface of organic colloidal surfaces, which contributed to increase the  
551 relative accessibility of both the  $\text{DOC}_{\text{K}_2\text{SO}_4}$  and  $\text{DOC}_{\text{buffer}}$  pools. Therefore, soil microbes  
552 may have satisfied their increasing energy demands at warmer temperatures by a  
553 proportionally higher solubilization of the C adsorbed in soil mineral surfaces.

554

555 Microbes increased their respiratory demands per unit of biomass in warmer soils  
556 (Marañón-Jiménez et al. 2018, Walker et al. 2018), probably as a consequence of  
557 increasing energy costs for metabolic maintenance and for the solubilization of  
558 adsorbed organic substrates. Soil warming did, however, not cause substantial shifts in

559 the C:N imbalance between SOM and microbial biomass (Fig. 4c) and the response of  
560 respiration to the substrate (C, N and P) addition was also equivalent across warming  
561 levels (Fig. 5a). Rather than increasing their C demands at the ecosystem level,  
562 microbes maintained accelerated rates of C consumption under conditions of low C  
563 accessibility by a reduction of the standing biomass (Walker et al. 2018, Fig. 5b), which  
564 provoked a coupled and equivalent loss of microbial N (Fig. 5c, d). These results again  
565 highlight the strict C:N stoichiometric needs of soil microbes and the tight coupling  
566 between N immobilization and biomass production. Warming can therefore lead to  
567 proportional soil C and N losses when increased N mineralization rates are not  
568 compensated by rapid plant N uptake and plant-derived C inputs to the soil.

569

## 570 **5. Conclusions**

571 Seven years of chronic exposure to warmer temperatures led to large and proportional  
572 losses of C and N from these high-latitude soils. These findings point to the strict C:N  
573 stoichiometric needs of soil microbes and the tight coupling between microbial N  
574 immobilization and biomass production as a key mechanism. The continuous exposure  
575 of soil microbes to higher temperatures for seven years increased their respiratory  
576 demands and provoked the depletion of a large fraction of the easily accessible C pools  
577 of these subarctic soils, where microbial C limitation was already strong. Soil warming  
578 constrained, as a result, the C retention in microbial biomass and the immobilization of  
579 mineralized N. A release of mineral N that is not rapidly compensated by plant N  
580 uptake is vulnerable to be lost through leaching in case of nitrification and gaseous  
581 fluxes in case of denitrification. The loss of N storage capacity of microbial biomass  
582 likely provoked a shift from a close to a leakier N cycle with a detrimental effect on soil  
583 N availability and C storage capacity. This mechanism may be key in soils where the  
584 low C availability can compromise the maintenance of microbial biomass under a  
585 warmer climate, particularly during periods of limited plant C inputs and N uptake. Our  
586 results also highlight the need to change the frequent misconception of the ubiquitous N  
587 limitation in high latitude ecosystems by a better framed concept of limitation for each  
588 specific process or ecosystem component. Accordingly, our findings suggest a strong  
589 control of microbial physiology and C:N stoichiometric needs on the retention of soil N  
590 and ultimately on the resilience of high-latitude soil C stocks to warming. Overlooking  
591 this may be the cause of the large divergences between the predicted response of soil C  
592 stocks from models and observations at high latitudes.

593

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610

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- 833

**834 Figure captions:**

835 Figure 1: A) Microbial respiration and B) microbial biomass C in unwarmed soils in  
836 response to the C, N and P additions. Microbial respiration was measured 12 h after the  
837 additions at the mean annual soil temperature (5 °C). Microbial biomass was measured  
838 six days after the substrate additions. Different letters indicate significant differences by  
839 Tukey's post hoc tests at  $\alpha=0.05$ .

840

841 Figure 2: A) Total microbial N, B) remaining  $\text{NH}_4^+$  and C) remaining dissolved  
842 organic N in unwarmed soils six days after the C, N and P additions. Triangles indicate  
843 the initial  $\text{NH}_4^+$  concentration in soil prior to the soil incubation. Different letters  
844 indicate significant differences by Tukey's post hoc tests at  $\alpha=0.05$ .

845

846 Figure 3: C:N ratios in A) soil microbes and B)  $\text{K}_2\text{SO}_4$ -extractable organic pools from  
847 unwarmed soils six days after to the C, N and P additions. Different letters indicate  
848 significant differences by Tukey's post hoc tests at  $\alpha=0.05$ .

849

850 Figure 4: A) Dissolved organic C pools, B) their relative accessibility and C) C:N ratios  
851 of  $\text{K}_2\text{SO}_4$ -extractable organic pools, microbial biomass and the C:N imbalance between  
852 these at the different intensities of soil warming. Data correspond to the initial values in  
853 soils before the incubation or substrates addition. The relative accessibility of  
854 extractable soil C pools was calculated as their ratio to the total organic C pool. The  
855 C:N imbalance was calculated as the ratio of C:N of soil organic pools over microbial  
856 C:N. Different letters indicate significant differences by Tukey's post hoc tests at  
857  $\alpha=0.05$ .

858

859 Figure 5: A) Microbial respiration, B) microbial biomass C, C) total microbial N and D)  
860 microbial C:N ratios in response to the C, N and P addition at the different intensities of  
861 soil warming. Microbial respiration was measured 12 h after the additions at the mean  
862 annual soil temperature (5 °C). Microbial biomass C and N were measured six days  
863 after the additions. Different letters indicate significant differences among the soil  
864 warming intensities according to two-way ANOVAs and Tukey's post hoc tests. \* and  
865 \*\* indicate significant differences between substrate addition levels within each soil  
866 warming intensity according to one-way ANOVAs:  $*0.01 < P \leq 0.05$ ,  $**0.001 \leq P \leq 0.01$ .

1 Table 1: Main soil parameters along the *in situ* soil warming levels at the time of  
 2 sampling.  $P_{0.05}$ - $P_{0.95}$ , range of mean soil temperature values between the 5<sup>th</sup> and 95<sup>th</sup>  
 3 percentiles; WHC, water holding capacity; SOC, total soil organic C; TN, total soil N;  
 4  $\text{DON}_{\text{K}_2\text{SO}_4}$ , dissolved organic N in  $\text{K}_2\text{SO}_4$ ;  $\text{P}_{\text{inorg}}$ , available inorganic P in  $\text{NaHCO}_3$ ;  $\text{P}_{\text{org}}$ ,  
 5 organic P in  $\text{NaHCO}_3$ ;  $\text{C}_{\text{micro}}$ , microbial biomass C;  $\text{N}_{\text{micro}}$ , total microbial N;  $\text{P}_{\text{micro}}$ , total  
 6 microbial P. Different letters indicate significant differences among sites (Tukey's post  
 7 hoc tests after one-way ANOVAs). Intervals indicate  $\pm$ standard errors.

8  
 9

| Soil parameter  | <i>In situ</i> soil warming ( $^{\circ}\text{C}$ above ambient) |  |  |  |   |  | F       | P             |
|---|---|--|--|--|---|--|---------|---------------|
|   | 0   | 0.5  | 1.8  | 3.4                                      | 8.7                                       | 15.9                                       |         |               |
| Mean annual soil T <sup>a</sup><br>( $^{\circ}\text{C}$ )†<br>( $P_{0.05}$ - $P_{0.95}$ ) | 5.6 $\pm$ 0.1 <sup>a</sup><br>(0.1-13.0)                        | 6.0 $\pm$ 0.1 <sup>a,b</sup><br>(0.2-13.4) | 7.3 $\pm$ 0.6 <sup>b,c</sup><br>(0.8-15.9) | 8.9 $\pm$ 0.2 <sup>c</sup><br>(2.3-17.1) | 14.3 $\pm$ 1.1 <sup>d</sup><br>(5.0-26.2) | 21.5 $\pm$ 0.4 <sup>e</sup><br>(11.7-33.8) | 110.99  | $\leq$ 0.0001 |
| WHC (%)   | 117.0 $\pm$ 1.7 <sup>a,b</sup>                                  | 129.8 $\pm$ 3.3 <sup>a</sup>               | 117.1 $\pm$ 4.9 <sup>a,b</sup>             | 112.2 $\pm$ 1.7 <sup>b</sup>             | 111.8 $\pm$ 4.5 <sup>b</sup>              | 109.1 $\pm$ 3.3 <sup>b</sup>               | 4.6080  | 0.0141        |
| SOC (%)†  | 5.78 $\pm$ 0.03 <sup>b</sup>                                    | 6.59 $\pm$ 0.02 <sup>a</sup>               | 5.28 $\pm$ 0.06 <sup>c</sup>               | 3.08 $\pm$ 0.03 <sup>d</sup>             | 2.81 $\pm$ 0.03 <sup>e</sup>              | 2.43 $\pm$ 0.04 <sup>f</sup>               | 2038.63 | $\leq$ 0.0001 |
| TN (%)†   | 0.483 $\pm$ 0.003 <sup>b</sup>                                  | 0.563 $\pm$ 0.003 <sup>a</sup>             | 0.4 $\pm$ 0 <sup>c</sup>                   | 0.257 $\pm$ 0.003 <sup>d</sup>           | 0.237 $\pm$ 0.003 <sup>e</sup>            | 0.223 $\pm$ 0.003 <sup>f</sup>             | 1840.80 | $\leq$ 0.0001 |
| SOC:TN  | 11.97 $\pm$ 0.07 <sup>b</sup>                                   | 11.7 $\pm$ 0.04 <sup>b</sup>               | 13.21 $\pm$ 0.15 <sup>a</sup>              | 12.01 $\pm$ 0.12 <sup>b</sup>            | 11.86 $\pm$ 0.12 <sup>b</sup>             | 10.87 $\pm$ 0.08 <sup>c</sup>              | 52.11   | $\leq$ 0.0001 |
| $\text{DON}_{\text{K}_2\text{SO}_4}$ (mg kg <sup>-1</sup><br>dry soil)                    | 12.41 $\pm$ 1.64 <sup>a,b</sup>                                 | 15.79 $\pm$ 2.01 <sup>a</sup>              | 10.81 $\pm$ 1.35 <sup>a,b</sup>            | 7.69 $\pm$ 1.27 <sup>b</sup>             | 7.70 $\pm$ 1.18 <sup>b</sup>              | 10.12 $\pm$ 3.15 <sup>a,b</sup>            | 3.49    | 0.0392        |
| $\text{NH}_4^+$ (mg kg <sup>-1</sup> dry<br>soil)†  | 2.72 $\pm$ 0.86 <sup>c</sup>                                    | 6.84 $\pm$ 0.36 <sup>a</sup>               | 9.15 $\pm$ 0.48 <sup>a</sup>               | 3.93 $\pm$ 0.16 <sup>b</sup>             | 2.64 $\pm$ 0.04 <sup>b,c</sup>            | 1.43 $\pm$ 0.05 <sup>d</sup>               | 50.93   | $\leq$ 0.0001 |
| $\text{NO}_3^-$ (mg kg <sup>-1</sup> dry<br>soil)†  | 0.490 $\pm$ 0.032 <sup>c</sup>                                  | 0.675 $\pm$ 0.043 <sup>b</sup>             | 1.221 $\pm$ 0.058 <sup>a</sup>             | 0.803 $\pm$ 0.026 <sup>b</sup>           | 0.301 $\pm$ 0.014 <sup>d</sup>            | 0.174 $\pm$ 0.001 <sup>e</sup>             | 206.56  | $\leq$ 0.0001 |
| $\text{P}_{\text{inorg}}$ (mg kg <sup>-1</sup> dry<br>soil)                               | 2.16 $\pm$ 0.18 <sup>b</sup>                                    | 2.24 $\pm$ 0.11 <sup>b</sup>               | 2.42 $\pm$ 0.04 <sup>b</sup>               | 2.93 $\pm$ 0.09 <sup>a</sup>             | 2.50 $\pm$ 0.02 <sup>b</sup>              | 2.40 $\pm$ 0.03 <sup>b</sup>               | 9.41    | $\leq$ 0.0001 |
| $\text{P}_{\text{org}}$ (mg kg <sup>-1</sup> dry<br>soil)†                                | 10.60 $\pm$ 0.26 <sup>b</sup>                                   | 14.12 $\pm$ 0.35 <sup>a</sup>              | 9.49 $\pm$ 0.41 <sup>b</sup>               | 5.43 $\pm$ 0.22 <sup>c</sup>             | 3.30 $\pm$ 0.23 <sup>d</sup>              | 3.83 $\pm$ 0.12 <sup>d</sup>               | 171.23  | $\leq$ 0.0001 |
| $\text{C}_{\text{micro}}$ (mg kg <sup>-1</sup> dry<br>soil)‡                              | 365.06 $\pm$ 10.86 <sup>a</sup>                                 | 413.84 $\pm$ 12.28 <sup>a</sup>            | 305.69 $\pm$ 25.02 <sup>a</sup>            | 153.63 $\pm$ 12.10 <sup>b</sup>          | 172.72 $\pm$ 16.73 <sup>b</sup>           | 139.15 $\pm$ 24.30 <sup>b</sup>            | 33.88   | $\leq$ 0.0001 |
| $\text{N}_{\text{micro}}$ (mg kg <sup>-1</sup> dry<br>soil)                               | 67.54 $\pm$ 2.57 <sup>a,b</sup>                                 | 82.35 $\pm$ 2.66 <sup>a</sup>              | 66.32 $\pm$ 6.16 <sup>b</sup>              | 34.20 $\pm$ 1.16 <sup>c</sup>            | 29.07 $\pm$ 1.87 <sup>c,d</sup>           | 17.95 $\pm$ 2.74 <sup>d</sup>              | 62.53   | $\leq$ 0.0001 |
| $\text{P}_{\text{micro}}$ (mg kg <sup>-1</sup> dry<br>soil)                               | 5.45 $\pm$ 0.89 <sup>a</sup>                                    | 4.34 $\pm$ 0.38 <sup>a,b</sup>             | 3.00 $\pm$ 0.60 <sup>b,c</sup>             | 2.80 $\pm$ 0.30 <sup>b,c,d</sup>         | 1.91 $\pm$ 0.32 <sup>c,d</sup>            | 0.74 $\pm$ 0.31 <sup>d</sup>               | 11.17   | $\leq$ 0.0001 |
| $\text{C}_{\text{micro}}:\text{P}_{\text{micro}}$ †                                       | 67.02 $\pm$ 1.99 <sup>b,c</sup>                                 | 95.43 $\pm$ 2.83 <sup>b</sup>              | 101.87 $\pm$ 8.34 <sup>b</sup>             | 54.90 $\pm$ 4.32 <sup>c</sup>            | 90.39 $\pm$ 8.75 <sup>b</sup>             | 188.05 $\pm$ 32.84 <sup>a</sup>            | 17.00   | $\leq$ 0.0001 |
| $\text{N}_{\text{micro}}:\text{P}_{\text{micro}}$   | 12.40 $\pm$ 0.47 <sup>c</sup>                                   | 18.99 $\pm$ 0.61 <sup>a,b,c</sup>          | 22.10 $\pm$ 2.05 <sup>a,b</sup>            | 12.22 $\pm$ 0.41 <sup>c</sup>            | 15.21 $\pm$ 0.98 <sup>b,c</sup>           | 24.26 $\pm$ 3.70 <sup>a</sup>              | 7.82    | 0.0018        |
| pH*   | 5.55 $\pm$ 0.01 <sup>b</sup>                                    | 5.48 $\pm$ 0.00 <sup>a</sup>               | 5.70 $\pm$ 0.01 <sup>c</sup>               | 5.96 $\pm$ 0.01 <sup>d</sup>             | 6.14 $\pm$ 0.00 <sup>e</sup>              | 6.20 $\pm$ 0.01 <sup>f</sup>               | 1350.3  | $\leq$ 0.0001 |

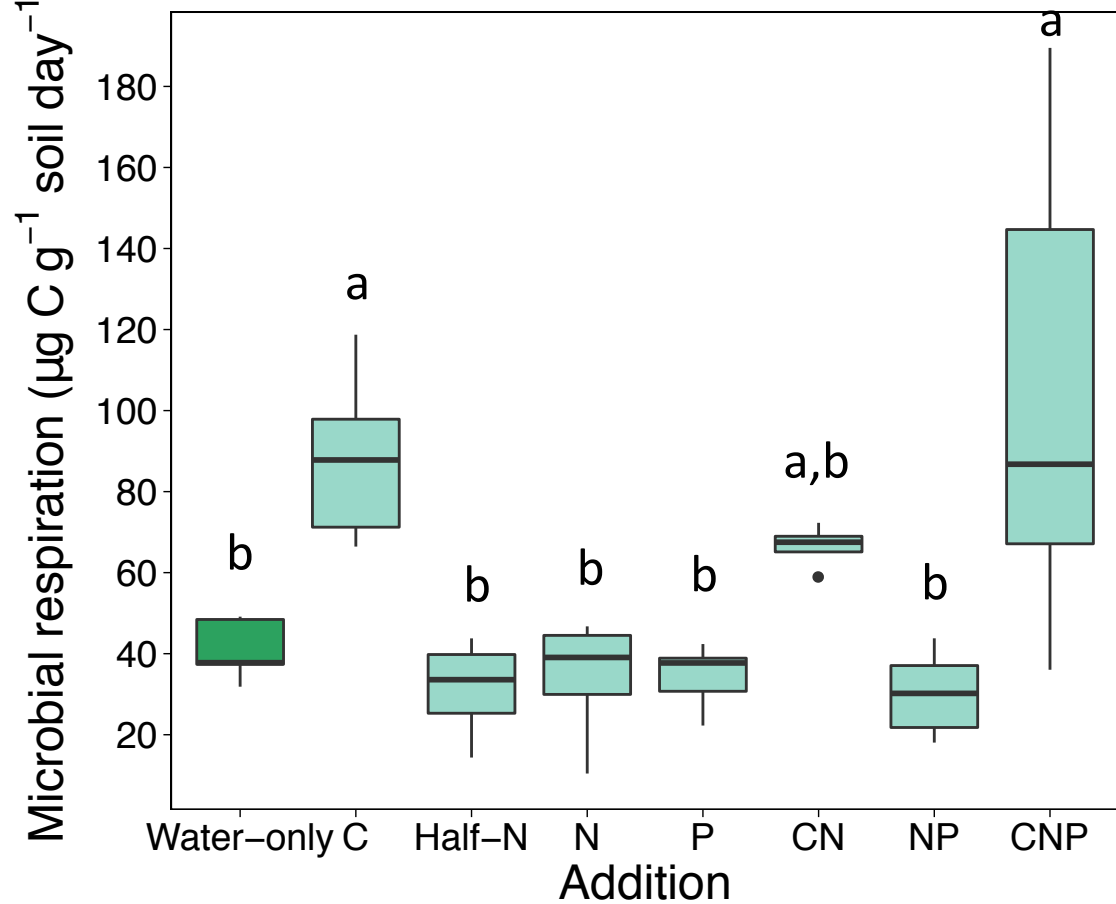
10 †Log-transformed data before ANOVAs

11 \*Exponential-transformed data before ANOVAs

12 ‡Square root-transformed data before ANOVAs

Figure 1

## A. Microbial respiration



## B. Microbial biomass C

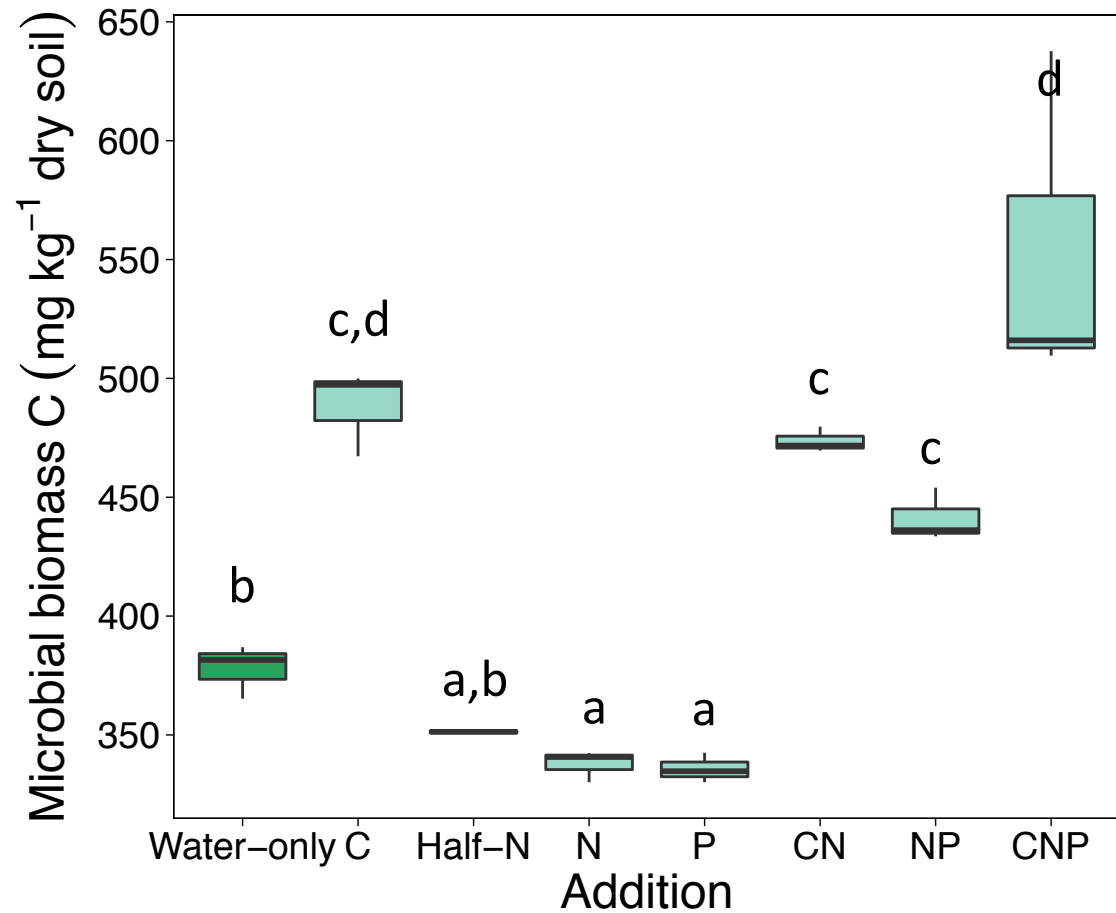
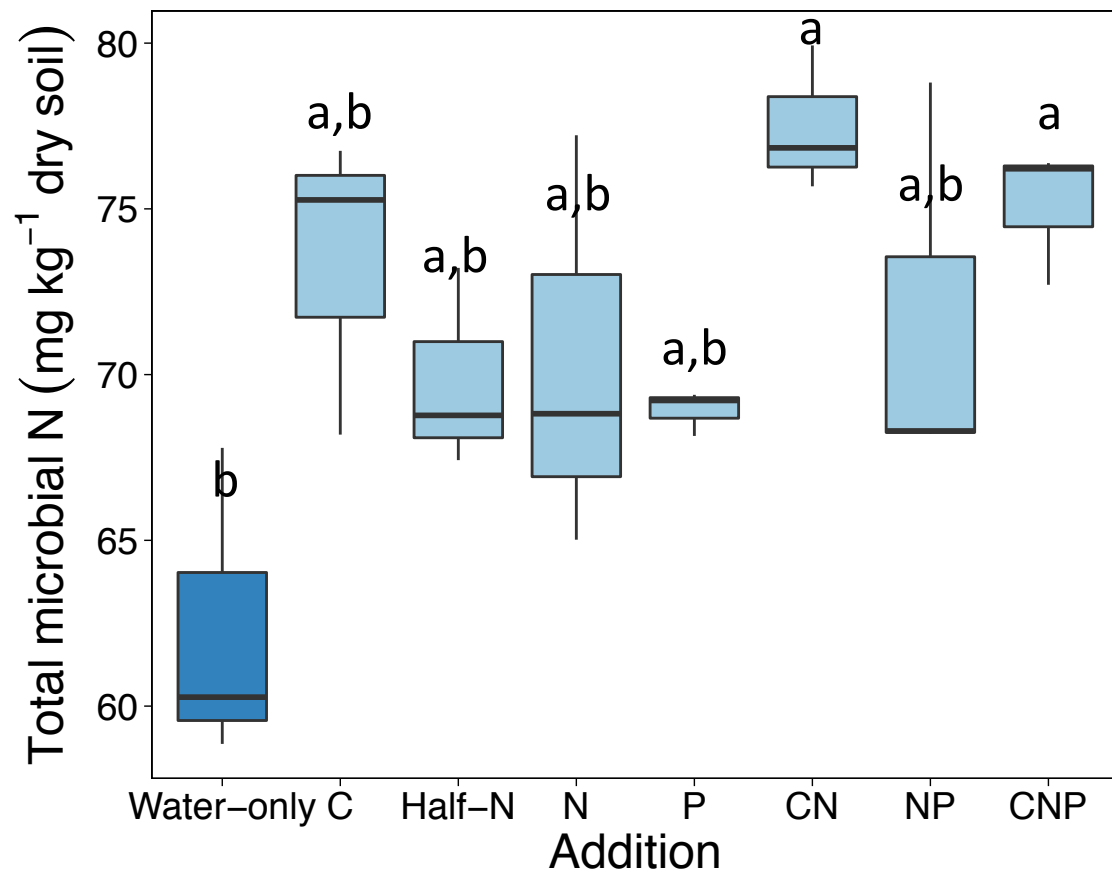
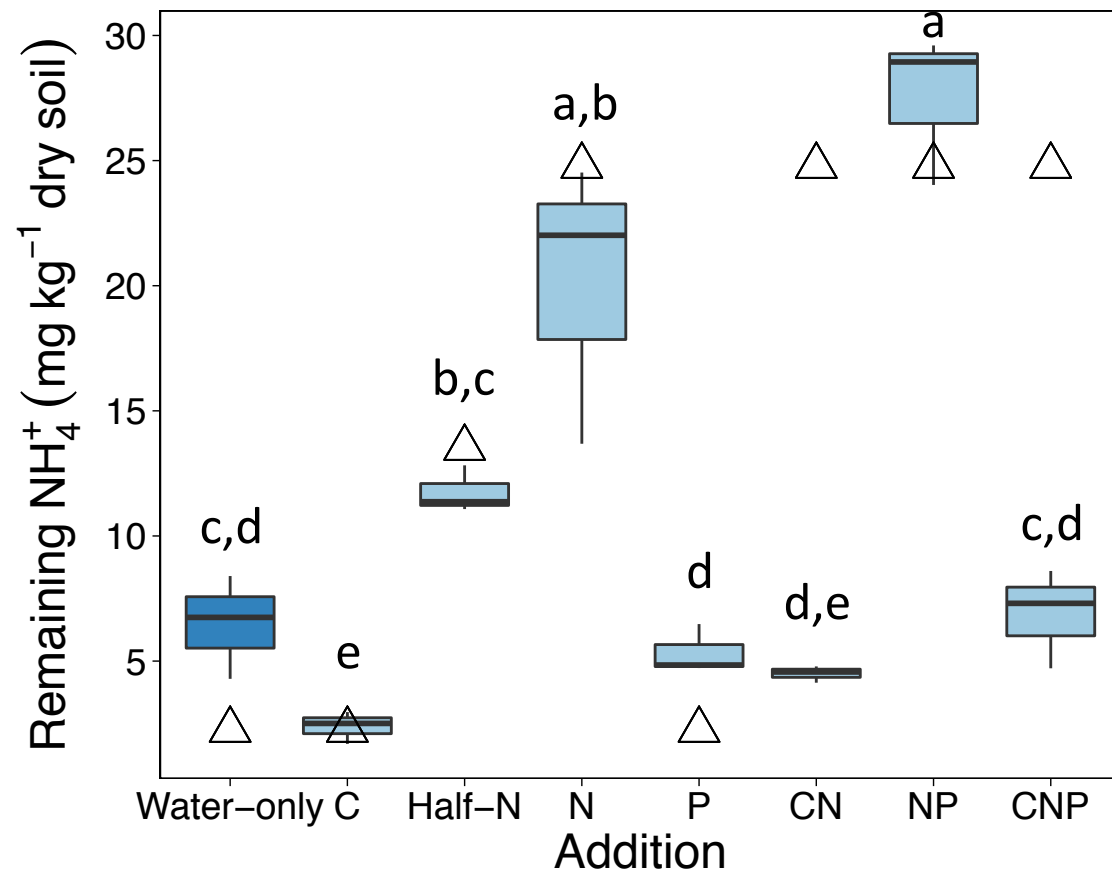


Figure 2

## A. Total microbial N

B. Remaining NH<sub>4</sub><sup>+</sup> in soil

## C. Remaining DON in soil

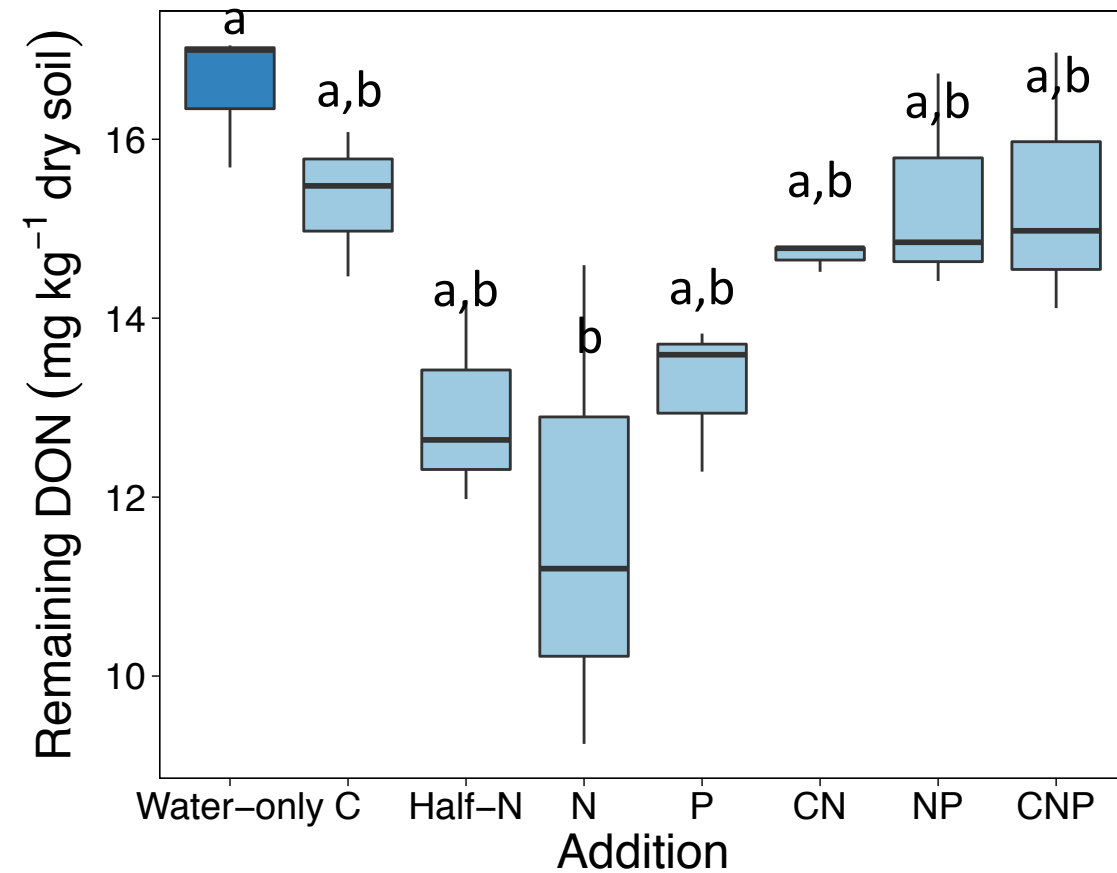
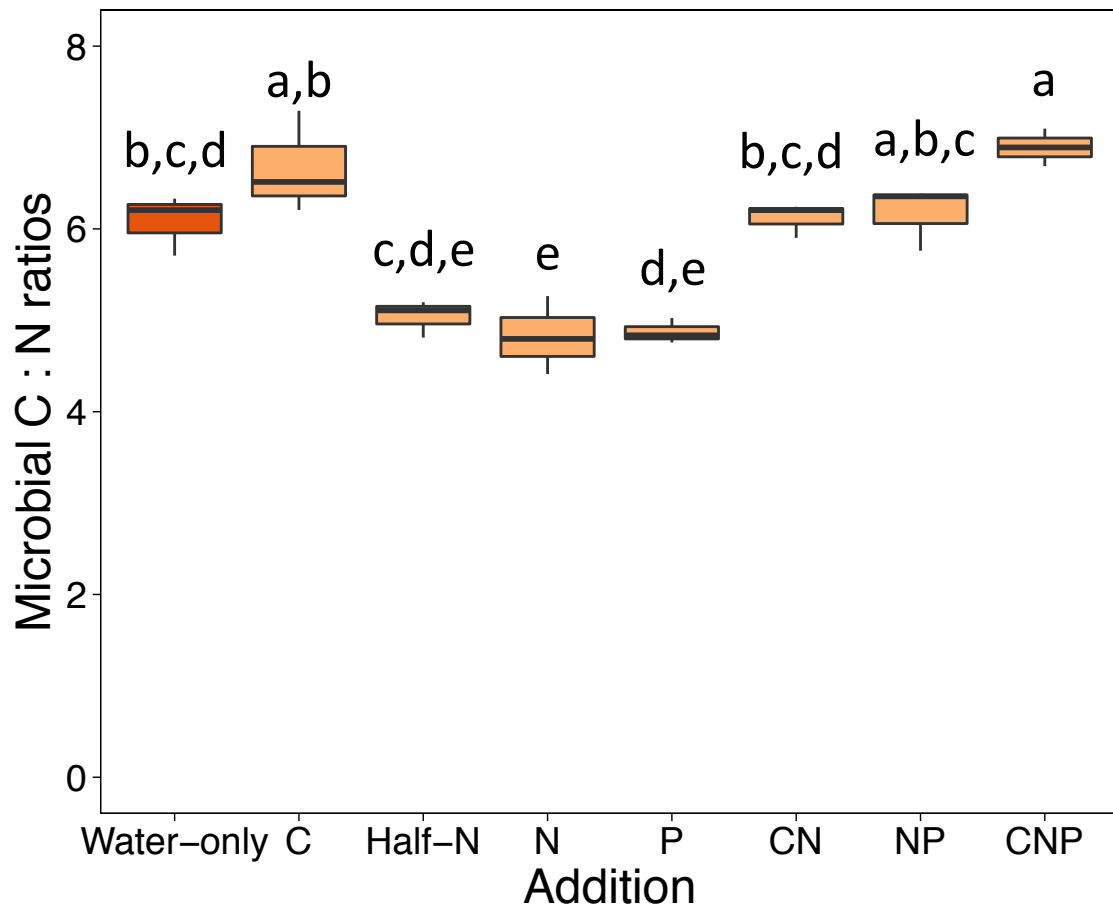




Figure 3

## A. Microbial C:N ratios



## B. DOC:Total extractable N in soil

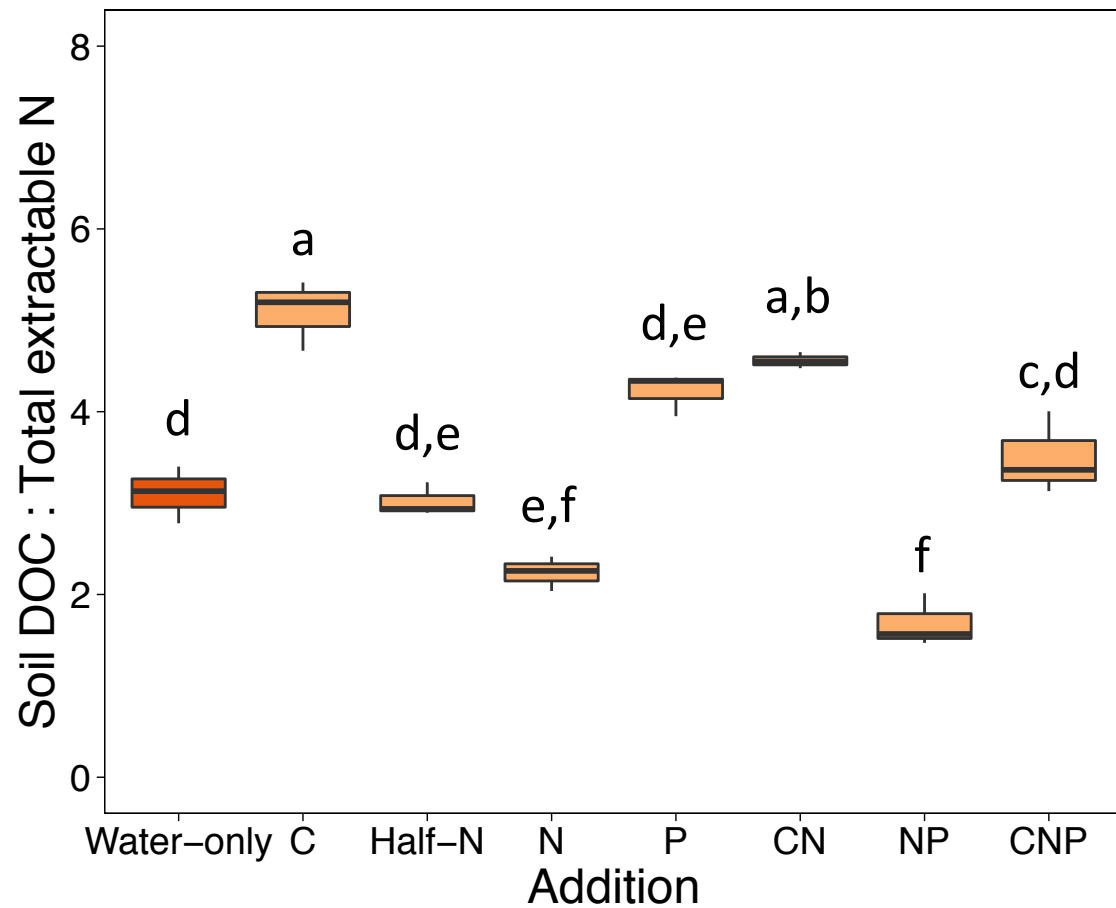
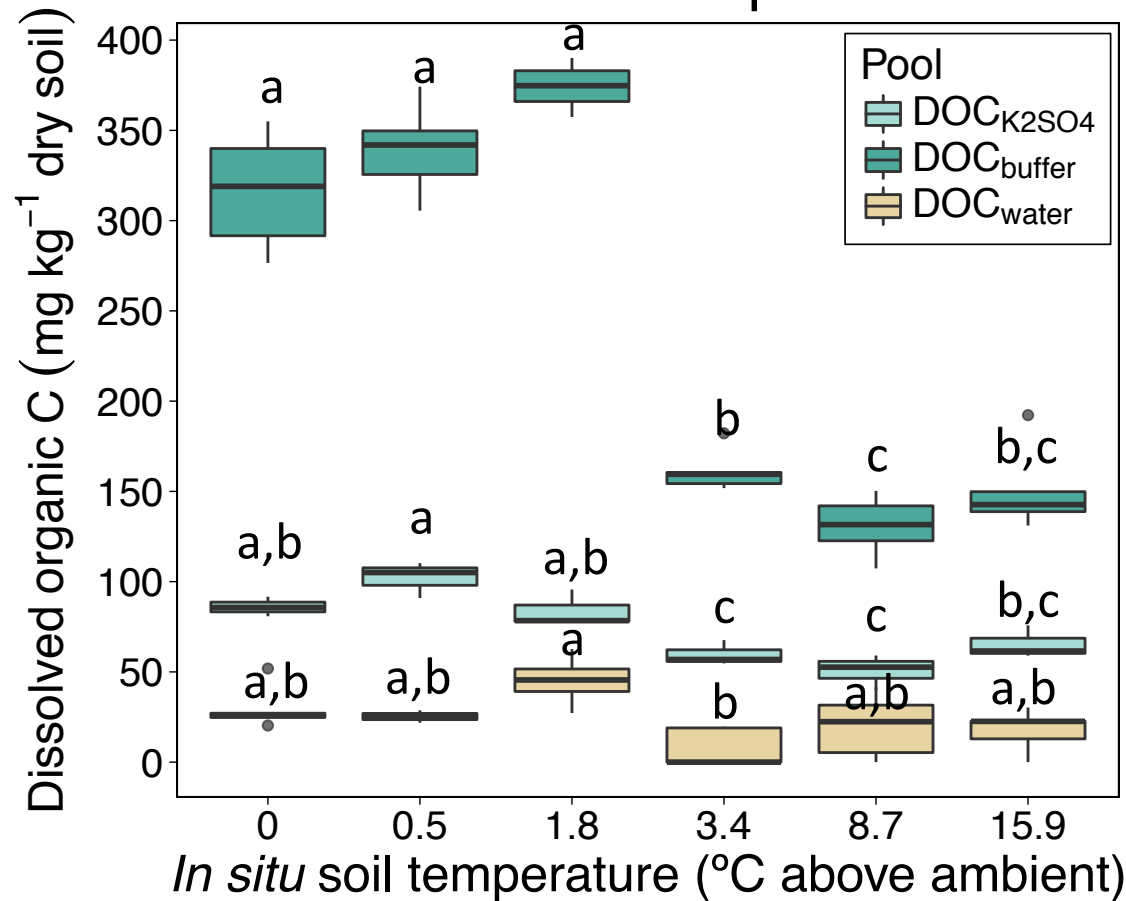
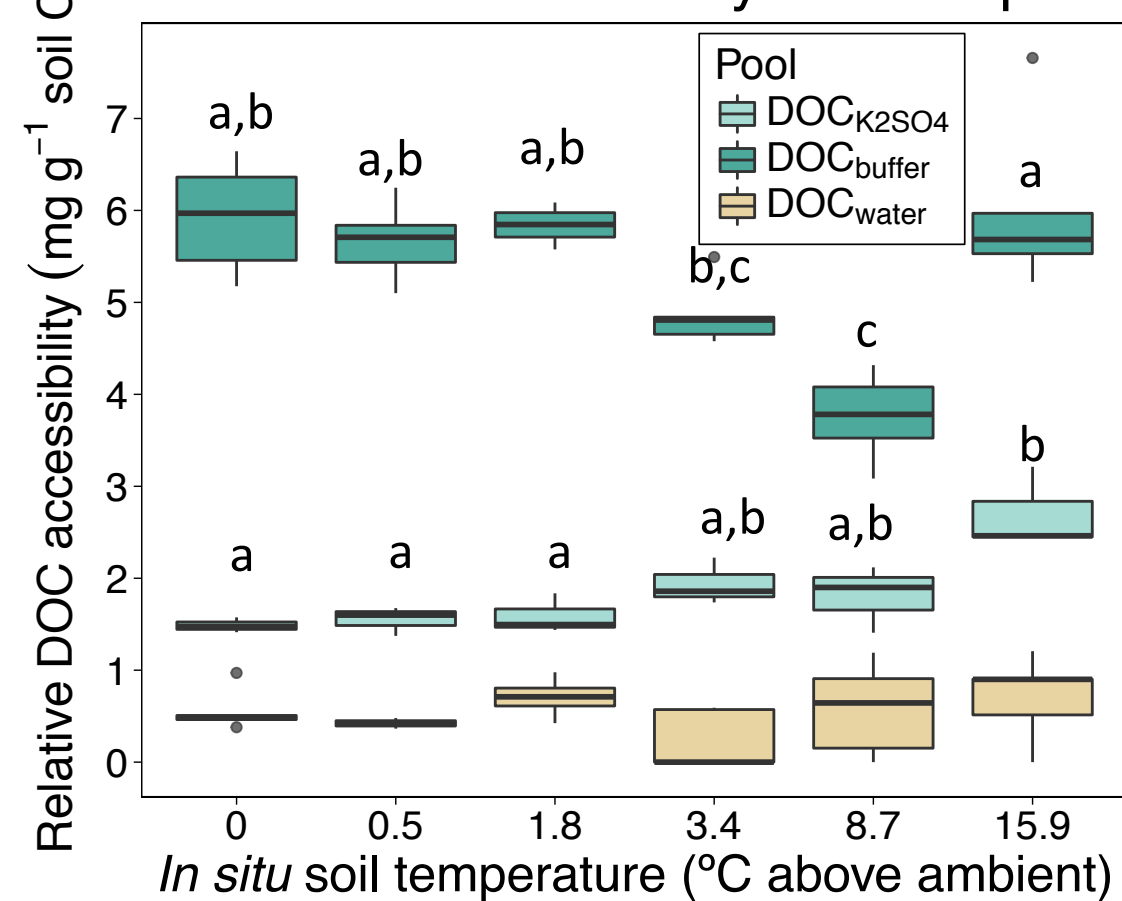


Figure 4

## A. Soil DOC pools



## B. Relative accessibility of DOC pools



## C. C:N ratios and C:N imbalance

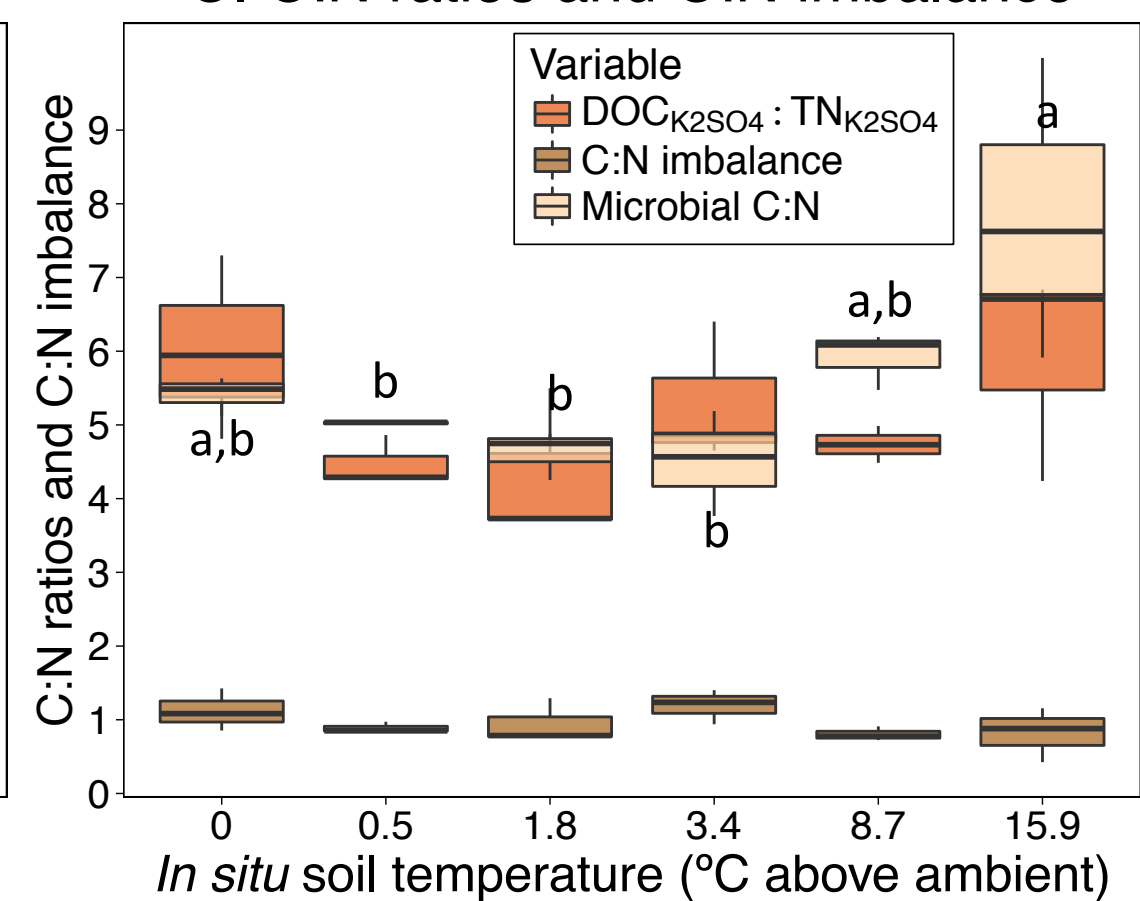
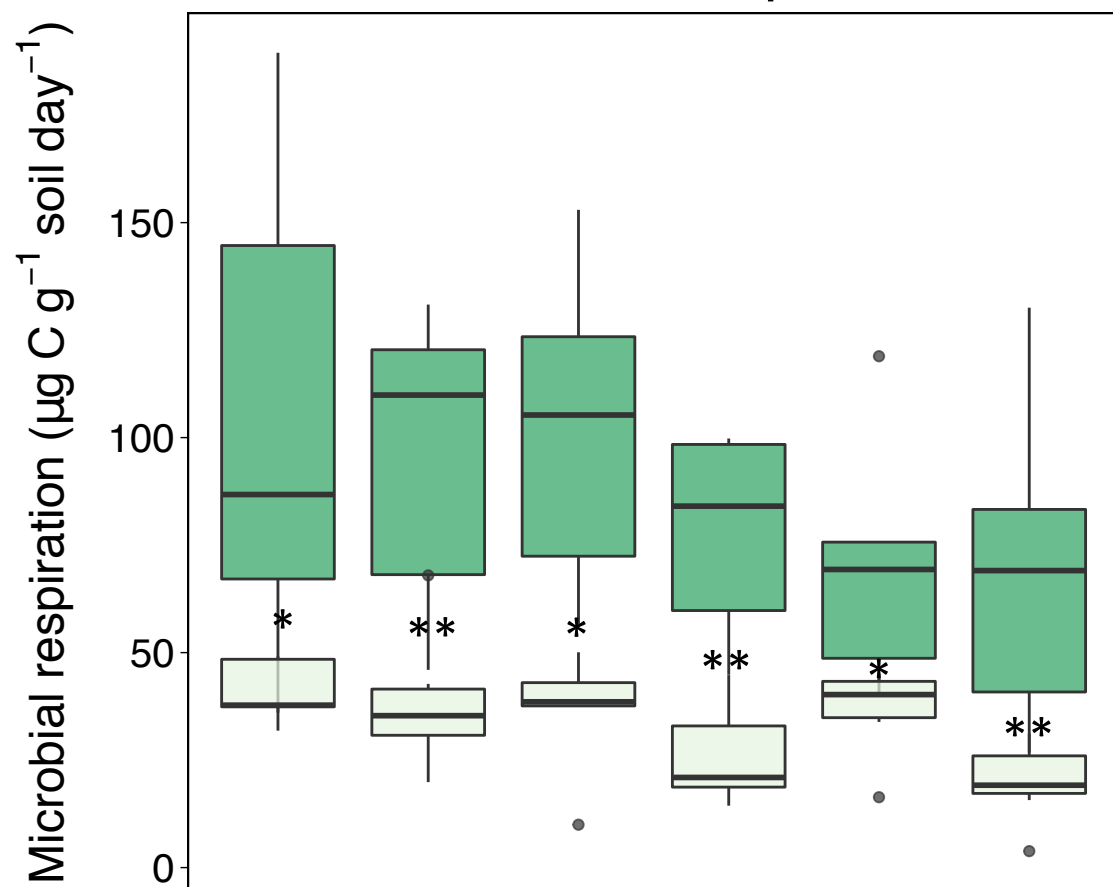
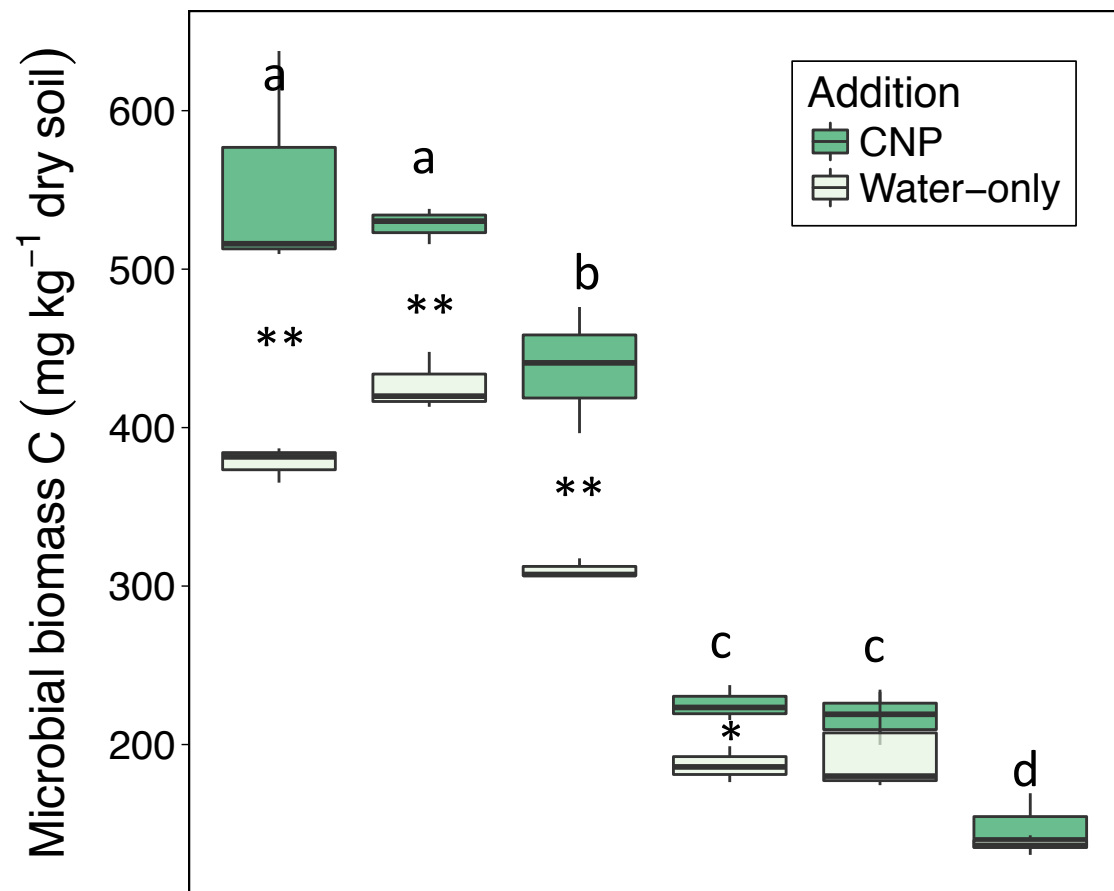


Figure 5

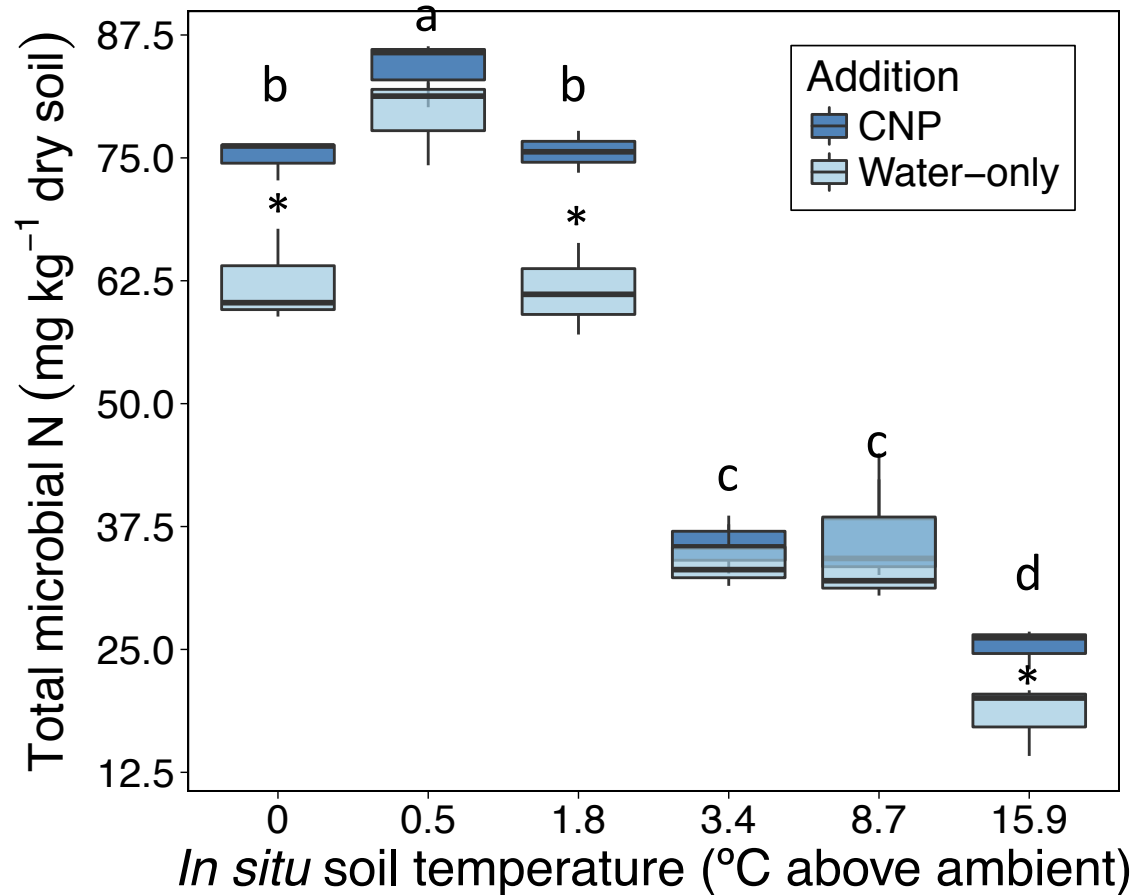
## A. Microbial respiration



## B. Microbial biomass C



## C. Total microbial N



## D. Microbial C:N ratios

