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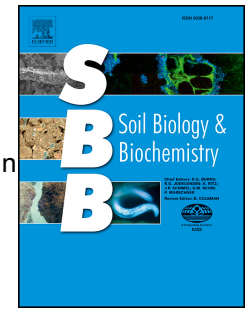

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S. Marañón-Jiménez, J. Peñuelas, A. Richter, B.D. Sigurdsson, L. Fuchslueger, N.I.W. Leblans, I.A. Janssens

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

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

Marañón-Jiménez S.[1,2,3], Peñuelas J.[1,2,4], Richter A.[5], Sigurdsson B. D.[6], Fuchslueger, L.[3], Leblans N.I.W.[3], Janssens I. A.[3]

[1]CREAF, Cerdanyola del Vallès, 08193 Barcelona, Spain

[2]CSIC, Global Ecology Unit CREAF-CSIC-UAB, Bellaterra, 08193 Barcelona, Spain

[3]Centre of Excellence PLECO (Plant and Vegetation Ecology), Department of Biology, University of Antwerpen, Campus Drie Eiken, Universiteitsplein 1, C. 203, BE- 2610, Wilrijk, Belgium.

[4]Institut d'Estudis Catalans, 08001 Barcelona, Spain

[5]Department of Microbiology and Ecosystem Science, University of Vienna, Althanstraße 14, 1090 Wien, Austria

[6]Agricultural University of Iceland, Hvanneyri 311, Borgarnes, Iceland

[‡]Address correspondence to S. Marañón-Jiménez, email: s.maranon@creaf.uab.es

Research Article

Abstract

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

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

1. Introduction

Global warming is expected to accelerate the decomposition of soil organic matter (SOM) more than its production, causing large releases of CO_2 to the atmosphere and positive feedbacks to the climatic system (Davidson and Janssens et al. 2006, Jenkinson et al. 1991). Soils at northern latitudes store more than half of the surface-soil carbon (C) (Tarnocai et al. 2009). As their SOM decomposition has been strongly limited by low temperatures and they are warming more rapidly, they are particularly vulnerable to temperature driven C losses (Smith et al. 2015, Crowther et al. 2016). As such, warming of northern soils may potentially increase global concentrations of atmospheric CO_2 (McGuire et al. 2009). Model predictions for future CO_2 emissions and climate change projections by the Intergovernmental Panel on Climate Change (IPCC) remain, nonetheless, largely uncertain (Friedlingstein et al. 2006, Todd-Brown et al. 2013), partly due to the lack of accurate representation of vegetation and soil microbial feedbacks (Bardgett et al. 2013, Friedlingstein et al. 2006) and interactions between C and nutrient cycles (Bardford et al. 2016, Friedlingstein et al. 2006).

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

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

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

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

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

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

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

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

2. Methods

2.1. Study site

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

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

2.2. Experimental design and soil sampling

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

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

2.3. Initial soil parameters

Three subsamples of 15, 7.5 and 7 g of fresh soil were extracted with 2 M KCl, 0.5 M $NaHCO_3$ and 0.5 M K_2SO_4, respectively, within 24 h of sampling. Ammonium (NH_4^+)

and nitrate (NO_3^-) were determined from the KCl extracts (Bremner and Keeney 1965). Half of the $NaHCO_3$ and K_2SO_4 extract volume was digested at 400 ºC with H_2SO_4 with selenium as a catalyst. Total phosphorus (P) and total extractable N (TN_{K2SO4}) were determined from the digested $NaHCO_3$ and K_2SO_4 extracts, respectively. Available inorganic P (P_{inorg}) was determined from the undigested $NaHCO_3$ extracts (Olsen et al. 1954) and dissolved organic C (DOC_{K2SO4}) and NH_4^+ from the undigested K_2SO_4 extracts. Organic P (P_{org}) and dissolved organic N (DON_{K2SO4}) were determined as the difference between digested and undigested $NaHCO_3$ and K_2SO_4 extracts, respectively (Jones and Willett 2006). Two other pools of soluble organic C were quantified using extractants of different ionic strengths. For this, two subsamples of 10 g of fresh soil were extracted with deionized water (DOC_{water}), which is a common measure of readily-soluble C, and a weak phosphate buffer at 10 mM (0.33 mM KH_2PO_4 and 6.67 mM Na_2HPO_4) adjusted to pH 7.0 (DOC_{buffer}), which extracts both the readily-soluble C and weakly adsorbed C in clay minerals (Nelson et al. 1994, Kaiser and Zech 1999). The lower ionic strength and pH of the buffer solution compared to the 0.5 M K_2SO_4 solution reduces the flocculation of organic colloids and the re-adsorption of the solubilized C onto the diffuse double layer surrounding clay particles (Haney et al. 2001). The relative accessibility of extractable soil C pools (DOC_{K2SO4}, DOC_{water}, DOC_{buffer}) was calculated as the ratio of DOC to SOC pools.

Another set of subsamples of the same mass of fresh soil were also extracted as described above for determining microbial biomass C and total microbial N and P by fumigation-extraction (Jenkinson and Powlson 1976). Microbial biomass C (C_{micro}), total microbial N (N_{micro}) and total microbial P (P_{micro}) were determined as the difference in DOC_{K2SO4}, TN_{K2SO4} and total P between the fumigated and unfumigated subsamples, respectively. All analyses were performed by colorimetric detection with a San^{++} Continuous Flow Analyzer (Skalar Analytical B.V., Breda, The Netherlands). NO_3^- was determined after reduction to NO_2^- and formation of the diazo complex at 540 nm wavelength (EN-ISO 13395). NH_4^+ was determined after reaction with salicylate, a catalyst and active chlorite solution to form a green colored complex at 660 nm wavelength (ISO 11732). TN_{K2SO4} and NH_4^+ in digested and undigested K_2SO_4 extracts respectively, were determined colorimetrically at 660 nm wavelength. DOC_{K2SO4} was determined after reaction with phenolphthalein at 550 nm wavelength (ISO 5667-3). P_{inorg} was determined colorimetrically as phospho-molybdic complex at 880 nm

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

We calculated the stoichiometric C:N imbalance between soil organic pools and microbial biomass following Mooshammer et al. 2014a, as the ratio of C:N in the SOM pools (SOC:TN and DOC_{K2SO4}:TN_{K2SO4}) over microbial biomass C:N (C_{micro}:N_{micro}). The C:N imbalance is then a measure of the divergence between the C:N stoichiometry of soil microbes and soil organic substrates, where C:N imbalance < 1 thus reflects a lack of C in SOM pools for soil microbes.

2.4. Substrate addition and soil incubation

Subsamples of 40 g (dry equivalent) of fresh soil from the unwarmed ambient plots (hereafter "incubation flasks") were distributed into flasks within 72 h after sampling. In order to determine the stoichiometric demands of soil microorganisms and their capacity of C storage and N immobilization (experiment of stoichiometric demands characterization), a 1-ml of deionized water solution with a source of C, N, P or their combinations (hereafter "addition") was added to each flask. We hypothesized that losses of soil N were associated with a restricted capacity of microbial N immobilization, so we tested the effect of two levels of N addition instead of the CP combination. C was added as glucose (1.73 mg of glucose g^{-1} dry soil, that is, 0.69 mg C g^{-1}), N was added as NH_4NO_3 (0.1 mg of NH_4NO_3 g^{-1}, that is, 34 µg N g^{-1} for the N addition level and 0.05 mg of NH_4NO_3 g^{-1}, 17 µg N g^{-1} for the "half-N" addition level) and P was added as KH_2PO_4 (0.101 mg KH_2PO_4 g^{-1}, 23 µg P g^{-1}). The amount of substrates added accounted for ca. 1 % of the initial soil C content and 0.7 and 0.35 % of the initial soil N content for N and "half-N", respectively (Table 1). Phosphorous retention is generally >90 % for Icelandic Andosols (Arnalds et al. 1995), so that the P added was ca. ten times the initial available inorganic P soil content to ensure that enough P was accessible to soil microbes. These amounts of substrates were chosen to ensure the alleviation of potential C and nutrient limitations of soil microbes while

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

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

The soils were then incubated at the mean annual soil temperature in the field (5 °C) and allowed to equilibrate for 12 h. This time lapse was determined in a preliminary assay using the same soils based on the time needed to obtain acceptable coefficients of variability (<20 %) of microbial respiration. Microbial respiration (i.e. substrate induced respiration) was then measured in all samples using an infrared gas analyzer (EGM-4/SRC-1, PP-Systems, Hitchin, UK) coupled to a custom-made chamber with a fan and vent. Incubation flasks were partially closed during the incubation to prevent drying but allow the gas exchange. The flasks were ventilated with a fan for ca. 2 minutes prior each respiration measurement to release the accumulated CO_2 in soil pores and in the air layer closed to the soil surface. Flasks were immersed in a water bath at a constant temperature of 5 ºC to maintain the targeted temperature during the respiration measurements. Temperature was continuously monitored during the measurements and incubation using TidbiT v2 HOBO Data Loggers (Onset Computer Corporation, Bourne, USA). Gravimetric soil moisture stayed constant at 60 % water-holding capacity throughout the experiment.

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

substrate uptake and growth (Nedwell 1999). C_{micro}, N_{micro} and the remaining DOC_{K2SO4}, NH_4^+ and DON_{K2SO4} in the soil were determined for all incubated samples as described above six days after the C and nutrient additions to allow soil microbes to take up the substrates. We were only interested in relative differences among treatments, so the concentrations in the microbial fraction presented here were not corrected for extraction efficiency. All fractions are presented relative to soil dry mass.

2.5. Data analyses

The effect of *in situ* soil warming on initial soil and microbial C and nutrient contents and ratios prior to the incubations was tested using one-way ANOVAs, and differences among warming levels were further tested by post hoc tests with Tukey correction for multiple testing. The effects of C, N and P substrate additions on microbial respiration, C_{micro}, N_{micro}, microbial C:N ratios; the remaining DOC_{K2SO4}, NH_4^+ and DON_{K2SO4} and the DOC_{K2SO4}:TN_{K2SO4} ratio in unwarmed soils (experiment of stoichiometric demands characterization) after the incubation were tested using one-way ANOVAs, and differences among addition levels were further tested by post hoc tests with Tukey correction for multiple testing. The differences from soils without any addition were also tested using post hoc Dunnett´s tests, using the "water-only" unamended soils as control. The effect of soil warming, substrate addition (C, N and P combined) and their interaction on microbial respiration, C_{micro}, N_{micro}, the microbial C:N ratio (experiment of warming impacts on soil C and N retention) were tested using two-ways ANOVAs, with "addition" and "*in situ* soil warming" as fixed factors. Differences among *in situ* warming levels were further tested by post hoc tests with Tukey correction for multiple testing. The effect of substrate addition on the above variables was also tested for each warming level separately by one-way ANOVAs. Data were transformed when required to improve normality and homoscedasticity (Quinn and Keough, 2009). Stoichiometric ratios were calculated on a mass basis. Statistical analyses and model construction were performed using JMP 13.0 (SAS Institute). All results are presented as means ± standard errors.

3. Results

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

Microbial biomass C (non-corrected for extraction efficiency) constituted only 0.63 % of the SOC in this subarctic soil but contained four times more C than the DOC_{K2SO4} pool (Table 1). Microbial respiration increased ca. 12 h after the C addition ($P<0.001$), but N addition and P addition did not cause any significant changes in the rate of microbial respiration (Fig. 1a), either alone or in combination with C.

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

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

The microbial N pool represented ca. three times the total extractable N in the soil (Table 1). Most of this extractable soil N (79 %) was in an organic form, while NH_4^+ and NO_3^- represented only 17 % and 3 % of this pool, respectively (Table 1). Total microbial N only increased significantly in response to the combined addition of C and N (Fig. 2a, $P=0.02$), although values also increased, but not significantly, in all the rest of the addition levels. Consequently, the C addition also caused a depletion of the NH_4^+ in soil (Fig. 2b, $P<0.001$). Circa 82 and 72 % of the NH_4^+ initially available was depleted from the soil when C and N were added in combination in the CN and CNP addition levels, respectively (Fig. 2b), while a large proportion (86, 81 and 111 % for "half-N", N and NP, respectively) still remained in the soil otherwise. In contrast, soil DON_{K2SO4} decreased in response to N-only addition (Fig. 2c, $P=0.007$).

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

The C:N ratios of K_2SO_4-extractable soil organic substrates decreased to lower values than in microbial biomass after six days of incubation (C:N imbalance <1, Fig. 3). Microbial C:N ratios increased significantly in response to the CNP addition and decreased after the addition of N and P only ($P<0.001$).

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

Seven years of continuous warming provoked a substantial depletion of the pools of DOC extracted with K_2SO_4 and with phosphate buffer (DOC_{K2SO4} and DOC_{buffer},

respectively, Fig. 4a), while the most readily-available DOC pool (DOC_{water}) did not show a consistent decreasing pattern with soil temperatures *in situ*. Moreover, the relative accessibility of the DOC_{buffer} pool, calculated as the ratio of DOC_{buffer} to SOC pools, decreased with the intensity of soil warming up to 8.7 ºC above ambient ($P<0.001$, Fig. 4b), while the relative accessibility of the DOC_{K2SO4} pool was not substantially affected below this soil warming intensity. Nonetheless, the non-extractable C pools (SOC) were depleted in a higher proportion at the highest warming level (15.9 ºC above ambient, Table 1), contributing to increase the relative accessibility of both the DOC_{K2SO4} and DOC_{buffer} pools. The relative accessibility of the DOC_{water} pool remained however unaffected by *in situ* soil warming.

Soil warming also decreased the pools of soil DOC_{K2SO4} and TN_{K2SO4} proportionally, without any significant shifts in DOC_{K2SO4}:TN_{K2SO4} ratios along the *in situ* temperature gradient (Fig. 4c). Even though the C:N ratios of soil organic matter (SOC:TN) were 2.3 times higher than the C:N ratios of microbial biomass, the imbalance from the C:N of the extractable fraction of organic substrates (DOC_{K2SO4}:TN_{K2SO4}) was initially close to one (Fig. 4c), since the C:N ratios of the extractable organic pools were much lower than the ratios of the total organic matter pools. Warming did not cause shifts in the stoichiometric imbalance between the extractable organic substrates and microbial biomass, given the coupled and proportional losses of C and N from both biomass and soil (Fig. 4c).

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

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

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

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

4. Discussion

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

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

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

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

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

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

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

Carbon limitation and the strict C:N stoichiometric needs of soil microbes (Zeichmeister-Boltenstern et al. 2015) actually constrained microbial N immobilization. Only the C addition provoked a significant increase in microbial N (Fig. 2a), and N immobilization was highest when C and N were added in combination, although the addition of inorganic N alone also stimulated microbial N immobilization slightly. A 86, 81 and 111 % of the total NH_4^+ initially available still remained in the soil six days after addition for the “half-N”, N and NP addition levels, respectively (Fig. 2b), while only 18 to 28 % remained when C was also added for the CN and CNP additions. The decrease of microbial biomass (Fig. 1b) and the predominant use of DON as C source when only N was added (Fig. 2c) are further evidences of C limitation for microbial growth and N immobilization (Farrell et al. 2014). Similar C constraints of microbial N demands have been observed in Siberia (Wild et al. 2015), reminding the need to frame the concept of C or nutrient limitation to specific ecosystem components or processes rather than generalizing to entire ecosystems. Sub-surface soils (>5 cm depth) also showed no capacity for net retention of increased N inputs after 20 years of fertilization experiment in Alaska, leading to a net C loss (Mack et al. 2004). Soils with relatively low C:N ratios may also present a secondary microbial P limitation. The addition of P in these soils may fuel the synthesis of P-rich mRNA for protein transcription (Elser et al. 1996), enhancing immobilization of soil DON for protein synthesis up to certain level, where the N immobilization is again saturated and limited by C availability (Hessen et al. 2007). This limitation was evidenced by the decrease in microbial biomass in

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

4.2. Response of microbial cycling to soil warming

Seven years of continuous soil warming led to a substantial loss of total soil C and N from the upper 10 cm (Table 1), but not all pools of SOC were depleted equally. Both DOC_{K2SO4} and DOC_{buffer} pools decreased significantly with *in situ* soil warming, while the DOC_{water} pool did not show a consistent decreasing pattern (Fig. 4a). In relative terms, soil warming provoked a predominant depletion of the DOC_{buffer} pool in relation to the total SOC up to +8.7 °C warming (Fig. 4b), indicating a proportional decrease of the soil organic C adsorbed within the soil minerals. Water-extractable C is known as the most readily-available C pool for soil microbes, but it has also shown a lower biodegradability compared to the buffer-extractable C pool when both pools are fully accessible to soil microbes (Nelson et al. 1994, Wagai and Sollings 2002). Soil microbes may have resorted on the weakly-adsorbed C fraction, the largest DOC pool in these soils, as a predominant C source as the water-extractable C pool was depleted at increasing soil temperatures. Increasing rates of depolymerization and solubilization from the weakly-adsorbed SOM fraction may have also contributed to increase the water-extractable C inputs, compensating the microbial consumption of this pool. Nonetheless, the non-extractable C pools (SOC) also experienced a predominant depletion at the most extreme warming level (15.9 ºC above ambient), probably causing a decrease in the surface of organic colloidal surfaces, which contributed to increase the relative accessibility of both the DOC_{K2SO4} and DOC_{buffer} pools. Therefore, soil microbes may have satisfied their increasing energy demands at warmer temperatures by a proportionally higher solubilization of the C adsorbed in soil mineral surfaces.

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

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

5. Conclusions

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

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Figure captions:

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

Figure 2: A) Total microbial N, B) remaining NH4+ and C) remaining dissolved organic N in unwarmed soils six days after the C, N and P additions. Triangles indicate the initial NH4+ concentration in soil prior to the soil incubation. Different letters indicate significant differences by Tukey´s post hoc tests at α=0.05.

Figure 3: C:N ratios in A) soil microbes and B) K_2SO_4 extractable organic pools from unwarmed soils six days after to the C, N and P additions. Different letters indicate significant differences by Tukey´s post hoc tests at α=0.05.

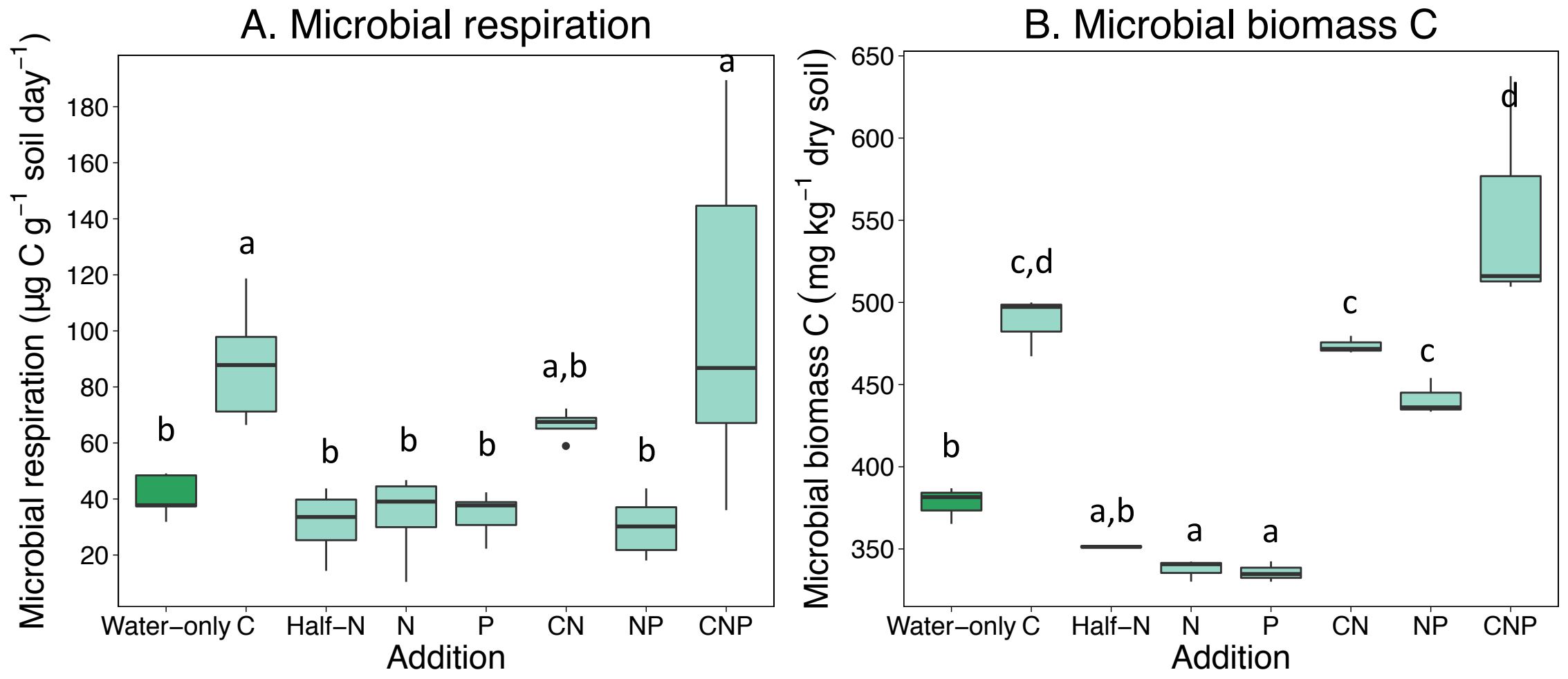
Figure 4: A) Dissolved organic C pools, B) their relative accessibility and C) C:N ratios of K_2SO_4 extractable organic pools, microbial biomass and the C:N imbalance between these at the different intensities of soil warming. Data correspond to the initial values in soils before the incubation or substrates addition. The relative accessibility of extractable soil C pools was calculated as their ratio to the total organic C pool. The C:N imbalance was calculated as the ratio of C:N of soil organic pools over microbial C:N. Different letters indicate significant differences by Tukey´s post hoc tests at α=0.05.

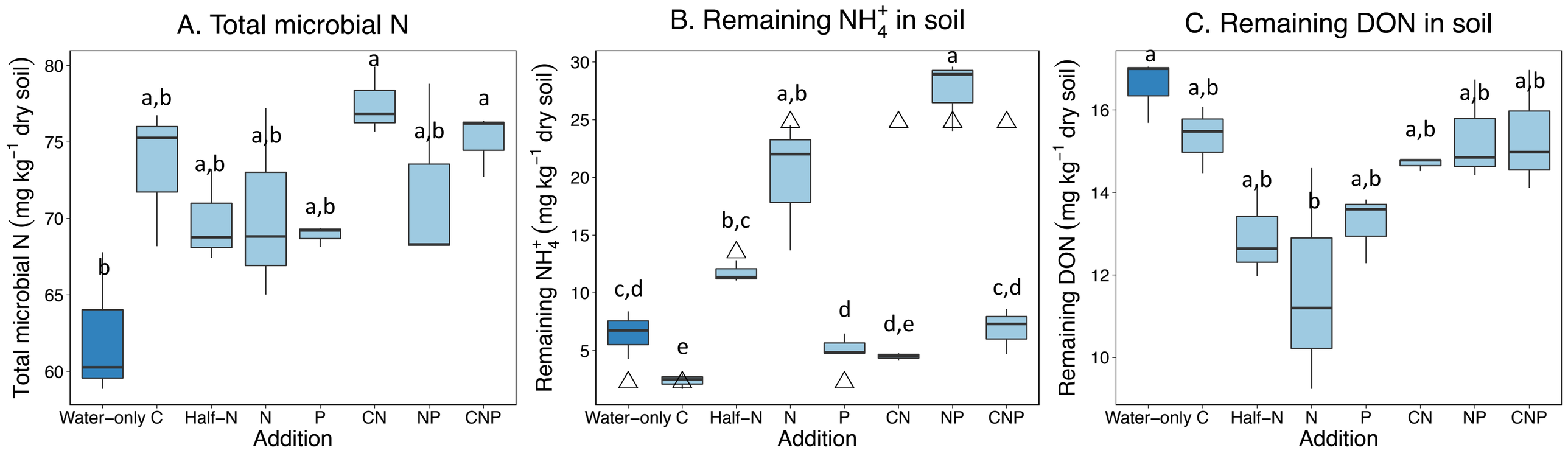
Figure 5: A) Microbial respiration, B) microbial biomass C, C) total microbial N and D) microbial C:N ratios in response to the C, N and P addition at the different intensities of soil warming. Microbial respiration was measured 12 h after the additions at the mean annual soil temperature (5 °C). Microbial biomass C and N were measured six days after the additions. Different letters indicate significant differences among the soil warming intensities according to two-way ANOVAs and Tukey´s post hoc tests. * and ** indicate significant differences between substrate addition levels within each soil warming intensity according to one-way ANOVAs: *$0.01<P\leq0.05$, **$0.001\leq P\leq0.01$.

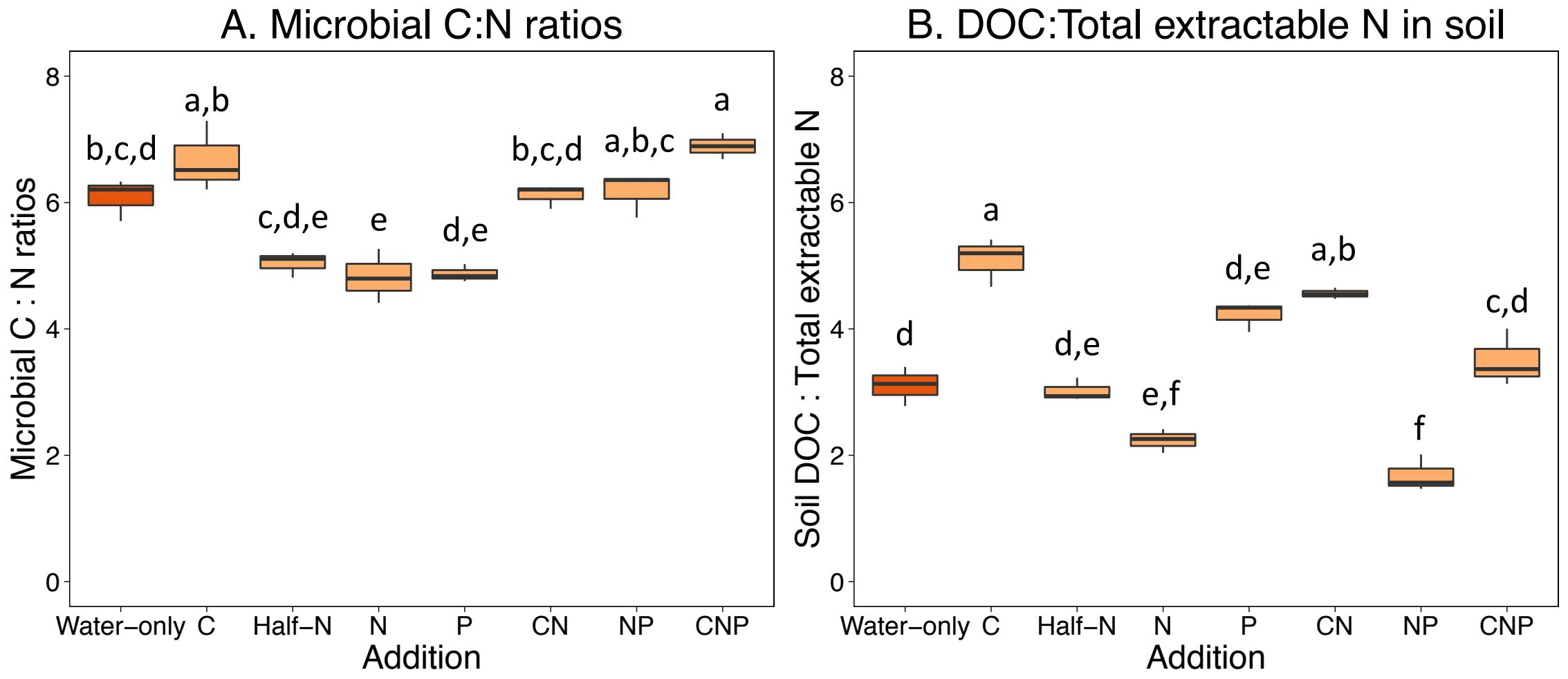
Table 1: Main soil parameters along the *in situ* soil warming levels at the time of sampling. $P_{0.05}$-$P_{0.95}$, range of mean soil temperature values between the 5th and 95th percentiles; WHC, water holding capacity; SOC, total soil organic C; TN, total soil N; DON_{K2SO4}, dissolved organic N in K_2SO_4; P_{inorg}, available inorganic P in $NaHCO_3$; P_{org}, organic P in $NaHCO_3$; C_{micro}, microbial biomass C; N_{micro}, total microbial N; P_{micro}, total microbial P. Different letters indicate significant differences among sites (Tukey´s post hoc tests after one-way ANOVAs). Intervals indicate ±standard errors.

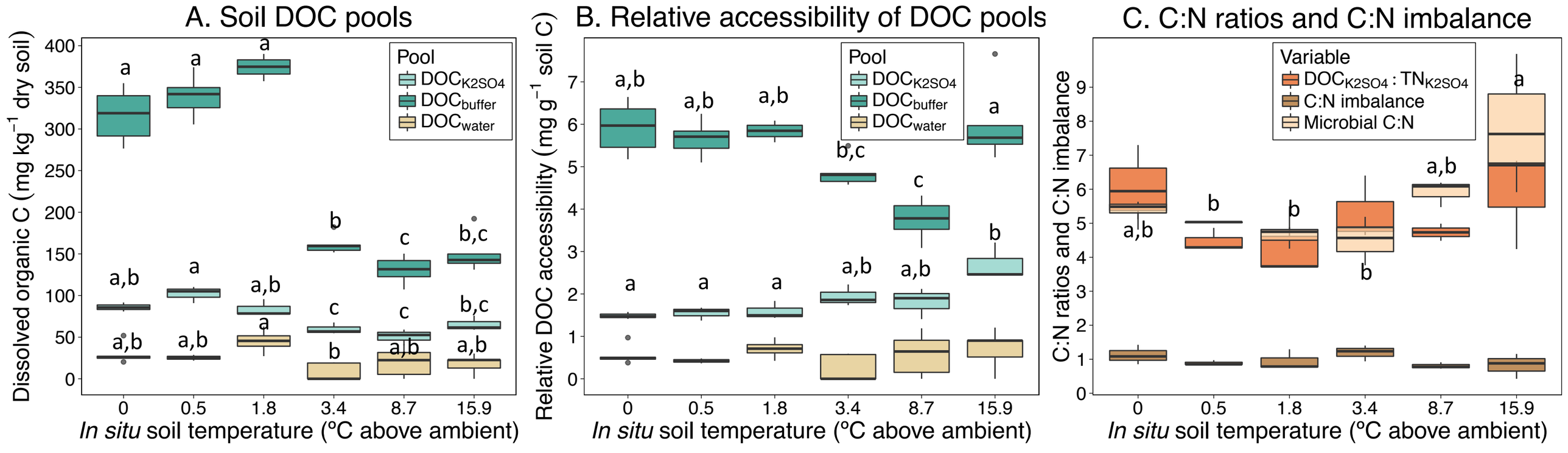
Soil parameter	*In situ* soil warming (ºC above ambient)						*F*	*P*
	0	0.5	1.8	3.4	8.7	15.9		
Mean annual soil T^a (ºC)† ($P_{0.05}$-$P_{0.95}$)	5.6±0.1^a (0.1-13.0)	6.0±0.1^{a,b} (0.2-13.4)	7.3±0.6^{b,c} (0.8-15.9)	8.9±0.2^c (2.3-17.1)	14.3±1.1^d (5.0-26.2)	21.5±0.4^e (11.7-33.8)	110.99	≤0.0001
WHC (%)	117.0±1.7^{a,b}	129.8±3.3^a	117.1±4.9^{a,b}	112.2±1.7^b	111.8±4.5^b	109.1±3.3^b	4.6080	0.0141
SOC (%)†	5.78±0.03^b	6.59±0.02^a	5.28±0.06^c	3.08±0.03^d	2.81±0.03^e	2.43±0.04^f	2038.63	≤0.0001
TN (%)†	0.483±0.003^b	0.563±0.003^a	0.4±0^c	0.257±0.003^d	0.237±0.003^e	0.223±0.003^f	1840.80	≤0.0001
SOC:TN	11.97±0.07^b	11.7±0.04^b	13.21±0.15^a	12.01±0.12^b	11.86±0.12^b	10.87±0.08^c	52.11	≤0.0001
DON_{K2SO4} (mg kg^{-1} dry soil)	12.41±1.64^{a,b}	15.79±2.01^a	10.81±1.35^{a,b}	7.69±1.27^b	7.70±1.18^b	10.12±3.15^{a,b}	3.49	0.0392
NH_4^+ (mg kg^{-1} dry soil)†	2.72±0.86^c	6.84±0.36^a	9.15±0.48^a	3.93±0.16^b	2.64±0.04^{b,c}	1.43±0.05^d	50.93	≤0.0001
NO_3^- (mg kg^{-1} dry soil)†	0.490±0.032^c	0.675±0.043^b	1.221±0.058^a	0.803±0.026^b	0.301±0.014^d	0.174±0.001^e	206.56	≤0.0001
P_{inorg} (mg kg^{-1} dry soil)	2.16±0.18^b	2.24±0.11^b	2.42±0.04^b	2.93±0.09^a	2.50±0.02^b	2.40±0.03^b	9.41	≤0.0001
P_{org} (mg kg^{-1} dry soil)†	10.60±0.26^b	14.12±0.35^a	9.49±0.41^b	5.43±0.22^c	3.30±0.23^d	3.83±0.12^d	171.23	≤0.0001
C_{micro} (mg kg^{-1} dry soil)‡	365.06±10.86^a	413.84±12.28^a	305.69±25.02^a	153.63±12.10^b	172.72±16.73^b	139.15±24.30^b	33.88	≤0.0001
N_{micro} (mg kg^{-1} dry soil)	67.54±2.57^{a,b}	82.35±2.66^a	66.32±6.16^b	34.20±1.16^c	29.07±1.87^{c,d}	17.95±2.74^d	62.53	≤0.0001
P_{micro} (mg kg^{-1} dry soil)	5.45±0.89^a	4.34±0.38^{a,b}	3.00±0.60^{b,c}	2.80±0.30^{b,c,d}	1.91±0.32^{c,d}	0.74±0.31^d	11.17	≤0.0001
C_{micro}:P_{micro}†	67.02±1.99^{b,c}	95.43±2.83^b	101.87±8.34^b	54.90±4.32^c	90.39±8.75^b	188.05±32.84^a	17.00	≤0.0001
N_{micro}:P_{micro}	12.40±0.47^c	18.99±0.61^{a,b,c}	22.10±2.05^{a,b}	12.22±0.41^c	15.21±0.98^{b,c}	24.26±3.70^a	7.82	0.0018
pH*	5.55±0.01^b	5.48±0.00^a	5.70±0.01^c	5.96±0.01^d	6.14±0.00^e	6.20±0.01^f	1350.3	≤0.0001

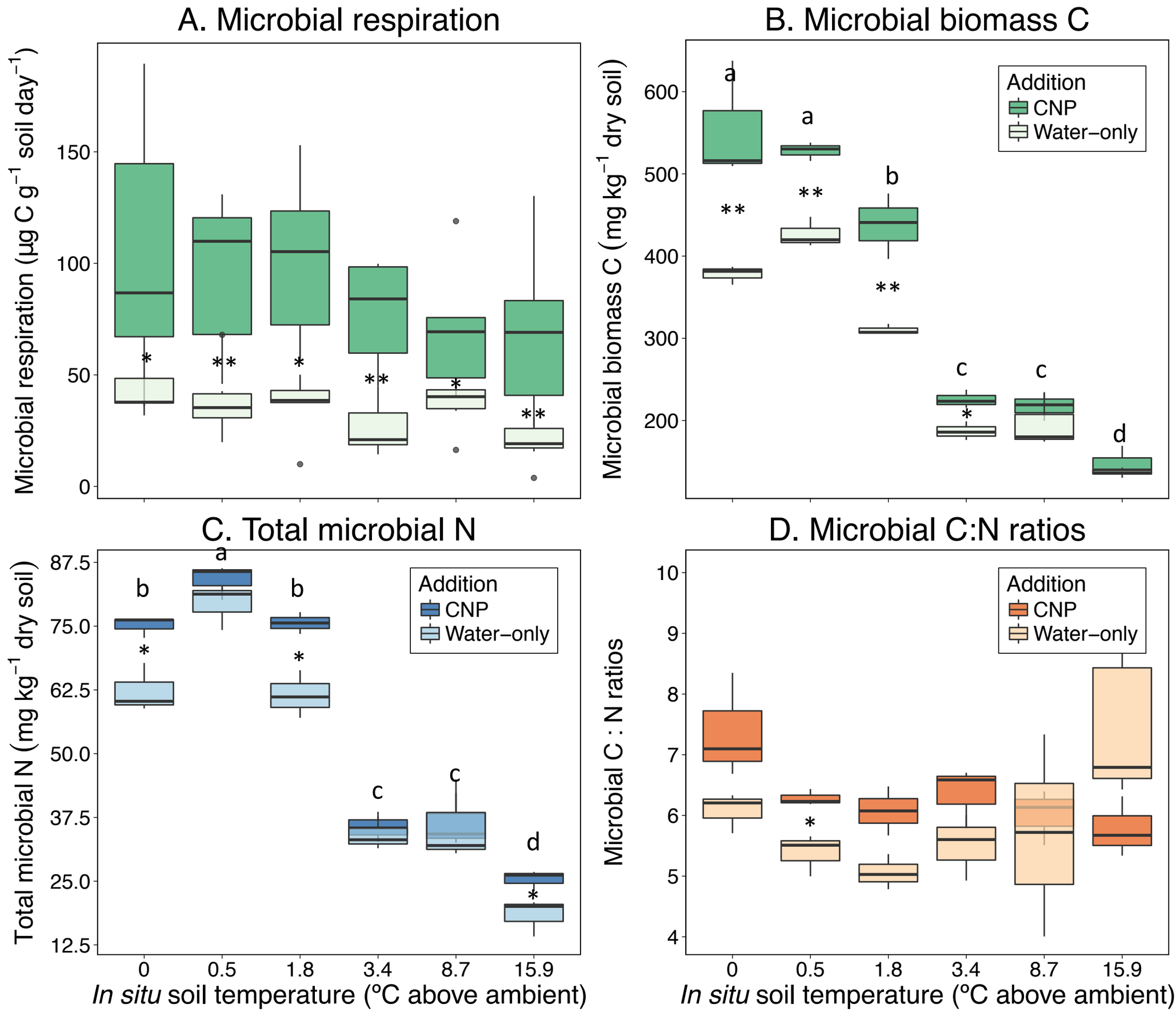
†Log-transformed data before ANOVAs
*Exponential-transformed data before ANOVAs
‡Square root-transformed data before ANOVAs


A. Microbial respiration
Microbial respiration (µg C g−1 soil day−1)
20
40
60
80
100
120
140
160
180
b
a
b
b
b
a,b
b
a
Water−only C
Half−N
N
P
CN
NP
CNP
Addition
B. Microbial biomass C
Microbial biomass C (mg kg−1 dry soil)
350
400
450
500
550
600
650
b
c,d
a,b
a
a
c
c
d
Water−only C
Half−N
N
P
CN
NP
CNP
Addition


A. Total microbial N
Total microbial N (mg kg−1 dry soil)
80
75
70
65
60
b
a,b
a,b
a,b
a,b
a
a,b
a
Water−only C
Half−N
N
P
CN
NP
CNP
Addition
B. Remaining NH4+ in soil
Remaining NH4+ (mg kg−1 dry soil)
30
25
20
15
10
5
c,d
e
b,c
a,b
d
d,e
a
c,d
Water−only C
Half−N
N
P
CN
NP
CNP
Addition
C. Remaining DON in soil
Remaining DON (mg kg−1 dry soil)
16
14
12
10
a
a,b
a,b
b
a,b
a,b
a,b
Water−only C
Half−N
N
P
CN
NP
CNP
Addition


A. Microbial C:N ratios
Microbial C : N ratios
0
2
4
6
8
b,c,d
a,b
c,d,e
e
d,e
b,c,d
a,b,c
a
Water−only
C
Half−N
N
P
CN
NP
CNP
Addition
B. DOC:Total extractable N in soil
Soil DOC : Total extractable N
0
2
4
6
8
d
a
d,e
e,f
d,e
a,b
f
c,d
Water−only
C
Half−N
N
P
CN
NP
CNP
Addition


A. Soil DOC pools
Dissolved organic C (mg kg^{-1} dry soil)
Pool
DOC$_{K2SO4}$
DOC$_{buffer}$
DOC$_{water}$
0
50
100
150
200
250
300
350
400
a
a
a
b
c
b,c
a,b
a
a,b
a
c
b,c
a,b
a,b
b
a,b
0
0.5
1.8
3.4
8.7
15.9
In situ soil temperature (°C above ambient)
B. Relative accessibility of DOC pools
Relative DOC accessibility (mg g^{-1} soil C)
Pool
DOC$_{K2SO4}$
DOC$_{buffer}$
DOC$_{water}$
0
1
2
3
4
5
6
7
a,b
a,b
a,b
b,c
a
a
a
a
a,b
a,b
b
0
0.5
1.8
3.4
8.7
15.9
In situ soil temperature (°C above ambient)
C. C:N ratios and C:N imbalance
C:N ratios and C:N imbalance
Variable
DOC$_{K2SO4}$: TN$_{K2SO4}$
C:N imbalance
Microbial C:N
0
1
2
3
4
5
6
7
8
9
a,b
b
b
b
a,b
a
0
0.5
1.8
3.4
8.7
15.9
In situ soil temperature (°C above ambient)


A. Microbial respiration
Microbial respiration (µg C g^{-1} soil day^{-1})
B. Microbial biomass C
Microbial biomass C (mg kg^{-1} dry soil)
Addition
CNP
Water−only
C. Total microbial N
Total microbial N (mg kg^{-1} dry soil)
D. Microbial C:N ratios
Microbial C : N ratios
In situ soil temperature (°C above ambient)
0
0.5
1.8
3.4
8.7
15.9

- Warming triggered large and proportional C and N losses from these subarctic soils
- Weakly sorbed organic substrates in soil minerals were depleted predominantly
- Warmed soils were able to sustain a lower microbial biomass
- Strict microbial C:N stoichiometric demands also constrained N retention
- This impaired soil N storage and increased its vulnerability to C losses