Unexpected microbial metabolic responses to elevated temperatures and nitrogen addition in subarctic soils under different land uses

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Abstract Subarctic regions are particularly affected by global warming. As vegetation periods lengthen, boreal forests could gradually be converted into agricultural land. How land use alters the susceptibility of soil organic matter decomposition to rising temperatures or how changes in nutrient availability, such as nitrogen (N) fertilisation, affect carbon (C) cycling is unknown. Microbial carbon use efficiency (CUE) defines how much of the decomposed soil organic carbon is directed to growth or lost to the atmosphere. Here, we investigated the response of CUE (24 h) and soil organic matter decomposition (50 days) to $+10\ °C$ warming and N addition in three subarctic soils derived from paired plots (forest, grassland, cropland) in the Yukon, Canada. Contrary to our literature-based expectations, boreal forest soils did not demonstrate the most sensitive response to warming and N addition. Temperature sensitivity was not affected by land-use type. In contrast to a generally assumed decline, short-term warming increased CUE by $+30\%$, which was positively correlated with microbial growth. N addition reduced overall CUE by -7%, in contrast to the expectation that CUE would rise due to the alleviation of nutrient limitations. The response to N addition was negatively correlated with the ratio of fungi to bacteria, and presumably depended on the prevailing N-fertilisation regime. The temperature sensitivity of microbial metabolism was driven by site-specific parameters rather than by land-use type. Our results indicate that it may not be necessary to consider land use-specific temperature sensitivities when modelling soil organic carbon dynamics under future climate conditions.

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Introduction

Microbial decomposition of soil organic matter (SOM) is expected to intensify with soil warming due to climate change (García-Palacios et al. 2021). Consequently, increased losses of CO_2 from the terrestrial C pool to the atmosphere will induce a positive feedback to climate change (Lloyd and Taylor 1994). Arctic and subarctic soils may particularly contribute to the soil carbon climate feedback (Karhu et al. 2014) since (1) high-latitude ecosystems with low mean annual temperatures are assumed to be more sensitive to warming (Lloyd and Taylor 1994; Bekku et al. 2003; Zhou et al. 2009), (2) temperature rises in these regions are higher than the global mean (IPCC 2014), and (3) northern ecosystems store large amounts of soil organic carbon (SOC) (Tarnocai et al. 2009). While the global mean temperature has increased by +1 °C compared with the 1951–1980 baseline, warming in Northern Canada has already reached +2.3 °C, with even larger temperature anomalies in winter (+4.3 °C) (https://climateatlas.ca). As a consequence, vegetation periods are becoming longer, which might encourage more agricultural activity in these subarctic regions (Tchebakova et al. 2011). In various climate zones, land-use changes from natural vegetation to agricultural land have been found to negatively affect SOC stocks (Guo and Gifford 2002; Grünzweig et al. 2004; Don et al. 2011). The combined effects of warming and land-use change could aggravate C losses from subarctic soils. However, there has been little investigation into the conversion of multi resource-limited subarctic forests to agricultural land. In particular, it is not known how land use alters the sensitivity of SOM decomposition to increasing temperatures or how changes in nutrient availability, due to N fertilisation for example, will affect C cycling since land-use change has a fundamental impact on soil chemistry, biology and organic matter quality (DeGryze et al. 2004; Poeplau and Don 2013; Szoboszlay et al. 2017). It can therefore be expected that the response of microbial communities to climate change varies across land-use and land-cover types. Models might need to consider varying temperature sensitivity with land use for better prediction of SOM decomposition and potential feedbacks.

The decomposition of SOM and its temperature sensitivity in mineral soils depend on the interplay of different factors (Davidson and Janssens 2006; Conant et al. 2011): the amount and quality of organic matter input to the soil, the physico-chemical protection of SOM and thus its availability for microbial decomposition, and the microbial community composition and its metabolic efficiency. The carbon-quality temperature theory assumes that the decomposition of low-quality substrates, i.e. with a more complex chemical structure, requires more exoenzymatic activity and higher total activation energy than the decomposition of a simple carbon substrate (Bosatta and Ågren 1999). Therefore, the decomposition of low-quality substances is thought to be more temperature dependent, with greater temperature sensitivity than the decomposition of more labile, high-quality substrates (Fierer et al. 2005; Leifeld and Fuhrer 2005; Wetterstedt et al. 2010). As the C quality of input differs with land-use type, this theory suggests potential differences in the temperature sensitivity of SOM decomposition between land-use types (Bosatta and Ågren 1999; Leifeld and Fuhrer 2005; Lehmann and Kleber 2015). In line with this theory, land-use type was found to explain 38% of variation in Q_{10} of SOM decomposition in temperate soils with Q_{10} values being highest in the order forest > grassland > cropland (Meyer et al. 2018). However, there has been limited investigation into the effect of land-use type (including agricultural use) on the temperature sensitivity of SOM decomposition in subarctic soils. This knowledge is needed to appropriately predict C dynamics under global change. We hypothesised that the response of SOM decomposition to elevated temperatures, as indicated by microbial respiration, differs between land-use types in subarctic soils, with forest soils being most affected due to their presumably greater levels of low-quality SOM. The extent of C losses due to warming may also depend on the temperature sensitivity of microbial carbon use efficiency (CUE), altering the share of C directed to growth to total uptake (Allison et al. 2010; García-Palacios et al. 2021). Based on theoretical considerations, it is often assumed in models that soil microbial CUE declines in response to warming, aggravating C losses (Manzoni et al. 2012; Sinsabaugh et al. 2013). However, in laboratory studies, warming has yielded contrasting effects on microbial CUE (e.g. Zheng et al. 2019; Simon et al. 2020; Li et al. 2021). Given the divergent literature and the complex dependence of microbial metabolism on SOM availability and its

temperature sensitivity, there is still a knowledge gap regarding the actual response of CUE to elevated temperatures and whether the type of land use influences the temperature sensitivity of CUE. To contribute to the discussion, we investigated the direct response of ^{18}O-CUE, microbial growth and respiration to warming in a laboratory incubation experiment. Since land use influences all the presented factors affecting SOM degradation and its potential temperature sensitivity, we hypothesised that the warming response of microbial CUE differ along the land-use intensity gradient of native forest, agricultural grassland and cropland soils.

Agricultural activities in subarctic soils alter the availability of nutrients such as nitrogen via direct fertilisation (cropland, agricultural grassland), atmospheric deposition and leaching (natural vegetation, i.e. boreal forest) which presumably affects SOM decomposition and thus C losses under global change. Increased N input is acknowledged to reduce heterotrophic respiration and thus SOM decomposition in soils of natural ecosystems (Zhou et al. 2014), such as boreal forest soils (Coucheney et al. 2013), and agricultural grassland soils (Spohn et al. 2016b; Poeplau et al. 2019; Widdig et al. 2020). However, N addition shows diverging effects on heterotrophic respiration in long-term fertilised cropland soils (Zhou et al. 2014; Geisseler and Scow 2014) and the response of heterotrophic respiration to N addition might depend on the prevailing N fertilisation (Poeplau et al. 2016). Three potential mechanisms explaining the reduction in heterotrophic soil respiration and SOM decomposition following N addition are discussed in the literature (Riggs and Hobbie 2016): (i) a reduction in microbial biomass, (ii) the direct inhibitory effect of N on oxidative enzymes, which decompose more complex C substrates such as lignin, and (iii) an alleviation of N mining resulting in increased microbial CUE. It is assumed that CUE is impaired in N-deficient soils, such as boreal forest soils, due to overflow respiration in order to access N sources from low-quality C sources with a high C: N ratio, i.e. 'N mining', (Schimel and Weintraub 2003), and more C has to be allocated to the production of extracellular enzymes required for nutrient acquisition (Manzoni et al. 2012; Sinsabaugh et al. 2013). Based on these mechanisms, we hypothesised that the inhibitory effect from N addition would be greatest in forest soils where lignin and ligninolytic microorganisms are typically more prevalent than in grassland and cropland soils (Riggs and Hobbie 2016). Furthermore, we expected that N addition would directly increase microbial CUE by altering nutrient stoichiometry and thus facilitating microbial metabolism in N-deficient soils (Sinsabaugh et al. 2016), and that this effect is most pronounced in nutrient-poor boreal forest soils. Consequently, we hypothesised that the N response differs between land-use types and that forest soils respond more sensitively to N addition, i.e. exhibit the greatest decreases in cumulative respiration, while cropland does not exhibit reduced respiration. In this case, N deposition would suppress the extent of C losses from boreal forest soils, but not from agricultural soils. To test the effect of altered N availability on C dynamics in subarctic soils under different land-use an N treatment was included in this study.

The objective of this study was to investigate whether land-use type affects the microbial metabolic response to warming and N addition in subarctic soils in order to clarify potential future C dynamics in these soils, which will be particularly affected by global change. To measure responses to warming and N addition, an incubation experiment was conducted including two temperature treatments (10 °C and 20 °C) and a laboratory N application treatment (20 °C + N). Based on state-of-the-art literature, we hypothesised that: (i) microbial metabolic response to warming and N addition differs between land-use types, with forest soils responding most sensitively, and that CUE (ii) declines with warming and (iii) increases with N addition. Furthermore, we aimed to identify drivers of warming and N response to better understand the mechanisms behind the metabolic responses.

Material and methods

Sites and sampling

To investigate the effect of warming and N fertilisation on metabolic parameters for subarctic soils under different land-use types, we chose three sites from a sampling campaign in the Yukon, Canada, conducted in June–August 2019. The study area has a subarctic climate with a climate gradient from Whitehorse via Mayo to Dawson (Table 1). Agricultural land-use history in the Yukon is relatively short as it started with

Table 1 Site information for the reference forest plot at the three sites: Circle D Ranch (*CD*), Minto Bridge Farm (*MB*) and Sister Island (*SI*)

Site	Land use	Nearest town	Latitude	Longitude	Altitude	MAT	MAP	Frost-free days[a]	Cumulative degree days	Soil type
			DD	DD	masl	°C	mm		(> 0 °C)	WRB
CD	Forest	Whitehorse	60.8604	− 135.5829	646	− 0.13	315	82.3	1909	Cambisol
MB	Forest	Mayo	63.7016	− 135.8577	582	− 3.52	331	95.3	1751	Cambisol
SI	Forest	Dawson	64.0932	− 139.4589	317	− 3.51	362	83.7	1874	Cryosol

Climate data were derived from Climate Atlas of Canada (2019) https://climateatlas.ca

MAT mean annual temperature; *MAP* mean annual precipitation

[a]Frost-free days refer to the period between the last spring frost and the first autumn frost, which determines the vegetation period

the Klondike gold rush at the end of the nineteen century, and the selected farms are amongst the oldest in the territory (30–100 years). The study was designed as a paired-plot experiment with three land-use types at each site: pristine boreal forest vegetation, agricultural grassland and cropland. Boreal forest is the natural vegetation representing the reference prior to land use change, while cropland and grassland represent the two major categories of agricultural use in Yukon. At the same time, those three land-use types represent a land-use intensity gradient. Grasslands and croplands were established after clearing of pristine forest. Grassland comprises meadow for hay production and pasture for livestock grazing (e.g. cattle, horses) under permanent use. Cropland refers to the small-scale farming systems found in the Yukon between market gardens and cropland growing vegetables, greens and herbs, as well as occasionally grains for local markets.

The Circle D Ranch (*CD*) is located close to Whitehorse on the bank of the Takhini River. The area has been under cultivation for 30 years. Most of the farm's area is used as pasture for cows. Cropland is cultivated with oats for animal fodder production and cow manure is used as organic fertiliser on the farm. The Minto Bridge Farm (*MB*) is located close to Mayo and was established in the 1970s when farmers started clearing the land. The pasture has been home to chickens and goats ever since. The cropland is cultivated in a crop rotation of peas, potatoes, carrots, kale, cauliflower and clover. Chicken and goat manure as well as eggshells are used as organic fertiliser for the cropland. No additional fertiliser is used. Sister Island (*SI*) is positioned in the middle of the Yukon River close to Dawson. While most of the

island was cleared a 100 years ago, except for a small forest strip at.

the west end of the island boreal forest has now grown back on most of the island. Grassland at *SI* is no longer used as pasture, but is regularly mown. The cropland area is cultivated in a crop rotation of potatoes, carrots, beans, brassicas, turnips, swedes, lettuces and beetroot. Organic fertiliser pellets are used for cropland fertilisation. The reference forests at all three sites have never been cleared and are mixed-wood forests of the boreal cordillera ecoregion (Smith et al. 2004), dominated by *Picea mariana* (MILL). The most important general soil parameters with regard to SOC dynamics are listed in Table 2. For more detailed information, we refer to the freely accessible dataset.

This study comprised a total of nine plots (3 sites × 3 land-use types). Three composite samples were collected per plot within a sampling area of approximately 10 × 10 m. To create a composite sample, a slide-hammer driven soil corer (6 cm diameter) was used to collect three individual soil cores from the top 10 cm after manual removal of the litter layer. After thorough homogenisation by hand, an aliquot of approximately 50 g fresh weight was taken as a composite sample. Samples were frozen at − 20 °C within 8 hours, stored and subsequently shipped frozen.

General soil parameters

Within the project, general soil parameters were determined on pooled soil core samples for each plot as described in Peplau et al. (in print). In brief, the following parameters were determined from oven-dried, 2 mm-sieved samples: soil pH$_{H2O}$, total organic

Table 2 Soil properties of the study plots: Circle D Ranch (*CD*), Minto Bridge Farm (*MB*), Sister Island (*SI*)

Site	Land use	Clay (%)	pH H_2O (1:5 w/v)	C_{org} (%)	N_{total} (%)	Olsen-P (%)	C:N ratio	C:P ratio	WHC (%)	C_{mic} (μg C g^{-1} soil)
CD	Forest	22.6	7.2	2.56	0.147	0.048	17	54	53	311 ± 39
CD	Grassland	22.9	6.7	1.78	0.148	0.063	12	28	44	211 ± 73
CD	Cropland	22.7	6.1	1.41	0.110	0.064	13	22	39	209 ± 22
MB	Forest	10.2	4.8	6.92	0.222	0.053	31	131	77	171 ± 134
MB	Grassland	10.9	6.9	10.44	0.683	0.240	15	44	98	1256 ± 248
MB	Cropland	16.6	7.4	7.17	0.623	0.896	12	8	71	875 ± 214
SI	Forest	14.1	7.3	9.30	0.415	0.034	22	273	111	801 ± 85
SI	Grassland	7.7	7.9	2.67	0.183	0.012	15	224	64	229 ± 25
SI	Cropland	8.2	7.8	4.37	0.247	0.041	18	107	55	155 ± 22

WHC water-holding capacity

carbon (C_{org}) and nitrogen (N_{total}) content, water-holding capacity (WHC), Olsen-P and soil texture. Soil pH was measured via a pH glass electrode in a 1:5 w/v ratio soil to H_2O after shaking for 1 h on a horizontal shaker at 200 rpm. Dried (40 °C to weight constancy) and milled aliquots of pooled samples were analysed for C and N content by dry combustion (LECO TruMac). All samples were also analysed for carbonates via stepwise combustion at 450 °C for 12 h (LECO RC612). WHC was quantified by soaking 10 g soil placed on a cotton wool-padded funnel with water. The state of 100% WHC was assumed when no water accumulated at the soil surface and runoff stopped. About 5 g of the water-holding soil were dried to weight constancy at 105 °C. WHC was calculated as the percentage of water in the water-holding soil. Extractable P was determined according to Olsen et al. (1954). In brief, 1 g soil dry weight (DW) was extracted in a 1:20 w/v ratio with 0.5 M NaHCO$_3$ solution adjusted to pH 8.5 by horizontal shaking (30 min, 180 rpm) and filtered (hw3, Sartorius Stedim Biotech). Olsen-P extracts were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES, iCAP 7400 Thermo Fisher Scientific) at a wavelength of 178.284 nm (mode of measurement: axial). Soil texture was measured according to ISO 11,277 (clay < 2 μm, silt 2–63 μm, sand > 63 and < 2000 μm), which is based on a combination of sieving and sedimentation of fine particles in a suspension (Köhn 1929).

Additionally, water content and the C:N ratio were determined for each of the 27 frozen composite samples. Water content was assessed by drying 5 g fresh weight to a constant mass at 105 °C. Analysis of C and N was performed as described above.

Experimental set-up

Short-term temperature sensitivity as well as N-input effects on microbial CUE, respiration, growth and turnover were assessed in a 1-day incubation experiment according to the ^{18}O-CUE approach (Spohn et al. 2016a). Short-term incubation was chosen to avoid the confounding effects of changes in microbial community composition or acclimation of microbial physiology in order to directly assess temperature effects on microbial CUE (Bradford 2013; Zheng et al. 2019). Furthermore, soils were incubated for 50 days, i.e. 7 weeks, to assess the temperature sensitivity and N response of microbial respiration and SOM decomposition. Temperature sensitivity was assessed according to the equal-time approach (Hamdi et al. 2013). Both experiments were conducted with the following three treatments: incubation at 10 °C, incubation at 20 °C, and incubation at 20 °C plus N-fertiliser addition at an amendment rate of 100 kg N ha^{-1}, all following 1 week of pre-incubation at 15 °C (Fig. 1). Due to limited resources and capacities the N fertilisation factor had to be restricted and we decided to include a N treatment incubated at 20 °C (instead of 10 °C) to represent increased N availability due to agricultural activity under a warmer climate. We also assumed that an effect would be more likely to be detectable at 20 °C, which was thus used as the reference temperature for investigating N effects. Pre-incubation temperature
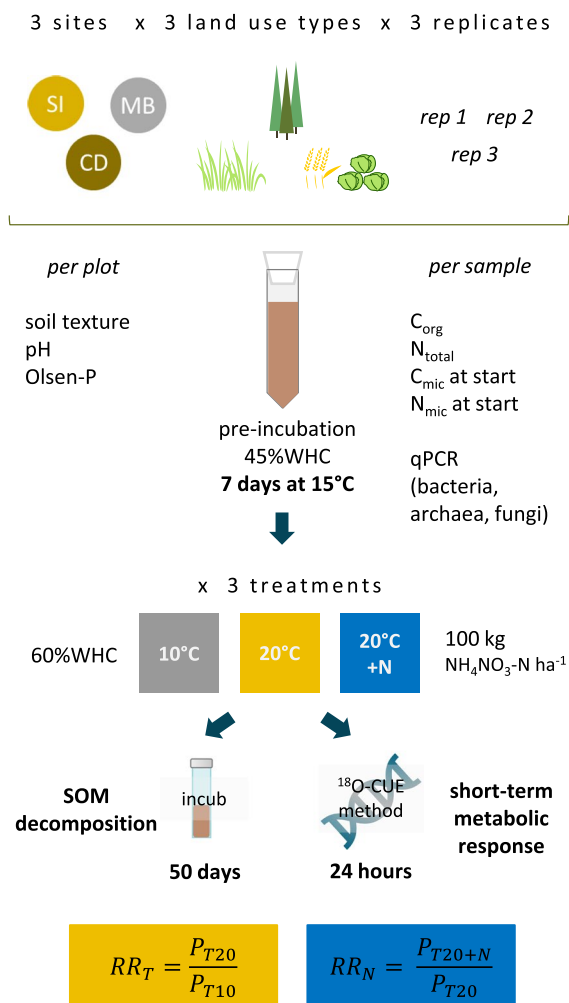
Fig. 1 Experimental set-up. Three sites were chosen, each comprising three land-use types. Per plot, three field replicate samples from the top 10 cm of mineral soil were adjusted to 45% WHC and pre-incubated for 7 days at 15 °C before adjustment of incubation treatments. Soils were incubated at 10 °C, 20 °C, and 20 °C plus N addition at an equivalent of 100 kg NH_4NO_3-N ha^{-1}. A 50 day incubation was run to assess the temperature sensitivity and N response of soil organic matter decomposition. The short-term microbial metabolic response was assessed in a 24 h incubation experiment. Temperature sensitivity and response to N addition were expressed as response ratios (RR) based on process rates (P)

was set equal to avoid uneven substrate depletion during pre-incubation. Strictly speaking, temperature treatments thus correspond to a cooling or warming of 5 °C compared to the pre-incubation conditions. However, cooling experiments have shown that the temperature sensitivity of microbial respiration is

independent of the prevailing temperature regime, as no thermal acclimation to cooling occurred, whereas the reduction of substrate C in warming experiments causes a decrease in temperature sensitivity over time (Hartley et al. 2008; Karhu et al. 2014). Therefore, it can be assumed that our approach of equal pre-incubation conditions and a temperature difference between treatments is representative of a 10 °C warming, as which it is referred to in the following. To calculate the amount of NH_4NO_3 needed to adjust 100 kg N ha^{-1} nitrogen fertilisation, we considered the mean density of all plots in the upper 20 cm (0.8 ± 0.3 g soil DW cm^{-3}), which represents the ploughing horizon in cultivated soils. In brief, the final concentration of added N in the soil was adjusted to 0.0625 mg NH_4NO_3-N g^{-1} soil DW.

Determination of ^{18}O CUE

The ^{18}O-labelling method was conducted according to the original protocol of Spohn et al. (2016a), with the same modifications as described in Poeplau et al. (2019) and Schroeder et al. (2021). In this method, microbial growth is determined by incorporation of ^{18}O from labelled water into the DNA. In brief, two aliquots of 300 mg fresh weight soil were weighed into Eppendorf vials, placed into 20 ml glass vials, and crimp sealed. Labelled water H_2^{18}O (97 at% ^{18}O) was added with a syringe (Hamilton) in the labelled aliquot to reach a labelling of 20 at% ^{18}O in final soil water while adjusting the water content to 60% WHC. The same amount of bi-distilled water was added to the natural abundance control. For the N treatment, water content was partly adjusted by first adding NH_4NO_3 solution, which was then considered in the calculation for addition of labelled or bi-distilled water. In order to equalise the starting conditions, vials with labelled samples were evacuated and flushed with standard gas of known CO_2 concentration (348 ppm) to a pressure of 1.3 bar within 1 min of ^{18}O water addition. A gas sample of 20 ml was taken from labelled samples with a manual gas syringe (SGE Syringe, Trajan Scientific and Medical) after 24 h incubation in the dark at the respective treatment temperature. Vials were de-crimped pairwise and soil samples were immediately frozen in liquid N$_2$. Non-labelled and labelled samples were stored at −80 °C until DNA extraction. Gas samples

were analysed and respiration flux was calculated as described in Eq. 1.

DNA was extracted from labelled and non-labelled soil samples using the FastDNA™ SPIN Kit for Soil (MP Biomedicals) following the standard protocol, with an extension of centrifugation to 15 min in step five (15,000 rpm, Sigma 4-16KS). DNA was eluted in 100 µl DNase-free water. The DNA concentration in the extracts was quantified with the QuantiT Pico-Green dsDNA Kit (Invitrogen). Then 60 µl of DNA eluate were transferred to silver capsules and oven-dried at 60 °C for 12 h. Isotopic analysis of ^{18}O in the dried DNA was performed on labelled and non-labelled samples using a high-temperature conversion/elemental analyser (TC/EA) (Thermo Fisher Scientific) coupled with a Delta V Plus isotope ratio mass spectrometer via a ConFloIV interface (Thermo Fisher Scientific).

The amount of DNA produced was calculated based on ^{18}O incorporation into microbial DNA from isotopic analysis of labelled and non-labelled samples and initial enrichment of 20 at% ^{18}O [for formulae see Schroeder et al. (2021)]. Microbial turnover [days] was calculated as quotient of total DNA extracted over the rate of DNA produced. The allocation rate to microbial biomass production C_{Growth} [ng C g^{-1} soil DW h^{-1}] is derived by using the conversion factor *fDNA* to transform the amount of total DNA produced into microbial biomass C produced, and related to soil mass and time. The *fDNA* values were derived for individual field samples.

The microbial CUE is defined as microbial biomass C produced (C_{Growth}) over the total uptake of C, as an approximation of the sum of microbial biomass C produced and C respired ($C_{Growth} + C_{Respiration}$) (Manzoni et al. 2012; Sinsabaugh et al. 2013). The CUE value has no entity.

Fifty-day incubation experiment

After pre-incubation, 6 g soil DW per treatment were weighed directly into 20 ml glass vials, which served as small closed chamber incubation vessels (20 mm Crimp Top Vial, Part No. WIC 43200/100, WICOM). This approach was taken as the amount of material was limited. The amount of soil was set to 6 g soil DW to limit the soil-to-headspace ratio in the incubation vessels, while still allowing the analysis of microbial biomass C at the end of the incubation.

The calculated amount of water and NH_4NO_3 solution (N treatment) needed to adjust water content to 60% WHC and 100 kg N ha^{-1} was added.

Gas samples were taken in order to assess respiration rates over a total incubation period of 50 days as follows: the first gas sample was taken approximately 4 h after addition of the water or NH_4NO_3 solution. Sampling took place once per day during the first week, every other day during the second and third weeks, and then twice a week until the end of the incubation period, resulting in a total of 18 sampling events. Glass vials were aerated every two to three days if not sampled to avoid O_2 depletion.

At each gas sampling event, vials were evacuated, the gas phase was exchanged with a standard gas of known concentration (348 ppm CO_2), and pressure was adjusted to 1.3 bar in order to equalise starting conditions. After a 1 h incubation time at the respective treatment temperature, a gas sample was taken manually from the vial using a gas-tight syringe (SGE Syringe, Trajan Scientific and Medical). After gas sampling, the gas phase was flushed again with standard gas adjusted to 1.0 bar.

Gas samples were analysed for CO_2 concentration via gas chromatography equipped with an electron capture detector (Agilent 7890A GC, Agilent Technologies). The total amount of respired CO_2-C was calculated from the blank-corrected CO_2 increase in concentration according to the ideal gas equation, and related to soil dry weight and time to obtain the respiration rate $C_{Respiration}$ [ng C g^{-1} soil DW h^{-1}]:

$$C_{Respiration} = \frac{p \times V}{R \times T} \times M \times \Delta CO_2 \times \frac{1}{\text{gsoil DW} \times t} \quad (1)$$

where p is the pressure [kPa] in the vial (1300 kPa), V is the volume [L] of the vial being replaced when evacuated and flushed with standard gas, R is the universal gas constant (8314 J mol^{-1} K^{-1}), T is the temperature [K] at which the standard gas was injected into the vial (20 °C = 293.15 K), M is the molecular mass of carbon (12.01 g mol^{-1}), and ΔCO_2 is the increase in CO_2 concentration [ppm] during the incubation time t [h]. In order to determine the gas volume of each individual vial, which includes the gas phase in soil pores and in the headspace, a defined volume of gas containing a known molar amount was added to the evacuated vial and the pressure increase was measured. The volume captured by the

gas was calculated via the ideal gas equation from the measured pressure increase, and the mean of three repeated measurements was considered as the volume for calculations of $C_{Respiration}$. Cumulative respiration per interval was calculated by multiplying the respiration rate by the time since the last measurement. Total cumulative respiration is thus the sum of respiration of all intervals.

Acidification by NH_4NO_3-amendment

The addition of ammonium fertilisers is acknowledged to induce soil acidification under field conditions (He et al. 1998). To test whether soil acidification was induced during the incubation experiment, we assessed soil pH at day 0, 1, 7 and 50 after NH_4NO_3 addition in the NH_4NO_3-amended and control soils. Therefore, 5 g DW aliquots were weighed into 50 ml PE vessels and NH_4NO_3 solution was added to adjust to 60% WHC and a concentration of 100 kg N ha^{-1}. Vessels were closed and incubated at 20 °C until pH was measured as described above, considering the actual water content of the soil.

Determination of microbial biomass

Microbial biomass C was assessed using the chloroform fumigation extraction method (Vance et al. 1987). Fumigation was conducted for 24 h at room temperature in the dark under an excess amount of chloroform ($CHCl_3$). Non-fumigated and fumigated aliquots of soil were extracted with 0.5 M K_2SO_4 solution at a ratio of 1:4 w/v soil to extractant by horizontal shaking (180 rpm, 30 min) and filtered. Non-purgeable organic carbon (NPOC) was analysed in a 1:4 v/v extract dilution with bi-distilled water after removing total inorganic C by adding 15% HCl solution, adjusting pH to 2–3, and outgassing emerging CO_2 for 6 min with artificial air (Dimatoc 2000, DIMATEC Analysentechnik). NPOC values were assumed to represent total organic carbon (TOC). All TOC values were blank-corrected. Microbial biomass C was calculated with a conversion factor of 0.45 (Joergensen 1996). Total N in non-fumigated and fumigated extracts was assessed in 1:10 v/v dilutions using a total nitrogen analyser TN-100 (Nittoseiko Analytech, formerly Mitsubishi Chemical Analytech). Microbial biomass N was calculated using a conversion factor of 0.54 (Joergensen and Mueller 1996).

Chloroform fumigation extraction was conducted on 5 g DW soil aliquots extracted with 20 ml 0.5 M K_2SO_4 at the end of the pre-incubation to determine microbial biomass C and N (Table S1) at the start of incubation and assess individual *fDNA* conversion factors for the calculation of microbial growth within the ^{18}O-labelling method. Additionally, the final amount of microbial biomass C after 50 days' incubation at the respective temperature was assessed on 2 g soil DM aliquots extracted with 8 ml 0.5 M K_2SO_4 solution. The assessment of microbial biomass N at the end of the incubation period was disturbed by added NH_4NO_3 signals and had to be rejected.

Microbial abundance by qPCR

Abundances of bacteria, archaea and fungi were estimated from the non-labelled DNA extracts by qPCR using the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories) (Hemkemeyer et al. 2015). In brief, the Taqman-probe approach was used for archaea and bacteria. Amplification of the 16S rRNA gene of archaea and bacteria was conducted using the primers ARC787F, ARC1059R and BAC338F, BAC805R. The probes ARC915F and BAC516F were used for quantification of the same gene (Yu et al. 2005). Fungal ITS1 sequences were amplified using the primers NSI1 and 58A2R and quantified by SYBR Green (Martin and Rygiewicz 2005). Reactions were carried out in duplicates from 50× to 100× dilutions of the DNA extracts. Standard curves for the respective domains were generated using DNA from pure cultures of *Bacillus subtilis*, *Methanobacterium oryzae* and *Fusarium culmorum*. The PCR efficiencies were $96.1 \pm 1.7\%$ ($R^2 = 0.998$) for archaea, $95.5 \pm 1.4\%$ ($R^2 = 0.999$) for bacteria, and $94.2 \pm 3.7\%$ ($R^2 = 0.997$) for fungi. Data are provided in Table S1.

Data analysis

We calculated the temperature sensitivity as a response ratio of the 20 °C treatment to the 10 °C treatment as a quotient of the microbial process rates at the respective incubation temperatures (P_{T20} and P_{T10}):

$$RR_T = \frac{P_{T20}}{P_{T10}} \qquad (2)$$

The effect of N input was calculated as a response ratio of the 20 °C + 100 kg NH_4NO_3-N treatment to the 20 °C treatment as a quotient of the microbial process rates of the respective treatments (P_{T20+N} and P_{T20}):

$$RR_N = \frac{P_{T20+N}}{P_{T20}} \qquad (3)$$

A response ratio (RR) equal to 1 indicates that the parameter observed does not change in response to warming or N addition.

All statistical analyses as well as data visualisation were conducted in R v3.6.3 (R Core Team 2020) using RStudio v1.1.463 (RStudio Team 2016) and the packages of *tidyverse* (Wickham et al. 2019), *lme4* (Bates et al. 2015), *lmerTest* (Kuznetsova et al. 2017), *emmeans* (Lenth 2021), *multcomp* (Hothorn et al. 2008), *multcompView* (Graves et al. 2019), *corrplot* (Wei and Simko 2021), *ggpmisc* (Aphalo 2021) and *cowplot* (Wilke 2020).

The values below are given as mean ± standard deviation unless otherwise stated.

The Student's t-test (t.test, *stats*) was considered to test whether RR_T and RR_N differed significantly from 1, with a significance level of $\alpha = 0.05$.

To test whether the responses to N addition and warming differed significantly between land-use types, a linear mixed-effects model approach (lmer, *lme4*) was used, which accounted for the paired character of the experimental design by setting land use as the fixed effect and site as the random effect, allowing for random intercept. A visual inspection of residual plots was used to check for deviations from homoscedasticity or normality, and data were log-transformed where necessary. Significance of the fixed effect was assessed at a significance level of $\alpha = 0.05$. Estimated marginal means (emmeans, *emmeans*) were calculated and differences between land-use types are given as a compact letter display in the respective figures at a significance level of $\alpha = 0.05$. The p values were adjusted according to Tukey. A table of estimated marginal means, standard errors and confidence intervals is provided in the supplementary material (Table S2).

Another linear mixed-effects model approach was used for the analysis of absolute values, with site and land use as fixed effects, allowing for interaction, and treatment as the random effect since treatments served as the replicate in this case (random intercept). Here, estimated marginal means were calculated for land use grouped by site, and differences between land-use types within a site are given as a compact letter display.

The acidifying effect of NH_4NO_3 on soil pH was tested using a linear mixed-effects model approach, with treatment (control and NH_4NO_3 addition) and time since start of the incubation (0, 1, 7, and 50 days) as fixed effects and plot as the random effect (random intercept).

Correlograms were used for correlation analysis based on Spearman's correlation coefficient ρ (cor, *stats*, and corrplot, *corrplot*). The correlation analysis included tests for the relationships between average extents of microbial metabolic parameters (e.g. cumulative respiration, CUE) as well as response ratios to N addition or warming and soil characteristics (e.g. abundance data, soil pH, texture). The regression curves displayed best describe the relationship between observed parameters, as indicated by adjusted R^2 (tested for linear, exponential, logarithmic and second grade polynomial).

All necessary raw data as well as the R code used to conduct statistical analysis and create figures are publicly accessible (https://doi.org/10.5281/zenodo.6457750).

Results

Average metabolic parameters and their drivers

The mean cumulative respiration after 50 days' incubation over all treatments was 1.9 ± 1.8 for *CD*, 7.9 ± 5.0 for *MB*, and 11.9 ± 13.4 mg CO_2-C g^{-1} soil DW for *SI*, showing significant differences between sites. To enable land-use comparison, cumulative respiration was normalised by SOC content (Fig. 2a). The mean SOC-normalised cumulative respiration was highest in forest soils except for *MB* forest, which demonstrated the lowest mean cumulative respiration per land-use type at the *MB* site.

Parameters of microbial activity such as C_{Growth}, $C_{Respiration}$ and cumulative respiration were

Fig. 2 Distribution of absolute values of **a** soil organic carbon normalised cumulative respiration after 50 days of incubation and **b** microbial carbon use efficiency (CUE) measured after 24 h incubation per land use at each site, including three field replicates and three treatments (incubation at 10 °C, 20 °C, and 20 °C + N addition) given as a box-and-whisker plot (n = 9). Letters indicate significant differences between land-use types at the respective site at a level of significance of p < 0.05. Black dots give the mean
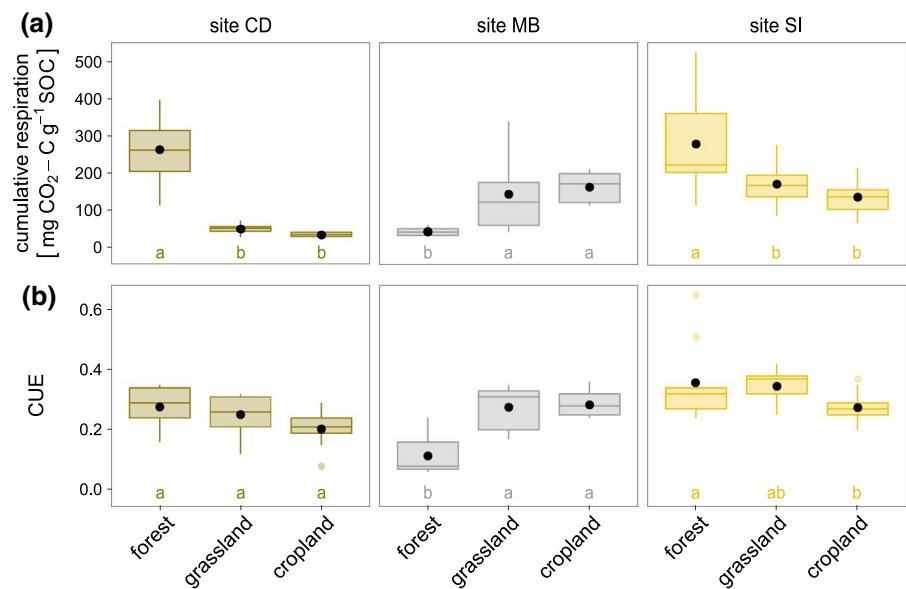
positively correlated with the amount of microbial biomass derived at the beginning of the incubation ($p < 0.001$) as well as the gene copy number of bacteria ($p < 0.01$), which in turn were both positively correlated with C_{org} ($p < 0.001$) (Fig. S1). Soil organic carbon content varied significantly between sites ($p < 0.001$) (Table 1). The low microbial activity at *MB* forest was related to: 1 lower C_{org} contents in comparison to the other land-use types at this site, 2 concurrently reduced microbial biomass C, and 3 a high relative abundance of fungi with a fungi-to-bacteria (F:B) ratio of 0.139 ± 0.041 (Table S1) compared with an overall median F:B ratio of 0.019.

Mean CUE was 0.25 ± 0.07 for *CD*, 0.23 ± 0.10 for *MB*, and 0.33 ± 0.09 for *SI*. CUE differed between land-use types at the *MB* and *SI* sites, without a consistent land-use effect across all sites (Fig. 2b).

Absolute CUE values were strongly positively correlated with soil pH_{H2O}, with higher mean CUE values per plot at a higher soil pH (Fig. 3). Additionally, CUE values were negatively correlated with the gene copy numbers of fungi ($p < 0.01$), F:B ratio ($p < 0.01$) and fungi-to-archaea (F:A) ratio ($p < 0.05$), which could be linked to a higher relative abundance of fungi at a low soil pH ($p < 0.05$ for F:B to pH_{H2O}) (Fig. S1). Differences in C_{Growth} were the major drivers of CUE, as can be seen from the significant positive correlation (p < 0.001).
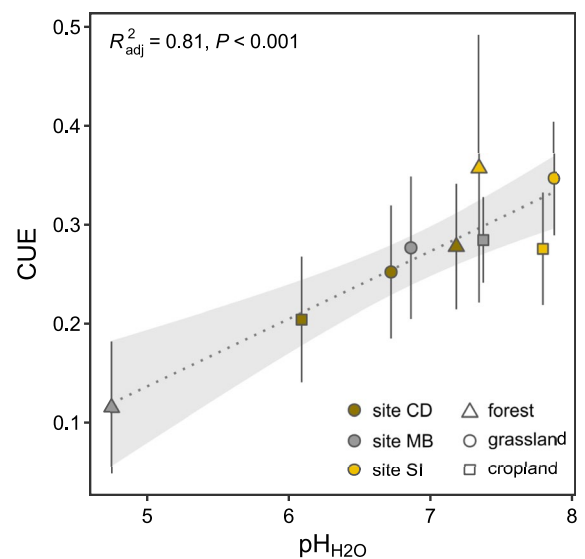

Fig. 3 Mean microbial carbon use efficiency (CUE) per site and land-use type (forest, grassland, cropland) over soil pH_{H2O}. Error bars give the standard deviation of CUE (n = 9)

RRT and RRN of microbial metabolism

To investigate the effects of warming and N addition on microbial metabolism, response ratios were calculated. These are displayed in Fig. 4. Corresponding estimated marginal means and confidence intervals from the linear mixed-effects analysis are given in Table S2. For the vast majority of tested parameters,
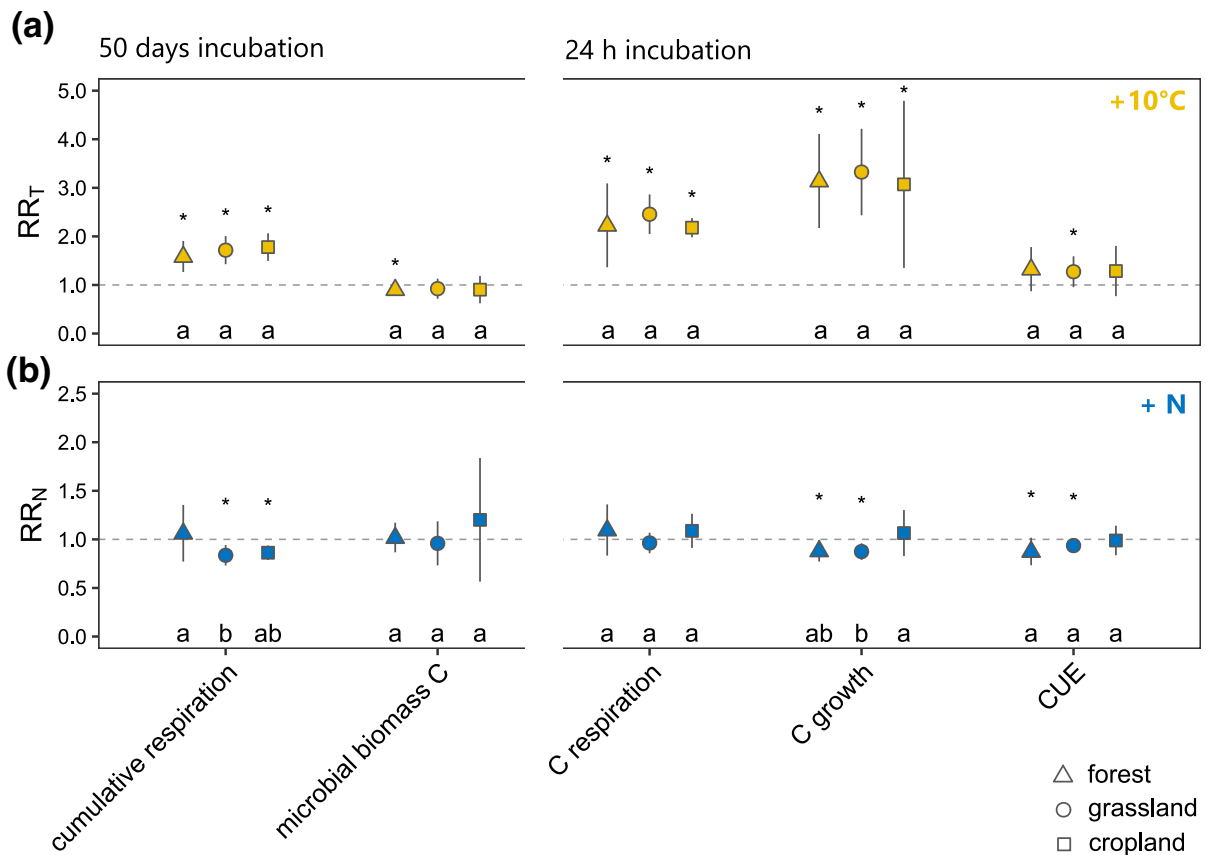
Fig. 4 Microbial response to **a** +10 °C warming and **b** N addition per land-use type (shape) expressed as the response ratio (RR) (mean ± sd, n = 9) of cumulative respiration and microbial biomass C after 50 days of incubation, and C directed to microbial respiration and growth, as well as carbon use efficiency (CUE) within 24 h of incubation. RR > 1 indicates an increase of the given parameter upon treatment in relation to the control. Significant responses to treatment (RR ≠ 1) per individual type of land use are marked by an asterisk. Different letters indicate different responses between land-use types at a level of significance of p < 0.05

the random effect of site only explained a small part of the variation. One exception was the warming response of C_{Growth}, which was higher overall at the *CD* site than at the other sites.

Overall, microbial activity was accelerated in response to +10 °C warming. Warming increased cumulative respiration by 69 ± 30%, with no significant differences between land-use types. Turnover of microbial biomass C was accelerated with warming from 172 ± 179 days at 10 °C to 64 ± 66 days at 20 °C (p < 0.001, 95% CI: 0.29–0.77) (Fig. S2). Meanwhile, microbial biomass C measured at the end of the 50 day incubation period was slightly (not significantly) reduced in warmed samples by 9 ± 20% (Fig. 4a). In comparison to 50 days' warming, short-term warming for 24 h resulted in a more pronounced increase in $C_{Respiration}$, i.e. by a factor of 2.3 ± 0.6. Concurrently, C_{Growth} increased to a greater extent with an RR_T of 3.1 ± 1.2, which explains why CUE, as the ratio of C_{Growth} to the sum of C_{Growth} and $C_{Respiration}$, was increased with warming (+30 ± 42%) (Fig. 4a). However, this trend in CUE was only significant when all land-use types were taken together (p < 0.001, 95% CI 1.13–1.45). Overall, land-use types did not show distinct responses to warming.

Compared with RR_T, the response to N addition was much less pronounced. Nitrogen addition had either an inhibitory effect or no effect at all on microbial parameters, with significant differences between land-use types for RR_N of cumulative respiration

and C_{Growth} (Fig. 4b). Cumulative respiration after 50 days' incubation was reduced by $-14 \pm 8\%$ in grassland and by $-16 \pm 11\%$ in cropland, while forest did not reveal a distinct reaction to N addition. The forest soil at the *CD* site was the only soil with increased cumulative respiration in response to N addition (RR_N cumulative respiration $= 1.3 \pm 0.4$) (Fig. S3). When the *CD* site was excluded from the analysis of RR_N cumulative respiration, differences between land-use types were no longer significant. Furthermore, N addition reduced C_{Growth} in the short term by $-22 \pm 11\%$ in forest soils and by $-23 \pm 9\%$ in grassland soils, but not in cropland soils (Fig. 4b). N amendment resulted in reduced CUE ($-7 \pm 13\%$) across all land-use types ($p < 0.001$, 95% CI:

0.88–0.98), without significant differences between land-use types. The addition of NH_4NO_3 reduced the soil pH significantly by on average 0.1 pH points ($p < 0.001$) on all measurement days (day 0, 1, 7, 50), without significant differences between land-use types. There was a tendency for increasing acidification during the 50 days of incubation, but this was not significant.

Soil parameters affecting RRT and RRN

To understand the drivers of warming and N response, RR_T and RR_N of metabolic parameters were tested for their correlation with soil characteristics, including general soil parameters such as soil pH and soil
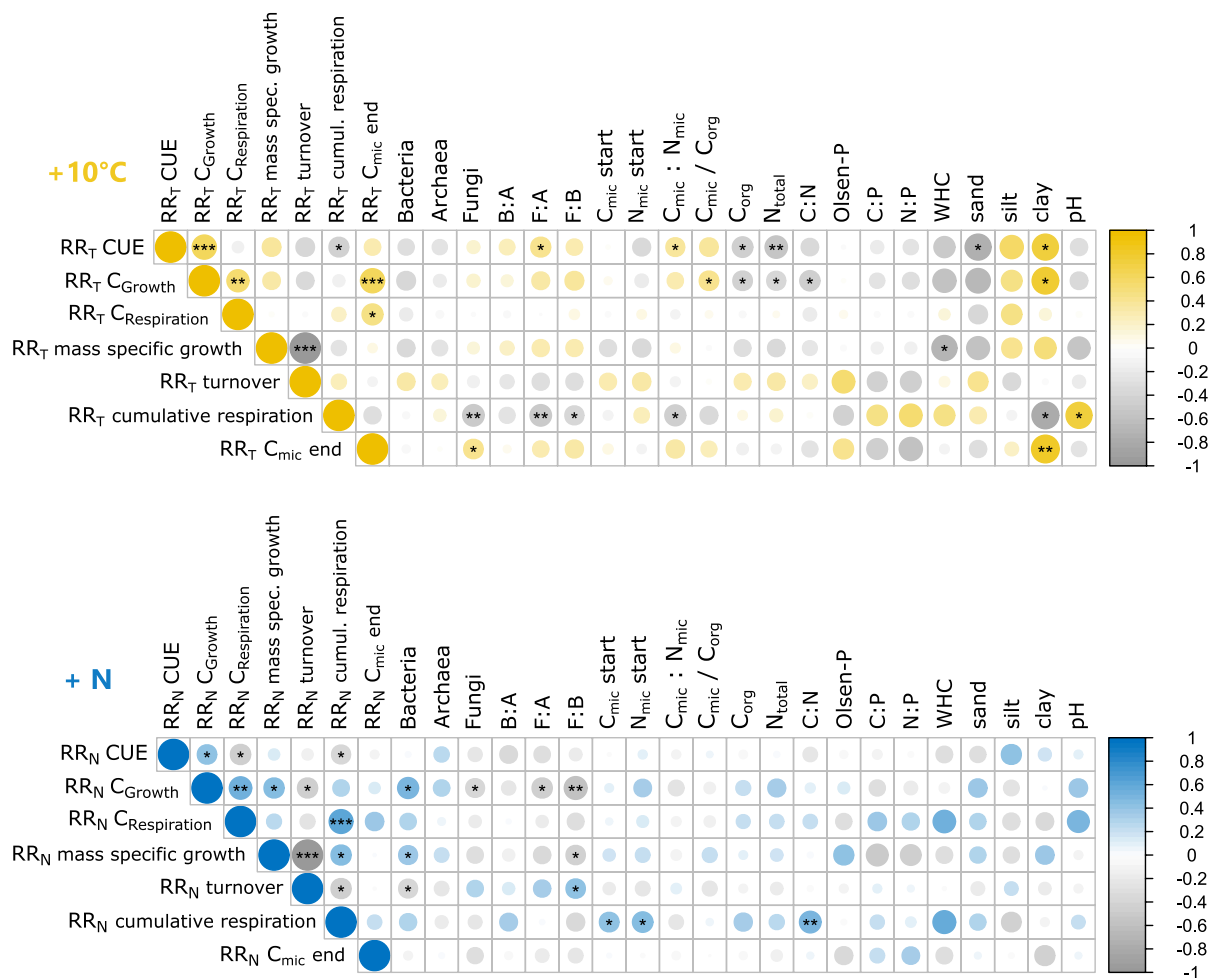
Fig. 5 Correlograms of the response ratios to warming or N addition and plot characteristics. Colours and circle size mark the Spearman's correlation coefficient ρ and asterisks indicate the level of significance (*<0.05, **<0.01, ***<0.001)

texture, SOC content and data on soil microbial community structure derived from qPCR analysis (Fig. 5).

In contrast to absolute values, the response of CUE to warming or N addition was not dependent on soil pH. RR_T and RR_N of CUE were significantly correlated with RR_T and RR_N of C_{Growth} ($p < 0.001$), indicating that the response of CUE to treatments was mainly driven by the response of C_{Growth}.

According to the correlation analysis, the responses of CUE and C_{Growth} to warming were positively correlated with clay content ($p < 0.01$), while RR_T of cumulative respiration was negatively correlated with clay content ($p = 0.01$) (Fig. 5). Regression analysis revealed that the correlations were mainly driven by the *CD* site, which showed the greatest temperature sensitivity of C_{Growth} at the highest clay content (Table 2). Soils with a higher estimated abundance of fungi revealed lower temperature sensitivity of cumulative respiration ($p < 0.001$).

Meanwhile, the responses of CUE and C_{Growth} to N addition were not significantly correlated with general soil parameters. However, the response of cumulative respiration to N addition was positively correlated with the soil C:N ratio ($p < 0.01$), indicating higher cumulative respiration with N addition when the soil C:N ratio was higher (Fig. 6a). Microbial growth response to N addition depended on the dominance of bacteria over fungi in soils, as seen from a negative correlation between RR_N of C_{Growth} and F:B ratio ($p < 0.01$) (Fig. 6b).

Discussion

Temperature sensitivity is driven by site characteristics, not land-use type

As expected, warming increased microbial activity and SOM decomposition, while microbial turnover time was greatly reduced (from 172 ± 179 days at 10 °C to 64 ± 66 days at 20 °C), indicating accelerated microbial metabolism. This finding corroborates that SOM decomposition will be stimulated under a warmer climate in subarctic soils (Karhu et al. 2014). It is assumed that microbial CUE declines in response to warming due to higher maintenance costs (Steinweg et al. 2008; Allison et al. 2010) or energy spilling (Bradford 2013), resulting in a greater relative increase in respiration as compared to growth and
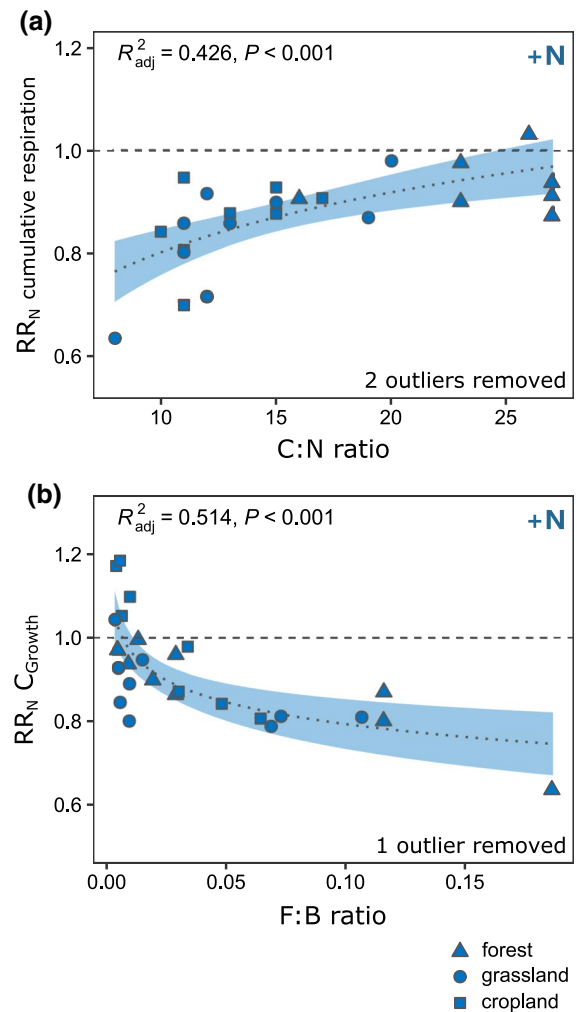

Fig. 6 Drivers of the microbial response to N addition given as the response ratio (RR_N). **a** Response of cumulative respiration after 50 days of incubation to N addition was significantly correlated with soil C:N ratio. **b** Response of microbial growth within the first 24 h of incubation was significantly correlated with the initial fungi-to-bacteria ratio of the soil

therefore aggravating losses from increased SOM decomposition with warming. Here, we found a 30% increase in CUE in response to higher temperatures across all three sites and land-use types, contradicting our hypothesis, that CUE would decline with warming. Within the increasing number of studies assessing the warming effects on ^{18}O CUE of SOM, some have found that CUE decreases (Li et al. 2021; Liu et al 2021), is unaltered (Walker et al. 2018; Poeplau et al. 2019; Simon et al. 2020) or increases (Zheng et al. 2019). We are adding a further study that shows

that the assumed decrease in CUE implemented in modelling approaches does not necessarily occur (Bölscher et al. 2020). While short-term warming increased both respiration and growth rate, the CUE response to warming was mainly driven by microbial growth. Therefore, we conclude that the observed increase in CUE might be associated to overall facilitated growth conditions with warming in the studied subarctic soils. It has recently been found that the temperature sensitivity of CUE is linked to CUE itself in bacterial isolates. Inefficient bacteria taxa increased their CUE with temperature, while those with high CUE showed a decrease in CUE with temperature (Pold et al. 2020), which could explain divergent observations across studies. Indeed, the average CUE values measured in this study were rather low in comparison to previous studies using the same method where CUE ranged from e.g. 0.26–0.66 (Zheng et al. 2019) or 0.2–0.7 (Li et al. 2021). However, CUE and its temperature response did not covary in our dataset, suggesting that environmental constraints may not only obscure the temperature response of SOM decomposition but also of CUE in soils as opposed to isolates.

An increase in CUE might alleviate CO_2-C losses due to accelerated SOM decomposition under future climate. However, directly linking the results from the 24 h and the 50 days incubations is difficult, as the slight, insignificant reduction in microbial biomass C with warming at the end of the 50 days incubation period may indicate a C depletion of easily available substrate in the warmer treatment. Indeed, unequal depletion of C sources at different incubation temperatures over time is acknowledged to be a major limitation of the equal time approach (Leifeld and Fuhrer 2005; Hamdi et al. 2013). The diverging C availability between incubation at 10 °C and 20 °C most likely explained the decline in the temperature response of microbial respiration, with a mean RR_T of $C_{Respiration}$ of 2.3 within the first 24 h of incubation (^{18}O-CUE) and RR_T of cumulative respiration after 50 days incubation of 1.7. This temporal dynamic was also observed in the temperature response of respiration rate over the course of the 50 day incubation period (Fig. S4). Extrapolating the results of short-term incubation experiments, as in the 50 days incubation, to ecosystem C dynamic under prolonged warming may be possible only to some extent. A meta-analysis on long-term warming experiments (1–15 years)

substantiated that the stimulating effect of warming on soil respiration often found in short-term incubation experiments decreases over time (Romero-Olivares et al. 2017). Suggested mechanisms for the apparent thermal adaptation of soil respiration to long-term warming are microbial acclimation, shifts in microbial community composition, evolutionary adaptation of microbes or depletion of labile C (Bradford 2013; Romero-Olivares et al. 2017). Thus, it is unclear, whether the observed increase in CUE would be long-lasting. However, there was no evidence for acclimation of microbial physiology, i.e. the CUE, in a geothermal warming gradient for a timespan of weeks up to 50 years (Walker et al. 2018). Instead, overall respiration decreased as microbial biomass declined due to C depletion, while warming increased microbial activity constantly, questioning the acclimation theory. Warming might facilitate microbial metabolic efficiency in subarctic soils not only in short-term but in the long-term. Indeed, a modelling approach found that a positive relationship between CUE and mean annual temperature—not a negative—can explain heterotrophic soil respiration globally (Ye et al. 2019).

We expected that the temperature sensitivity of SOM decomposition and CUE would differ significantly between land-use types. Based on the carbon-quality temperature theory, we hypothesised that the most sensitive response to warming is found in subarctic forest soils, which we suspected contain the highest amounts of low-quality SOM. However, the warming response of cumulative respiration was not linked to soil C:N ratio as one indicator of soil C quality, which contradicted our hypothesis. Furthermore, the 10 °C warming increased metabolic efficiency to a similar relative proportion regardless of the land-use type, contrasting recent studies that reported the highest temperature sensitivity of CUE in forest soils (Zheng et al. 2019; Bölscher et al. 2020; Li et al. 2021). We found the warming response of CUE was also not influenced by the relative proportion of microbial domains, i.e. bacteria, archaea or fungi, which is largely influenced by land-use type (Szoboszlay et al. 2017). The diverging warming responses of CUE in literature (increase, unaltered, reduction), and the absence of a land-use type effect on temperature sensitivity of CUE in our findings, suggest that there might not be a general warming response in CUE that is dependent on land-use type.

Environmental constraints, e.g. binding of SOM to mineral surfaces which is also dependent on temperature, can mask the intrinsic temperature sensitivity, resulting in lower apparent temperature sensitivity (Davidson and Janssen 2006; Conant et al. 2011). Divergent trends with land-use type in overall metabolic parameters between sites (Fig. 2) suggest that microbial decomposition as well as microbial CUE is hampered by different environmental conditions at each site, which may explain the absence of a general land-use type dependent temperature sensitivity pattern. For example, while SOC-normalised cumulative respiration was in general higher in forest soils than agricultural soils, it was lowest in forest soil at the *MB* site, which was associated with low microbial biomass and acidic conditions. It is therefore unclear whether the absence of a general land-use type dependent response to warming is related to the limited replication of three sites, with management-related differences within land-use type. Nevertheless, our findings support the conclusion that site-specific properties rather than land use-type related characteristics drive temperature sensitivity. The correlation analysis indicated significant correlations between the warming response of cumulative respiration (negative), CUE (positive) and microbial growth (positive) with clay content. However, clay content was one of the major soil characteristics clustering the three sites. The warming response of C_{Growth} was significantly higher at *CD* than at the other sites. Furthermore, the regression analysis revealed that the correlations between C_{Growth} and CUE with clay content were driven by the *CD* site alone, indicating a site effect but not necessarily a link to clay content. Due to the importance of adsorption and desorption processes to mineral surfaces for SOM availability, it is suspected that the temperature sensitivity of SOM decomposition depends on soil texture (Wei et al. 2014; Ding et al. 2018; Li et al. 2021), fraction size (Leifeld and Fuhrer 2005; Plante et al. 2009; Ding et al. 2018), and clay mineralogy (Zhang 2010; Singh et al. 2017). However, higher Q_{10} values found in the clay fraction were not long-lasting and rather associated with SOM quality (Ding et al. 2018). No correlations between the Q_{10} of SOM decomposition and clay content were found in artificial soils (Wei et al. 2014) nor in a meta-analysis (Hamdi et al. 2013), thus literature does not support such a causality. In conclusion, our results suggest that site specific soil properties may override land-use type effects, which might thus not be the most important to consider for improving model predictions of subarctic SOM dynamics under climate change.

Response to N addition is driven by soil microbial community composition

As expected, the cumulative respiration declined upon N addition in the 50 days incubation experiment. This finding was in line with observations from similar laboratory studies (Poeplau et al. 2016; Silva-Sánchez et al. 2019) and long-term fertilisation field experiments (e.g. Coucheney et al. 2013; Riggs and Hobbie 2016; Spohn et al. 2016b; Poeplau et al. 2019; Widdig et al. 2020). While a reduction in microbial biomass explained the reduction in heterotrophic respiration upon long-term N fertilisation (Riggs and Hobbie 2016; Spohn et al. 2016b), the observed reduction in 50 day cumulative respiration was not associated with a reduction in C_{mic} in this study. In long-term fertilisation experiments, ammonium fertilisers have been shown to induce soil acidification in relation to N load and duration (Chien et al. 2008; Zhou et al. 2017). Given the positive correlation between absolute CUE and soil pH observed here and noted in the literature (Malik et al. 2018), we tested the acidifying effect of NH_4NO_3 addition on soil pH to assess whether potential shifts in soil pH could explain the reduction in CUE or cumulative respiration in response to NH_4NO_3 addition. We found a significant, albeit small reduction in soil pH with NH_4NO_3 addition of 0.1 pH points over the entire 50 day incubation, irrespective of land-use type. However, the metabolic response was not correlated to overall soil pH, suggesting that soil pH is not a driver of N response. Another hypothesised mechanism behind reduced respiration with N fertilisation is the alleviation of stoichiometric constraints and thus a reduction in overflow respiration for N-mining (Riggs and Hobbie 2016). Therefore, we hypothesised that increased N availability stimulates microbial CUE and expected a direct positive response of microbial metabolism upon N addition within the first 24 h of incubation. Instead, we observed a reduction in ^{18}O-CUE of -7% in response to N addition. The N response of CUE was linked to reduced microbial growth rate, as seen by the significantly positive correlation between the response ratio of C_{Growth} and

CUE, whereas microbial respiration was only slightly, but not significantly, increased within 24 h of N addition. While long-term effects of N fertilisation on CUE might be attributed to shifts in the microbial community composition, e.g. in the F:B ratio (Frey et al. 2004; Soares and Rousk 2019) or shifts from oligotroph to copiotroph microbes (Riggs and Hobbie 2016), it can be assumed that in this study microbial CUE was affected directly by added N suppressing microbial growth. Ammonium fertilisers are assumed to have a direct inhibitory effect on bacterial growth, which might be linked to enhanced osmolarity and increased ionic strength rather than to acidification (Müller et al. 2006). The addition of NH_4NO_3 might potentially have shifted microbial metabolism from growth to a stress response, explaining the short-term reduction in C_{Growth} under the unaltered respiration rate (slight, but not significant increase), and thus reduced CUE.

It was hypothesised that boreal forest soils would have the most sensitive response to N addition, i.e. show the greatest decline in respiration. Instead, forest soils revealed the lowest relative decreases in microbial cumulative respiration in response to N addition, even when *CD* forest soil—the only soil exhibiting positive RR_N of cumulative respiration—was excluded. We considered nutrient constraints—affecting unfertilised forest soils most—to be alleviated by N addition and thus N mining to be reduced, while inhibition of ligninolytic enzymes would further limit SOM decomposition in forest soils. Although depicting higher C:N ratios, indicating stronger stoichiometric limitations, forest soils exhibited the smallest relative changes in cumulative respiration in response to N addition (Fig. 6a). Similarly, N addition did not affect CO_2-C losses from Alaskan boreal forest soils, and there was no evidence for microbial feedbacks to C cycle under climate warming and N addition (Allison et al. 2008). In contrast to our predictions, the short-term response of CUE to N addition was not significantly affected by land-use type. Interestingly, C_{Growth} was not altered in response to N addition in cropland soils, which are generally exposed to a larger N supply, whereas C_{Growth} decreased by -22% in forest and grassland soils. Differences between forest soils and cropland soils were linked to a stronger inhibition of microbial growth upon N addition when the relative abundance of fungi was higher (Fig. 6b), suggesting that bacteria

were less suppressed by N addition than fungi. This was further supported by a positive correlation between the RR_N of C_{Growth} and the initial number of bacterial gene copies as an indicator of absolute abundance. Our results therefore suggest that the soil microbial community composition, which is strongly linked to land use (Szoboszlay et al. 2017), was driving the response to N addition. In contrast to our findings, reduced substrate use efficiency (^{13}C-vanillin, ^{13}C-glucose) upon $(NH_4)_2SO_4$ addition were linked to shifts in fungal and bacterial growth dominance in two boreal forest soils, where bacterial growth was inhibited while fungal growth appeared unaffected (Silva-Sánchez et al. 2019). However, we did not investigate the proportions of bacterial and fungal growth and can only refer to initial relative abundance data, which suggests that microbial communities with higher bacterial dominance responded less sensitive. It should be noted that the findings of Silva-Sánchez et al. (2019) refer to boreal forest soil microbial communities from formerly unfertilised soils, and their results may only apply to systems under natural vegetation. Bacterial communities adapted to high N availability might potentially be less sensitive to N additions as they are directly able to handle large amounts of N. A short-term depressing effect of N addition on respiration and heat production was found for Swedish arable soils, showing microbial metabolism (calorespirometry) to be dependent on a long-term fertilisation regime, where N-deficient soils exhibited a reduction in respiration upon in-vitro N addition, while respiration increased in long-term fertilised soils (Poeplau et al. 2016). These results suggest that the observed differences in our study are due to the prevailing N availability. The cropland soils considered here were regularly fertilised organically, in contrast to grassland soils, and thus exposed to higher nutrient supplies. It is likely that the presence of microbial strains or taxa adapted to higher mineral N inputs affected the immediate response of the microbiome to NH_4NO_3 addition. Even though N addition might inhibit microbial growth in the short term and thus reduce CUE, the microbial community may adapt over time to altered N availability (Silva-Sánchez et al. 2019), limiting the extrapolation of our results to a long-term period.

Conclusions

Our study was able to offer valuable insights into C dynamics in subarctic soils in a changing world. While SOM decomposition will initially increase under warming, the extent to which CO_2-C will be lost could be alleviated by facilitated microbial metabolic efficiency. At least in the short term and for the chosen temperature range of 10–20 °C, warming stimulated microbial growth more than respiration, resulting in increased CUE. This was unexpected and questions the widespread assumption that CUE is generally reduced with warming. Thus, predictions on C dynamics assuming fixed or reduced CUE with warming might not be generalisable. Most interestingly, there is also no general land-use type dependency of temperature sensitivity. Here, site-specific soil characteristics were found to affect the warming response of microbial metabolism rather than soil properties specific to land-use type, e.g. the local microbial community structure. Based on our results, we see no need to per se consider land-use type specific temperature sensitivities in modelling approaches for SOC dynamics under future climate conditions. Instead, further research should focus on the role of abiotic soil properties for the temperature sensitivity of microbial metabolism. Agricultural activity, especially market gardening, is accompanied by increased N inputs, and these inputs may also trigger increased N deposition in their surrounding ecosystems. Our findings suggest that a potential increase in agricultural activity in nutrient-limited subarctic biomes with warmer climate might initially not affect SOM decomposition in boreal forests. While N addition reduced heterotrophic respiration in arable soils, boreal forests were unaffected. In contrast to the assumption that CUE increases in response to the alleviation of nutrient limitations in N-deficient soils, CUE declined in direct response to N addition. Nitrogen addition suppressed microbial growth depending on the community structure and the prevailing N input, showing that management has a fundamental impact on the metabolic response to N amendment.

Author contributions Not applicable.

Data availability All necessary raw data as well as the R code used to conduct statistical analysis and create figures are publicly accessible (https://doi.org/10.5281/zenodo.6457750). Note: will be made publicly available when accepted for publication.

Declarations

Competing interests The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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