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

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Received: 6 January 2022 / Accepted: 17 May 2022 / Published online: 17 June 2022
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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. 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.

Keywords  18O-labelling · Carbon use efficiency · Incubation experiment · Nitrogen fertilisation · Temperature sensitivity · qPCR
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 CO₂ 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://climatetlas.ca). As a consequence, vegetation periods are becoming longer, which might encourage more agricultural activity in these subarctic regions (Tchébakova 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 Q₁₀ of SOM decomposition in temperate soils with Q₁₀ 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 18O-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
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 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$_{H_2O}$, total organic
carbon (C$_{\text{org}}$) and nitrogen (N$_{\text{total}}$) content, waterholding 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$_2$O 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

### 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$_2$O (1:5 w/v) | C$_{\text{org}}$ (%) | N$_{\text{total}}$ (%) | Olsen-P (%) | C:N ratio | C:P ratio | WHC (%) | C$_{\text{mic}}$ (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        | 23        | 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

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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 NH₄NO₃ 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 NH₄NO₃-N g⁻¹ soil DW.

Determination of ¹⁸O CUE

The ¹⁸O-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 ¹⁸O 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 H₂¹⁸O (97 at% ¹⁸O) was added with a syringe (Hamilton) in the labelled aliquot to reach a labelling of 20 at% ¹⁸O 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 NH₄NO₃ 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 CO₂ concentration (348 ppm) to a pressure of 1.3 bar within 1 min of ¹⁸O 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 N₂. 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 18O 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 18O incorporation into microbial DNA from isotopic analysis of labelled and non-labelled samples and initial enrichment of 20 at% 18O [for formula 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 $C_{\text{Growth}}$ [ng C g$^{-1}$ soil DW h$^{-1}$] is derived by using the conversion factor $f_{\text{DNA}}$ to transform the amount of total DNA produced into microbial biomass C produced, and related to soil mass and time. The $f_{\text{DNA}}$ values were derived for individual field samples.

The microbial CUE is defined as microbial biomass C produced ($C_{\text{Growth}}$) over the total uptake of C, as an approximation of the sum of microbial biomass C produced and C respired ($C_{\text{Growth}} + C_{\text{Respiration}}$) (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. WICOM 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$_3$NO$_3$ 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$_3$NO$_3$ 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 $C_{\text{Respiration}}$ [ng C g$^{-1}$ soil DW h$^{-1}$]:

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

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 $\Delta CO_2$ 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 $C_{\text{Respiration}}$. 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$_4$NO$_3$-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$_4$NO$_3$ addition in the NH$_4$NO$_3$-amended and control soils. Therefore, 5 g DW aliquots were weighed into 50 ml PE vessels and NH$_4$NO$_3$ 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$_2$SO$_4$ solution at a ratio of 1:4 v/w 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 Analysetechnik). 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$_2$SO$_4$ 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$_2$SO$_4$ solution. The assessment of microbial biomass N at the end of the incubation period was disturbed by added NH$_4$NO$_3$ 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 ± 1.7% ($R^2=0.998$) for archaea, 95.5 ± 1.4% ($R^2=0.999$) for bacteria, and 94.2 ± 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 $P_{T10}$):
The effect of N input was calculated as a response ratio of the 20 °C + 100 kg NH₄NO₃-N treatment to the 20 °C treatment as a quotient of the microbial process rates of the respective treatments ($P_{T20+N}$ and $P_{T20}$):

$$RR_T = \frac{P_{T20}}{P_{T10}}$$  \hspace{1cm} (2)

$$RR_N = \frac{P_{T20+N}}{P_{T20}}$$  \hspace{1cm} (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 NH₄NO₃ on soil pH was tested using a linear mixed-effects model approach, with treatment (control and NH₄NO₃ 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 $\rho$ (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 CO₂-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 $C_{\text{Growth}}$, $C_{\text{Respiration}}$ and cumulative respiration were
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 $0.139 \pm 0.041$ (Table S1) compared with an overall median F:B ratio of 0.019.

Mean CUE was $0.25 \pm 0.07$ for CD, $0.23 \pm 0.10$ for MB, and $0.33 \pm 0.09$ 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 ($p < 0.001$).

\[ R^2_{adj} = 0.81, P < 0.001 \]

**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,
the random effect of site only explained a small part of the variation. One exception was the warming response of \( C_{\text{Growth}} \), 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 (\( p < 0.001, 95\% \text{ CI}: 0.29–0.77 \)) (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 \( C_{\text{Respiration}} \) i.e. by a factor of 2.3 ± 0.6. Concurrently, \( C_{\text{Growth}} \) increased to a greater extent with an \( RRT \) of 3.1 ± 1.2, which explains why CUE, as the ratio of \( C_{\text{Growth}} \) to the sum of \( C_{\text{Growth}} \) and \( C_{\text{Respiration}} \), was increased with warming (+30 ± 42%) (Fig. 4a). However, this trend in CUE was only significant when all land-use types were taken together (\( p < 0.001, 95\% \text{ CI } 1.13–1.45 \)). Overall, land-use types did not show distinct responses to warming.

Compared with \( RRT \), 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 indicates an increase of the given parameter upon treatment in relation to the control. Significant responses to treatment (\( RR \neq 1 \)) 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 \( p < 0.05 \)

\[ \begin{align*}
\text{(a) 50 days incubation} & \quad \text{(b) 24 h incubation} \\
\text{RR}_T & \quad \text{RR}_N \\
\text{cumulative respiration} & \quad \text{microbial biomass C} \\
\text{C respiration} & \quad \text{C growth} \\
\text{CUE} & \quad \text{forest, grassland, cropland} \\
\end{align*} \]

\[ \begin{align*}
\text{a a a a a a a a a} & \quad \text{a a a a a a a a a} \\
\end{align*} \]

\[ \begin{align*}
\text{a a a a a a a a a} & \quad \text{a a a a a a a a a} \\
\end{align*} \]

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
and $C_{\text{Growth}}$ (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 $C_{\text{Growth}}$ 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 an average of 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
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 \( C_{\text{Growth}} \) (\( p < 0.001 \)), indicating that the response of CUE to treatments was mainly driven by the response of \( C_{\text{Growth}} \).

According to the correlation analysis, the responses of CUE and \( C_{\text{Growth}} \) 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 \( C_{\text{Growth}} \) 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 \( C_{\text{Growth}} \) 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 \( C_{\text{Growth}} \) 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 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 \( ^{18} \text{O} \) 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 CO₂-C losses due to accelerated SOM decomposition under future climate. However, directly linking the results from the 24 h and the 50 days incubation 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 RRₜ of C_{respiration} of 2.3 within the first 24 h of incubation (¹⁸O-CUE) and RRₜ 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 sitespecific 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 $C_{\text{growth}}$ was significantly higher at CD than at the other sites. Furthermore, the regression analysis revealed that the correlations between $C_{\text{growth}}$ 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 $C_{\text{mic}}$ 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 $\text{NH}_4\text{NO}_3$ addition on soil pH to assess whether potential shifts in soil pH could explain the reduction in CUE or cumulative respiration in response to $\text{NH}_4\text{NO}_3$ addition.

We found a significant, albeit small reduction in soil pH with $\text{NH}_4\text{NO}_3$ 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 $^{18}\text{O}$-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 $C_{\text{growth}}$ 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 NH₄NO₃ might potentially have shifted microbial metabolism from growth to a stress response, explaining the short-term reduction in CGrowth 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 RRN of cumulative respiration—was excluded. We considered nutrient constraints—acting 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 CO₂-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, CGrowth was not altered in response to N addition in cropland soils, which are generally exposed to a larger N supply, whereas CGrowth 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 RRₙ of CGrowth 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 (¹³C-vanillin, ¹³C-glucose) upon (NH₄)₂SO₄ 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 NH₄NO₃ 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 CO₂-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.

Acknowledgments We are very grateful to the Yukon farmers Barbara and Bill Drury from the Circle D Ranch, Norma and Ralph Mease from the Minto Bridge Farm, and Louise Tyacke and Gary Masters from Sister Island for participating in our study. Thanks go to the Geological Survey Yukon and Whitehorse local Jeannie McLorie for offering facilities for soil sample drying and sieving. This study was conducted under the Yukon Scientists and Explorers Act Licence No. 6800-20-1151, and we thank all Yukon First Nations for granting us permission to conduct our scientific work on their traditional land. We would also like to thank the technical staff of the Thünen Institute involved in this study: Frank Hegewald (sampling), Kerstin Gilke (GC), Claudia Wiese (CN), Arne Heidkamp and the team of the Soil Monitoring Laboratory (soil texture, Olsen-P), Sabine Wathsack (Cmic), Andrea Niemeyer (Nmic), Britta Müller, Karin Trescher and Jana Usarek (DNA extraction, qPCR). Special thanks go to Dr. Jens Dyckmans and his colleagues at the Centre for Stable Isotope Research and Analysis of the University of Göttingen. This study was part of the “Breaking the Ice” project funded by the German Research Foundation (DFG) (Grant No. 401106790). Funding for E.G. was provided by the Science & Technology Branch of Agriculture & Agri-Food Canada [Project J-001756 ‘Biological Soil Carbon Stabilisation’].

Author contributions Not applicable.

Funding Open Access funding enabled and organized by Projekt DEAL. This study was part of the “Breaking the Ice” project funded by the German Research Foundation (Grant No. 401106790). Funding for E.G. was provided by the Science & Technology Branch of Agriculture & Agri-Food Canada [Project J-001756 ‘Biological Soil Carbon Stabilisation’].

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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References

Allison SD, Czimczik CI, Treseder KK (2008) Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Glob Change Biol 14(5):1156–1168. https://doi.org/10.1111/j.1365-2486.2008.01549.x

Allison SD, Wallenstein MD, Bradford MA (2010) Soil-carbon response to warming dependent on microbial physiology. Nat Geosci 3(5):336–340. https://doi.org/10.1038/ngeo846

Aphalo PJ (2021) ggpmisc: miscellaneous extensions to ‘ggplot2’. R Package Version 0.3.9

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67(1):1–48. https://doi.org/10.18637/jss.v067.i01

Bekku YS, Nakatsubo T, Kume A, Adachi M, Koizumi H (2003) Effect of warming on the temperature dependence of soil respiration rate in arctic, temperate and tropical soils. Appl Soil Ecol 22(3):205–210. https://doi.org/10.1016/S0929-1393(02)00158-0

Bölscher T, Ågren GI, Herrmann AM (2020) Land-use alters the temperature response of microbial carbon-use efficiency in soils—a consumption-based approach. Soil Biol Biochem 140:107639. https://doi.org/10.1016/j.soilbio.2019.107639

Bosatta E, Ågren GI (1999) Soil organic matter quality interpreted thermodynamically. Sci Total Environ 31(13):1889–1891. https://doi.org/10.1016/S0048-9697(99)00105-4

Bradford MA (2013) Thermal adaptation of decomposer communities in warming soils. Front Microbiol 4:333. https://doi.org/10.3389/fmicb.2013.00333

Chien SH, Gearhart MM, Collamer DJ (2008) The effect of different ammonical nitrogen sources on soil acidification. Soil Sci 173(8):544–551. https://doi.org/10.1097/SS.0b013e318179d17

Climate Atlas of Canada. version 2 (July 10, 2019). using BCCAQv2 climate model data. Available at www.climata.ca. Accessed 19 Aug 2021

Conant RT, Ryan MG, Ågren GI, Birge HE, Davidson EA, Eliasson PE, Evans SE, Frey SD, Giardina CP, Hopkins FM, Hyvönen R, Kirschbaum MUF, Lavallee JM, Leifeld J, Parton WJ, Megan Steinweg J, Wallenstein MD, Martin Wetterstedt JÅ, Bradford MA (2011) Temperature and soil organic matter decomposition rates—synthesis of current knowledge and a way forward. Glob Change Biol 17(11):3392–3404. https://doi.org/10.1111/j.1365-2486.2011.02496.x

Coucheney E, Strömgren M, Lerch TZ, Herrmann AM (2013) Long-term fertilization of a boreal Norway spruce forest increases the temperature sensitivity of soil organic carbon mineralization. Ecol Evol 3(16):5177–5188. https://doi.org/10.1002/ece3.895

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440(7081):165–173. https://doi.org/10.1038/nature04514

DeGryze S, Six J, Paustian K, Morris SJ, Paul EA, Merckx R (2004) Soil organic carbon pool changes following land-use conversions. Glob Change Biol 10(7):1120–1132. https://doi.org/10.1111/j.1529-8817.2003.00786.x

Ding F, Sun W, Huang Y, Hu X (2018) Larger Q 10 of carbon decomposition in finer soil particles does not bring long-lasting dependence of Q 10 on soil texture. Eur J Soil Sci 69(2):336–347. https://doi.org/10.1111/ejss.12530

Don A, Schumacher J, Freibauer A (2011) Impact of tropical land-use change on soil organic carbon stocks—a meta-analysis. Glob Change Biol 17(4):1658–1670. https://doi.org/10.1111/j.1365-2486.2010.02336.x

Fierer N, Craine JM, McLaughlin K, Schimel JP (2005) Litter quality and the temperature sensitivity of decomposition. Ecology 86(2):320–326. https://doi.org/10.1890/04-1254

Frey SD, Knorr M, Parrent JL, Simpson RT (2004) Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. For Ecol Manag 196(1):159–171. https://doi.org/10.1016/j.foreco.2004.03.018

García-Palacios P, Crowther TW, Dalac M, Hartley IP, Reinsch S, Rinnan R, Rouk J, van den Hoogen J, Ye J-S, Bradford MA (2021) Evidence for large microbial-mediated losses of soil carbon under anthropogenic warming. Nat Rev Earth Environ 2(7):507–517. https://doi.org/10.1038/s43017-021-00178-4

Geisseler D, Scow KM (2014) Long-term effects of mineral fertilizers on soil microorganisms—a review. Soil Biol Biochem 75:54–63. https://doi.org/10.1016/j.soilbio.2014.03.023

Graves S, Piepho H-P, Selzer L, with help from Dorai-Raj, Sundar (2019) multcompView: visualizations of paired comparisons. R package version 0.1-8

Grünzweig JM, Sparrow SD, Yakir D, Chapin FS (2004) Impact of agricultural land-use change on carbon storage in boreal Alaska. Glob Change Biol 10:452–472. https://doi.org/10.1111/j.1529-8817.2003.00738.x

Guo LB, Gifford RM (2002) Soil carbon stocks and land use change: a meta analysis. Glob Change Biol 8(4):345–360. https://doi.org/10.1046/j.1365-1393.2002.00486.x

Hamdi S, Moyano F, Sall S, Bernoux M, Chevallier T (2013) Synthesis analysis of the temperature sensitivity of soil respiration from laboratory studies in relation to incubation methods and soil conditions. Soil Biol Biochem 58:115–126. https://doi.org/10.1016/j.soilbio.2012.11.012

Hartley IP, Hopkins DW, Garnett MH, Sommerkorn M, Woomer PA (2008) Soil microbial respiration in arctic soil does not accclimate to temperature. Ecol Lett 11(10):1092–1100. https://doi.org/10.1111/j.1461-0248.2008.01223.x

He ZL, Alva AK, Calvert DV, Li YC, Banks DJ (1998) Effects of nitrogen fertilization of grapefruit trees on soil acidification and nutrient availability in a Riviera fine sand. Plant Soil 206(1):11–19. https://doi.org/10.1023/A:100436805789

Hemkemeyer M, Christensen BT, Martens R, Tebbe CC (2015) Soil particle size fractions harbour distinct microbial communities and differ in potential for microbial mineralisation of organic pollutants. Soil Biol Biochem 90:255–265. https://doi.org/10.1016/j.soilbio.2015.08.018
Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50(3):346–363. https://doi.org/10.1002/bimj.200810425

IPCC (2014) Climate change 2014. Synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. IPCC, Geneva, Switzerland

Joergensen RG (1996) The fumigation-extraction method to estimate soil microbial biomass: calibration of the $k_{EC}$ value. Sci Total Environ 281(1):25–31. https://doi.org/10.1016/S0048-9697(95)00102-6

Joergensen RG, Mueller T (1996) The fumigation-extraction method to estimate soil microbial biomass: calibration of the $k_{EN}$ value. Soil Biol Biochem 28(1):33–37. https://doi.org/10.1016/0038-0717(95)00101-8

Karhu K, Auffret MD, Dungait JAJ, Hopkins DW, Prosser JI, Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerT – Package: tests in linear mixed effects models. J Stat Software 82:13. https://doi.org/10.18637/jss.v082.i13

Kleber M (1929) Korngrößenanalyse Vermittels Pipettenanalyse. Tonindustrie-Zeitung 53:729–731

Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerT–Est Package: tests in linear mixed effects models. J Stat Softw 82:13. https://doi.org/10.18637/jss.v082.i13

Lloyd J, Taylor JA (1994) On the temperature dependence of soil respiration. Funct Ecol 8(3):315. https://doi.org/10.2307/2389824

Malik AA, Puissant J, Buckeridge KM, Goodall T, Jemhlíček N, Chowdhury S, Gweon HS, Peyton JM, Mason KE, van Agtmaal M, Blaud A, Clark IM, Whitaker J, Pywell RF, Ostle N, Gleixner G, Griffiths RI (2018) Land use driven change in soil pH affects microbial carbon cycling processes. Nat Commun 9(1):3591. https://doi.org/10.1038/s41467-018-05980-1

Manzoni S, Taylor P, Richter A, Porporato A, Agren GI (2012) Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol 196(1):79–91. https://doi.org/10.1111/j.1469-8137.2012.04225.x

Martin KJ, Rygiewicz PT (2005) Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiol 5:28. https://doi.org/10.1186/1471-2180-5-28

Meyer N, Welp G, Ameling W (2018) The temperature sensitivity (Q10) of soil respiration: controlling factors and spatial prediction at regional scale based on environmental soil classes. Global Biogeochem Cycles 32(2):306–323. https://doi.org/10.1002/2017GB005644

Müller T, Walter B, Wirtz A, Burkovski A (2006) Ammonium toxicity in bacteria. Curr Microbiol 52(5):400–406. https://doi.org/10.1007/s00284-005-0370-x

Olsen SR (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate, vol 939. US Department of Agriculture, Washington, D.C.

Peplau T, Schroeder J, Gregorich E, Poepplu C (in print) Subarctic soil carbon losses after deforestation for agriculture depend on permafrost abundance. Glob Change Biol

Plante AF, Six J, Paul EA, Conant RT (2009) Does physical protection of soil organic matter attenuate temperature sensitivity? Soil Sci Soc Am J 73(4):1168–1172. https://doi.org/10.2136/sssaj2008.0351

Poepplu C, Don A (2013) Sensitivity of soil organic carbon stocks and fractions to different land-use changes across Europe. Geoderma 192(3):189–201. https://doi.org/10.1016/j.geoderma.2012.08.003

Poepplu C, Herrmann AM, Kätterer T (2016) Opposing effects of nitrogen and phosphorus on soil microbial metabolism and the implications for soil carbon storage. Soil Biol Biochem 100:83–91. https://doi.org/10.1016/j.soilbio.2016.05.021

Poepplu C, Helfrich M, Dechow R, Szoboszlai M, Tebbe CC, Don A, Greiner B, Zopf D, Thumm U, Korevaar H, Geerts R (2019) Increased microbial anabolism contributes to soil carbon sequestration by mineral fertilization in temperate grasslands. Soil Biol Biochem 130:167–176. https://doi.org/10.1016/j.soilbio.2018.12.019

Pold G, Domeignoz-Horta LA, DeAngelis KM (2020) Heavy and wet: the consequences of violating assumptions of measuring soil microbial growth efficiency using the $^{18}$O water method. Elem Sci Anthr 8:1. https://doi.org/10.1525/elementa.069

R Core Team (2020) R: a language and environment for statistical computing. R Found Stat Comput, Vienna

Riggs CE, Hobbie SE (2016) Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. Soil Biol Biochem 99:54–65. https://doi.org/10.1016/j.soilbio.2016.04.023

Romero-Olivares AL, Allison SD, Treseder KK (2017) Soil microbes and their response to experimental warming over time: a meta-analysis of field studies. Soil Biol Biochem 107:32–40. https://doi.org/10.1016/j.soilbio.2016.12.026

RStudio Team (2016) RStudio: integrated development environment for R. RStudio Inc, Boston

Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35(4):549–563. https://doi.org/10.1016/s0038-0717(03)00015-4

Springer
Schroeder J, Kammler M, Helfrich M, Tebbe CC, Poeplau C (2021) Impact of common sample pre-treatments on key soil microbial properties. Soil Biol Biochem 160:108321. https://doi.org/10.1016/j.soilbio.2021.108321

Silva-Sánchez A, Soares M, Rousk J (2019) Testing the dependence of microbial growth and carbon use efficiency on nitrogen availability, pH, and organic matter quality. Soil Biol Biochem 134:25–35. https://doi.org/10.1016/j.soilbio.2019.03.008

Simon E, Canarini A, Martin V, Séneca J, Böckle T, Reinthaler D, Pötsch EM, Piepho H-P, Bahn M, Wanek W, Richter A (2020) Microbial growth and carbon use efficiency show seasonal responses in a multifactorial climate change experiment. Commun Biol 3(1):584. https://doi.org/10.1038/s42255-020-01317-1

Singh M, Sarkar B, Biswas B, Bolan NS, Churchman GJ (2017) Relationship between soil clay mineralogy and carbon protection capacity as influenced by temperature and moisture. Soil Biol Biochem 109:95–106. https://doi.org/10.1016/j.soilbio.2017.02.003

Sinsabaugh RL, Manzoni S, Moorhead DL, Richter A (2013) Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. Ecol Lett 16(7):930–939. https://doi.org/10.1111/ele.12113

Sinsabaugh RL, Turner BL, Talbot JM, Waring BG, Powers JS, Kuske CR, Moorhead DL, Fallstad Shah JJ (2016) Stoichiometry of microbial carbon use efficiency in soils. Ecol Monogr 86(2):172–189. https://doi.org/10.1890/15-2110.1

Smith CA, Meikle JC, Roots CF (eds) (2004) Ecoregions of the Yukon Territory: biophysical properties of Yukon landscapes. Agriculture and Agri-Food Canada, PARC Technical Bulletin No. 04-01, Summerland, British Columbia

Soares M, Rousk J (2019) Microbial growth and carbon use efficiency in soil: Links to fungal-bacterial dominance, SOC-quality and stoichiometry. Soil Biol Biochem 134:25–35. https://doi.org/10.1016/j.soilbio.2020.01.010

Spohn M, Klaus K, Wanek W, Richter A (2016a) Microbial carbon use efficiency and biomass turnover times depending on soil depth—implications for carbon cycling. Soil Biol Biochem 96:74–81. https://doi.org/10.1016/j.soilbio.2016.01.016

Spohn M, Pötsch EM, Eichorst SA, Woebken D, Wanek W, Richter A (2016b) Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. Soil Biol Biochem 97:168–175. https://doi.org/10.1016/j.soilbio.2016.03.008

Steinweg JM, Plante AF, Conant RT, Paul EA, Tanaka DL (2008) Patterns of substrate utilization during long-term incubations at different temperatures. Soil Biol Biochem 40(11):2722–2728. https://doi.org/10.1016/j.soilbio.2008.07.002

Szoboszlai M, Dohrmann AB, Poeplau C, Don A, Tebbe CC (2017) Impact of land-use change and soil organic carbon quality on microbial diversity in soils across Europe. FEMS Microbiol Ecol 93:12. https://doi.org/10.1093/femsse/fix146

Tarnocai C, Canadell JG, Schuur EAG, Kuhry P, Mazhitova G, Zimov SA (2009) Soil organic carbon pools in the northern circumpolar permafrost region. Glob Biogeochem Cycles 23(2):1–11. https://doi.org/10.1029/2008GB003327

Tchibakova NM, Parfenova EI, Lysanova GI, Soja AJ (2011) Agroclimatic potential across central Siberia in an altered twentieth-first century. Environ Res Lett 6(4):45207. https://doi.org/10.1088/1748-9326/6/4/045207

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19(6):703–707. https://doi.org/10.1016/0038-1038(78)90052-6

Walker TW, Kaiser C, Strasser F, Herbold CW, Leblans NI, Woebken D, Janssens IA, Sigurdsson BD, Richter A (2018) Microbial temperature sensitivity and biomass change explain soil carbon loss with warming. Nat Clim Chang 8(10):885–889. https://doi.org/10.1038/s41586-018-0259-x

Wei H, Guenet B, Vicca S, Nunan N, Asard H, Abdelgawad H, Shen W, Janssens IA (2014) High clay content accelerates the decomposition of fresh organic matter in artificial soils. Soil Biol Biochem 77:100–108. https://doi.org/10.1016/j.soilbio.2014.06.006

Wei T, Simko V (2021) corplot: visualization of a correlation matrix. R Package Version 0.90

Wetterstedt JÅM, Persson T, Ågren GI (2010) Temperature sensitivity and substrate quality in soil organic matter decomposition: results of an incubation study with three substrates. Glob Change Biol 16(6):1806–1819. https://doi.org/10.1111/j.1365-2486.2009.02112.x

Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, Gromelec G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen T, Miller E, Bache S, Müller K, Ooms J, Robinson D, Seidel D, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019) Welcome to the Tidyverse. J Open Source Softw 4(43):1686. https://doi.org/10.21105/joss.01686

Widdig M, Schleuss P-M, Biederman LA, Borer ET, Crawley MJ, Kirkman KP, Seabloom EW, Wragg PD, Spohn M (2020) Microbial carbon use efficiency in grassland soils subjected to nitrogen and phosphorus additions. Soil Biol Biochem 146:107815. https://doi.org/10.1016/j.soilbio.2020.107815

Wilke CO (2020) cowplot: streamlined plot theme and plot annotations for ‘ggplot2.’ R Package Version 1.1

Ye J-S, Bradford MA, Dalal M, Maestre FT, García-Palacios P (2019) Increasing microbial carbon use efficiency with warming predicts soil heterotrophic respiration globally. Glob Change Biol 25(10):3354–3364. https://doi.org/10.1111/gcb.14738

Yu Y, Lee C, Kim J, Hwang S (2005) Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol Bioeng 89(6):670–679. https://doi.org/10.1002/bit.20347

Zhang J (2010) Temperature sensitivity of soil organic matter decomposition and the influence of soil carbon pools and attributes. Graduate Theses and Dissertations, Iowa State University

Zheng Q, Hu Y, Zhang S, Noll L, Böckle T, Richter A, Wanek W (2019) Growth explains microbial carbon use efficiency across soils differing in land use and geology. Soil
Zhou T, Shi P, Hui D, Luo Y (2009) Global pattern of temperature sensitivity of soil heterotrophic respiration (Q10) and its implications for carbon-climate feedback. J Geophys Res Biogeosci 114(G2):G02016. https://doi.org/10.1029/2008JG000850

Zhou L, Zhou X, Zhang B, Lu M, Luo Y, Liu L, Li B (2014) Different responses of soil respiration and its components to nitrogen addition among biomes: a meta-analysis. Glob Change Biol 20(7):2332–2343. https://doi.org/10.1111/gcb.12490

Zhou Z, Wang C, Zheng M, Jiang L, Luo Y (2017) Patterns and mechanisms of responses by soil microbial communities to nitrogen addition. Soil Biol Biochem 115:433–441. https://doi.org/10.1016/j.soilbio.2017.09.015

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