Soil Biology and Biochemistry 184 (2023) 109109

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Long-term warming of a forest soil reduces microbial biomass and its carbon and nitrogen use efficiencies

Ye Tian [a,b,*], Andreas Schindlbacher [c], Carolina Urbina Malo [a,d], Chupei Shi [a,e], Jakob Heinzle [c], Steve Kwatcho Kengdo [f], Erich Inselsbacher [g], Werner Borken [f], Wolfgang Wanek [a,**]

[a] Division of Terrestrial Ecosystem Research, Department of Microbiology and Ecosystem Science, Center of Microbiology and Environmental Systems Science, University of Vienna, Djerassiplatz 1, A-1030, Vienna, Austria
[b] Doctoral School in Microbiology and Environmental Science, University of Vienna, Djerassiplatz 1, A-1030, Vienna, Austria
[c] Department of Forest Ecology and Soil, Federal Research and Training Centre for Forests, Natural Hazards and Landscape-BFW, Seckendorff-Gudent Weg 8, 1131, Vienna, Austria
[d] Institute of Soil Science, Leibniz Universität Hannover, Herrenhäuser Straße 2, 30419, Hannover, Germany
[e] Department of Ecosystem and Landscape Dynamics, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, Netherlands
[f] Department of Soil Ecology, University of Bayreuth, Dr. Hans-Frisch-Straße 1-3, 95448, Bayreuth, Germany
[g] Institute of Soil Research, Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences Vienna (BOKU), Peter Jordan-Straße 82, 1190, Vienna, Austria

ARTICLE INFO

Keywords:
Soil warming
soil carbon (C) cycling
soil nitrogen (N) cycling
isotope pool dilution
microbial carbon use efficiency (CUE)
microbial nitrogen use efficiency (NUE)

ABSTRACT

Global warming impacts biogeochemical cycles in terrestrial ecosystems, but it is still unclear how the simultaneous cycling of carbon (C) and nitrogen (N) in soils could be affected in the longer-term. Here, we evaluated how 14 years of soil warming (+4 °C) affected the soil C and N cycle across different soil depths and seasons in a temperate mountain forest. We used $H_2^{18}O$ incorporation into DNA and ^{15}N isotope pool dilution techniques to determine gross rates of C and N transformation processes. Our data showed different warming effects on soil C and N cycling, and these were consistent across soil depths and seasons. Warming decreased microbial biomass C (−22%), but at the same time increased microbial biomass-specific growth (+25%) and respiration (+39%), the potential activity of β-glucosidase (+31%), and microbial turnover (+14%). Warming reduced gross rates of protein depolymerization (−19%), but stimulated gross N mineralization (+63%) and the potential activities of N-acetylglucosaminidase (+106%) and leucine-aminopeptidase (+46%), and had no impact on gross nitrification (+1%). Microbial C and N use efficiencies were both lower in the warming treatment (−15% and −17%, respectively). Overall, our results suggest that long-term warming drives soil microbes to incorporate less C and N into their biomass (and necromass), and to release more inorganic C and N to the environment, causing lower soil C and N storage in this forest, as indicated by lower soil C and total N contents. The decreases in microbial CUE and NUE were likely triggered by increasing microbial P constraints in warmed soils, limiting anabolic processes and microbial growth and promoting pervasive losses of C and N from the soil.

1. Introduction

Global atmospheric temperatures are predicted to increase by 1.0–5.7 °C above pre-industrial levels until the end of the century (Masson-Delmotte et al., 2021), resulting in higher soil temperatures and thereby impacting biogeochemical processes in terrestrial ecosystems (Davidson and Janssens, 2006; García-Palacios et al., 2021; Li et al., 2021; Melillo et al., 2002). How soil carbon (C) and nitrogen (N) cycling respond to warming is central, as C and N are pivotal for the growth and functioning of all soil organism and as their bioavailability governs the release of greenhouse gases that could feed back on climate (Allison et al., 2010; Bai et al., 2013; Melillo et al., 2002). Since soil C

* Corresponding author. Division of Terrestrial Ecosystem Research, Department of Microbiology and Ecosystem Science, Center of Microbiology and Environmental Systems Science, University of Vienna, Djerassiplatz 1, A-1030, Vienna, Austria.
** Corresponding author.
E-mail addresses: ye.tian@univie.ac.at (Y. Tian), wolfgang.wanek@univie.ac.at (W. Wanek).

https://doi.org/10.1016/j.soilbio.2023.109109
Received 14 March 2023; Received in revised form 17 June 2023; Accepted 21 June 2023
Available online 23 June 2023

and N cycling are strongly interconnected (Fuchslueger et al., 2019; Gruber and Galloway, 2008; Melillo et al., 2011; Yue et al., 2017), only a comprehensive view on their pools and processes and their interrelationships can provide an integrated understanding of how forest soil biogeochemistry responds to global warming.

In-situ soil warming experiments are the prime experimental approach to investigate warming effects in tall forests, where whole ecosystem warming can hardly be established at representative spatial scales (Carey et al., 2016; Cavaleri et al., 2015; Chung et al., 2013; Dorrepaal et al., 2009; Melillo et al., 2002; Rousk et al., 2013; Schindlbacher et al., 2015a). Artificial soil warming typically increases soil respiration, causing C losses from soils to the atmosphere, especially in the early years of warming (Melillo et al., 2002; Romero-Olivares et al., 2017; Rustad et al., 2001). Elevated soil temperatures can accelerate soil C cycling not only by direct stimulation of microbial and root activities, but also via an up-regulation of extracellular enzyme activities which speed up the breakdown of soil organic matter (SOM) (Fanin et al., 2022; Melillo et al., 2011; Schindlbacher et al., 2009; Zuccarini et al., 2020). In conjunction with this stimulation of SOM decomposition, concurrent increases in soil N availability and potential interactions with C cycling may, however, modulate the overall warming response. Higher N availability, on the one hand, can favor soil C sequestration via increasing soil aggregation and C occlusion in macro-aggregates (Riggs et al., 2015), and by forming chemically protected compounds, such as heterocyclic forms of N (Janssens et al., 2010; Nommik and Vahtras, 1982; Thorn and Mikita, 1992). On the other hand, higher soil available N was shown to decrease SOM decomposition through decreasing soil microbial biomass and soil respiration, and by suppressing chemically complex SOM degrading (oxidative) enzymes (Carreiro et al., 2000; Janssens et al., 2010; Lu et al., 2021; Riggs et al., 2015; Saiya-Cork et al., 2002). Thus, changes in N availability can potentially strongly affect and thereby composensate the warming effects on the soil C cycle.

Regarding direct warming effects on soil N cycling, meta-analyses have suggested that warming amplified net N mineralization (by 46–52%), net nitrification (by 32–56%), and potential denitrification (by 38–91%), because of intensified soil microbial and enzymatic activities (Bai et al., 2013; Lu et al., 2013; Zhang et al., 2015a). Gross N transformation rates, e.g. gross mineralization and gross nitrification, which give more direct insight into the specific production and consumption processes of nitrogenous compounds in soils, however, generally received much less attention in soil warming experiments (Bai et al., 2013), therefore obviating meta-analytical generalizations. Especially gross protein depolymerization rates have hardly been addressed so far (Maxwell et al., 2021; Wild et al., 2018), though depolymerization processes play a crucial role as being the rate-limiting process for the subsequent soil inorganic N transformation processes (Jan et al., 2009; Mooshammer et al., 2014a; Schimel and Bennett, 2004).

Whether soil microbial biomass increases or decreases can play a key role in the overall biogeochemical cycling response to warming (Walker et al., 2018). Thus, the ability of soil microorganisms to efficiently incorporate C and N into their biomass is of great significance in a warming environment. Microbial C and N use efficiencies (CUE and NUE) depict the partitioning of acquired organic C and N between incorporation into microbial biomass (anabolic processes) and the release as inorganic C (CO_2) and N (NH_4^+) to the environment (catabolic processes) (Allison et al., 2010; Manzoni et al., 2012; Mooshammer et al., 2014a). Theoretically, microbial CUE decreases with elevated temperatures, because of increased respiration to meet the inflating microbial energy demands at higher temperatures (Li et al., 2019; Pold et al., 2017). However, unchanged or even increased microbial CUE under warming has also been reported due to other drivers than temperature (e.g. substrate availability) affecting microbial respiration and growth simultaneously (Simon et al., 2020; Wang et al., 2022; Ye et al., 2019). The warming effects on microbial NUE have received less attention than microbial CUE and therefore remained unclear (Maxwell et al., 2021; Wild et al., 2018). Dai et al. (2020), in their meta-analysis, reported that increased temperatures drive N dynamics to become more catabolically dominated. Microbial NUE can thus be expected to decrease under warming, denoting higher potential losses of soil N and less potential for soil organic N storage, which in turn may negatively affect soil C cycling and storage.

The supply, quality and accessibility of C and nutrients may change with the duration of warming. This gives the long-term response of soil C dynamics a higher level of complexity than the short-term response, and makes it difficult to predict (Melillo et al., 2017; Nottingham et al., 2020; Wilson et al., 2021). Some long-term soil warming studies observed a down-regulation of warming effects over time, resulting from total and labile C depletion, microbial acclimation and/or thermal adaptation (Melillo et al., 2002; Romero-Olivares et al., 2017). On the other hand, it also has been shown that the response on single process rates such as soil CO_2 efflux can oscillate considerably during decadal time-scales, as a matter of substrate supply and microbial community adaptations (Lim et al., 2019; Melillo et al., 2017). Few studies, contrarily, did not see this down-regulation, but instead observed consistent stimulatory warming effects on the soil C cycle in the mid-to longer-run (Reth et al., 2009; Schindlbacher et al., 2015a; Soong et al., 2021; Teramoto et al., 2016).

The objective of this study therefore was to investigate how long-term (14 years) warming affects a large suite of soil C and N cycling processes, and to evaluate possible changes in soil C and N conditions in a temperate mountain forest in central Europe. In a previous study, a set of C and N cycling parameters was analyzed after nine years of soil warming. At this point in time, no significant alterations or adaptation in microbial biomass, soil CO_2 efflux, and soil enzyme activities were evident (Schindlbacher et al., 2015a), eventually due to high SOC stocks in forest topsoils developed on dolomite and limestone bedrock (Prietzel and Christophel, 2014; Wiesmeier et al., 2013). However, this might have changed since then as a matter of continued mineralization of available SOM. We here hypothesized that (i) long-term soil warming accelerates SOM decomposition (microbial respiration, enzyme activities), since organic C stocks in the topsoil are high and partly poorly protected in the calcareous soil. We further hypothesized that (ii) warming reduces microbial CUE as a matter of increased C investment in maintenance processes. With regard to N transformation processes, we hypothesized that (iii) long-term soil warming accelerates gross N processes, but (iv) reduces microbial NUE and thereby increases the risk of potential N losses from soil. Moreover, the expected warming effects on C and N pools and cycling processes were tested across soil depth and season to evaluate their consistency and/or their spatiotemporal context-dependency.

2. Methods

2.1. Site description and soil sampling

The research site is a ~130-year-old mountain forest in the Northern Limestone Alps, near Achenkirch, Austria (47°34′50″N, 11°38′21″E; 910 m a.s.l.). The forest consists of 80% *Picea abies* L., 15% *Fagus sylvatica* L., and 5% *Abies alba* Mill. The understory is dominated by regeneration of *Fagus sylvatica* L. The site has a cool humid climate. Mean annual air temperature and precipitation were 7.0 °C and 1493 mm (1988–2017), respectively, and the mean air temperature and total precipitation in 2019 were 6.7 °C and 1908 mm, respectively (data from Zentralanstalt für Meteorologie und Geodynamik (ZAMG), Austria). The bedrock is formed of dolomite. According to the World Reference Base for Soil Resources (FAO, 2015), the soils are characterized as a mosaic of Chromic Cambisols and Rendzic Leptosols, with high carbonate content and near-neutral pH (Schindlbacher et al., 2015b). The carbonate contents ranged between 144 and 175 g kg^{-1} d.s. In 0–10 cm and 212 and 276 g kg-1 d.s. In 10–20 cm soil depth. Soils are shallow with soil depths

ranging between 10 cm and 25 cm. (Schindlbacher et al., 2010, 2015b).

This work is based on a long-term soil warming manipulation experiment (the Achenkirch soil warming experiment). Six blocks with paired 2 × 2 m warmed and control plots were randomly distributed in the forest. Resistance heating cables (0.4 cm diameter, TECUTE – 0.18 Ohm-1 UV-1, Etherma, Salzburg, Austria) or dummy cables were buried at a depth of 3 cm with a spacing of 7–8 cm in warmed and control plots, respectively. In every plot, temperature sensors were installed (PT100; EMS, Brno, Czech Republic) at a depth of 5 cm between two heating cables to ensure a 4 °C higher temperature in warmed plots than in their adjacent control plots during the snow-free season (April to December; to avoid warming-induced snow melting and adverse effects on soil temperature and hydrology). The first three blocks were established in 2004, which was extended by further three blocks in 2007.

We sampled soils three times in 2019 (spring: 2nd May, summer: 6th August, and autumn: 15th October). In each plot, 6–7 soil cores were taken per season by stainless steel corers (diameter 2.5 cm). We separated soils in 0–10 cm and 10–20 cm depth increments. Soil samples were pooled per plot, soil depth, and season to form 72 soil samples in total. All soil samples were sieved (2 mm) and homogeneously mixed, and the extracted roots and stones collected for further washing, drying, and further measurements. Following this, warmed and control soil samples were incubated at their corresponding *in-situ* temperatures before and during the following measurements (warmed samples: 14 °C, 17 °C, and 14 °C; control samples: 10 °C, 13 °C, and 10 °C for spring, summer, and autumn collections, respectively). Thereby we studied the possible future state of this system in terms of C- and N-related biogeochemical multifunctionality in a warmer world, by comparing ambient conditions with long-term, warmed conditions.

2.2. Soil parameters, root parameters, and microbial biomass

We measured soil pH using an ISFET pH sensor (Sentron, The Netherlands) in a 1:5 (w:v) mix of air-dried soil and ultra-pure water. For soil hydraulic parameters, we weighed 10 g fresh soil in funnels with ash-free cellulose filter paper, and measured soil water holding capacity (WHC) gravimetrically by saturating the soil with ultra-pure water and draining for 2.5 h. Soil water content (SWC) was measured gravimetrically via weighing 5 g fresh soil and drying at 105 °C for 48 h. Then, we took two aliquots of this oven-dried soil. One aliquot was acidified with 2 M HCl to remove carbonate and thereafter re-dried, and both aliquots were then ground in a ball mill for determination of SOC, soil total N (TN), soil C:N ratios, and soil $\delta^{13}C$ and $\delta^{15}N$ with an elemental analyzer (EA1110, CE Instrument) coupled by a ConFlo III interface to an isotope ratio mass spectrometer (IRMS, Delta^{PLUS}, all Thermo Scientific, Austria). We calculated SOC from soil C:N measured in acidified soils times soil TN in non-treated soils. Root samples were ground, and root C and N contents, root C:N ratios, and root $\delta^{13}C$ and $\delta^{15}N$ determined with the same IRMS setting as for soils.

We took two aliquots of fresh soil. One aliquot was extracted with 1 M KCl (1:7.5 (w:v)) and analyzed for NH_4^+ and NO_3^- contents colorimetrically (Hood-Nowotny et al., 2010; Kandeler and Gerber, 1988; Miranda et al., 2001) and for free amino acid (FAA) content fluorimetrically (Jones et al., 2002; Prommer et al., 2014). The same soil KCl extracts were also used for dissolved organic C (measured as non-purgeable organic C) and total dissolved N (TDN) measurements via a TOC/TN-Analyzer (TOC-VCPH/CPNT-NM-1, Shimadzu, Japan). Then, dissolved organic N (DON) was calculated as the difference between TDN and the sum of NH_4^+ and NO_3^-. To determine microbial biomass C and N (MBC and MBN) (Brookes et al., 1985; Haubensak et al., 2002; Jenkinson et al., 2004; Vance et al., 1987), we fumigated the other aliquot of fresh soil with chloroform in the dark for 48 h, and extracted (1 M KCl) and measured DOC and TDN (TOC/TN-Analyzer) the same way as done for non-fumigated samples. Then, microbial biomass C and N (MBC and MBN) were calculated as follows:

$$MBC = \frac{DOC_{fumigated} - DOC_{non-fumigated}}{k_{EC}} \quad (1)$$

$$MBN = \frac{TDN_{fumigated} - TDN_{non-fumigated}}{k_{EN}} \quad (2)$$

where $DOC_{fumigated}$, $DOC_{non\text{-}fumigated}$, $TDN_{fumigated}$, and $TDN_{non\text{-}fumigated}$ are the results of DOC and TDN from the fumigated and the non-fumigated soil aliquots, respectively. k_{EC} and k_{EN} are the correction factors of 0.45 and 0.45 for MBC and MBN, respectively (Jenkinson et al., 2004). Microbial C:N ratios are expressed on a mass basis.

2.3. Soil C cycling and microbial CUE

We used $H_2^{18}O$ incorporation into DNA and CO_2 evolution to determine microbial growth, respiration, microbial turnover rates, and microbial CUE (Blazewicz and Schwartz, 2011; Spohn et al., 2016a; Spohn et al., 2016b; Zheng et al., 2019). In short, two aliquots of fresh soil (0.4 g) were weighed into 2 mL screw cap vials (without caps), which were placed in 50 mL glass serum bottles (Supelco, Sigma-Aldrich Chemie GmbH, Germany). $H_2^{18}O$ (Campro Scientific) was added to one aliquot to reach approximately 20 at% ^{18}O in soil water, while adjusting the soil moisture to 60% WHC. The other aliquot was amended with the same volume of ultra-pure water as natural ^{18}O abundance control. Then, the serum bottles were crimped with rubber seals gas-tightly (Supelco, Sigma-Aldrich Chemie GmbH, USA). Right after sealing the bottles, 5 mL gas samples were taken and replaced by 5 mL synthetic air of known CO_2 concentration. The CO_2 levels of these gas samples were measured with a portable IRGA (EGM-4, PP Systems, USA). After 24 h of incubation at the respective temperatures, a second gas collection and CO_2 measurement was performed. Then, the screw cap vials were closed, frozen in liquid N, and stored in a −80 °C freezer.

DNA was extracted from both soil aliquots using a DNA extraction kit (FastDNA™ SPIN Kit for Soil, MP Biomedicals, Germany). The concentration of dsDNA was measured fluorimetrically according to the PicoGreen® Assay (Quant-iT™ PicoGreen® dsDNA Reagent, Thermo Fisher, Germany). For determination of the ^{18}O:^{16}O ratio of DNA, 50 μL of the DNA extracts were pipetted into silver capsules and dried at 60 °C for 48 h to remove water. The ^{18}O abundance and total oxygen content in the DNA aliquots were measured by a Thermochemical Elemental Analyzer (TC/EA, Thermo Fisher) coupled to an IRMS (Delta V Advantage, Thermo Fisher, Germany). Then, microbial growth rates (expressed as μg carbon per gram soil dry weight per hour) were calculated as follows:

$$Growth = \frac{DNA_p \times MBC}{DNA \times DW \times t} \quad (3)$$

where MBC (μg g^{-1} DW) is the microbial biomass C, DNA (μg) is the soil DNA concentration, DW (g) is the soil dry weight, t (h) is the incubation period, and DNA_p is the DNA produced during the incubation period. The latter was calculated as the difference in ^{18}O abundance between the labeled samples and the natural abundance controls using a factor of 31.21, which represents the proportional mass of oxygen in an average DNA molecule (Zheng et al., 2019). Microbial respiration (CO_2, expressed as μg C per gram soil dry weight per hour) was calculated based on the ideal gas equation:

$$CO_2 = \frac{conc._{dCO2} \times p \times molecular\ weight_C \times V_{headspace}}{DW \times t \times R \times T} \quad (4)$$

where conc. $_{dCO2}$ (ppm) is the difference of the CO_2 concentration between the beginning and the end of the incubation, while p is the air pressure in kPa, molar weight in g mol^{-1},R is the universal gas constant in J mol^{-1} K^{-1}, T in kelvin, and $V_{headspace}$ in liter. The microbial turnover rate (in d) and microbial CUE were calculated as follows:

$$Microbial\ turnover = \frac{DNA}{DNA_p \times \frac{24}{t}} \quad (5)$$

$$CUE = \frac{Growth}{C_{uptake}} = \frac{Growth}{Growth + CO_2} \quad (6)$$

We also calculated microbial biomass-specific growth (qGrowth) and respiration (qCO_2) by dividing microbial growth and respiration by MBC.

2.4. Soil N cycling and microbial NUE

^{15}N isotope pool dilution (IPD) assays were applied to determine gross rates of protein depolymerization, FAA uptake, N mineralization, NH_4^+ uptake, and nitrification (Kirkham and Bartholomew, 1954; Lachouani et al., 2010; McIlvin and Altabet, 2005; Noll et al., 2019; Wanek et al., 2010; Wild et al., 2013; Zhang et al., 2019). Briefly, 200 μL ^{15}N-labeled algal FAA mix (20 amino acids, Cambridge Isotope Laboratories), $^{15}NH_4Cl$, or $K^{15}NO_3$ tracers (10 atom% ^{15}N) were added to 4 g duplicate fresh soil samples. The amount of tracers that we added was approximately 20% of the target pool sizes based on the previously measured background FAA, NH_4^+, and NO_3^- contents. The samples were incubated at their corresponding *in-situ* temperatures. Incubations were terminated by adding 4 °C 1 M KCl (1:7.5 (w:v)) after 15 min (t_1) and 45 min (t_2) for the measurements of protein depolymerization and FAA uptake, and by adding room temperature 1 M KCl (1:7.5 (w:v)) after 4 h (t_1) and 24 h (t_2) to the samples for mineralization, NH_4^+ uptake, and nitrification determinations. Then, the extracts were filtered after 30 min of shaking and frozen at −20 °C until further processing.

For protein depolymerization, FAA uptake, N mineralization, and NH_4^+ uptake, we firstly used the micro-diffusion method to remove NH_4^+ from the extracts while recovering NH_4^+ in the acid traps (Brooks et al., 1989; Zhang et al., 2015b). Then, the conversion of FAA to N_2O was done as described in Noll et al. (2019), and the conversions of NH_4^+ and NO_3^- to N_2O were performed as described by Zhang et al. (2019). After the reactions were terminated, the N_2O concentrations and ^{15}N:^{14}N ratios of all samples were measured by a purge-and-trap IRMS (PT-IRMS), consisting of a Gasbench II headspace analyzer with a cryofocusing unit coupled to a Finnigan Delta V Advantage IRMS (all Thermo Fisher, Germany) (Lachouani et al., 2010). We calculated the gross rates of production and uptake (of FAA, NH_4^+, and NO_3^-; expressed as μg N per gram soil dry weight per day) according to the following two equations (Kirkham and Bartholomew, 1954):

$$Gross\ production = \frac{N_2 - N_1}{t_2 - t_1} \times \frac{\ln\left(\frac{APE_1}{APE_2}\right)}{\ln\left(\frac{N_2}{N_1}\right)} \quad (7)$$

$$Gross\ uptake = \frac{N_1 - N_2}{t_2 - t_1} \times \left[1 + \frac{\ln\left(\frac{APE_2}{APE_1}\right)}{\ln\left(\frac{N_2}{N_1}\right)}\right] \quad (8)$$

where t_1 and t_2 are the termination time points 1 and 2, N_1 and N_2 are the corresponding concentrations (μg g^{-1} DW) of FAA, NH_4^+, and NO_3^- at t_1 and t_2, and APE_1 and APE_2 (at% ^{15}N) are the differences in the at% ^{15}N values of FAA, NH_4^+, and NO_3^- between the natural abundance ^{15}N content and the ^{15}N content measured in the samples at the two-time points. Microbial NUE was estimated as follows:

$$NUE = \frac{N_{growth}}{N_{uptake}} = \frac{N_{growth}}{N_{growth} + M_N} \quad (9)$$

where M_N is the gross rate of N mineralization, and N_{growth} was calculated by dividing C-based microbial growth by the respective microbial C:N ratio (Zhang et al., 2019).

2.5. Soil extracellular enzyme activities

The potential activities of four hydrolytic enzymes, i.e., β-glucosidase (BG), N-acetylglucosaminidase (NAG), leucine-aminopeptidase (LAP), and acid phosphatase (AP), were measured using the substrate addition method (Kaiser et al., 2010). For each sample, we prepared soil slurries by mixing and ultrasonicating (energy input = 350J ± 20J) fresh soils with 50 mM sodium acetate buffer (pH = 5.5, 1:100 (w:v)). Then, the soil slurries were transferred to black microtiter-plates (12 columns, 8 rows), one sample per column (i.e., eight spots per sample). For the first three rows of each column, we mixed soil slurries with their corresponding substrates (i.e., 4-methylumbelliferyl-β-D-glucopyranoside for BG, 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide for NAG, L-leucine-7-amido-4-methylcoumarin hydrochloride for LAP, and 4-methylumbelliferyl-phosphate for AP). In the last five rows of each column, we added a series of different concentrations of methylumbelliferone (MUF) as the standards for BG, NAG, and AP, and of aminomethylcoumarin (AMC) as the standard for LAP. All black microtiter-plates were then incubated at their corresponding temperatures in the dark for 3 h with repeated measurements of fluorescence yield every 30 min with a TECAN Infinite® M200 spectrophotometer to measure the concentrations of released MUF or AMC, at an excitation wavelength of 365 and an emission wavelength of 450 nm. We calculated the potential enzyme activities as the difference of released MUF or AMC during the measuring period, and calibrated the potential activities by the sample-specific standards, which corrects for potential quenching effects.

Enzyme vector analysis was performed based on the measured potential activities of four hydrolytic enzymes as described by Moorhead et al. (2016). Enzyme vector length and enzyme vector angle were calculated as follows:

$$Enzyme\ vector\ length = SQRT\left(x^2 + y^2\right) \quad (10)$$

$$Enzyme\ vector\ angle = DEGREE[ATAN\ 2(x, y)] \quad (11)$$

where x is the proportional activity of the C- versus P-acquiring enzymes (BG/(BG + AP)), and y is the proportional activity of the C- versus N-acquiring enzymes (BG/(BG + NAG + LAP)).

2.6. Data analysis

All statistical analyses were performed in R 3.6.3 (RC Team, 2021). We used linear mixed-effects models (lme4 package) with warming, soil depth, and season as fixed factors, and block and warming duration as random factors, to check the individual effects and interactions for all measured parameters (Bates et al., 2014). Moreover, we assessed multiple comparisons for season using Tukey post hoc tests (Hothorn et al., 2016). Besides, Pearson coefficient correlation analysis was performed to investigate possible univariate regressions. Data were log- or sqrt-transformed prior to the analyses mentioned above to meet the assumptions of homoscedasticity and normal distribution. We assessed homoscedasticity via the Bartlett test, and normal distribution via diagnostic plots. The significance threshold was set to 0.05 for all analyses.

3. Results

3.1. Basic soil and root parameters

Warmed soils were characterized by significantly lower soil pH and SWC (Table 1; Fig. 1). Warming increased soil $\delta^{13}C$, but soil $\delta^{15}N$ and soil C:N ratios remained unaltered by the warming treatment (Table 1; Fig. 1). Regarding fine roots, warming increased root $\delta^{15}N$, but had no effects on root $\delta^{13}C$ and root C:N ratios (Table 1; Fig. 1).

Table 1
Results of mixed-effects models showing the effects of warming (W) and its interactions with soil depth (D) and/or season (S) on basic soil and root parameters. Warming, soil depth, and season were fixed factors, while block and warming duration were inserted as random factors. Data presented are means ± standard errors for treatments, averaged across soil depths and seasons. Significance levels are represented as asterisks: *p < 0.05; **p < 0.01; ***p < 0.001; - not significant. Prior to applying mixed-effects models, soil C:N, DOC (dissolved organic C), FAA (free amino acid) were log-transformed, and NH_4^+ and NO_3^- were sqrt-transformed. Other abbreviations: SWC (soil water content), SOC (soil organic C), MBC (microbial biomass C), TN (soil total N), MBN (microbial biomass N), TDN (total dissolved N), DON (dissolved organic N), and d.s. (dry soil).

Parameter		Unit	Treatment						Significance Level			
			Warming			Control			W	W x D	W x S	W x D x S
Soil	pH		6.63	±	0.05	7.07	±	0.04	***	–	–	–
	SWC	g H_2O g d.s.$^{-1}$	0.67	±	0.03	0.82	±	0.04	***	–	–	–
	Soil $\delta^{13}C$		−26.0	±	0.08	−26.2	±	0.07	**	–	–	–
	SOC	%	8.62	±	0.61	9.68	±	0.55	**	–	–	–
	DOC	μg C g d.s.$^{-1}$	113	±	16.8	76.1	±	5.87	–	–	***	–
	MBC	μg C g d.s.$^{-1}$	1734	±	134	2221	±	158	***	–	–	*
	Soil $\delta^{15}N$		0.68	±	0.17	0.59	±	0.16	–	–	–	–
	TN	%	0.58	±	0.04	0.66	±	0.04	**	–	–	–
	TDN	μg N g d.s.$^{-1}$	27.4	±	2.02	29.1	±	1.71	–	*	–	–
	DON	μg N g d.s.$^{-1}$	8.87	±	1.29	7.98	±	1.28	–	**	–	*
	NH_4^+	μg N g d.s.$^{-1}$	2.68	±	0.28	1.93	±	0.16	**	–	–	–
	NO_3^-	μg N g d.s.$^{-1}$	16.0	±	1.10	19.0	±	1.69	*	–	–	–
	FAA	μg N g d.s.$^{-1}$	3.83	±	0.26	3.46	±	0.31	**	–	***	–
	MBN	μg N g d.s.$^{-1}$	325	±	27.8	393	±	27.6	**	–	–	–
	Soil C:N		14.7	±	0.25	14.8	±	0.27	–	*	–	***
	Microbial C:N		5.58	±	0.19	5.70	±	0.16	–	–	*	–
Root	Root C:N		54.5	±	1.90	56.6	±	2.04	–	–	–	–
	Root $\delta^{13}C$		−27.8	±	0.17	−27.7	±	0.21	–	–	–	–
	Root $\delta^{15}N$		−3.44	±	0.19	−4.54	±	0.18	***	–	–	–

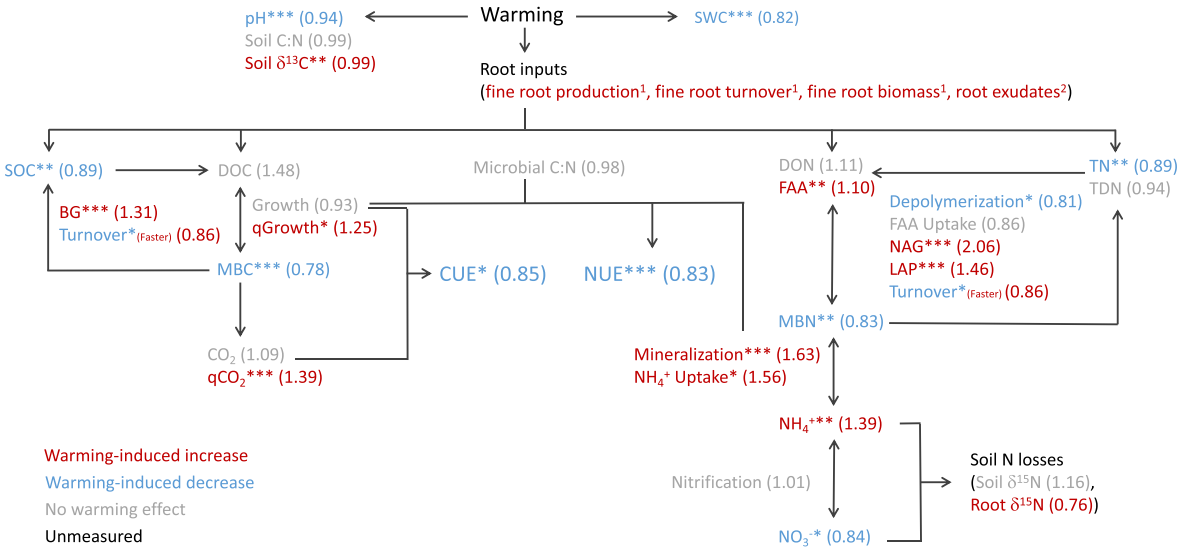

Fig. 1. Schematic illustration showing long-term warming effects on belowground C and N cycling. Red, blue, and grey texts indicate long-term warming increased, decreased, or did not affect parameters, respectively. Black text indicates unmeasured parameters. Asterisks show the significance level (*p < 0.05; **p < 0.01; ***p < 0.001) of warming effects based on the results of the linear mixed-effects models. The values in brackets after each parameter indicate the response ratio, which was calculated by dividing the value of the warming treatment by the value of the control treatment. Fine root data are from studies that have been performed at the same site: [1]Kengdo et al., 2022; [2]Heinzle et al. (unpublished data). Abbreviations: SWC (soil water content), TN (soil total nitrogen), SOC (soil organic carbon), BG (β-glucosidase), NAG (N-acetylglucosaminidase), LAP (leucine-aminopeptidase), AP (acid phosphatase), DOC (dissolved organic C), MBC (microbial biomass C), TDN (total dissolved N), MBN (microbial biomass N), CO_2 (microbial respiration), CUE (microbial C use efficiency), qGrowth (microbial biomass specific growth), qCO_2 (microbial biomass specific respiration), FAA (free amino acids), DON (dissolved organic N), protein depoly. (protein depolymerization), and NUE (microbial N use efficiency).

3.2. Warming effects on carbon cycling pools and processes

Soil organic C content (in %) was lower (−11%) in the warming treatment (Table 1; Figs. 1 and 2). Dissolved organic C was 48% higher in warmed soil but the increase was not statistically significant (Table 1; Fig. 1). Warming reduced MBC (−22%), but accelerated the microbial turnover, i.e., turnover time decreased by 14% (Tables 1 and 2; Figs. 1 and 2). Warming increased the potential activity of BG (+31%; Table 2; Figs. 1 and 2). Normalized to per unit of dry soil, both microbial respiration and growth were not different between treatments (Table 2; Figs. 1 and 2). However, qGrowth (+25%) and qCO_2 (+39%) were higher in warmed soils than in control soils (Table 2; Figs. 1 and 2). Microbial CUE decreased in warmed soils by 15% relative to controls (Table 2; Figs. 1 and 2).

3.3. Warming effects on nitrogen cycling pools and processes

Regarding N pools, warming had positive effects on FAA (+10%) and

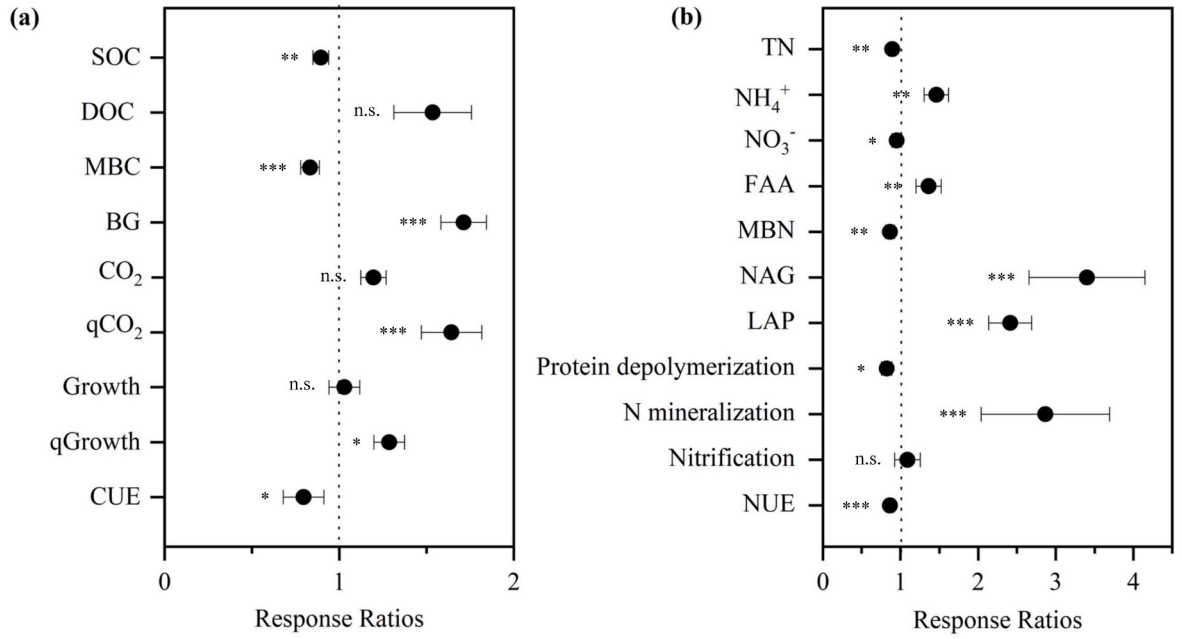

Fig. 2. Response ratios of warming effects on soil C (a) and N (b) pools and processes. Response ratios were calculated by dividing the values from the warming treatment by the values from the correspondingly paired control samples (applying no ln or log transformation). Black dots indicate the mean values of response ratios, and error bars show standard errors. Asterisks and n. s. show the significance level (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; n. s. not significant) of warming effects based on the results of the linear mixed-effects models. Abbreviations: SOC (soil organic carbon), DOC (dissolved organic C), MBC (microbial biomass C), BG (β-glucosidase), CO_2 (microbial respiration), qCO_2 (microbial biomass specific respiration), qGrowth (microbial biomass specific growth), CUE (microbial C use efficiency), TN (soil total nitrogen), FAA (free amino acid), NAG (N-acetylglucosaminidase), LAP (leucine-aminopeptidase), and NUE (microbial N use efficiency).

Table 2
Results of mixed-effects models showing the effects of warming (W) and its interactions with soil depth (D) and season (S) on soil C and N processes. Mean values ± SE are shown for warmed and control soils. Warming, soil depth, and season were fixed factors while block and warming duration were inserted as random factors. Data presented are means ± standard error averaged across soil depths and seasons. Significance levels are represented as asterisks: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; - not significant. Prior to applying mixed-effects models, BG (β-glucosidase), NAG (N-acetylglucosaminidase), LAP (leucine-aminopeptidase), AP (acid phosphatase), turnover, uptake, FAA uptake, mineralization, and NH_4^+ uptake were log-transformed, and CO_2 (respiration) and protein depolymerization were sqrt-transformed. Other abbreviations: qCO_2, qGrowth, CUE, NUE, and d.s. Are microbial biomass specific respiration, microbial biomass specific growth, microbial C use efficiency, microbial N use efficiency, and dry soil, respectively.

Parameter	Unit	Treatment						Significance Level			
		Warming			Control			W	W x D	W x S	W x D x S
CO_2	ng C g d.s.$^{-1}$ h^{-1}	2473	±	201	2266	±	212	–	–	–	–
qCO_2	μg C mg MBC^{-1} d^{-1}	36.3	±	2.30	26.2	±	2.04	***	–	–	–
Growth	ng C g d.s.$^{-1}$ h^{-1}	747	±	64.1	803	±	74.4	–	–	–	–
qGrowth	μg C mg MBC^{-1} d^{-1}	10.8	±	0.60	8.68	±	0.55	*	–	–	–
C uptake	ng C g d.s.$^{-1}$ h^{-1}	3184	±	249	3068	±	266	–	–	–	–
Microbial turnover	d	103	±	6.91	119	±	6.38	*	–	–	–
Microbial CUE		0.22	±	0.01	0.26	±	0.02	*	–	–	–
Protein depolymerization	μg N g d.s.$^{-1}$ d^{-1}	223	±	24.1	277	±	27.1	*	–	–	–
FAA uptake	μg N g d.s.$^{-1}$ d^{-1}	243	±	28.2	284	±	33.0	–	–	–	–
N mineralization	μg N g d.s.$^{-1}$ d^{-1}	2.91	±	0.58	1.79	±	0.37	***	–	*	–
NH_4^+ uptake	μg N g d.s.$^{-1}$ d^{-1}	3.48	±	0.60	2.23	±	0.46	*	–	–	–
Nitrification	μg N g d.s.$^{-1}$ d^{-1}	1.58	±	0.22	1.57	±	0.30	–	–	–	–
Microbial NUE		0.54	±	0.03	0.66	±	0.03	***	–	–	–
BG	nmol g d.s.$^{-1}$ h^{-1}	104	±	10.3	79.1	±	14.9	***	–	**	–
NAG	nmol g d.s.$^{-1}$ h^{-1}	152	±	24.7	74.0	±	13.7	***	–	–	–
LAP	nmol g d.s.$^{-1}$ h^{-1}	25.4	±	3.38	17.4	±	3.86	***	–	***	–
AP	nmol g d.s.$^{-1}$ h^{-1}	590	±	54.2	474	±	72.6	**	–	*	–
Enzyme vector length		0.45	±	0.01	0.50	±	0.02	*	–	–	–
Enzyme vector angle		67.9	±	1.54	73.9	±	0.81	***	–	–	–

NH_4^+(+39%), negative effects on soil TN (−11%) and NO_3^- (−16%), and no effects on TDN and DON (Table 1; Figs. 1 and 2). Warming reduced MBN (−17%) to a similar extent as MBC, leaving microbial C:N ratios unaffected between warming and control treatments (Table 1; Figs. 1 and 2). Elevated temperatures notably increased N-related extracellular enzyme activities (EEAs; Table 2; Figs. 1 and 2). The potential activity of LAP was 46% higher and that of NAG 106% higher in warmed compared to control soils. On the other hand, the response of soil warming on gross N transformation rates differed among processes. Warmed soils showed lower gross rates of protein depolymerization than control soils (−19%), while the gross rates of FAA uptake decreased by 14% by elevated temperature (although not statistically significant; Table 2; Figs. 1 and 2). Warming accelerated both gross rates of N mineralization (+63%) and NH_4^+ uptake (56%; Table 2; Figs. 1 and 2). In contrast, gross rates of

nitrification were comparable between warming and control treatments (Table 2; Figs. 1 and 2). In line with microbial CUE, warming triggered a decrease in microbial NUE (−17%; Table 2; Figs. 1 and 2).

3.4. Warming effects on soil enzyme allocation

Enzyme vector analysis was used to assess the relative C to nutrient limitations of the soil communities. Long-term soil warming decreased enzyme vector length (Table 2), indicating a more intensive limitation by N and/or P relative to C in warmed soils. Regarding enzyme vector angles, both warmed and control soils had very high values (Table 2), with higher values in control than warmed soils. The average angle was 71°, implying a considerable phosphorus (P) limitation relative to N limitation at this site.

4. Discussion

4.1. Warming effects on soil C cycling processes

Contrary to our first hypothesis, microbial respiratory CO_2 efflux (per g soil dry mass) from warmed soil was insignificantly (by ~10%; Table 2; Figs. 1 and 2) higher than from control soil. This small difference was surprising, as the total soil CO_2 efflux in the field was about ~40% higher during the soil sampling campaigns (Schindlbacher, personal communication). The small difference further does not align with previously conducted laboratory incubations, during which the soil CO_2 efflux increased to a similar magnitude as in the field (Schindlbacher et al., 2015a). In this previous Achenkirch study, Q_{10} values of microbial respiration were 2.5 in warmed and control soils, soil respiration therefore roughly increasing by 50% for a 4 °C increase in temperature, but MBC was unaffected at that time (9 years soil warming). It cannot be excluded that the soil treatment (sieving, storage) and the small amount of soil used for the incubation (0.4 g) plus the comparable long CO_2 accumulation times (24 h) had affected the CO_2 efflux estimates. However, the bias would have been similar for warmed and control soil, not affecting the relative differences. Accordingly, the similar respiration rates from warmed and control plots would, for the first time in this long-term experiment, argue that the warming effect on microbial respiration decreased over time. A simultaneously performed study on fine root biomass in the warmed soil adds evidence to such a decline. Kengdo et al. (2022, 2023) observed 17% higher fine root biomass stocks and faster fine root turnover in the warmed plots than in control plots, implying a shift towards greater contributions of autotrophic compared to heterotrophic respiration to soil CO_2 efflux. The increase in fine root activity in the warmed soil might therefore explain a larger fraction of the warming effect on the field soil CO_2 efflux, especially if root respiration is more sensitive to soil warming than heterotrophic respiration (Boone et al., 1998).

The mechanisms for declining respiration rates in warmed soil could be gradual substrate depletion and/or related changes in the microbial community structure after long-term warming (Kirschbaum, 2004; Melillo et al., 2017). With regard to substrate availability, we assessed DOC concentrations as well as SOC contents of the samples. DOC concentrations were higher (but not statistically significant) while the SOC content was significantly lower in the warmed soil (Table 1; Figs. 1 and 2). The sustained DOC levels could be owing to an accelerated microbial turnover rate and/or to higher potential activities of extracellular enzymes mining SOC in the warming treatment (Bhatia et al., 2002; Zuccarini et al., 2020). However, considering the soil-only warming approach in this experiment (due to technical and financial constraints in mature forest ecosystems), plant C inputs are unlikely to have increased as would be expected in soil + canopy warming experiments, especially from aboveground litter inputs, eventually causing reduced SOC contents. The significantly lower SOC contents, however, are a hint towards increasing substrate depletion, but it has to be noted that the SOC contents still remained generally high in this carbonate soil (9.7% for control soils and 8.6% for warmed soils), indicating that there is still sufficient SOM left for decomposition and microbial assimilation. Regarding microbial related changes, long-term warming significantly reduced MBC (−22%; Table 1; Figs. 1 and 2) in all three seasons, dampening any warming induced stimulation of gross microbial respiration significantly. Sustained microbial growth (on a soil dry mass basis) in combination with reduced microbial CUE and accelerated microbial turnover rates can lead to such a medium-term reduction in microbial biomass. Besides, Liu et al. (2017), who performed their experiment at the same site in 2012, observed changes in microbial functions based on meta-proteomics analyses, e.g., an increased abundance of proteins linked to microbial energy production. We speculate that such functional changes may develop over time, along with decreased MBC and microbial respiration and increased soil C losses in the warming treatment (Walker et al., 2018). Moreover, long-term soil warming changed the community composition of ectomycorrhizal fungi and of root-associated bacterial diversity (Kwatcho Kengdo et al., 2022), with great potential to mediate some of the observed changes in microbial functions, yet hard to directly link.

When normalized to microbial biomass, both C-related process rates, i.e., qGrowth and qCO_2, were faster in warmed soils (Table 2; Figs. 1 and 2). Warming-increased qGrowth and qCO_2 and accelerated microbial turnover rates highlight a faster soil C cycle with a more active microbial community in warmed soils (Fuchslueger et al., 2019). Moreover, due to stronger warming effects on qCO_2 than qGrowth, warming significantly reduced microbial CUE (Table 2; Figs. 1 and 2). This result accords with our second hypothesis and theoretical considerations as higher soil temperatures promote microbial activity, which increases microbial energy consumption by inflating the costs of protein and lipid turnover, membrane charge maintenance, etc. (Manzoni et al., 2012; Pold et al., 2017; Sinsabaugh et al., 2013). Therefore, soil microbes allocate more C to respiration to provide energy rather than investing into growth, causing a reduction in microbial CUE. Moreover, according to the results of enzyme vector analysis, control soils had a longer enzyme vector length than warmed soils (Table 2), indicating that C is a more limiting factor than nutrients in the control treatment compared to the warming treatment (Moorhead et al., 2016). This aligns well with the results of a substrate addition experiment performed at our site in the same year (Shi et al., 2023), which showed a shift of microbial limitation from being predominantly C limited in the control treatment to become CP co-limited in the warming treatment. Thus, microbes likely allocated more C (energy) to acquire increasingly restricted nutrients in warmed soils than in control soils, further resulting in a lower microbial CUE.

Overall, our results indicate that long-term soil warming accelerated microbial C cycling, but that the reduced microbial CUE and faster turnover caused a decline in microbial biomass and thereby reduced the warming effect on microbial respiration in the long-term, causing an apparent acclimation response. Therefore, it appears that, the first time after 14 years of intensive warming, there is evidence that heterotrophic respiration had “adapted” to the warmer conditions.

4.2. Warming effects on soil N cycling processes

Our results only partly confirmed our third hypothesis that long-term soil warming would accelerate gross N transformation and uptake rates. While we found a significant increase in gross mineralization and NH_4^+ uptake rates, gross nitrification rates were not affected by warming, and gross protein depolymerization rates were lower in the warming treatment than in controls (Table 2; Figs. 1 and 2).

The latter finding of reduced protein depolymerization rates contrasts with the theoretical expectation that warming should increase the breakdown of N-bearing polymers, due to higher microbial activity and higher EEA under warming (Maxwell et al., 2021; Wild et al., 2018). We found significantly higher potential activities of LAP in warmed soils (Table 2; Figs. 1 and 2), which fitted our hypothesis, but they were inconsistent with reduced gross protein depolymerization rates. This

discrepancy is most likely related to the fact that LAP activities are determined as potential rates while gross protein depolymerization reflects actual rates. Thus, while based on LAP activities, protein depolymerization potentially should have been higher through upregulation, they were actually lower, most likely due to decreased SWC and proteolytic substrate limitation. N-related processes are more strongly controlled by water availability compared to soil C cycling (Fuchslueger et al., 2019). Warming concurrently decreased SWC at our site (Table 1 and Fig. 1). Lower SWC can not only impair the *in situ* activities of extracellular hydrolytic enzymes, e.g., LAP, but also hinder substrate diffusion, reducing protein depolymerization (Fig. 3a). Besides, long-term warming decreased TN contents (Fig. 1; Fig. 2). So far, only two other warming studies have measured gross protein depolymerization (Maxwell et al., 2021; Wild et al., 2018). Maxwell et al. (2021) reported inconsistent warming effects on protein depolymerization across seasons, due to seasonal changes in N substrate availability and temperature. Wild et al. (2018), on the other hand, found no warming effect on protein depolymerization, probably because of the small temperature difference between warmed and ambient treatments (0.4 °C) in their field study. Besides, after 8 years of soil warming, unlike in our study, soil TN concentrations remained the same among their treatments, providing an ample substrate supply to sustain protein depolymerization. Moreover, Tian et al. (2023) reported higher total and crystalline iron (Fe) oxide contents in the warmed soil of the Achenkirch experiment. Higher Fe oxide contents increase the soils' sorption strength for organic N, and therefore further reduce substrate availability for protein depolymerization (Gu et al., 1994; Noll et al., 2022; Sowers et al., 2019), resulting in decreased protein depolymerization rates in the warming treatment.

Contrarily, and consistent with our hypothesis, warming accelerated the gross rates of N mineralization (Table 2; Figs. 1 and 2). While lower protein depolymerization should have reduced substrate supply for N mineralization, we found no decrease in DON and even a slight, but significant, increase in FAA in warmed soils (Table 1; Figs. 1 and 2). This may have been caused by the simultaneously decreased (not statistically significant) gross FAA uptake (Table 2; Fig. 1), which could have led to FAA accumulation in warmed soil. Moreover, more than doubled potential activities of NAG in the warming treatment than in the control treatment can potentially increase the amino sugar release from bacterial and fungal necromass in warmed soils, fueling N mineralization processes. Regarding the potential effects of warming reduced soil moisture, we did not observe a significant correlation between SWC and gross N mineralization rate (log-transformed). These results align with Zhang et al. (2019) where they reported that increasing soil moisture from 30 to 60% WHC had no stimulatory effect on gross N mineralization. Owing to the temperate moist climate at our site, all soil samples across the three seasons sustained sufficient soil moisture, ranging from 33 to 57% of soil water holding capacity (WHC), i.e., no severe drought situation nor sub- nor anoxic conditions were recognizable. Hence, owing to the sustained substrate supply without recognizable soil moisture stress, elevated soil temperatures facilitated microbial metabolism and enzymatic activities, thereby stimulating the soil N mineralization process (Björsne et al., 2014; Larsen et al., 2011).

Regarding gross nitrification, there was no significant difference between the warming treatment and controls (Table 2; Figs. 1 and 2). This was surprising, especially considering the increase in concentrations and supply rates of soil NH_4^+ caused by increased gross N mineralization rates, which should alleviate the common substrate limitation of nitrifiers (Song et al., 2021). Further, optimal temperatures for nitrification are around 25–30 °C (Saad and Conrad, 1993), which is higher than the soil temperatures in the warming treatment during all three seasons (14, 17, and 14 °C) at our site. Thus, warming should potentially promote nitrification during all three experimental seasons, as it rose soil temperatures closer to the optimum. However, nitrification can also be regulated by other abiotic factors, such as soil moisture and soil pH. We observed a positive correlation between SWC and gross nitrification (log-transformed; Fig. 3c). Warming induced reductions in SWC can hinder substrate accessibility, especially considering that NH_4^+ is not very mobile in most soils due to its positive charge, thereby counteracting the temperature induced stimulation of biochemical prcesses. Besides, nitrifiers are sensitive to acidic conditions (Gieseke et al., 2006). At our site, the soil pH in the warming treatment was significantly lower than in controls (Table 1; Fig. 1), though still in the pH-neutral range where pH changes are not expected to affect nitrifiers. Alternatively, any stimulation of autotrophic nitrifiers might have been masked by negative, inverse responses by their heterotrophic counterparts, which may not respond or negatively react to increased ammonium, though this is speculative (Elrys et al., 2021; Martikainen, 2022; Zhang et al., 2023). For example, in a meta-analysis the contribution of heterotrophic nitrification to total nitrification in soils declined with increasing mean annual temperature (Zhang et al., 2023).

In line with our fourth hypothesis, microbial NUE was lower in the warming treatment (Table 2; Figs. 1 and 2). Gross N mineralization and microbial growth are critical drivers of microbial NUE (Zhang et al., 2019). At our site, increased gross N mineralization rates in combination with comparable microbial growth rates between warming and control treatments caused the lower microbial NUE in the warming treatment. Besides, a reduced microbial NUE might also result from an imbalanced soil nutrient availability (Mooshammer et al., 2014a). Based on the enzyme vector analysis, the mean value of the vector angle was 71°, which is way larger than 45°. This large vector angle implies a substantial microbial P constraint compared to N (Moorhead et al., 2016). Therefore, it is possible that N is not limiting or is even sufficient to microbes at the study site, probably due to the ongoing atmospheric inorganic N deposition (~15 kg ha^{-1} $year^{-1}$) at our study site (Heinzle et al., 2021; Herman et al., 2002). When N is not limiting, microbial NUE

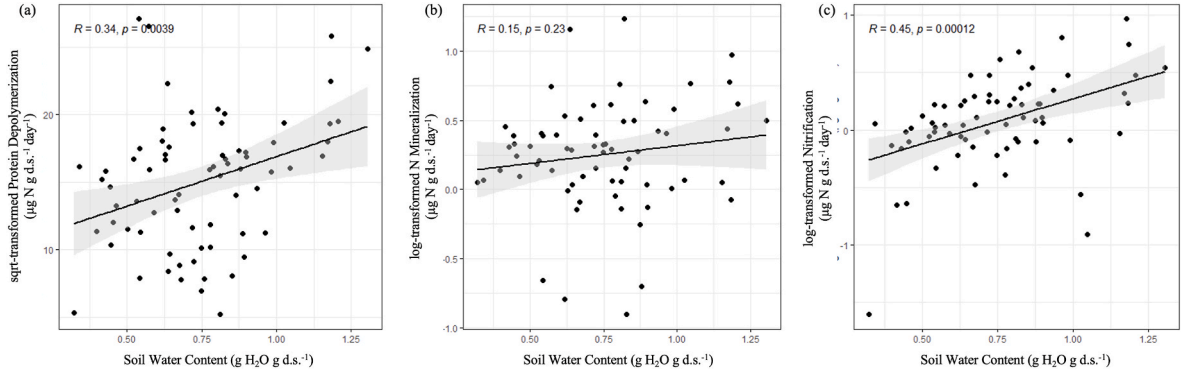

Fig. 3. Pearson correlation between soil water contents and (a) sqrt-transformed gross protein depolymerization rates, (b) log-transformed gross N mineralization rates, and (c) log-transformed gross nitrification rates. Abbreviation: d.s. (dry soil weight).

decreases with increasing N supply (Mooshammer et al., 2014a). In this study, warmed soils had a larger pool size of the substrate available for N mineralization, i.e., FAA, compared to controls. Hence, soil microbes in warmed soils likely incorporated less N into their biomass relative to the N released to the environment as NH_4^+, demonstrated by a lower MBN and a larger NH_4^+ pool in warmed soils (Fig. 1; Fig. 2), and eventually leading to a lowering of microbial NUE.

4.3. Further biogeochemical consequences for soil carbon and nitrogen cycling

Soil C and N cycles responded largely similar to long-term soil warming, in terms of the responses of anabolic processes (increased C- and N-based growth), catabolic processes (increased N mineralization and microbial respiration), soil enzymes (increased C- and N-mining soil enzymes), soil microbial biomass (decreased soil microbial biomass C and N) and in the respective element use efficiencies (decreased microbial CUE and NUE). Only nitrification (non-significantly) and protein depolymerization (inhibition by soil warming) responded in a different way. Given the corresponding responses in both biogeochemical cycles we did not find a significant decoupling of soil C and N cycling, which was mirrored in unaltered C:N stoichiometries in soil organic matter, extractable pools, microbial biomass, and roots.

The underlying causes of parallel losses of C and N from the system are reflected in decreased CUE and NUE. Warming-induced decreases in microbial CUE and NUE (both decreased by ~16%) indicate that less organic C and N were utilized by microbes for their growth (Manzoni et al., 2012; Mooshammer et al., 2014a; Sinsabaugh et al., 2016). In turn, more C and N were diverted to catabolic processes, which intermittently decouples C and N cycling by cutting the C–N bonds in organic matter. During microbial catabolic processes, organic C is converted to CO_2, which is released via soil CO_2 efflux to the atmosphere (Allison et al., 2010; Manzoni et al., 2012). Nitrogen catabolism converts organic N into NH_4^+, which shows very little mobility in soils and therefore remains in the soil system, or further to NO_3^-, which is prone to partial hydrological or gaseous loss pathways (Bai et al., 2013; Dijkstra et al., 2012; Heinzle et al., 2023; Mooshammer et al., 2014a; Rustad et al., 2001). Depending on the extent of inorganic N losses versus inorganic N recycling, the soil C and N cycles vary in their decoupling. Notably, in the Achenkirch experiment, long-term warming caused soil C and N losses at similar magnitudes, as demonstrated by comparable soil, microbial, and fine root C:N ratios between treatments (Table 1; Fig. 1). This highlights that a large fraction of mineralized N left the soil system via NO_3^- leaching or gaseous emissions, the losses thereby following the stoichiometric ratios of C and N catabolic processes and the C:N ratios of microbial resources.

Stoichiometric controls on microbial CUE and NUE would trigger increases in CUE and decreases in NUE with decreasing resource C:N, i.e. CUE and NUE would respond inversely to an environmental trigger that causes increases in N availability (Mooshammer et al., 2014a, 2014b). Increased N availability in warmed soils would increase CUE due to increasing C limitation, but decrease NUE due to N becoming in excess. This clearly does not match the pattern that we observed, with increased soil N losses, but concurrent decreases in microbial CUE and NUE, but hints at an underlying stronger effector masking these C:N stoichiometric effects. Enzyme vector analysis – with decreased vector length - indicated a decrease in microbial C limitation and an increase in overall nutrient limitation, the rising nutrients constraints being largely driven by increased P limitation (indicated by an averaged vector angle >70°). Both trends are corroborated by recent publications on the Achenkirch experiment, showing decreasing C constraints and increasing microbial P (co)limitation based on in-depth P process analysis and the analysis of growth stimulation with substrate amendments (Shi et al., 2023; Tian et al., 2023). The increasing P constraints likely triggered decreases in microbial CUE and NUE by limiting anabolic processes and microbial growth, promoting pervasive losses of C and N from the soil compartments.

In conclusion, our results provide empirical evidence that long-term soil warming generally accelerated the soil C cycle while having contrasting effects on soil N cycling processes. Total soil C and N as well as microbial biomass uniformly decreased after 14 years of warming. Labile C and N pools were sustained or changed variously, likely due to quicker microbial turnover rates and increased C- and N-related EEAs. Moreover, according to the results of mixed-effects models, most of the measured parameters showed no interactions between warming and soil depth, warming and season, and warming, soil depth, and season (Tables 1 and 2, and Supplementary Table 1). Hence, the long-term soil warming effects on different processes were consistent across different soil depths and seasons. Given the large SOC content and enhanced root litter input, these changes may also persist for some more years. Our results suggest that SOC and TN may further decrease with ongoing soil warming, but this might be accompanied and dampened by a reduction in microbial biomass and in gross C and N process rates, eventually down-regulating soil C and N cycling processes.

Declaration of competing interest

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

Data availability

The data has been uploaded to an online depository. The link has been shared in the main text document.

Acknowledgement

This study was funded by the Austrian Science Fund - FWF (project I 3745). We sincerely thank Christian Holtermann for field site maintenance, Margarete Watzka, Sabine Maringer, Sabrina Pober, and Ludwig Seidl for technical and material support, Shasha Zhang for experimental guidance, Tania L. Maxwell for her guidance in the data analysis, and Marilena Heitger for laboratory assistance. Moreover, we acknowledge the inspirational communications and warm support from people in the Terrestrial Ecosystem Research laboratories, University of Vienna.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2023.109109.

References

Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience 3 (5), 336–340. https://doi.org/10.1038/ngeo846.

Bai, E., Li, S., Xu, W., Li, W., Dai, W., Jiang, P., 2013. A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. New Phytologist 199 (2), 441–451. https://doi.org/10.1111/nph.12252.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting Linear Mixed-Effects Models Using Lme4. *ArXiv Preprint ArXiv:1406.5823*.

Bhatia, Y., Mishra, S., Bisaria, V.S., 2002. Microbial β-glucosidases: cloning, properties, and applications. Critical Reviews in Biotechnology 22 (4), 375–407. https://doi.org/10.1080/07388550290789568.

Björsne, A.-K., Rütting, T., Ambus, P., 2014. Combined climate factors alleviate changes in gross soil nitrogen dynamics in heathlands. Biogeochemistry 120 (1), 191–201.

Blazewicz, S.J., Schwartz, E., 2011. Dynamics of 18O incorporation from H218O into soil microbial DNA. Microbial Ecology 61 (4), 911–916. https://doi.org/10.1007/s00248-011-9826-7.

Boone, R.D., Nadelhoffer, K.J., Canary, J.D., Kaye, J.P., 1998. Roots exert a strong influence on the temperature sensitivityof soil respiration. Nature 396 (6711), 570–572.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17 (6), 837–842.

Brooks, P.D., Stark, J.M., McInteer, B.B., Preston, T., 1989. Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Science Society of America Journal 53 (6), 1707–1711.

Carey, J.C., Tang, J., Templer, P.H., Kroeger, K.D., Crowther, T.W., Burton, A.J., Dukes, J.S., Emmett, B., Frey, S.D., Heskel, M.A., Jiang, L., Machmuller, M.B., Mohan, J., Panetta, A.M., Reich, P.B., Reinschj, S., Wang, X., Allison, S.D., Bamminger, C., et al., 2016. Temperature response of soil respiration largely unaltered with experimental warming. Proceedings of the National Academy of Sciences of the United States of America 113 (48), 13797–13802. https://doi.org/10.1073/pnas.1605365113.

Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81 (9), 2359–2365.

Cavaleri, M.A., Reed, S.C., Smith, W.K., Wood, T.E., 2015. Urgent need for warming experiments in tropical forests. Global Change Biology 21 (6), 2111–2121. https://doi.org/10.1111/gcb.12860.

Chung, H., Muraoka, H., Nakamura, M., Han, S., Muller, O., Son, Y., 2013. Experimental warming studies on tree species and forest ecosystems: a literature review. Journal of Plant Research 126 (4), 447–460. https://doi.org/10.1007/s10265-013-0565-3.

Dai, Z., Yu, M., Chen, H., Zhao, H., Huang, Y., Su, W., Xia, F., Chang, S.X., Brookes, P.C., Dahlgren, R.A., Xu, J., 2020. Elevated temperature shifts soil N cycling from microbial immobilization to enhanced mineralization, nitrification and denitrification across global terrestrial ecosystems. Global Change Biology 26 (9), 5267–5276. https://doi.org/10.1111/gcb.15211.

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

Dijkstra, F.A., Pendall, E., Morgan, J.A., Blumenthal, D.M., Carrillo, Y., Lecain, D.R., Follett, R.F., Williams, D.G., 2012. Climate change alters stoichiometry of phosphorus and nitrogen in a semiarid grassland. New Phytologist 196 (3), 807–815. https://doi.org/10.1111/j.1469-8137.2012.04349.x.

Dorrepaal, E., Toet, S., Van Logtestijn, R.S.P., Swart, E., Van De Weg, M.J., Callaghan, T. V., Aerts, R., 2009. Carbon respiration from subsurface peat accelerated by climate warming in the subarctic. Nature 460 (7255), 616–619.

Elrys, A.S., Wang, J., Metwally, M.A.S., Cheng, Y., Zhang, J.B., Cai, Z.C., Chang, S.X., Müller, C., 2021. Global gross nitrification rates are dominantly driven by soil carbon-to-nitrogen stoichiometry and total nitrogen. Global Change Biology 27 (24), 6512–6524. https://doi.org/10.1111/gcb.15883.

Fanin, N., Mooshammer, M., Sauvadet, M., Meng, C., Alvarez, G., Bernard, L., Bertrand, I., Blagodatskaya, E., Bon, L., Fontaine, S., Niu, S., Lashermes, G., Maxwell, T.L., Weintraub, M.N., Wingate, L., Moorhead, D., Nottingham, A.T., 2022. Soil enzymes in response to climate warming: mechanisms and feedbacks. Functional Ecology 36 (6), 1378–1395. https://doi.org/10.1111/1365-2435.14027.

FAO, 2015. World Reference Base for Soil Resources 2014 International Soil Classification System.

Fuchslueger, L., Wild, B., Mooshammer, M., Takriti, M., Kienzl, S., Knoltsch, A., Hofhansl, F., Bahn, M., Richter, A., 2019. Microbial carbon and nitrogen cycling responses to drought and temperature in differently managed mountain grasslands. Soil Biology and Biochemistry 135 (May), 144–153. https://doi.org/10.1016/j.soilbio.2019.05.002.

García-Palacios, P., Crowther, T.W., Dacal, M., Hartley, I.P., Reinsch, S., Rinnan, R., Rousk, J., Van den Hoogen, J., Ye, J.-S., Bradford, M.A., 2021. Evidence for large microbial-mediated losses of soil carbon under anthropogenic warming. Nature Reviews Earth & Environment 2 (7), 507–517.

Gieseke, A., Tarre, S., Green, M., De Beer, D., 2006. Nitrification in a biofilm at low pH values: role of in situ microenvironments and acid tolerance. Applied and Environmental Microbiology 72 (6), 4283–4292. https://doi.org/10.1128/AEM.00241-06.

Gruber, N., Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. Nature 451 (7176), 293–296. https://doi.org/10.1038/nature06592.

Gu, B., Schmitt, J., Chen, Z., Liang, L., McCarthy, J.F., 1994. Adsorption and desorption of natural organic matter on iron oxide: mechanisms and models. Environmental Science and Technology 28 (1), 38–46. https://doi.org/10.1021/es00050a007.

Haubensak, K.A., Hart, S.C., Stark, J.M., 2002. Influences of chloroform exposure time and soil water content on C and N release in forest soils. Soil Biology and Biochemistry 34 (11), 1549–1562.

Heinzle, J., Kitzler, B., Zechmeister-Boltenstern, S., Tian, Y., Kwatcho Kengdo, S., Wanek, W., Borken, W., Schindlbacher, A., 2023. Soil CH4 and N2O response diminishes during decadal soil warming in a temperate mountain forest. Agricultural and Forest Meteorology 329 (December 2022), 109287. https://doi.org/10.1016/j.agrformet.2022.109287.

Heinzle, J., Wanek, W., Tian, Y., Kengdo, S.K., Borken, W., Schindlbacher, A., Inselsbacher, E., 2021. No effect of long-term soil warming on diffusive soil inorganic and organic nitrogen fluxes in a temperate forest soil. Soil Biology and Biochemistry 158 (April), 108261. https://doi.org/10.1016/j.soilbio.2021.108261.

Herman, F., Smidt, S., Englisch, M., Feichtinger, F., Gerzabek, M., Haberhauer, G., Jandl, R., Kalina, M., Zechmeister-Boltenstern, S., 2002. Investigations of nitrogen fluxes and pools on a limestone site in the Alps. Environmental Science and Pollution Research 9 (2), 46–52.

Hood-Nowotny, R., Umana, N.H.-N., Inselbacher, E., Oswald-Lachouani, P., Wanek, W., 2010. Alternative methods for measuring inorganic, organic, and total dissolved nitrogen in soil. Soil Science Society of America Journal 74 (3), 1018–1027.

Hothorn, T., Bretz, F., Westfall, P., Heiberger, R.M., Schuetzenmeister, A., Scheibe, S., Hothorn, M.T., 2016. Package 'multcomp.' *Simultaneous Inference In General Parametric Models*. Project for Statistical Computing, Vienna, Austria.

Jan, M.T., Roberts, P., Tonheim, S.K., Jones, D.L., 2009. Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. Soil Biology and Biochemistry 41 (11), 2272–2282.

Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.D., Tang, J., Law, B.E., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nature Geoscience 3 (5), 315–322. https://doi.org/10.1038/ngeo844.

Jenkinson, D.S., Brookes, P.C., Powlson, D.S., 2004. Measuring soil microbial biomass. Soil Biology and Biochemistry 36 (1), 5–7.

Jones, D.L., Owen, A.G., Farrar, J.F., 2002. Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. Soil Biology and Biochemistry 34 (12), 1893–1902.

Kaiser, C., Koranda, M., Kitzler, B., Fuchslueger, L., Schnecker, J., Schweiger, P., Rasche, F., Zechmeister-Boltenstern, S., Sessitsch, A., Richter, A., 2010. Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. New Phytologist 187 (3), 843–858.

Kandeler, E., Gerber, H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biology and Fertility of Soils 6 (1), 68–72.

Kirkham, D.O.N., Bartholomew, W.V., 1954. Equations for following nutrient transformations in soil, utilizing tracer data. Soil Science Society of America Journal 18 (1), 33–34.

Kirschbaum, M.U.F., 2004. Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? Global Change Biology 10 (11), 1870–1877. https://doi.org/10.1111/j.1365-2486.2004.00852.x.

Kwatcho Kengdo, S., Ahrens, B., Tian, Y., Heinzle, J., Wanek, W., Schindlbacher, A., Borken, W., 2023. Increase in carbon input by enhanced fine root turnover in a long-term warmed forest soil. The Science of the Total Environment 855, 158800.

Kwatcho Kengdo, S., Peršoh, D., Schindlbacher, A., Heinzle, J., Tian, Y., Wanek, W., Borken, W., 2022. Long-term soil warming alters fine root dynamics and morphology, and their ectomycorrhizal fungal community in a temperate forest soil. Global Change Biology. https://doi.org/10.1111/gcb.16155. March.

Lachouani, P., Frank, A.H., Wanek, W., 2010. A suite of sensitive chemical methods to determine the δ15N of ammonium, nitrate and total dissolved N in soil extracts. Rapid Communications in Mass Spectrometry 24 (24), 3615–3623.

Larsen, K.S., Andresen, L.C., Beier, C., Jonasson, S., Albert, K.R., Ambus, P.E.R., Arndal, M.F., Carter, M.S., Christensen, S., Holmstrup, M., 2011. Reduced N cycling in response to elevated CO2, warming, and drought in a Danish heathland: synthesizing results of the CLIMAITE project after two years of treatments. Global Change Biology 17 (5), 1884–1899.

Li, J., Wang, G., Mayes, M.A., Allison, S.D., Frey, S.D., Shi, Z., Hu, X.M., Luo, Y., Melillo, J.M., 2019. Reduced carbon use efficiency and increased microbial turnover with soil warming. Global Change Biology 25 (3), 900–910. https://doi.org/10.1111/gcb.14517.

Li, Z., Zeng, Z., Song, Z., Wang, F., Tian, D., Mi, W., Huang, X., Wang, J., Song, L., Yang, Z., Wang, J., Feng, H., Jiang, L., Chen, Y., Luo, Y., Niu, S., 2021. Vital roles of soil microbes in driving terrestrial nitrogen immobilization. Global Change Biology 27 (9), 1848–1858. https://doi.org/10.1111/gcb.15552.

Lim, H., Oren, R., Näsholm, T., Strömgren, M., Lundmark, T., Grip, H., Linder, S., 2019. Boreal forest biomass accumulation is not increased by two decades of soil warming. Nature Climate Change 9 (1), 49–52. https://doi.org/10.1038/s41558-018-0373-9.

Liu, D., Keiblinger, K.M., Schindlbacher, A., Wegner, U., Sun, H., Fuchs, S., Lassek, C., Riedel, K., Zechmeister-Boltenstern, S., 2017. Microbial functionality as affected by experimental warming of a temperate mountain forest soil—a metaproteomics survey. Applied Soil Ecology 117–118 (April), 196–202. https://doi.org/10.1016/j.apsoil.2017.04.021.

Lu, M., Zhou, X., Yang, Q., Li, H., Luo, Y., Fang, C., Chen, J., Yang, X., Li, B., 2013. Responses of ecosystem carbon cycle to experimental warming: a meta-analysis. Ecology 94 (3), 726–738. https://doi.org/10.1890/12-0279.1.

Lu, X., Vitousek, P.M., Mao, Q., Gilliam, F.S., Luo, Y., Turner, B.L., Zhou, G., Mo, J., 2021. Nitrogen deposition accelerates soil carbon sequestration in tropical forests. Proceedings of the National Academy of Sciences of the United States of America 118 (16), 1–7. https://doi.org/10.1073/pnas.2020790118.

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

Martikainen, P.J., 2022. Heterotrophic nitrification – an eternal mystery in the nitrogen cycle. Soil Biology and Biochemistry 168 (September 2021), 108611. https://doi.org/10.1016/j.soilbio.2022.108611.

Masson-Delmotte, V., Zhai, P., Pirani, A., L, S., Connors, C. Péan, Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., Huang, M., Leitzell, K., Lonnoy, E., R, J.B., Matthews, T.K., Maycock, T., Waterfield, O., Yelekçi, R.Yu, Z, B. (Eds.), 2021. IPCC, 2021: Climate Change 2021: the Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate change.Cambridge University Pres. Cambridge, United Kingdom and New York, USA. doi:10.3724/sp.j.7103161536.

Maxwell, T.L., Canarini, A., Bogdanovic, I., Böckle, T., Martin, V., Noll, L., Prommer, J., Séneca, J., Simon, E., Piepho, H.P., Herndl, M., Pötsch, E.M., Kaiser, C., Richter, A., Bahn, M., Wanek, W., 2021. Contrasting drivers of belowground nitrogen cycling in a montane grassland exposed to a multifactorial global change experiment with elevated CO2, warming, and drought. Global Change Biology 1–17. https://doi.org/10.1111/gcb.16035. October 2021.

McIlvin, M.R., Altabet, M.A., 2005. Chemical conversion of nitrate and nitrite to nitrous oxide for nitrogen and oxygen isotopic analysis in freshwater and seawater. Analytical Chemistry 77 (17), 5589–5595.

Melillo, J.M., Frey, S.D., DeAngelis, K.M., Werner, W.J., Bernard, M.J., Bowles, F.P., Pold, G., Knorr, M.A., Grandy, A.S., 2017. Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. Science 358 (6359), 101–105. https://doi.org/10.1126/science.aan2874.

Melillo, J.M., Steudler, P.A., Aber, J.D., Newkirk, K., Lux, H., Bowles, F.P., Catricala, C., Magill, A., Ahrens, T., Morrisseau, S., 2002. Soil warming and carbon-cycle feedbacks to the climate system. Science 298 (5601), 2173–2176. https://doi.org/10.1126/science.1074153.

Melillo, Jerry M., Butler, S., Johnson, J., Mohan, J., Steudler, P., Lux, H., Burrows, E., Bowles, F., Smith, R., Scott, L., Vario, C., Hill, T., Burton, A., Zhouj, Y.M., Tang, J., 2011. Soil warming, carbon-nitrogen interactions, and forest carbon budgets. Proceedings of the National Academy of Sciences of the United States of America 108 (23), 9508–9512. https://doi.org/10.1073/pnas.1018189108.

Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5 (1), 62–71.

Moorhead, D.L., Sinsabaugh, R.L., Hill, B.H., Weintraub, M.N., 2016. Vector analysis of ecoenzyme activities reveal constraints on coupled C, N and P dynamics. Soil Biology and Biochemistry 93, 1–7. https://doi.org/10.1016/j.soilbio.2015.10.019.

Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K.M., Zechmeister-Boltenstern, S., Richter, A., 2014a. Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling. Nature Communications 5 (1), 3694. https://doi.org/10.1038/ncomms4694.

Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., 2014b. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Frontiers in Microbiology 5 (FEB), 1–10. https://doi.org/10.3389/fmicb.2014.00022.

Noll, L., Zhang, S., Wanek, W., 2019. Novel high-throughput approach to determine key processes of soil organic nitrogen cycling: gross protein depolymerization and microbial amino acid uptake. Soil Biology and Biochemistry 130, 73–81. https://doi.org/10.1016/J.SOILBIO.2018.12.005.

Noll, L., Zhang, S., Zheng, Q., Hu, Y., Hofhansl, F., Wanek, W., 2022. Climate and Geology Overwrite Land Use Effects on Soil Organic Nitrogen Cycling on a Continental Scale, vol. 8, pp. 5419–5433. https://doi.org/10.5194/bg-2022-41.

Nommik, H., Vahtras, K., 1982. Retention and fixation of ammonium and ammonia in soils. Nitrogen in Agricultural Soils 22, 123–171.

Nottingham, A.T., Meir, P., Velasquez, E., Turner, B.L., 2020. Soil carbon loss by experimental warming in a tropical forest. Nature 584 (7820), 234–237. https://doi.org/10.1038/s41586-020-2566-4.

Pold, G., Grandy, A.S., Melillo, J.M., DeAngelis, K.M., 2017. Changes in substrate availability drive carbon cycle response to chronic warming. Soil Biology and Biochemistry 110, 68–78. https://doi.org/10.1016/j.soilbio.2017.03.002.

Prietzel, J., Christophel, D., 2014. Organic carbon stocks in forest soils of the German alps. Geoderma 221–222, 28–39. https://doi.org/10.1016/j.geoderma.2014.01.021.

Prommer, J., Wanek, W., Hofhansl, F., Trojan, D., Offre, P., Urich, T., Schleper, C., Sassmann, S., Kitzler, B., Soja, G., 2014. Biochar decelerates soil organic nitrogen cycling but stimulates soil nitrification in a temperate arable field trial. PLoS One 9 (1), e86388.

Reth, S., Graf, W., Reichstein, M., Munch, J.C., 2009. Sustained stimulation of soil respiration after 10 years of experimental warming. Environmental Research Letters 4 (2). https://doi.org/10.1088/1748-9326/4/2/024005.

Riggs, C.E., Hobbie, S.E., Bach, E.M., Hofmockel, K.S., Kazanski, C.E., 2015. Nitrogen addition changes grassland soil organic matter decomposition. Biogeochemistry 125 (2), 203–219. https://doi.org/10.1007/s10533-015-0123-2.

Romero-Olivares, A.L., Allison, S.D., Treseder, K.K., 2017. Soil microbes and their response to experimental warming over time: a meta-analysis of field studies. Soil Biology and Biochemistry 107, 32–40. https://doi.org/10.1016/j.soilbio.2016.12.026.

Rousk, J., Smith, A.R., Jones, D.L., 2013. Investigating the long-term legacy of drought and warming on the soil microbial community across five E uropean shrubland ecosystems. Global Change Biology 19 (12), 3872–3884.

Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J.H.C., Gurevitch, J., Alward, R., Beier, C., Burke, I., Canadell, J., Callaghan, T., Christensen, T.R., Fahnestock, J., Fernandez, I., Harte, J., Hollister, R., John, H., et al., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126 (4), 543–562. https://doi.org/10.1007/s004420000544.

Saad, O.A.L.O., Conrad, R., 1993. Temperature dependence of nitrification, denitrification, and turnover of nitric oxide in different soils. Biology and Fertility of Soils 15 (1), 21–27.

Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology and Biochemistry 34 (9), 1309–1315. https://doi.org/10.1016/S0038-0717(02)00074-3.

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85 (3), 591–602.

Schindlbacher, A., Borken, W., Djukic, I., Brandstätter, C., Spötl, C., Wanek, W., 2015b. Contribution of carbonate weathering to the CO2 efflux from temperate forest soils. Biogeochemistry 124 (1–3), 273–290. https://doi.org/10.1007/s10533-015-0097-0.

Schindlbacher, A., De Gonzalo, C., Díaz-Pinés, E., Gíorra, P., Matthews, B., Inclán, R., Zechmeister-Boltenstern, S., Rubio, A., Jandl, R., 2010. Temperature sensitivity of forest soil organic matter decomposition along two elevation gradients. Journal of Geophysical Research: Biogeosciences 115 (3), 1–10. https://doi.org/10.1029/2009JG001191.

Schindlbacher, A., Schnecker, J., Takriti, M., Borken, W., Wanek, W., 2015a. Microbial physiology and soil CO2 efflux after 9 years of soil warming in a temperate forest - no indications for thermal adaptations. Global Change Biology 21 (11), 4265–4277. https://doi.org/10.1111/gcb.12996.

Schindlbacher, A., Zechmeister-Boltenstern, S., Jandl, R., 2009. Carbon losses due to soil warming: do autotrophic and heterotrophic soil respiration respond equally? Global Change Biology 15 (4), 901–913. https://doi.org/10.1111/j.1365-2486.2008.01757.x.

Shi, C., Urbina-Malo, C., Tian, Y., Heinzle, J., Kwatcho Kengdo, S., Inselsbacher, E., Borken, W., Schindlbacher, A., Wanek, W., 2023. Does long-term soil warming affect microbial element limitation? A test by short-term assays of microbial growth responses to labile C, N and P additions. Global Change Biology 29 (8), 2188–2202. https://doi.org/10.1111/gcb.16591.

Simon, E., Canarini, A., Martin, V., Séneca, J., Böckle, T., Reinthaler, D., Pötsch, E.M., 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. Communications Biology 3 (1). https://doi.org/10.1038/s42003-020-01317-1.

Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. Ecology Letters 16 (7), 930–939.

Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R., Moorhead, D.L., Shah, J.J.F., 2016. Stoichiometry of microbial carbon use efficiency in soils. Ecological Monographs 86 (2), 172–189. https://doi.org/10.1890/15-2110.1.

Song, L., Li, Z., Niu, S., 2021. Global soil gross nitrogen transformation under increasing nitrogen deposition. Global Biogeochemical Cycles 35 (1), 1–12. https://doi.org/10.1029/2020GB006711.

Soong, J.L., Castanha, C., Hicks Pries, C.E., Ofiti, N., Porras, R.C., Riley, W.J., Schmidt, M.W.I., Torn, M.S., 2021. Five years of whole-soil warming led to loss of subsoil carbon stocks and increased CO2 efflux. Science Advances 7 (21), 1–9. https://doi.org/10.1126/sciadv.abd1343.

Sowers, T.D., Holden, K.L., Coward, E.K., Sparks, D.L., 2019. Dissolved organic matter sorption and molecular fractionation by naturally occurring bacteriogenic iron (Oxyhydr)oxides. Environmental Science and Technology 53 (8), 4295–4304. https://doi.org/10.1021/acs.est.9b00540.

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 Biology and Biochemistry 96, 74–81. https://doi.org/10.1016/j.soilbio.2016.01.016.

Spohn, M., Pötsch, E.M., Eichorst, S.A., 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 Biology and Biochemistry 97, 168–175. https://doi.org/10.1016/j.soilbio.2016.03.008.

Team, R.C., 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, 2012.

Teramoto, M., Liang, N., Takagi, M., Zeng, J., Grace, J., 2016. Sustained acceleration of soil carbon decomposition observed in a 6-year warming experiment in a warmerate forest in southern Japan. Scientific Reports 6 (October), 1–14. https://doi.org/10.1038/srep35563.

Thorn, K.A., Mikita, M.A., 1992. Ammonia fixation by humic substances: a nitrogen-15 and carbon-13 NMR study. Science of the Total Environment 113 (1–2), 67–87.

Tian, Y., Shi, C., Malo, C.U., Kwatcho Kengdo, S., Heinzle, J., Inselsbacher, E., Ottner, F., Borken, W., Michel, K., Schindlbacher, A., 2023. Long-term soil warming decreases microbial phosphorus utilization by increasing abiotic phosphorus sorption and phosphorus losses. Nature Communications 14 (1), 864.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19 (6), 703–707.

Walker, T.W.N., Kaiser, C., Strasser, F., Herbold, C.W., Leblans, N.I.W., Woebken, D., Janssens, I.A., Sigurdsson, B.D., Richter, A., 2018. Microbial temperature sensitivity and biomass change explain soil carbon loss with warming. Nature Climate Change 8 (10), 885–889. https://doi.org/10.1038/s41558-018-0259-x.

Wanek, W., Mooshammer, M., Blöchl, A., Hanreich, A., Richter, A., 2010. Determination of gross rates of amino acid production and immobilization in decomposing leaf litter by a novel 15N isotope pool dilution technique. Soil Biology and Biochemistry 42 (8), 1293–1302.

Wang, M., Dungait, J.A.J., Wei, X., Ge, T., Hou, R., Ouyang, Z., Zhang, F., Tian, J., 2022. Long-term warming increased microbial carbon use efficiency and turnover rate under conservation tillage system. Soil Biology and Biochemistry 172 (July), 108770. https://doi.org/10.1016/j.soilbio.2022.108770.

Wiesmeier, M., Prietzel, J., Barthold, F., Spörlein, P., Geuß, U., Hangen, E., Reischl, A., Schilling, B., von Lützow, M., Kögel-Knabner, I., 2013. Storage and drivers of organic carbon in forest soils of southeast Germany (Bavaria) - implications for carbon sequestration. Forest Ecology and Management 295 (February 2018), 162–172. https://doi.org/10.1016/j.foreco.2013.01.025.

Wild, B., Ambus, P., Reinsch, S., Richter, A., 2018. Resistance of soil protein depolymerization rates to eight years of elevated CO2, warming, and summer drought in a temperate heathland. Biogeochemistry 140 (3), 255–267. https://doi.org/10.1007/s10533-018-0487-1.

Wild, B., Schnecker, J., Bárta, J., Čapek, P., Guggenberger, G., Hofhansl, F., Kaiser, C., Lashchinsky, N., Mikutta, R., Mooshammer, M., 2013. Nitrogen dynamics in turbic cryosols from Siberia and Greenland. Soil Biology and Biochemistry 67, 85–93.

Wilson, R.M., Tfaily, M.M., Kolton, M., Johnston, E.R., Petro, C., Zalman, C.A., Hanson, P.J., Heyman, H.M., Kyle, J.E., Hoyt, D.W., Eder, E.K., Purvine, S.O., Kolka, R.K., Sebestyen, S.D., Griffiths, N.A., Schadt, C.W., Keller, J.K., Bridgham, S. D., Chanton, J.P., Kostka, J.E., 2021. Soil metabolome response to whole-ecosystem

warming at the spruce and peatland responses under changing environments experiment. Proceedings of the National Academy of Sciences of the United States of America 118 (25), 1–11. https://doi.org/10.1073/pnas.2004192118.

Ye, J.S., Bradford, M.A., Dacal, M., Maestre, F.T., García-Palacios, P., 2019. Increasing microbial carbon use efficiency with warming predicts soil heterotrophic respiration globally. Global Change Biology 25 (10), 3354–3364. https://doi.org/10.1111/gcb.14738.

Yue, K., Fornara, D.A., Yang, W., Peng, Y., Li, Z., Wu, F., Peng, C., 2017. Effects of three global change drivers on terrestrial C:N:P stoichiometry: a global synthesis. Global Change Biology 23 (6), 2450–2463. https://doi.org/10.1111/gcb.13569.

Zhang, X.Z., Shen, Z.X., Fu, G., 2015a. A meta-analysis of the effects of experimental warming on soil carbon and nitrogen dynamics on the Tibetan Plateau. Applied Soil Ecology 87, 32–38. https://doi.org/10.1016/j.apsoil.2014.11.012.

Zhang, S., Fang, Y., Xi, D., 2015b. Adaptation of micro-diffusion method for the analysis of 15N natural abundance of ammonium in samples with small volume. Rapid Communications in Mass Spectrometry 29 (14), 1297–1306.

Zhang, S., Zheng, Q., Noll, L., Hu, Y., Wanek, W., 2019. Environmental effects on soil microbial nitrogen use efficiency are controlled by allocation of organic nitrogen to microbial growth and regulate gross N mineralization. Soil Biology and Biochemistry 135 (May), 304–315. https://doi.org/10.1016/j.soilbio.2019.05.019.

Zhang, Y., Cai, Z., Zhang, J., Müller, C., 2023. The controlling factors and the role of soil heterotrophic nitrification from a global review. Applied Soil Ecology 182 (August 2022), 104698. https://doi.org/10.1016/j.apsoil.2022.104698.

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 Biology and Biochemistry 128 (October 2018), 45–55. https://doi.org/10.1016/j.soilbio.2018.10.006.

Zuccarini, P., Asensio, D., Ogaya, R., Sardans, J., Peñuelas, J., 2020. Effects of seasonal and decadal warming on soil enzymatic activity in a P-deficient Mediterranean shrubland. In: Global Change Biology. https://doi.org/10.1111/gcb.15077.