universität wien

# MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Long-term responses of soil microbial activities to soil warming in a temperate forest demonstrate strong changes in element cycling and microbial element limitation."

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2020 / Vienna, 2020

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|---|---|
| Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet: | UA 066 833 |
| Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet: | Masterstudium Ecology and Ecosystems |
| Betreut von / Supervisor: | Univ.-Prof. Mag. Dr. Wolfgang Wanek |

**Acknowledgments**

First and foremost, I would like to thank my supervisor Wolfgang Wanek, for his kind invitation to participate in this great experiment. Thanks for always been there for me, for sharing your interest and passion for science, your knowledge, and your fascination for understanding the world we live in, for your patience, for been always encouraging, helpful, and supportive.

I am grateful to Vicky, Jörg, and Andi for introducing me to the wonderful world of soil ecology. Thanks to Andreas Richter for allowing me to belong to the great TER family, for letting me in with no further inquire, for your trust in my motivation and curiosity. Thanks, Vicky, for teaching me the basics and for making me feel comfortable in the lab. Thank you, Jörg for allowing me to work with you, and train myself as a researcher.

Thanks to Sabine for always been helpful. Thanks to Ludwig for always have the best Viennese attitude when helping me out, and of course the jokes. Thanks to Grete for the interest and disposition to help, and for sharing your adventures in Spanish. Thanks to Sabrina for being so understanding, fun, beyond helpful, interested, curious. Without all of you, this work would have been impossible.

Thanks to Julia Horak for being there since the very beginning, thanks for being my family, for making the world a kinder place. Thanks to Moritz for all your help since the very beginning. Thank you, guys, for that crazy lab summer love.

Thanks to Eva for all the elegance, to Julia W for the bright optimism, to Johan for the micro-empathy, to Judith for all your help and good advice, to Johana for being an example, to Stefan for the territorial encounters at the lab, to Tina for being a role model for me, to Lucy for your sweetness, to Theresa for your kind coolness, to Phillip for the rhythm. Thanks to Marilena for your help in the lab. Thanks, Alberto for your career advice, and all the punches.

Thanks to my friends Hunter, Magda, David, Sonja, Dani, Zully, and Sara for making me feel at home.

Thanks to Qingfang and Shasha, for sharing your knowledge and experience, for your incommensurable empathy and love. Thanks for being there for me in the hard moments, thanks for letting me be more than a colleague.

Thanks to my mother and father for always cheering up for me, for supporting in all my endeavors, from the Jungle to the lab. Thanks for being hilarious and wise. I would not be half the person I am today if I had not been lucky enough to be born into such a loving home. Thank you, mom, for teaching me to always act out of love, thank you, Dad, for instilling in me the love for science.

Thanks to my team and family Ye and Chupei. I am aware I was extremely lucky to find you in my way, to live and have fun with you, you two hold my back until the last minute, you teach me the value of unconditional friendship, you are my second family. Thanks for all the late lab dinners, the fancy dinners, the cozy dinners, in summary, thanks for feeding me. Thanks for making even the most boring task fun. Thanks for all the weirdness and silliness, for enduring, thanks for helping me go through the hardship. Thank you Chupei for being my sister and loving me not despite but because of our differences. Thanks, Ye for being the absolute perfect match for me at this time in life.

Last but not least, I like to thank the monkeys… my monkeys, being able to live in their world changed me forever, and made me realize how small we are, and motivated me to understand the fundamental connections between all forms of life.

## Contents

# 1. GENERAL INTRODUCTION

Forests make up to 30% of the total global land area and have an important role as C sinks in terms of C storage in aboveground biomass and soil organic matter. Climate change associated perturbations can impact nutrient cycles in a way that forests might turn from a C sink to a source, climate models predict an increasing decomposition rate of SOM with increasing temperatures (DeAngelis et al., 2019). However, the expected losses of C due to enhanced microbial decomposition of SOC in a warmer world might be partially compensated by increased plant growth. Thermal adaptations of microbial processes could either facilitate or mitigate warming effects on soil C losses. The effects of climate change on soil C dynamics, and therefore the feedback between soil C sequestration and C liberation to the atmosphere in the short and long term is complex and uncertain. Soil microbial communities play a key role as decomposers, they impact soil C turnover and the availability of other nutrients. Understanding their physiological responses to rising temperatures is therefore vital for predicting the response of the forest C cycle to future environmental conditions (Jansson & Hofmockel, 2020; Lladó et al., 2017).

The impact of chronic warming on soil C dynamics is diverse and ranges from direct effects such as immediate acceleration of microbial metabolism, to indirect effects like changes in substrate quality and quantity via modification of the microbial community structure and function or by soil drying (DeAngelis et al., 2019). Soil microorganisms are important regulators of soil biogeochemical cycles, and microbially mediated processes might be altered by warmer temperatures; the inherent biological dependence on temperature makes the soil microbial communities subject to potential physiological stress. Processes such as heterotrophic respiration, organic matter decomposition, C, and nutrient mineralization have been found to be impacted by warming. The overall response of decomposition of soil organic matter to warming, however, depends on the temperature response and sensitivity of the decomposers, on substrate quality and quantity, on other environmental drivers like soil moisture, on interactions with aboveground plant processes and potential adaptations of microbial physiology (Schindlbacher et al., 2011).

The typical response of soil respiration to long-term warming is an initial rise in soil $CO_2$ efflux and a later decline in soil respiration until achieving pre-warming levels (Romero-Olivares et al., 2019). To explain this response to warming two main hypotheses have been proposed, (i) the observed response being the result of the fast consumption and subsequent depletion of the readily

available organic carbon pool or (ii) by thermal acclimation defined as the return of microbial activity towards pre-warmed rates over time (Walker et al., 2018). Thermal acclimation involves changes in active biochemical systems within microbial cells, which are intermediate timescale physiological adjustments, that modify preexisting biochemical systems through the synthesis of new or different quantities of cellular machinery (Bradford, 2013). Sustained exposure to higher temperatures can involve increases in enzyme efficiency, in maintenance respiration, and microbial mortality. Soil microorganisms may adapt to different temperature conditions, as they exhibit short generation times, high mutation rates, and horizontal gene transfer, which allows them to adapt to warming on a contemporary scale (Romero-Olivares et al., 2017). Microbial responses to chronic warming are complex and can be driven by a combination of substrate depletion and thermal acclimation which are not mutually exclusive, and in the long term could involve evolutionary adaptation.

Extracellular enzymes break down complex organic macromolecules and provide soluble low molecular weight products for microbial uptake (Xu & Yuan, 2017). As the release of extracellular enzymes is the first step for nutrient and energy acquisition, microorganisms invest in the acquisition of limiting elements and release the elements present in excess. According to the resource allocation theory, an increase in the demand for certain elements should be evident in the increased production of corresponding extracellular enzymes, and therefore microbial nutrient demand is reflected in enzyme allocation patterns and allows microbes to obtain limiting nutrients from complex substrates (Sinsabaugh & Follstad Shah, 2012). Warming can have diverse effects on extracellular enzyme production and activity in soils; for example, warming associated substrate depletion might shift the enzymatic expression towards enzymes with higher substrate affinity, and higher temperatures directly affect the enzyme kinetics of enzymes as long as water is not limiting. The $Q_{10}$ coefficient has been widely used to describe the relationship between the rate of biological processes like extracellular enzyme activities and SOM decomposition, and temperature (Jansson & Hofmockel, 2020; Kätterer et al., 1998). The temperature sensitivity or $Q_{10}$ values of a process reflects the change of a process rate with an increase in temperature by 10°C. A $Q_{10}$ value of 1 denotes a stable rate with temperature increase, a value below 1 means a decrease in the rate of the process at higher temperatures, and most biological processes exhibit $Q_{10}$ values around or above 2. A difference in the $Q_{10}$ value of a biogeochemical process between heated and control soils would be a clear sign of thermal acclimation.

Microorganisms have the dual role to decompose and respire SOC and to stabilize plant C inputs via assimilation and necromass formation (Jansson & Hofmockel, 2020). Microbial C use efficiency (CUE) depicts the partitioning of organic C taken up which is allocated to growth relative to the proportion being mineralized to $CO_2$. The efficiency of soil organic matter decomposition and microbial CUE are temperature- and substrate-dependent. For instance, in temperate soils, the substrate use efficiency of recalcitrant compounds decreased with temperature, while it remained the same for labile compounds (Frey et al., 2013). Moreover, in the same study, the impact of chronic warming was stronger for more stable substrates compared to labile substrates for which no difference in the thermal sensitivity was found between chronically warmed and control soils. Microbial CUE is expected to decrease with rising temperature, due to the increasing maintenance costs for activities like protein synthesis and maintenance of ionic gradients across membranes (Bradford, 2013). The recalcitrant C pool becomes an alternative energy resource for a C limited soil microbial community, and subject to warming-induced labile substrate depletion, the decomposition of this stable C pool which comprised of more complex compounds, has a higher temperature sensitivity (Frey et al., 2013). With warming labile substrates become depleted while the decomposition of recalcitrant substrates is facilitated (DeAngelis et al., 2019). The limitation of easily accessible C, together with an increase in soil N mineralization (Romero-Olivares et al., 2017), might even increase soil C sequestration due to a reduction in microbial biomass and activity causing a reduction in the decomposition of SOM (Lladó et al., 2017)

Asymmetric C and N mineralization responses to warming have been observed, for example in (Beier et al., 2008), where the temperature sensitivity of N mineralization was unaltered by warming in contrast to a strong increase in SOC decomposition and soil respiration. These results were explained by calling different drivers governing the response for each element, the soil N cycle being more water-dependent and responding slower than the soil C cycle which is more strongly affected by temperature. In their meta-analysis (Bai et al., 2013) found that warming stimulated soil N processes like mineralization and nitrification but did not impact microbial biomass N, indicating that warming might lead to a leakier soil N cycle. To better understand the links between thermal responses of soil C and N processes, the microbial use efficiency of these elements should be considered. Like microbial CUE, microbial NUE reflects the share of organic N taken up that is allocated to microbial biomass growth (Mooshammer et al., 2014). A certain element use efficiency is expected to be high when an element is scarce but should be low when

this element is in ample supply, and microorganisms can adjust the allocation to anabolic processes (growth) and catabolic processes (N mineralization, respiration) accordingly. Microbial CUE and NUE are expected to respond inversely to changes in relative C:N availability and therefore to mirror the C:N stoichiometry of the soil system. CUE is expected to decrease with the shift from labile to more complex, recalcitrant substrates due to long term warming. In the absence of short-term thermal stimulation of gross N mineralization microbial NUE is expected to increase, a possible reason being the warming-induced increase in the release of SOC relative to organic N, and thus an increase in microbial N demand (Schindlbacher et al., 2015). (Zhang et al., 2019) found the most important factors affecting NUE were microbial C:N and C availability. The balance between the thermal response of energy (C) and nutrient use efficiency might also depend on the characteristics of the study site like substrate quality and quantity and moisture stress, as well as with the resource C:N imbalances generated by chronic warming.

There is a large uncertainty when it comes to predicting the future response of forest ecosystems, to future warming; further long-term warming experiments and comprehensive analysis of the available information on soil warming responses are therefore highly needed. To improve predictions of the future fate of soil C and N, metabolic parameters such as microbial CUE and NUE, and their temperature sensitivities, need to be incorporated in global climate models.

## 2. MANUSCRIPT

# “Long-term responses of soil microbial activities to soil warming in a temperate forest demonstrate strong changes in element cycling and microbial element limitation”

## 2.1 Introduction

Soils are the largest repository of organic C in the terrestrial biosphere as they contain twice as much C as the atmosphere and terrestrial vegetation combined. They are an important source of carbon dioxide to the atmosphere driven by microbial decomposition of soil organic matter (SOM) (Melillo et al., 2002). A warmer climate threatens to turn global soils from a C sink to a C source, and as soil microorganisms are key regulators of soil organic matter decomposition and storage (DeAngelis, 2019) they will play a crucial role in the future fate of soil C. Soil warming has multiple direct effects (e.g. immediate increases in microbial metabolism, microbial physiological responses to stress) and indirect effects (decrease in soil moisture, labile substrate depletion, plant responses) which impact the soil microbial community in terms of structure and function and therefore its response to short-term and long-term warming.

Temperature is the main environmental factor impacting the decomposition of SOM and plant detritus in temperate ecosystems (Schindlbacher et al., 2015), and is a strong selective force acting on the structure of microbial cells and populations, triggering as a consequence individual and community reactions (Bradford et al., 2019). Soil warming experiments and meta-analyses on the effects of warming on soil microbial processes showed varied and contrasting results. In their meta-analysis (Song et al., 2019) found that experimental warming increased soil respiration, aboveground, and root biomass while net primary productivity remained unchanged across terrestrial ecosystems. In contrast, the Harvard forest warming experiment and their literature synthesis (Melillo et al., 2002) found increased warming-induced N mobilization leading to a substantial increase in C storage in woody tissues of trees.

There are three main mechanisms of how the soil microbial community regulates the C feedback to climate warming: (i) changes in microbial community composition, (ii) differential stimulation of microbial functional groups, and (iii) by promoting nutrient cycling (Zhou et al., 2012).

The Achenkirch forest soil warming experiment, which was established in 2005, provides an interesting study case for the effects of chronic warming on soil C dynamics, showing prolonged stimulation of soil $CO_2$ efflux after decadal warming. In a meta-genomics study (Schindlbacher et al., 2011b) did not observe warming-related changes in microbial community composition, but an increase in the expression of microbial stress biomarkers. At the same site (Liu et al., 2017) found a shift in microbial community function in warmed plots, with an increase in proteins related to

microbial energy production and conversion, and a decline in functions related to growth, based on soil meta-proteomics. A first sign of change in microbial community composition was found by (Liu et al., 2017), with a shift towards a more fungi-dominated microbial community in warmed plots, which might, in turn, lead to a reduced temperature response of microbial respiration in the future (Bradford et al., 2008). The temperature sensitivity of microbial processes, such as soil and heterotrophic respiration, soil enzymatic activity, microbial growth, organic N mineralization, and substrate use efficiency remained unchanged after 9 years of warming (Schindlbacher et al., 2015). As no significant changes in SOC composition and availability were found at the site (Schnecker et al., 2016), the main mechanisms by which the microbial community regulates the warming - C feedback seems to be related to direct physiological responses to soil warming so far. Since the duration of warming can have a dramatic effect on soil microbial responses (Xu et at, 2013) we re-assessed the temperature response of microbially mediated processes at this site after 15 years of warming, to study whether chronic warming of temperate forest soils has more long-lasting effects on soil biogeochemistry. In this study, we aimed to evaluate the microbial C and N use dynamics and the short-term temperature responses of microbial mediated C and N processes in chronically warmed and control soils at the Achenkirch site.

Hypotheses

Given the still open question of whether decadal warming causes decreases in soil $CO_2$ efflux and SOM loss or not, and if yes, whether microbial responses are due to thermal acclimation or due to labile C depletion, we formulated three alternative hypotheses with related predictions, to be tested in this experiment. The predictions relate to biogeochemical rates measured at *in situ* temperature, $R_{field}$ (rate in control and heated soils at field temperature in the manipulation experiment, indicating whether a biochemical process increases or decreases in a warmer world compared to ambient), to chronic responses to warming in terms of biogeochemical rates extrapolated to the current temperature, $R_{10}$ (rate at 10 °C of control soils, indicating whether a process was up- or down-regulated), and short-term temperature responses of biochemical rates, $Q_{10}$ (rate stimulation by a 10 °C increase).

(I) **No substrate depletion**: In several long-term warming experiments no substrate depletion has been evident, SOC did not change, and soil $CO_2$ efflux remained stimulated. In this case, we would predict $R_{10}$ to be similar in warmed and control soils, while $R_{insitu}$ would be higher in heated soils. $Q_{10}$ would not respond.

(II) **Substrate depletion** causing microbial element limitation: This has been found in other long-term warming studies causing e.g. SOC loss and soil $CO_2$ efflux to decline in warmed plots towards levels in control soils. Here we would expect decreased $R_{10}$ and similar $R_{field}$ in warmed compared to control soils. $Q_{10}$ might remain unaltered, increase, or decrease. If element limitation causes microbes to allocate more into enzymes mining the scarce elements, $R_{10}$ of specific soil enzymes might however also increase in warmed compared to controls, while in situ process rates related to the limiting element will not respond or decrease.

(III) **Thermal acclimation**: This has been mostly found in cultures and less in situ studies. Here we would expect $R_{14}$ to decrease, causing converging $R_{insitu}$ in warmed and control soils. $Q_{10}$ decreases due to altered soil enzyme complement with lowered thermal sensitivity.

## 2.2 Materials and methods

Site description and soil sampling

The sampling site is located in the Austrian Limestone Alps, in Achenkirch, Austria at (47°34'50" N; 11°38'21" E), in an approximately 130-year old temperate forest, which is dominated by spruce (*Picea abies*), with interspersed European beach (*Fagus sylvatica*). The soils developed on dolomite bedrock, represent a mosaic of shallow Chromic Cambisols and Rendzic Leptosols, and are characterized by high carbonate content and near-neutral pH. The mean annual air temperature and precipitation are 6.9 °C and 1506 mm (1992-2012) at Achenkirch village (~7 km away at similar altitude).

The Achenkirch warming experiment consists of 6 plots, three of them have been established in 2004 and three in 2008, with each plot consisting of an adjacent control and a heated subplot (2 x 2 m). Heated subplots are warmed, during the snow-free season, to 4 °C above ambient soil temperature measured in the control plots, with resistance heating cables (0.4 cm diameter, TECTA, 0.18 Ohm $m^{-1}$). The dummy cables in control and heating cables in heated plots were buried in 3 cm deep trenches and had a spacing of 7-8 cm (Schindlbacher et al., 2015).

Soils were collected in October 2019 with a soil corer of 5 cm diameter to 20 cm depth and separated for topsoils (0-10 cm) and subsoils (10-20 cm), due to the shallow nature of the soils there. Each soil sample was obtained from a mixture of 10 soil cores per subplot. Soils were sieved to 2 mm and incubated at their respective average ambient temperature in the field at the time of sampling, being 10 °C (control soils) and 14 °C (heated soils).

Basic soil parameters

Soil organic C (SOC), total soil N, and C and N isotope ratios were measured on finely ground dried soils using an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS; EA1110 coupled to Delta$^{PLUS}$ IRMS via ConFlo III interface, Thermo Fisher, Germany). For SOC measurements carbonates were removed by treatment with successive additions of 2 M HCl, before drying. Photometric assays were used to determine the concentrations of soil ammonium, nitrate, and free amino acids in 1 M KCl extracts (Hood-Nowotny et al., 2010; Jones et al., 2002; Kandeler & Gerber, 1988), respectively.

Soil water content (SWC) was measured gravimetrically after drying sample aliquots (5 g) at 105 °C for two days. Water holding capacity (WHC) was determined by repeatedly saturating 10 g of fresh soil with water in a funnel with an ash-free cellulose filter, draining for 2 hours, and weighing water-saturated soil weight.

Temperature sensitivity incubation experiment

Before any measurement of temperature sensitivities of biogeochemical processes were conducted aliquots of control and warmed topsoil and subsoil samples were incubated for 24 hours, in triplicates for each sample, at three different temperatures per warming treatment: control soils (10°C, 14 °C, 20 °C) and heated soils (14 °C, 18 °C, 24 °C).

Microbial biomass C and N

Parallel to the beginning of the experiment, dissolved C and N pools were determined in 1 M KCl extracts of fresh soils (1:7.5 (w/v)). Dissolved organic C (DOC) and total dissolved N (TDN) were determined with a TOC/TN-Analyzer (Shimadzu TOC-VCPH with TNM1 and ASI Autosampler, Shimadzu, Korneuburg, Austria). At the same time chloroform-fumigation extraction (CFE) was applied, where duplicates of fresh soils were fumigated with chloroform (Brookes et al., 1985) for 48 hours to obtain microbial biomass C (MBC) and microbial biomass N (MBN). After fumigation they were extracted with 1 M KCl as above. MBC and MBN were calculated as the difference in DOC and TDN between fumigated and not fumigated soils, applying a correction with the extraction efficiency factor $k_{eC}$ and $k_{eN}$ = 0.45.

Microbial C metabolism

After pre-incubation at the three temperatures per warming treatment soil microbial activity, MBC, basal respiration and $^{18}O$ incorporation from 18O labelled soil water into genomic, double-stranded DNA (dsDNA) were measured to calculate microbial growth, respiration, C uptake, growth normalized to MBC (qGrowth), respiration normalized to MBC (qRespiration), C uptake normalized to MBC (qUptake), microbial carbon use efficiency (CUE) and microbial biomass turnover time. The applied method (Zheng et al, 2019; Spohn et al., 2016) provides a measurement of gross microbial growth rates, by measuring the incorporation of $^{18}O$ from isotopically enriched soil water into dsDNA. Duplicate fresh soil aliquots (400 mg) were weighed in 2 ml screw cap

vials and were transferred to 50 mL glass serum bottles (Supelco, Sigma-Aldrich Chemie GmbH, Germany) and incubated for 24 hours at the different incubation temperatures after adding $^{18}O$-$H_2O$ to one set of duplicates. The $^{18}O$ content of soil water was adjusted to 20.0 at%$^{18}O$ by addition of $^{18}O$-$H_2O$ (97.0 at%, Campro Scientific, Germany; diluted to lower $^{18}O$ enrichments as needed) while adjusting the volume of water added to reach a WHC of 60%. To the second set of duplicates the same amount of Milli Q water was added to be used as natural $^{18}O$ abundance (NA) controls. Directly after the water was added, the serum bottles were crimped with aluminum caps and butyl rubber stoppers (Supelco, Sigma-Aldrich Chemie GmbH, USA), an air sample of 5 ml was taken from the headspace with a gas-tight syringe, and $CO_2$ levels were measured with a portable IRGA (EGM-4, PP Systems, USA). Directly after retrieving the 5 mL headspace gas sample 5 ml of air with a known $CO_2$ concentration were injected into the serum bottles to replace the sampled air. NA and labelled samples were incubated at their respective temperatures for 24 hours. Afterwards a second gas measurement was performed (5 mL headspace sample), then 2 ml screw cap vials were removed, closed with screw caps, shock-frozen in liquid nitrogen, and stored at −80 °C until further usage. DNA was extracted with a kit (FastDNA™ SPIN Kit for Soil, MP Biomedicals, Germany), and DNA concentrations were quantified by the Picogreen fluorescence assay (Quant-iT™ PicoGreen® dsDNA Reagent, Thermo Fisher, Germany) using a Microplate spectrophotometer (Infinite® M200, Tecan, Austria). Afterwards, aliquots (50 µL) of the DNA extracts were pipetted into silver capsules (70 µL nominal volume: IVA Analysentechnik, Germany), and dried in a drying oven at 60 °C for 48 h. Finally, the silver capsules were folded, and total oxygen content and $^{18}O$ abundance in the DNA extracts were determined by a Thermochemical Elemental Analyzer (TCEA; glassy carbon reactor temperature at 1350 °C) coupled to an IRMS (Delta V Advantage, Thermo Fisher, Germany).

The following calculations were made following the detailed description in (Zheng et al., 2019). Final solution $^{18}O$ enrichment as $\%\, at_{label}$was calculated by the following formula where $\%\, at_{added}$ is the at% of the enriched water added to the soil and A is the amount added, W is the SWC and $\%\, at_{NA}$ is the $^{18}O$ from the NA samples which is close to 0.2 at%:

$$\%\, at_{label} = \frac{\%\, at_{added} * A + \%\, at_{NA} * W}{W + A}$$

The amount of newly produced DNA within 24 h ($DNA_{produced}$) was obtained as the difference in $^{18}O$ abundance between the labelled and the NA samples and using the factor of 31.21 which represents the proportional mass of O in DNA. $O_{Total}$ is the total O in the dried DNA extracts and $\%\ at_{excess}$ is the difference between the at%$^{18}O$ between NA and labelled samples.

$$DNA_{produced} = O_{Total} * \frac{\%\ at_{excess}}{100} * \frac{100}{\%\ at_{label}} * \frac{100}{31.21}$$

Microbial growth rate, as microbial C produced by gram of soil dry matter per hour ($C_{growth}$), was calculated using a conversion factor ($f_{DNA}$= $C_{mic}/DNA_{mic}$) which describes the relationship between microbial C and microbial DNA based on soil dry matter, and the quantity of newly produced DNA (Spohn et al., 2016). DW is the soil sample dry weight and t is the exact incubation time.

$$C_{growth} = \left( \frac{\left(\frac{C_{mic}}{DNA_{mic}}\right) * DNA_{produced}}{DW * t} \right)$$

Microbial uptake was calculated as the sum of mineralized C ($C_{respiration}$) and C invested into microbial growth ($C_{growth}$).

$$C_{uptake} = C_{respiration} + C_{growth}$$

Microbial CUE was then calculated as the ratio of C allocated to growth over microbial C uptake (Manzoni et al., 2012).

$$CUE = \frac{C_{growth}}{C_{uptake}}$$

Microbial turnover time was calculated as the ratio of microbial carbon over microbial growth and is therefore given in hours or days.

$$Turnover\ time = \frac{C_{mic}}{C_{growth}}$$

Basal respiration rates ($C_{respiration}$) were calculated considering the difference between the initial and final $CO_2$ concentration after the incubation period of approximately 24 hours. The C respired was calculated per gram dry weight; for further details please refer to (Zheng et al., 2019).

Microbial respiration, growth and uptake were also normalized per unit of microbial biomass, and refered as qGrowth, qRespiration and qUptake.

$$qGrowth = \frac{C_{growth}}{C_{mic}} \qquad qRespiration = \frac{C_{respiration}}{C_{mic}} \qquad qUptake = \frac{C_{uptake}}{C_{mic}}$$

15N Pool dilution assays

Gross rates of N mineralization, ammonium immobilization and nitrification were measured with isotope pool dilution methods as modified in (Wanek et al., 2010). The analyses were conducted by labeling the ammonium and nitrate pools with $^{15}N$ enriched tracers, and the gross rates of influx and efflux of the targeted pools (either ammonium or nitrate) were calculated using the difference in the isotopic composition and pool size between two time points. Low amounts of inorganic $^{15}N$ were applied, representing no more than 20% of the native pool size. For a detailed description of the isotope pool dilution methods see (Zhang et al., 2019). Gross protein depolymerization and amino acid uptake were measured with the isotope pool dilution method described in (Noll et al., 2019).

Nitrogen Use Efficiency

Microbial N growth ($N_{growth}$) was obtained by multiplying $C_{growth}$ by the microbial biomass C:N ratio, and subsequently microbial NUE was calculated as in (Zhang et al., 2019):

$$NUE = \frac{N_{growth}}{N_{growth} + N_{mineralized}}$$

Extracellular enzymatic activity

Potential activities of four soil hydrolytic enzymes: β-glucosidase (BG), chitinase in the form of N-acetylglucosaminidase (NAG), acid phosphatase (AP), and leucine-aminopeptidase (LAP), were measured fluorometrically using of 4-methylumbelliferone (MUF) and 7-amino-4-methylcoumarine (AMC) conjugated substrates. The substrates were respectively 4-methylumbelliferyl-β-D-glucopyranoside, 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide, 4-methylumbelliferyl-phosphate and L-leucine-7-amido-4-methylcoumarin hydrochloride. Soil slurries were prepared from one-gram fresh soil in 100 ml 50 mM sodium acetate buffer (Ph 5.5) by ultrasonication. Soil slurries (200 µl) were pipetted in five technical replicates into black microtiter plates and subsequently the corresponding substrates (50 µl) were added. Sample specific standard rows were prepared by adding aliquots of standards of declining concentration of methylumbelliferon (MUF) as a standard for BG, NAG and AP, whereas aminomethylcoumarin (AMC) was used for LAP. Plates were incubated in the dark for 15 minutes at the different treatment temperatures, and fluorescence was measured at an excitation/emission wavelength of 365/450 nm by the TECAN Infinite® M200 spectrophotometer every 30 minutes for 3 hours. Potential enzymatic activities were determined considering the increase in fluorescence between measuring times and soil specific quenching.

Enzyme vector analysis

Enzymatic vector angle and length were calculated using the following functions in MS Excel

$$LENGTH = SQRT\,(x^2 + y^2)$$
$$ANGLE = DEGREES\,(ATAN2(x, y))$$

x represents the relative C (BG) vs. P (AP) acquiring enzyme activities and y represents the relative C vs. N (LAP, NAG) acquiring enzyme activities (Moorhead et al., 2016). Enzymatic C:N and N:P acquisition ratios were calculated as the natural logarithm of the enzymatic activity of C over N and of N over P acquiring enzyme activities.

Statistical analysis

All statistical analyses were performed using the statistical software R version 3.6.3. The temperature sensitivities of potential enzymatic activities, and of C and N processes were computed by means of the $Q_{10}$ function as in (Schindlbacher et al., 2015):

$$R = R_{10} * Q_{10}^{(\frac{T-10}{10})}$$

In which R is the measured process/parameter rate at a specific temperature, $R_{10}$ is the equation derived rate at 10 °C, $Q_{10}$ is the temperature sensitivity of the process, and T is the soil incubation temperature in °C. $R_{10}$ and $Q_{10}$ were fitted to the measured R and T, using R (R Core Team, 2020). For statistical analysis, the extrapolated rates of processes at a common temperature, here at 10 °C ($R_{10}$), was used to evaluate basal process rate differences between treatments. Two-way ANOVAs were run to assess the effect of treatment and depth on the temperature response ($Q_{10}$ and $R_{field}$) of the processes. Linear models were fitted, with the process as independent variable and warming treatment (control/heated) and soil depth (0-10 cm and 10-20 cm) as predictors. Normality and homoscedasticity were assessed visually checking the frequency and distribution of residuals, and via Shapiro and Levene tests, and data failing to meet these assumptions were log transformed. Based on the $Q_{10}$ curve fitting procedure only summary statistics (average, standard error, number of replicates) are retrieved which are only amenable to one-way ANOVA tests using the program Sigmaplot 14. One-way ANOVA was therefore carried out separately for topsoils and subsoils, for the factor warming treatment.

## 2.3 Results

Chronic responses of soil parameters and functions to long-term soil warming were assessed by $R_{10}$, as a proxy for the up- or down-regulation of a process as long-term response to warming. Changes in the temperature sensitivity through thermal acclimation, measured as a short-term (24 hours) response to temperature increases by 10 °C, were measured based on $Q_{10}$ changes. The product of both parameters then provides the integral ($R_{field}$), showing whether soils in a warmer world will increase or decrease process rates, and allows to decipher where the eventual response of $R_{field}$ originates from ($R_{10}$ or $Q_{10}$).

Soil chemistry

Most labile soil C and N fractions did not differ between heated and control soils at the time of sampling (October 2019), such as DOC, MBC, free amino acids, nitrate and MBN, while ammonium increased in heated soils (Table 1). Soil organic C and soil total N tended to decrease by 15% and 9% (data from May 2019) (Appendix 1). All these parameters differed significantly by soil depth, i.e. decreased from topsoil to subsoil, except for ammonium. Soil $\delta^{15}N$ also tended to increase due to warming and increased with soil depth (Appendix 1).

R10: Down- (up-)regulation of microbial processes due to chronic warming

We found no down-regulation of microbial anabolic and catabolic processes and other parameters related to C and N cycling, except for changes in $R_{10}$ in soil enzyme activities and microbial biomass. Topsoils responded very little to long-term warming; only the $R_{10}$ of microbial biomass C:N and leucine amino peptidase increased, and that of acid phosphatase tended to increase (Table 2). Subsoils were more sensitive to long-term warming, and showed a decrease of $R_{10}$ in microbial biomass C and N, and an increase in microbial biomass C:N. Moreover, subsoils showed increases in the $R_{10}$ of leucine aminopeptidase and N:P acquisition, a trend towards an increase in β-glucosidase, but a decreasing trend of enzyme vector angles (Table 2, Fig. 2). The decreases in microbial NUE, amino acid consumption and nitrification as well as increases in chitinase were large but non-significant, due to large spatial heterogeneity between the six replicate plots. Here only one-way ANOVA results are shown as the curve fitting procedure only produces summary statistics (average, standard error, number of replicates) that only allow testing for single effects (here: treatment) using the program Sigmaplot 14.

Q10: Thermal acclimation of microbial processes and soil enzymes

Highest thermal sensitivities ($Q_{10}$) were found for microbial C-related processes like growth, respiration and organic C uptake (based on soil dry mass and on microbial biomass), ranging between (1.2) 1.6 and 3.4 (Table 3, Fig. 1). A similar range was found for soil enzymes, $Q_{10}$ values ranging from 1.5 to 3.2. With the exception of microbial N growth and nitrification ($Q_{10}$ 1.5-2.3), microbial N processes had $Q_{10}$ values close to or below 1, indicating that in the short-term these process rates decline at higher temperatures. Chronic warming caused a decrease in the thermal sensitivities of many processes and functions (Table 3), including microbial C growth, microbial CUE and NUE, protein depolymerization, and the enzymes β-glucosidase, leucine aminopeptidase, and chitinase. In contrast, $Q_{10}$ values of N mineralization and $NH_4^+$ consumption increased. We found no thermal acclimation in microbial respiration and C uptake, and in microbial N growth.

Rfield: In situ (field) process rates in warmed soils compared to ambient conditions

In field situations, in warmed soils (due to rare down-regulation and some but limited thermal acclimation of processes) many microbial C and N processes as well as soil enzymes were accelerated, even after decadal soil warming (Table 4). Respiration and all microbial biomass-based C process rates were higher in warmed than in control soils. Moreover, all soil enzymes showed higher $R_{field}$ in heated soils, and N:P acquisition ratios increased. In contrast, $R_{field}$ of protein depolymerization decreased and microbial biomass turnover times decreased, indicating faster microbial biomass turnover. In warmer soils we, however, did not find changes in microbial C and N growth, C uptake, N mineralization, $NH_4^+$ consumption, amino acid consumption, nitrification, enzyme vector length and angle, and in enzymatic C:N acquisition ratios (Fig. 3).

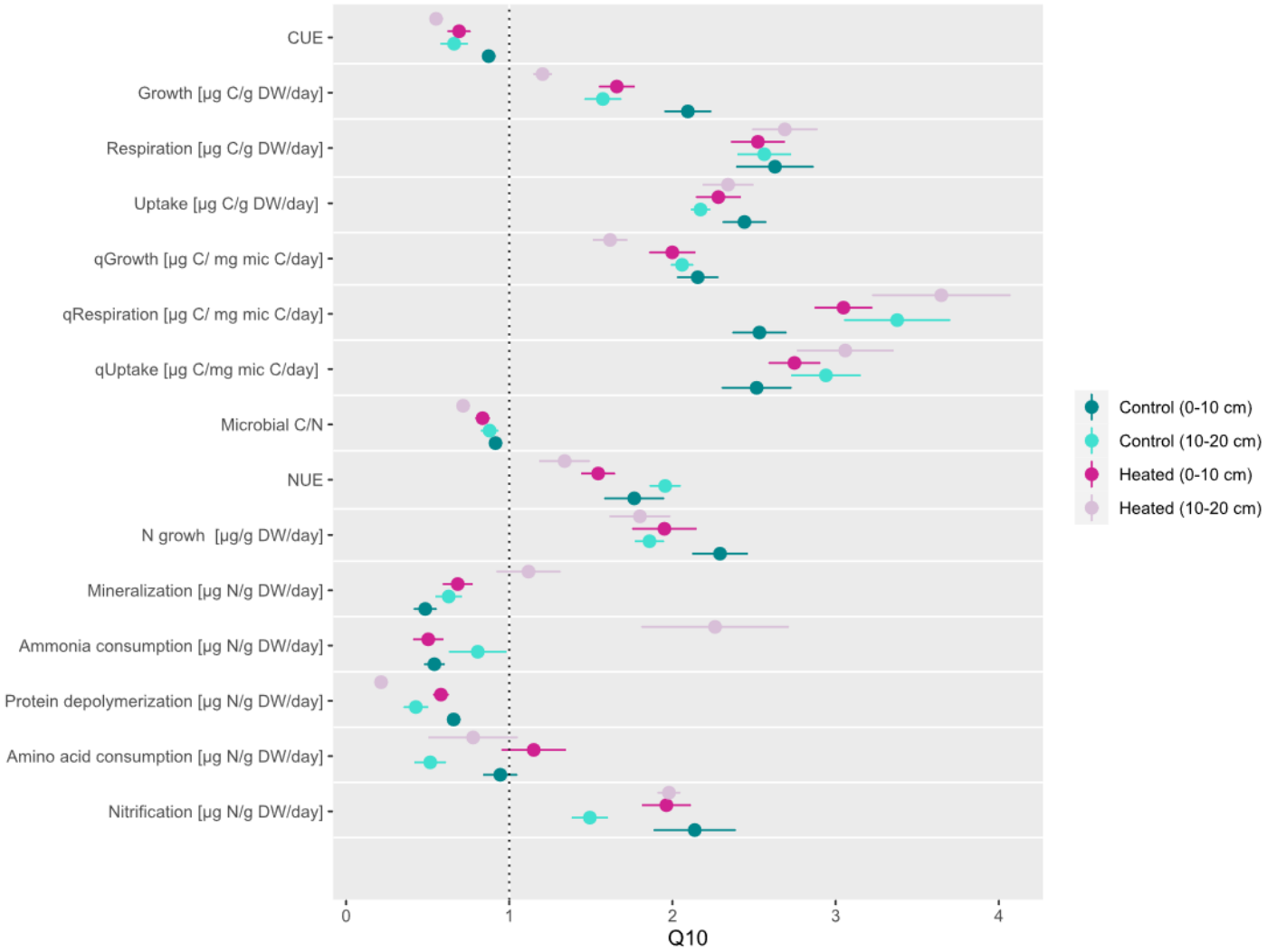


**Fig. 1.** Mean temperature sensitivity ($Q_{10}$) of C and N processes, bars show the standard error.

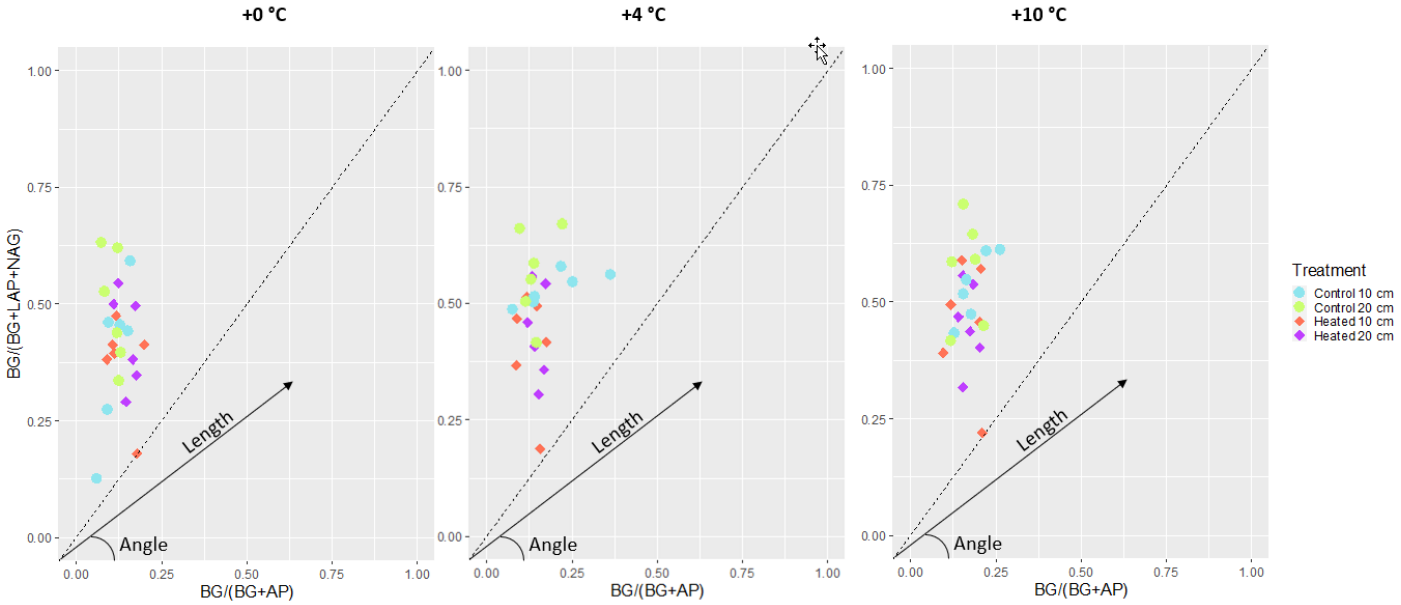


**Fig. 2.** Vector analysis for potential enzyme activity, showing relative C:N vs relative C:P acquisition. Control and heated subplots are shown at ambient temperature, +4 °C, and +10 °C. The length of the vector represents relative C limitation, and the vector angle relative P limitation.

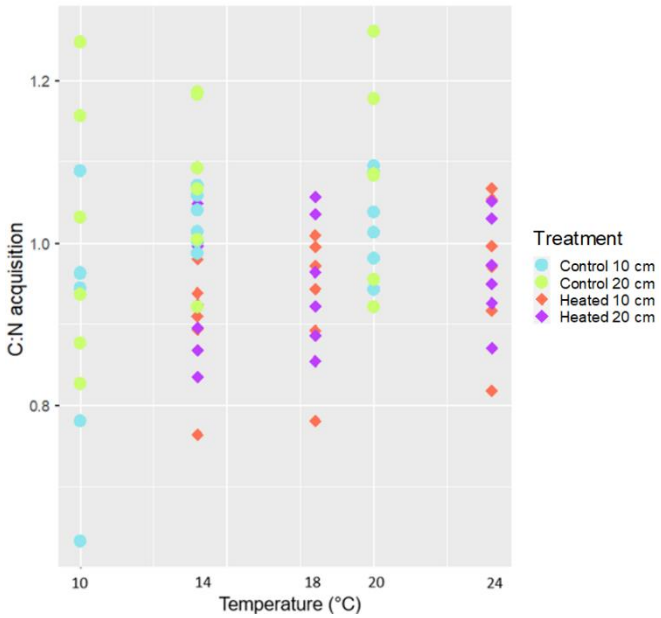


**Fig. 3.** C:N acquisition ratio, calculated as the log transformed potential activities of (BG) over (LAP + NAG) by incubation temperature. Circles represent values for control soils, blue for topsoils and green for subsoils, and diamonds represent heated soils, red indicating topsoils (0-10 cm) and purple subsoils (10-20 cm).

| $\bar{X} \pm$ STD | (0-10) cm | | (10-20) cm | | ANOVA | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Heated | Control | Heated | P Treatment | F Treatment | P Depth | F Depth | P interaction | F interaction |
| Water content (% fresh soil) | 52 ± 5 | 57 ± 3 | 61 ± 5 | 65 ± 5 | * | 4.69 | ** | 23.47 | n.s | 0.01 |
| Root mass [g/m$^2$] | 42.73 ± 10.36 | 44 ± 9.79 | 13.4 ± 7.11 | 18.16 ± 14.36 | n.s | 0.47 | *** | 39.70 | n.s | 0.16 |
| Disolved organic C [µg C/g DW] | 80 ± 10 | 90 ± 50 | 50 ± 20 | 40 ± 10 | n.s$^ǂ$ | 0.00 | **$^ǂ$ | 14.60 | n.s$^ǂ$ | 1.25 |
| Free amino acids [µmol N/g DW] | 0.27 ± 0 | 0.27 ± 0.09 | 0.15 ± 0.03 | 0.13 ± 0.05 | n.s$^ǂ$ | 0.71 | ***$^ǂ$ | 42.80 | n.s$^ǂ$ | 0.37 |
| Ammonium [µmol N/g DW] | 0.23 ± 0.06 | 0.25 ± 0.04 | 0.17 ± 0.03 | 0.26 ± 0.1 | *$^ǂ$ | 4.92 | n.s$^ǂ$ | 2.59 | n.s$^ǂ$ | 1.34 |
| Nitrate [µmol N/g DW] | 1.58 ± 0.18 | 1.33 ± 0.46 | 1.06 ± 0.37 | 0.93 ± 0.38 | n.s | 1.66 | ** | 9.28 | n.s | 0.16 |
| Total dissolved N [µg N/g DW] | 30 ± 10 | 40 ± 10 | 30 ± 10 | 20 ± 10 | n.s | 0.03 | * | 4.42 | * | 5.33 |
| Soil δ15N | -0.21 ± 0.41 | 0.02 ± 0.53 | 1.21 ± 0.57 | 1.67 ± 0.52 | n.s | 2.71 | *** | 54.05 | n.s | 0.30 |

**Table 1**. Mean ± 1 standard deviation for edaphic parameters of heated and control subplots, in topsoils (0-10 cm) and subsoils (10-20 cm). Two-way Anova analysis of the factors treatment and depth are presented. Variance homogeneity was confirmed for all data, n.s indicates a non-significant result, * corresponds to a $P < 0.05$, ** to a $P < 0.01$, and *** to a $P < 0.001$. ǂ Indicates results for log transformed data.

| $R_{10}$ $\bar{X}$ & SE | (0-10) cm | | | | | | (10-20) cm | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | Heated | | ANOVA | | Control | | Heated | | ANOVA | |
| | $R_{10}$ | SE | $R_{10}$ | SE | F | P | $R_{10}$ | SE | $R_{10}$ | SE | F | P |
| CUE | 0.26 | 0.02 | 0.27 | 0.03 | 0.06 | n.s | 0.35 | 0.02 | 0.31 | 0.05 | 0.68 | n.s |
| Growth [µg C/g DW/day] | 22.4 | 2.5 | 23.5 | 6.2 | 0.03 | n.s | 14.9 | 1.4 | 12.3 | 2.8 | 0.73 | n.s |
| Respiration [µg C/g DW/day] | 64.5 | 6.1 | 63.1 | 14.2 | 0.01 | n.s | 29.1 | 3.6 | 26.9 | 4.8 | 0.14 | n.s |
| Uptake [µg C/g DW/day] | 86.9 | 6.7 | 85.5 | 18.9 | 0.01 | n.s | 43.7 | 4.6 | 38.4 | 6.2 | 0.49 | n.s |
| qGrowth [µg C/ mg mic C/day] | 8.4 | 0.7 | 8.3 | 1.7 | 0.00 | n.s | 7.8 | 0.7 | 9.1 | 1.0 | 1.13 | n.s |
| qRespiration [µg C/ mg mic C/day] | 24.9 | 2.9 | 22.0 | 2.8 | 0.51 | n.s | 16.2 | 1.7 | 19.5 | 6.5 | 0.24 | n.s |
| qUptake [µg C/ mg mic C/day] | 33.3 | 3.2 | 29.8 | 3.2 | 0.58 | n.s | 23.9 | 2.2 | 27.8 | 7.5 | 0.25 | n.s |
| Turnover time [days] | 125.1 | 6.9 | 138.6 | 24.1 | 0.29 | n.s | 141.4 | 8.8 | 123.6 | 12.6 | 1.34 | n.s |
| Dissolved organic C [µg C/g DW] | 97.9 | 12.5 | 100.2 | 26.2 | 0.01 | n.s | 66.5 | 9.7 | 51.3 | 13.8 | 0.81 | n.s |
| Microbial C [µg C/g DW] | 2619.9 | 12.8 | 2833.4 | 482.7 | 0.20 | n.s | 2220.4 | 301.3 | 1321.2 | 308.4 | 4.35 | n.s |
| Microbial N [µg N/g DW] | 437.9 | 36.8 | 444.0 | 78.2 | 0.01 | n.s | 331.7 | 54.8 | 166.2 | 48.9 | 5.08 | * |
| Microbial C:N | 6.0 | 0.0 | 6.6 | 0.2 | 9.44 | * | 7.0 | 0.1 | 8.5 | 0.2 | 44.63 | *** |
| NUE | 0.45 | 0.05 | 0.38 | 0.08 | 0.65 | n.s | 0.39 | 0.07 | 0.31 | 0.08 | 0.73 | n.s |
| N Growth [µg N/g DW/day] | 156.8 | 18.8 | 158.2 | 43.3 | 0.00 | n.s | 91.5 | 10.4 | 65.4 | 18.6 | 1.50 | n.s |
| N Mineralization [µg N/g DW/day] | 5.6 | 1.1 | 6.2 | 1.3 | 0.16 | n.s | 3.9 | 0.7 | 3.4 | 0.9 | 0.20 | n.s |
| $NH_4^+$ Consumption [µg N/g DW/day] | 6.1 | 1.0 | 7.8 | 1.7 | 0.77 | n.s | 4.3 | 0.9 | 3.2 | 0.9 | 0.63 | n.s |
| Protein depolymerization [µg N/g DW/day] | 278.7 | 36.5 | 292.3 | 52.1 | 0.05 | n.s | 219.6 | 16.4 | 198.3 | 48.7 | 0.17 | n.s |
| Amino acid consumption [µg N/g DW/day] | 298.1 | 63.5 | 203.9 | 43.3 | 1.50 | n.s | 177.7 | 19.6 | 139.3 | 36.1 | 0.87 | n.s |
| Nitrification [µg N/g DW/day] | 1.1 | 0.3 | 0.9 | 0.3 | 0.40 | n.s | 1.3 | 0.1 | 0.9 | 0.3 | 1.60 | n.s |
| b-Glucosidase [nmol/h/g DW] | 62.8 | 12.4 | 81.4 | 25.4 | 0.43 | n.s | 35.5 | 6.7 | 56.9 | 9.9 | 3.23 | n.s |
| Aminopeptidase [nmol/h/g DW] | 6.6 | 1.0 | 17.3 | 2.0 | 22.77 | *** | 3.0 | 0.6 | 10.0 | 1.3 | 22.74 | *** |
| Chitinase [nmol/h/g DW] | 78.4 | 15.3 | 169.8 | 111.6 | 0.66 | n.s | 30.2 | 6.7 | 80.8 | 33.2 | 2.23 | n.s |
| Phosphatase [nmol/h/g DW] | 426.9 | 73.4 | 615.2 | 73.4 | 3.30 | n.s | 266.8 | 44.1 | 359.3 | 52.2 | 1.84 | n.s |
| Length | 0.45 | 0.04 | 0.37 | 0.05 | 1.57 | n.s | 0.52 | 0.04 | 0.44 | 0.05 | 1.93 | n.s |
| Angle | 71.8 | 2.0 | 69.7 | 6.3 | 0.10 | n.s | 76.7 | 1.7 | 70.5 | 3.0 | 3.11 | n.s |
| C:N acquisition | 0.93 | 0.04 | 0.88 | 0.04 | 0.85 | n.s | 1.03 | 0.05 | 0.93 | 0.04 | 2.39 | n.s |
| N:P acquisition | 0.74 | 0.02 | 0.78 | 0.06 | 0.49 | n.s | 0.62 | 0.03 | 0.74 | 0.04 | 5.79 | * |

**Table 2.** Mean $R_{10}$ values ± SE of microbial C and N processes and parameters, enzymatic activity, and vector analysis, in heated and control subplots, in topsoils (0-10 cm) and subsoils (10-20 cm). Results of one-way Anova analysis (F values, P) for the factor treatment are given separately for topsoils and subsoils.

| $Q_{10}$ $\bar{X}$ ± STD | (0-10) cm | | (10-20) cm | | Lineal model | | | | ANOVA | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Heated | Control | Heated | P Treatment | P Depth | Adjusted $R^2$ | P model | P Treatment | F Treatment | P Depth | F Depth | P interaction | F interaction |
| CUE | 0.9 ± 0.1 | 0.7 ± 0.2 | 0.7 ± 0.2 | 0.6 ± 0.1 | * | ** | 0.36 | ** | * | 5.95 | ** | 8.71 | n.s | 0.35 |
| Growth [µg C/g DW/day] | 2.1 ± 0.3 | 1.7 ± 0.3 | 1.6 ± 0.3 | 1.2 ± 0.1 | *** | *** | 0.61 | *** | *** | 16.44 | *** | 24.43 | n.s | 0.03 |
| Respiration [µg C/g DW/day] | 2.6 ± 0.6 | 2.5 ± 0.4 | 2.6 ± 0.4 | 2.7 ± 0.5 | n.s | n.s | -0.09 | n.s | n.s | 0.00 | n.s | 0.07 | n.s | 0.35 |
| Uptake [µg C/g DW/day] | 2.4 ± 0.3 | 2.3 ± 0.3 | 2.2 ± 0.1 | 2.3 ± 0.4 | n.s | n.s | -0.06 | n.s | n.s | 0.00 | n.s | 0.67 | n.s | 1.65 |
| qGrowth [µg C/ mg mic C/day] | 2.2 ± 0.3 | 2 ± 0.3 | 2.1 ± 0.2 | 1.6 ± 0.3 | * | n.s | 0.28 | * | * | 6.78 | n.s | 4.33 | n.s | 1.54 |
| qRespiration [µg C/ mg mic C/day] | 2.5 ± 0.4 | 3 ± 0.4 | 3.4 ± 0.8 | 3.6 ± 1 | n.s | * | 0.21 | * | n.s | 1.78 | * | 6.02 | n.s | 0.17 |
| qUptake [µg C/ mg mic C/day] | 2.5 ± 0.5 | 2.7 ± 0.4 | 2.9 ± 0.5 | 3.1 ± 0.7 | n.s | n.s | 0.06 | n.s | n.s | 0.60 | n.s | 2.64 | n.s | 0.06 |
| Turnover time [days] | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.6 ± 0.1 | n.s | n.s | 0.11 | n.s | n.s | 3.60 | n.s | 2.01 | * | 4.90 |
| Dissolved organic C [µg C/g DW] | 1.1 ± 0.2 | 1.1 ± 0.2 | 1 ± 0.3 | 1.2 ± 0.3 | n.s | n.s | -0.06 | n.s | n.s | 0.60 | n.s | 0.05 | n.s | 0.77 |
| Microbial C [µg C/g DW] | 1 ± 0.1 | 0.9 ± 0.1 | 0.7 ± 0.1 | 0.8 ± 0 | n.s | *** | 0.50 | *** | n.s | 1.64 | *** | 34.43 | ** | 10.39 |
| Microbial N [µg N/g DW] | 1.1 ± 0.1 | 1 ± 0.1 | 0.9 ± 0.1 | 1.2 ± 0.3 | n.s | n.s | 0.05 | n.s | n.s | 3.50 | n.s | 0.43 | ** | 9.74 |
| Microbial C:N | 0.9 ± 0 | 0.8 ± 0.1 | 0.9 ± 0.1 | 0.7 ± 0 | ** | * | 0.37 | ** | ** | 11.11 | * | 4.57 | n.s | 1.32 |
| NUE | 1.8 ± 0.5 | 1.5 ± 0.3 | 2 ± 0.2 | 1.3 ± 0.4 | ** | n.s | 0.22 | * | ** | 8.90 | n.s | 0.00 | n.s | 1.98 |
| N Growth [µg N/g DW/day] | 2.3 ± 0.4 | 2 ± 0.5 | 1.9 ± 0.2 | 1.8 ± 0.5 | n.s | n.s | 0.10 | n.s | n.s | 1.44 | n.s | 3.04 | n.s | 0.71 |
| N Mineralization [µg N/g DW/day] | 0.5 ± 0.2 | 0.7 ± 0.2 | 0.6 ± 0.2 | 1.1 ± 0.5 | * | * | 0.33 | ** | * | 8.00 | * | 5.64 | n.s | 1.42 |
| $NH_4^+$ Consumption [µg N/g DW/day] | 0.5 ± 0.2 | 0.5 ± 0.2 | 0.8 ± 0.4 | 2.3 ± 1.1 | *ǂ | ***ǂ | 0.48 | *** | * | 8.06 | *** | 16.44 | ** | 8.93 |
| Protein depolymerization [µg N/g DW/day] | 0.7 ± 0 | 0.6 ± 0.1 | 0.4 ± 0.2 | 0.4 ± 0.3 | **ǂ | ***ǂ | 0.66 | *** | ** | 9.73 | *** | 41.25 | n.s | 2.11 |
| Amino acid consumption [µg N/g DW/day] | 0.9 ± 0.3 | 1.1 ± 0.5 | 0.5 ± 0.2 | 0.8 ± 0.7 | n.sǂ | ***ǂ | 0.25 | * | n.s | 0.65 | ** | 8.48 | n.s | 0.02 |
| Nitrification [µg N/g DW/day] | 2.1 ± 0.6 | 2 ± 0.4 | 1.5 ± 0.3 | 2 ± 0.2 | n.s | n.s | 0.08 | n.s | n.s | 0.93 | n.s | 3.78 | n.s | 4.17 |
| b-Glucosidase [nmol/h/g DW] | 2.3 ± 0.3 | 2.2 ± 0.3 | 2.4 ± 0.3 | 1.9 ± 0.2 | * | n.s | 0.16 | n.s | * | 6.11 | n.s | 0.88 | n.s | 3.53 |
| Aminopeptidase [nmol/h/g DW] | 3.2 ± 0.5 | 2.3 ± 0.2 | 2.7 ± 0.4 | 2.3 ± 0.2 | ***ǂ | n.sǂ | 0.43 | ** | *** | 18.95 | n.s | 2.11 | n.s | 2.76 |
| Chitinase [nmol/h/g DW] | 2.1 ± 0.2 | 1.6 ± 0.2 | 1.7 ± 0.3 | 1.6 ± 0.2 | ** | n.s | 0.37 | ** | ** | 13.84 | * | 4.72 | * | 5.46 |
| Phosphatase [nmol/h/g DW] | 2 ± 0.4 | 1.7 ± 0.1 | 1.5 ± 0.1 | 1.6 ± 0.2 | n.sǂ | ***ǂ | 0.31 | ** | n.s | 1.12 | ** | 11.66 | n.s | 3.99 |
| Length | 1,4 ± 0,7 | 1,2 ± 0,1 | 1,2 ± 0,3 | 1,1 ± 0,1 | n.s | n.s | 0.03 | n.s | n.sǂ | 0.61 | n.sǂ | 0.61 | n.sǂ | 0.26 |
| Angle | 1 ± 0 | 1 ± 0 | 1 ± 0 | 1 ± 0 | n.s | n.s | 0.00 | n.s | n.s | 1.30 | n.s | 1.30 | n.s | 0.51 |
| C:N acquisition | 1,1 ± 0,2 | 1,1 ± 0 | 1,1 ± 0,2 | 1 ± 0 | n.s | n.s | -0.01 | n.s | n.sǂ | 0.44 | n.sǂ | 0.44 | n.sǂ | 0.21 |
| N:P acquisition | 1 ± 0,1 | 1 ± 0 | 1,1 ± 0,2 | 1 ± 0,1 | n.s | n.s | -0.01 | n.s | n.s | 0.50 | n.s | 0.50 | n.s | 0.18 |

**Table 3.** Mean $Q_{10}$ values ± 1 SD of microbial C and N processes and parameters, enzymatic activity, and vector analysis, in heated and control subplots, in topsoils (0-10 cm) and subsoils (10-20 cm). Results of simple linear models using treatment and depth as predictors for $Q_{10}$, and results of two-way Anova analysis of the factors treatment and depth as well as their interaction. Variance homogeneity was tested for all data, and ǂ Indicates results for log transformed data. n.s indicates a non-significant result, * corresponds to a $P < 0.05$, ** to a $P < 0.01$, and *** to a $P < 0.001$.

| $R_{field}$ $\bar{X}$ ± SD | (0-10) cm | | (10-20) cm | | Lineal model | | | | ANOVA | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Heated | Control | Heated | P Treatment | P Depth | Adjusted $R^2$ | P model | P Treatment | F Treatment | P Depth | F Depth | P interaction | F interaction |
| CUE | 0.24 ± 0.03 | 0.24 ± 0.05 | 0.36 ± 0.10 | 0.25 ± 0.08 | * | * | 0.27 | * | * | 4.93 | * | 6.71 | n.s | 3.01 |
| Growth [µg C/g DW/day] | 20 ± 3.4 | 24.5 ± 10.4 | 15.6 ± 5.5 | 12.3 ± 19.1 | n.s‡ | ***‡ | 0.37 | **‡ | n.s‡ | 0.16 | ***‡ | 16.75 | n.s‡ | 2.75 |
| Respiration [µg C/g DW/day] | 66.9 ± 13.7 | 83.8 ± 22.8 | 29 ± 9.6 | 39.7 ± 11.7 | * | *** | 0.68 | *** | * | 4.91 | *** | 43.07 | n.s | 0.25 |
| Uptake [µg C/g DW/day] | 86.9 ± 15.7 | 108.3 ± 31.3 | 44.6 ± 11 | 52 ± 11.1 | n.s | *** | 0.64 | *** | n.s | 3.37 | *** | 39.74 | n.s | 0.80 |
| qGrowth [µg C/ mg mic C/day] | 8.2 ± 139.9 | 9.5 ± 434.4 | 7.2 ± 227.6 | 10.2 ± 796.6 | * | n.s | 0.17 | n.s | * | 6.83 | n.s | 0.01 | n.s | 1.05 |
| qRespiration [µg C/ mg mic C/day] | 27.5 ± 6.9 | 32.8 ± 5.4 | 14.2 ± 5.8 | 33.6 ± 12 | ** | n.s | 0.37 | ** | ** | 14.42 | n.s | 3.68 | * | 4.73 |
| qUptake [µg C/ mg mic C/day] | 35.7 ± 8.1 | 42.3 ± 6.8 | 21.4 ± 7 | 43.9 ± 12.1 | ** | n.s | 0.39 | ** | *** | 16.40 | n.s | 3.12 | * | 4.89 |
| Turnover time [days] | 125.7 ± 22.4 | 113.6 ± 36.3 | 144.7 ± 34.5 | 101.8 ± 22.8 | * | n.s | 0.12 | n.s | n.s | 5.14 | n.s | 0.09 | n.s | 1.62 |
| Dissolved organic C [µg C/g DW] | 74.9 ± 12 | 93.4 ± 51.6 | 50.8 ± 24.2 | 41.5 ± 15.1 | n.s‡ | **‡ | 0.35 | **‡ | n.s‡ | 0.00 | **‡ | 14.19 | n.s‡ | 0.73 |
| Microbial C [µg C/g DW] | 2514.3 ± 568.1 | 2600 ± 829.3 | 2349.5 ± 1296.5 | 1205.1 ± 117.6 | *‡ | **‡ | 0.37 | **‡ | *‡ | 5.66 | **‡ | 13.61 | *‡ | 6.22 |
| Microbial N [µg N/g DW] | 416.9 ± 98.6 | 426.3 ± 150.3 | 353.8 ± 243.9 | 161.9 ± 19.9 | *‡ | ***‡ | 0.44 | ***‡ | *‡ | 5.49 | ***‡ | 19.10 | *‡ | 5.40 |
| Microbial C:N | 6 ± 0.2 | 6.2 ± 0.6 | 7 ± 0.8 | 7.5 ± 0.4 | n.s‡ | ***‡ | 0.55 | ***‡ | n.s‡ | 3.72 | ***‡ | 57.15 | n.s‡ | 1.43 |
| NUE | 0.42 ± 0.14 | 0.41 ± 0.16 | 0.44 ± 0.26 | 0.32 ± 0.08 | n.s | n.s | -0.04 | n.s | n.s | 0.94 | n.s | 0.23 | n.s | 0.65 |
| N Growth [µg N/g DW/day] | 138.6 ± 22.8 | 169.6 ± 82.7 | 96.5 ± 40.8 | 68.8 ± 16 | n.s‡ | ***‡ | 0.45 | ***‡ | n.s‡ | 0.42 | ***‡ | 21.48 | n.s‡ | 2.46 |
| N Mineralization [µg N/g DW/day] | 5.6 ± 3.6 | 5.7 ± 1.4 | 3.6 ± 2.5 | 3.7 ± 1.1 | n.s | * | 0.09 | n.s | n.s | 0.00 | n.s | 4.16 | n.s | 0.00 |
| $NH_4^+$ Consumption [µg N/g DW/day] | 5.2 ± 3.4 | 5.9 ± 1.8 | 3.3 ± 2.1 | 3 ± 1.6 | n.s | * | 0.17 | n.s | n.s | 0.03 | * | 6.59 | n.s | 0.31 |
| Protein depolymerization [µg N/g DW/day] | 261.4 ± 36.2 | 230.1 ± 84.5 | 230.3 ± 45.3 | 149.2 ± 48.5 | * | * | 0.30 | ** | * | 5.91 | * | 5.85 | n.s | 1.16 |
| Amino acid consumption [µg N/g DW/day] | 261 ± 57.8 | 222.2 ± 96.3 | 201.2 ± 33.4 | 135.5 ± 62.9 | n.s | * | 0.29 | * | n.s | 3.71 | * | 7.28 | n.s | 0.25 |
| Nitrification [µg N/g DW/day] | 1.3 ± 0.8 | 1.1 ± 0.3 | 1.6 ± 0.3 | 1.1 ± 0.2 | n.s | n.s | 0.05 | n.s | n.s | 2.71 | n.s | 0.44 | n.s | 0.71 |
| b-Glucosidase [nmol/h/g DW] | 63.1 ± 31.5 | 113.1 ± 59.9 | 28 ± 9.1 | 68.9 ± 18.2 | ***‡ | **‡ | 0.55 | ***‡ | ***‡ | 18.67 | **‡ | 10.99 | n.s‡ | 0.85 |
| Aminopeptidase [nmol/h/g DW] | 6.4 ± 1.6 | 24.4 ± 4 | 3.2 ± 1 | 14.2 ± 2.6 | *** | *** | 0.87 | *** | *** | 191.57 | *** | 40.94 | ** | 11.02 |
| Chitinase [nmol/h/g DW] | 95.3 ± 49.4 | 202.6 ± 200 | 28.9 ± 16.8 | 91.4 ± 61.8 | **‡ | **‡ | 0.43 | **‡ | **‡ | 8.17 | **‡ | 11.37 | n.s‡ | 1.23 |
| Phosphatase [nmol/h/g DW] | 480 ± 160.6 | 700 ± 153.3 | 233.1 ± 77.6 | 397 ± 94.8 | ** | *** | 0.64 | *** | ** | 13.76 | *** | 28.21 | n.s | 0.29 |
| Length | 0.4 ± 0.2 | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 | n.s | n.s | 0.03 | n.s | n.s | 0.32 | n.s | 2.25 | n.s | 0.23 |
| Angle | 72.4 ± 4.7 | 68.7 ± 12.3 | 76.5 ± 5.5 | 69.7 ± 6.6 | n.s | n.s | 0.06 | n.s | n.s | 2.63 | n.s | 0.64 | n.s | 0.23 |
| C:N acquisition | 0.90 ± 0.16 | 0.90 ± 0.07 | 1.01 ± 0.16 | 0.94 ± 0.09 | n.s | n.s | 0.03 | n.s | n.s | 0.39 | n.s | 2.21 | n.s | 0.56 |
| N:P acquisition | 0.74 ± 0.07 | 0.79 ± 0.11 | 0.62 ± 0.13 | 0.76 ± 0.09 | * | n.s | 0.22 | * | * | 4.91 | n.s | 3.61 | n.s | 1.24 |

**Table 4.** Mean $R_{field}$ values ± 1 SD of microbial C and N processes and parameters, enzymatic activity, and vector analysis, in heated and control subplots, in topsoils (0-10 cm) and subsoils (10-20 cm). Results of simple linear models using treatment and depth as predictors for $Q_{10}$, and results of two-way Anova analysis of the factors treatment and depth as well as their interaction. Variance homogeneity was tested for all data, and ‡ Indicates results for log transformed data. n.s indicates a non-significant result, * corresponds to a $P < 0.05$, ** to a $P < 0.01$, and *** to a $P < 0.001$.

## 2.4 Discussion

Temperature is a key driver of SOM decomposition and can explain large proportions of the variations in SOM decomposition across sites (Li et al., 2015). Temperature driven changes in microbial C and N processes will strongly impact the response of the terrestrial ecosystems to climate warming. Our results showed that chronic warming can induce changes in soil C and N cycling i.e. thermal acclimation, and such effects became only evident after 12-15 years of consecutive soil warming. This study contrasts with the one from (Schindlbacher et al., 2015) at the same experimental site, in which no differences in the temperature sensitivity of microbial substrate use efficiency, soil enzyme activity, gross N mineralization, and microbial respiration were observed after 8 years of warming.

Microbial carbon use efficiency and C cycling in response to chronic warming

The response of microbial CUE to warming is a complex one, where ecosystem type, successional history, microbial community composition, substrate quality and availability, among others, need to be considered when assessing the thermal response of microbial CUE. We found an overall decrease in microbial CUE ($Q_{10}$ and $R_{field}$), depending on a strong warming-induced increase of microbial respiration, although the $R_{10}$ of microbial respiration indicated no up- or down-regulation in warmed soils. At our study site warming has initially enhanced soil $CO_2$ efflux by 40% since 2005 (Schindlbacher et al., 2008), and this stimulation continues nowadays (Jakob Heinzle and Andreas Schindlbacher et al., manuscript in preparation). Usually the effect of warming on soil respiration is an immediate increase in the soil $CO_2$ efflux, followed by a deceleration until returning to pre-warming levels (Walker et al., 2018). In our experiment we did not observe any down-regulation in terms of decreases in $R_{10}$ and $R_{field}$ (Tables 2 and 5) nor in the $Q_{10}$ (Table 2) of microbial respiration between heated and control subplots. Our results therefore show a lack of physiological acclimation in soil $CO_2$ efflux and in microbial respiration after 12-15 years of chronic soil warming. Dolomite soils are rich in organic C (12-14% organic C in topsoils), and much of this C is not strongly mineral-bound, making it relatively labile and available for decomposition and microbial utilization. The decrease in SOC by 15% was not significant, due to high spatial variability, but indicates the absence of strong labile substrate depletion.

When incubated at the same temperature ($R_{10}$), there were no differences between control and heated soils in microbial CUE, although the ability to respond to warming differed among treatments. Decadal warming has impacted the capacity of the microbial community to respond to transient, diurnal warming and cooling (decrease in $Q_{10}$). The change in the thermal sensitivity of microbial CUE was largely driven by $Q_{10}$ responses of microbial growth than respiration. Our results therefore show that the long-term warming effect on the physiological response of soil microbes to short-term warming was only evident in microbial growth (Table 3). We found a consistent decrease in $Q_{10}$ and $R_{field}$ of microbial growth with warming across treatments and soil depths, although we observed an increasing soil enzymatic potential with warming. However, this did not translate in a greater resource availability for microbial cell growth in our case and as reported by others (Pold et al., 2017). The temperature sensitivity of microbial growth was relatively low compared ($Q_{10}$ 1.2-2.1) compared to other studies where it ranged between 2.1 and 3.2 (Zheng et al., 2019). Warming is expected to increase respiratory costs, and cause microbial heat stress responses, increasing cellular maintenance costs, and therefore deflecting resources from growth (Manzoni et al., 2012). At our site warming has diminished the capacity of the microbial community to grow faster in warmed soils, as well as reduced its temperature sensitivity. Microbial growth limitation at our site could be additionally triggered by increasing microbial phosphorus limitation, e.g. gross rates of soil P mobilization decreased while soil P sorption increased, both limiting the availability of P (Ye Tian et al., manuscript in preparation). Enzymatic vector analysis showed a strong relative P over N limitation (vector angles greater than 45 °C) at all our plots and a higher relative C limitation in control soils (Fig 4.). Microbial P limitation can strongly impact microbial growth and metabolism as microorganisms need to sustain a balanced composition of elements and the homeostasis of the microbial biomass (Cui et al., 2020).

The tradeoff between substrate quality and temperature sensitivity of decomposition has been widely documented, where the decomposition of more recalcitrant SOM is more temperature sensitive than labile compounds (Alster et al., 2018; Knorr et al., 2005; Pold et al., 2017). Chronic warming can impact the quality and quantity of available resources via their divergent production and consumption (Pold et al., 2020). We found a trend towards higher $\delta^{15}N$ signatures and lower soil C:N ratios in heated than in control subplots, indicating a

stronger microbial decomposition and aging of SOM (Ye Tian et al, manuscript in preparation). These are proxies of increased microbial processing (Melillo et al., 2002) and might be an indication of warming-induced reduction of labile soil organic matter, which can lead to a decrease in microbial biomass, as found by others (Pold et al., 2017).

Microbial nitrogen use efficiency and N cycling in response to chronic soil warming

Microbial NUE increased with short term warming, $Q_{10}$ ranging between 1.3 and 2.0. Though microbial NUE was down-regulated ($R_{10}$), this was not significant. $R_{field}$ of microbial NUE did not significantly respond to warming, even though the temperature sensitivity decreased. The decrease in the Q10 of microbial NUE was controlled by the response of gross N mineralization that increased from control to heated subplots. Gross N mineralization had a low temperature sensitivity ($Q_{10}<1$), causing increases in microbial NUE at rising temperatures in the short-term, while chronic warming increased its $Q_{10}$ and basal rates ($R_{10}$) did not change. A microbial NUE positively stimulated by temperature, translate in a decrease of the fraction of organic N taken up by microorganisms that is mineralized, this can be therefore reconciled with a lack of a short-term stimulation of gross N mineralization by increasing temperature (Schindlbacher et al, 2015). Enzyme ratios revealed an increase in enzymatic N:P acquisition strategy in warmed soils, both in terms of $R_{10}$ and $R_{field}$, indicating a relatively higher investment to mine for N compounds in heated subplots. Protein depolymerization is a key step for N acquisition and is controlled by substrate (protein) availability and enzymatic activity (Reuter et al., 2020). Despite the strong up-regulation of leucine aminopeptidase ($R_{10}$) we observed an overall negative response of protein depolymerization to warming, with $Q_{10}$ values lower than 1 indicating that in the short-term temperature does not stimulate this process. Moreover, protein depolymerization responded negatively to chronic soil warming, showing down-regulation (lower $R_{10}$), thermal acclimation (decreasing $Q_{10}$) and therefore lower in situ activities in warmed soils (lower $R_{field}$). This might be explained by protein depolymerization becoming more substrate limited when temperatures increase in the short- and the long- term, due to factors such as limited substrate bioavailability via increased sorption (Noll et al., 2019, Vinolas et al., 2001). The latter would also coincide with the stronger sorption of reactive phosphate (and protein) and

indicates changes in soil mineralogy due to soil warming, though such a mechanism has remained elusive and speculative as we did not measure them.

Coupling of warming responses of microbial C and N processes

Microbial NUE showed the inverse pattern as microbial CUE, i.e. in the short-term NUE increased with temperature ($Q_{10} > 1$) while CUE decreased ($Q_{10} < 1$). Microbial CUE can decline when nutrients are limited and C is available, but also if microbial C limitation becomes severe with warming. This can cause a decline in microbial biomass production as a result (Billings & Ballantyne, 2013), which in our case together with an increase in microbial respiration, caused the observed low microbial CUE values. The warming-related changes in the temperature sensitivity of microbial NUE and CUE were coupled, showing a significant decrease of the temperature sensitivity of microbial CUE and NUE in chronically warmed soils. Responses of microbial C processes were mostly coupled, especially for microbial growth and mineralization, not so much for microbial C uptake, similar to the results found by others (Thompson, 2019). The short-term increase in microbial NUE (Q10 > 1) with temperature could possibly be triggered by the increase in the short-term temperature response of C respiration relative to N mineralization, causing an increased microbial N demand. The microbial biomass C:N ratios that we report here are much higher than the ones from (Schindlbacher et al., 2015) from the same site, indicating that prolonged warming had impacted the cellular stoichiometry of soil microbes. We also observed an increase in microbial biomass C:N in chronically warmed soils, however, the trend towards increasing soil $\delta^{15}N$ in warmed subplots rather indicates a more open soil N cycle, with increasing N losses through leaching or gaseous N emissions.

With increasing depth, substrate quality and quantity usually decrease (Schütt et al., 2013). Interestingly the temperature response of C mineralization did not vary between depths, while it was higher in subsoils for N mineralization. At the study site we also found a strong increase $\delta^{15}N$ values from topsoils to subsoils, indicating a lower substrate quality and stronger microbial reprocessing of subsoil SOM. Furthermore, microbial biomass dynamics and activity probably affected gross N mineralization and the connection between C and N mineralization (Tian et al., 2017).

Chronic warming effect on extracellular enzyme activity

Enzyme activity controls the first step of resource acquisition by the soil microbial community. The complement of soil enzymes therefore represents a proxy for the demands and investment strategies of the decomposer community. The temperature sensitivities of leucine aminopeptidase, chitinase and β-glucosidase mostly decreased in response to chronic warming, in contrast to (Schindlbacher et al., 2015), who did not find changes in $Q_{10}$ values after 8 years at the same site. While soil enzymes have been shown to be relatively unresponsive to experimental warming in other studies and in a meta-analysis, we found a strong up-regulation of these enzymes including acid phosphatase ($R_{10}$ increased) in the warming treatment. Changes in the temperature sensitivity of enzymes may be due to a warming-related difference in the expressed isoenzyme complement in heated and control soils, while other factors that could influence the enzymatic response to temperature changes are changes in SOM abundance and composition, in soil texture, and in mineralogy (Wallenstein et al., 2010).

Different to the metanalysis of (Meng et al., 2020) and consistent with the results of (Mori, 2020) we found a strong and consistent stimulation of all enzymes with warming (Table 3), especially for N and P enzymes. In our study the warming treatment, together with high-water content of the soil, nutrient deficiency, and increased microbial activity, may have stimulated the activity of hydrolytic extracellular enzymes (Zuccarini et al., 2020). However, though soil enzymes were up-regulated, their stimulation was slightly attenuated by decreases in $Q_{10}$ in warmed soils, and was – most important - not reflected in any change in C and N process rates in warmed soils relative to control soils.

## 2.5 Conclusions

Coming back to our alternative scenarios for decadal soil warming effects on soil microbial function we can conclude that:

- Though SOC decreased by 15% (non-significantly), DOC as well as labile N forms were not affected. This sustained a strong positive response of soil microbial C and N processes ($R_{field}$) while it did not trigger a down-regulation of the same processes ($R_{10}$). Labile SOM depletion therefore did not play a strong role in the responses of soil microbes to long-term soil warming.
- Nonetheless, we detected thermal acclimation of soil microbial communities, as reflected in decreasing $Q_{10}$ values of microbial C growth, and soil N processes such as N mineralization, protein depolymerization, and microbial CUE and NUE, indicating the complex regulation of microbial C and N metabolism in a warmer world.
- Opposite to the patterns above we found soil enzymes to be strongly upregulated ($R_{10}$), and despite thermal acclimation ($Q_{10}$ decreased), warmer soils had significantly higher soil enzyme activities ($R_{field}$). These higher potential enzyme activities, however, did not translate into higher C and N process rates ($R_{10}$), indicating a wide-spread substrate (and not enzyme) limitation of soil C and N processes of the studied processes.
- After 12-15 years, the experimentally warmed forest soils at Achenkirch still did not show substrate depletion but expressed already strong microbial thermal acclimation. The soils are therefore at an interphase between all three mentioned alternative mechanisms of soil warming effects on soil microbial activity.

## 2.6 Additional information

| $\bar{X}$ ± STD | (0-10) cm | | (10-20) cm | | ANOVA | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Heated | Control | Heated | P Treatment | F Treatment | P Depth | F Depth | P interaction | F interaction |
| Soil d13C | -26.46 ± 0.3 | -26.29 ± 0.28 | -25.91 ± 0.34 | -25.84 ± 0.27 | n.s | 0.86 | ** | 13.83 | n.s | 0.15 |
| Soil organic N [mg N/g DW] | 0.85 ± 0.17 | 0.77 ± 0.19 | 0.46 ± 0.09 | 0.4 ± 0.1 | n.s | 1.32 | ** | 35.74 | n.s | 0.03 |
| Soil organic C [mg C/g DW] | 14.07 ± 2.85 | 11.92 ± 1.87 | 6.95 ± 1.29 | 6.08 ± 1.62 | n.s | 2.84 | ** | 52.77 | n.s | 0.52 |
| Soil C/N | 16.7 ± 1.63 | 15.95 ± 1.92 | 15.23 ± 0.6 | 15.29 ± 0.53 | n.s | 0.34 | n.s | 3.24 | n.s | 0.46 |

**Appendix 1.** Soil Parameters at in situ temperature 10 °C and 14°C for Control and Heated subplots respectively, data were taken at our site in Spring (Tian et al, manuscript in preparation). Mean ± Standard deviation from parameters for Heated and control subplots, at top (0-10 cm) and mid (10-20) soils. Two-way Anova analysis of the factors Treatment at Depth. Variance homogeneity applied to all data, n.s indicates a non-significant result, * corresponds to a $P < 0.05$, ** to a $P < 0.01$, and *** to a $P < 0.001$. † Indicates results for log transformed data.

# 3. SUMMARIES

## 3.1 Summary

Despite the intensified efforts to understand the impacts of climate change on forest soil C dynamics, few studies have addressed the long-term effects of warming on microbial mediated soil C and nutrient processes. In long-term soil warming experiments the initial stimulation of soil C cycling diminished with time, due to thermal acclimation of the microbial community or due to depletion of labile soil C as the major substrate for heterotrophic soil microbes. Thermal acclimation can arise because of prolonged warming and is defined as the direct organism response to elevated temperature across annual to decadal timescales which manifest as a physiological change of the soil microbial community. This mechanism is clearly different from apparent thermal acclimation, where the attenuated response of soil microbial processes to warming is due to the exhaustion of the labile soil C pool.

The Achenkirch experiment, situated in the Northern Limestone Alps, Austria (47°34’ 50’’ N; 11°38’ 21’’ E; 910 m a.s.l.) is a long term soil warming experiment (>15 yrs, +4 °C warming above ambient) that has provided key insights into the effects of global warming on the forest soil C cycle. At the Achenkirch site, we have observed a sustained positive response of heterotrophic soil respiration and of soil $CO_2$ efflux to warming after nine years (2013), making it an appropriate setting for testing hypotheses about continued or decreasing warming effects at decadal scales. We collected soil from six heated and six control plots in October 2019, from 0-10 cm and 10-20 cm soil depth, and incubated them at three different temperatures: ambient, +4 °C, and +10 °C. We measured potential soil enzyme activities with fluorometric assays, gross rates of protein depolymerization, N mineralization, and nitrification with $^{15}N$ isotope pool dilution approaches, and microbial growth, respiration, and C and N use efficiencies based on the $^{18}O$ incorporation in DNA.

Our results show that the potential enzyme activities including leucine aminopeptidase, N-acetylglucosaminidase, β-glucosidase, and acid phosphatase were significantly up-regulated by decadal soil warming (1.3- to 3.3-fold in $R_{10}$). In contrast, their temperature sensitivity ($Q_{10}$) decreased with soil warming, particularly for C- and N-related enzyme activities,

indicating thermal acclimation of the microbes and the production of new isoenzymes. For microbial C processes, we found no down-regulation ($R_{10}$) due to chronic warming while soil warming notably decreased the $Q_{10}$ of microbial anabolic processes (growth) but not of catabolic processes (respiration). This effectively caused a decrease in the thermal sensitivity of microbial CUE in warmed soils. At the same time, microbial CUE had $Q_{10}$ values lower than one, demonstrating that in the short-term microbial CUE decreases with short-term temperature increases. This triggered a decrease in microbial CUE under warmed soil conditions in a future world, while microbial biomass turnover time decreased, pointing to faster microbial turnover and necromass formation. Soil N cycle processes were also not down-regulated ($R_{10}$) though we found thermal acclimation on protein depolymerization (decrease in $Q_{10}$). Under field conditions ($R_{field}$), warmer soils did not show differences in soil N processes other than protein depolymerization (decrease). Overall, our results indicate a complex response of microbial C and N cycle processes. Microbial C and N processes were not down-regulated ($R_{10}$), but the temperature sensitivity ($Q_{10}$) of most of them decreased, leading to a slight attenuation but still a stimulation of these processes in warmed soils ($R_{field}$). The stimulation of soil enzymes therefore did not translate into higher C and N process rates, indicating increased substrate limitation in warmer soils.

## 3.2 Zusammenfassung

Trotz der verstärkten Bemühungen, die Auswirkungen des Klimawandels auf die Kohlenstoff (C) Dynamik des Waldbodens zu verstehen, haben sich nur wenige Studien mit den langfristigen Auswirkungen der Erwärmung auf durch Mikroben vermittelte Boden-C- und Nährstoffprozesse befasst. Aufgrund der thermischen Akklimatisierung der mikrobiellen Gemeinschaft oder der Erschöpfung des labilen Boden-C als Hauptsubstrat für heterotrophe Bodenmikroben, lässt die anfängliche Stimulation des C-Zyklus des Bodens mit der Zeit in Langzeitversuchen zur Bodenerwärmung nach. Die thermische Akklimatisation kann wegen einer längeren Erwärmung auftreten und ist definiert als die direkte Reaktion des Organismus auf erhöhten Temperaturen über jährliche bis dekadische Zeitskalen, die sich als physiologische Veränderungen der mikrobiellen Gemeinschaft im Boden manifestieren. Dieser Mechanismus unterscheidet sich deutlich von der offensichtlichen thermischen Akklimatisation, bei der die abgeschwächte Reaktion von mikrobiellen Prozessen im Boden auf die Erwärmung auf die Erschöpfung der labilen C-Pools im Boden zurückzuführen ist. Das Achenkirch-Experiment in den nördlichen Kalksteinalpen in Österreich (47° 34' 50" N; 11° 38' 21" O; 910 m ü.M.) ist ein langfristiges Experiment zur Bodenerwärmung (> 15 Jahre, +4 °C Erwärmung über der Umgebung), die wichtige Einblicke in die Auswirkungen der globalen Erwärmung auf den C-Zyklus des Waldbodens liefert. Am Standort Achenkirch haben wir nach neun Jahren (2013) eine anhaltend positive Reaktion der heterotrophen Bodenatmung und des $CO_2$-Ausflusses auf die Erwärmung beobachtet, die zu einer geeigneten Umgebung wurde für die Prüfung von Hypothesen über anhaltende oder abnehmende Erwärmungseffekte auf dekadischen Skalen. Wir haben im Oktober 2019 Erdeproben von sechs beheizten und sechs Kontrollflächen mit Bodentiefe von 0-10 cm und 10-20 cm gesammelt und bei drei verschiedenen Temperaturen inkubiert: Außentemperatur, +4 ° C und +10 ° C. Wir haben die potenzielle Bodenenzymaktivitäten mit fluorometrischen Assays, die Bruttoraten der Protein-depolymerisation, Stickstoff (N)-Mineralisierung und Nitrifikation anhand den 15N-Isotopenpool-Verdünnungsansätzen sowie die mikrobiellen Wachstum- und $CO_2$ Produktion- und die Effizienz der C- und N-Nutzungen basierend auf dem $^{18}O$-Einbau in DNA gemessen. Unsere Ergebnisse zeigten, dass die potenziellen Enzymaktivitäten, einschließlich Leucinaminopeptidase, N-Acetylglucosaminidase, □-Glucosidase und saurer Phosphatase, durch dekadische Bodenerwärmung signifikant

hochreguliert wurden (1,3- bis 3,3-fach in $R_{10}$). Im Gegensatz dazu nahm ihre Temperaturempfindlichkeit ($Q_{10}$) mit der Erwärmung des Bodens ab, insbesondere für C- und N-bezogene Enzymaktivitäten, was auf eine thermische Akklimatisierung der Mikroben und die Produktion neuer Isoenzyme hinweist. Bei mikrobiellen C-Prozessen fanden wir keine Herunterregulierung ($R_{10}$) mit chronischer Erwärmung, während die Bodenerwärmung die $Q_{10}$ von mikrobiellen anabolen Prozessen merklich verringerte (Wachstum), blieb diese Verringerung bei $Q_{10}$ von katabolen Prozessen (Atmung) aus. Dies führte effektiv zu einer Abnahme der thermischen Empfindlichkeit von mikrobiellem CUE in erwärmten Böden. Gleichzeitig hatten die $Q_{10}$-Werte der mikrobiellen CUE weniger als eins, was zeigt, dass die mikrobielle CUE mit kurzfristigen Temperaturerhöhungen abnimmt. Dies löste in der Zukunft unter erwärmten Bodenbedingungen eine Abnahme des mikrobiellen CUE aus, während die Umschlagsdauer für mikrobielle Biomasse abnahm, was auf einen schnelleren mikrobiellen Umsatz und eine schnellere Bildung von Nekromassen hinweist. Boden-N-Prozesse wurden ebenfalls nicht herunterreguliert ($R_{10}$), obwohl wir eine thermische Akklimatisierung der Protein-Depolymerisation (Abnahme von $Q_{10}$) fanden. Unter Feldbedingungen ($R_{field}$) zeigten

wärmere Böden keine Unterschiede in den Boden-N-Prozessen außer der Protein-depolymerisation (Abnahme). Insgesamt deuteten unsere Ergebnisse auf komplexe Reaktionen von mikrobiellen C- und N-Prozessen hin. Mikrobielle C- und N-Prozesse wurden nicht herunterreguliert ($R_{10}$), aber die Temperaturempfindlichkeit ($Q_{10}$) der meisten mikrobiellen C- und N-Prozessen nahm ab, was zu einer leichten Abschwächung aber dennoch zu einer Stimulierung dieser Prozessen in erwärmten Böden ($R_{field}$) führte. Die Stimulation von Bodenenzymen führte daher nicht zu höheren C- und N-Prozessraten, was auf eine erhöhte Substratbegrenzung in wärmeren Böden hinweist.

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