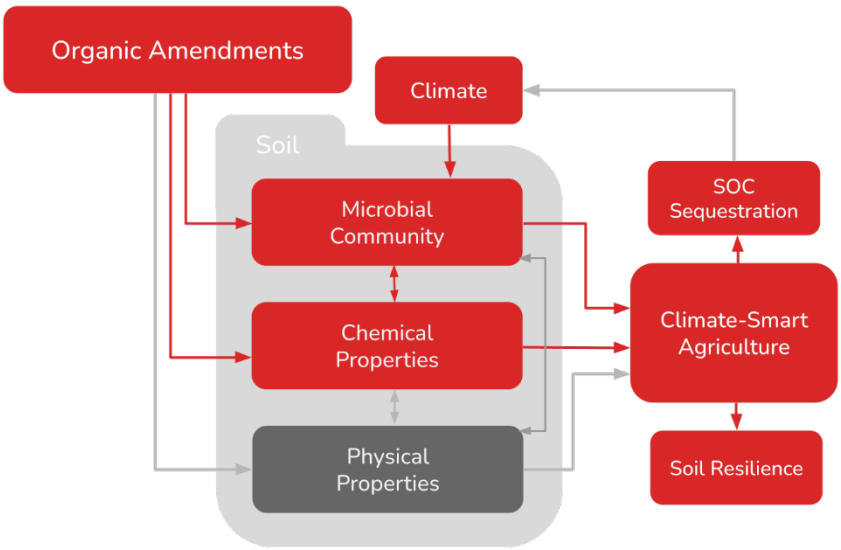
Soil organic amendments for climate-smart agriculture

Kok, D.D.


Organic Amendments
Climate
Soil
Microbial Community
Chemical Properties
Physical Properties
SOC Sequestration
Climate-Smart Agriculture
Soil Resilience

CHAPTER 4

Contrasting Effects of Different Organic Amendments on the Microbial Responses to Extreme Temperature Changes

Dirk-Jan Kok
Laura Scherer
Wim de Vries
Peter van Bodegom

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ABSTRACT

Soil organic amendments can change microbial communities, potentially impacting their resilience to environmental stresses and, consequently, their transformation of soil carbon and nitrogen. While several studies have investigated the impact of a few organic amendments on microbial resilience to stresses in the form of *absolute change* in environmental conditions, the potential stress effects of different *rates of change* have received much less attention. The rate of change in environmental properties such as temperature may induce different microbial stress responses, given the reliance of microbial defensive mechanisms on time-dependent signalling and activation processes. In this research, we explored the relevance of the rate of temperature change as a microbial stressor and evaluated the potential use of organic amendments in steering soil ecological response. We monitored the changes in microbial properties and concentrations of different carbon and nitrogen fractions to temperature change treatments of 2.5 and 30 °C d^{-1} after the application of three commonly available rural organic amendments to a podzol soil in a laboratory incubation study. Results showed significant effects of the temperature treatments on bacterial and total microbial DNA concentrations, as well as on dissolvable and insoluble carbon and nitrogen ratios regardless of the organic amendment treatment. Temperature impacts on respiration rates, priming rates and the concentration of soil hot water extractable carbon and nitrogen differed depending on the organic amendment treatment. Results demonstrated that compost-amended soils were least sensitive to the temperature treatments, while grasses and fermented grasses treatments were generally more sensitive and showed opposite responses. Our findings indicate that organic amendments might be utilized to manipulate the impact of environmental stresses on soil carbon and nitrogen concentrations, and highlight the need for future research to investigate the relevant ecological properties and mechanisms.

The data of this chapter been made publicly accessible in the DANS-Easy data repository under 10.17026/dans-zgj-n8r5.

Keywords: *Soil microbial community, soil carbon fractions, soil nitrogen fractions, microbial stress response, rate of temperature change*

4.1 INTRODUCTION

The survival and reproduction of soil microorganisms are often challenged by weather-induced stresses such as droughts (Manzoni, Schimel, et al. 2012), heat (Riah-Anglet et al. 2015), and freeze-thaw cycles (Sharma et al. 2006). To cope with these stresses, microorganisms rely on several adaptation and acclimation mechanisms aimed at relieving the associated heat shock or osmotic and oxidative pressures (Schimel, Balser, and Wallenstein 2007). However, enabling these mechanisms comes at a cost, forcing shifts in metabolism from growth and maintenance to survival pathways. Organisms that do not possess the relevant survival mechanisms or organisms that cannot energetically afford to maintain them (Kempes et al. 2017) are likely to perish.

When exposed to physiological challenges that threaten their function or survival, different microorganisms have demonstrated different vulnerabilities to stress partly due to differences in survival strategies (Chodak et al. 2015; de Vries et al. 2018). Many fungi, for instance, are capable of forming physical barriers between their cells and an intolerable environment by forming calcium oxalate crystals on the surface of their hyphae (Dutton et al. 1993). Bacteria, instead, mostly rely on dormancy (Manzoni, Schimel, et al. 2012), sporulation, the cytoplasmic accumulation of osmoregulatory solutes (Landesman and Dighton 2010) and the formation of exopolysaccharide protection layers (Kohler, Caravaca, and Roldán 2009). Survival mechanisms also vary at lower taxonomic levels (Chandra et al. 2021), where biochemical adaptation of different phyla reveals different trade-offs in enzymes and cell membrane structures (Hochachka and Somero 2002). The efficacy and costs of enabling these different survival mechanisms determine whether a microbial community can maintain or recover its composition and function and will thereby indirectly influence the impact a stress event has on local carbon (C) and nitrogen (N) transformations (Chodak et al. 2015; Crowther et al. 2015; Hueso, García, and Hernández 2012). A microbial community that remains unaffected during a specific stress, showing no changes in microbial properties or carbon and nitrogen transformations, could in this respect be regarded as resistant, whereas communities demonstrating significant changes in their properties and/or transformation of carbon and nitrogen, appear to instead be more sensitive to the stress. These stress-sensitive communities might also be regarded as resilient if they are able to recover to a similar state as compared to an unstressed community, after the stress has subsided (Huang et al. 2020).

Organic amendments may affect the resistance of a microbial community by changing the microbial community composition and its activity. Organic amendments may preferentially stimulate the growth of microorganisms whose metabolic efficiencies and/or life strategies (e.g. K- vs. r-strategists; copiotrophic and oligotrophic functional groups) are better aligned to the nutrient composition of the organic amendment applied (Balser, Kinzig, and Firestone

2013; Cotrufo et al. 2013; Kallenbach, Frey, and Grandy 2016; Lalande et al. 2005; Lehmann and Kleber 2015; Marschner et al. 2003). In this way, organic amendments may lead to a divergence in the growth of different taxonomic phyla, resulting in changes in microbial community composition and, thus, potentially, changes in the community stress response. Organic amendments may also change soil nutrient availability, thereby stimulating or suppressing the production of specific extracellular enzymes that may benefit or harm microbial survival (Allison et al. 2011; Mooshammer et al. 2017; Waldrop, Balser, and Firestone 2000). Organic amendments can thus render a microbial community more or less resistant or resilient to environmental stressors in multiple ways.

The adaptive sustenance of resilient cells, and fatality of vulnerable cells, developed under different OA regimes can subsequently affect microbial functioning with consequences for microbial-nutrient dynamics such as the microbial transformation of soil carbon and nitrogen (Chodak et al. 2015; Crowther et al. 2015; Hueso et al. 2012). Soil carbon and nitrogen transformations may be affected by microbial resilience at the physiological level due to the direct effects of activated survival mechanisms on nutrient flows. For instance, calcium oxalate crystal formation by fungi may, post-mortem, lead to the stabilization of recalcitrant carbon (Crowther et al. 2015). Furthermore, stress-enhanced enzyme activity by bacteria has been observed to reduce soil cellulosic and hemicellulosic carbon concentrations (Bouskill et al. 2016). Soil carbon and N may also be affected indirectly by changes in microbial resilience at a community level. For instance, microbes with specific growth strategies that are also capable of posing an efficient defence to a stress event might temporarily gain a competitive advantage over microorganisms that depend on different growth strategies and/or are incapable or inefficient in their defence. These organic amendment impacts likely operate at different temporal scales, where physiological changes mainly regulate short-term biogeochemical processes and shifts in community composition likely regulate them over a longer time period (Schimel et al. 2007). Thus, the impact of stress events on communities developed under different organic amendment regimes may result in temporally variable changes in microbial dynamics that can differently impact soil biogeochemistry. Yet, despite our long tradition of using organic amendments to improve soil fertility, few studies have attempted to identify and assess the value of different organic amendments in potentially steering soil ecological resilience or only do so for only a limited number of microbial or soil chemical variables (Fujino et al. 2008; Hueso et al. 2012; Ng et al. 2015; Sun et al. 2022).

Understanding microbial stress response and its implications for microbially-driven ecosystem functions is becoming increasingly important given the increasing prevalence of climate change-related environmental stresses (Allison et al. 2010; IPCC et al. 2021; Liang et al. 2017; Malik et al. 2020; Mooshammer et al. 2017; Wieder et al. 2015). Yet, while much research has been done on the microbial response to different magnitudes and exposure times of stresses such as temperature (Bérard et al. 2011; Kumar et al. 2014;

Morley et al. 1983; Riah-Anglet et al. 2015), water (Bastida et al. 2017; Canarini et al. 2016; Griffiths et al. 2003; Hueso et al. 2012; Landesman and Dighton 2010; Manzoni, Schimel, et al. 2012), and predation (Gao et al. 2019; Jiang et al. 2018; Thakur et al. 2021), the literature presents no research on soil microbial responses to the rate of stressor change - let alone the impacts of organic amendments thereupon. The rate of stressor change may be important given the costs for soil microorganisms in activating different physiological stress-response mechanisms (e.g. formation of calcium oxalate crystals vs. triggered dormancy). Consequentially, not only the absolute change in an environmental property might be relevant in driving a microbial stress response, but also the rate of change of that property (i.e. the speed at which the environment changes).

A potential microbial stressor may be the increasing rate of temperature change as a result of increases in the diurnal temperature range (DTR), i.e. the difference between maximum and minimum temperatures within a 24-hour day. Climate change has increased regional DTR in surface air by more than 2 °C, for instance, in Mexico (Englehart and Douglas 2005), Western Himalaya (Yadav et al. 2004), central Eurasia (Wang and Dillon 2014), western North America and Australia (Vose, Easterling, and Gleason 2005). The DTR in other areas of the world is similarly changing, as a number of studies have reported regionally variable increases and decreases in DTR over the past decades (Jhajharia and Singh 2011; Vose et al. 2005; Wang and Dillon 2014). Such changes in DTR can significantly impact ecosystems, as has been demonstrated by the high mortality response observed in a study with zebra finches (Briga and Verhulst 2015). Furthermore, changes in DTR can impact the soil, as soil temperatures are directly affected by air temperature (JiKang et al. 2009). Desert soils already exhibit high DTRs, where surface soil temperatures change at an average of 38 °C d^{-1} (Gupta and Gupta 1982). The effect of air temperature oscillations for these soils can be traced down at least to a depth of 30 cm (Gupta and Gupta 1982). As very little is known about the potential impacts that rates of temperature change may have on soil microorganisms, it is of interest, especially in the context of a globally changing climate, to explore how microorganisms respond to such changes and to evaluate the role that organic amendments can play in remediating any negative effects.

The objective of this study was to investigate the effect of organic amendments on the microbial stress response to different rates of temperature change in an agricultural podzol soil. By means of a laboratory incubation study, we evaluated changes in microbial functioning in terms of soil carbon and nitrogen transformations, as well as changes in several microbial properties, i.e. microbial biomass, biomass growth, community composition (bacterial to fungal DNA ratios), carbon-use efficiencies (CUE), metabolic quotients (qCO_2), priming rates and nitrogen and carbon mineralization activity. We determined the sensitivity of a microbial community developed under different organic amendment treatments based on differences in microbial functions (i.e. carbon and nitrogen

transformations) and properties of amended and unamended soils in temperature change stressed systems versus amended and unamended soils in an unstressed control system (i.e. no temperature change). We hypothesized that the application of organic amendments would increase the sensitivity of the microbial community to more extreme temperature changes, as organic amendments are likely to increase microbial biomass and diversity, consequently allowing for a more extreme or diverse microbial response.

4.2 MATERIALS AND METHODS

4.2.1 Soil preparation

The incubation experiment was performed on Podzol soil (WRB-FAO classification; 0-30 cm depth; bulk density = 1.15 g cm^{-3}; pH = 5.19; soil moisture = 20.38%; 1.64% carbon; 90.94% sand and 9.06% silt and clay particle size fractions) acquired from an agricultural site with a maize cropping history from Haarlo, the Netherlands (52.10 N, 6.59 E). The site has an annual mean temperature of 10°C, an average minimum of 0°C and maximum of 21°C, and an average annual precipitation of 825 mm for the period 1991-2020 (KNMI, 2022). A podzol was chosen given the prevalence of agricultural production on podzol in much of Europe (Lee 1987) and the likely vulnerability of this agricultural production to environmental stresses given the sensitivity of, for instance, vegetation in podzol grasslands to extreme climate events (Mulder et al. 2019). The podzol was sieved at 2 cm and thoroughly homogenized before 50-gram dry weight equivalent (d.w.e.) aliquots were filled into sterile, polypropylene incubation pots. Three incubation pots (n=3) were prepared for each sampling moment and each temperature-amendment treatment (96 pots total).

4.2.2 Organic amendments

The incubation pots were amended with isotopically enriched i) roadside grasses, ii) compost from roadside grasses, or iii) Bokashi-fermented roadside grasses. Isotopic enrichment of the organic amendments was achieved by cultivating an excavated plot of roadside grasses in a climate chamber while pulse-labelling with ^{13}C-CO_2. The details of composting and Bokashi fermenting are more elaborately described in Kok *et al.* (2022), and involve conventional aerobic composting of the grasses after inoculation with household compost leachate, and an anaerobic fermentation of grasses after inoculation with commercial Bokashi 'effective microorganisms' (EMs). We decided to apply roadside grasses given its abundant availability, its potential for agricultural application in the Netherlands (Elbersen and Spijker 2014), its ease of isotopic labelling and processability into compost and Bokashi, and because of its relatively unknown and underexplored impact on soils. Images of the plant diversity in the roadside grasses mixture are presented in

Appendix G. The chemical properties of the different organic amendments are described in Appendix H.

For each treatment, 0.5 g d.w.e. of organic amendment was manually mixed into each 50 g pot of soil (1%, 0.01 g OA g-1 soil). Unamended control pots were also mixed to ensure equal physical disturbance across samples. To account for potentially confounding effects such as the immediate microbial response to soil disturbance (i.e. mixing), samples were subjected to a pre-incubation of approximately 2 months (59 days) at 60% water-holding capacity and 20°C.

4.2.3 Temperature change treatments

After pre-incubation, samples were exposed to either i) a gradual temperature change ($T_{Gradual}$=2.5 °C d^{-1}), ii) a rapid temperature change (T_{Rapid}=30 °C d^{-1}), or iii) no temperature change, i.e. a constant 20°C, ($T_{Control}$=0 °C d^{-1}). To negate as much as possible the effect of absolute temperature change and thereby isolate the effect of the rate of temperature change, we designed the temperature fluxes such that the net temperature dose at the end of the experiment (final average temperature) would be approximately equal for all treatments (20 °C). To achieve this, the temperatures of the gradual and rapid change treatment were initially reduced to 2°C at 2.5°C d^{-1} before being increased to 32°C at their respective rates (Figure 1). Minimum and maximum temperatures were constrained at 2°C and 32°C to avoid freezing effects while remaining within the range of realistic maximum temperatures observed in the field. The temperature pulse treatments were maintained at maximum temperature for different durations to ensure approximately equal temperature 'doses' among treatments (i.e. same area under the temperature curve; Figure 1). Consequentially, the rapid temperature change treatment was maintained at 32°C for four days longer than the gradual temperature change treatment (11 versus 7 days). During the experiment, incubation pots exposed to a temperature stress were destructively sampled four times: i) prior to stress treatment (baseline/start/directly after preincubation), ii) at the attainment of peak temperature (presumed peak stress), iii) 10-14 days post-stress (after return to 20 °C), and iv) approximately two months post-stress (after return to 20 °C, experiment end). Focusing on the rate of temperature change, and not the occurrence/non-occurrence of a temperature pulse, only the gradual and rapid temperature change treatments were tested for significant differences. Nevertheless, the soils subjected to a constant temperature treatment were sampled at the start and end of the experiment for reference. Soil moisture was maintained at 60% by weighting the incubation pots every 2 to 3 days and compensating for the amount of water evaporated.

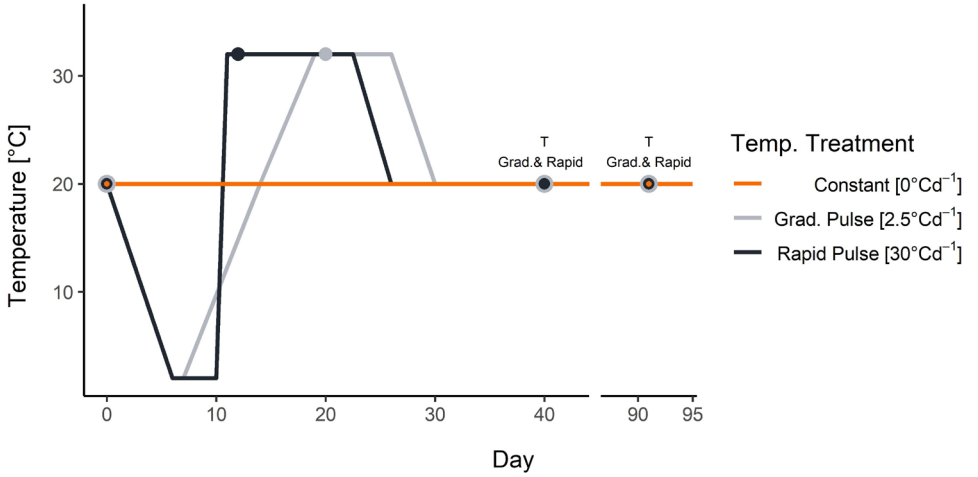

Figure 4.1 Overview of temperature treatments and sampling times, where the differently coloured lines represent the different temperature treatments and the differently coloured points indicate the sampling time for each treatment. 'T' marks when the data was subjected to statistical testing (section 2.5).

4.2.4 Determination of microbial response variables

Five microbial properties and processes were measured, from which seven others were additionally derived. Total microbial biomass carbon, community composition based on bacterial and fungal DNA concentrations, mineralization activity as respired CO_2-carbon, and mineral nitrogen concentration (sum NH_4^+-nitrogen + NO_3^--nitrogen + NO_2^--nitrogen) were measured. Amendment-derived biomass carbon ($\delta^{13}MBC$), total microbial DNA concentrations, bacterial to fungal DNA ratios (B:F), biomass yield efficiencies (YE) and metabolic quotients (qCO_2), and priming rates were calculated based on the aforementioned measured microbial properties and their isotopic signatures (see section 2.4).

The measured properties were determined following methods outlined in Kok et al. (2022). In summary, microbial biomass carbon was determined based on the chloroform-fumigation technique (Brookes et al. 1985; Vance et al. 1987). Carbon respiration was determined by headspace sampling after 24 hours, one day prior to the destructive chemical sampling of the incubation jars, and by use of a gas chromatograph (Thermo Finnigan Trace) coupled to an isotope-ratio mass spectrometer (IRMS; Thermo Scientific Delta 5). The B:F DNA ratio was quantified through droplet digital polymerase chain reaction (ddPCR), a demonstrably sensitive and accurate tool for the absolute quantification of DNA (Doi et al. 2015; Hindson et al. 2013; Nathan et al. 2014; Trimbos et al. 2021). For the ddPCR quantification, microbial DNA was extracted from 250 mg of soil from each sample using a DNeasy

PowerSoil Pro DNA Extraction Kit (Qiagen). ddPCR was performed following the manufacturer's standard EvaGreen® protocol for the QX200™ ddPCR™ system (Bio-Rad Laboratories, Hercucles, CA, USA). Bacterial 16S rRNA subunit genes were targeted for amplification using 341 forward (CCTACGGGNGGCWGCAG) and 785 reverse (GACTACHVGGGTATCTAATCC) primers (Klindworth et al. 2013; Thijs et al. 2017). Fungal ITS gene amplification was achieved using ITS1 forward (TCCGTAGGTGAACCTGCGG) and 5.8s reverse (CGC TGC GTT CTT CAT CG) primers (Fierer et al. 2005). The protocol for quantification of the B:F DNA ratio by ddPCR is further described in Appendix A.

4.2.5 Determination of soil organic carbon and nitrogen fractions

Dissolvable (DO), hot-water extractable (HW), and non-hydrolysable (NH) soil organic fractions were determined by stepwise, wet-chemical extraction (schematically outlined in Appendix I). The DO fraction was extracted with 0.05M potassium sulphate (K_2SO_4) at a ratio of 36 ml per 12.00 g of soil. The mixes were vortexed at room temperature on a vortex shaker for 1 hour. The HW fraction was extracted from the residue of the K_2SO_4 extraction with 36 ml of 80°C deionised water for 18 hours. Both the DO and HW soluble extracts were separated from the residue by centrifugation for 10 minutes at 3000 rpm. The supernatant of each was recovered and centrifuged a second time for 10 minutes, filtered at 2.5 µm porosity using cellulose syringe filters, and dehydrated in a ventilated oven at 70°C. The NH fraction was recovered through two-stage hydrolysis of the hot-water residue. In the first hydrolysis step, the residue was combined with 2 ml of 26N sulfuric acid (H_2SO_4) and shaken for 17 hours at room temperature. In the second hydrolysis step, the mixture was diluted to 2N and left to further hydrolyse at 90°C for 3 hours. The sample was then centrifuged, and the supernatant discarded. The residue was washed thrice with demineralised water and oven-dried at 70°C. Approximately 25-70 mg of each dried extract was analysed for total carbon and nitrogen contents and $\delta^{13}C$ and $\delta^{15}N$ signatures through an elemental analyser coupled to an isotope ratio mass spectrometer (EA-IRMS). Soil mineral nitrogen concentrations were determined from 12.00 g of soil through a colorimetric procedure based on the salicylate method (Mulvaney 1996) and reduction-Greiss reactions (Miranda, Espey, and Wink 2001). The concentration of carbon and nitrogen in each fraction was gravimetrically corrected for the water content of the originally extracted soil.

4.2.6 Calculations

4.2.6.1 Organic amendment and soil-derived carbon fractions

The determination of CUE and priming effects requires a distinction between exogenous carbon derived from organic amendments and endogenous carbon derived from the soil. This separation was achieved through isotope tracing and the application of a two-end-member mixing model. Firstly, the $\delta^{13}C$ (‰) enrichment of each carbon pool or flux was determined in reference to the international Vienna-Pee Dee Belemnite (VPDB) standard following eq. 4.1:

$$\delta^{13}C = \left[\left(R_{sample}/R_{VPDB}\right) - 1\right] \cdot 1000 \qquad (4.1$$

Where R_{sample} is the measured isotope ratio of the sample, and R_{VPDB} is the isotope ratio of the International Vienna-Peedee Belemnite standard (R_{VPDB}= 0.011802). Subsequently, the two end-member mixing model was applied following (eq. 4.2; Amelung et al. 2008):

$$F_{OA} = \frac{\delta^{13}_{final} - \delta^{13}_{initial}}{\delta^{13}_{OA} - \delta^{13}_{initial}} \qquad (4.2$$

Where F_{OA} is the fraction of organic amendment derived carbon; δ^{13}_{final} is the bulk measured isotope ratio; $\delta^{13}_{initial}$ is the original isotope ratio of the unamended soil, and δ^{13}_{OA} is the isotope ratio of the enriched organic amendment. Final concentrations of amendment-derived carbon were calculated by multiplying F_{OA} with the total carbon concentration.

4.2.6.2 Organic-amendment-derived biomass, carbon-use efficiency, and metabolic quotient

Amendment-derived biomass carbon, $\delta^{13}MBC$, is the amount of carbon from organic amendments that is incorporated into microbial biomass (MBC: µgMBC-δ^{13}C g^{-1}), and was calculated following eq. 4.3 and eq. 4.4 by Geyer et al. (2019).

$$at\%MBC = \frac{at\%DOC_F \cdot DOC_F - at\%DOC_{NF} \cdot DOC_{NF}}{(DOC_F - DOC_{NF})} \quad (4.3$$

$$\delta^{13}MBC = (DOC_F - DOC_{NF}) \cdot \frac{at\%MBC_t - at\%MBC_c}{at\%OA - at\%MBC_c} \quad (4.4$$

where $at\%DOC_F$, DOC_F, $at\%DOC_{NF}$, and DOC_{NF} represent the atom % and total carbon concentrations (µg C g^{-1} soil) of fumigated (F) and non-fumigated (NF) K_2SO_4 soil extracts, respectively; $at\%MBC_t$ and $at\%MBC_c$ are the atom % of sample treatments and natural abundance controls, and $at\%OA$ is the atom % of the organic amendment.

Carbon-use efficiency (CUE; %) is the ratio of carbon incorporated into biomass to the sum of carbon incorporated into biomass and respired as CO_2. It is calculated following eq. 4.5 (Geyer et al. 2019):

$$CUE\% = \delta^{13}MBC / [\delta^{13}MBC + \delta^{13}R_C] \cdot 100 \quad (4.5$$

Where $\delta^{13}R_C$ is the cumulative respired amendment-derived CO_2-carbon (µgCO_2-δ^{13}C). The microbial metabolic quotient (µg CO_2-C μg^{-1} MBC d^{-1}) is the microbial respiration per unit microbial biomass and is calculated as: $Q_{CO2} = R/MBC$ where R represents the respiration rate (µgCO_2-C $g^{-1}d^{-1}$) and MBC (µgC g^{-1}) the total microbial biomass carbon concentration.

4.2.6.3 Priming rates

The priming rate (PR; µgCO_2-C g^{-1}soil d^{-1}) represents the additional respiration derived from the soil endogenous carbon pool after the application of an organic amendment when compared to an unamended control soil. We calculated priming rates by eq. 4.6:

$$PR = R_{Soil}^{trt} - R_{Soil}^{control} \quad (4.6$$

where R_{Soil}^{trt} (μgCO$_2$-C g^{-1}d^{-1}) is the rate of soil-derived CO_2 respiration for a given organic amendment treatment, and $R_{Soil}^{control}$ (μgCO$_2$-C g^{-1}d^{-1}) is the rate of soil-derived CO_2 respiration for the unamended control soil. The R_{Soil}^{trt} was calculated following eq. 4.7

$$R_{Soil}^{trt} = (1 - F_{R,OA}) \cdot R_{Total}^{trt} \quad (4.7$$

where $F_{R,OA}$ is the fraction of amendment-derived CO2 as calculated per eq. 4.2 and R_{Total}^{trt} (μgCO2-C g−1d−1) is the total respiration rate (soil and amendment derived) for a given organic amendment-treated soil.

4.2.7 Statistical analysis

Three-way analysis of variance (ANOVA) was performed to evaluate for significant main and interaction effects of organic amendments, temperature treatments and sampling times on soil microbial and chemical properties for the temperature pulse treatments ($T_{Gradual}$ and T_{Rapi}) on day 40 and day 91. Tukey's post-hoc test of pairwise comparisons was applied when significant, temperature-related main or interaction effects were detected by the ANOVA ($\alpha = 0.1$). In such a case, all statistically non-significant higher effect terms (if any) were dropped from the model. The data was then plotted as the estimated marginal means (EMMs) for significant main and 2-way interaction effects and by observed means for 3-way interaction effects. Significant differences between groups or treatments were labelled with the results from the Tukey's pairwise comparisons ($\alpha = 0.1$). Plots of observed means and pairwise comparisons for all data, regardless of their significance, are presented in Appendix J. The statistical analyses were executed using the package 'emmeans' (Lenth 2020) in RStudio (RStudio Team 2020).

4.3 RESULTS

4.3.1 Response of microbial and soil properties to organic amendments and temperature treatments

ANOVA results show that the temperature change treatments significantly impacted the majority (13/20) of soil and microbial properties in the days following the temperature pulses (Table 4.1). For all OAs and both sampling moments (day 40 and 91), temperature treatments impacted dissolvable carbon (C_{DO}), non-hydrolysable carbon (C_{NH}) and nitrogen (N_{NH}), and their ratio, C_{NH}:N_{NH} ($p(T)<0.1$). Temperature impacts were significant, but

differently affected by sampling time, for total microbial and bacterial DNA concentrations, the B:F DNA ratio and C_{do}:N_{do} (p(S:T)<0.1). Temperature impacts on CO_2 respiration rates, priming rates (PR), and the hot water extractable carbon to nitrogen ratio (C_{HW}:N_{HW}) varied depending on the organic amendment treatments (p(A:T)<0.1). Finally, temperature impacts on C_{HW} and N_{HW} were influenced by both the organic amendment treatment and the sampling day (p(A:S:T)<0.1). All of the twenty measured properties were significantly affected by the organic amendment treatments (p(A)<0.1). Complete ANOVA results are presented in Appendix .K

Table 4.1 ANOVA F-statistics and significance for the impacts of amendment (A), sampling time (S), temperature treatment (T), and associated interaction terms on the investigated microbial and soil properties for the last two sampling days (40 and 91). Symbols represent the significance of the differences between pairs: p<0.001 = '***'; p<0.01 = '**'; p<0.05 = '*'; p<0.1 = '•'. Highest order, statistically significant effect related to temperature are emboldened per property. Microbial and soil properties in italics show significant differences with temperature and are discussed in further detail below.

ANOVA-III		**Effect Term (F-statistic)**						
Microbial Properties		**A**	**S**	**T**	**A:S**	**S:T**	**A:T**	**A:S:T**
Biomass	Carbon	31.9***	2.70	0.58	1.17	0.06	1.24	0.79
	DNA-Conc.	11.0***	1.58	0.27	4.01*	**15.2*****	1.60	1.72
	δ^{13}MBC	129.***	14.6***	0.75	1.65	0.06	1.04	1.53
Structure	Fung. DNA	24.9***	0.51	0.21	0.80	1.33	0.42	0.64
	Bact. DNA	5.03**	1.56	0.26	3.70*	**13.4*****	1.26	1.07
	B:F DNA	17.8***	0.00	0.43	0.57	**3.68•**	0.26	0.03
Respiration	CO_2-Rate	6.60**	1.10	0.70	0.02	1.89	**2.70•**	0.59
Metabolism	Efficiency	6.48**	2.09	0.51	0.85	0.14	0.93	0.86
	Quotient	10.1***	3.09•	0.10	1.54	0.66	0.02	0.77
Priming	Priming Rate	3.27•	0.03	0.02	0.02	0.71	**3.46***	0.67
Soil Properties								
Mineral	Nitrogen	384.***	14.6***	0.11	1.51	0.93	1.96	1.09
Dissolvable	Carbon	67.0***	8.58**	**3.33•**	0.91	0.84	0.35	0.10
	Nitrogen	39.1***	1.09	0.07	2.9•	0.69	0.75	0.04
	C:N	15.9***	0.19	0.56	1.65	**3.42•**	0.42	0.14
Hot water-	*Carbon*	95.0***	8.29**	2.39	1.21	0.06	1.04	**2.87•**
Extractable	*Nitrogen*	38.9***	0.06	0.04	0.20	0.01	3.81*	**3.12***
	C:N	7.46***	4.30*	1.89	1.26	0.19	**4.02***	0.77
Non-	*Carbon*	12.7***	0.00	**5.05***	0.08	0.17	0.52	2.16
Hydrolysable	*Nitrogen*	15.7***	2.80	**7.46***	0.42	0.16	0.57	1.10
	C:N	19.1***	4.16*	11.0**	0.48	0.12	**4.60****	1.03

4.3.2 Temperature main effects

Tukey post-hoc testing of soil properties with significant main temperature effects, and no higher-order temperature-related interaction effects, revealed significantly lower concentrations of C_{DO} (Figure 4.2A), and higher concentrations of C_{NH} (Figure 4.2B) and N_{NH} (Figure 4.2C) in soils subjected to the rapid temperature change treatment (T_{Rapid}) versus the gradual temperature change treatment ($T_{Gradual}$). This effect was present for both sampling days, on days 40 and 91, and for all organic amendments.

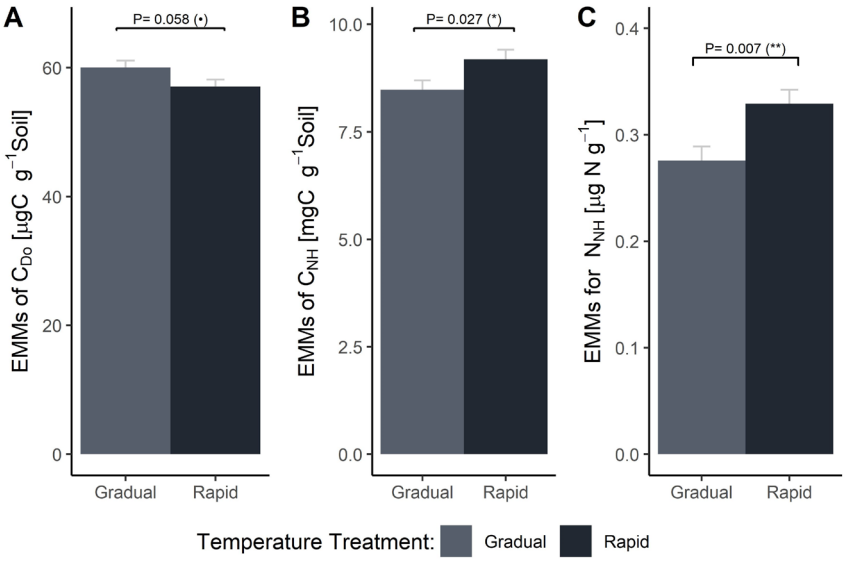

Figure 4.2 EMMs (Estimated Marginal Means; n=24 for each bar) for soil properties where the ANOVA identified significant main effects of temperature treatments. A) dissolvable carbon concentrations, B) non-hydrolysable carbon concentrations, and C) non-hydrolysable nitrogen concentrations. Symbols represent the significance of the differences between pairs: $p<0.01$ = '**'; $p<0.05$ = '*'; $p<0.1$ = '•'.

4.3.3 Temperature impacts per sampling day

Pairwise comparisons for temperature impacts per sampling day show that total microbial DNA concentrations (Figure 4.3A), bacterial DNA concentrations (Figure 4.3B), B:F DNA ratios (Figure 4.3C) and C_{DO}:N_{DO} ratios (Figure 4.3D) show opposite changes over time for T_{Rapid} versus $T_{Gradual,}$ as concentrations. Each of these microbial indicators was markedly lower for T_{Rapid} versus $T_{Gradual}$ on day 91, but respectively higher on day 40. These differences, however, were not statistically significant on day 91 for the B:F DNA ratio (Figure 4.3C) and not on day 40 for the C_{DO}:N_{DO} ratios (Figure 4.3D).

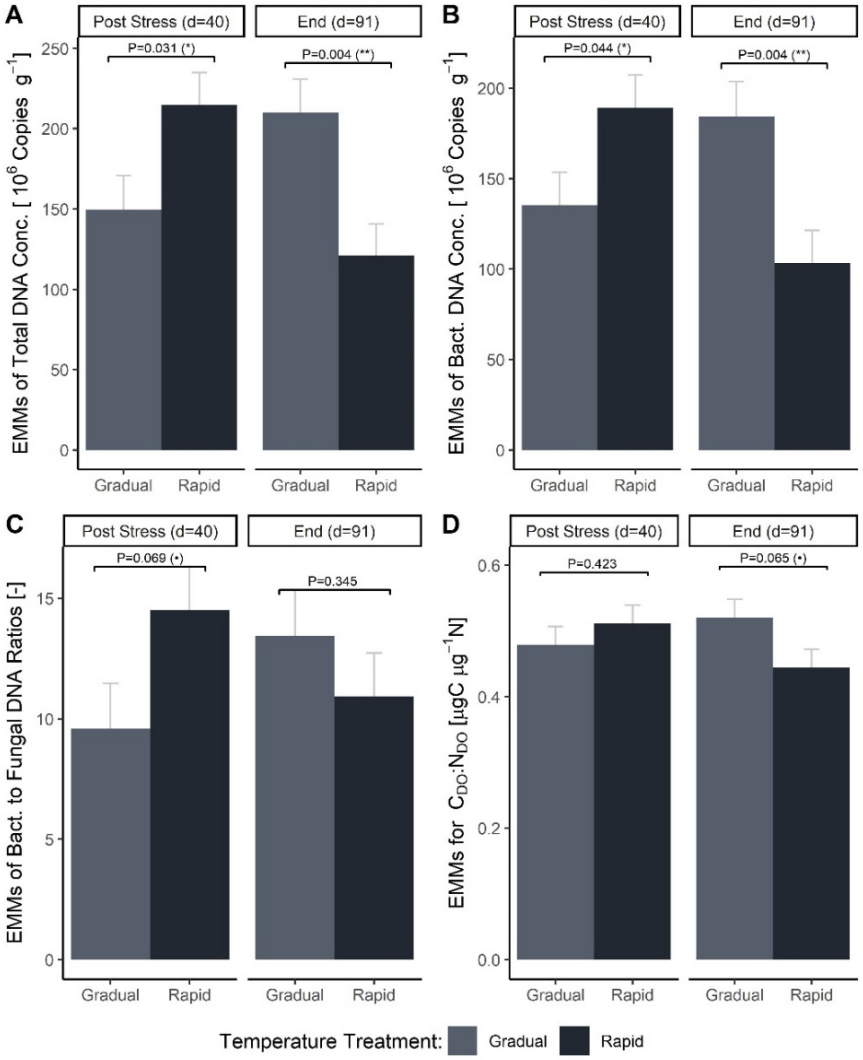

Figure 4.3 EMMs (Estimated Marginal Means; n=12 for each bar) for soil properties where the ANOVA identified significant interaction effects of temperature treatments with sampling day. A) total microbial DNA concentrations, B) bacterial DNA concentrations, C) ratio of bacterial to fungal DNA (B:F) and D) dissolvable carbon to nitrogen ratio (C_{DO}:N_{DO}). Symbols indicate the significance of the differences between pairs: $p<0.01$ = '**'; $p<0.05$ = '*'; $p<0.1$ = '•'.

4.3.4 Temperature interaction effects with organic amendments

Post-hoc testing of soil properties with significant temperature-amendment interaction effects showed significantly lower CO_2-respiration rates (Figure 4.4A), priming rates (Figure 4.4B) and C_{HW}:N_{HW} (Figure 4.4C) in T_{Rapid} versus $T_{Gradual}$ for soil amended with the grasses organic amendment. Pairwise comparisons showed no significant differences in EMMs at this level for C_{NH}:N_{NH} (Figure 4.4D). Inversely, higher CO_2-respiration rates (Figure 4.4A), priming rates (Figure 4B) and C_{HW}:N_{HW} (Figure 4.4C) were found in T_{Rapid} versus $T_{Gradual}$ for the fermented grasses treatment, while the effects on priming rates were not significant (though nearly so; p=0.104).

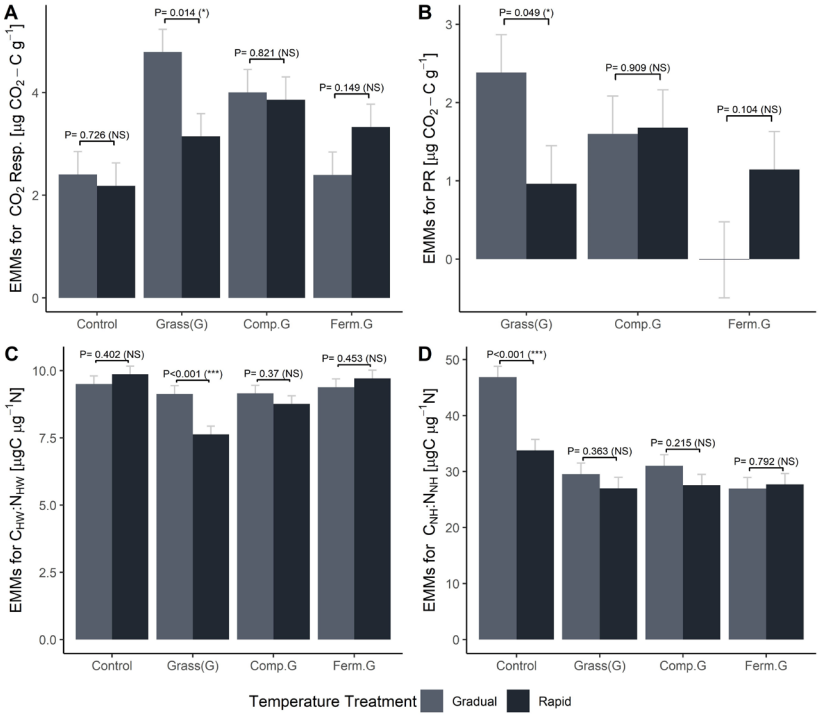

Figure 4.4 EMMs (Estimated Marginal Means; n=6 for each bar) for soil properties where the ANOVA identified significant interaction effects of temperature treatments with organic amendment treatments. A) carbon respiration rates (CO_2-resp.), B) priming rates (PR), C) hot water carbon to nitrogen ratio (C_{HW}:N_{HW}) and D) non-hydrolysable carbon to nitrogen ratio (C_{NH}:N_{NH}). 'Comp. G.' stands for composted grasses, and 'Ferm. G.' for fermented grasses. Symbols indicate the significance of the differences between pairs: p<0.001 = '***'; p<0.05 = '*'; p>0.1 = 'NS' (Not Significant).

4.3.5 Temperature impacts per organic amendment and sampling day

Post-hoc testing of the significant three-way interaction effects revealed that, at the end of the experiment on day 90, T_{Rapid} resulted in significantly lower C_{HW} (Figure 4.5) and N_{HW} (Figure 4.6) than $T_{Gradual}$ for the fermented grasses treatment. Contrastingly, for the regular grasses treatment, N_{HW} was significantly higher for T_{Rapid} than $T_{Gradual}$ (Figure 4.6). Differences between the temperature treatments for these and other organic amendments were not statistically significant shortly after the temperature pulse, on day 40 of the experiment. Other amendments did not show significant differences between $T_{Gradual}$ and T_{Rapid} on any day for C_{HW} and N_{HW}.

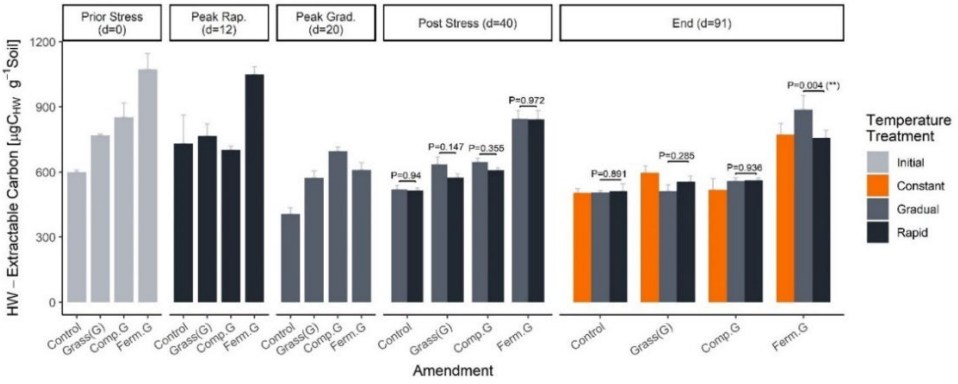

Figure 4.5 EMMs (Estimated Marginal Means; n=3 for each bar) for hot water (HW) extractable carbon. Symbols indicate the significance of the differences between pairs: p<0.01 = '**'. 'Comp. G.' stands for composted grasses, and 'Ferm. G.' for fermented grasses. Data for days 0, 12 and 20, as well as any data for the constant temperature treatment, were not tested for significant differences as it would have resulted in an unbalanced ANOVA.

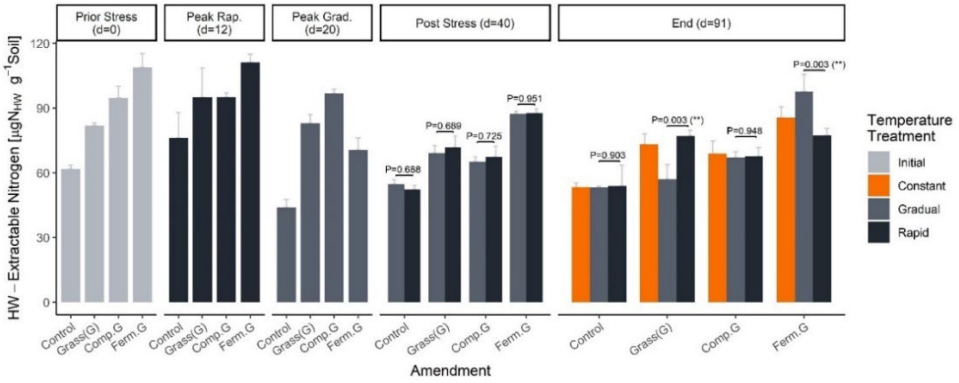

Figure 4.6 EMMs (Estimated Marginal Means; n=3 for each bar) for hot water (HW) extractable nitrogen, as the ANOVA identified significant 3-way interaction effects of temperature treatments with organic amendment and sampling day for this soil property. Symbols indicate the significance of the differences between pairs: p<0.01 = '**'. 'Comp. G.' stands for composted grasses, and 'Ferm. G.' for fermented grasses. Data for days 0, 12 and 20, as well as any data for the constant temperature treatment, were not tested for significant differences as it would have resulted in an unbalanced ANOVA.

4.4 DISCUSSION

4.4.1 Microbial response to temperature treatments

Our results show that microbial communities responded to different rates of temperature changes as we measured differences in bacterial DNA concentrations and total microbial DNA concentrations between $T_{Gradual}$ and T_{Rapid}, regardless of the organic amendment treatment (Figure 4.3). These differences in DNA concentrations and ratios were significantly higher in T_{Rapid} than $T_{Gradual}$ shortly after the temperature treatments and significantly lower approximately two months thereafter.

To our knowledge, no other studies have explored the impact of the rate of change of temperature on soil microbial properties, which complicates the evaluation of our results. However, similarities and differences with studies exploring microbial responses to *absolute* temperature changes allow explaining some of the responses observed in the current study. For instance, meta-analyses have shown that the vast majority of soil-warming experiments observe increases in bacterial abundances as well as changes in microbial community structure (Allison and Martiny 2008). Increases in microbial growth with temperature are well established in quadratic and exponential equations such as Arrhenius and Q_{10} functions (Schipper et al. 2014) and have been observed empirically in both environmental (Donhauser et al. 2020; Rinnan et al. 2009; Rousk, Frey, and Bååth 2012) as well as pure culture studies (Ratkowsky et al. 1983). Such non-linear responses of microbial growth may have partly affected the differences in responses to T_{Rapid} vs. $T_{Gradual}$, given that – to obtain an equal temperature dose - T_{Rapid} was exposed to 32°C temperatures for four days longer than $T_{Gradual}$. Our findings nevertheless reveal that any potential negative effects of the rapid 30°C d^{-1} rate of change certainly did not outweigh the significantly greater benefit for T_{Rapid} of remaining at 32°C for four days longer than $T_{gradual}$.

Approximately two months after the temperature treatments, bacterial DNA concentrations, total microbial DNA concentrations and B:F DNA ratios were significantly lower in T_{Rapid} than $T_{Gradual}$, reflecting more closely the changes that we had expected to see on day 40, two weeks after the treatments. While T_{Rapid}, likely due to its prolonged exposure to 32°C, appears to have benefited the microbial community in the short term, it also had impacts that appear more negative in the longer term. Lower microbial abundances on day 91 of the current study might be explained by over-consumption of substrates during earlier growth phases, leaving limited substrates remaining to sustain future growth. These findings are supported by the lower C_{DO} and higher C_{NH} and N_{NH} concentrations found in T_{Rapid} for all sampling moments after the pulses (Figure 4.2). Dissolvable compounds (i.e. C_{DO} and N_{DO}) encompass predominantly low molecular weight organic substances such as amino acids, carboxylic acids, and carbohydrates, which are of higher bioavailability (Fischer et al.

2007). Acid non-hydrolysable compounds (i.e. C_{NH} and N_{NH}) are instead believed to largely consist of difficultly decomposable ligneous substances (Melillo et al. 1989), substances that have been transformed over the course of microbial decomposition (Baskaran et al. 2019), and can include microbial biomass in the form of cell wall melanins and the molecules complexed within them (Nosanchuk, Stark, and Casadevall 2015). Higher concentrations of non-hydrolysable fractions in T_{Rapid} might, therefore, originate from a microbial transformation of more readily decomposable compounds at near-optimal growth temperatures, potentially also including some contributions of cell wall material from microbes that have since deceased.

Sustained growth in T_{Rapid} soils may furthermore have been constrained by arising stoichiometric imbalances. Stoichiometric theory postulates that decomposition by microorganisms is significantly affected by the microbial need to maintain stoichiometric homeostasis (Buchkowski, Schmitz, and Bradford 2015; Cleveland and Liptzin 2007; Manzoni et al. 2010; Spohn 2016). Significantly lower C_{DO}:N_{DO} ratios measured in T_{Rapid} coincide with differences observed in DNA concentrations, which might be evidence that microbial growth was stoichiometrically constrained. Overall, our findings show that the temperature treatments can have temporally variable impacts on soil microbial properties by either modulating microbial growth conditions directly, i.e. by stimulating microbial activity under higher temperature, or indirectly, through potential substrate depletion effects or stoichiometric imbalances arising thereafter.

4.4.2 Effect of organic amendments on the impact of the temperature treatments

We hypothesized that the application of organic amendments would increase the sensitivity of the microbial community to temperature changes, given that organic amendments are likely to increase microbial biomass and diversity, consequently allowing for a more extreme or diverse microbial response. Evidence for an increased sensitivity of microbial communities in amended soils can be sought in the greater differences between T_{Rapid} and T_{Grad} compared to unamended control soils for CO_2 respiration rates and C_{HW} and N_{HW} concentrations in soils amended with grasses and/or fermented grasses. However, the hypothesis cannot be convincingly accepted as temperature impacts on microbial biomass and DNA concentrations were not affected by the application of any organic amendment type. Of the organic amendments investigated, grasses and fermented grasses treatments demonstrated greater sensitivity to T_{Rapid} for a number of soil properties than for the unamended control soil. Compost, instead, was hardly impacted temperature treatments and showed a mostly similar response as was observed for the unamended control soil. Surprisingly, grasses and fermented grasses treatments altered soil sensitivity in different ways. In the grasses treatment, CO_2 respiration and priming rates were consistently lower

for T_{Rapid} versus $T_{Gradual}$, whereas for the fermented grasses treatment, they were consistently higher (Figure 4.4). Similar opposite effects were observed for C_{HW} and N_{HW} on day 91 of the experiment, where grass-amended soils exposed to T_{Rapid} showed higher concentrations of C_{HW} while fermented grass-amended soil showed much lower concentrations in C_{HW} (as well as N_{HW};Figure 4.4, Figure 4.5 and Figure 4.6).

Overall, our experimental results demonstrate that *microbial properties* (e.g. biomass and diversity; Figure 4.3) were not affected by temperature-amendment interaction effects. However, *microbial functions,* in terms of respiration and priming rates (Figure 4.4), as well as soil C_{HW} and N_{HW} transformations, were (Figure 4.4, Figure 4.5 and Figure 4.6). A disconnect between changes in microbial properties with changes in microbial processes and soil carbon and nitrogen interactions has also been observed in other studies. Three months after a nine-day heat-stress cycle (23°C to 30°C), Mooshammer et al. (2017) measured significant differences in the temperature impact on N/P mineralization rates, cellobiosidase activity and PO_4^{3-} consumption for soils amended with different types of beech litter despite observing no temperature-litter interaction effects for different microbial indicator lipids. Because parallel impacts on both microbial properties and processes were detected for earlier sampling moments, i.e. three days after the heat stress in Mooshammer et al. (2017), microbial impacts on microbial properties had potentially already recovered by the time our amended soils were first sampled, i.e. two weeks after the temperature treatment - while impacts on microbial processes and soil carbon and nitrogen had not. These results support the notion that a community's disturbance history can potentially have longer-lasting effects on ecosystem functioning (Evans and Wallenstein 2012), and also show that this effect can occur without long-lasting changes in microbial properties.

Differences in the concentration of hot water extractable compounds potentially reflect changes in the microbial community that respond differently to T_{Rapid}. C_{HW}, specifically in combination with C_{DO}, has shown a stronger correlation to microbial properties such as respiration rates, priming effects, biomass growth, and nitrogen mineralization rates than any other carbon or nitrogen fraction (Kok et al. 2022). Since C_{HW} effectively extracts amino acids and denatured enzymes (Ghani, Dexter, and Perrott 2003), subtle differences therein may potentially reflect changes in microbial community composition. These changes are expected given that organic amendments had major impacts on microbial communities (Table 4.1) and probably also at lower phylogenetic levels than the B:F DNA ratios measured in this study (Hernández and Hobbie 2010; Ji et al. 2018; Maietta et al. 2020; Sun et al. 2022). These changes likely impacted the sensitivity to the temperature disturbances (Riah-Anglet et al. 2015), given that, even at the high level of gram-positive versus gram-negative bacteria, differences in growth and survival strategies are recognized to result in differences in their stress resilience (Atlas 1998; Ramos et al. 2001; Ventura et al. 2006). Of course, C_{HW} can contain many more and diverse compounds (Bu et al. 2010) and enzyme activities were not actually measured in this experiment. Therefore, further

study is necessary to determine whether changes in community composition at such levels actually occurred or if there were other pathways that led to substantial changes in microbial processes and soil properties.

4.4.3 Priming effects in response to temperature treatments

We observed contrasting impacts of T_{Rapid} on the priming rates in differently amended soils, where, relative to $T_{Gradual,}$ T_{Rapid} significantly decreased priming rates in the grasses treatment and increased them in the bokashi fermented grasses treatments (Figure 4.4B). Heat stress studies have demonstrated general insensitivity of priming rates to temperature changes of up to 30 °C for glucose (Ghee et al. 2013) and increasing priming rates with increasing temperature for biochar and leaf litter (Creamer et al. 2015; Fang et al. 2017). Differences in priming effects, and the impacts of temperature thereupon, are potentially related to soil nitrogen concentrations, changes in sorption capacity and/or differences in microbial community composition. Higher soil nitrogen concentrations, as influenced by the applied organic amendments, might result in increased production of hydrolytic enzymes (Cusack et al. 2011; Waldrop et al. 2004) or more efficient enzyme types (Stone et al. 2012) that subsequently become more effective in decomposing soil carbon with increasing temperatures (Creamer et al. 2015). Increasing temperatures may furthermore release soil organic carbon previously sorbed to smectite minerals, thereby stimulating priming by making it available for microbial utilization (Conant et al. 2011; Fang et al. 2017; Lützow et al. 2006). Finally, also changes in microbial community composition might explain differences in the priming effects and its temperature response, as priming effects have demonstrated a closer association with the activity of certain microbes more than others (Bird, Herman, and Firestone 2011; De Graaff et al. 2010; Fontaine et al. 2011; Garcia-Pausas and Paterson 2011; Paterson and Sim 2013). Of these three pathways, only changes in microbial community composition can explain decreasing priming effects for T_{Rapid} in the fermented grasses treatment. The application of Bokashi-fermented grasses (anaerobically fermented) induced shifts in the soil microbial community composition that potentially led to the production of different enzyme types. Possibly, these enzymes, and the microbial production thereof, had lower temperature optima and were subsequently more vulnerable to becoming denatured under higher temperatures resulting in reduced priming effects.

4.4.4 Implications and Future Research

Results show large differences in the impact of gradual and rapid temperature change treatments on measured soil microbial and chemical properties and furthermore demonstrate that organic amendments can influence these temperature impacts. However, they also show that the potential benefits or harm of organic amendments to the resilience of soils should

be of no serious concern given that carbon and nitrogen fractions in amended soils were, at all sampling moments and across all temperature treatments, always higher for the organically amended soils than in their unamended counterparts. Should the thermal resilience of a soil nevertheless be a concern, then compost shows the smallest differences between temperature treatments and, therefore, has the least impact on the sensitivity of a podzol to temperature changes. While our compost exhibited similar chemical characteristics to other composts (e.g. low C_{DO} and high C_{NH}; Appendix H), compost can significantly vary in quality, and it is therefore uncertain whether results for the current, small-scale produced compost can be extrapolated to traditional composts. Extrapolation of these findings to other soils should anyway be done with care, as a comparison of results with other studies, e.g. on the thermal sensitivity of mineral nitrogen concentrations (Donhauser et al. 2020), demonstrated that the effect of organic amendments and temperature (Pérez-Guzmán et al. 2020) likely depends on endemic soil properties also.

The impact of rates of temperature change on soil microbial-nutrient interactions has received limited attention thus far despite the many potential mechanisms that could result in significant changes in soil properties, and despite the implications that such interactions would have for microbially driven carbon and nitrogen models. Here, we have shown how temperature treatments involving rates of temperature change of up to 30°C d^{-1} can impact soil carbon and nitrogen fractions differently depending on the organic amendment treatment at an equal temperature dose. Experiments on rates of temperature change will always need to compromise on either differences in exposure times to maximum or minimum temperatures or differences in the rate of change of temperature decreases, and thus it would be interesting for future experiments to make a different trade-off and compare the results.

4.5 CONCLUSION

We have shown that temperature treatments involving different rates of temperature change can affect bacterial and total microbial abundances and C_{DO}:N_{DO} ratios even two months after returning to stable temperatures. The impacts of temperature on CO_2 respiration and priming rates, and the microbial transformation of C_{HW} and N_{HW} were influenced by the application of organic amendments. We hypothesized that the application of organic amendments would increase the sensitivity of the microbial community to the temperature treatments, resulting in greater differences between temperature treatments for the soil properties of amended soils compared to the unamended control soil. Results show evidence for and against the hypothesis, where larger differences between temperature treatments for amended soils were observed for CO_2 respiration rates and C_{HW} and N_{HW} concentrations, while no organic amendment impacts were found for microbial biomass and DNA

concentrations. The observed differences in respiration rates, priming rates and concentrations in carbon and nitrogen pools suggest that microbial communities developed under different organic amendment regimes were sensitive to different rates of temperature change, but resilient enough to prevent large shifts in major microbial properties such as B:F DNA ratios and total biomass carbon. Of the organic amendments applied, compost showed the smallest differences between temperature treatments, indicating that compost applications might result in the development of a relatively more resilient community than had developed for the grasses or fermented grasses treatments. Grasses and fermented grasses treatments demonstrated contrasting effects on the impact of temperature treatments on priming rates. The significantly greater benefits that organic amendments provided in terms of their immediate and direct increases in the soil concentration of different soil carbon and nitrogen fractions outweigh, however, at least in the short term and for the investigated podzol, the trade-offs in enhanced thermal sensitivity. Current findings hint at the existence of yet another pathway by which organic amendments might be utilized to manipulate the climate resilience and climate mitigation potential of agricultural soils.