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The susceptibility of soils to priming: the impact of litter addition, nitrogen fertilisation, and heat shock

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(Thrice.)

Abstract

Climate change is expected to alter soil nutrient availability and microbial community functionality. Inputs of labile carbon (C) from root exudates can stimulate the decomposition of soil organic matter (SOM) in a phenomenon known as "priming", which is thought to be driven by microbial demand for limiting resources. Climate change may therefore influence the susceptibility of soils to priming, with important implications for long-term soil C storage.

This thesis investigated how climate change-associated shifts in resource availability – via increased litter inputs, nitrogen (N) addition, and heat-shock treatments – influenced microbial activity and subsequent SOM priming in response to glucose addition, in temperate beech forest soils. Several specific hypotheses were tested related to the following two themes: (H1) The climate-simulation treatments will have an effect on the functioning of the microbial community, (H2) The susceptibility of soils to priming will vary depending on the climate-simulation treatments.

Soils were first subjected to the respective treatments, followed by ^{13}C-labelled glucose addition simulating root exudation, to assess the effect on "priming" of SOM decomposition. Microbial respiration (glucose- and SOM-derived CO_2), growth (via isotope incorporation), and carbon use efficiency (CUE) were measured to understand the mechanisms behind the priming dynamics.

All initial treatments altered microbial functioning and influenced the susceptibility of soils to priming, in accordance with hypotheses H1 and H2. Glucose addition consistently reduced CUE across all treatments, indicating a microbial shift towards respiration over growth. Preferential substrate use was observed in control, litter, and N-amended soils immediately after glucose addition, which suppressed initial SOM mineralisation. However, over time there was a shift towards increased SOM mineralisation (i.e. "priming"). The strong priming response in the litter-amended soil was likely driven by both elevated microbial demand for nutrients, induced by the high C:nutrient imbalance of the added litter, and the greater microbial biomass, promoting SOM decomposition through mechanisms such as selective N-mining. N addition increased microbial CUE while reducing overall growth and respiration, suggesting more efficient but reduced resource use in these soils. Surprisingly, priming was also enhanced, but likely via stoichiometric decomposition, resulting from the increased N availability. Heat-shock reduced microbial biomass and bacterial growth, as expected, but unexpectedly left fungi largely unaffected, shifting the community composition. Respiration also increased despite the lower microbial biomass, while CUE declined, indicating a stress response. In contrast to the other treatments, heat-shock reduced the susceptibility of soils to priming.

Overall, these findings highlight how microbial responses to changing resource regimes can impact priming susceptibility and microbial C-use strategies, with implications for soil C dynamics under future climate scenarios.

Contents

List of abbreviations

Abbreviation	Meaning	Unit
C	Carbon	
CO_2	Carbon dioxide	
CUE	Carbon use efficiency	
DPM	Disintegration per minute	Becquerel (Bq)
dwt	Dry weight	g
fwt	Fresh weight	g
GC	Gas chromatography *(or gas chromatographer)*	
H	Hydrogen	
LOI	Loss on ignition	
N	Nitrogen	
SE	Standard error	
SIR	Substrate induced respiration	µg CO_2 g^{-1} fwt soil h^{-1}
SOC	Soil organic carbon	
SOM	Soil organic matter	

Treatment Types

Abbreviation	Initial Treatment	Stimulant Addition
C	Control	-
L	Litter addition	-
N	Nitrogen addition	-
H	Heat-shock	-
c	-	Control
g	-	Glucose
Cc	Control	Control
Lc	Litter addition	Control
Nc	Nitrogen addition	Control
Hc	Heat-shock	Control
Cg	Control	Glucose
Lg	Litter addition	Glucose
Ng	Nitrogen addition	Glucose
Hg	Heat-shock	Glucose

1. Introduction

Beech forests (*Fagus sylvatica L*) in southern Sweden play a crucial role in carbon (C) sequestration, particularly due to their high litter-to-humus fraction, meaning that much C is stored as soil organic matter (SOM) (Akselsson et al., 2005). As such, Skåne exhibits Sweden's highest C sequestration rate, but its soils are vulnerable to changes in SOM mineralisation and nutrient cycling under climate warming scenarios (Kirschbaum, 1995).

Soil priming – increases in pre-existing SOM mineralisation in response to new substrate (e.g. plant-derived C) input (Kuzyakov et al., 2000) – may lead to soil C loss. Climate change will increase plant productivity and the input of C inputs belowground, with these inputs potentially stimulating the decomposition of SOM through priming. Various mechanisms have been proposed to explain "priming", with one widely held explanation being that microbial demand for resources may trigger microbes to decompose SOM to acquire limiting resources (Kuzyakov et al., 2000). Such a mechanism is "microbial N mining", where microorganisms may decompose SOM to specifically acquire nitrogen (N), resulting in increased C fluxes from soils to the atmosphere. As such, it is essential to assess the impact of resource availability on the sensitivity of soils to priming, in order to fully understand the impact of climate change on the soil C budget, and subsequently, the net global flux of C.

Climate change is expected to induce shifts in resource availability in soils, potentially impacting soil priming sensitivity and consequent priming effects. Elevated atmospheric CO_2, due to climate change and increased fossil fuel emissions, increases plant productivity leading to increased leaf litter (Lloyd, 1999), as well as increased root production (and consequent root exudation) (Pritchard, 2011), enhancing the resource availability for soil microorganisms. Warmer temperatures will also increase microbial activity, resulting in an increased SOM turnover, and an increase in N (and other nutrient) availability in the soil (Broadbent, 1947). Another consequence of climate change is the anticipated increase in the frequency of extreme temperature events (Intergovernmental Panel on Climate Change [IPCC], 2014). Although the specific effects of these heat-wave events on resource availability are not yet fully understood, they are expected to influence nutrient cycling in soils. This could occur through elevated microbial activity, which may accelerate the turnover of SOM, or through increased microbial mortality, where the resulting necromass becomes available for surviving microorganisms to use (Milner et al., 2012).

Microbes are responsible for the mineralisation of SOM and thus are critical in understanding the mechanisms behind the priming of SOM mineralisation. In studies to date, fungi have been found to be more associated with priming responses (Rousk et al., 2016; Soares et al., 2017). Soil bacteria and fungi occupy distinct ecological niches, with bacteria specialising in the rapid

use of more simple, labile compounds (low C:N ratio), and fungi being better at decomposing more chemically and structurally complex, recalcitrant materials, implying a high C:N ratio (Wang and Kuzyakov, 2024). A low C:N ratio, means that the amount of C and N in the compound is similar, however, substrates characterised by a high C:N ratio implies that there is far more C than N present in the compound. Following leaf litterfall, an initial flush of labile C (e.g. as sugars and amino acids) stimulates microbial growth (Torres et al., 2005). As these labile compounds are depleted, fungi increasingly dominate due to their ability to utilise the remaining resistant substrates (Wang and Kuzyakov, 2024).

The fungal-to-bacterial ratio is also often found to be impacted by the addition of inorganic N compounds. Inorganic N additions can stimulate microbial growth in N-limited soils such as beech forests (Michopoulos et al., 2008), with bacteria responding more strongly than fungi (Chen et al., 2022), potentially explained by the greater nutrient requirements of bacteria compared to fungi (Strickland and Rousk, 2010), leading to more bacterially dominated decomposition.

As mentioned earlier, temperature has a large impact on soil nutrient availability, and thus the expected susceptibility of soils to priming. However, in addition to a general increase in average annual temperatures, climate change will also result in an increase in the frequency of extreme heatwave (hereafter; 'heat-shock') events. Experiments exposing soils to short-term heat exposure (40–60°C) found an increase soil respiration (Luxhøi et al., 2008; Schnecker et al., 2024; Andrews et al., 2000), as well as a reduction microbial biomass and increase in mortality, particularly among fungi, which were found to be more heat-sensitive than bacteria (Riah-Anglet et al., 2015; Frey et al., 2008).

The impact of predicted climate change on ecosystem services suggest that climate change will decrease the C sequestration and climate regulation provided by soils in northern Europe, resulting in a subsequent decrease in soil C stocks (IPCC, 2014). Furthermore, it was predicted that from 2050 there will be a reduction in terrestrial C due to a climate-driven loss of soil C (IPCC, 2014).

Understanding the sensitivity of soils to priming will be important in predicting responses to future climate change. Previous studies in (sub-)Arctic (e.g., Hicks et al., 2020; Rousk et al., 2016) and subtropical (e.g., Wang et al., 2016) regions have found that soils are sensitive to microbial N-mining, but it remains understudied in temperate beech forests which are considered to be N-limited ecosystems. Furthermore, most research to date has focussed on gradual warming (e.g. Zheng et al., 2023), while heat-hock events, representing increasingly frequent climate extremes (Dunn et al., 2020), could lead to microbial death, and a resultant pool of SOM that may be sensitive to priming when conditions return to normal. However, this process remains poorly investigated.

1.1. Aim of study

This project aims to fill these gaps by evaluating how heat-shock, increased leaf litter inputs, and N enrichment affect the susceptibility of soils to priming by labile C input (using glucose to simulate root exudates). I will specifically resolve responses in soil C mineralisation and microbial growth, using isotope labelling (the standard method for testing priming responses); and by measuring these endpoints, it will be possible to determine the consequences of such climate change factors for soil C sequestration. The study does not test combined effects of simulated climate change treatments (e.g. litter and nitrogen addition applied together), but instead applies each treatment individually. This design allows for clearer attribution of observed changes in C mineralisation and the priming effect to specific environmental drivers, thereby contributing to mechanistic understanding.

1.2. Hypotheses

To address these study aims, this thesis tests two overarching hypotheses, each with multiple components:

(H1) The initial treatments will have an effect on the functioning of the microbial community:

- (H1.i) The litter addition treatment will provide substantial C input to the soil (alongside N and other nutrients), therefore stimulating microbial growth – particularly fungal growth, given fungi's greater ability to decompose complex organic matter (Wang and Kuzyakov, 2024).
- (H1.ii) Given the N-limited nature of beech forest soils (Michopoulos et al., 2008), N addition is expected to enhance microbial growth – particularly for bacteria, which have narrower C:N biomass ratio than fungi and are therefore more sensitive to N limitation (Chen et al., 2022).
- (H1.iii) It can be expected that the heat-shock treatment would initially reduce microbial activity and growth due to increased microbial mortality, leading to a reduction in growth rates (with fungi being more sensitive to heat-shock than bacteria), and lower microbial biomass.

(H2) The susceptibility of soils to priming will vary depending on the initial treatments:

- (H2.i) Glucose addition will stimulate priming of microbial growth and SOM mineralisation in the control soils, as microbial demand for N in these N-limited soils may trigger decomposition of SOM to access N (‘microbial N mining’).
- (H2.ii) The addition of litter with a wide C:N ratio will increase microbial N demand, thereby enhancing the susceptibility of SOM to priming. As fungi are frequently associated

with priming responses (Rousk et al., 2016; Soares et al., 2017), and litter inputs are likely to favour a more fungal-dominated community, a stronger priming effect is anticipated in the litter-amended soils.

- (H2.iii) In the N fertilised soils, the addition of N will result in a shift towards a more bacterially dominated composition, so no or reduced priming can be expected, i.e. N addition will alleviate the priming.
- (H2.iv) The expectation for the heat-shock treated soils are the most difficult to theorise and one of two options will be examined in this study. Heat-shock could initially reduce microbial activity due to increased microbial mortality; subsequent microbial biomass turnover could enhance SOM priming and C mineralisation. Alternatively, dead microbes may provide a flush of nutrients to the soil, so mineralisation rates might decrease as SOM decomposition will not be required to obtain nutrients, reducing the likelihood of priming of SOM mineralisation.

2. Theoretical background

Climate change is expected to have a profound effect on the terrestrial C cycle, particularly through alterations in temperature, moisture (Chatterjee and Saha., 2018; Sehgal et al., 2025) and nutrient availability (see section 2.3). Forest soils serve as critical C sinks, capturing and storing atmospheric C dioxide (CO_2) through organic matter accumulation. However, increasing global temperatures can enhance microbial activity, potentially accelerating decomposition rates and increasing C release to the atmosphere (Mack et al., 2004). This process can suppress the terrestrial C sink and contribute to a positive feedback loop, exacerbating global warming (Cox et al., 2000).

In northern Europe, including southern Sweden, climate models predict increasing temperatures and more frequent extreme weather events, which could impact soil C sequestration (IPCC, 2014). These changes highlight the importance of understanding how soil processes, particularly microbial activity and decomposition, responds to climate change.

2.1. The role of soil microbes in the terrestrial C cycle

The term 'C sequestration' is commonly defined as the processes, natural or otherwise, of the capture, removal, and resultant storage of CO_2 in a carbon pool, from the atmosphere (IPCC, 2021). CO_2 is regarded as the most important anthropogenic contributor to climate change induced global warming (IPCC, 2023), as such sequestration of atmospheric CO_2 is one of the most effective methods to help maintain stabilisation of the climate (Schulze et al., 2010). In forests, the main source of C uptake is through assimilation by plants via photosynthesis, where large quantities of C are stored in woody biomass, leaf tissues and root networks belowground (Lloyd, 1999). In addition to assimilation by photosynthesis, forest soils are also responsible for the sequestration of C through soil organic matter and the consequent soil C stock, resulting in forest soil being a significant sink of C in the terrestrial soil C balance (Schulze et al., 2010). However, forests and their soils are not only sinks for atmospheric CO_2. The decomposition of organic matter via heterotrophic respiration by soil micro-organisms on and within the soil, as well as autotrophic soil respiration by roots and their associated rhizosphere (i.e. rhizosphere bacteria and mycorrhiza), are major contributors to soil–atmosphere CO_2 fluxes.

Ultimately, soil C storage reflects the balance between C inputs, primarily from plant material, and C losses driven largely by microbial activity. When microbes metabolise a C source, part of the C is respired as CO_2 and returned to the atmosphere, while the remainder is incorporated into microbial biomass, potentially contributing to longer-term C storage (Liang et al., 2017). The proportion of C allocated to microbial growth relative to total C uptake is known as the microbial C use efficiency (CUE). As such, microbial CUE is a key parameter for

understanding how ecosystems respond to, and influence, climate change, as a higher CUE reflects a greater potential for soil C storage (Adingo et al., 2021).

Together, microbial growth and CUE offer insights into how much C can be effectively 'locked away' in soils. When microbial cells die, some of the C in their biomass, particularly C that is more chemically stable or physically protected by soil minerals, may become stabilised within the soil matrix, contributing to long-term C storage (Hu et al., 2023).

2.2. Priming as a key concept in soil organic matter (SOM) dynamics

A crucial factor influencing soil C sequestration is the priming effect. The priming effect refers to the phenomenon whereby new labile C inputs (e.g. root exudates) stimulate microbial activity, leading to transient accelerated decomposition of pre-existing recalcitrant SOM (Kuzyakov et al., 2000). These labile C inputs through root exudates can be easily broken down, providing easily available energy for soil microbes. As such, new labile C inputs may stimulate microbial mineralisation of C and N from recalcitrant native SOM – it is this phenomenon that is termed the "priming effect" (Kuzyakov et al., 2000; Bingemann et al., 1953).

Mineralisation is a critical component of the C and N cycles, transforming organic compounds into inorganic forms that are accessible for microbial and plant uptake. In C mineralisation, microbes break down organic C into primarily CO_2, which is released back into the atmosphere through outgassing. In N mineralisation, organic nitrogen, which is inaccessible to plants, is converted into inorganic forms such as ammonium (NH_4^+), which can be taken up by plants and directly supports plant growth and productivity. Consequently, mineralisation is fundamental to nutrient cycling, soil fertility, and overall ecosystem function.

Understanding of priming in beech forest soils may be particularly important due to their high litter-to-humus fraction, which means significant portions of C are stored as SOM (Akselsson et al., 2005). Understanding how priming responds to climate-induced shifts in resource availability, such as increased litter deposition, N availability and heat stress, is critical for predicting changes in forest soil C balance as the extent to which priming occurs can determine whether forest soils act as long-term C sinks or sources under future climate scenarios.

2.2.1. Mechanisms behind priming

The input of labile C sources (e.g. root exudates) can strongly influence the mineralisation of native SOM (Kuzyakov, 2010; Kuzyakov et al., 2000). Changes in environmental conditions (e.g. temperature, rainfall, CO_2 concentration, etc.) are likely to influence the plant growth, and this plant growth often reflects in changes of below-ground root biomass, which is responsible for the exudation of these labile compounds into the rhizosphere.

In experimental practice, glucose is commonly used as a labile C source simulating the rhizosphere input, and has consistently been shown to induce the priming effect, with the rate and frequency of labile C addition appearing to significantly influence SOM mineralisation and associated priming effects (Hartley et al., 2009; Hicks et al., 2020; Na et al., 2022; Rousk et al., 2016).

Priming effects are hypothesised to be driven by several key microbial mechanisms, including:

1. Microbial nitrogen ('N') mining: the microbial decomposition of SOM to obtain N in N-limited soils. More specifically, changes in the availability of N and labile organic compounds can induce a shift in microbial strategies towards the utilisation of N-rich components of SOM, inducing an increased mineralisation of SOM (Hicks et al., 2022).
2. Stoichiometric Decomposition: when C and nutrient inputs meet microbial demands leading to a balanced microbial activity and growth, and consequent acceleration of organic matter mineralisation (Chen et al., 2014).
3. Co-metabolism: the concurrent microbial degradation of native (recalcitrant) SOM and labile organic matter. In other words, the energy obtained by microbes from the labile compounds enables the simultaneous breakdown of the more recalcitrant SOM (Broadbent, 1947).
4. Preferential Substrate Utilisation: involves the shift in microbial C usage from SOM to more labile sources. This results in a reduction in SOM mineralisation ("negative priming") (Kuzyakov, 2002).

Bacterial and fungal growth is vital to measure when understanding the priming phenomena, as it is these microbes that control the mineralisation of SOM, and consequently the priming of SOM mineralisation (Kuzyakov, 2010). As such, microbial responses under different climate treatments will likely impact the priming of SOM mineralisation after the addition of labile C. A recent paper by Wang and Kuzyakov (2024) illustrates the niche differentiation between bacteria and fungi, specifically, bacteria are more efficient in the use of simpler organic compounds which are easier to breakdown (i.e. labile compounds), whereas fungi are more advantageous in the utilisation of more complex compounds, such as SOM, and thus are more associated with the priming of SOM mineralisation (Rousk et al., 2016; Soares et al., 2017).

2.3. The influence of climate impacts on the microbial community, and the consequent susceptibility of SOM mineralisation to priming

2.3.1. Litter input

In European forests, litterfall from trees contributes the primary natural source of nutrients, energy and organic matter to the forest soils, and as such has a strong influence on soil microbial activity and decomposition rates (Akselsson et al., 2005; Lui et al., 2004). Litter deposition is closely linked to climate variables, such as temperature and moisture (Aerts, 1997). In this thesis, litter addition was used to simulate the anticipated increase in litter input resulting from increased plant productivity associated with climate warming scenarios.

At the initial stage of leaf litter decomposition, high-quality C inputs (in the form of sugars, amino acids, and other labile compounds) are released into the soil through the leaching. These labile substrates are utilised by both bacteria and fungi, stimulating microbial growth (Torres et al., 2005). However, as these labile compounds are assimilated by microbes, the remaining litter becomes increasingly dominated by recalcitrant compounds (e.g. lignin, chitin etc.). Thus, at this stage, fungal growth and activity typically dominates over bacterial processes (Wang and Kuzyakov., 2024). These recalcitrant substrates of litter are characterised by a high C:N ratio which can induce high microbial demand for N (Hicks et al., 2022). Consequently, soils may be made more susceptible to priming as microbes accelerate the decomposition of SOM to acquire N – a phenomenon known as microbial N mining. Studies have shown that beech litter typically induces a positive priming effect (Di Lonardo et al., 2018).

Plant litter quality appears to influence both the timing and magnitude of soil priming responses. Fanin et al. (2020) demonstrated that high-quality litter (defined as being labile and nutrient rich) induced an immediate priming effect on SOM mineralisation, whereas low-quality (recalcitrant and nutrient poor) litter led to a delayed response, likely as a result of the differing microbial community responses to litter C and nutrient accessibility. Furthermore, Yang et al. (2021) found that litter with a high C:N ratio (indicative of lower quality) tended to induce a stronger priming effect.

Several field studies have examined how increased litter inputs from shrubs affect C mineralisation in Arctic soils. Contrary to expectations, litter addition generally did not enhance SOM-derived C mineralisation. Instead, indirect mechanisms, such as shifts in microbial community composition, played a more significant role (Rinnan et al., 2007). These variations in outcomes highlight the importance of studying diverse litter types and soil conditions, as the susceptibility to priming may vary regionally and by litter quality, with important implications for future C fluxes.

2.3.2. N availability

It is predicted that there will be increases in the availability of N, due to warming induced accelerated SOM mineralisation, resulting in increased N availability in soils (Salazar et al., 2020). Subsequently, these changes in N availability have the potential to impact the decomposition activities of microorganisms, as well as stimulate plant productivity (in nutrient limited species). A study by Michopoulos et al. (2008) found evidence for low N-availability in both the organic and mineral soil horizons of beech forest soils. As a result, it is likely that the addition of inorganic N will stimulate microbial growth in these N-limited soils, consequently resulting in enhanced SOM decomposition.

Moreover, studies have shown that bacteria are more sensitive to additions of N (and other limiting nutrients) than fungi due to bacteria being more N-limited (e.g. Chen et al., 2022). As a result, N addition to the soils is likely to decrease the fungal-to-bacterial ratios, thereby resulting in shifted decomposition pathways.

Despite these findings, there remains a notable gap in the literature regarding the direct effects of N addition on SOM priming in temperate beech forest soils. The present study aims to address this gap and provide insights into consequences of N enrichment in such ecosystems.

2.3.3. Heat-shock

Since the 1980s, Scandinavia has experienced some of the strongest global warming, particularly in winter (IPCC, 2014). In addition, extreme temperature events such as heatwaves (defined as 5 days above the 90th percentile of historical climatology (IPCC, 2021)), are to become increasingly frequent across Europe (IPCC, 2014). Heatwaves can directly affect microbial activity, potentially accelerating decomposition rates (Dunn et al., 2020), while also altering soil moisture levels, which influences nutrient availability and microbial responses (Na et al., 2025).

One of the key research gaps this thesis addresses is the impact of short-term heat-shock events on soil susceptibility to priming. Most previous studies have examined the combined effects of extreme temperatures ("heat-shock") and drying on microbial communities and the subsequent mineralisation of SOM, with few isolating heat-stress alone. When heat stress is examined in isolation, the focus is often on changes in microbial community structure, rather than on priming effects themselves. While microbial dynamics are intrinsically linked to priming, this still represents a critical area of uncertainty.

Experiments exposing soils to temperatures between 40-50°C, have reported strong increases in microbial mortality, reductions in microbial biomass, and elevated respiration rates (Schnecker et al., 2024; Andrews at al., 2000). Furthermore, fungal species appear to be more

sensitive to heat, thus often resulting in a shift towards either more heat-tolerant fungal species (Allison and Treseder, 2008) or a dominance of bacterial communities (Riah-Anglet et al., 2015; Frey et al., 2008).

In addition, studies that do investigate the effects of warming on the susceptibility to priming typically use long-term heat treatments – either small temperature increases sustained over several years or more intense heating over weeks to months. These approaches do not represent the short, intense temperature spikes characteristic of heat-shock events (IPCC, 2014).

A paper by Ding et al. (2025) concluded that experimental warming generally reduces positive priming effects in forest soils. In contrast, a study by Zhou et al. (2024) found that increased microbial turnover, and the subsequent accumulation of microbial necromass actually enhanced the priming of SOM mineralisation, as the necromass provided an accessible C source.

In summary, it is definite that more frequent heatwaves will occur in the future, however few studies actually investigate the impact that these short heat-wave ('heat-shock') events will have on the microbial community and functionality, and the consequent influence this will have on the susceptibility of soils to priming.

3. Materials and Methods

3.1. General overview

Soil samples were collected from a Beech forest in southern Sweden and subjected to three experimental pre-treatments ('initial treatments') simulating different effects of climate change: 1) litter addition, 2) inorganic N addition, and 3) heat-shock treatment.

Using these treated soils, a priming experiment was then conducted, where labile plant-derived C input was simulated by the addition of ^{13}C-labelled glucose (or deionised H_2O for control samples). By adding ^{13}C-labelled glucose it was possible evaluate the priming effect, as CO_2 respired from the added substrate could be distinguished from CO_2 coming from pre-existing SOM using an isotope mixing model (see section 3.7).

Priming effects were assessed by measuring the impact of simulated rhizosphere inputs on soil organic C (SOC) mineralisation rates, as well as bacterial and fungal growth rates over time, using isotope labelling. The difference in C mineralisation and growth rates between soils with glucose addition and without (control) were used to determine the priming effect. In addition, I also measured responses in gross N mineralisation, however, these results are not included in this thesis (not possible to get data in time).

3.2. Soil sampling and experimental design

Soil samples were collected from a beech-dominated forest located in a natural reserve near Knivsås-Borelund, Skåne, southern Sweden (55°41′ N, 13°24′ E), in mid-February 2025 under frost-free conditions. The site is located in a temperate oceanic climate (Koppen-Geiger class Cfb), with a mean annual temperature of 8°C and an average annual precipitation of 680 mm. The site is primarily composed of European beech (*Fagus sylvatica*), as well as scattered occurrences of oak (*Quercus* sp.), birch (*Betula* sp.), and hazel (*Corylus*sp.). Labile C additions to soils from this site have previously been shown to induce priming associated with microbial N-mining (Na et al. 2025). I tested how shifts in resource availability associated with different aspects of climate change will affect the susceptibility to priming.

Soils were sampled from the organic horizon (ca. 5 to 15 cm depth), avoiding mineral soil and excluding surface litter. Sampling followed a random stratified design. Three replicate plots were established (5m by 5m), at least 20 m apart, and within each plot 15 to 20 individual soil samples were collected and combined to form a composite sample per replicate. The composite samples were sieved through a < 4 mm mesh to remove larger plant debris and stones, leaving behind combined bulk and rhizosphere components. Samples were stored in plastic bags,

ensuring that replicates remained distinct, and transported to the laboratory, where they were kept in the fridge until further processing.

Litter samples were also collected from all replicate sites, pooled to form a composite litter sample, and subsequently dried at 40°C until a constant weight was achieved (~48 hours). Non-beech litter was manually removed, and the remaining material was ground into a homogenous fine powder using an electric coffee grinder. This processed litter was later used for experimental litter additions. The C:N ratio of the beech litter used in this study was unable to be determined, so the typical literature beech litter C:N ratio of 50 to 70 (Zeller et al., 2000; Jacob et al., 2010) was used in this study to infer conclusions.

The experiment was conducted over a total in-laboratory duration of 12 weeks, with replicates processed in three consecutive rounds. Each round followed a fortnightly cycle, consisting of one week of climate simulation treatments followed by one week of priming experiment. Within each round, all climate treatments were run in parallel to ensure consistency across replicates. See Figure 1 for a schematic diagram of the methodology.

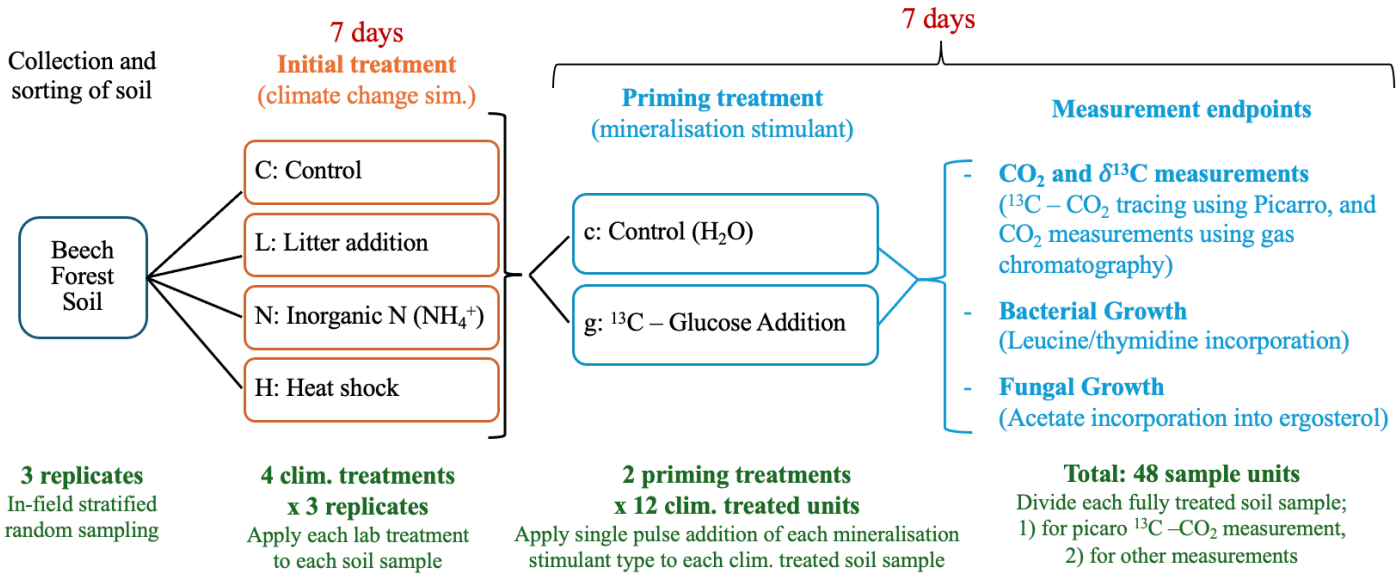

Figure 1. Schematic diagram of project containing simulated climate treatments ('initial treatments') (orange), measurement endpoint variables with their associated methods ('priming treatment') (blue), replicate and sample unit quantities (green), and procedure duration (red).

Each replicate soil sample was divided into four subsamples (ca. 580 g fwt each) and placed into 1L plastic microcosms with lids. The microcosms were then subjected to one of three environmentally relevant climate change treatments, or a control (no-change) treatment, for seven days – hereafter referred to as the 'initial treatments' (see Figure 1):

I. Control ('C'): Soils were maintained at 16°C without additional treatment for the seven-day period.

II. Litter Addition ('L'): Dried ground beech litter (*Fagus sylvatica*) was added at a rate of 40 mg litter g^{-1} fwt soil, following Silva-Sánchez et al. (2019), and approximately corresponding to the annual litter input in beech forest soils. Litter was added in layers and then the microcosms were gently shaken to ensure even distribution.

III. Inorganic N Addition ('N'): NH_4Cl was added to achieve a concentration of 0.1 mg N g^{-1} fwt soil, following Yuan et al. (2022), equivalent to ca. 50 kg N ha^{-1} addition, and approximately corresponding to the N release expected from a moderate < 3°C warming scenario (Mack et al., 2004). NH_4Cl was dissolved in the minimal volume of deionized water required to form a saturated solution and applied to the soil via pipetting. Microcosms were gently shaken to ensure even distribution.

IV. Heat-shock ('H'): Microcosms were incubated at 50°C for three days, followed by storage at 16°C for the remaining treatment period. To ensure uniform heating while minimising desiccation, microcosms were placed in a water bath.

All microcosms were opened/flushed daily throughout the initial treatment period to allow a replenished microcosm headspace and avoid anaerobic conditions within the microcosm. All microcosms (except those undergoing heat-shock) were stored in a climate-controlled room at 16°C throughout the initial treatment period.

The temperatures used in the heat-shock treatment are unlikely to occur regularly or in the near future in southern Sweden. However, an extreme but environmentally plausible temperature was chosen to illustrate the potential impacts of short-term extreme warming on soil microbes. Even if such temperatures are unlikely to be reached, understanding their consequences and underlying mechanisms is crucial, which is why they were tested.

3.3. Priming treatments and labile ^{13}C-glucose addition

Following the seven-day climate simulation period, soils from each microcosm were divided into two 1 L microcosms to assess their susceptibility to priming effects. One set of microcosms received a labile C addition in the form of ^{13}C-enriched glucose, while the other received an equivalent mass of deionised water as a control.

Labile C was introduced as a solution of ^{13}C-enriched (3% atom) glucose at a concentration of 400 µg C g^{-1} fwt soil. The glucose was dissolved in deionised water (10 µl g^{-1} soil) and applied uniformly by gently shaking the closed microcosms. This addition rate was chosen following Hicks et al. (2020), approximately corresponding root exudation rates from tree seedlings measured under comparable experimental conditions (Bengtson et al., 2012).

Each microcosm was then further subdivided, resulting in two replicates per treatment, leading to a total of 16 microcosms (4 initial treatments × 2 priming treatments × 2 microcosms), all of equal soil weight. One replicate of each microcosm type was stored in a dark, climate-controlled room at 16°C for repeated soil sampling over a one-week period to measure bacterial growth, fungal growth and gross N mineralisation. The second replicate was connected to a Picarro isotope analyser (under the same climate-controlled conditions) to continuously monitor CO_2 production and ^{13}C enrichment in the headspace over the priming exposure period.

3.4. Soil characteristics

Fresh soil subsamples were oven dried at 105°C for 24 hours to assess the gravimetric water content (w), the mass of water per mass of dry soil, for each replicate pre- and post-initial treatment and priming simulant exposure. Gravimetric water content was calculated using equation 1:

$$w = \frac{M_w}{M_s} \quad \textbf{[1]}$$

Where M_s is the 'dry soil mass' (mass of soil after exposure to 105°C, in grams), and M_w is the 'water mass' (fwt soil weight $- M_s$, in grams).

The same soil samples (after 105°C exposure and w measurements were taken) were then used to determine SOM content through loss on ignition (LOI) (600°C overnight, ensuring all organic matter had been oxidised). LOI was then calculated as the percentage fraction of oxidised C from the dried soil (see equation 2).

$$LOI\ (\%) = \frac{M_s - burned\ soil\ mass}{M_s} * 100 \quad \textbf{[2]}$$

Where $burned\ soil\ mass$ is the soil mass after exposure to 600°C (g).

Soil acidity (pH) and electrical conductivity were measured in a 1:5 (w:V) soil-water solution (5.0 g soil + 25 mL H_2O) using their respective electrodes, following a 1-hour extraction and 20 min sedimentation.

3.5. Microbial Biomass

Microbial biomass was determined through substrate induced respiration (SIR). To do so, 1.0 g of fwt soil was transferred into 20ml glass vials and mixing with ca. 25 mg glucose mix in the proportion 1:4 (glucose: talcum) per gram of fresh weight soil. After 30 minutes the vials' headspace was purged with pressurised air, sealed, and incubated for 3h, in a 16°C room, without light. The amount of evolved CO_2 was measured with a gas chromatograph (GC). μg

CO_2 produced was then calculated from the sample's GC peak area using equation 3. The values of SIR were then used for an estimation of microbial C biomass, where 1 mg CO_2 per hour, at 22°C, corresponds to 20 mg biomass-C (Anderson and Domsch, 1978). Microbial biomass was measured in soils after the initial climate change simulation treatments.

3.6. Microbial growth

Bacterial growth was assessed using ^{3}H-leucine incorporation method (Bååth et al., 2001) and ^{14}C-acetate-in-ergosterol incorporation method was used to determine fungal growth (Newell and Fallon, 1991), adapted for soil (Bååth et al., 2001; Rousk et al., 2009). Using both methods enables distinction between the different microbial groups' response to climate change simulation treatments and labile C additions. Furthermore, by measuring microbial growth, the amount of C processed by microbes and staying in the soil (rather than being respired to the atmosphere) can be determined, helping inform about C sequestration potential, as more microbially processed C may be more effectively stabilised in soils (Liang et al. 2017).

Fungal and bacterial growth rates were measured at 4h, 24h, 48h, 72h, 168h in all soils.

3.6.1. Bacterial growth

Bacterial growth was determined by using the ^{3}H-Leucine (Leu) incorporation method (Bååth, 1994; Bååth et al., 2001). This method uses the idea that by determining the rate of protein synthesis you can obtain a measure of bacterial growth. As such, this method uses a radioactive precursor molecule (3^{H}-Leucine, an amino acid) which is incorporated into proteins during the incubation period. The growth rate is then indicated by the amount of incorporated radioactive precursor.

At each time point, ca. 1.0 g of fwt soil and 20 ml of distilled water was added to a 50 ml centrifuge tube and centrifuged for 8 minutes at 1000 rcf (relative centrifugal force, equivalent to 1000 times gravitational acceleration, g). 1.5 ml of the resultant supernatant (bacterial suspension) was taken from a predetermined height of the centrifuge tube and transferred into a 2 ml eppendorph-vial. Then, 20 µl of ^{3}H-leucine solution was added, resulting in a final leucine concentration of 275 nM in the bacterial suspension, in accordance with the leucine saturation curve from Bååth (1994). The solution was mixed before being incubated at 16°C for 2h after which 75 µl of 100% trichloroacetic acid (TCA) was added to terminate growth. The samples were then centrifuged (16 200 x *g* for 8 minutes), after which the supernatant was removed, leaving behind the small radioactive pellet. Multiple washings steps were then carried out as followed; 1.5 ml 5% TCA was added, the sample vortexed briefly, before centrifuging and the supernatant was removed (as previously). 1.5 ml 80% ethanol was added, and the same vortexed, centrifuge, supernatant removal was performed. 200 µl 1.0 M NaOH was the added, the sample vortexed until the pellet was dissolved, and then the samples were placed in a 90°C

oven for ca. 45 minutes. Once the samples were removed and cooled (sampled placed 5-10 minutes in the freezer), 1 ml of scintillation cocktail (Ultimer Gold; PerkinElmer, USA) was added, and the sample vortexed until clear. The samples were then placed into scintillation vials, and the amount of radiolabelled leucine incorporated into bacterial proteins was measured as disintegration per minute (DPM) using a liquid scintillation counter.

Bacterial growth rate was estimated using the incorporation of radiolabelled leucine into bacterial proteins. First, DPM values were normalised to soil dry weight and incubation time, to obtain values expressed in terms of Leu DPM g^{-1} h^{-1}. These values were then converted to pmol Leu g^{-1} h^{-1} to represent the amount of leucine incorporated into the extracted bacterial biomass. A conversion factor, as described by Soares and Rousk (2019), was applied to estimate DNA synthesis by converting leucine incorporation to its equivalent in thymidine (pmol TdR g^{-1} h^{-1}). Finally, the growth rate of bacteria (µg C g^{-1} soil h^{-1}) was estimated from the rate of DNA synthesis using an empirical factor relating DNA synthesis to C incorporation into bacterial biomass, whereby 5.5 pmol incorporated TdR corresponded to 1 mg bacterial biomass C, also described by Soares and Rousk (2019).

3.6.2. Fungal growth

Fungal growth was measured using ^{14}C-acetate incorporation into ergosterol, a method by Newell and Fallon (1991) adapted for soil use by Bååth (2001) and Rousk et al. (2009). Radiolabelled ^{14}C-acetate, a 'building block' of ergosterol (a fungal-specific membrane lipid) can be used to estimate fungal growth by tracing the incorporation of ^{14}C-acetate into newly synthesised ergosterol. By quantifying the incorporation of acetate into newly synthesised ergosterol over a defined incubation period, fungal biomass production could be estimated.

At each measurement point, 1.0 g fwt soil was measured into a 10 ml test tube along with 1.95 ml distilled water and 50 µl ^{14}C-acetate solution (20 µl ^{14}C-acetate and 30 µl unlabelled acetate, forming a 220 µM ^{14}C-acetate concentration). After incubation for 4h at 16°C 0.5 ml 10% formalin was added to terminate fungal growth.

To extract the lipids from the soils, the samples were centrifuged (5 min at 800 x g), and the supernatant discarded. 5 ml of 10% KOH dissolved in methanol was added to each sample, vortexed, and then placed into a sonicator for 15 minutes. The samples were then placed in a water bath (70°C) for 60 minutes, vortexed, then left to cool. Once cooled, 1 ml distilled H_2O and 2 cyclohexane was added to each sample and vortexed to induce phase separation. The phases were separated by centrifugation (5 min, 800 x *g*) and the 'upper' hydrophobic cyclohexane phase was transferred into new sampling tubes. The 'original' sampling tubes had another 2 ml cyclohexane added, with repeat vortexing and centrifuging, and the newly formed upper phase was also transferred to the new sampling tubes – resulting in the new sampling tubes having a combination of the two transferred upper phases.

The cyclohexane in the new sampling tubes was evaporated under N gas on a 40°C heating block, and the remaining precipitate was dissolved in 200 µl methanol and vortexed. The new sampling tubes were then placed in a water bath (40°C) for 15 minutes. The samples were then filtered through a 0.45µm Teflon syringe filter into new in autosampler vials, ready for High performance (pressure) liquid chromatography (HPLC) analysis, which enables the separation of substances which would otherwise be hard to separate. The separated ergosterol fraction from the HPLC was collected into 5 ml scintillation vials, and 3 ml scintillation cocktail (Ultimer Gold; PerkinElmer, USA) was added to each vial. The samples were then vortexed and then and vortex run on the liquid scintillation counter to determine ^{14}C-derived DPM radioactivity.

Fungal growth rates (µg C h^{-1} g^{-1} soil) were estimated by tracing the incorporation of ^{14}C -acetate into fungal ergosterol. DPM values were first normalised to incubation time (DPM h^{-1}), and a conversion factor (0.1242331 × DPM h^{-1}) was applied to calculate acetate incorporation rates (picomol acetate h^{-1}). These rates were then adjusted for dry soil mass, resulting in picomol acetate h^{-1} g^{-1} soil. Finally, fungal growth rates were obtained by applying a second conversion factor whereby 2.6 pmol incorporated acetate corresponded to 1 mg fungal biomass C, resulting in fungal growth rates expressed as µg C h^{-1} g^{-1} soil (Soares and Rousk., 2019).

3.6.3. Cumulative microbial growth and priming of microbial growth

The same method to calculate cumulative microbial growth, and priming of microbial growth applies to both bacteria and fungi. Cumulative microbial growth (bacterial or fungal) was calculated by integrating the microbial growth rates over time using the trapezoidal rule (equation 3). This approach estimates the area under the microbial growth curve, resulting in the total microbial C production (µg C g^{-1} soil) over the incubation period. The general formula is:

$$\text{Cumulative Growth} = \sum_{i=1}^{n-1} \frac{G_i + G_{i+1}}{2} \times (t_{i+1} - t_i) \qquad \textbf{[3]}$$

Where G_i and G_{i+1} are the microbial (bacterial or fungal) growth rates (µg C g^{-1} h^{-1}) at consecutive timepoints t_i and t_{i+1} (in hours), $(t_{i+1} - t_i)$ is time interval between measurements, n is the total number of timepoints.

Priming of microbial growth was calculated as the difference in microbial growth rates between glucose-amended soils and their corresponding control soils (non-glucose amended) at each time point. The same difference-based approach was applied to cumulative microbial growth values; whereby cumulative priming was determined by subtracting the cumulative microbial growth in control soils from that in glucose-amended soils.

3.7. CO_2 and ^{13}C measurements

Originally, measurements of evolved CO_2 production (respiration) and ^{13}C were to be obtained solely from the use of a Picarro G2201-i Isotopic Analyzer. However, due to a technical error after the first replicate, respiration was measured using traditional gas sampling and gas chromatography (GC), while the Picarro measurements were used to obtain ^{13}C values.

3.7.1. Gas sampling measurements and total respiration

This method for measuring respiration rate provides an estimate of total microbial activity within the soil, from bacteria and fungi; it is not selective for any particular organism group as obtaining a value for a specific group activity is very difficult. This method was undertaken only in replicates two and three.

In summary, ca. 1g of fwt soil was weighed into a 20 ml glass vial. The headspace of each vial was then flushed with pressurised air (so sample headspaces start from ambient CO_2). Vials were then sealed with crimp caps to maintain a closed system.

The samples were then left to incubate for 6 to 98 hours depending on the measurement timepoint (see Figure 2).

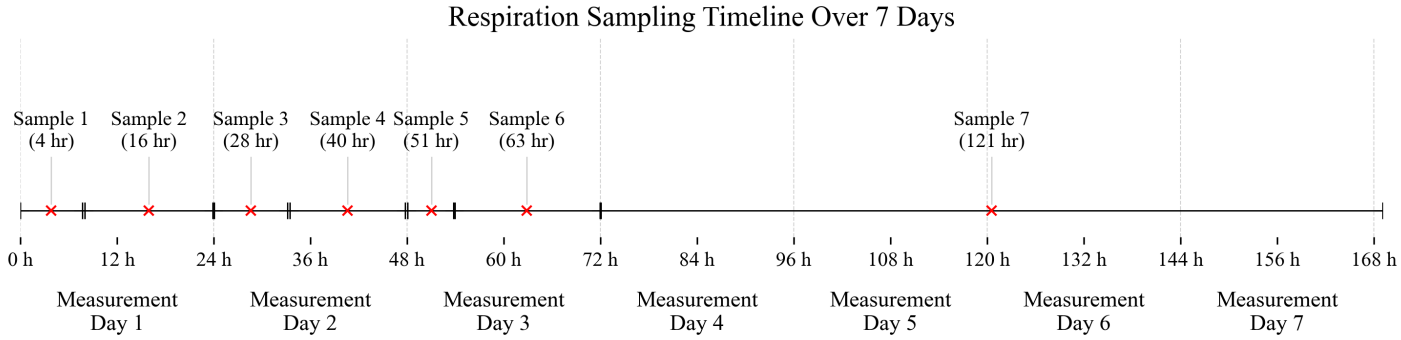

Figure 2. Respiration sampling timeline over 7 days following substrate addition, for replicates 2 and 3. Red crosses indicate the midpoints of each gas sampling event, calculated as the time halfway between sealing of the respiration sampling valve and measurement by gas chromatography. Vertical ticks denote 24-hour intervals from the start of the experiment.

Following incubation, the amount of CO_2 in the headspace was quantified using the GC. The GC software provided the peak area corresponding to the concentration of CO_2 present. CO_2 quantity in each sample was calculated using the equation 4:

$$\mu g\ CO_2 = \frac{A_{sample} - A_{background}}{Cal} \quad \textbf{[4]}$$

Where A_{sample} is the GC CO_2 peak area of the sample, $A_{background}$ is the GC CO_2 peak area of the background (empty vial), and Cal is the calibration factor (gradient of the GC standard curve; approx. 1.68 in this study).

The resulting CO_2 produced (in µg) was then normalised to both dwt soil weight (in grams) and incubation time (h), using equation 5, resulting in the final respiration rate:

$$Respiration\ rate = \frac{\mu g\ CO_2}{g\ dwt\ soil\ \times\ h} \quad \textbf{[5]}$$

This value represents the rate of microbial CO_2 production per unit dry soil mass per hour, and was used to measure C mineralisation from SOM under the different treatment conditions.

3.7.2. Picarro analyser and $\delta^{13}C$ measurements

To distinguish between C respired from added glucose and native SOM, a stable C isotope (^{13}C) labelling approach was used. The isotopic composition of CO_2 was measured throughout the incubation using a Picarro G2201-i cavity ring-down spectrometer, which recorded $\delta^{13}C$ values (‰) of each treatment microcosm over the priming treatment duration. These $\delta^{13}C$ values represent the degree of ^{13}C enrichment in the respired CO_2, expressed in per mil (‰), relative to a standard reference.

The reference standard used was the Vienna Pee Dee Belemnite (VPDB), a fossil carbonate with a known and stable isotope ratio of ^{13}C to ^{12}C (R_{std}).

A Picarro analyser was used due to its high temporal resolution (see Figure A 1) compared to traditional gas sampling methods, and has been used in studies with similar measurement endpoints (e.g. nutrient mineralisation and microbial activity) (Na et al., 2025).

Raw measurement files from the Picarro were processed in Python (v3.8.3) using Jupyter Notebook, involving systematic data cleaning, aggregation, and structuring for analysis. The $\delta^{13}C$ values were corrected for the background $\delta^{13}C$ signal, using an empty microcosm following standard procedures (Paterson and Sim, 2013), ensuring that only biologically respired CO_2 was analysed. The $\delta^{13}C$ values were then converted to Atom% ^{13}C values, using the $^{13}C/^{12}C$ ratio provided by the Picarro analyser and the VPDB standard reference.

3.7.3. Calculating the respiration of C derived from SOM and glucose

The resultant $At\%\ ^{13}C$ represents the isotopic signature of CO_2 respired by microbes at each timepoint and treatment. These $At\%\ ^{13}C$ values (smoothed using a Savitzky-Golay filter, and interpolated in order to obtain Atom% ^{13}C values for specific timepoints, (see Appendix A, Figure A 1) were then used in an isotope mixing model – a widely used approach to quantify priming effects (Paterson and Sim, 2013). This approach involves partitioning the total CO_2

flux into the fraction of total C from the added glucose (equation 6) and the C respired from native SOM (equation 7). The priming of C mineralisation was then calculated as the difference between respired C from glucose amended soils, and respired C from soils not amended with glucose, see equation 8 for the priming of SOM-derived C mineralisation, and equation 9 for glucose-derived C mineralisation.

At% ^{13}C values were smoothed using a to reduce noise. From these smoothed curves, values were interpolated at defined post-addition timepoints (4, 16, 28, 40, 51, 63, and 121 hours (matching those from the manual respiration headspace measurements).

$$f_{glu} = \frac{At\%^{13}C_{glucose} - At\%^{13}C_{control}}{\delta^{13}C_{glu} - At\%^{13}C_{control}} \quad \textbf{[6]}$$

Where f_{glu} is the fraction of the total C that was glucose-derived, $At\%^{13}C_{glucose}$ is the isotopic signature of the glucose-treated sample at each time point, $At\%^{13}C_{control}$ is the isotopic signature of the control treatment (without glucose addition), and $\delta^{13}C_{glu}$ is the isotopic signature of the added glucose (=3).

$$S_{R,\ SOM} = \left(1 - f_{glu}\right) \times T_R \quad \textbf{[7]}$$

Where $S_{R,\ SOM}$ is the SOM-derived respired C in soils with added glucose (µg CO_2 g^{-1} dwt soil h^{-1}), and T_R is the total respired C from soils with glucose added (glucose (µg CO_2 g^{-1} dwt soil h^{-1}).

$$PE_{SOM} = S_{R,\ SOM} - C_R \quad \textbf{[8]}$$

Where PE_{SOM} is the priming of SOM-derived C mineralisation (µg CO_2 g^{-1} dwt soil h^{-1}), and C_R is the respired C from the control soils (µg CO_2 g^{-1} soil h^{-1}).

$$R_{glu} = f_{glu} \times T_R \quad \textbf{[9]}$$

Where R_{glu} is the respiration of glucose-derived C (µg CO_2 g^{-1} soil h^{-1}).

3.8. Microbial carbon use efficiency (CUE)

Microbial CUE was estimated using measurements of total microbial growth and respiration values, all in units of C (see equation 10). Respiration values for the respective microbial measurement times (4, 24, 48, 72, 168 hours) were extracted from the interpolated results from section 3.7.1.

$$\mathrm{CUE} = \frac{bacterial\ growth + fungal\ growth}{bacterial\ growth + fungal\ growth + respiration} \quad \textbf{[10]}$$

Overall microbial CUE was obtained from the CUE values during the 168 hours.

3.9. Gross N mineralisation

N mineralisation was estimated using the ^{15}N isotope pool-dilution method following Rousk et al. (2016). While not directly related to C sequestration, these measurements offer mechanistic insights into microbial processes underpinning priming dynamics. However, due to time and equipment constraints, data processing was not completed in time for inclusion in this thesis.

3.10. Data analysis and statistical methods

All data processing, statistical analysis, and visualisation were performed using Python (v3.8.3) within Jupyter Notebook.

Standard error (SE) of the mean was used to quantify the variability among replicates and the precision of calculated means. SE bars were included in all figures to represent the spread of replicate data (n = 3 for all data analyses, except for respiration-related analyses where n = 2 due to a technical error following the first replicate).

3.10.1. Hypothesis Testing

To determine the effects of climate change-associated treatments (control, litter addition, nitrogen addition, and heat-shock) and glucose addition on microbial activity and priming responses, the following statistical approaches were applied:

- One-way ANOVA was used to test for differences between treatment groups at individual timepoints or for single-value outcomes (e.g. cumulative microbial growth, respiration, and CUE). Where ANOVA revealed significant effects, Tukey's Honestly Significant Difference (HSD) post hoc tests were applied to identify pairwise differences between treatments.

- Two-way ANOVA was used to evaluate interactions between treatment type and time or glucose addition, e.g. for time series of microbial growth, respiration, and CUE. These models tested for main effects and interaction terms, with Tukey post hoc comparisons applied where appropriate.

- A significance level of $P < 0.05$ was used as the threshold for statistical significance. However, due to limited replication (again, n = 3 for all data analyses, except for

respiration-related analyses where n = 2 due to a technical error following the first replicate), the statistical power was low. As such, where patterns were evident despite a lack of formal significance, these trends are discussed as potentially meaningful biological effects.

To better satisfy the assumptions of ANOVA, specifically the requirement of data having equal variances, log-transformations on the data were trialled, as the data in this study exhibited increasing variance with higher values. However, these transformations did not substantially alter the resulting P-values. Therefore, the original (untransformed) data were used for the final analyses to maintain consistency across datasets.

3.10.2. Calculation of Priming Effects

To quantify the priming effect of glucose addition on microbial growth and SOM mineralisation, a difference-based approach was used. For each replicate and timepoint, values from control (non-glucose) treatments were subtracted from those of the glucose-amended samples, resulting in priming effects expressed as absolute or relative changes (see Equation 8). Positive values indicate stimulation, while negative values indicate suppression of microbial processes due to substrate addition. This approach aligns with standard practices in priming effect quantification in soil biogeochemistry.

4. Results

Treatment abbreviations are defined under 'Treatment Types' in the abbreviations list.

4.1. Initial physicochemical and microbial characteristics after initial treatment exposure

4.1.1. Soil physiochemistry

The initial treatment had no significant effect on soil water content (SWC), being around 0.80 g H_2O g^{-1} dwt soil in all soils. Additionally, no significant effect was seen for SOM (0.25 ± 0.02 to 0.29 ± 0.02 g g^{-1} dwt soil), although SOM was marginally higher in the litter treatment and approached significance ($P = 0.09$), Table 1.

In contrast, both soil pH_{H2O} and electrical conductivity were significantly affected by the treatments ($P < 0.001$ and $P < 0.01$, respectively). Soil pH was higher in the litter and heat-shock treatments (both 4.4 ± 0.02), compared to the control (4.1 ± 0.05) and N (4.1 ± 0.03) treatments. Electrical conductivity was highest in the N treated soils (357 ± 17 µS cm^{-1}), followed by the control (229 ± 25 µS cm^{-1}), heat-shock (206 ± 4 µS cm^{-1}), and litter (174 ± 10 µS cm^{-1}) treatments.

Table 1. Soil physiological and microbial characteristics (mean ± SE) in response to litter addition, inorganic N and heat-shock treatments (i.e. 'initial' treatments).

	Control		**L**		**N**		**H**	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Water content (g H_2O g^{-1} dwt soil)	0.79	0.02	0.77	0.03	0.83	0.03	0.83	0.03
Soil organic matter (g g^{-1} dwt soil)	0.24	0.02	0.29	0.02	0.25	0.02	0.25	0.02
Soil pH_{H2O}	4.1	0.05	4.4	0.02	4.1	0.03	4.4	0.02
Electrical conductivity (µS cm^{-1})	229	25	174	10	357	17	206	4

Post hoc groupings based on these results are as follows:

Soil pH: Control (A), Litter (B), Nitrogen (A), and Heat-shock (B)

EC: Control (A), Litter (B), Nitrogen (C), and Heat-shock (B)

4.1.2. Soil microbial growth, respiration, SIR-Biomass and CUE

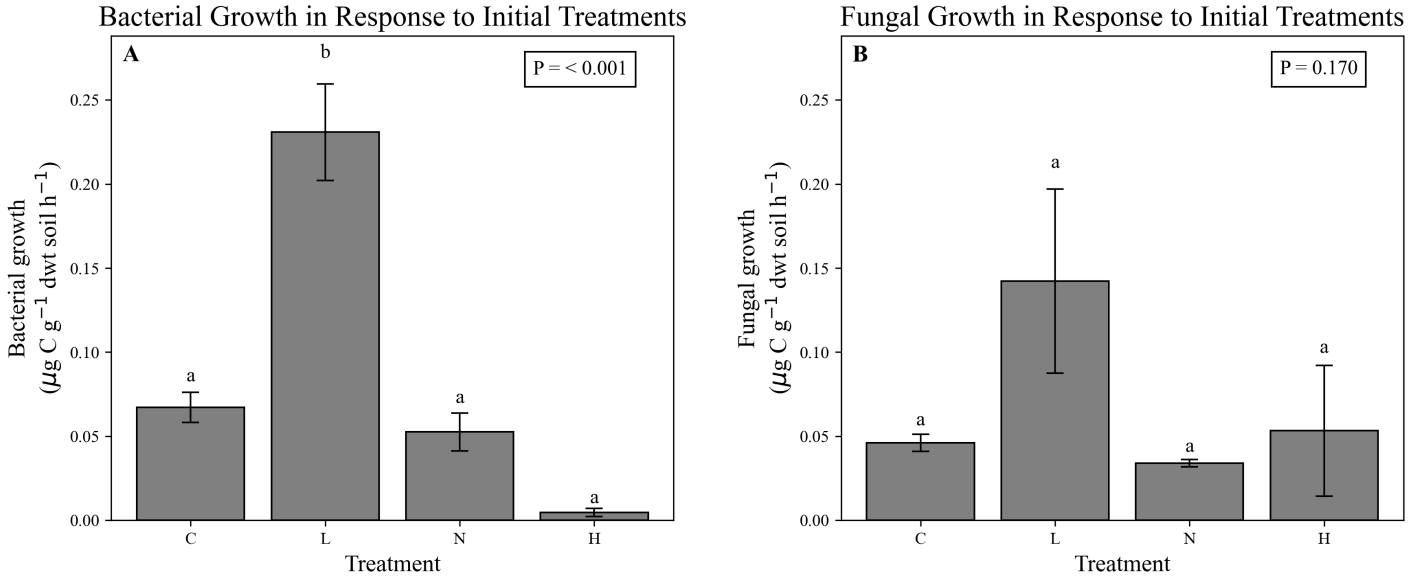

Figure 3. Microbial growth in response to initial treatments for (A) Bacterial growth, and (B) Fungal growth. Data shows mean ± SE (n = 3). Different letters above bars indicate statistically significant differences between treatments (Tukey's HSD, $p < 0.05$), applied consistently across all bar graphs. P-values from one-way ANOVA tests are shown in the black boxes within each panel.

Initial treatments had a significant effect on bacterial growth ($P < 0.001$; Figure 3a). The litter treatment resulted in the highest bacterial growth rate (0.23 ± 0.03 µg C g^{-1} dwt soil h^{-1}), which was significantly greater than all other treatments. The control and N addition treatments showed moderate bacterial growth (0.07 ± 0.01 and 0.05 ± 0.01 µg C g^{-1} dwt soil h^{-1}, respectively), while bacterial growth in the heat-shock treatment tended to be lower than in the other treatments, although the effect was not significant (0.01 ± 0.002 µg C g^{-1} dwt soil h^{-1}).

Fungal growth did not differ significantly across treatments ($P = 0.170$; Figure 3b), despite the litter treatment showing the highest mean fungal growth (0.14 ± 0.06 µg C g^{-1} dwt soil h^{-1}). All treatments, including the control (0.05 ± 0.01), N (0.03 ± 0.002), and heat-shock (0.05 ± 0.04), were grouped together in Tukey's HSD test, reflecting high variability and lack of statistical separation.

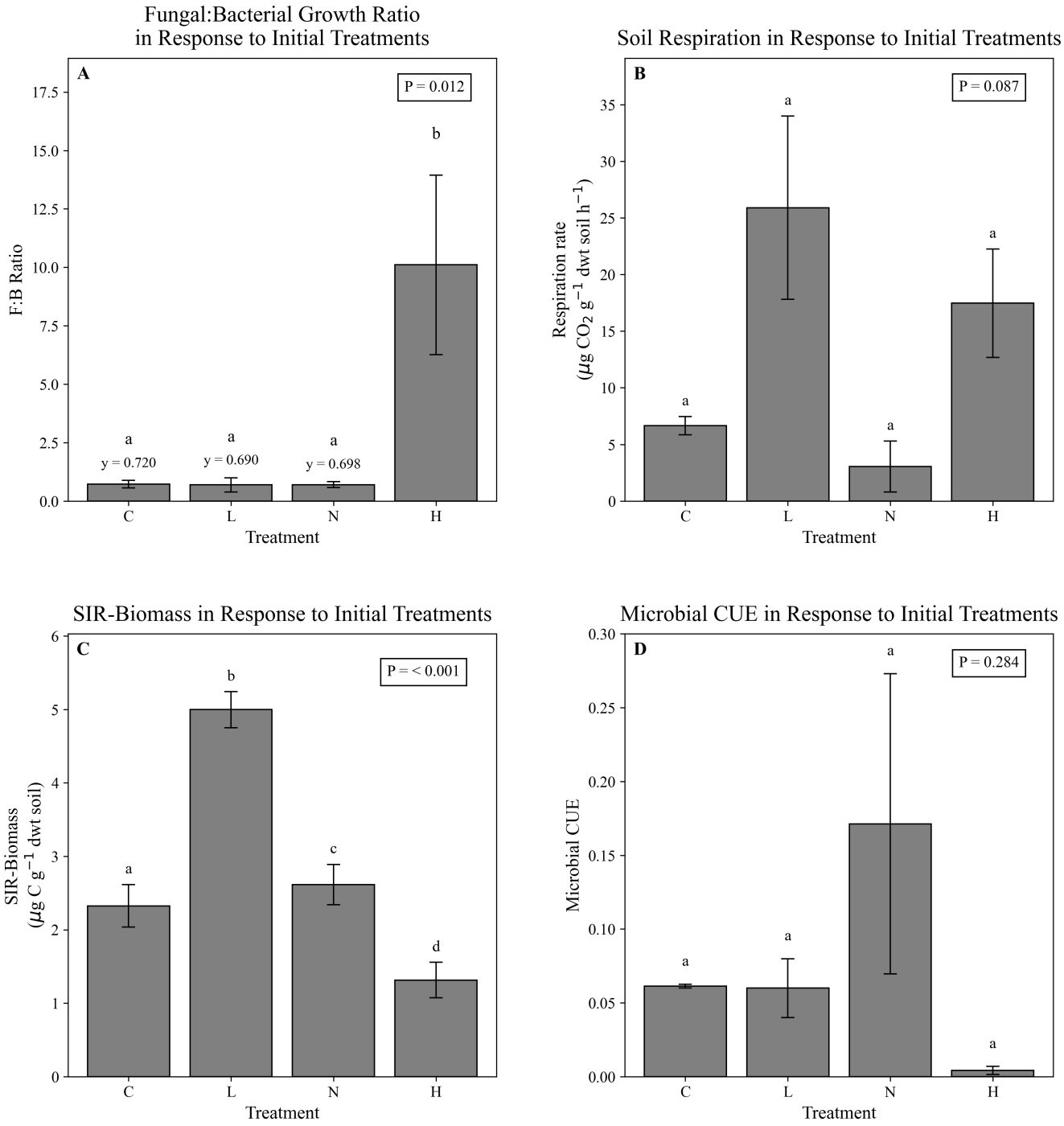

Figure 4. Microbial response to initial treatments: (A) Fungal-to-bacterial growth ratio, with values for C, L, and N displayed above bars (B) Soil respiration rate (C) SIR-Biomass (D) Microbial CUE. Data shows mean ± SE (n = 3 for (A) and (C); n = 2 for (B) and (D)). Different letters above bars indicate statistically significant differences between treatments (Tukey's HSD, $p < 0.05$), applied consistently across all bar graphs. P-values from one-way ANOVA tests are shown in black boxes within each panel.

The F:B growth ratio differed significantly among treatments ($P < 0.001$; Figure 4a). The heat-shock treatment resulted in a markedly higher F:B ratio (10 ± 3.8), being significantly greater than the control (0.72 ± 0.16), litter (0.69 ± 0.31), and N (0.70 ± 0.13) treatments, which did not differ from each other (all grouped as 'a' with Tukey HSD).

Soil respiration rates varied across treatments but did not reach statistical significance ($P = 0.087$; Figure 4b). The litter treatment showed the highest mean respiration rate (26 ± 8.1 µg CO_2 g^{-1} dwt soil h^{-1}), followed by heat-shock (17 ± 4.8 µg CO_2 g^{-1} dwt soil h^{-1}), control (6.7 ± 0.80 µg CO_2 g^{-1} dwt soil h^{-1}), and N (3.0 ± 2.2 µg CO_2 g^{-1} dwt soil h^{-1}). Although the litter treatment had the numerically highest respiration, high variability likely prevented statistical significance between the litter treatment and the control.

Microbial biomass differed significantly among treatments ($P < 0.001$; Figure 4c). The litter treatment exhibited the highest microbial biomass (5.0 ± 0.25 µg C g^{-1} dwt soil), significantly greater than all other treatments. The N treatment (2.6 ± 0.27 µg C g^{-1} dwt soil) had higher biomass than control (2.3 ± 0.29), while the heat-shock treatment had the lowest biomass (1.3 ± 0.24 µg C g^{-1} dwt soil).

Microbial CUE did not differ significantly among treatments ($P = 0.28$; Figure 4d), however, mean values showed noticeable trends. The N addition had the highest CUE (0.17 ± 0.10), followed by control (0.06 ± 0.001), litter (0.06 ± 0.02), and heat-shock (0.004 ± 0.003).

4.2. The effect of the 'initial' treatment on microbial characteristics in response to simulated root exudates

4.2.1. Priming of microbial growth

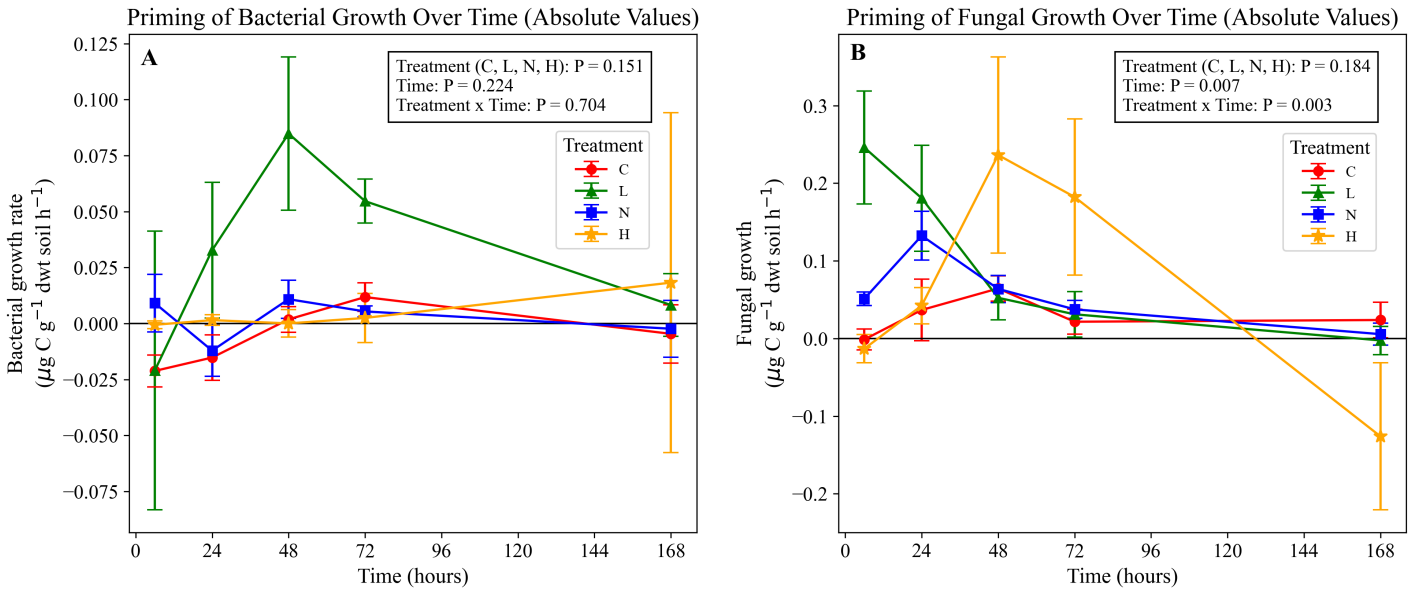

Figure 5. Priming of microbial growth over priming-exposure duration across treatments (absolute values). (A) Bacterial growth and (B) Fungal growth. Treatments: C = red circles, L = green triangles, N = blue squares, H = yellow stars Data shows mean ± SE (n=3). P-values from two-way ANOVA tests (factors: treatment, time, and their interaction) are shown in black boxes within each panel.

Bacterial growth responses to glucose addition showed variable patterns over time across treatments, though bacterial growth did not differ significantly between treatments or over time, and no interaction effect was observed (Figure 5a). For the litter treatment, bacterial growth peaked at 48 hours (0.09 ± 0.03 µg C g^{-1} dwt h^{-1}), before declining towards zero growth by 168 hours. The control and N treatments exhibited only slight positive priming effects throughout the incubation, both with peak values below 0.02 µg C g^{-1} dwt h^{-1}, at 72 hours and 48 hours respectively. The heat-shock treatment showed minimal bacterial growth priming at most timepoints, with high variability at 168 hours.

Priming of fungal growth varied across treatments and over time (Figure 5b). While overall treatment effects were not statistically significant (Treatment (C, L, N, H): P value = 0.184), fungal growth changed significantly over time (Time: P value = 0.007), and the influence of treatments varied across timepoints, illustrated by the Treatment x Time P value of 0.003. The heat-shock treatment showed a pronounced peak in fungal growth priming at 48 hours (0.24 µg ± 0.13 C g^{-1} dwt h^{-1}), followed by a steep decline to negative values by 168 hours. The litter treatment also peaked early (0.26 ± 0.07 µg C g^{-1} dwt h^{-1} at 24 hours), declining exponentially afterwards. The control treatment peaked in fungal growth at 48 hours (0.06 ± 0.02 µg C g^{-1} dwt h^{-1}), with the N treatment peaking earlier and more pronounced than the control at 24 hours, 0.13 ± 0.03 µg C g^{-1} dwt h^{-1}. Both the control and N treatment declined gradually until 72 hours, with no substantial change thereafter.

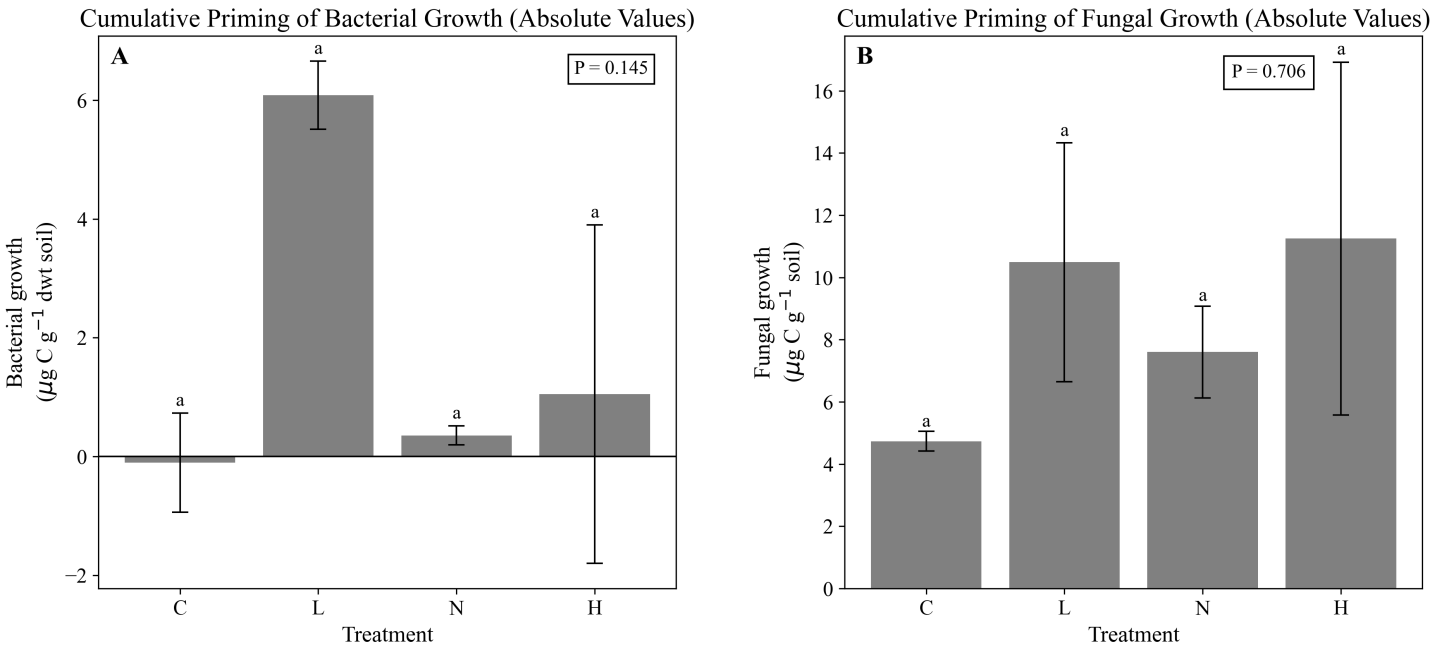

Figure 6. Cumulative priming of microbial growth across treatments induced by glucose addition, shown as absolute values. (A) Bacterial growth and (B) Fungal growth. Data shows mean ± SE (n=3). Treatments: C = control, L = litter addition, N = nitrogen addition, H = heat shock. Different letters above bars indicate statistically significant differences between treatments (Tukey's HSD, $p < 0.05$), applied consistently across all bar graphs. P-values from one-way ANOVA tests are shown in black boxes within each panel.

The cumulative priming of bacterial growth induced by glucose addition did not differ significantly among treatments (P = 0.15; Figure 6a), despite the litter treatment showing a substantially higher cumulative priming (6.1 ± 0.57 µg C g^{-1} dwt soil), while the control exhibited a slightly negative response (-0.10 ± 0.83 µg C g^{-1} dwt soil). The N and heat-shock treatments showed moderate positive cumulative values (0.36 ± 0.16 and 1.05 ± 2.8 µg C g^{-1} dwt soil, respectively), with high variability in the heat-shock treatment.

Cumulative priming of fungal growth also showed no significant differences among treatments (P = 0.71; Figure 6b). Mean cumulative values ranged from 4.7 ± 0.32 µg C g^{-1} dwt soil in the control to 11 ± 6.7 µg C g^{-1} soil in the heat-shock treatment. Despite higher means observed in the litter (10 ± 3.8 µg C g^{-1} dwt soil), N (7.6 ± 1.5 µg C g^{-1} soil), and heat-shock treatments, large standard errors resulted in no statistical significance.

4.3. Effects of the 'initial' treatment on the mineralisation of C in response to simulated root exudates

4.3.1. Source of respired C

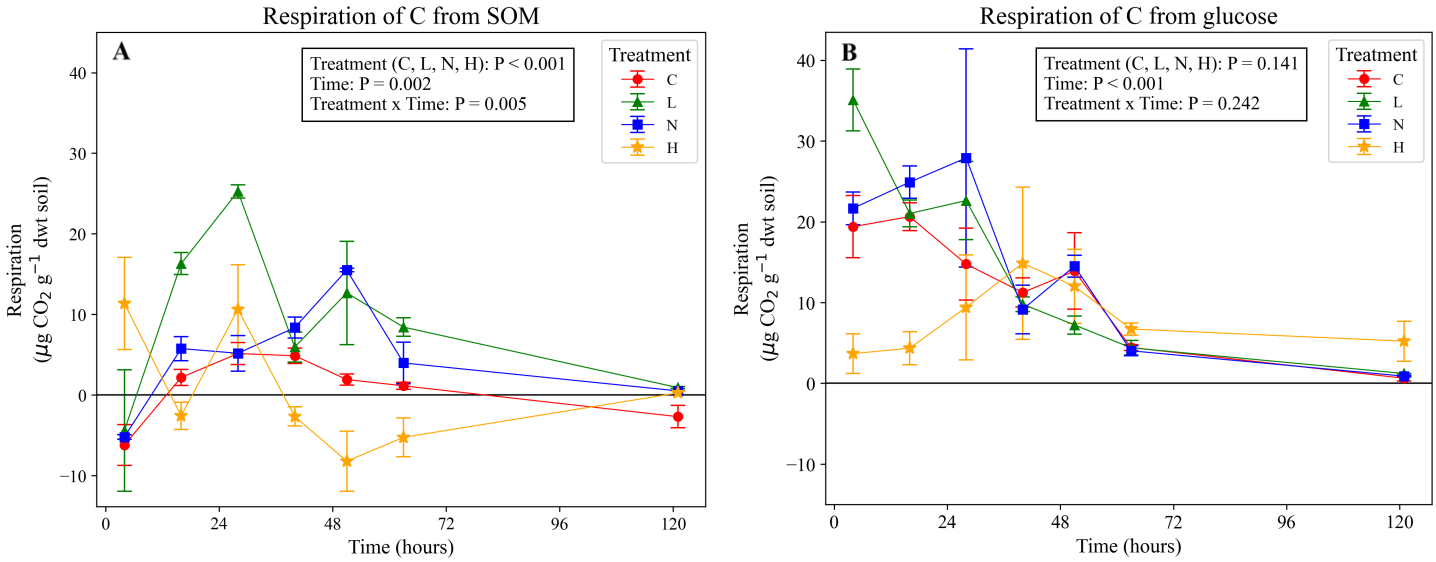

Figure 7. (A) Primed soil CO_2-C mineralisation induced by glucose addition and (B) glucose-derived CO_2-C mineralisation, over time across all treatment types. Data shows mean ± SE (n=2). Values correspond to the midpoint of each incubation period, as illustrated in Figure 2. Treatments: C = red circles, L = green triangles, N = blue squares, H = yellow stars. P-values from two-way ANOVA tests (factors: treatment, time, and their interaction) are shown in black boxes within each panel.

Respiration of C derived from SOM induced by glucose addition (i.e. "primed C mineralisation") differed significantly among treatments (P < 0.001; Figure 7a), and over time (P = 0.002), with a significant treatment × time interaction (P = 0.005), indicating that treatment effects varied across timepoints. The litter treatment exhibited the highest priming of C

mineralisation, peaking at 24 hours (25 ± 0.83 µg CO_2 g^{-1} dwt soil h^{-1}) before declining, with fluctuation, over time. The N treatment also showed a peak (16 ± 0. µg CO_2 g^{-1} dwt soil h^{-1} at 51 hours), followed by a gradual decline, with the control showing a broad low peak of respiration at 28 hours (5.1 ± 1.4 µg CO_2 g^{-1} dwt soil h^{-1}), decreasing gradually thereafter to negative respiration values. The heat-shock treatment fluctuated more widely, including negative values at 51 hours (-8.2 ± 3.7 µg CO_2 g^{-1} dwt soil h^{-1}). The litter, N and heat-shock treatments all displayed a convergence towards zero respiration after 63 hours.

Respiration of glucose-derived carbon did not differ significantly among treatments (P = 0.14; Figure 7b), but did vary significantly over time (P < 0.001). No significant interaction was observed between treatment and time (P = 0.24). However, several trends were observed: the control, litter and N treatments showed high initial glucose respiration rates of 19 ± 3.8, 35 ± 3.8 and 22 ± 2.0 µg CO_2 g^{-1} dwt soil h^{-1}, respectively. These values declined progressively over time, with some fluctuation, approaching zero respiration of C from glucose by 120 hours. In contrast, the heat-shock treatment began with a lower respiration rate (3.7 ± 2.5 µg CO_2 g^{-1} dwt soil h^{-1}) but exhibited a delayed peak at 40 hours (15 ± 9.4 µg CO_2 g^{-1} dwt soil h^{-1}), before gradually declining towards zero respiration of glucose-derived C.

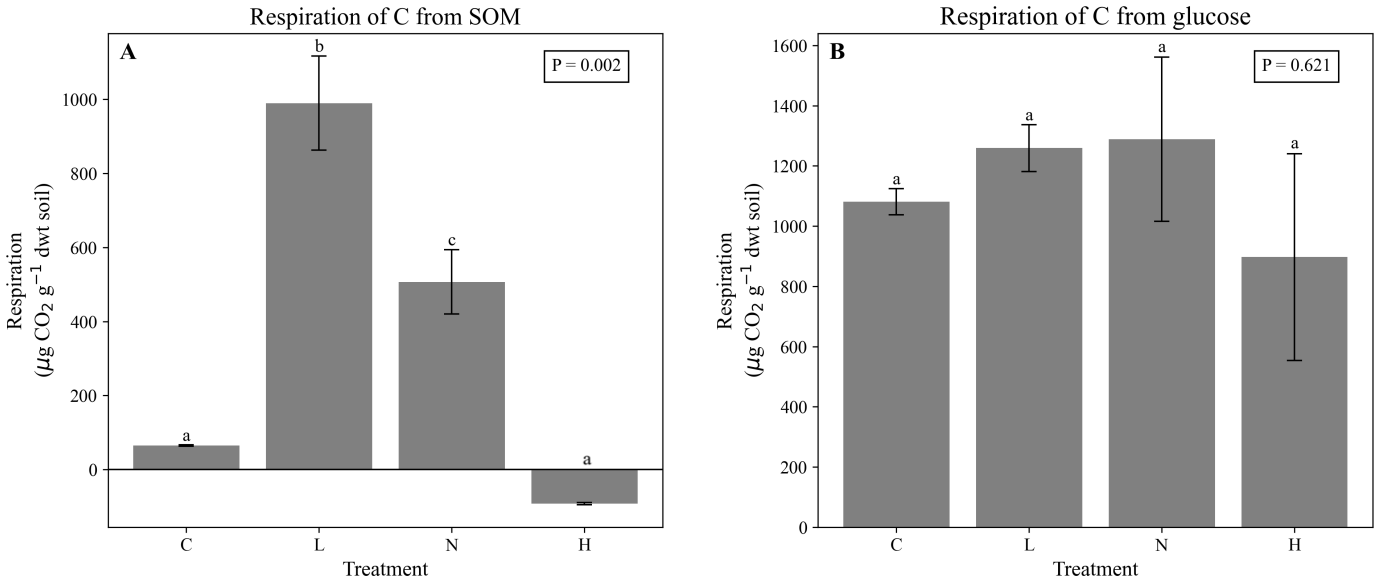

Figure 8. (A) Cumulative primed soil CO_2 mineralisation induced by glucose addition during 168 hours and (B) cumulative glucose-derived CO_2 mineralisation during 168 hours. Data shows mean ± SE (n=2). Different letters above bars indicate statistically significant differences between treatments (Tukey's HSD, p < 0.05), applied consistently across all bar graphs. P-values from one-way ANOVA tests are shown in black boxes within each panel.

Cumulative priming of C mineralisation from SOM differed significantly among treatments (P = 0.002; Figure 8a). The litter treatment showed the highest SOM-derived C respiration (990 ± 130 µg CO_2 g^{-1} dwt soil), significantly greater than all other treatments. The N treatment followed with a moderate level of respiration (510 ± 87 µg CO_2 g^{-1} dwt soil), while the control

exhibited much lower values (65 ± 1.5 µg CO_2 g^{-1} dwt soil). The heat-shock treatment showed net negative primed SOM-derived respiration of -92 ± 3.2 µg CO_2 g^{-1} dwt soil.

In contrast, cumulative respiration of glucose-derived C did not differ significantly among treatments (P = 0.62; Figure 8b). All treatments showed similar cumulative glucose respiration, with mean values ranging from 900 ± 340 to 130 ± 270 µg CO_2 g^{-1} dwt soil, even though the temporal dynamics of glucose-derived C mineralisation varied among soils (Figure 6b).

4.3.2. Microbial CUE

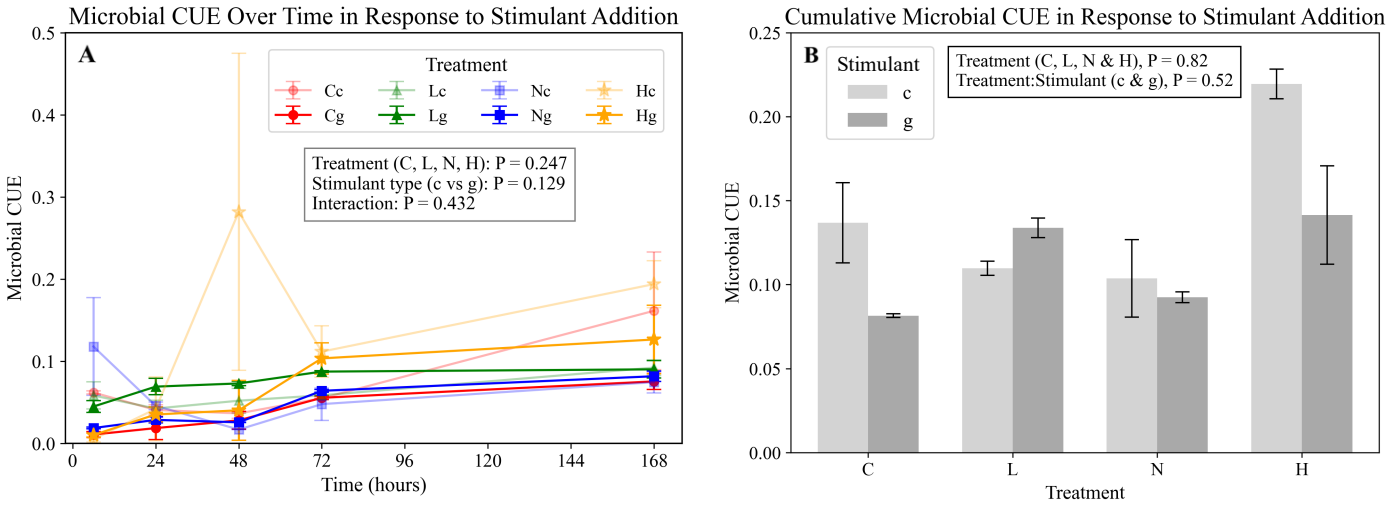

Figure 9. (A) Microbial CUE over time across for the different soil treatments and stimulant types. Treatments: C = red circles, L = green triangles, N = blue squares, H = yellow stars. P-values from two-way ANOVA tests (factors: treatment, stimulant type, and their interaction) are shown in the black box within the panel. (B) Cumulative CUE for the different soil treatments and stimulant types during 168 hours. Stimulant: c = control (no glucose), g = glucose-amended. P-values from two-way ANOVA tests (factors: treatment and stimulant type) are shown in the black box within the panel. Data shows mean ± SE (n=2)

Microbial CUE changed significantly over time following stimulant addition (P < 0.001), with a marginal effect of treatment (P = 0.057; Figure 9a). Across all treatments, CUE generally increased between 0 and 168 hours. The litter and control treatments exhibited steady, moderate increases in CUE over time in both control and glucose-amended soils. The heat-shock treatment showed greater variability, particularly in glucose-amended soils, where CUE peaked sharply at 48 hours (0.21 ± 0.17) before stabilising. Control soils maintained comparatively low and stable CUE across all treatments.

Cumulative microbial CUE varied across treatments but was not significantly influenced by either treatment type (control, litter, nitrogen, heat-shock; P = 0.40, stimulant addition (control vs. glucose; P = 0.68) (Figure 9b). Across all treatments, CUE was generally higher under control conditions than under glucose amended soils, except for the litter addition treatment, though the difference was not statistically significant.

5. Discussion

5.1. The effect of the initial treatments on microbial community function

5.1.1. Litter addition stimulated microbial growth

The litter treatment stimulated both bacterial and fungal growth (Figure 3), however, the fungal-to-bacterial growth ratio remained unchanged compared to the control (Figure 4a), indicating that both microbial groups were stimulated to a similar extent. This suggests that both bacteria and fungi were able to use and respond similarly to the added litter, rather than it particularly benefitting fungal growth, contrary to the initial hypothesis (H1.i). Although fungi are usually associated with litter decomposition due to the wide C:N ratio of litter and presence of more resistant compounds (e.g. lignin) (Wang and Kuzyakov, 2024), the loss of more simple soluble C compounds immediately following the addition of the added litter may have also supported higher bacterial growth (Rousk et al., 2016).

The increase in microbial biomass under the litter treatment (Figure 4c) suggests significant growth of the microbial community during the initial 7-day incubation period, consistent with the observed increases in microbial growth (Figure 3). This supports the interpretation that the litter addition not only enhanced microbial activity but also promoted biomass accumulation. Elevated microbial respiration in the litter-treated soils further confirms this (Figure 4b), although this difference was not statistically significant ($P = 0.15$), likely due to limited replication ($n = 2$). However, it is important to recognise that while an increase in biomass may reflect greater microbial activity, it could also indicate the presence of a large but very inactive microbial population, since biomass merely reflects the size of the microbial pool, not its activity level. Therefore, microbial growth measurements offer a more direct insight into microbial functioning, and their active role in contributing to the decomposition processes during the incubation period.

As such, the increase in microbial growth (Figure 3) provides strong evidence for enhanced microbial activity in response to the litter addition treatment, making the concurrent rise in microbial respiration unsurprising. However, despite the increased microbial respiration and growth, CUE remained similar between the litter and control treatments (Figure 4). This suggests that the added litter stimulated microbial activity (increased both respiration and growth), but it did not significantly increase the efficiency with which microbes incorporated the C into biomass. This suggests that increased litter inputs as a result of climate change could accelerate microbial processing of C but will not substantially affect the fate of this C, with a similar fraction released back to the atmosphere as CO_2.

In summary, litter addition stimulated both bacterial and fungal growth equally, with no change in their growth ratio. Microbial biomass and respiration increased, but CUE remained unchanged. This suggests litter boosts microbial activity without altering the proportion of C retained versus lost as CO_2.

5.1.2. N addition increased microbial CUE

Contrary to expectation, the N addition treatment did not significantly enhance microbial growth compared to the control (H1.ii), despite the N-limited nature of beech forest soils (Michopoulos et al., 2008). Both bacterial and fungal growth showed a slight, non-significant decline in the N-treated soils (Figure 3), if anything, indicating an inhibitory effect rather than a stimulatory one. However, this result does align with findings from previous studies where high levels of inorganic N, 50-100 kg ha^{-1} yr^{-1}, have been shown to inhibit microbial, especially in ecosystems where microbes are adapted to lower N availability (Wang et al., 2024).

In terms of community structure, the fungal-to-bacterial growth ratio remained largely unchanged in the N-treated soils (Figure 4a). This is contrary to the hypothesis (H1.ii) where it was believed that bacterial growth would be more enhanced than fungal growth due to bacteria having a narrower C:N ratio than fungi, and thus are more sensitive to nitrogen limitation (Xu et al., 2013; Strickland and Rousk, 2010; Hu et al., 2023). Microbial respiration was also reduced by N addition (Figure 4b). Consequently, despite a tendency towards reduced microbial growth, the CUE of the microbial community in N-treated soils was significantly higher than in the control (Figure 4d), as the reduction in respiration exceeded the reduction in growth. This suggests that, under N addition, microbes became more efficient at allocating assimilated C to biomass rather than respiring it as CO_2. This shift likely reflects a prioritisation of biomass synthesis over respiration under the high N conditions – rather than growing more rapidly, as microbes are using C more efficiently for biomass production rather than losing it to respiration. This contradicts the initial hypothesis where the N addition treatment was expected to have increased microbial growth, leading to higher respiration due to increased microbial activity (Silva-Sanchez, 2019).

Overall, N addition led to an increase in microbial CUE (Figure 4d), but a decrease in overall growth (Figure 3) and respiration (Figure 4b). The unchanged F:B growth ratio further suggests that both bacterial and fungal groups were similarly affected, and that the community composition remained the same, despite functional changes. These results show that microbial communities responded to the N inputs not by expanding in size, but by reallocating resources to become more efficient under the changed nutrient conditions.

5.1.3. Heat-shock reduced overall microbial biomass but favoured fungal growth

The heat-shock treatment was expected to reduce overall microbial activity, particularly fungal growth. However, the results revealed mixed results: while bacterial growth was almost entirely suppressed, the fungal growth remained stable or even slightly increased (Figure 3). These microbial growth responses contradict both the initial hypothesis (H1.iii) and previous literature, which generally suggests that bacteria are more tolerant to elevated temperatures (e.g. 50°C, Riah-Anglet et al., (2015)) than fungi (Riah-Anglet et al., 2015; Frey et al., 2008). The observed increase in fungal growth, despite its variability, may reflect a selective advantage for more heat-tolerant fungal taxa (Allison and Treseder, 2008), or a competitive release of fungi after the killing off of bacteria, enabling fungi to better exploit available resources (Hicks et al., 2019).

Moreover, while control soils exhibited a fungal-to-bacterial growth ratio of 0.7:1, the heat-treated soils displayed a significantly elevated ratio of approximately 10:1 (Figure 4a), clearly illustrating a substantial shift in microbial community structure. As fungal growth remained relatively stable, this shift can be attributed primarily to the near-total collapse of bacterial activity.

In accordance with the hypothesis (H1.iii), respiration increased significantly following heat exposure (Figure 4b). This rise is likely driven by high fungal metabolic activity or potentially by the decomposition of microbial necromass resulting from bacterial mortality (Andrews et al., 2000).

The observed reduction in microbial biomass under heat-shock conditions (Figure 4c) further supports the implication of high microbial mortality in these soils, agreeing with the hypothesis (H1.iii). Despite elevated respiration rates, the decline in active microbial biomass indicates that fewer living cells were responsible for the observed respiration. Additionally, the significant decrease in microbial CUE in heat-treated soils (Figure 4d) suggests a marked shift in microbial C allocation strategies. The remaining microbial community appears to have prioritised respiration over biomass synthesis, likely as a stress response to maintain cellular integrity and energy balance under extreme thermal conditions (Beugnon et al., 2025).

To summarise, heat-shock suppressed bacterial growth but had little effect on fungi, leading to a sharp increase in the fungal-to-bacterial growth ratio. Despite reduced microbial biomass, respiration increased, likely due to fungal activity or bacterial necromass decomposition. Microbial CUE declined, indicating a stress response favouring respiration over growth.

Together, these results confirm that all experimental treatments induced changes in the microbial community (albeit not always matching hypotheses), enabling me to test the question of how shifts in resource availability associated with climate change would affect the susceptibility of soils to priming by labile C input.

5.2. The susceptibility of soils to priming differed depending on the initial treatment

5.2.1. Dynamic priming of SOM in control soils after glucose addition

A recent study of the same beech forest soils found that semi-continuous additions of glucose stimulated ("primed") microbial growth and SOM mineralisation (Na et al., 2025). I therefore expected that glucose addition would also stimulate microbial growth and SOM mineralisation in the control soils used here (H2.i). However, my results show a dynamic and transient priming response. SOM mineralisation was initially suppressed (Figure 7a), likely due to preferential substrate utilisation, where microbes initially prioritised using the added glucose over SOM (Kuzyakov, 2002). This was supported by the high early rates of glucose-derived respiration (Figure 7b). As glucose availability declined, primed SOM mineralisation briefly increased, suggesting a short-lived positive priming phase (Figure 8a). This response is likely due to the single pulse nature of the glucose addition. Other pulse addition experiments have also shown transient priming responses with similar dynamics (Hicks et al., 2020). Despite the short-lived nature of the response, the priming of SOM mineralisation was still substantial in the glucose-amended soils when compared to the amount of SOM mineralisation in the control (non-glucose amended) soils, indicating that labile C addition will overall trigger a loss of pre-existing SOM.

Microbial growth mirrored this pattern. Bacterial growth showed no clear priming (Figure 5a), contrasting with previous studies reporting transient stimulation (Na et al., 2025). A slight bacterial peak coincided with the peak glucose respiration, consistent with bacterial preference for labile C. In contrast, fungal growth was briefly stimulated (Figure 5b), aligning with the timing of SOM mineralisation, suggesting that fungi contributed more to the observed priming. The previous study also measured gross N mineralisation in response to glucose addition, and found that the priming of SOM mineralisation was specifically associated with a targeted decomposition of more N-rich SOM (Na et al., 2025). This was interpreted as "microbial N mining", suggesting that the priming response was driven by microbial demand for N. It could therefore be assumed that a similar mechanism may also be driving the priming response observed here. This can later be verified as I also measured gross N mineralisation in my soils (awaiting data).

The lower CUE values for the glucose treated soils, compared to the non-glucose treated soils, is often seen in literature (Hicks et al., 2020) (Figure 9). Initially, the glucose addition likely causes microbes to focus on rapid energy production (via respiration) rather than investing in growth. This results in a lower CUE because more C is lost through respiration, and less is allocated to biomass production. In contrast, the non-glucose soils use SOM (a more complex C source) for slowed but more efficient and sustained growth, leading to higher CUE. The reduction in microbial growth but increase in CUE can be explained by the shift toward more efficient C use in a response to nutrient depletion, leading to higher CUE over time.

Overall, glucose addition triggered a brief, fungal-driven priming of SOM, but microbial activity remained largely focused on the labile substrate. The initial negative priming of SOM mineralisation suggests preferential substrate utilisation. Glucose-treated soils showed lower CUE than non-glucose soils due to initial microbial prioritisation of respiration over growth, while non-glucose soils exhibited more efficient C use over time.

5.2.2. Litter addition strengthened the priming of SOM following glucose addition

The litter treatment led to a larger and more active microbial community (Figure 3, Figure 4c), which appears to have driven the greater priming of SOM mineralisation observed after glucose addition, which is in agreement with the hypothesis (H2.ii). A larger microbial biomass enhances the potential for substrate uptake and metabolic activity, explaining the significantly increased cumulative respiration of C from SOM in litter-treated soils compared to the control following glucose addition (Figure 8a).

This response may be driven by a combination of increased microbial biomass due to the litter treatment, and intensified nutrient limitation. While the litter provided abundant C, its wide C:N ratio and relatively low nutrient content likely imposed stronger N limitations on microbial activity (Hicks et al., 2022). As a result, once microbes had exhausted the added glucose - used initially due to preferential substrate utilisation (Figure 7b) – they increasingly turned to SOM to mine nutrients (through such mechanisms as 'selective microbial N-mining'; see section 2.2), resulting in amplified SOM decomposition in the litter soils compared to the control.

An alternative, but complementary explanation is the greater stimulation of microbial growth in litter-treated soils. A larger microbial biomass can metabolise more substrate, accelerating turnover and respiration. As in agreement with the initial hypothesis, the cumulative priming of bacterial and particularly fungal growth (Figure 6) further supports the idea of increased microbial community leading to increased priming of SOM mineralisation, especially as fungi are frequently associated with priming responses (Rousk et al., 2016; Soares et al., 2017). The temporal alignment between bacterial growth and SOM respiration suggests that bacteria, despite typically preferring labile C, may have contributed substantially to SOM decomposition. This could be due to their greater N demand (i.e. narrower C:N ratio, Chen et al., (2022)), potentially driving them to mine SOM for N.

Notably, glucose-derived respiration in the litter-treated soils was initially very high, then declined to near-zero over the week (Figure 7b), reinforcing that microbial activity shifted away from labile C use and toward SOM decomposition. The delayed SOM response and microbial growth pattern point to stronger nutrient mining in litter-treated soils, perhaps driven by the need to access limiting resources not supplied by the glucose pulse.

One would normally expect the CUE of the glucose-exposed soils to be lower than its control counterpart as more of the labile C (glucose) is easily respired off, without contributing to

microbial growth (Paterson and Sim, 2013). However, this is not the case for the litter-treated soils; instead, microbes appeared to use glucose efficiently early on, supporting a burst of growth. However, glucose became depleted, and microbial activity shifted toward SOM decomposition, likely to mine nutrients, as mentioned previously. During this phase, both growth and respiration declined proportionally, maintaining a stable or slightly elevated CUE (Figure 9). This pattern suggests C limitation following glucose exhaustion, rather than nutrient limitation, which would typically lower CUE due to higher respiration relative to growth.

In summary, the strong priming of SOM in litter-treated soils likely reflects both a larger and more active microbial biomass resulting from the recent history of litter addition coupled with a heightened demand for nutrients due to the litter's high C:nutrient imbalance. Following glucose addition, microbes first utilised the labile C but were then forced to intensively decompose SOM to acquire C and nutrients, resulting in a stronger priming effect compared to control soils.

5.2.3. N addition supported an increase in microbial activity resulting in greater priming of SOM mineralisation

It was hypothesised that N addition would reduce microbial priming of SOM due to alleviation of N limitation (Hatley et al., 2009) and a potential shift toward a more bacterially dominated community (which are less associated with priming) (H2.iii). However, the results contradict this expectation. Cumulative primed respiration of C from SOM was significantly higher in the N-treated soils compared to the control (Figure 8a), indicating a clear priming effect. This suggests that N addition did not suppress SOM mineralisation but may have rather facilitated it, potentially by supporting microbial growth or altering community function. This indicates the occurrence of stoichiometric decomposition, whereby the C from the added glucose, together with the N from the N addition, were together used to support microbial growth, which then decomposed more SOM (Hessan et al., 2004). Like the control and litter treated soils, the N addition treatment initially exhibited signs of preferential substrate utilisation.

The priming of C mineralisation occurs later and more pronounced than the control. One explanation may be that in the N-treated soils, the added N sources initially supress the nutrient mining behaviour, but once the labile C input has been consumed, microbes may begin decomposing SOM for other limiting micronutrients (e.g. phosphorus), producing a delayed priming response. Additionally, the delay may be due to stoichiometric decomposition, where it takes a while for the microbial community to grow, before initiating SOM mineralisation – hence, the priming response emerges gradually rather than immediately (Chen et al., 2014).

Bacterial growth exhibited a small, non-significant positive priming response, with error bars just above zero (Figure 6a). In contrast, fungal growth showed a more pronounced priming, peaking earlier and more sharply than in the control (Figure 5b), and remaining higher overall

in the cumulative response (Figure 6b). Although statistically insignificant, this pattern suggests that fungal activity, not bacterial, was more responsive to glucose addition under N-enriched conditions, which is contrary to the original hypothesis (H2.iii). Furthermore, the earlier priming of the fungal growth, compared to the priming of SOM mineralisation, also aligns with the aforementioned stoichiometric decomposition theory. In addition, as stated earlier in the discussion, fungi are frequently associated with priming responses; as such, this could be an explanation for the unexpected priming response.

CUE was lower in the glucose-treated N addition soils than in the controls (Figure 9b), which matches the expected decrease due to rapid respiration of labile C. However, these differences were not statistically significant, and large error bars, particularly in the time series data, limit strong conclusions.

Overall, N addition did not alleviate priming as hypothesised. Instead, the apparent stoichiometric decomposition and a modest increase in microbial activity, particularly in fungi, likely lead to greater SOM mineralisation,

5.2.4. Heat-shock resulted in negative priming of SOM mineralisation

The effects of heat-shock treatment on SOM priming were difficult to predict. While microbial mortality from high temperatures might reduce activity and suppress SOM mineralisation, it could also release nutrients from dead biomass, potentially reducing the need to decompose SOM (H2.iv). Alternatively, biomass turnover could stimulate microbial recovery and enhance priming.

In this study, heat-shocked soils showed a trend for negative priming effect (although not statistically significant), as indicated by the reduced cumulative respiration of SOM-derived C compared to control soils (Figure 8a). This suggests that SOM decomposition was not stimulated by glucose addition, possibly due to a smaller active microbial community following the initial harsh heating disturbance (Figure 3, Figure 4c).

There was also limited evidence of priming in microbial growth in these soils. Bacterial growth showed a small response, but large error bars limit conclusions about the size or direction of priming. Fungal growth showed slightly more stimulation than the control (Figure 6b), though this was not statistically significant. As discussed earlier, the higher F:B growth ratio after heat-shock suggests that fungi may have been more resilient to the disturbance, potentially dominating recovery. Notably, after the glucose addition, fungal growth peaked at 48 hours (Figure 5b), coinciding with the peak in CUE in non-glucose-treated soils (Figure 9a). This indicates that C was being directed toward microbial growth rather than respiration at that time, possibly explaining the dip in SOM-derived respiration at this time. This suggests a trade-off between growth and decomposition: as fungi recovered and invested in biomass production, SOM mineralisation was deprioritised, contributing to the observed negative priming.

The pattern of glucose-derived respiration in heat-shocked soils also deviated from other treatments. Rather than peaking immediately, as seen in other soils, glucose respiration peaked at 48 hours (Figure 7b). This delay likely reflects a "bottlenecked" microbial response, constrained by the reduced size and metabolic capacity of the microbial community (Anderson and Domsch, 1975), meaning that they could not use the added glucose as quickly and to the same extent as in the other soils. As the community (particularly fungal) recovered, glucose utilisation increased, and by the end of the 7-day study period, there was no difference in cumulative glucose-C mineralisation among treatments (Figure 8b). The corelation between fungal growth and glucose respiration peaks supports this, suggesting fungi were actively metabolising glucose once the community regained functionality.

Although fungi are often linked to SOM priming (Rousk et al., 2016; Soares et al., 2017), these results imply that under heat-stress, fungi also exhibit preferential substrate utilisation, prioritising glucose metabolism over SOM decomposition. It is particularly striking, however, that fungi favoured SOM over glucose while community size was still limited, particularly as glucose is a better source of C for fungal growth for short term growth than SOM, due to its labile nature. A possible reason is that the heat-shock treatment may have induced N limitation in the soil – this may explain why fungi prioritised SOM decomposition over glucose metabolism despite the latter being a more readily available C source. Furthermore, part of the observed SOM decomposition may have resulted from the breakdown of the microbial necromass, which is rich in N. If fungi were N-limited, they may have targeted this recently dead microbial biomass within SOM as a nutrient source.

In summary, heat-shocked soils showed a trend toward negative SOM priming, likely due to reduced microbial activity after heating. Fungal growth was more resilient than bacterial growth, with delayed glucose respiration and a shift towards biomass production over decomposition. This suggests fungi prioritised glucose for growth, while early SOM respiration may indicate N mining from SOM to aid in the initial recovery of microbial activity.

5.3. Limitations and future outlook

A central limitation of this study was the low replication (n = 3 or n = 2, as discussed previously) limiting statistical power. This very likely contributed to cases where biologically meaningful trends did not reach statistical significance.

One limitation of this study was the use of separate microcosms for the Picarro δ^{13}C measurements, and for the measurements of microbial growth and N-mineralisation. The study assumes identical environments between the measurement microcosms (for the same treatment exposure); however, in reality slightly different practices were carried out with the different microcosms. The Picarro microcosm remained closed for the duration of the treatment exposures (except for headspace flushing) with no mixing of the soil, whereas the microbial

growth/N-mineralisation microcosms were opened and stirred to ensure homogeneous soils when sampling. This could influence soil-headspace moisture dynamics, as well as exposure of micro-organisms to C and nutrients withing the soil (it can be assumed that mixing the soil will increase exposure), and consequent differences in the mineralisation of SOM.

A silver lining of the Picarro's unreliable functionality was the requirement of manual C measurements. The samples used for the respiration measurements were taken from the same microcosm as the ones used for measurements of soil characteristics, microbial growth and N mineralisation, improving consistency between microbial activity and respiration measurements.

In addition, the chemical composition of the litter used in the litter addition treatment was not analysed. Information on C:N ratio and the proportion of labile vs. recalcitrant compounds could have provided context for interpreting microbial responses and priming mechanisms especially given the importance of litter quality on priming effects (Fanin et al., 2020).

Microbial community shifts were deduced from bacterial and fungal growth rates (e.g. fungal-to-bacterial growth ratios), rather than measured directly through molecular or biochemical methods. Direct characterisation (e.g phospholipid fatty acid (PLFA) profiling) would be necessary to confirm actual microbial community changes; for example, to determine whether the apparent fungal resilience to heat-shock was truly due to the selection of more heat-tolerant fungal species.

Another limitation is that the priming measurements only spanned 168 hours (7 days) following a single pulse addition of glucose, capturing only short-term microbial responses and priming effects. However, longer-term dynamics remain untested and may differ substantially in magnitude or direction, especially in response to root exudates which occur in a semi-continuous nature in soils. As a result, any extrapolation of these results to long-term ecosystem processes/projections should be taken with caution, as the responses observed should first be verified in intact plant-soil systems.

Moreover, this study investigated the effects of root exudation (labile C input), litter, N addition, and heat-shock individually. Yet in reality, these factors often co-occur and may interact in complex ways. For instance, Di Lonardo et al. (2018) showed that N addition actually suppressed litter-induced priming. Future work should explore such interactions to better reflect climate change impacts on soil C cycling.

Finally, while I did carry out the measurements for gross N mineralisation, I haven't acquired data yet. This would enable me to verify the mechanisms related to microbial N mining, which at the moment are inferred based on responses in soil C mineralisation.

While I believe my study provides valuable insights into how different climate-related treatments affect microbial growth and SOM priming, the limitations discussed show where further research could help support my conclusions. These include longer-term experiments, measuring microbial community composition directly, and testing the interactive-effects of treatments that would give a more realistic picture of how these processes play out in natural systems.

5.4. The implication for impact of climate effects on the changes in SOM mineralisation for regional C sequestration in southern Sweden

Changes in microbial CUE following glucose addition offers valuable insights into how climate-induced shifts in root exudation might influence soil-atmosphere C dynamics. Glucose addition, simulating increased root exudation expected under warming scenarios, tended to reduce microbial CUE in most treatments (Figure 9), despite lack of statistical significance.

This general trend towards a reduction in CUE, following glucose addition, suggests that more C is being lost as CO_2 rather than retained within the microbial biomass pool, which is as expected (Na et al., 2022). My results could indicate a shift toward reduced soil C under future conditions of enhanced labile C inputs; consequently, this decrease in CUE (i.e. increase in CO_2 to the atmosphere) could result in a positive feedback loop, increasing emissions of CO_2, increasing temperatures, and further increasing root exudation rates. However, although minimal, under glucose addition the litter, N and heat-shock treatments all tended to have greater CUE than the control soils indicating a greater efficiency of microbial C use and suggesting that a greater fraction of C used by microorganisms may stay in soil. As a result, a possible implication is that the impacts of climate change might actually decrease the C flux from the soils to the atmosphere, inducing a negative C cycle feedback.

Interestingly, a notable exception emerged in the litter addition treatment, where glucose addition tended to increase microbial CUE. This suggests that the mechanism of co-metabolism was taking place under glucose addition; where the glucose provided the energy needed for microbes to simultaneously decompose more recalcitrant SOM, while also supporting biomass synthesis (Broadbent, 1947). In this case, the energy from the labile C input (glucose) could be used not only for respiration, but for also building more microbial biomass which might ultimately become stabilised in soils as necromass – a process that contributes to SOM formation and consequent increases in soil C storage (Liang et al., 2017; Miltner et al., 2012).

Another interesting result is the higher CUE values observed for the heat-shock treated samples across both glucose and control soils (Figure 9). The lack of priming and higher CUE suggests a stress-resilient but low-activity microbial community that conserves C rather than actively mineralising it, thereby reducing the overall C flux from soil to atmosphere – at least temporarily, until microbial communities recover.

It is also important to consider how these belowground responses might interact with plant growth. For instance, increased SOM priming (as exhibited for the litter and N treatments, Figure 8) may release N from SOM that is otherwise inaccessible to plants, potentially stimulating plant growth – particularly in N-limited systems such as beech forests. Subsequently, this could enhance CO_2 uptake through photosynthesis, offering a potential offset to microbial respiration losses. However, if microbial turnover leads to nutrient immobilisation or reduced mineralisation, this could constrain plant productivity, (as nutrients are not released through decomposition of necromass), creating a positive feedback loop that reduces nutrient availability and plant growth.

All together, these findings suggest that climate-driven increases in labile C inputs may generally lower microbial CUE, potentially reducing soil C storage. However, certain treatments (e.g., litter addition or heat-shock) highlight that the microbial response is complex and context-dependent. Understanding how microbial communities allocate C – especially under stress or varying nutrient resource conditions – will be crucial for predicting soil C dynamics in a warming world.

6. Conclusions

This study suggests that climate-driven alterations of beech forest soils will induce changes in microbial community and functionality, which will also affect the susceptibility of soils to priming by increased labile C inputs.

These findings outline the importance of stochiometric balance of C and nutrients in the soil, and the effect that alterations in the availability of resources (either through increased labile C exudation from root biomass, litter inputs, N addition, or heat-shock treatments) has on the susceptibility of SOM to priming. Notably, all treatments exhibited some degree of preferential substrate utilisation following glucose addition, and stronger priming effects in litter and nitrogen treatments were linked to increased microbial biomass and potential microbial nitrogen mining and stoichiometric decomposition. In addition, the heat-shock treatment revealed unexpected fungal dominance in glucose-derived respiration, and a tendency towards inhibited priming of SOM mineralisation.

Together, these results suggest that nutrient stoichiometry and microbial community size are key regulators of priming in temperate beech forest soils. Furthermore, treatments that enhance priming, such as the addition of labile C substrates, could lead to accelerated soil C losses, particularly via microbial N-mining or biomass-driven SOM turnover. While a limitation in this study is the low statistical power (due to the limited number of replicates), observed trends in the data can give us an insight into the future C balance of terrestrial ecosystems, specifically beech forests.

In summary, while the findings from this controlled laboratory-based study must be validated in intact plant-soil systems, if climate change continues to shift resource availability and stress regimes in forest soils, the balance between C inputs and losses may increasingly favour C mineralisation over sequestration, transforming these critical forest ecosystems from C sinks to potential sources in the global C budget.

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Appendix

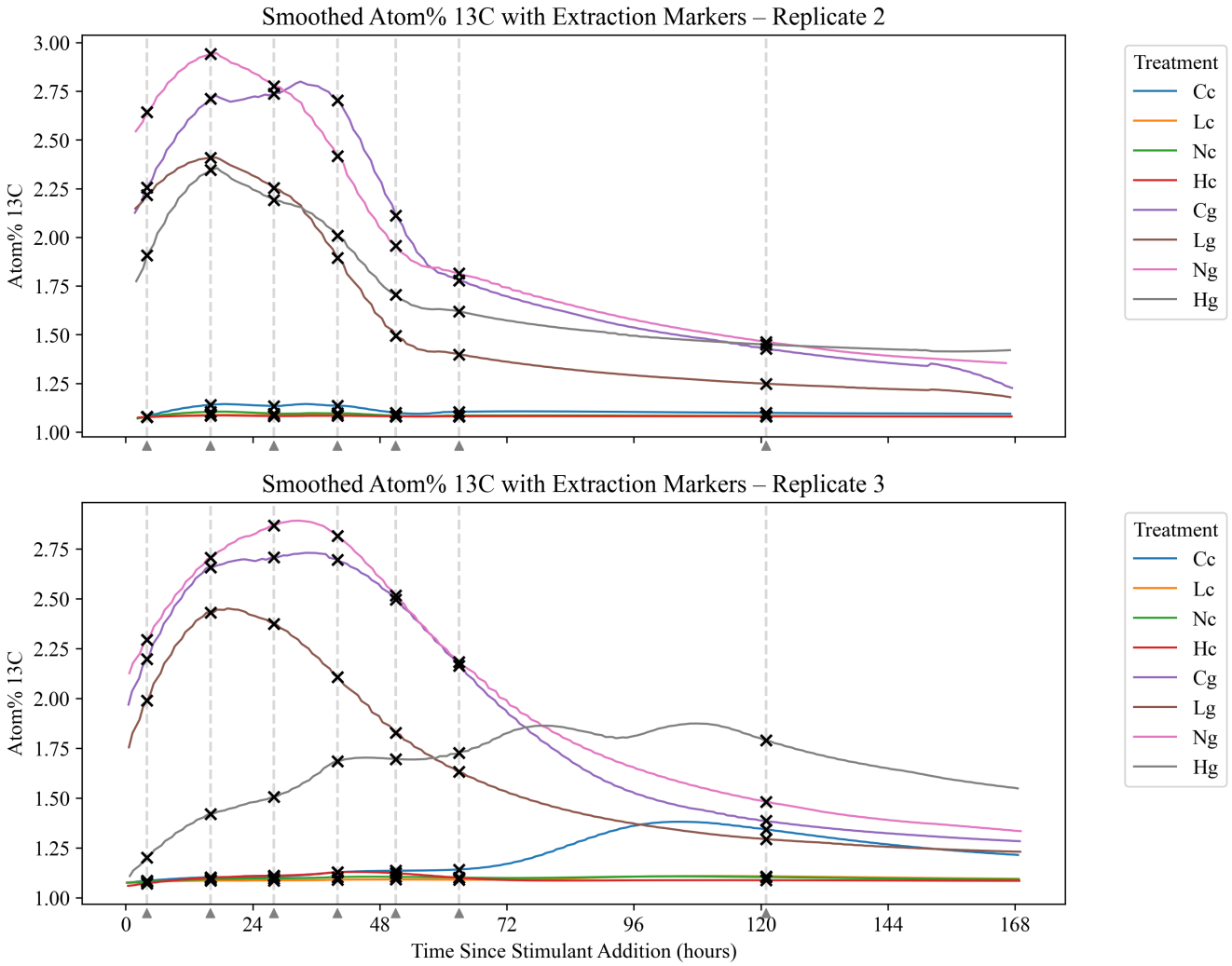

Figure A 1. Smoothed Atom% ^{13}C values of CO_2 respired over time following stimulant addition for replicates 2 and 3, across all treatments. Values were smoothed using a Savitzky–Golay filter and interpolated to extract Atom% ^{13}C at specific timepoints corresponding to manual respiration sampling. Grey ticks indicate sampling times, and black crosses show the interpolated timepoints used in the isotope mixing model to quantify priming effects. Treatment abbreviations are defined under 'Treatment Types' in the abbreviations list.