THESIS

WHAT HAPPENS DURING SOIL INCUBATIONS? EXPLORING MICROBIAL BIOMASS, CARBON AVAILABILITY AND TEMPERATURE CONSTRAINTS ON SOIL RESPIRATION

Submitted by

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ABSTRACT

WHAT HAPPENS DURING SOIL INCUBATIONS? EXPLORING MICROBIAL BIOMASS, CARBON AVAILABILITY AND TEMPERATURE CONSTRAINTS ON SOIL RESPIRATION

Decomposition of soil organic matter (SOM) is one of earth's most important and dynamic biogeochemical cycles. Much research is devoted to separating and studying individual controls on SOM decomposition. A commonly used approach is to incubate soils under controlled conditions to understand the drivers of SOM decomposition. In chapter 1, I explore the use of soil incubations to investigate SOM-temperature dynamics, and emphasize the importance of testing the assumptions of laboratory soil incubations. In chapter 2, I describe how I tested whether depletion of available SOM, soil microbial biomass, or extra-cellular enzyme pools drive the decline in soil respiration over the course of a long-term incubation in soils from two sites (a cultivated plot and a forested plot at Kellogg Biological Station, Hickory Corners, MI USA). I found that the availability of SOM was the key determinant of respiration, and the loss of microbial biomass and extra-cellular enzymes over the course of a long-term incubation did not limit the ability of the remaining microbial biomass to respire available SOM. I observed a sharp increase in respiration when the soils were mixed, which supports availability as a key driver of soil respiration. My results support a paradigm in which physico-chemical drivers are the primary determinant of soil respiration over the course of a long-term incubation. In chapter 3, I describe how I investigated the validity of using constant temperatures – a departure from diurnal temperature oscillations soils experience *in situ* – in laboratory soil incubations. The effect of oscillating versus constant temperature in incubation experiments designed to measure

soil organic matter (SOM) decomposition response to temperature is not well studied in the laboratory. I investigated the impact of oscillating versus constant temperature incubation regimes on soils from the two sites listed above with varying levels of available SOM, microbial biomass and extra-cellular enzymes. Over 42 days of incubation I measured changes in soil respiration, changes in the existing microbial biomass and extra-cellular enzyme pools, and shifts in the thermal optima of four common soil extra-cellular enzymes in response to oscillating (shifting between 25°C and 35°C every 12 hours) and constant (30°C) temperature treatments. I found that none of these soil pools were significantly affected by incubation temperature oscillations. My results justify the use of soils depleted of microbial biomass and a constant temperature regime to investigate SOM decomposition in laboratory soil incubations.

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CHAPTER 1

INTRODUCTORY CHAPTER: THE USE OF SOIL INCUBATIONS TO INVESTIGATE SOIL ORGANIC MATTER DECOMPOSITION

INTRODUCTION

From their inception in the early 20th century, laboratory soil incubations have been a mainstay in soil science research. My master's thesis tests fundamental assumptions of soil incubations to provide information about the factors driving soil organic matter (SOM) decomposition.

Terrestrial decomposition models like CENTURY (Kelly et al., 1997) and ROTH-C (Jenkinson, 1990) make use of data from field and laboratory incubations to forecast the quality and quantity of terrestrial SOM stocks under different climatic scenarios. Dynamics of SOM respiration are of great interest to ecologists due to SOM's ecological role as a global carbon sink, as a source of soil fertility through nutrient release, its role as a driver of soil structure, and its ability to enhance moisture retention (Gupta & Larson, 1979; Oades, 1984; Saini, 1966; Tisdall & Oades 1982; Tiessen et. al, 1994). In a world with changing climatic patterns, growing human populations, and an increasing demand for ecosystem services (i.e. food and textile production, water filtration, decomposition, etc) from swelling human populations (Dominati et al, 2010), it is all the more important to understand the complex and dynamic nature of SOM and to test the validity of laboratory incubations as surrogates for field conditions (Haile-Mariam et al., 2008; Jenkinson, 1966; Trumbore, 2006).

Even though soil incubation studies are plentiful, there is disagreement among experts regarding terminology, experimental design/data collection methods, and interpretation of results

(Conant et al., 2011). Resolving these contentions will strengthen the power of soil incubations to provide us with useful information about *in situ* processes and projections of future SOM stocks and flows.

For example, the terms "labile" and "recalcitrant" frequently appear in scientific literature to describe SOM fractions but lack clear, universally accepted definitions. Resistance to decomposition is attributable to a variety of physico-chemical factors such as isolation within aggregates, temperature/water limitations, the availability and electron affinity of terminal electron acceptors, etc. (Davidson and Janssen, 2006). Due to its slow decomposition rate, SOM bound to or occluded within minerals could be labeled as "recalcitrant", without implicating biochemical structure (Hartley & Ineson, 2008) despite the fact that "recalcitrant" is frequently used to imply SOM compounds with high biochemical complexity (i.e. lignin) (Sollins et al., 2006). Therefore, the term "recalcitrant" and "labile" are broad terms that tell us little about the actual SOM fractions aside from their apparent decomposition rate. That these terms are often used in peer-reviewed publications to mean different things likely causes confusion and disagreement based on semantics alone.

Further complicating our understanding of lability and recalcitrance, SOM exists not in clearly delineated fractions, but as a continuous range of biochemical complexities with varying levels of physico-chemical protection.

Additionally, SOM incubation results meant to describe the same phenomenon can differ due to diverse methodological approaches, and a data set from a singular incubation can be vastly interpreted. For example, Kemmitt et al. (2008) used a laboratory incubation to cull an estimated 90% of the microbial biomass to determine if microbial biomass size limits SOM decomposition. They concluded that SOM decomposition was not limited by the size of the

microbial biomass and rather was governed solely by abiotic processes. Kuzykov et al. (2009) responded that Kemmitt et al.'s results reflected their methods, not an actual phenomenon, arguing that their approach failed to account for the significant contribution of microbial extra-cellular enzymes in the soil matrix to SOM decomposition. Brookes et al. (2010) responded in defense of the Kemmit et al. (2008) and argued that any extra-cellular enzymes present were unlikely to account for the amount of CO_2 respired by the greatly reduced microbial biomass pool.

While there is evidence that microbial extra-cellular enzymes persist in the soil and contribute to decomposition independently of their source cell (Maire et al., 2012), the role of extra-cellular enzymes in limiting SOM decomposition specifically is not well studied, in part due to the difficulties associated with their size and the heterogeneous and opaque nature of soil. Clearly, both laboratory methods and varied interpretation of results contribute to the heated debate. While scientific debate is important to furthering our understanding of the accuracy of decomposition models, reducing disagreement based solely on spurious laboratory artifacts is fundamental to our continued reliance on decomposition models under changing climatic paradigms (Mcguire & Treseder, 2010).

Another instance of laboratory decomposition data causing dissent among scientists comes from a paper published in *Nature* by Giardina and Ryan (2000). The authors conducted a meta-analysis of data from 82 decomposition experiments, including long-term incubation data on soils from five continents. They concluded that warmer temperatures do not drive faster decomposition of forest-derived soil carbon. This is major divergence from a more standard understanding of the relationship between temperature and decomposition, and was immediately critiqued by Davidson et al. (2000). They argued that Ryan and Giardina's conclusions failed to

distinguish between the impacts of temperature on different SOM fractions and that the more slowly decomposing fraction could be larger and more temperature sensitive than the small, rapidly cycling temperature-insensitive pool. Davidson et al. 2000 concluded that discerning temperature response from individual soil fractions versus that from the whole soil may reveal the true nature of the SOM decomposition-temperature relationship. So, while there are still technological limitations to distinguishing CO_2 derived from different SOM fractions during incubation, there is not even consensus among scientists that this distinction is needed to understand the relationship between soil temperature and SOM decomposition.

Laboratory incubations are valuable and widely used tools for studying SOM decomposition in soils, but they require a set of assumptions that enable soils to be removed from their *in situ* context. These assumptions have generated methodological and interpretive disagreement among scientists. Yet science continues to rely heavily on data from laboratory incubations to forecast future terrestrial carbon stores and fluxes. This clearly shows the need to rigorously examine laboratory incubation assumptions and their impact on the validity of incubation data.

My master's research focuses on two of the common assumptions of laboratory soil incubations: (1) decreases in SOM availability – rather than a shrinking microbial biomass – drive declines in soil respiration over the course of a long-term incubation, and (2) constant temperature regimes employed in laboratory incubations are adequate replacements for diurnal temperature fluctuations ubiquitous in natural systems.

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CHAPTER 2

SOIL RESPIRATION IS DETERMINED BY SUBSTRATE AVAILABILITY, NOT MICROBIAL BIOMASS: INSIGHTS FROM A LONG-TERM INCUBATION

INTRODUCTION

Soil respiration rates in long-term incubations or field experiments decline over time following cessation of labile carbon (C) inputs (Cambardella & Elliott, 1992; Conant et al., 2011; Kelly et al., 1997; Schmidt et al., 2011; Six & Jastrow, 2002). Whether this decline in respiration is predominantly driven by a reduction of the available soil organic matter (SOM) pool as labile inputs diminish or a depletion of microbial activity due to substrate limitation is not thoroughly understood. A simplified conceptual model (Figure 2.1) shows the physico-chemical and biotic pools and flows of on SOM decomposition. Available SOM is defined as low molecular weight organic compounds in the soil that are physico-chemically unbound and susceptible to enzymatic depolymerization. Whether the rate limiting step of SOM decomposition is physico-chemical release of OM to the available pool or depolymerization of available OM is not well established.

Kemmitt et al. (2008) reported that when soils were incubated under controlled conditions, there was no significant decrease in soil respiration despite a 90% reduction in microbial biomass. They suggested that physico-chemical processes of physical stabilization in microaggregates and chemical associations with clay and silt particles (Six & Jastrow, 2002) release a slow "trickle" of SOM from the protected to the available SOM pool where it is rapidly depolymerized, assimilated, and then respired at a rate that is independent of the microbial

biomass pool size. Kemmit et al. (2008) thus concluded that SOM respiration is controlled by physico-chemical, rather than biological, drivers.

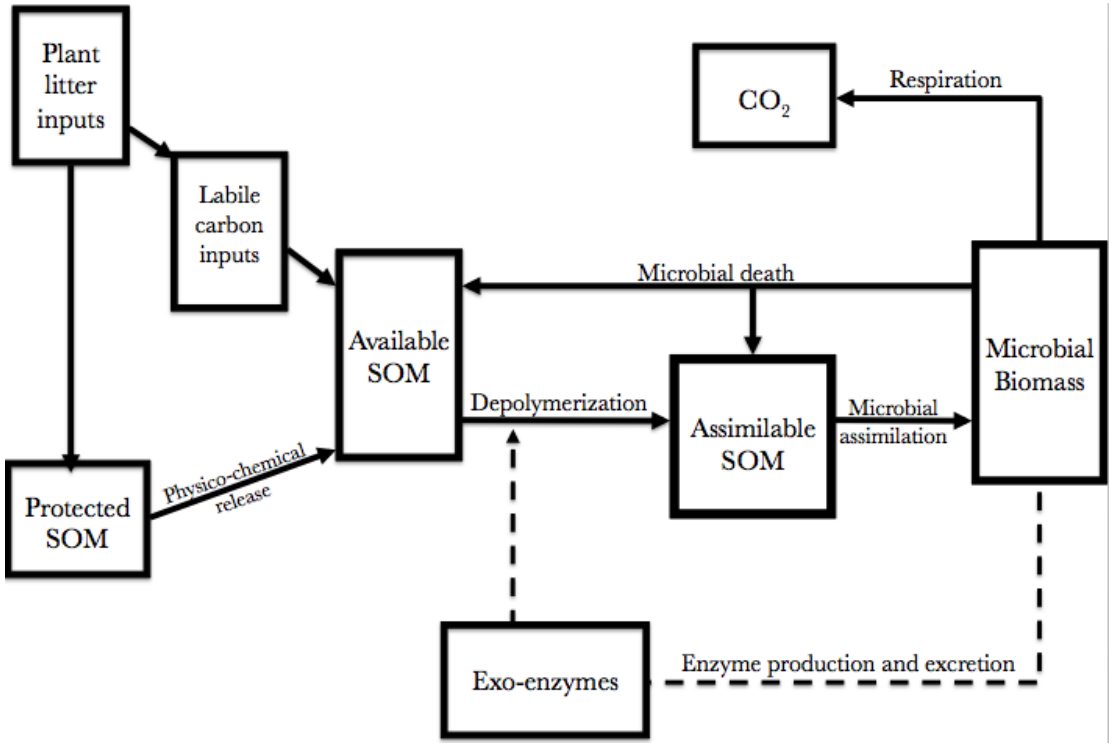


Figure 2.1 This conceptual diagram describes pools and flows of soil organic matter (SOM). Carbon enters the soil matrix through plant litter inputs and is either physico-chemically protected through occlusion in aggregates and adsorption to mineral surfaces or becomes immediately available for depolymerization. Protected SOM can be made available to depolymerization through physico-chemical release. Once SOM is available, it is depolymerized into smaller, assimilable SOM by extra-cellular enzymes excreted by the microbial biomass. The microbial biomass takes up the depolymerized, assimilable SOM, incorporating carbon into their biomass or respiring it as CO_2. Adapted from Conant et al., 2011.

Conversely, there is evidence in the literature that changes in the size of the microbial biomass and extra-cellular enzyme pools control soil respiration rates. Kuzyakov (2000) and others reported that an increase in respiration in response to substrate addition was unexplainable by the amount of substrate addition alone, and attributed the additional respiration to an increase in microbial activity, yielding increased turnover of SOM, a phenomenon often referred to as

"priming" (Blagodatskaya et al., 2011; Kuzyakov, 2006; Kuzyakov et al., 2009; Yakov & Kuzyakov, 2010; Neff et al., 2002). There is also evidence that soil respiration is limited when enzymes targeting specific, abundant substrate (e.g. hemicelluloses) are present at prohibitively low levels (German et al., 2011). This work suggests that the rate-limiting step in SOM decomposition in laboratory incubations is the depolymerization of available SOM by extra-cellular enzyme pool.

Experiments that measure soil respiration during the depletion of low molecular weight, unprotected SOM (defined here as the available SOM pool) show that respiration rates decline over time, whether conducted in the field (as bare fallow experiments; Cambardella & Elliott, 1992; Six et al., 2002) or in laboratory incubations (Conen et al., 2008; Creamer et al., 2011; Haile-Mariam et al., 2008; Liu et al., 2006; Plante et al., 2010). Whether depletion of the microbial biomass or extra-cellular enzyme pools drive this reduction in respiration rates is still contested. The purpose of my work was to investigate whether a decline in respiration rate observed over the course of a long-term laboratory incubation was attributable to reduced inputs to the available SOM pool or to the depletion of extra-cellular enzyme and/or microbial biomass pools. By isolating these two mechanisms, I hope to provide further support for either physico-chemical or microbial controls for determining soil respiration over the course of a long-term incubation.

METHODS

A long-term soil incubation was used to deplete soils of both SOM and microbial biomass to distinguish the effects of available SOM limitation and reduced microbial activity on respiration rates. A subsequent incubation was used to test the alternative hypotheses that SOM decomposition rates are limited by (1) substrate availability or (2) microbial biomass. To assess

whether substrate availability limits SOM decomposition rates, I alleviated potential substrate limitation by adding labile substrate to soil depleted of microbial biomass and with low extra-cellular enzyme activity. To test whether microbial biomass drives a reduction of respiration rates, I alleviated potential microbial constraints by evaluating respiration of soil containing undisturbed, full microbial biomass and extra-cellular enzyme pools.

Depleting soils of organic matter and microbial biomass

In late 2007, soil samples were collected (20cm-deep cores; dia.=1.8cm) at the Kellogg Biological Station Long-Term Ecological Research site (Robertson, 1991) from cultivated and forested sites (Table 2.1). The cultivated plot was converted from deciduous hardwood forest in the early 1900s and was cropped in a mixed small grain corn–soybean rotation for many years and under alfalfa just prior to 1993, when it was converted to the current continuous, conventionally-tilled corn crop. The forested plot was reforested in the late 1800s and is currently a late-successional deciduous hardwood forest.

Table 2.1 KBS site descriptions, including total % carbon respired after 707 days of incubation at 30°C. Percent carbon values are averages (±SE). KBS MAT is 9.7°C and MAP is 890mm.

Site	Vegetation	Total C (%)	C:N	C respired after 707d (% of total soil C)
Cultivated	Corn crop	0.76 (±0.03)	12.8	20.34 (±0.64)
Forested	Deciduous forest	1.22 (±0.05)	8.5	18.02 (±1.61)

After collection, soil samples were transported to the laboratory and stored at 4°C in sterile, plastic bags for roughly one week. Soils were then passed through a 2mm-mesh sieve and large (>2mm) surface and belowground plant matter was removed. Samples of sieved, air-dried soil were analyzed for carbonates (none were detected, using a standard technique of 1M HCl drops to detect effervescence at ambient temperature) and total organic carbon and nitrogen

using a LECO CHN-1000 analyzer (LECO Corp., St. Joseph, MI).

The processed soils were then subjected to incubation under constant 30°C temperature and moisture for 707 days. Soil samples (200g) were placed in 250mL un-covered glass beakers, which were placed within sealed, half-gallon sized jars. The soils were maintained at 50% water filled pore space throughout the incubation. Air samples from the headspace of the sealed mason jars were drawn through septa, transferred to evacuated vials, and brought to laboratory facilities at the Natural Resource Ecology Laboratory (NREL) to measure CO_2 concentration using a Li-Cor LI-6252 (Li-COR Biosciences, Lincoln, NE) infrared gas analyzer.

CO_2 in the headspace of each jar was measured every two to five days at the outset of the incubation, when respiration rates were at their highest. From days 14-707, samples were taken every 7 to 28 days. Jars were flushed with CO_2–free air after every measurement and lost moisture and worn septa were replenished as needed, roughly six times throughout the incubation (modified from Haddix et al., 2011 and Follett et al., 1997). Incubating the soils for 707 days significantly depleted them of soil carbon (Table 2.1).

After 707 days of incubation, all soil samples were removed from the incubator, with some destructively harvested and some subject to further experimentation (hereafter referred to as the "*incubation-depleted*" soil samples). In 2010, new, "*fresh*" samples were collected from the same sites (cultivated and forested) at the Kellogg Biological Station and processed in the same manner as the samples collected in 2007.

Substrate amendment experiments

I added substrate to a subset of incubation-depleted and fresh soil samples to assess microbial constraints on respiration in samples with low (incubation-depleted) and high (fresh) microbial biomass. I evaluated substrate constraints on respiration by adding new substrate to a

subset of samples ("*amended*") and no substrate to others ("*unamended*").

Substrate amendments were added as 600µg C g^{-1} dry soil of finely ground plant material. This amount of substrate addition is roughly enough to produce new biomass equivalent to the amount of microbial biomass C of the fresh soil. All soils samples (amended and unamended, incubation-depleted and fresh) were mixed thoroughly by stirring and subjected to incubation at 30°C for 42 days. Soil samples (27g dry-weight equivalent) were placed in 250mL un-covered glass jars, which were placed within sealed, pint-sized jars with rubber septa sealed in their lids. Approximately 20mL of water was placed in the bottom of each jar to maintain a humid headspace and 50% water filled pore space. Throughout the incubation, air samples from the headspace of the jars were collected as described above for the 707d incubation, with a similar frequency (daily for five days, every 2-3d thereafter).

After 21 days of incubation, half of the samples were harvested ("*Day 21*" samples) and analyzed for microbial biomass and extra-cellular enzyme activity. I measured chloroform-extractable carbon using a chloroform fumigation-extraction method (based on methods used by Wu et al., 1994) and potential soil extra-cellular enzyme activity using a fluorescence microplate assay method developed by Steinweg and McMahon (2012). These were measured as indices of microbial biomass and activity, respectively.

I added a second pulse of substrate to the amended samples at day 21 to assess how the re-growing microbial biomass was respiring labile substrate, or, if the microbial biomass pool was not regenerating, whether the incubation-depleted extra-cellular enzyme and biomass pools were capable of depolymerizing and respiring an additional round of labile substrate. At day 21, all remaining soils (amended and unamended alike) were well mixed and returned to the incubation for another 21 days. At the end of the incubation, samples were analyzed for

microbial biomass and extra-cellular enzyme activity.

Investigating the soil extra-cellular enzyme pool using enzyme assays

I investigated the activity of three hydrolytic C acquisition extra-cellular enzymes: α-glucosidase (AG), β-glucosidase (BG), β-D-cellobiohydrolase (CB); and one nitrogen acquisition extra-cellular enzyme: N-acetyl-β-d-glucosaminide (NAD) to investigate whether respiration of SOM and the added labile substrate was controlled by the potential activity of the soil extra-cellular enzyme pool, and if the size of the extra-cellular enzyme pool is coupled to biomass and/or substrate availability.

Following a protocol developed by Steinweg and McMahon (2012), I mixed 1.375g of soil with 45mL of 50mM sodium acetate buffer with a pH of 5.5 in a soil blender (Waring 8011G) on high for 60 seconds. The soil solution was then pipetted into deep-well plates and fluorescently-labeled substrate was added in excess. The deep-well plates containing the soil sample solution and substrate were covered and incubated at 30ºC for 2hr 15min. The fluorescent tags on the substrates only absorb electromagnetic radiation and fluoresce when separated from the substrate, which occurs when extra-cellular enzymes catalyze the mineralization of the fluorescently labeled substrate. Using a plate reader, I analyzed the amount of fluorescence emitted by the incubated slurries.

Statistical analyses

Four field replicates were collected from the cultivated site and three from the forested site at both sampling dates (pre-incubated and fresh soils). In the lab, each replicate was split into four equal sub-samples, two of which were given a substrate addition. Half of the samples were harvested at day 21, and the other half at day 42. I used analysis of variance (ANOVA) to test the effects of depletion (soil carbon and biomass) and substrate addition on respiration, microbial

biomass and extra-cellular enzyme activity. Differences were considered statistically significant for $P < 0.05$.

RESULTS

Cumulative respiration throughout the 42-day incubation was, on average, roughly two times greater in the fresh amended versus fresh unamended soil, and 4-6 times greater in the incubation-depleted amended versus incubation-depleted unamended soils (Figure 2.2). Cumulative respiration was also greater from the unamended fresh forested and cultivated soils than from corresponding incubation-depleted soils, respiring an average 3-5 times more CO_2 throughout the incubation. A notable exception to this pattern is the forested, amended, incubation-depleted soil, which respired as much as the forested, amended, fresh soil. Respiration from the forested soil in all treatments was much more variable than from the cultivated soil (Figure 2.2).

Respiration of the added substrate – calculated by subtracting the control (non-amended) soil respiration from the substrate-amended soil respiration –increased between day 21 and 42, but was not significantly different among fresh or incubation-depleted soils from either site at day 21 or day 42 (Figure 2.3). At day 21, roughly 62% of the added substrate had been respired from both the incubation-depleted forested soils and the fresh cultivated soils, with 28% and 49% of substrate respired from the fresh forested and incubation-depleted cultivated soils, respectively. At day 42, roughly 65% and 60% of the added substrate was respired from the incubation-depleted cultivated and fresh forested soils, respectively, with 50% and 40% respired from the incubation-depleted forested and fresh cultivated soils, respectively.

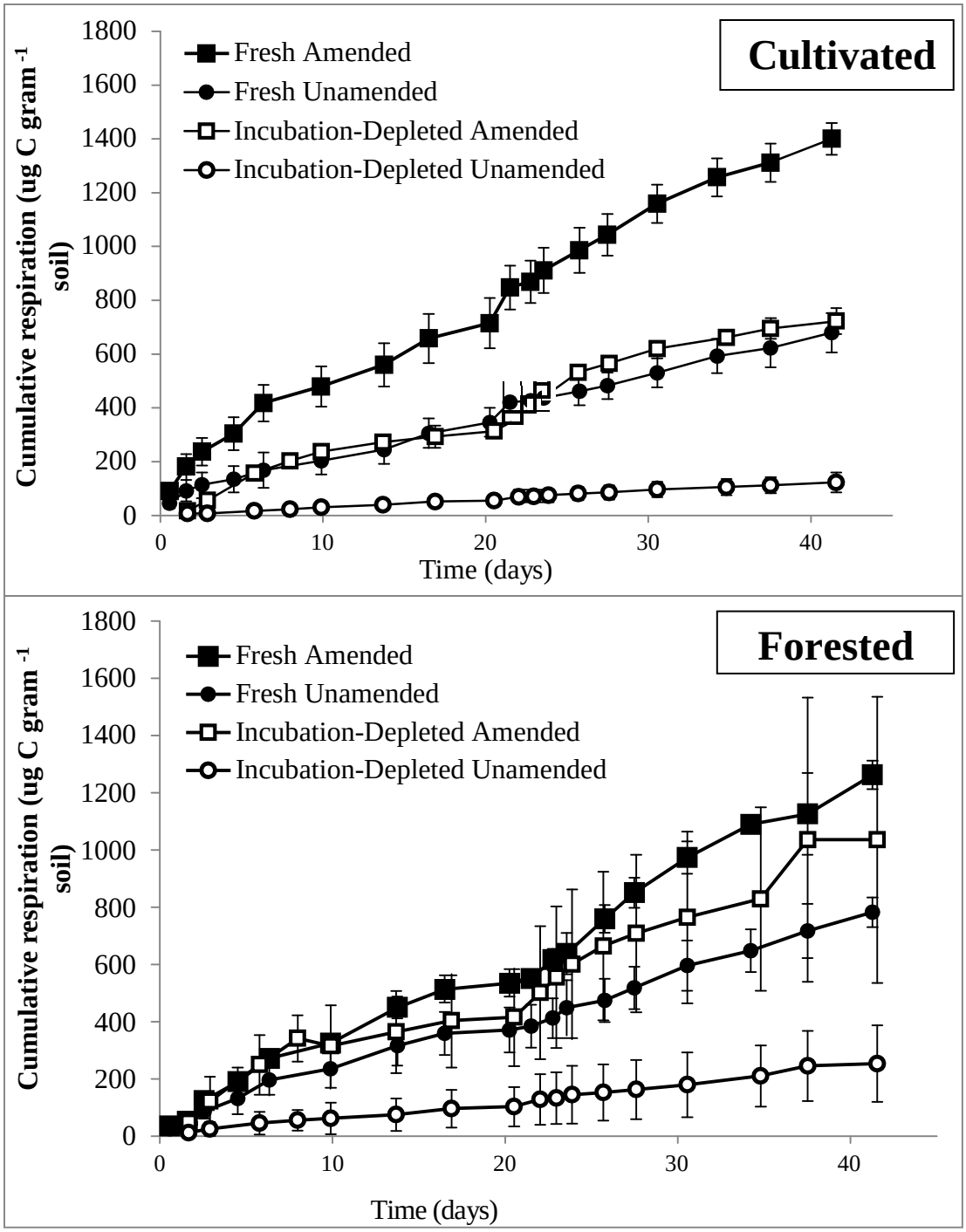


Figure 2.2 Cumulative soil respiration over 42 days from cultivated (top) and forested (bottom) land use, with varying levels of labile substrate depletion: fresh amended (solid squares), fresh unamended (solid circles), depleted amended (unfilled squares), and depleted unamended (unfilled circles). Values are averages (±SE).

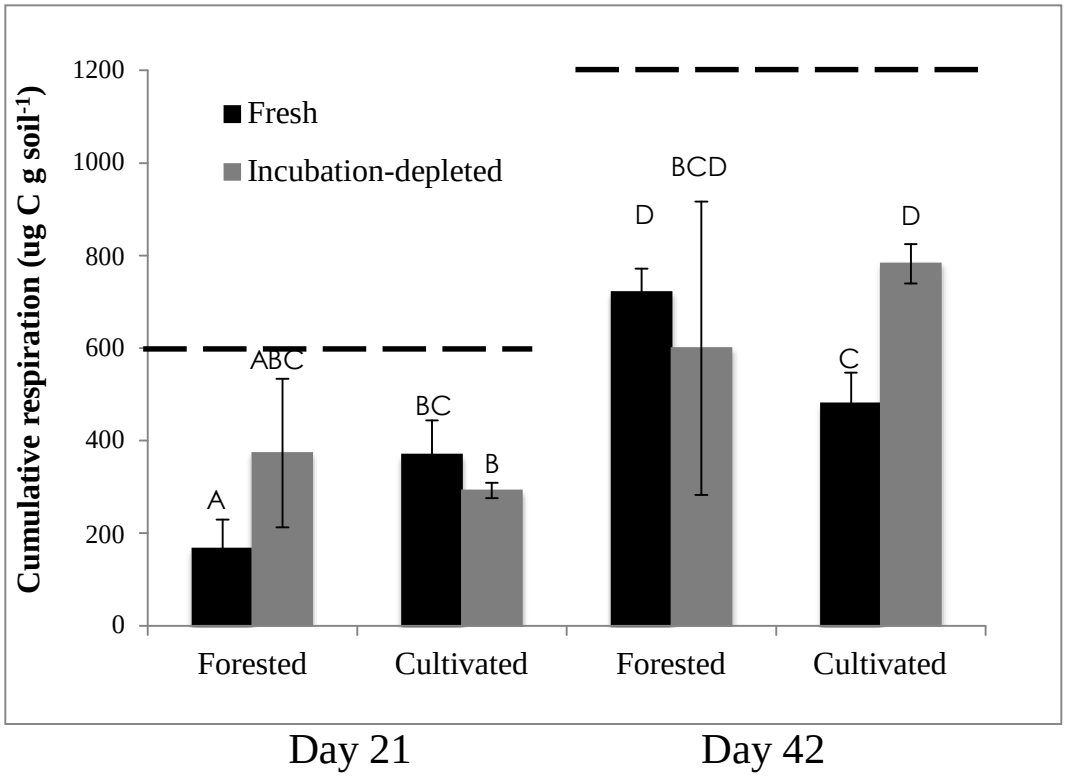


Figure 2.3 Amount of respiration resulting from substrate addition (600µg C gram soil at day 0, and an additional 600µg C at day 21; represented by the broken lines) for depleted and fresh soils from both sites, calculated as the difference between the substrate amended and control (unamended) cumulative soil respiration at day 42. Values are averages (±SE) and the same letters denote statistically similar values.

Chloroform-extractable organic carbon (here a surrogate for microbial biomass) decreased from field levels over the course of both the 707 and 42-day incubation by an average 60-70% (Figure 2.4). Substrate amendments had no effect on the microbial biomass pool size of the incubation-depleted soils, which remained unchanged between days 21 and 42 and between amended and unamended samples. Substrate similarly did not affect this decline in the fresh soils. By the end of the 42-day incubation, there was no significant difference in the amount of chloroform-extractable organic carbon among any of the treatments.

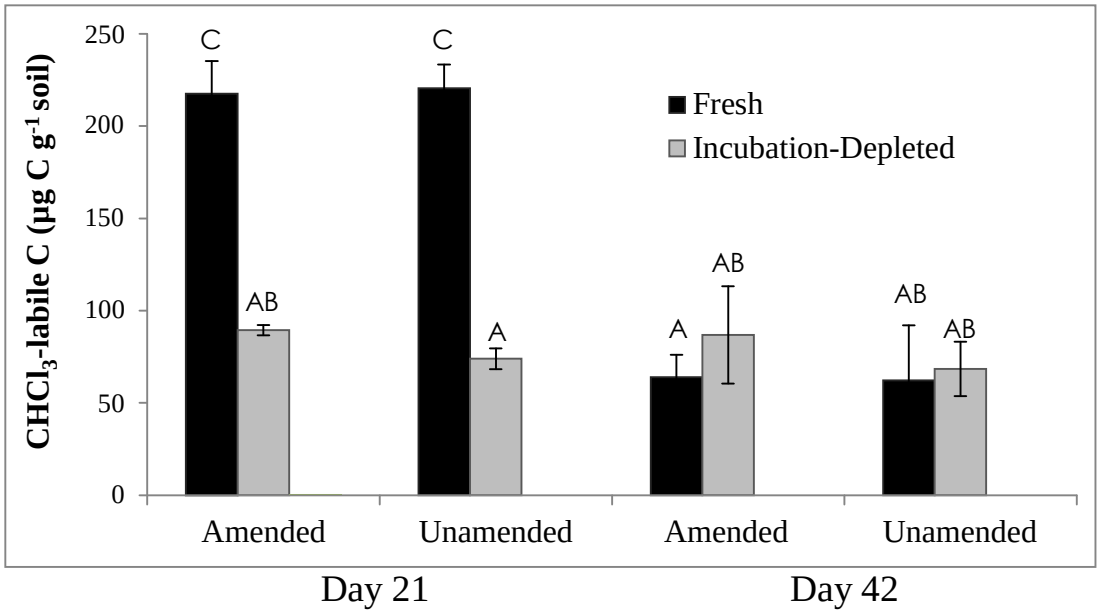


Figure 2.4 Chloroform-extractable organic carbon (a surrogate for microbial biomass) at day 21 and 42 from the substrate amended and unamended, fresh and depleted, cultivated plots. Values are averages (±SE) and the same letters denote statistically similar values.

Activity of the three carbon cycling extra-cellular enzymes (α-glucosidase (AG), β-glucosidase (BG), and β-D-cellobiohydrolase (CB)) in both the cultivated and forested soils was sometimes significantly greater in the fresh soils than the incubation-depleted soils, and substrate amendment had no impact on the potential extra-cellular enzyme activity in the fresh or depleted soils at either day 21 or day 42 (Figure 2.5). Land use (i.e., cultivated versus native) was the strongest determinant of changes in potential extra-cellular enzyme activity, with much higher NAG activity and generally lower overall activity of the other three extra-cellular enzymes in the forested plots. Variability was also greater in the forested versus cultivated plots. The activity of nitrogen-acquisition extra-cellular enzyme I studied (N-acetyl-β-d-glucosaminide) was slightly greater in the forested plots compared to other studies, but was not affected by any of the other experimental treatments.

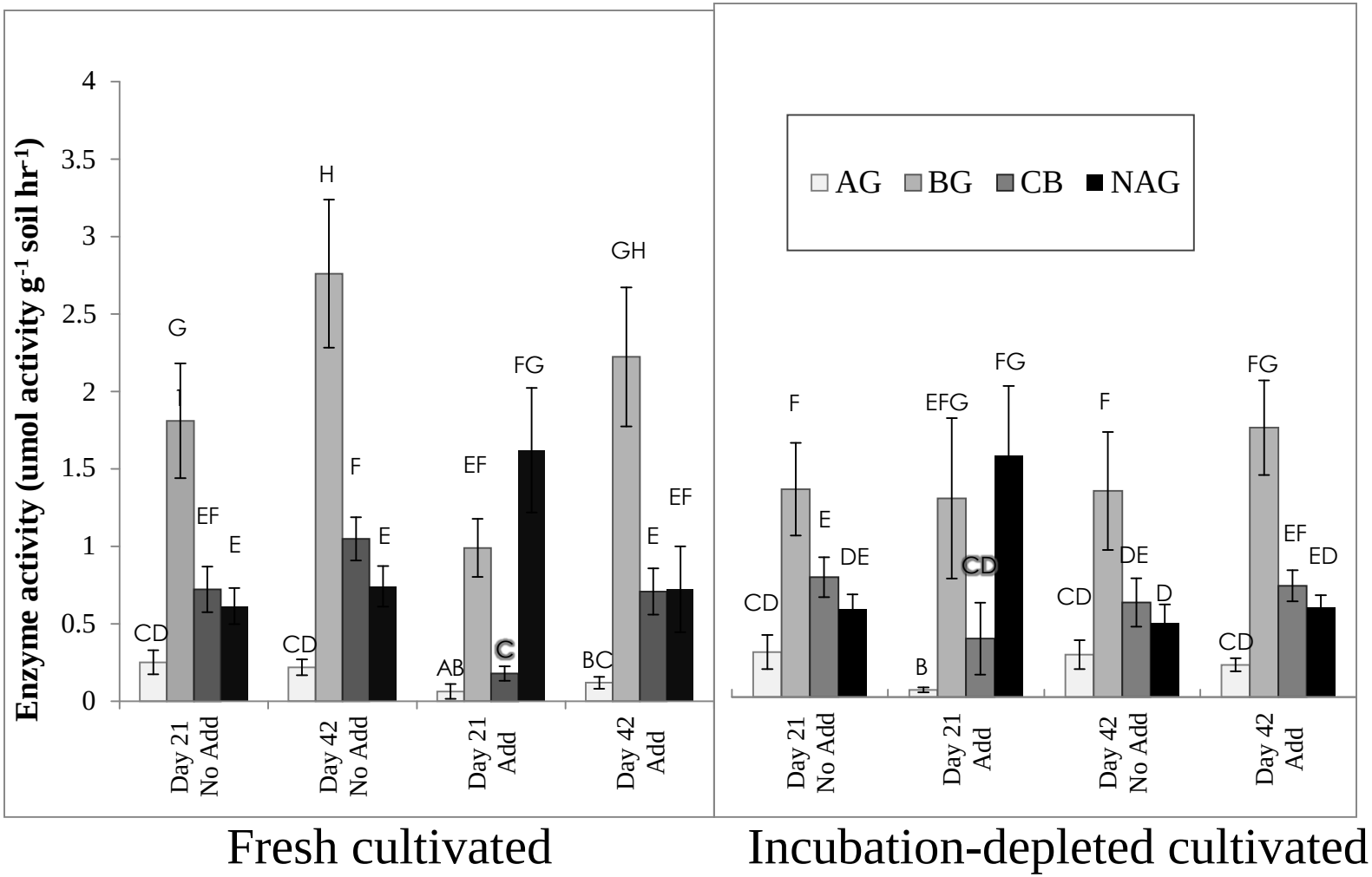
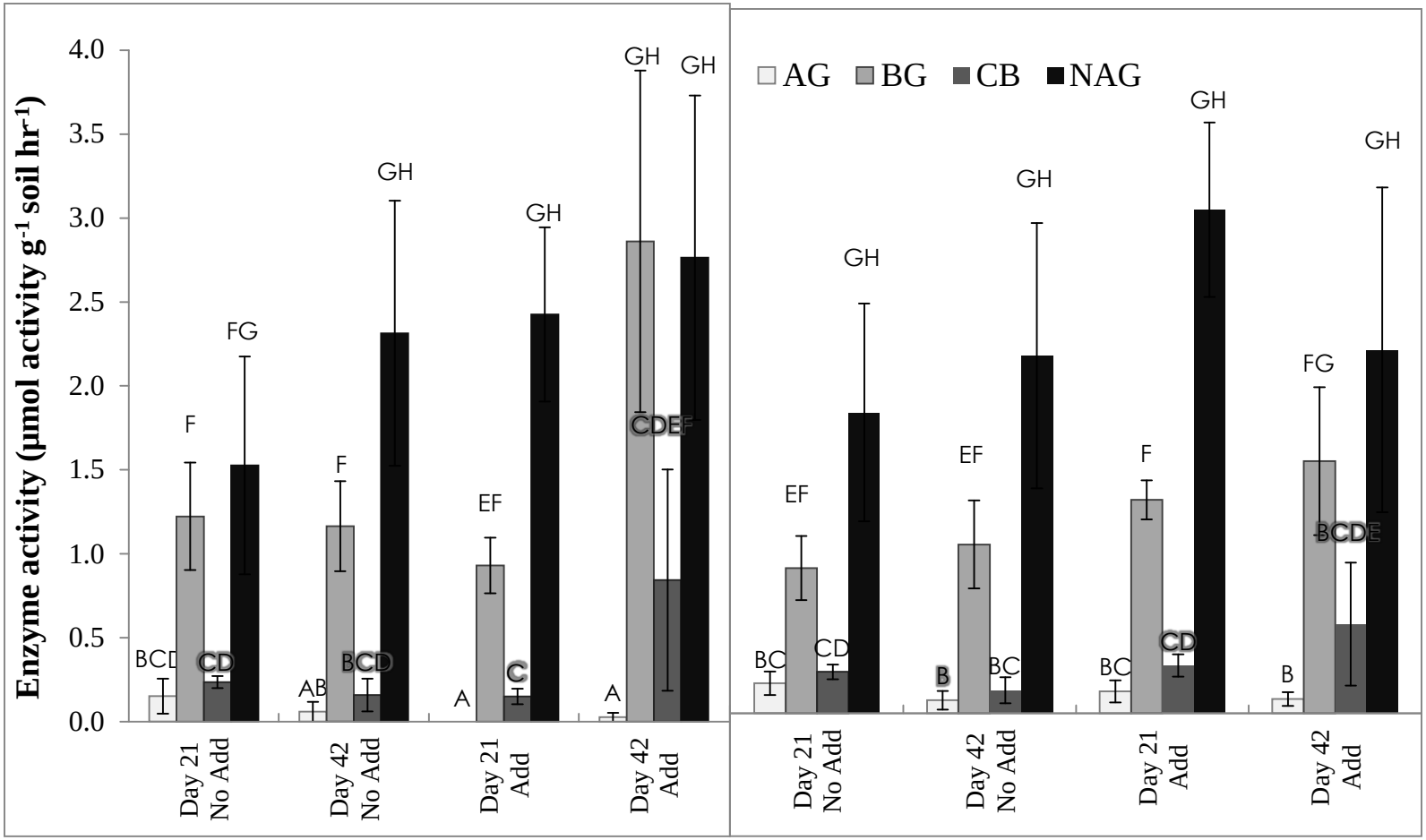


Figure 2.5 Extra-cellular enzyme activity of AG, BG, CB and NAG in the depleted and fresh amended ("Add") and unamended ("No Add") soil from the cultivated and forested soils at days 21 and 42 at an assay temperature of 30°C. Values are averages (±SE) and the same letters denote statistically similar values.



Fresh forested Incubation-depleted forested

Figure 2.5 (continued). Extra-cellular enzyme activity of AG, BG, CB and NAG in the depleted and fresh amended ("Add") and unamended ("No Add") soil from the cultivated and forested soils at days 21 and 42 at an assay temperature of 30°C. Values are averages (±SE) and the same letters denote statistically similar values.

Physically disturbing the soil through mixing contributed significantly to cumulative soil respiration, increasing respiration rates in the incubation-depleted soils by roughly three-fold. Following 707 days of incubation, I observed a spike in respiration following the mixing (and substrate addition to amended soils) of soils at days 0 and 21 (Table 2.2). The largest disturbance-induced spike in respiration occurred in the unamended, incubation-depleted cultivated soil (31.2 ±1.2% of the cumulative 42 day respiration occurred during days 22-25) and in the unamended, depleted, forested soil (22.6 ±5.5%). Substrate addition generally mitigated this "mixing effect", especially in the cultivated soils where the amended soils (depleted and fresh) effectively respired the same proportion of total respiration between days 22 and 25 (15%).

Table 2.2. The contribution of mixing and substrate amendment to soil respiration rates (μC/g soil/day) from the incubation-depleted cultivated and forested soils following an initial disturbance (following 707 days of incubation) at day 0, and a second disturbance (and second substrate amendment) at day 21. Days -35 and -1 represent time points during the 707-day respiration and before the 42-day incubation. Values are averages (±SE).

Treatment	**Day -35**	**Day -1**	**Day 2**	**Day 6**	**Day 20**	**Day 22**	**Day 24**
Cultivated Unamended	1.06 (±0.31)	1.22 (±0.10)	5.14 (±1.20)	4.16 (±0.83)	1.38 (±0.61)	4.67 (±1.36)	5.73 (±2.01)
Cultivated Amended	--	--	24.5 (±11.30)	37.85 (±2.44)	5.57 (±1.01)	37.33 (±3.43)	48.16 (±1.83)
Forested Unamended	2.51 (± 0.52)	2.42 (± 0.48)	6.92 (±1.53)	38.69 (±17.79)	2.35 (±0.91)	5.88 (±1.36)	11.5 (±2.16)
Forested Amended	--	--	22.89 (±6.86)	71.80 (±22.76)	2.38 (±1.23)	35.20 (±6.82)	48.38 (±4.96)

DISCUSSION

My results show that added substrate prompts more respiration but no microbial biomass response, and that incubation depletes both substrate and biomass but does not affect the microbial biomass' ability to respire added labile substrate. This suggests that the rate-limiting step of SOM decomposition in the two soils I investigated is the physico-chemical flow of SOM inputs to the available SOM pool. While respiration was significantly greater in the fresh soils and the amended soils than in the depleted soils and unamended soils, alleviating substrate limitation in the depleted soils yielded respiration rates similar to or even greater than those from the fresh, unamended soil in both the cultivated and forested soils.

Chloroform-extractable C (the index of microbial biomass I used) and extra-cellular enzyme pools were diminished after 707 days of incubation, but neither limited microbial capacity to respire added substrate. The incubation-depleted soils respired a similar or greater proportion of the added substrate than the fresh soils. Additionally, substrate amendment did not influence the size of either the microbial biomass or extra-cellular enzyme pools. My results show that the diminished microbial biomass and extra-cellular enzyme pools remaining after 707 days of incubation were sufficient to process added substrate – which was added at levels similar to that available over the course of 42 days under field conditions without concurrent replenishment of their own pools. This result is noteworthy because it suggests that a significant decrease of the microbial biomass does not limit soil respiration.

While microbial biomass did not change in the incubation-depleted soils, it did significantly decrease in the fresh soils over the course of the 42-day incubation. The exact mechanism for biomass declines due to incubation is unknown, but perhaps sample processing

and the controlled incubation setting diminished the variation and quantity of microhabitats in the soil, driving a reduction in microbial biomass.

The levels of potential extra-cellular enzyme activity I observed in both the forested and cultivated soil were comparable to other studies (Bailey et al., 2010; Trasarcepeda et al., 2007; Waldrop & Firestone, 2006), as were the levels of β-glucosidase (Bell & Henry, 2011; German et al., 2011; Steinweg & Wallenstein, 2009; Waldrop & Firestone, 2006), which were consistently higher than the three other extra-cellular enzymes I measured. Extra-cellular enzyme pools were smaller in the depleted soils than in the fresh soils, but did not significantly diminish in the fresh soils over the course of the 42 days. This pattern of extra-cellular enzyme activity appears to be decoupled from microbial biomass and respiration, and could be explained by extra-cellular enzyme turnover rates slower than those of microbial biomass, or that the diminished microbial biomass was sufficiently large enough to produce enough carbon-cycling extra-cellular enzymes to depolymerize the available SOM.

That there was no microbial growth response to substrate addition in the incubation-depleted soil was surprising, and an important contribution of my work: while the size of the microbial biomass decreased over the course of both incubations regardless of substrate availability, the remaining extra-cellular enzyme and biomass pools were sufficient to respire the added SOM. This could be that decomposition of labile substrate is a general microbial process unlikely to be lost if a significant portion of the microbial community dies, shrinks, or become inactive. It could also result from a shift in the microbial biomass over the long-term incubation to a smaller community with an altered life strategy promoting low substrate utilization efficiency, i.e., assimilated substrate is preferentially used for maintenance and energy (more carbon is respired as CO_2 than stored in biomass) over anabolism and reproduction (when more

carbon is fixed as biomass than respired as CO_2) (Steinweg et al., 2008). The "Exomet Pathway" might be another explanation of my results. In this pathway, intracellular oxidative enzymes released to the soil matrix following cell lysis persist in the soil independently of the microbial biomass, oxidizing SOM directly to CO_2 , bypassing assimilation and respiration by the microbial biomass (hence "Exomet") (Maire et al., 2012).

The large effect of mixing on respiration shows that while there was significant depletion of available SOM, spatial isolation of existing available SOM pools from microbial decomposers protected it from decomposition. This suggests that spatial proximity/accessibility of the existing available SOM to microbial decomposers and extra-cellular enzymes is an important control on soil respiration limits (Dungait et al., 2012). The physical act of mixing the soil and redistributing the pockets of labile SOM likely served to release physico-chemically protected and/or isolated, low-molecular weight SOM so that it came into contact with soil extra-cellular enzymes, relieving zones of substrate limitation and yielding a sudden bump of soil respiration. By mixing the soil, we increased the accessibility of the existing available SOM while keeping the labile inputs, microbial biomass and extra-cellular enzyme pools the same. Further, the degree of respiration response to mixing reflected the level of substrate depletion and not the size of the microbial biomass, further supporting the supposition that it is SOM availability –and not the size of the microbial biomass – that limits respiration.

Decreases in microbial biomass and extra-cellular enzyme pool size occurred in concert with a depletion of labile carbon, but I found no evidence that the pool of microbial biomass and extra-cellular enzymes were reduced enough to limit respiration of labile carbon. The mixing effect and respiration response to added substrate I observed support a paradigm in which the physico-chemical controls of accessibility and flow of SOM into the available pool are the rate

limiting step of soil respiration (Kemmit et al., 2008) over the course of a long-term incubation, as long as some critical threshold of microbial biomass and extra-cellular enzyme pools – unmet in my investigation – are maintained.

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CHAPTER 3

THE USE OF CONSTANT VERSUS DIURNALLY OSCILLATING TEMPERATURE IN LABORATORY INCUBATIONS TO INVESTIGATE SOIL ORGANIC MATTER (SOM) RESPIRATION

INTRODUCTION

In the field, soils experience dramatic diurnal temperature flux. Laboratory incubations almost exclusively use a constant temperature treatment, which is a departure from diel temperature fluctuations, which are ubiquitous in natural systems.

The decomposition rate of recalcitrant, slowly decomposing SOM pools are sensitive to increases in temperature (Conant et al., 2008; Davidson & Janssens, 2006; Xu et al., 2010). However, the majority of CO_2 (roughly 80%; Schimel et al., 1994) respired from soil evolves from the smaller (5-10% of the total SOM pool; Townsend et al., 1995; Trumbore, 1997), easily decomposed labile SOM pool. While the respiration of this labile pool responds to changes in temperature, the sensitivity intensifies with increasing biochemical recalcitrance. As a result, an average temperature treatment may not elicit the anticipated curvilinear response for either pool. It is this sensitivity of the large, biochemically recalcitrant SOM that is likely of exceptional importance to terrestrial-atmospheric climate feedbacks as mean annual temperatures continue to rise. As a result, the nuanced relationship between temperature and recalcitrant SOM respiration may not be elicited using a constant temperature treatment free of temperature maximums and minimums.

It is well established that temperature and SOM quality drive respiration rates. However, respiration is a mediated by microbiota which are in turn influenced by temperature and SOM

(Barccenas-Moreno et al., 2009; Koch et al., 2007; Waldrop & Firestone, 2006). More specifically, temperature may elicit changes in microbial growth rates and efficiencies (Steinweg et al., 2008; Zogg et al., 1993) and enzyme activity rates (Balser & Wixon, 2009; Wallenstein & Weintraub, 2008). Long-term *in situ* soil temperature regimes are also drivers of microbial community structure (Walker et al., 2008) and thermal optima for decomposing SOM (Wallenstein et al., 2009; Zogg et al., 1993). However, whether oscillating versus constant temperature significantly affects SOM decomposition in laboratory incubations remains unknown. Although temperature regimes drive soil microbial community structure and function, there is evidence that microbial communities rapidly adapt to new temperature regimes through collective shifts in physiology and/or natural selection (Bradford et al., 2008). This adaptation depends on the rapidity of the community's response to a new temperature treatment. Capturing any microbial responses to changing incubation conditions is therefore key to understanding the whole soil respiration response to different temperature and labile SOM regimes during incubation.

METHODS

Sample collection and processing

Soil samples were collected (20cm-deep cores; dia.=1.8cm) at the Kellogg Biological Station Long-Term Ecological Research site (Robertson, 1991) from cultivated (three field replicates) and forested (four replicates) plots in late 2007. The cultivated plot was converted from deciduous hardwood forest in the early 1900s. It was cropped in a mixed small grain corn–soybean rotation for many years and under alfalfa just prior to 1993, when it was converted to the current continuous, conventionally-tilled corn crop. The forest plot was reforested in the late 1800s and is currently a late-successional deciduous hardwood forest.

After collection, soil samples were transported to the laboratory and stored at 4°C in sterile, plastic bags for roughly one week. Soils were then passed through a 2mm-mesh sieve and large (>2mm) surface and belowground plant matter was removed. Samples of sieved, air-dried soil were analyzed for carbonates (none were detected, using a standard technique of 1M HCl drops to detect effervescence at ambient temperature) and total carbon and nitrogen using a LECO CHN-1000 analyzer (LECO Corp., St. Joseph, MI).

Creating four levels of substrate availability

To reveal the effect of oscillating versus constant temperature on SOM with a range of lability, I created four levels of labile SOM depletion using a combination of long-term incubation and substrate amendment. The processed soils were subjected to incubation under constant 30°C temperature and moisture for 707 days. Soil samples (200g) were placed in 250mL uncovered glass beakers, which were placed within sealed, half-gallon sized jars. The soils were maintained at 50% water filled pore space throughout the incubation. Air samples from the headspace of the sealed mason jars were drawn through septa, transferred to evacuated vials, and brought to laboratory facilities at the Natural Resource Ecology Laboratory (NREL) to measure CO_2 concentration using a Li-Cor LI-6252 (Li-COR Biosciences, Lincoln, NE) infrared gas analyzer.

The CO_2 in the headspace was measured every two to five days at the outset of the incubation, when respiration rates were at their highest. From days 14-707, CO2 concentration was measured every 7 to 28 days. Jars were flushed with CO_2–free air after every measurement and lost moisture and worn septa were replenished as needed, roughly six times throughout the incubation (modified from Haddix et al., 2011 and Follett et al., 1997). After 707 of incubation,

soils were significantly depleted of labile C, losing 20.34 and 18.02 of their total % soil C in the fresh forested and cultivated soils, respectively.

After 707 days of incubation, all soil samples (herein referred to as "*incubation-depleted*") were removed from the incubator, with some destructively harvested and the rest subjected to further experimentation. In 2010, new samples ("*fresh*") were collected from the same sites (cultivated and forested) at the Kellogg Biological Station and processed in the same manner as the samples collected in 2007.

To achieve the two additional levels of labile SOM depletion, I added substrate to a subset of both the incubation-depleted and fresh soils and subjected all soil to a 42-day incubation. Those soils receiving a substrate amendment are herein referred to as "*amended*" and those that did not receive an amendment referred to as "*unamended*". Substrate additions were added as 600µg C g^{-1} dry soil of finely ground plant material. This level of substrate addition was selected in an attempt to promote soil respiration in the incubation-depleted samples similar to that of the fresh soil, while mimicking amounts of labile substrate input under field conditions. I added a second pulse of 600µg C g^{-1} soil of substrate to the amended samples at day 21 in an effort to maintain the level of labile substrate established at the outset of the 42-day incubation. At day 21, all soils (amended and unamended alike) were well mixed before being exposed to another 21 days of incubation.

Measuring the effect of oscillating temperature on soil respiration

Soil samples of 27g dry-weight equivalent were placed in 250mL uncovered glass jars, which were placed within sealed, pint-sized jars with rubber septa sealed in their lids. Approximately 20mL of water was placed in the bottom of each jar to maintain a humid headspace and 50% water filled pore space. All soil samples (depleted and fresh, amended and

unamended) were well mixed and subjected to a 42 day incubation at either constant 30°C or temperatures oscillating between 25°C and 35°C in twelve hour increments repeated daily. I achieved an oscillating temperature treatment using a tabletop incubator set at 35°C with an automatic timer in a constant temperature room set to 25°C. The timer turned the 35°C incubator on at 8:00 and off at 16:00 every day throughout the incubation. Leaving the incubator door cracked open allowed the inside of incubator to rapidly heat and maintain 35°C between 8:00 and 16:00 and then cool to 25°C after it turned off at 16:00. The regulation of the 25°C constant temperature room allowed the room to stay at 25°C while the inside of the incubator fluctuated by 10°C every twelve hours. I used an iButton (DS1921G Thermochron iButton, Maxim Innovation, Sunnyvale, CA, USA) to record the temperature inside the incubator.

The oscillating temperature treatment I used was imperfect because it exposed the soils to both oscillations and a 0.09°C higher temperature (Figure 3.1).

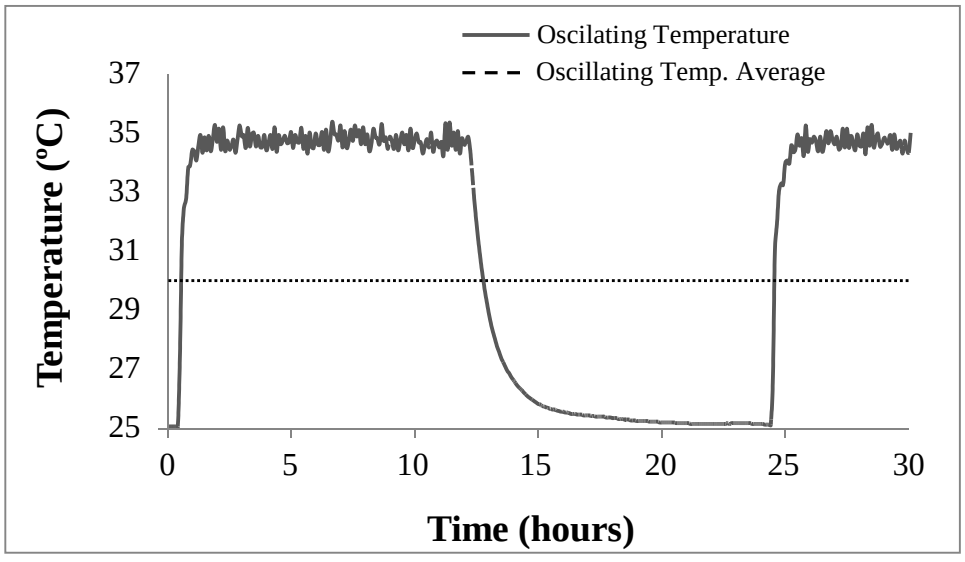

Figure 3.1 Temperature data from the oscillating temperature treatments over a representative thirty hour period, shown by the solid gray line. The black solid and broken lines represent the constant incubation temperature and the average temperature of the oscillating treatment, respectively. The lag following the end of the 12hr 35ºC cycle resulted in a 0.09ºC higher average temperature as compared to the constant temperature 30ºC incubation.

Before correcting for the +0.09°C difference, the oscillating temperature treatment yielded slightly higher respiration. Using a Q_{10} value of 2, I estimated the increase in cumulative respiration at day 42 expected as a result of the +0.09°C difference in the oscillating treatment. I calculated a range of expected values by transforming the upper and lower 95% confidence interval values (Table 3.1).

Throughout the incubation, samples from the headspace of the jars were collected as described above for the 707-day incubation, with a similar frequency (roughly daily for five days, and every 2-3 days thereafter).

Measuring changes in microbial biomass and extra-cellular enzyme pools

At day 21 and 42 of the incubation, samples were harvested and analyzed for microbial biomass and extra-cellular enzyme activity. I measured chloroform-extractable carbon and potential soil extra-cellular enzyme activity as indices of microbial biomass and activity using a chloroform fumigation-extraction method (based on methods from Wu et al., 1994) and a fluorescence microplate assay method developed by Steinweg and McMahon (2012).

I measured the activity of three hydrolytic C-acquisition extra-cellular enzymes: α-glucosidase (AG), β-glucosidase (BG), β-D-cellobiohydrolase (CB); and one nitrogen acquisition extra-cellular enzyme, N-acetyl-β-d-glucosaminide (NAD), to investigate whether respiration of SOM and the added labile substrate was controlled by the potential activity of the soil extra-cellular enzyme pool, and if the size of the extra-cellular enzyme pool is coupled to microbial biomass and/or substrate availability. To determine if either field or long-term incubation temperature regimes (oscillating in the field and constant in the lab) selected for a different thermal optima (calculated as energy of activation "E_a" using the Arrhenius equation) in extra-cellular enzyme activity, I measured potential extra-cellular enzyme activity at 25, 30 and 35°C.

Following a protocol developed by Steinweg and McMahon (2012), I mixed 1.375g of soil with 45mL of 50mM sodium acetate buffer with a pH of 5.5 in a soil blender (Waring 8011G) on high for 60 seconds. The soil slurry was then pipetted into deep-well plates and fluorescently labeled substrate was added in excess. The deep-well plates containing the soil slurry and substrate were covered and incubated at 30ºC for 2hr 15min. The fluorescent tags on the substrates only absorb electromagnetic radiation and fluoresce when separated from the substrate, which occurs when extra-cellular enzymes catalyze the mineralization of the fluorescently labeled substrate. Using a plate reader, I analyzed the amount of fluorescence emitted by the incubated slurries-substrate solution.

Statistical analyses

Each field replicate from the initial incubation or freshly-collected from the field was split into eight equal sub-samples, four of which were given a substrate addition, and four of which were exposed to oscillating temperature. Half of the samples were harvested at day 21, and the other half at day 42. I used analysis of variance (ANOVA) to determine if mean soil respiration, microbial biomass, and extra-cellular enzyme activity significantly varied among treatments and substrate addition on respiration. Differences were considered statistically significant for $P < 0.05$.

RESULTS

After adjusting for the 0.09°C difference using a Q10 of 2, my results suggest no significant differences in respiration attributable to oscillating versus constant temperature (Table 3.1).

Table 3.1 Cumulative respiration at day 42 from soil under constant temperature, oscillating temperature, and the range of predicted respiration based on a Q10 of 2 to account for the +0.09°C higher average temperature under the oscillating treatment. The range of values adjusted for Q10 were calculated using constant temperature average value ± their standard error. Respiration is in µg C g^{-1} soil (±SE in the first two columns).

Site and Treatment	Substrate Addition	Respiration under constant temperature	Respiration under oscillating temperature	Range of constant temperature respiration values adjusted for Q10
Fresh, forested	Yes	1262 ±50	1223 ±118	1224 -1220
	No	782 ±52	888 ±58	735 -840
Fresh, cultivated	Yes	1400 ±60	1334 ±70	1349-1483
	No	679 ±73	769 ±22	610-706
Incubation-depleted, forested	Yes	1036 ±500	873 ±157	539-1546
	No	253 ±22	430 ± 221	233-277
Incubation-depleted, cultivated	Yes	723 ±48.	814 ±57	679-775
	No	123 ±37	147 ±9	86 -161

After correcting for the 0.09°C difference in incubation temperatures, the key determinant of soil respiration was substrate availability. The soils with higher soil carbon (fresh) and substrate inputs (substrate amended) consistently respired more than their incubation-depleted and unamended counterparts (Figure 3.2). The effect of the oscillating temperature on soil respiration from soils with different levels of available carbon yielded no significant differences in soil respiration versus respiration from constant temperature incubated soil (Table 3.1, Figure 3.2)

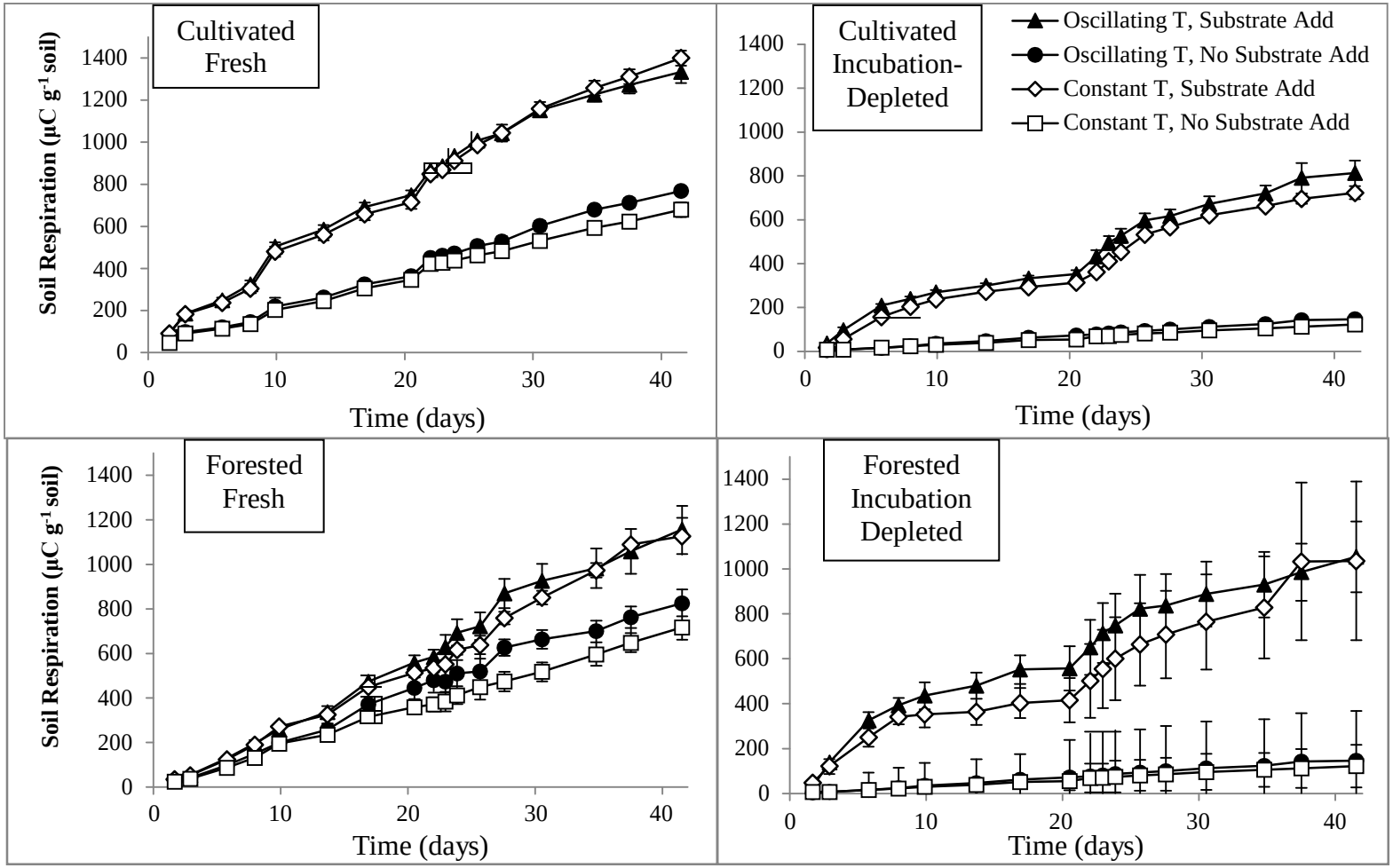

Figure 3.2. The impact of oscillating versus constant temperature on cumulative respiration from the four levels of labile-SOM depleted soil from the (a) cultivated soil fresh and incubation-depleted soils and (b) forested fresh and incubation-depleted forested soils. Values are averages (±SE)

Following the initial long-term incubation, microbial biomass (measured as chloroform-extractable carbon) was significantly depleted (Figure 3.3). There was also a trend for the microbial extra-cellular enzyme pool to be lower in the incubation-depleted soils, but the result was only slightly significant (P<0.1). However, neither oscillating temperature nor substrate addition had a significant impact on microbial biomass in the fresh or incubation-depleted soil. I found exposure to incubation to be the only determinant of microbial biomass size, with microbial biomass decreasing with incubation time (Figure 3.3).

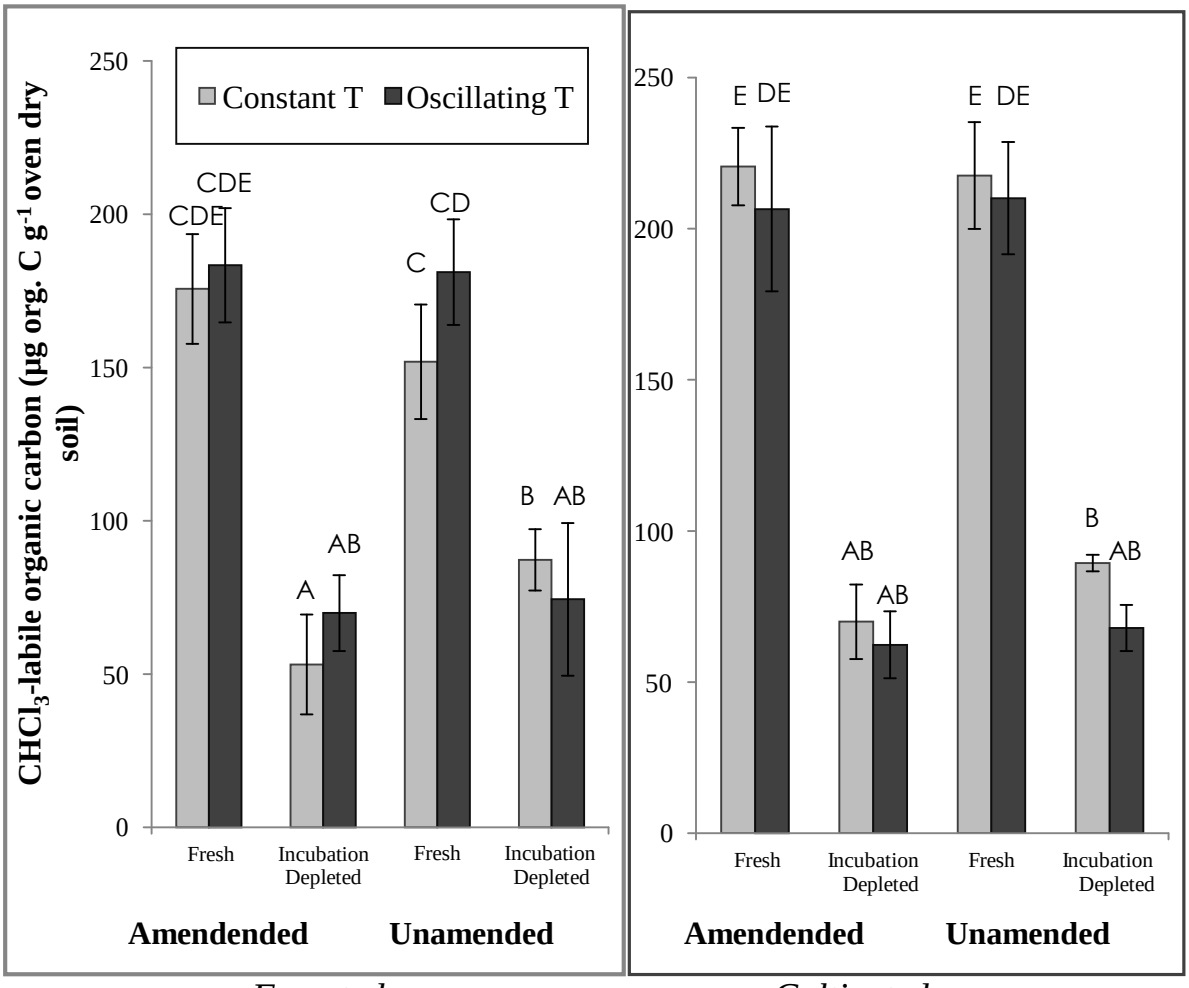

Figure 3.3 Chloroform-labile organic C (the index I used for microbial biomass) in the forested (left panel) and cultivated (right panel) soils under constant versus oscillating temperatures with or without substrate amendment. Data are from day 21. Values are averages (±SE) and the same letters denote statistically similar values.

The potential activity of the four extra-cellular enzymes I measured did not vary with oscillating versus constant temperature treatment (Figure 3.4). Only land use (forested versus cultivated) showed a relationship with potential extra-cellular enzyme activity: activity of the carbon cycling extra-cellular enzymes was higher in the cultivated plots, while the sole nitrogen cycling extra-cellular enzyme activity was higher in the forested soil, across fresh and incubation-depleted and amended and unamended soils. Activity was also slightly lower, with some variation, in the incubation-depleted versus fresh soils.

Extra-cellular enzyme activity increased with assay temperature (25, 30 and 35°C) in all soils, and did not vary in response to oscillating versus constant temperature treatment. Neither site nor long-term incubation had a significant effect on the E_a, which was similarly unaffected by the oscillating temperature treatment (Figure 3.4). The same pattern of response was observed at both harvest dates.

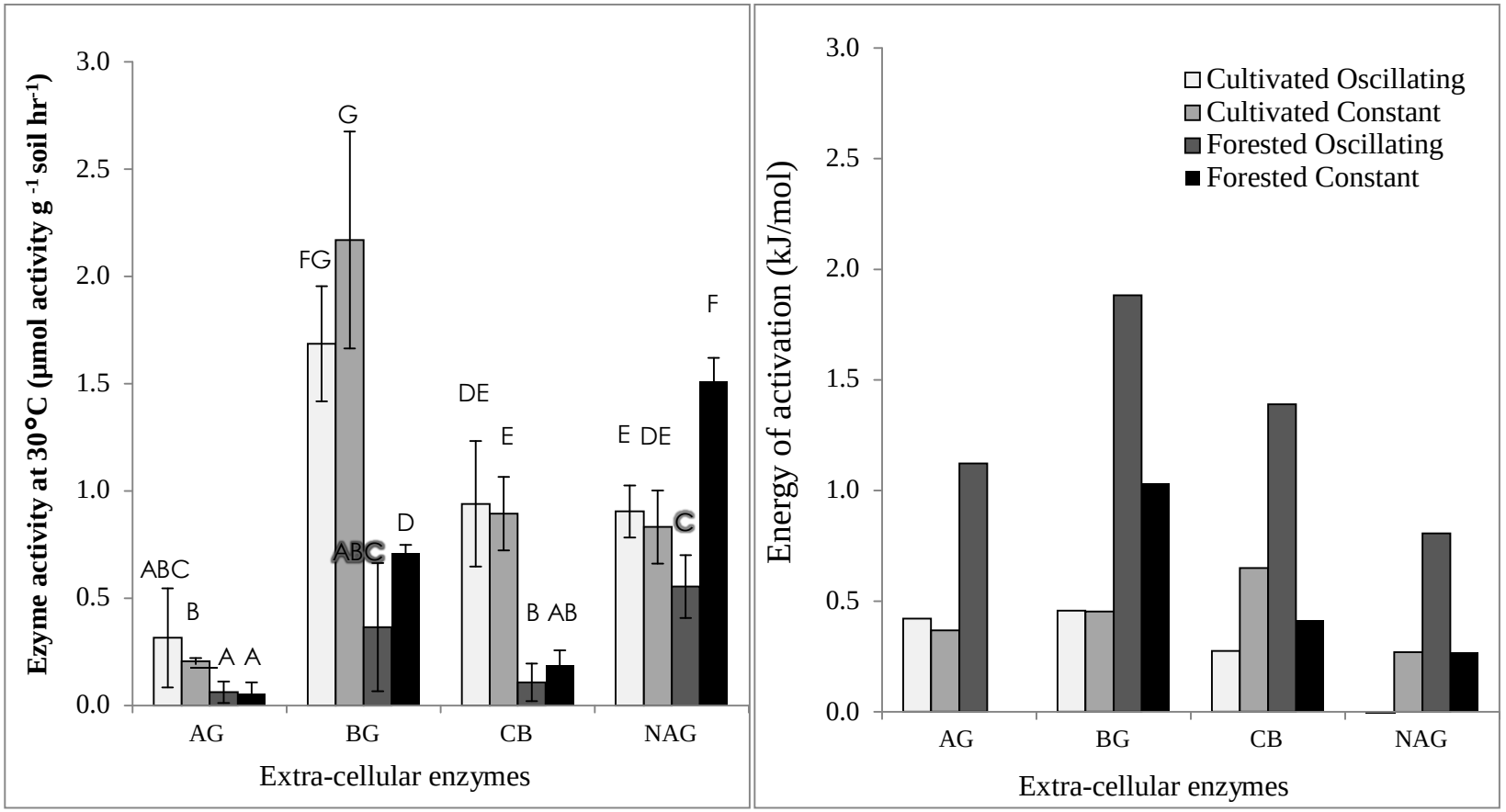

Figure 3.4 Potential enzyme activity at 30°C and energy of activation (kJ/mol) and of all four extra-cellular enzymes I investigated (α-glucosidase (AG), β-glucosidase (BG), β-D-cellobiohydrolase (CB) and N-acetyl-β-d-glucosaminide (NAD)) from both temperature treatments, sites (cultivated and forested) from the fresh soils. Data are shown for the unamended soils (amendment had no significant impact on enzyme activity or E_a). Values are averages (±SE for enzyme activity) and the same letters denote statistically similar values

DISCUSSION

My results show that constant temperature is adequate for laboratory incubations measuring soil respiration. The lack of respiration response to oscillating versus constant temperature treatments was not explained by adaptive shifts of the microbial indices I measured: oscillating temperature did not prompt changes in chloroform-extractable carbon (the index of microbial biomass I used) or extra-cellular enzyme activity. Changes in chloroform-extractable carbon did occur in response to other experimental treatments: site (forested versus cultivated) reliably explained differences in potential extra-cellular enzyme activity, and the incubation-depleted soils had consistently low microbial biomass, even when amended with a labile substrate. At every level of labile substrate availability, the forested soils had higher levels of potential NAG – the sole nitrogen acquisition extra-cellular enzyme I measured– activity. This is likely a reflection of lower nitrogen availability in the unfertilized, untilled forested soils. Although nitrogen is relatively more limiting in the forested soils, there was no difference in the forested soils' ability to decompose the added substrate; nitrogen limitation does not appear to control respiration rates in our study.

Intriguingly, NAG activity in the incubation-depleted soils remained at the same level throughout the long-term incubation even as mineral nitrogen accumulated in the soil, showing that extra-cellular enzymes may persist for long periods (roughly 707 days, in this case) in the soil. The persistence of extra-cellular enzymes in the soil is also supported by only a slight and inconsistent decrease in potential extra-cellular enzyme activity over the course of a long-term incubation in which a large portion of the microbial biomass was culled. Overall, even though microbial biomass and extra-cellular enzyme pools varied with other experimental factors, oscillating temperature did not prompt any measurable microbial response.

My results also show that a constant (30ºC) versus oscillating (12hr 10ºC oscillations around 30ºC) incubation temperature regime does not drive differences in cumulative soil respiration in laboratory incubation – and that this lack of response is unlikely to be the result of microbial functional response. My results could alternatively be explained by my methods. The incubation length I employed may have failed to capture real changes that occurred on a slower time scale. In addition, the amplitude of temperature oscillation I used may have been too small to elicit a clear and measurable response. It is therefore important to note that use of oscillating temperature could still be more appropriate than constant temperature, depending on factors like *in situ* temperature flux and the scale of the investigation (e.g. whole soil respiration versus that from very specific SOM fraction, microbial adaptation, etc.).

Overall, however, my results support the use of constant incubation temperatures for investigating SOM-temperature respiration in soils with varying levels of available carbon, microbial biomass and microbial extra-cellular enzyme pools.

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CHAPTER 4

SUMMARY AND REFLECTIONS

The results of my work are striking for three reasons. First, that a depleted microbial biomass both fails to recover its initial size once substrate limitation is relieved and is able to respire labile substrate as readily as a non-depleted microbial biomass was unexpected. This finding is important because it illustrates how much we have to discover about soil microbial dynamics, and confirms that conventional biological paradigms may not apply to the mysterious realm of microbes. It also calls into question how well we are able to study these organisms in the opaque, heterogeneous soil matrix. Methods to elucidate indices of soil microbial community structure and function rely on the observation of indirect and imperfect surrogates. For example, we often measure inputs and outputs (i.e. substrate amendment and CO_2 evolution) to infer microbial process rates such as respiration. Enzyme assays measure the fluorescence of markers depolymerized from synthetic substrates by soil extracellular enzymes in a liquid matrix rather than the enzymes directly.

This finding also calls into question whether a consideration of microbial dynamics is needed to address larger scale ecological questions. While a deeper understanding of microbial dynamics is fundamental from a basic science approach, its relevancy to pressing applied ecological questions remains unknown. My results show that, while a significant depletion in the microbial biomass took place, its capacity to carry out an essential process of organic carbon respiration remained unchanged. This result is exceedingly interesting from a basic science perspective, but I would argue that it supports an interpretation of the microbial biomass as a static "black box" at scales relevant to applied scientific questions.

Secondly, my results show that SOM accessibility limits soil respiration. Numerous investigations show that tillage elicits greatly accelerated SOM losses; my work shows that it occurs on a small scale independent of the microbial biomass. Regardless of the size of the microbial biomass and level of substrate depletion, physical mixing of the soil yielded a bump in respiration. In fact, this brief increase in respiration following mixing was most pronounced relative to total CO_2 lost in those soils that were most depleted of substrate. This further supports our finding that respiration is independent of the microbial biomass: even a considerable loss in microbial biomass is grossly overwhelmed by the availability of SOM in terms of respiration response. That a significantly depleted microbial biomass is identical to a non-depleted microbial biomass in terms of its ability to processes available SOM supports a paradigm in which physico-chemical –rather than biotic –controls limit SOM respiration.

Thirdly, my results show that simulating diel temperature fluctuations during an incubation is not necessary. Although plant activity is sensitive to changes in temperature and sunlight and drives soil dynamics (e.g. soil moisture, exudation) the soil devoid of plants showed no signs of functional response to diel temperature fluctuations. In other words, a departure from a thermal regime characterized by daily oscillations in the field to a constant temperature in a laboratory incubation did not affect the response of cumulative respiration, microbial biomass or extra-cellular activity to varied substrate availability.

Overall, the experience of uncovering and communicating these findings was difficult but highly rewarding. My master's work taught me – first and foremost – how to think more like a scientist, how to self-motivate, and how to write. Science writing requires a clear, rigorous thought process followed by careful documentation of that process. Even looking back at drafts of my thesis from a year ago, I see how far I've come. While far from perfect, my writing is

vastly less confused and muddled than it was when I started my Master's degree. To combine the ability to write clearly about complex ideas with the ability to ask important, interesting questions is something that marks great scientists, in my own opinion. I hope that as I continue down my career path I continue to hone both of these skills so that I might reach that level of success.