Soil Environ. 44(2): 331-344, 2025
DOI: doi.org/10.25252/SE/2025/253868
Online ISSN: 2075-1141
Print ISSN: 2074-9546

Drying-rewetting cycles and temperature combine to affect farmland soil microbial biomass carbon and metabolism: A laboratory study in Indiana, US

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***[Received:** June 23, 2025 **Accepted:** September 30, 2025 **Published Online:** October 03, 2025]*

Abstract

Soil moisture and temperature significantly influence soil microbial activity. Climate change is expected to increase the frequency of soil drying-rewetting cycles (DWC) and temperature fluctuations. While studies have examined the individual effects of DWC or temperature on soil microbial activity, their combined impact remains unclear. We conducted a 60-day laboratory simulation to investigate how DWC and varying temperatures together affect soil microbial biomass carbon (MBC) and related metabolic processes. The experiment included four 15-day DWC with five water treatments: 5-day wetting plus 10-day drying (W5-D10), 10-day wetting plus 5-day drying (W10-D5), air-dried (AD), constant moisture at 60% water-holding capacity (OPT), and saturated (ST). These were combined with five temperature treatments: 0°C, 10°C, 20°C, 30°C, and 40°C. Results showed that both water regime and temperature significantly influenced MBC and enzymatic activities, with significant interactive effects except on Arylamidase activity. DWCs generally increased MBC and enzymatic activity along the 0-20°C temperature gradient compared to constant drying or saturation treatments. No significant difference was observed between W5-D10 and W10-D5 for MBC and fluorescein diacetate hydrolysis activity (FDA) across the 0-40°C range. However, β-glucosidase activity (BG) and acid phosphatase activity were higher in W5-D10 than in W10-D5 throughout the 0-30°C range, especially in topsoil layers. These findings highlight the importance of effective soil moisture and temperature management strategies for farmland to enhance MBC and soil enzyme activity, particularly under changing environmental conditions.

Keywords: Soil microbial biomass carbon (MBC), soil enzyme activity, drying-rewetting cycles (DWC), farmland

Introduction

The Intergovernmental Panel on Climate Change (IPCC) reports that climate change is not only causing warming but also altering the global hydrologic cycle and precipitation patterns, leading to more frequent extreme droughts and heavier rainstorms (IPCC, 2013; Zhang *et al*., 2023). These changing climatic conditions, particularly during rainy seasons, often result in pulse precipitations (Feldman *et al*., 2024). Such events typically cause intermittent runoff in farmland rills and channels, especially when multiple storms occur within a short timeframe. This exposes farmland soils to frequent drying-rewetting cycles, creating complex soil moisture conditions (Ribeiro *et al*., 2024). Soil moisture and temperature are crucial for microbial activity, which significantly affects the decomposition and fate of soil organic matter (SOM) (Davidson and Janssens, 2006; Moyano *et al*., 2013; Karhu *et al*., 2014). Consequently, the biogeochemical cycling of carbon, often intertwined with other nutrients in farmland soils, could be significantly impacted by altered rainfall patterns, specifically the induced soil DWC and varying air temperatures (Xiao *et al*., 2018). However, understanding of this impact remains limited (Zhang *et al*., 2020).

Soil DWCs, indicating alternate shifts of soil moisture from saturation to desiccation or vice versa, physiologically stress microbial communities by altering structural compositions, causing partial microbial death or lysis, and triggering cytoplasmic component efflux from surviving

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Cite This Paper: Yang, L.X., L.J. Hu, D.E. Stott and C.H. Huang. 2025. Drying-rewetting cycles and temperature combine to affect farmland soil microbial biomass carbon and metabolism: A laboratory study in Indiana, US. *Soil Environ*. 44(2): 331-344.

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cells (Birch, 1958; Semenov *et al*., 2014). Two main mechanisms explain DWC effects on soil microbial communities: (1) Physical disruption of soil aggregates, which exposes fresh surfaces for microbial colonization (Denef *et al*., 2001), and (2) Degradation of microbial biomass, leading to soil mineralization (Birch, 1958; Soulides and Allison, 1961; Sørensen, 1974). During the DWC period, desiccation (*i.e.*, from saturation or moist to dry) suppresses microbial activity and substrate diffusion, reducing soil respiration and biomass (Halverson *et al*., 2000; Schimel, 2018). In contrast, rewetting (*i.e.*, from dry or non-saturated to saturation) rapidly increases microbial biomass and activity due to newly available substrates from microbial and physical processes, resulting in a carbon dioxide (CO_2) pulse release (Zhao *et al*., 2000; Guo *et al*., 2012; Blazewicz *et al*., 2014). These DWC effects on soil metabolic processes are significant (Zhang *et al*., 2023). Research across grasslands, forests, and farmlands shows varied DWC effects on microbial communities and carbon decomposition (Alvarez *et al*., 1998; Placella *et al*., 2012; Aanderud *et al*., 2015; Barnard *et al*., 2015; Salazar-Villegas *et al*., 2016). Xiang *et al*. (2008) found increased soil enzyme activity in grasslands due to DWC, while Fierer *et al*. (2003) observed decreased bacterial communities in forest soils with few DWCs but not in grassland soils with many DWCs. Mazzei *et al*. (2019) reported an initial decrease in enzyme activity that stabilized after three weeks. A meta-analysis by Zhang *et al*. (2020) concluded that CO_2 pulses from rewetting fully compensate for reduced CO_2 release during drying periods, with cumulative carbon loss depending solely on DWC intensity.

Soil microorganisms are essential for biogeochemical cycling in terrestrial ecosystems (Liang *et al*., 2011). Soil microbial biomass, which includes both living organisms and necromass, constitutes a significant portion of the active annual cycling soil organic carbon (SOC). With a rapid turnover time of weeks to years, this biomass makes up approximately 0-5% of the global SOC pool (Parton *et al*., 1987; Grandy and Neff, 2008; Manzoni and Porporato, 2009). During the initial, rate-limiting stage of SOM mineralization, microorganisms secrete enzymes that break down and solubilize large organic compounds (Wallenstein and Weintraub, 2008). This enzymatic process is vital for catalyzing numerous biochemical reactions in soils (Aon and Colaneri, 2001; Baum *et al*., 2003; Sardans *et al*., 2008; Jin *et al*., 2009).

Temperature has been recognized as a critical factor in SOC mineralization (Magid *et al*., 1999; Davidson and Janssens, 2006; Karhu *et al*., 2014). It influences enzyme activity either by directly altering enzymes' conformational flexibility or indirectly shifting microbial communities (Bárcenas-Moreno *et al*., 2009; Allison *et al*., 2010; Rousk *et al*., 2012; Salazar-Villegas *et al*., 2016). However, a key challenge in research is that natural air temperatures fluctuate constantly, while laboratory enzyme assays typically use fixed temperatures. This discrepancy has resulted in significant methodological variations across studies. For instance, Steinweg *et al*. (2013) incubated at 20-25°C, Koyama et al. (2017) used 5-35°C, and Sardans *et al*. (2008) employed a higher temperature of 37°C. Conversely, Dominguez *et al*. (2017) used a lower temperature of 10°C. Some researchers simply used average summer soil temperatures for all treatments, while others failed to specify their incubation temperatures for enzyme assays.

While the individual effects of DWC or temperature on soil ecosystems are often discussed, their combined impact is rarely addressed. Yet a single factor seldom fully explains an ecological phenomenon (Marchin *et al*., 2015). To investigate how carbon cycling and soil microbial metabolism depend on multiple climatic factors, we conducted a laboratory study in Indiana, USA. We hypothesized that soil metabolic activity levels, measured by microbial biomass carbon (MBC) and enzyme activity, would: (1) be influenced by DWC, (2) vary with temperature, and (3) be lower in DWC-treated soils compared to soils maintained at a constant optimal moisture of 60% water-holding capacity (OPT), but higher than in air-dried soils (AD, indicating extreme water limitation) or saturated soils (ST, indicating anaerobic conditions). Given the importance of soil enzymes as indicators of ecological processes, we selected five enzymes to test their activity: β-glucosidase (BG - indicating fiber decomposition), N-acetyl-β-D-glucosaminidase (NAG - indicating carbohydrate and protein metabolic conditions), Arylamidase (indicating protein metabolism), acid phosphatase (indicating phosphorus metabolism), and fluorescein diacetate hydrolysis (FDA - indicating potential microbial activity in soil with organic amendments).

Materials and Methods

Site description

Soil samples were collected from three locations along a slope transect at the Throckmorton Purdue Agriculture Center (40°17'44"N, 86°54'05"W; elevation 218 m) in Lafayette, Indiana. The area has an average annual rainfall of 988 mm and an average temperature of 10.7°C. Over the past 30 years, the recorded highest and lowest annual temperatures were 16.0°C and 5.5°C, respectively. The

hilltop and mid-slope areas consist of Octagon silt loams (fine-loamy, mixed, active, mesic Mollic Oxyaquic Hapludalfs) with 2-6% slopes and eroded surfaces. The toe-slope area comprises a Toronto-Millbrook silt loam complex (fine-silty, mixed, super-active, mesic Udollic Epiaqualfs) with a 0-2% slope. This toe-slope area frequently experiences waterlogging after rainfall events.

Soil sampling

Before spring planting operations, soil samples were collected from a depth of 0 to 15 cm below the soil surface, with three replications for each location. The samples were air-dried indoors at room temperature (around 20°C), sieved through a 2.0 mm mesh, and inspected to remove any remaining roots. They were then stored at 4°C in a cooling

Table 1: Soil physicochemical properties

Slope position	Soil series	Texture	θ_{FC}	θ_{PWP}	θ_s	EC	pH	SOC	TN	DOC	AP
			%			dS m^{-1}		g kg^{-1}		mg kg^{-1}	
Hilltop	Octagon	Silt loam	21.3	8.5	28.6	68.0 a	7.2b	16.4b	1.4b	242.6a	55.9c
Mid-slope	Octagon	Sandy loam	13.6	5.4	18.2	48.0 b	7.9c	15.6a	1.3a	256.8a	41.8a
Toe-slope	Toronto-Millbrook	Silt loam	21.1	8.4	28.1	44.0 b	6.8a	18.0c	1.5c	245.9a	50.3b

EC - electrical conductivity, SOC - soil organic C, TN - total N, DOC - dissolved organic C, AP - available P, θ_{FC} - field capacity of soil water holding, θ_s – saturated soil moisture, θ_{PWP} - plant withering point.
Different letters within a column represent means that are significantly different as determined by the Fisher's LSD test at $P < 0.05$

Table 2: Initial MBC and enzymes' activity of soils

Slope position	MBC	BG	NAG	Arylamidase	acid phosphatase	FDA
	mg kg^{-1} h^{-1}					mg kg^{-1} $3h^{-1}$
Hilltop	142.0 a	171.0 a	28.5 a	0.017 a	322.0 b	318.0 b
Mid-slope	138.0 a	181.0ab	31.7 a	0.022 b	287.0 a	278.0 a
Toe-slope	135.0 a	187.0 b	36.0 b	0.019 a	390.0 c	297.0 ab

BG - β-glucosidase activity, FDA - fluorescein diacetate hydrolysis, MBC - microbial biomass carbon, NAG - *N*-acetyl-β-d-glucosaminidase
Different letters within a column represent means that are significantly different as determined by the Fisher's LSD test at $P < 0.05$

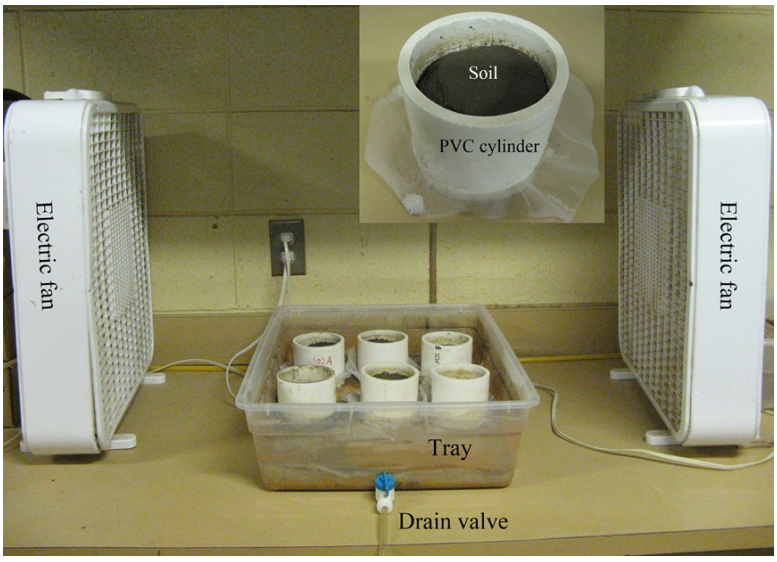


Figure 1: Setup of the experimental trials

chamber for later use. This initial air-drying pretreatment ensured that all soil samples were comparable before undergoing subsequent incubation processes. Table 1 shows the physicochemical properties of the soils.

Experimental design

Sampling locations may have effects on soil microbial activity and enzymatic performance due to the heterogeneity existing in soil properties, micro-geomorphology and hydrology amid the locations. Therefore, we collected soil from three slope positions, *i.e.* hilltop, mid-slope, and toe-slope, which may help better present and enhance accuracy of the examined effects. Each soil type (with three replicated samples) received 25 different treatments (five water regimes × five temperature levels) with three replications per treatment. The water regimes included: a) 5-day wetting to field capacity followed by 10-day drying (W5-D10, where drying meant no water addition in open conditions); b) 10-day wetting to field capacity followed by 5-day drying (W10-D5); c) air-dried conditions (AD); d) optimal moisture maintained at 60% water-holding capacity (OPT; Lovieno and Bååth, 2008—field capacity and initial soil moisture were measured beforehand to enable precise water control); and e) saturated conditions (ST). Incubation temperatures were 0°C, 10°C, 20°C, 30°C, and 40°C. The experiment ran for 60 days at respective treatment temperatures, completing four cycles of water-regime treatments, including two DWC scenarios.

The samples were prepared for incubation as follows: Each soil sample (350 g) was placed into a PVC cylinder (8 cm in diameter and height). A piece of Whatman filtering paper #1 and a cheese cloth were tied at the cylinder's bottom. These cylinders were then set in trays with a sand layer at the bottom and drain valves installed (Figure 1). During the drying days of relevant treatments, an electric fan was used to blow away vapor and facilitate the desiccation process. Soil moisture status was monitored by Time-Domain-Reflectometry (TDR) real-time detection once per half day throughout the experiment, where water was controlled to be evenly recharged before achieving saturation.

Preceding soil analysis

Soil texture was determined using the hydrometer method (Gee and Or, 2002). Silt percentage was calculated by subtracting the combined percentages of sand and clay from 100%. We measured soil electrical conductivity (EC) and pH using standard meters with 20g of air-dried soil passed through a 2 mm sieve (Watson and Brown, 1998; Whitney, 1998). This same soil was used to measure total carbon (TC) and total nitrogen (TN) through dry combustion with a Tru-Mac CN Analyzer. We quantified soil inorganic carbon following Sherrod *et al*. (2002), and determined SOC by subtracting inorganic carbon from total carbon. To measure dissolved organic carbon (DOC), we leached 10 g of soil with 100 mL of deionized water, then analyzed the solution using a TOC-Vws Analyzer after UV-persulfate digestion. For available phosphorus (AP), we first extracted soil solute with a Mehlich III solution (Mehlich, 1984), then measured phosphorus concentrations using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) with an Optima 2000 device.

Microbial activity analyses

We analyzed microbial biomass carbon (MBC) and the activity of five enzymes in fresh soil samples collected before and after incubation (Table 2; Figures 2-7). For MBC measurement, we followed the standard soil fumigation-incubation method (Horwath and Paul, 1994). After sieving samples through an 8mm mesh, we used KOH base traps to absorb CO_2. We then acidified aliquots from these traps and measured CO_2 concentration using a gas chromatograph (Model 3800, Varian, Inc., Walnut Creek, CA) equipped with a CombiPal autosampler (CTC Analytics AG, Zwingen, Switzerland) and a thermal conductivity detector. We expressed MBC results in mg C per kg soil.

We conducted enzyme activity analyses using fresh soil samples with appropriate standards and controls in triplicate. We measured β-glucosidase (BG, EC 3.2.1.21) activity using *p*-nitrophenyl-β-D-glucopyranoside as substrate in a modified universal buffer (MUB) at pH 6.0 (Tabatabai, 1994). For N-acetyl-β-D-glucosaminidase (NAG, EC 3.2.1.30) activity, we used *p*-nitrophenyl-N-acetyl-β-D-glucopyranoside as substrate in MUB at pH 5.5 (Parham and Deng, 2000). Arylamidase activity was determined using L-leucineβ-Naphthylamine as substrate in Tris (hydroxymethy) aminomethane buffer at pH 8.0 (Acosta-Martínez and Tabatabai, 2000). We measured acid phosphatase activity using disodium *p*-nitrophenol phosphate in MUB at pH 6.5 (Tabatabai, 1994). Fluorescein diacetate hydrolysis (FDA) was determined following Green *et al*. (2006), using a modified buffer (Prosser *et al*., 2011). We reported BG, NAG, and acid phosphatase activities in mg *p*-nitrophenol (PNP) released per kg soil per hour of incubation. Arylamidase activity was reported in mg β-naphthylamine released per kg soil per hour, and FDA hydrolysis in mg fluorescein released per kg soil every three hours of incubation.

Data analysis

We performed a two-way ANOVA (Analysis of Variance) using SPSS 16.0 for Windows (IBM, New York) to assess the combined effects of water regimes and incubation temperatures on soil biological variables (Table 3). Significant differences between means were identified using Fisher's least significant difference (LSD) method at $P < 0.05$. Since MBC showed no significant difference across the three sampling slope positions (Table 2), we analyzed and presented the effects of water regimes and temperatures on soil MBC by averaging data from all three positions (Figure 2). All figures were created using SIGMAPLOT 14.0 (Systat Software Inc.).

Table 3: ANOVA for the effects of temperatures, water regimes, and their interactions on MBC and enzymes' activity of soils using multivariate analysis of General Linear Model (GLM)

	Parameter	df	F	p
MBC	Temperature (T)	4	18.73	＜0.001
	Water regime (W)	4	36.73	＜0.001
	T × W	16	4.34	＜0.001
BG	Temperature (T)	4	103.83	＜0.001
	Water regime (W)	4	40.11	＜0.001
	T × W	16	8.26	＜0.001
NAG	Temperature (T)	4	21.30	＜0.001
	Water regime (W)	4	3.74	0.010
	T × W	16	1.13	0.056
Arylamidase	Temperature (T)	4	4.35	0.004
	Water regime (W)	4	5.24	0.001
	T × W	16	0.64	0.836
acid phosphatase	Temperature (T)	4	14.87	＜0.001
	Water regime (W)	4	9.49	＜0.001
	T × W	16	1.88	0.046
FDA	Temperature (T)	4	8.17	＜0.001
	Water regime (W)	4	8.20	＜0.001
	T × W	16	2.17	0.022

Results

Soil MBC

The ANOVA results (Table 3) showed that water regimes (including DWC treatments and comparisons), temperatures, and their interactions significantly influenced soil MBC in our study ($P < 0.001$). These findings support Hypotheses 1 and 2. The combined effects of DWC treatments and temperature on MBC were highest at 20°C, followed by 30°C, and then 10°C (Figure 2). MBC values under DWC treatments were significantly lower than those under OPT across the 10-40°C range. However, these values exceeded those under AD or ST throughout the 10-30°C range, partially supporting Hypothesis 3 (Figure 2).

At both the start and end of incubation, soil MBC levels were higher at the hilltop than at the mid-slope and toe-slope positions. However, no significant differences existed among these positions, especially under all water regimes at 0°C (Table 2; Figure 2). Similarly, MBC levels showed no significant differences between the two DWC treatments (W5-D10 and W10-D5) across the entire temperature range of 0-40°C ($P < 0.05$). MBC values reached their minimum at extreme temperatures (0°C and 40°C) for all water treatments except OPT at 40°C. Furthermore, both AD and ST conditions consistently produced lower MBC levels across the entire 0-40°C temperature range.

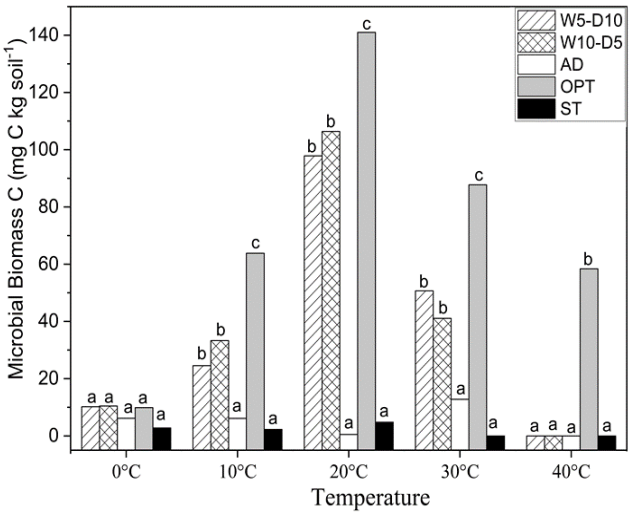


Figure 2: Response of MBC to water regimes and temperatures. No significant differences with the soil position. Means were calculated across three position soils. Different letters indicate significant differences between water regimes at the same temperature based on Fisher's LSD test at $p< 0.05$

Soil enzymes activity

The ANOVA results (Table 3) showed that both water regimes (including two DWC treatments and three comparisons) and temperatures significantly affected all five soil enzymes: BG ($P < 0.001$), NAG ($P < 0.01$), Arylamidase ($P < 0.004$), acid phosphatase ($P < 0.001$), and FDA ($P < 0.001$). These findings support Hypotheses 1 and 2. The interaction between water regimes and temperatures was significant for BG ($P < 0.001$), acid phosphatase ($P = 0.046$), FDA ($P = 0.022$), and NAG ($P = 0.056$), but not for Arylamidase ($P = 0.836$). Only NAG and FDA values under DWC treatments were lower than those under OPT but higher than those under AD or ST, particularly at 20°C,

partially supporting Hypothesis 3. The other enzymes showed more complex patterns, contradicting Hypothesis 3 (Figures 3 to 7).

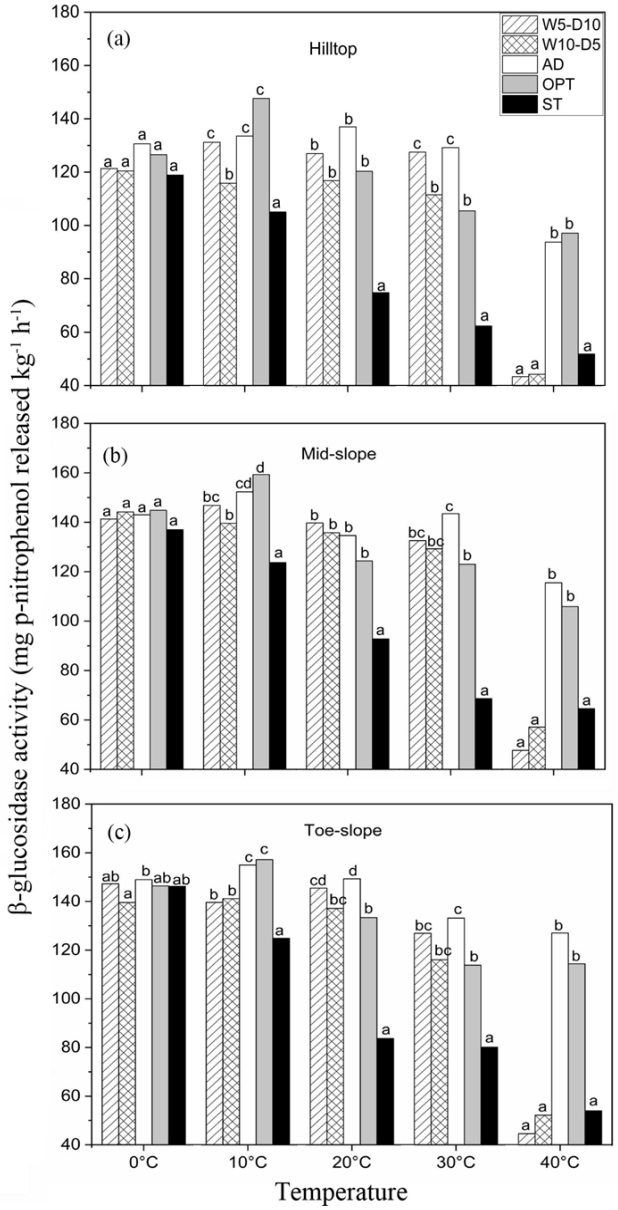


Figure 3: Response of BG activity to water regimes and temperatures in soils from three positions. (a)Hilltop; (b)Mid-slope; (c)Toe-slope. For each temperature, different lower case letters indicate significant differences between water regimes within the temperature, based on Fisher's LSD test at $p < 0.05$

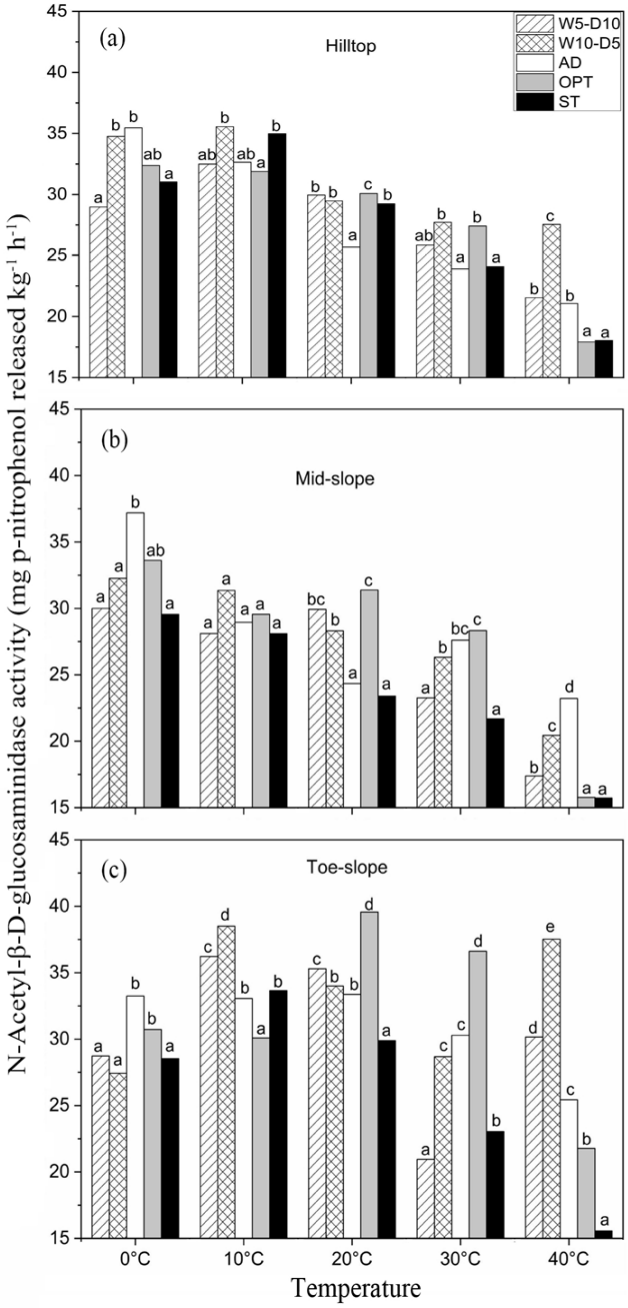


Figure 4: Response of NAG activity to water regimes and temperatures in soils from three positions. (a)Hilltop; (b)Mid-slope; (c)Toe-slope. For each temperature, different lower case letters indicate significant differences between water regimes within the temperature, based on Fisher's LSD test at $p < 0.05$

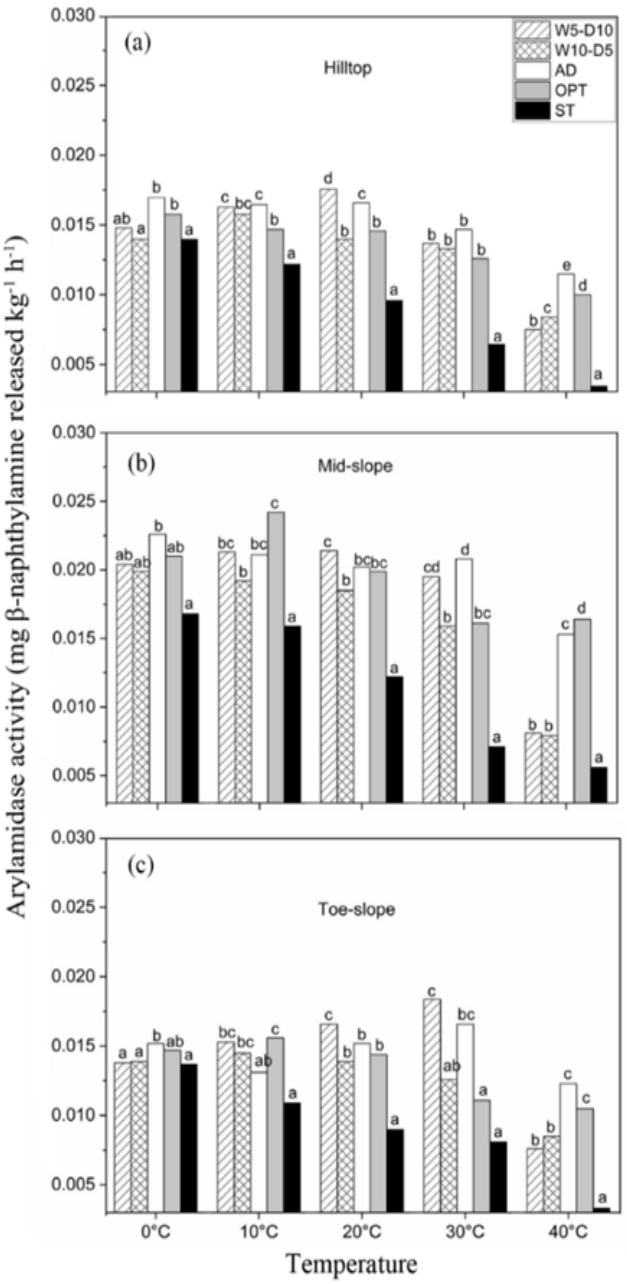


Figure 5: Response of Arylamidase activity to water regimes and temperature in soils from three positions. (a)Hilltop; (b)Mid-slope; (c)Toe-slope. For each temperature, different lower case letters indicate significant differences between water regimes within the temperature, based on Fisher's LSD test at $p < 0.05$

Before incubation, BG activity was lowest in the hilltop soils, intermediate in mid-slope soils, and highest in toe-slope soils, with a significant difference between hilltop and toe-slope locations (Table 2). After incubation, BG values peaked at 10°C, particularly for OPT and AD treatments, while reaching their lowest at 40°C, especially for W5-D10, W10-D5, and ST treatments across all positions. BG activity decreased significantly in response to ST treatment as temperatures rose at all three locations (Figure 3). No significant differences in BG values were observed among water-regime treatments at 0°C for hilltop and mid-slope soils (Figure 3a; Figure 3b). Similarly, BG activity showed no substantial difference between W5-D10 and W10-D5 treatments across all locations throughout the 0-40°C range, except in hilltop soils at 10°C and 30°C, where W5-D10 exhibited higher BG values than W10-D5 (Figure 3).

NAG activity was higher in the toe-slope soils both before and after incubation (Table 2; Figure 4). The most significant decrease in NAG activity occurred at 40°C, particularly for the ST treatment, with a 56.8% reduction in the toe-slope soils. The AD treatment combined with 0°C temperature produced the highest NAG values. However, W10-D5 treatment yielded the highest activity at 10°C and 40°C. At 20°C and 30°C, OPT led in activity across all slope locations. W10-D5 consistently showed higher NAG activity than W5-D10 across temperature gradients at all locations, except at 20°C and 0°C in the toe-slope soils. Significant differences in NAG activity between the typical DWC treatments (W10-D5 and W5-D10) were observed at 0°C and 40°C for hilltop soils, at 30°C and 40°C for mid-slope soils, and throughout the 10-40°C range for toe-slope soils (Figure 4).

Before and during soil incubation, Arylamidase activity was highest at the mid-slope position, followed by the toe-slope and hilltop (Table 2; Figure 5), which aligned with the distribution of DOC across the locations (Table 1). ST treatments showed the lowest Arylamidase activity across all locations, with activity decreasing as temperature increased. Consequently, the lowest Arylamidase activity occurred in ST treatments at 40°C in toe-slope soils. For hilltop soils, the W5-D10 DWC treatment combined with 20°C temperature produced the highest Arylamidase activity, while the lowest activity was observed at 40°C. In contrast, the AD treatment maintained relatively high Arylamidase activity throughout the entire temperature range. For mid-slope and toe-slope soils, significant differences in Arylamidase activity were observed between the two DWC treatments at 20°C and 30°C, with W5-D10 generally showing higher activity than W10-D5 across the

0-30°C range, though these differences were not statistically significant (Figure 5).

Before incubation, acid phosphatase activity was highest in the toe-slope soils, followed by hilltop, and then minimum at mid-slope, which aligned with the distribution of SOC and TN but showed opposite to soil pH across these positions (Table 1). The combined effect of ST treatment and temperature on acid phosphatase activity was lowest among all water treatments across the three positions, except at 40°C. At this higher temperature, the two DWC treatments showed the lowest acid phosphatase activity, reaching only 23.7 to 33.2% of the values observed under OPT. Notably, the combination of DWC treatment W5-D10 and 20°C temperature produced the highest acid phosphatase activity across all locations (Figure 6).

Before incubation, FDA hydrolysis activity was highest in soils from the hilltop position, followed by the toe-slope and mid-slope (Table 2). After incubation, the ST treatment consistently produced lower FDA values across the 0-40°C temperature range, with the exception at 20°C. The lowest FDA value occurred at 40°C under ST conditions, approximately 41% lower than under OPT at the same temperature. Generally, FDA activity under DWC treatments decreased as temperature increased, with no significant differences between W5-D10 and W10-D5 treatments throughout the entire 0-40°C range (Figure 7).

Discussion

As anticipated, our findings show that both soil water conditions—including two DWC treatments and three comparisons—and temperature significantly affected soil MBC and enzyme activity. These results align with established consensus (Moyano *et al*., 2013; Karhu *et al*., 2014). Beyond their individual effects on soil biological variables, their combined influence was significant for all measurements except Arylamidase. The interaction between both DWC scenarios (W5-D10 and W10-D5) and temperature showed similar effects on MBC across the 0-40°C range. However, significant differences in MBC emerged between DWC treatments and the three comparisons (AD, ST, and OPT) throughout the 10-30°C range. The interaction between DWC treatments and temperature on specific enzyme activity and associated metabolisms proved complex, closely linked to soil sampling locations, enzyme types, water conditions, and temperature levels. Overall, these findings highlight the critical role of DWC and temperature in jointly influencing soil microbial activity, metabolism, and SOM degradation.

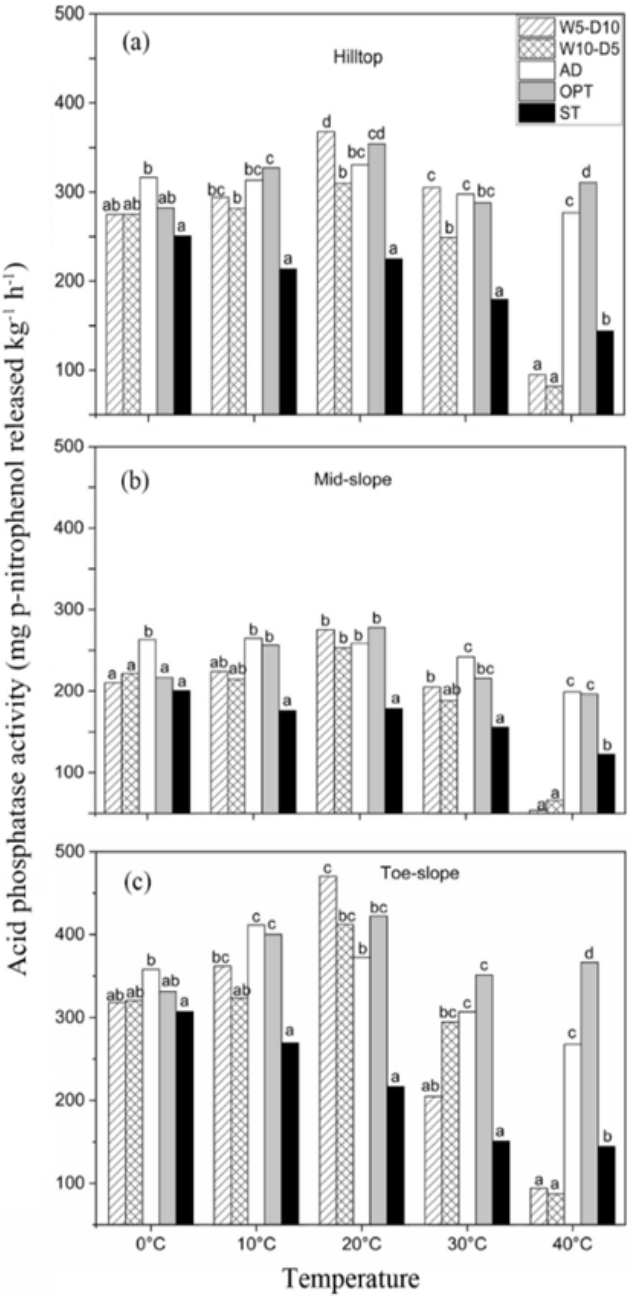


Figure 6: Response of acid phosphatase activity to water regimes and temperatures in soils from three positions. (a)Hilltop; (b)Mid-slope; (c)Toe-slope. For each temperature, different lower case letters indicate significant differences between water regimes within the temperature, based on Fisher's LSD test at $p < 0.05$

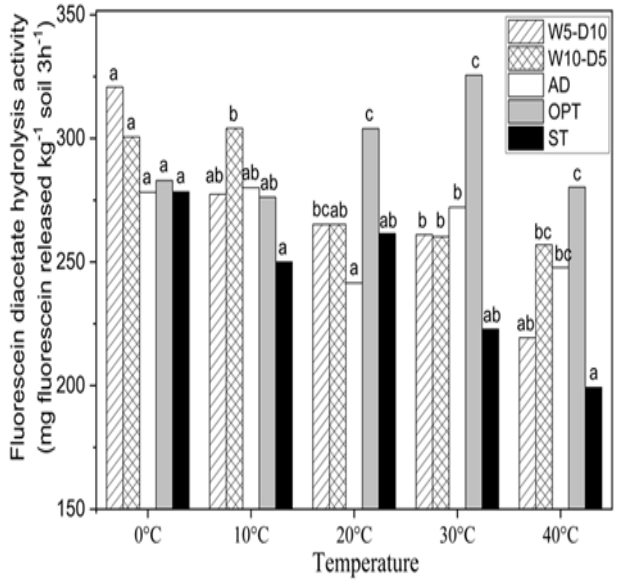


Figure 7: Response of FDA hydrolysis activity to water regimes and temperatures. Different letters indicate significant differences between water regimes at the same temperature based on Fisher's LSD test at $p < 0.05$

Regarding DWC, wetting typically enhances microbial activity within minutes or hours (Borken *et al*., 2003; Sponseller, 2007; Priemé and Christensen, 2001; Pulleman and Tietema, 1999). In contrast, drying can kill up to one-third of microbial biomass within 24 hours at 40°C (Bottner, 1985), and reduce biomass by approximately 14-30% over 10 days under these conditions (Sparling *et al*., 1986). The drying duration significantly influences how soil microbial processes respond to rewetting events during DWC (Fierer and Schimel, 2002; Xiang *et al*., 2008; Borken and Matzner, 2009; Fromin, 2010). Warmer conditions lead to increased evaporation and decreased soil moisture, which suppress microbial enzyme activity (Allison and Treseder, 2008; Henry, 2012). The soil microbial community faces greater stress under shorter-wetting-and-longer-drying treatment (W5-D10). This stress inhibits microbial growth, decreases microbial biomass, and makes soil enzymes more susceptible to denaturation. However, microbial communities can adapt to drought stress over time, resulting in fewer changes in enzyme activity under consistently lower moisture or even air-dried conditions (AD). This explains our results showing significant decreases in MBC, BG, Arylamidase, and acid phosphatase activity under hot conditions (40°C) × W5-D10, with only slight reductions under higher temperature × AD conditions (Figures 2, 3, 5, 6).The response of soil enzyme activity to DWC treatments can be attributed to two balanced processes: enzymes released into soil from dying microorganisms, and the desorption and reactivation of enzymes previously accumulated in soils. During soil drying, microorganisms accumulate compatible solutes (osmolytes) to lower their internal water potential, preventing dehydration and death (Schimel *et al*., 2007; Zhao *et al*., 2010). During rewetting, these osmolytes must be quickly cleared or diluted to prevent cell flooding, which could cause cell rupture or death (Xiang *et al*., 2008).

Numerous studies have explored the impacts of temperature on soil microbial activity, soil respiration rates, and soil carbon decomposition (Davidson and Janssens, 2006; Kirschbaum, 2006; Karhu *et al*., 2014). Our research introduces a fresh perspective on these issues. We discovered that under modified water (*i.e.* DWC) and temperature conditions, the effects of extreme temperatures on soil MBC and associated metabolisms were more diverse and specific than previously acknowledged. Under freezing conditions (0°C), the effect of soil moisture treatment on enzyme activity is nonsignificant. This is because low temperatures greatly slow the diffusion of substrates and enzymes within soils (Davidson and Janssens, 2006). As a result, the rate of SOM decomposition is typically reduced by lower temperatures (Bragazza *et al*., 2006; Moore *et al*., 2006), particularly around 0°C (Bowman *et al*., 2001; Yang *et al*., 2016). These findings align with our results showing no significant differences in MBC, BG, and FDA activity at 0°C among the water treatments (Figures 2, 3, 7). Typically, soil MBC affects the supply of available carbon within the soil (Blagodatskaya *et al*., 2011); BG determines the turnover of saccharide fractions of SOM in soil carbon cycling (Busto and Perez-Mateos, 2000; Parham and Deng, 2000); NAG and Arylamidase are nitrogen-acquiring enzymes (Acosta-Martinez and Tabatabai, 2001; Ekenler and Tabatabai, 2002); and FDA can be hydrolyzed by numerous enzymes and organisms (Bandick and Dick, 1999; Green *et al*., 2006). Therefore, due to water freezing and hindered diffusion of substrates within the soil (Davidson and Janssens, 2006), these enzymes and microorganisms likely reach a balance at 0°C, resulting in no detectable difference in activity. By contrast, soil MBC and enzyme activity significantly decline at higher temperatures (40°C) due to inhibited SOM decomposition and mineralization under extreme high temperatures that usually hinder microbial activity, despite differences among diverse water conditions. This decline is attributed to decreases in soil microbial nitrogen and carbon, which are typically consumed by microorganisms under extremely high temperature stress (Laurén *et al*., 2019). In our results, soil MBC and activity of the five enzymes generally reached their lowest values at 40°C, even lower

than at 0°C across the water treatments (Figures 2 to 7). For instance, at 40°C, only OPT affected MBC; OPT and AD affected BG; DWC and AD consistently affected NAG; AD and OPT affected Arylamidase and acid phosphatase; and OPT significantly affected FDA.

Based on our mechanism analysis, we can identify optimal combinations of temperature and water regimes for enhancing soil MBC and specific enzyme activity. MBC reached its peak at 20°C with OPT moisture, followed by DWC treatments, while remaining minimal with AD and ST treatments across the entire 0-40°C range (Figure 2). BG activity was highest at 10°C with OPT, followed by AD and then W5-D10 (Figure 3). NAG activity peaked at 10°C with W10-D5, with variable activity under AD, OPT, or ST at lower temperatures (Figure 4). Arylamidase activity was highest at 10°C with OPT in mid-slope soils, with notable activity also observed under W5-D10 and AD conditions (Figure 5). Acid phosphatase reached maximum activity at 20°C with W5-D10, followed by OPT and AD treatments (Figure 6). FDA showed peak activity at 30°C with OPT, followed by W5-D10 at 0°C, while showing minimum activity with OPT at 20°C (Figure 7).

Interestingly, Arylamidase activity exhibited highest while acid phosphatase activity showed minimum at mid-slope with sandy loam soil texture differing from the others with silt loam texture in our case (Figures 5 and 6). This might be explained by the effect of soil texture and other properties on diverse enzymes as are associated with specific metabolisms in the soils. For instance, Arylamidase indicates metabolism of proteins, which is often enhanced in sandy loam soils (*i.e.* mid-slope in our case) where mobility is usually increased (Creamer et al., 2013; Silver at al., 2000); while acid phosphatase indicates metabolism of phosphorus in soils, which is significantly hindered in an alkali environment by soil pH (Han et al., 2021), *e.g.*, pH 7.9 at mid-slope in our case (Table 1).

Finally, our soil samples underwent initial air-drying before incubation, which may raise questions about using air-dried samples versus intact fresh field soils. Due to the heterogeneity commonly found in intact soils, we used this initial air-drying process to ensure homogeneity, making all treated soils comparable throughout the incubation and measurement process. In other words, all water × temperature treatments in our study were conducted on these initially air-dried soils.

Conclusions

This research experimentally investigated the combined effects of DWC and varying temperatures on farmland soil MBC and related metabolisms. We collected soil from three slope positions at a Midwest agricultural research station and conducted laboratory incubation simulations. Our findings revealed that soil water conditions and temperatures collectively influenced soil MBC and associated metabolisms. We observed significant main effects of these factors on all measurements except Arylamidase activity, with interactive effects across all parameters. Significant differences in MBC emerged between DWC and other typical soil water treatments across the 10-30°C temperature range. However, no significant differences were detected between the two DWC treatments (W5-D10 and W10-D5) throughout the 0-40°C range. The combined impacts of DWC and temperature on specific enzyme activities were complex, varying with soil sampling locations, enzyme types, soil moisture, and temperature levels. Notably, higher extreme temperatures (40°C) reduced soil metabolic activity more severely than the freezing temperature (0°C). These findings enhance our understanding of how farmland soil microbial and biochemical processes respond to climate change, including altered precipitation patterns, soil moisture fluctuations, and temperature variations. This knowledge may support more accurate modeling of carbon cycling in response to multiple climate change factors and can inform strategies to mitigate climate change through proper regulation of soil moisture and temperature. For more reliable results, additional DWC scenarios or longer-term investigations will be necessary in future studies.

Acknowledgement

We thank Rhonda Graef for her technical assistance in field work and laboratory analysis. This work was financially supported by the USDA Agricultural Research Service research funds, the Chinese Scholarship Council grants awarded to L.X.Y. and L.J.H., and the National Natural Science Foundation of China program as well as the Jilin Provincial Department of Education program awarded to L.J.H. (No. 41071189; No. JJKH20250341CY).

References

Aanderud, Z.T., S.E. Jones, N. Fierer and J.T. Lennon. 2015. Resuscitation of the rare biosphere contributes to pulses of ecosystem activity. *Frontiers in Microbiology* 6: 24.

Acosta-Martinez, V. and M.A. Tabatabai. 2000. Arylamidase activity of soils. *Soil Science Society of America Journal* 64: 215-221.

Acosta-Martinez, V. and M.A. Tabatabai. 2001. Arylamidase activity in soils: effect of trace elements and relationships to soil properties and activities of

amidohydrolases. *Soil Biology and Biochemistry* 33: 17-23.

Allison, S.D. and K.K. Treseder. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology* 14: 2898-2909.

Allison, S.D., M.D. Wallenstein and M.A. Bradford. 2010. Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience*s 3: 336–340.

Alvarez, C., R. Alvarez, M. Grigera and R. Lavado. 1998. Associations between organic matter fractions and the active soil microbial biomass. *Soil Biology and Biochemistry* 30: 767-773.

Aon, M. and A. Colaneri. 2001. II. Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. *Applied Soil Ecology* 18: 255-270.

Bandick, A.K. and R.P. Dick. 1999. Field management effects on soil enzyme activities. *Soil Biology and Biochemistry* 31: 1471-1479.

Bárcenas-Moreno, G., G. Maria, J. Rousk and E. Bååth. 2009. Adaptation of soil microbial communities to temperature, microbial communities to temperature: comparison of fungi and bacteria in a laboratory experiment. *Global Change Biology* 15: 2950-2957.

Barnard, R.L., C.A. Osborne and M.K. Firestone. 2015. Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate. *The ISME Journal* 9: 946–957.

Baum, C., P. Leinweber and A. Schlichting. 2003. Effects of chemical conditions in re-wetted peats on temporal variation in microbial biomass and acid phosphatase activity within the growing season. *Applied Soil Ecology* 22: 167-174.

Birch, H.F. 1958. Th effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil* 10: 9-31.

Blagodatskaya, E., T. Yuyukina, S. Blagodatsky and Y. Kuzyakov. 2011. Three-source-partitioning of microbial biomass and of CO_2 efflux from soil to evaluate mechanisms of priming effects. *Soil Biology and Biochemistry* 43: 778-786.

Blazewicz, S.J., E. Schwartz and M.K. Firestone. 2014. Growth and death of bacteria and fungi underlie rainfall-induced carbon dioxide pulses from seasonally dried soil. *Ecology* 95: 1162-1172.

Borken, W.D., E. Savage, K. Gaudinski and S.J. Trumbore. 2003. Drying and wetting effects on carbon dioxide release from organic horizons. *Soil Science Society of America Journal* 67: 1888-1896.

Borken, W. and E. Matzner. 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Global Change Biology* 15: 808-824.

Bottner, P. 1985. Response of microbial biomass to alternate moist and dry conditions in a soil incubated with ^{14}C-and ^{15}N-labelled plant material. *Soil Biology and Biochemistry* 17: 329-337.

Bowman, W., T. Seastedt and Program LTER. 2001. Structure and function of an alpine ecosystem: Niwot ridge, Colorado. Oxford University Press, USA.

Bragazza, L., C. Freeman, T. Jones, H. Rydin, J. Limpens, N. Fenner, T. Ellis, R. Gerdol, M. Hajek, T. Hajek, P. Iacumin, L. Kutnar, T. Tahvanainen and H. Toberman. 2006. Atmospheric nitrogen deposition promotes carbon loss from peat bogs. *Proceedings of National Academy of Sciences USA* 103 (51): 19386-19389.

Busto, M.D. and M. Perez-Mateos. 2000. Characterization of β-D glucosidase extracted from soil fractions. *European Journal of Soil Sciences* 51: 193-200.

Creamer, C.A., T.R. Filley, D.C. Olk, D.E. Stott, V. Dooling and T.W. Boutton. 2013. Changes to soil organic N dynamics with leguminous woody plant encroachment into grasslands. *Biogeochemistry* 113: 307-321.

Davidson, E.A. and I.A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440: 165-173.

Denef, K., J. Six, H. Bossuyt, S.D. Frey, E.T. Elliott, R. Merckx and K. Paustian. 2001. Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. *Soil Biology and Biochemistry* 33: 1599-1611.

Dominguez, M.T., E. Holthof, A.R. Smith, E. Koller and B.A. Emmett. 2017. Contrasting response of summer soil respiration and enzyme activities to long-term warming and drought in a wet shrubland (NE Wales, UK). *Applied Soil Ecology* 110: 151-155.

Ekenler, M. and M. Tabatabai. 2002. β-Glucosaminidase activity of soils: Effect of cropping systems and its relationship to nitrogen mineralization. *Biology and Fertility of Soils* 36: 367-376.

Feldman, A.F., X. Feng, A.J. Felton, A.G. Konings, A.K. Knapp, J.A. Biederman and B. Poulter. 2024. Plant responses to changing rainfall frequency and intensity. *Nature Review Earth Environment* 5: 276-294.

Fierer, N. and J.P. Schimel. 2002. Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry* 34: 777-787.

Fierer, N., J.P. Schimel and P.A. Holden. 2003. Influence of drying-rewetting frequency on soil bacterial community structure. *Microbial Ecology* 45: 63-71.

Fromin, N., G. Pinay, B. Montuelle, D. Landais, J.M. Ourcival, R. Joffre and R. Lensi. 2010. Impact of seasonal sediment desiccation and rewetting on microbial processes involved in greenhouse gas emissions. *Ecohydrology* 3: 339-348.

Gee, G.W. and D. Or. 2002. Particle-size analysis. p. 255-293. In: *Methods of Soil Analysis. Part 4. Physical Methods*. J.H. Dane and G.C.Topp (eds.). Soil Science Society of America, Madison, WI, USA.Grandy, A.S. and J.C. Neff. 2008. Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. *Science of the Total Environment* 404: 297–307.

Green, V.S., D.E. Stott and M. Diack. 2006. Assay for fluorescein diacetate hydrolytic activity: Optimization for soil samples. *Soil Biology and Biochemistry* 38: 693-701.

Guo, X., C.F. Drury, X. Yang, W.D. Reynolds and R. Zhang. 2012. Impacts of wet-dry cycles and a range of constant water contents on carbon mineralization in soils under three cropping treatments. *Soil Science Society of America Journal* 76: 485-493.

Halverson, L.J., T.M. Jones and M.K. Firestone. 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. *Soil Science Society of America Journal* 64: 1630–1637.

Han, B., J. Li, K. Liu, H. Zhang, X. Wei and X. Shao. 2021. Variations in soil properties rather than functional gene abundances dominate soil phosphorus dynamics under short-term nitrogen input. *Plant and Soil* 469 (1/2): 227-241.

Henry, H.A.L. 2012. Soil extracellular enzyme dynamics in a changing climate. *Soil Biology and Biochemistry* 47: 53-59.

Horwath, W.R. and E.A. Paul. 1994. Microbial biomass. p. 753-773. In: *Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties.* R.W.Weaver, J.S. Angle and P.S. Bottomley (eds.). Soil Science Society of America, Madison, WI. .

IPCC. 2013. Climate change 2013: The physical science basis: Working Group I contribution to the Fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press.

Jin, K., S. Sleutel, D. Buchan, S. De Neve, D.X. Cai, D. Gabriels and J.Y. Jin. 2009. Changes of soil enzyme activities under different tillage practices in the Chinese Loess Plateau. *Soil and Tillage Research* 104: 115-120.

Karhu, K., M.D. Auffret, J.A.J. Dungait, D.W. Hopkins, J.I. Prosser, B.K. Singh, J.A. Subke, P.A. Wookey, G.I. Agren, M.T. Sebastia, F. Gouriveau, G. Bergkvist, P. Meir, A.T. Nottingham, N. Salinas and I.P. Hartley. 2014. Temperature sensitivity of soil respiration rates enhanced by microbial community response. *Nature* 513: 81-84.

Kirschbaum, M.U.F. 2006 The temperature dependence of organic-matter decomposition-still a topic of debate. *Soil Biology and Biochemistry* 38: 2510-2518.

Koyama, A., S.J. Megan, M.L. Haddix, J.S. Dukes and M.D. Wallenstein. 2017. Soil bacterial community responses to altered precipitation and temperature regimes in an old field grassland are mediated by plants. *Microbial Ecology* 94: 1-15.

Laurén, A., M. Lappalainen, A.J. Kieloaho, K. Karhu and M. Palviainen. 2019. Temperature sensitivity patterns of carbon and nitrogen processes in decomposition of boreal organic soils - Quantification in different compounds and molecule sizes based on a multifactorial experiment. *PLoS ONE* 14(10): e0223446.

Lovieno, P. and E. Bååth. 2008. Effect of drying and rewetting on bacterial growth rates in soil. *FEMS Microbiological Ecology* 65(3): 400-407.

Liang, C., G. Cheng, D.L. Wixon and T.C. Balser. 2011. An absorbing Markov Chain approach to understanding the microbial role in soil carbon stabilization. *Biogeochemistry* 106: 303-309.

Magid, J., C. Kjaergaard, A. Gorissen and P.J. Kuikman. 1999. Drying and rewetting of a loamy sand soil did not increase the turnover of native organic matter, but retarded the decomposition of added 14C-labelled plant material. *Soil Biology and Biochemistry* 31: 595–602.

Manzoni, S. and A. Porporato. 2009. Soil carbon and nitrogen mineralization: Theory and models across scales. *Soil Biology and Biochemistry* 41: 1355–1379.

Marchin, R.M., C.F. Salk, W.A. Hoffmann and R.R. Dunn. 2015. Temperature alone does not explain phenological variation of diverse temperate plants under experimental warming. *Global Change Biology* 21: 3138-3151.

Mazzei, P. and A. Piccolo. 2019. Reduced catalytic activity of an exogenous extracellular β-D-glucosidase due to adsorption on a model humic-clay complex and different soils under wetting and drying cycles. *Biology and Fertility of Soils* 55(6): 617-627.

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis* 15(12): 1409-1416.

Moore, T. and N. Basiliko. 2006. Decomposition in boreal peatlands. P. 125-140. In: Boreal Peatland Ecosystems. R.K.Wieder and D.H. Vitt (eds.). Springer-Verlag, Berlin.

Moyano, F.E., S. Mazoni and C. Chenu. 2013. Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. *Soil Biology and Biochemistry* 59: 72-85.

Parham, J. and S.P. Deng. 2000. Detection, quantification and characterization of β-glucosaminidase activity in soil. *Soil Biology and Biochemistry* 32: 1183-1190.

Parton, W.J., D.S. Schmel, C.V. Cole and D.S. Ojima. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Science Society of America Journal* 51: 1173-1179.

Placella, S.A., E.L. Brodie and M.K. Firestone. 2012. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. *Proceedings of National Academy of Sciences USA* 109: 10931-10936.

Priemé, A. and S. Christensen. 2001. Natural perturbations, drying-wetting and freezing-thawing cycles, and the emission of nitrous oxide, carbon dioxide and methane from farmed organic soils. *Soil Biology and Biochemistry* 33: 2083-2091.

Prosser, J.A., T.W. Speir and D.E. Stott. 2011. Soil oxidoreductases and FDA hydrolysis, In: Dick, R.P. (Ed.), Methods of soil enzymology. Soil Science Society of America, Madison, WI. pp. 107-110.

Pulleman, M. and A. Tietema. 1999. Microbial C and N transformations during drying and rewetting of coniferous forest floor material. *Soil Biology and Biochemistry* 31: 275-285.

Ribeiro, P.L., A. Singh, A. Sagervanshi, A. Naeem and K.H. Mühling. 2024. High soil moisture rather than drying-rewetting cycles reduces the effectiveness of nitrification inhibitors in mitigating N_2O emissions. *Biology and Fertility of Soils* 60: 627-638.

Rousk, J., S.D. Frey and E. Bååth. 2012. Temperature adaptation of bacterial communities in experimentally warmed forest soils. *Global Change Biology* 18: 3252-3258.

Sardans, J., J. Penuelas and M. Estiarte. 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. *Applied Soil Ecology* 39: 223-235.

Salazar-Villegas, A., E. Blagodatskaya and J.S. Dukes. 2016. Changes in the size of the active microbial pool explain short-term soil respiratory responses to temperature and moisture. *Frontiers in Microbiology* 7: 524.

Schimel, J.P. 2018. Life in dry soils: Effects of drought on soil microbial communities and processes. *Annual Review of Ecology, Evolution and Systematics* 49: 409-432.

Schimel, J., T.C. Balser and M. Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88: 1386-1394.

Semenov, V.M., B.M. Kogut and S.M. Lukin. 2014. Effect of repeated drying-wetting-freezing-thawing cycles on the active soil organic carbon pool. *Eurasian Soil Science* 47: 276-286.

Sherrod, L.A., G. Dunn, G.A. Peterson and R.L. Kolberg. 2002. Inorganic carbon analysis by modified pressure-calcimeter method. *Soil Science Society of America Journal* 66: 299-305.

Silver, W.L., J. Neff, M. McGroddy, E. Veldkamp, M. Keller and R. Cosme. 2000. Effects of soil texture on belowground carbon and nutrient storage in a lowland Amazonian forest ecosystem. *Ecosystems* 3(2): 193-209.

Soulides, D.A. and F.E. Allison. 1961. Effect of drying and freezing soils on carbon dioxide production, available mineral nutrients, aggregation, and bacterial population. *Soil Science* 91(5): 291-298.

Sparling, G., T.W. Speir and K.N. Whale. 1986. Changes in microbial biomass C, ATP content, soil phospho-monoesterase and phospho-diesterase activity following air-drying of soils. *Soil Biology and Biochemistry* 18: 363-370.

Sponseller, R.A. 2007. Precipitation pulses and soil CO_2 flux in a Sonoran Desert ecosystem. *Global Change Biology* 13: 426-436.

Steinweg, J.M., J.S. Dukes, E.A. Paul and M.D. Wallenstein. 2013. Microbial responses to multi-factor climate change: effects on soil enzymes. *Frontiers in Microbiology* 4: 146.

Sørensen, L. 1974. Rate of decomposition of organic matter in soil as influenced by repeated air drying-rewetting and repeated additions of organic material. *Soil Biology and Biochemistry* 6(5): 287-292.

Tabatabai, M.A. 1994. Soil enzymes. P. 775-834. In: Methods of Soil Analysis. Part 2, Microbiological and Biochemical Properties. Bottomley. P.J., J.S. Angle, and R.W. Weaver, (eds.). Soil Science Society of America, Madison.

Wallenstein, M.D. and M.N. Weintraub. 2008. Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. *Soil Biology and Biochemistry* 40: 2098–2106.

Watson, M.E. and J.R. Brown. 1998. pH and lime requirement. p. 13-16. In: *Recommended Chemical Soil Test Procedures for the North Central Region.* J.R. Brown (ed.). NCR Publ. 221 (revised). Missouri Agricultural Experiment Station Bulletin, SB1001.

Whitney, D.A. 1998. Soil salinity. P. 59-60. In: Recommended Chemical Soil Test Procedures for the

North Central Region. J.R. Brown, (Ed.). NCR Publ. 221 (revised). Missouri Agricultural Experiment Station Bulletin, SB1001.

Xiang, S.R., A. Doyle, P.A. Holden and J.P. Schimel. 2008. Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. *Soil Biology and Biochemistry* 40: 2281-2289.

Xiao, W., X. Chen, X. Jing and B. Zhu. 2018. A meta-analysis of soil extracellular enzyme activities in response to global change. *Soil Biology and Biochemistry* 123: 21-32.

Yang, Z., S.D. Wullschleger, L. Liang, D.E. Graham and B. Gu. 2016. Effects of warming on the degradation and production of low-molecular-weight labile organic carbon in an Arctic tundra soil. *Soil Biology and Biochemistry* 95: 202–211.

Zhang, S., J. Lin, P. Wang and B. Zhu. 2023. The direct and legacy effects of drying-rewetting cycles on active and relatively resistant soil carbon decomposition. *Land Degradation and Development* 34: 2124-2135.

Zhang, S., Z. Yu, J. Lin and B. Zhu. 2020. Responses of soil carbon decomposition to drying-rewetting cycles: A meta-analysis. *Geoderma* 361: 114069.

Zhao, B., J. Chen, J. Zhang and S. Qin. 2010. Soil microbial biomass and activity response to repeated drying-rewetting cycles along a soil fertility gradient modified by long-term fertilization management practices. *Geoderma* 160: 218-224.