Differential Organic Carbon Mineralization Responses to Soil Moisture in Three Different Soil Orders Under Mixed Forested System

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Soil microbial respiration is one of the largest sources of carbon (C) emissions to the atmosphere in terrestrial ecosystems, which is strongly dependent on multiple environmental variables including soil moisture. Soil moisture content is strongly dependent on soil texture, and the combined effects of texture and moisture on microbial respiration are complex and less explored. Therefore, this study examines the effects of soil moisture on the mineralization of soil organic C Soil organic carbon in three different soils, Ultisol, Alfisol and Vertisol, collected from mixed forests of Georgia, Missouri, and Texas, United States , respectively. A laboratory microcosm experiment was conducted for 90 days under different moisture regimes. Soil respiration was measured weekly, and destructive harvests were conducted at 1, 15, 60, and 90 days after incubation to determine extractable organic C (EOC), phospholipid fatty acid based microbial community, and C-acquiring hydrolytic extracellular enzyme activities (EEA). The highest cumulative respiration in Ultisol was observed at 50% water holding capacity (WHC), in Alfisol at 100% water holding capacity, and in Vertisol at 175% WHC. The trends in Extractable Organic Carbon were opposite to that of cumulative microbial respiration as the moisture levels showing the highest respiration showed the lowest EOC concentration in all soil types. Also, extracellular enzyme activities increased with increase in soil moisture in all soils, however, respiration and EEA showed a decoupled relationship in Ultisol and Alfisol soils. Soil moisture differences did not influence microbial community composition.

Keywords: microbial respiration, soil moisture content, soil texture, mineralization rate, extracellular enzyme activity 3

INTRODUCTION

Soil organic carbon (SOC) is the largest and most dynamic terrestrial C reserve (Jobbágy and Jackson, 2000) and understanding SOC decomposition in response to environmental changes is critical for predicting CO_2 feedbacks driving future climates (Davidson and Janssens, 2006; Schmidt et al., 2011; Hao et al., 2017; Su et al., 2020). Globally, 60 to 80 Pg of C is emitted to the atmosphere by microbial soil respiration, which is one of the largest C fluxes between the terrestrial ecosystem and atmosphere (Raich and Schlesinger, 1992; Jian et al., 2018; Warner et al., 2019). Approximately 10% of the atmospheric CO_2 cycles through soils each year and environmental changes that influence C cycling in soil impart a strong effect on atmospheric CO_2 concentrations (Rodeghiero and Cesscatti, 2005). Given the large amounts of soil C stocks and flux to the atmosphere, a major concern is that future climate change will create a positive feedback loop by increasing soil respiration (Trumbore, 1997; Davidson et al., 2000; Schlesinger and Andrews, 2000; Brangari et al., 2020).

Soil moisture plays a very important role in regulating microbial activity (Yan et al., 2018; Schimel et al., 2018). The existing literature shows that the soil moisture-respiration relationship is complex and site-specific, and is strongly controlled by porosity, bulk density, texture, and SOC concentration (Franzluebbers, 1999; Thomsen et al., 1999; Moyano et al., 2012; Herbst et al., 2016; Homyak et al., 2018; Li et al., 2020). However, such studies have focused mainly on determining the index of soil moisture that best predicts the microbial respiration (e.g., water-filled pore space, water potential) or elucidating the unified function that best describes the relationship between soil moisture and microbial respiration (e.g., linear, polynomial, etc.) (Ilstedt et al., 2000; Paul et al., 2003). Soil C models tend to rely on empirical soil moisture-respiration functions which are developed and validated based on site-specific studies (Bauer et al., 2008; Falloon et al., 2011). Moreover, these functions represent an average response of the moisture-respiration relationships, thus can introduce uncertainties into predictions of the SOC budget (Sierra et al., 2015).

Soil physico-chemical properties such as soil texture, can also impart a significant control on how moisture influences microbial mineralization of SOC (Franzluebbers et al., 1996; Moyano et al., 2012; Li et al., 2020). While clay content does not affect the mineralization of so-called labile SOC early in the decomposition process, during the later stages when non-labile SOC is mineralized, high clay content slows down decomposition rates as clay protects SOC (Wang et al., 2003). On the contrary, sandy soils poorly protect SOC against microbial respiration (Mutuo et al., 2006). The soil water content, a function of soil pore space and pore size distribution, is also governed by soil texture (Childs, 1940). Coarse textured soils tend to exhibit a lower water holding capacity (WHC) as the pore size distribution is dominated by macropores with minimum ability to retain water while fine textured soils, dominated by micropores, have the highest WHC and tend to retain much more water than coarse textured soils at same water potentials.

The low and high extremes of soil moisture content are not congenial for microbial respiration. Low soil moisture levels hinder microbial respiration by decreasing substrate accessibility to microbes. Under extremely dry conditions, microbes may undergo a state of low metabolic activity or dormancy (Manzoni et al., 2012; Manzoni et al., 2014). On the contrary, high soil moisture levels impede microbial respiration by reducing O_2 diffusion (Moyano et al., 2013). Extracellular enzyme activities that provide information on soil microbial metabolic functioning (Stott et al., 2010) are also strongly influenced by soil moisture (Acosta-Martinez et al., 2007) and soil texture (Günal et al., 2018). Despite the critical role of soil moisture in regulating SOC mineralization, the soil moisture sensitivity of terrestrial C cycling has received less attention in comparison to soil temperature sensitivity (Wang et al., 2019). Therefore, this paper aims to experimentally establish soil moisture-soil respiration relationships and determine how microbial respiration changes under changing soil moisture conditions in three different soils with distinctly different textures and other attributes. With these objectives in mind, we conducted a laboratory-scale microcosm experiment using three soils for 90 days. Our main aim was to determine if the measured soil properties would interact with soil moisture levels and this interactive response serves as the dominant factor controlling the microbial respiration.

MATERIALS AND METHODS

Study Sites

Three different soils-Ultisol, Alfisol, and Vertisol-were collected from mixed forests located in the southern United States. The Ultisol belonged to the Cowart series (fine-loamy, kaolinitic, thermic Typic Kanhapludults) and was collected from Taylor County in Georgia (32.54°N, 84.22°W). The predominant tree species comprising the forest are *Quercus alba, Acer saccharum, Quercus velutina, Juniperus virginiana, Carya* spp., *Pinus clausa, Nyssa sylvatica, Magnolia grandiflora,* and *Liriodendron tulipifera*. Mean annual temperature and precipitation at this site is 20°C and 1,200 mm, respectively. The Alfisol belonged to the Weller series (fine, smectitic, mesic Aquertic Chromic Hapludalfs) and was collected from the Missouri Ozark AmeriFlux (MOFLUX) site in central Missouri. This site is located at the University of Missouri Baskett Wildlife Research and Education Center (38.74°N, 92.20°W) in an upland oak-hickory forest with major tree species consisting of *Quercus alba, Carya ovata, Acer saccharum* and *Juniperus virginiana* (Gu et al., 2015; Wood et al., 2018). Mean annual temperature and precipitation at this site is 12°C and 986 mm, respectively. The Vertisol belonged to the Buxin series (very-fine, smectitic, thermic Aquic Hapluderts) and was collected from Bowie County, Texas (33.44°N, 94.48°W). At this site the predominant tree species are *Pinus taeda, Pinus echinata, Quercus* spp, *Carya* spp, and *Cupressus* spp. Mean annual temperature and precipitation at this site is 17°C and 900 mm, respectively. Basic properties of these three soils are reported in **Table 1**.

TABLE 1 | Properties of the soils used in the study.

Properties	Ultisol	Alfisol	Vertisol
Location	Taylor county, GA	Boone county, MO	Bowie county, TX
Soil series	Cowart	Weller	Buxin
Soil order	Ultisols	Alfisols	Vertisols
Sand (%)	77 ± 0.3	13 ± 0.2	15 ± 0.1
Silt (%)	7 ± 0.2	51 ± 0.6	14 ± 0.5
Clay (%)	16 ± 0.4	36 ± 0.6	71 ± 0.2
Texture	Sandy	Silt loam	Clayey
Porosity (%)	73.1 ± 0.003	67.5 ± 0.004	73.4 ± 0.001
Bulk density (Mg m^{-3})	0.71 ± 0.01	0.86 ± 0.01	0.70 ± 0.01
pH	4.4 ± 0.11	5.0 ± 0.04	5.9 ± 0.11
Water holding capacity (g kg^{-1})	400 ± 7	500 ± 12	650 ± 16
Soil organic C (g kg^{-1})	13 ± 0.10	9.3 ± 0.08	16 ± 0.12
Extractable organic C (mg kg^{-1})	88 ± 2.7	86 ± 0.9	80 ± 1.8
Microbial biomass C (mg kg^{-1} dry soil)	156 ± 7.8	206 ± 8.5	378 ± 27

Values are mean ± standard error (n = 3).

Soil Sampling and Characterization

Soil cores of 5 cm diameter were collected from several random locations at each site to a depth of 15 cm, after removing the litter layer, and combined to obtain a composite sample per site (Mavi et al., 2012; Jagadamma et al., 2014). Three additional undisturbed cores per site were collected to determine WHC. The soil samples were transported to the research laboratory in coolers with dry ice. The composite samples were immediately sieved through a 2 mm sieve. One section of the sieved samples was air-dried for the initial soil characterization (n = 3), and the remainder was stored at 4°C for 48 h to use for the microcosm experiment. Soil texture was determined by the Bouyoucos hydrometer method (Gee and Or, 2002). Soil pH was measured in a 1:2 soil:water suspension (Thomas, 1996) using a pH meter (Mettler Toledo, Columbus, Ohio). Total C was determined on finely ground samples by the dry combustion method using a Vario TOC cube CN analyzer in solid mode (Elementar, Hanau, Germany). As all the soils were acidic (pH < 6), total C was assumed to be equal to SOC (Al-Kaisi et al., 2005). Extractable organic C (EOC) was determined based on the method by Jones and Willett (2006). Briefly, soils were mixed with 0.5 M K$_2$SO$_4$ (1:4 soil:solution), shaken at 200 revolutions per minute for 1 h, and centrifuged at 3,500 rpm for 3 min. The supernatant was passed through a 0.45 μm filter paper (Whatman No. 42) and analyzed using a Vario TOC cube CN analyzer in liquid mode (Elementar, Hanau, Germany).

Soil Moisture Treatments and Incubation Experiment

This study included five soil moisture treatments: air-dried, 25, 50, 100, and 175% WHC. The WHC of the three soils was determined using pressure plate extractor method (Klute and Dirksen, 1986) by placing the saturated soil cores in the pressure plate extractors at −0.33 bar and then measuring the gravimetric soil moisture content of the core (Mavi et al., 2012; Zhou et al., 2014; Bao et al., 2016). We also determined the WHC using the funnel method (Jenkinson and Powlson, 1976). As the WHC values obtained from both methods were similar, the WHC obtained using pressure plate extractors is reported. Except for

the air-dried treatments, the moisture treatment manipulations involved adding MilliQ water to the soils based on the moisture treatments (Zhou et al., 2014). Prior to the start of incubation, soil samples were pre-incubated for 1 week at environmental conditions similar to the experiment (i.e., at room temperature (20°C) in the dark). Thirty grams of soil sample (on a dry weight basis) was added to plastic cups and MilliQ water was added to manipulate corresponding soil moisture treatments followed by gently mixing with a spatula. The cups were then placed in 1 L glass jars and closed with lids fitted with sampling ports at the center. A total of 12 replicates per treatments were prepared for each soil type to destructively harvest three replicates at days 1, 15, 60, and 90 of the incubation. Soil collected from the destructive harvests were frozen at −20°C pending analysis. To maintain constant soil moisture during the course of the incubation, water loss was checked on a weekly basis by weighing each soil container and corrected by adding MilliQ water as necessary. At the most, 0.1–0.2 ml of water was added every week.

Gas Sampling and CO$_2$ Measurement

Gas samples (15 ml) from the headspace of the jars (n = 3) were collected through the sampling port on the lids on days 0, 1, 2, 5, 7, 12, 19, 26, 34, 41, 48, 55, 62, 69, 76, 83, and 90 using a syringe-needle assembly and transferred to evacuated exetainer vials (Labco, United Kingdom). The jars were sampled before opening the lids and after gas sample collection, the jars were opened, and electrical fans were used for blowing air into the jars to maintain an aerobic incubation environment. For blank correction, gas samples were collected from triplicate empty (without soil) jars at all time points. The CO$_2$ concentration in the gas samples was measured using a flame ionization detector on a gas chromatograph (Shimadzu GC-2014, Japan). Microbial respiration rate was calculated using the linear response curve of CO$_2$ concentrations versus time (Curiel-Yuste et al., 2007).

Soil Extracellular Enzyme Assays

The activities of four C-acquiring hydrolytic enzymes, α-glucosidase (AG), β-glucosidase (BG), cellobiohydrolase

(CBH), and β-xylosidase (XYL), were determined from all treatments at all the destructive sampling time points (day 1, 15, 60, and 90) in 96-well plates according to German et al. (2011b). Each column of 12 wells on each plate corresponded to one soil sample. Another plate was used to create a standard curve for each sample at 25°C. Each column in the standard curve plate contained a soil slurry with a different concentration of the 4-methylumbelliferone (MUB) standard (0, 2.5, 5, 10, 25, 50, and 100 μM) in each of the wells. The reference standards for the enzymes AG, BG, CBH, and XYL are 4-MUB-α-D-glucopyranoside, 4-MUB-β-D-glucopyranoside, 4-MUB-β-D-cellobioside, and 4-MUB-β-D- xylopyranoside, respectively. A 2.75 g of frozen soil sample from each treatment was thawed to room temperature and homogenized with 91 ml of 50 mM sodium acetate buffer (pH adjusted according to the pH of the soil types shown in **Table 1**) for 1 min in a Waring laboratory grade blender on high speed to prepare a slurry. Homogenization was followed by adding 800 μL of the soil slurry into each of the eight wells of one column. After adding 12 samples in a plate, 200 μL of 200 μM respective substrates were added. The plates were incubated for 3 h at 25°C. After incubation, the plates were centrifuged at 350 × g for 3 min and 250 μL of the supernatant from each well was transferred to a 96-well black plate. A plate reader (Synergy-BioTek Instruments, Inc, Vermont, United States) was used to measure fluorescence at wavelengths 365 and 450 nm for excitation and emission, respectively. The standard curve plates were used to construct a linear standard curve to determine each enzymes' activity for each sample as nmol g^{-1} dry soil h^{-1}. Total activity of the C acquiring hydrolytic enzymes was defined as the sum of CBH, AG, BG, and XYL.

Phospholipid Fatty Acid Extraction and Analysis

Microbial community composition was determined using the phospholipid fatty acid (PLFA) and neutral fatty acid (NLFA) analyses (Buyer and Sasser, 2012). Briefly, 1.5–2 g of freeze-dried soil were used for Bligh-Dyer lipid extraction (Bligh and Dyer, 1959). The extracted fatty acids were dried and analyzed using a gas chromatograph (Agilent 7890A, Agilent Technologies, Wilmington, DE, United States) after *trans*-esterification for quantitative analysis relative to an internal standard. Fatty acid profiles were identified using MIDI PLFAD1 calibration mix and software SHERLOCK version 6.2 (MIDI, Inc, DE, United States). Bacteria, fungi, and arbuscular mycorrhizal fungi (AMF) were targeted using the fatty acid markers, where gram-positive bacteria markers were 15:0, i15:0, a15:0, i16:0, 16:0ω9, i17:0, and cy17:0, and gram-negative bacteria markers were 16:1ω7, 18:1ω7, and cy19:0 (Frostegård and Baath, 1996). The amount of PLFA 18:2ω6 was used as a marker of non-mycorrhizal fungal abundance and NLFA 16:1ω5 for AMF (Olsson et al., 1995; Olsson, 1999).

Exponential Decay Modeling

The CO_2 data corresponding to each moisture treatment in each soil was best fitted using a double pool exponential decay model based on the best fit model parameters among single, double, and triple-pool models (lowest Akaike Information Criterion values and dependencies, and highest R^2) (Farrar et al., 2012; Jagadamma et al., 2014; Das et al., 2019).

$$C_t = C_1\left(1 - e^{-k_1 t}\right) + C_2\left(1 - e^{-k_2 t}\right)$$

where C_t is the total CO_2-C production per unit soil weight (μg C g^{-1} dry soil), t is the time in days, C_1 is the fast mineralizing SOC pool (active pool), C_2 is the slow mineralizing SOC pool (slow pool) within time t, and k_1 and k_2 are the mineralization rates for C_1 and C_2, respectively. For each set of data, the model was fit using Sigma plot v14 (Systat Software Inc, IL, United States). Parameter constraints while fitting the model were $k_1 > 0$ and $k_2 > 0$ and $C_1 + C_2 = 100\%$ (Das et al., 2019).

Statistical Analyses

Statistical analyses were conducted using SAS software v 9.4 (SAS Institute Inc., 2002). The effect of moisture on CO_2 and EOC over the incubation period for each soil was analyzed using repeated measures ANOVA with incubation length as the repeated measure. Post hoc comparisons for determining the effect of soil moisture on respiration was performed using PROC GLIMMIX in SAS. The mean separation was done using Tukey's test. The dependence of observed microbial respiration to various soil properties including soil pore space, bulk density, SOC, pH, MBC, EOC and contents of sand, silt and clay was determined using multiple linear regression analysis by applying stepwise model selection procedure. In all statistical tests, the mean differences were considered significant if $p \le 0.05$. Moisture sensitivity for enzyme assays was estimated using regression analyses of measured enzyme activities for the moisture treatments, soil types, each destructive sampling time, and the total incubation length. To compare enzyme activities among soil moisture treatments on each day of destructive sampling, one-way ANOVA was used.

To understand how the microbial community composition varies across various moisture levels in all soil types, permutational multivariate analysis of variance (PERMANOVA) was used in R software (R Development Core Team, 2011) on the matrix of abundances of each microbial type for all soil types and moisture treatments. The Bray-Curtis distance metric and 9,999 permutations of residuals for the model were used (Kivlin et al., 2019). The Mantel test was also performed in R to assess correlations between enzyme activity and microbial community composition.

RESULTS

Microbial Respiration

The CO_2 respiration rate showed significant responses to soil moisture content in all soils (**Figure 1**) ($p < 0.0001$–0.032 for Ultisol; < 0.0001–0.004 for Alfisol; < 0.0001 for Vertisol across the time points). The lowest respiration rate was observed at the lowest moisture content (air-dried soils) in all the three soils throughout the incubation. However, the highest CO_2 respiration
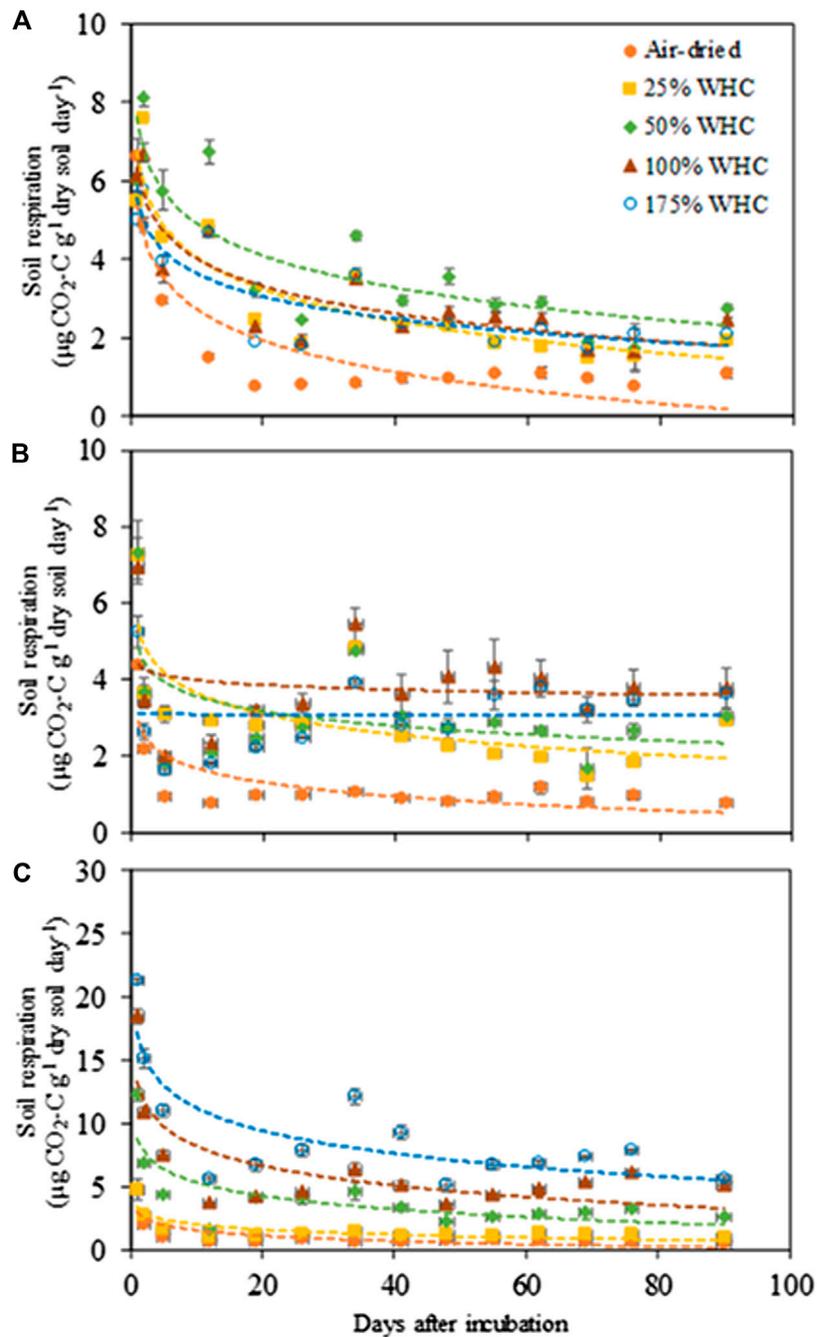
FIGURE 1 | Microbial respiration rate in response to soil moisture levels in **(A)** Ultisol, **(B)** Alfisol, and **(C)** Vertisol soils. Error bars represent standard error ($n = 3$). The regression functions are logarithmic for all soil moisture treatments in all soil types. Note the y axis scale is $3 \times$ larger for clay soils.

rate was observed at different moisture levels for the different soil types. For Ultisol, after the first 5 days of incubation, the highest respiration rate was consistently observed at 50% WHC throughout the incubation (**Figure 1A**). For Alfisol, after a week of incubation, the highest respiration was observed at 100% WHC for the remaining incubation period (**Figure 1B**). For Vertisol, the highest respiration rate was observed at 175% WHC consistently throughout the incubation period (**Figure 1C**). Also, we observed a

slight increase in respiration rate around day 34 in all soils, however, the trendline over time did not show an increase in any of the soils. The repeated measures ANOVA results showed significant effects of soil moisture ($p < 0.0001$), time of sampling ($p < 0.0001$), and their interaction ($p < 0.001$) on soil respiration for all soil types (**Table 2**). Cumulative CO_2 production at the end of incubation (day 90) was also the lowest in the air-dried treatments for all the three soils (**Figure 2**). However, across the moisture

TABLE 2 | Repeated measures ANOVA statistics (p value) for soil moisture and time effects on microbial respiration.

Soil type	Moisture	Time	Moisture × time
Ultisol	<0.0001	<0.0001	<0.001
Alfisol	<0.0001	<0.0001	<0.001
Vertisol	<0.0001	<0.0001	<0.001

levels, cumulative CO_2 production showed a bell-shaped second-order polynomial response with increased soil moisture was observed for Ultisol and Alfisol soils (**Figures 2A,B**), and an increasing second-order polynomial response for the Vertisol soil (**Figure 2C**). The second order polynomial curves were made using the actual soil moisture values, which are continuous variable. For Ultisol, soil respiration varied from 89 μg C g^{-1} dry soil to 265 μg C g^{-1} dry soil with the lowest respiration observed in air-dried treatment and the highest at 50% WHC (**Figure 2A**). Similarly, for Alfisol, the cumulative CO_2 production was the highest in the 100% WHC treatment which was over 200% higher than the CO_2 production in the air-dried treatment (**Figure 2B**). The cumulative CO_2 production from the 175% WHC treatment also averaged 17% lower than that at 100% WHC. In the Vertisol, the cumulative CO_2 increased with increased moisture (**Figure 2C**). Multiple linear regression of microbial respiration to soil properties revealed that respiration was significantly dependent on clay content, soil moisture, and SOC content (**Table 3**).

Soil Organic Carbon Pool Sizes and Mineralization Rates

Microbial respiration data was modeled by a double pool exponential decay model to determine SOC fractions that are actively decomposing (active pool, C_1) as well as relatively slowly decomposing (slow pool, C_2) at different moisture levels. Regardless of soil texture and soil moisture differences, < 1.5% of the initial SOC respired was partitioned as C_1 (**Table 4**). For both loamy and clayey soils, C_1 decreased with increasing moisture content. However, for Ultisol, C_1 was similar for all moisture levels except the air-dried treatment. The size of C_2 only varied between 99.2 and 99.7% of initial SOC among all soil types and soil moisture levels, and no significant differences were observed among soil moisture treatments.

Mineralization rate, k_1 of C_1 pool was the highest at 50% WHC (0.23 days^{-1}) for Ultisol, at 100% WHC (0.21 days^{-1}) and at 175% WHC (0.23 days^{-1}) moisture treatments for Alfisol, and at 175% WHC moisture content (0.17 days^{-1}) for Vertisol (**Figure 3A**). Mineralization rate, k_2, of C_2 pool was one to two order of magnitude lower than k_1 and showed no significant differences across moisture levels in all the three soils (**Figure 3B**). Comparing the soil types, Ultisol had comparatively higher k_2 followed by Alfisol and Vertisol.

Extractable Organic Carbon

The EOC values were generally higher in the drier treatments compared to the wetter treatments (**Figure 4**). For Ultisol, EOC

was statistically influenced by moisture ($p < 0.001$), length of incubation ($p < 0.0001$), and their interaction ($p < 0.001$) while for Alfisol and Ultisol soils, moisture and length of incubation significantly influenced the EOC and the interaction between moisture and length of incubation was not significant ($p > 0.05$). The EOC concentration at day 1 for all moisture levels and soil types was significantly higher (60–115 mg kg^{-1}) than that at days 15 (33–103 mg kg^{-1}), 60 (24–94 mg kg^{-1}), and 90 (22–89 mg kg^{-1}). As incubation progressed, for Ultisol and Alfisol soils, we observed no significant differences in EOC content of the driest treatment with time but for other treatments EOC decreased with time. However, for Vertisol, EOC in the driest treatment also decreased with incubation length. More importantly, beginning day 15, the lowest EOC content in each soil type was recorded from the moisture treatment which produced the highest cumulative respiration: at 50, 100, and 175% WHC for Ultisol, Alfisol, and Vertisol soils, respectively, (**Figures 2, 4**).

Extracellular Enzyme Activity and Microbial Community Composition Responses

There was a consistent increase in total C acquisition enzyme activities (sum of BG, AG, CBH, and XYL) with increase in soil moisture. The highest enzyme activity was observed at the highest soil moisture content for all the three soils at all time points (**Figure 5**). Also, regardless of soil types, air-dried treatments consistently showed the lowest enzyme activity and there was a decrease in enzyme activity with the length of incubation. For Ultisol, on day 1, we observed a significant increase in enzyme activity with an increase in moisture treatments but on day 90, no statistical difference in enzyme activity was observed in the moist treatments (only air-dried treatment showed lowest activity). The regression analysis showed a significant positive relationship between soil moisture and enzyme activity (**Table 5**). However, the PERMANOVA analysis revealed that soil moisture treatments did not influence the microbial community composition derived by PLFA in any soil type ($p = 0.35$ for Ultisol, 0.41 for Alfisol, and 0.12 for Vertisol) (**Supplementary Table S2**). Our Mantel test results showed that the microbial community composition did not influence the enzyme activity ($p > 0.05$) (**Supplementary Table S1**).

DISCUSSION

In accordance with our hypothesis, soil microbial respiration was influenced by soil moisture levels and showed variable responses in the three different soils. In all soils, significantly lower microbial respiration was observed in the drier treatments (**Figure 1**), likely due to limited substrate diffusion in the soil matrix that impeded microbial metabolism (Bell et al., 2009; Manzoni et al., 2012; Evans et al., 2014; Zhou et al., 2014, Yan et al., 2018). The slight increase in respiration rate around day 34 was due to moisture addition in soils on day 30 to maintain a steady moisture level. The moisture content at which the maximum soil respiration occurred varied across soil types (**Figure 2**). Over the incubation length of 90 days, we observed
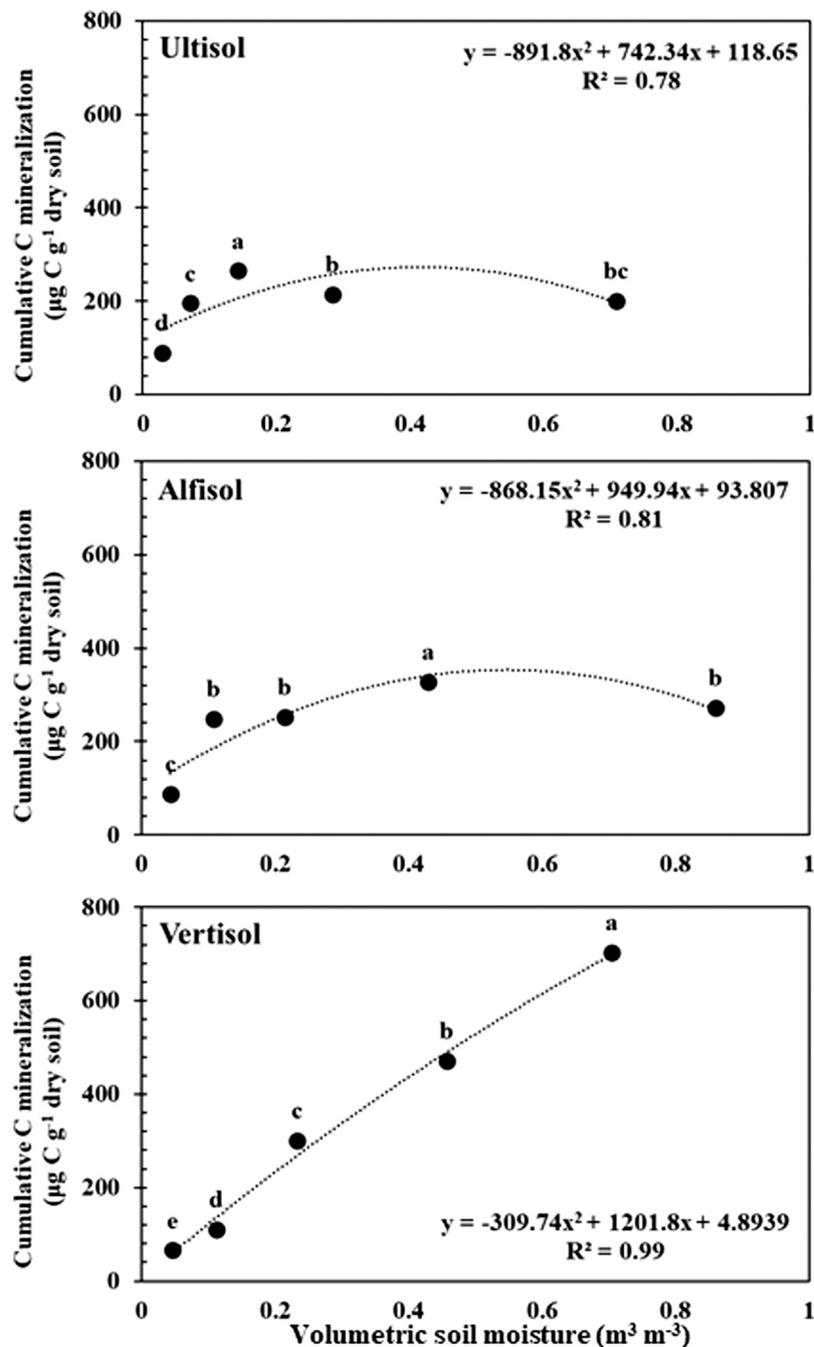
FIGURE 2 | Cumulative C mineralization per unit dry soil after day 90 from Ultisol **(A)**, Alfisol **(B)**, and Vertisol **(C)** soils in response to increasing soil moisture. Error bars represent standard errors (n = 3). Different letters represent statistical significance at $p < 0.05$. The curves represent the second-order polynomial response of cumulative C mineralization to soil moisture levels.

consistently highest respiration at 50% WHC for Ultisol, at WHC for Alfisol, and at 100% saturation for Vertisol. This clearly reflects persistently high microbial activity at respective moisture contents for all soil types which overrides the effect of substrate depletion over time (Zhou et al., 2014). Vertisol showed consistent increase in CO_2 efflux with 3-4 fold higher cumulative CO_2 at moisture optima (175% WHC) compared to

moisture optima for Alfisol (100% WHC) and Ultisol (50% WHC) soils. The Vertisol soil may have remnant air-filled micropores even at highly moist conditions to support aerobic microbial metabolism (Taboada, 2003). It is also important to note that Vertisol has an exceptional shrink-swell capacity that could promote the trapping of air in micropores (Taboada, 2003). The significant increase in microbial respiration from the Vertisol

TABLE 3 | Multiple linear regression metrics for microbial respiration.

Parameters	Partial R^2	Slope (μg C g^{-1} dry soil)
Clay content	0.08	2.3
Soil moisture content	0.04	1.2
Soil organic carbon	0.01	3.7

with increased moisture could also be explained mechanistically by the zonal theory of organic C layering on clay minerals (Kleber et al., 2007). Based on this theory, increased moisture may facilitate easy exchange of loosely adsorbed C molecules with the soil solution, making them available for microbial acquisition (Kögel-Knabner et al., 2008). For Ultisol and Alfisol soils, the decrease in respiration beyond optimum soil moisture treatment (i.e., peak respiration) is probably due to the decreased diffusion of O_2, thereby limiting aerobic microbial respiration (Prado and

Airoldi, 1999; Keiluweit et al., 2016). Also, Ultisol was sandy in texture which exhibits very high proportion of macropores leading to lower moisture retention and availability to microbes. This was probably the reason why the Ultisol exhibited the highest microbial respiration at 50% WHC. Li et al. (2020) also reported strong control of particle size distribution on C mineralization.

The microbial respiration data from this 90-day incubation was satisfactorily described by a double-pool exponential decay model. The air-dried soils showed the lowest k_1 and k_2, reflecting the lowest microbial activity. The moisture treatments which showed the highest cumulative microbial respiration (**Figure 2**) in all soil types also showed the highest k_1 values (**Figure 3**) indicating the tight coupling across soil texture, moisture content, and microbial activity. The k_2 was one to two orders of magnitude lower than k_1 and did not show significant differences among the moisture treatments for all the soil types despite following the order Ultisol > Alfisol > Vertisol. This is in line with our

TABLE 4 | Effects of soil moisture on soil organic carbon pool sizes as a percentage of initial soil organic carbon.

Soils	Moisture treatments	Active SOC pool (C_1)	Slow SOC pool (C_2)
		------------(%)--------------	
Ultisol	Air-dried	0.61 ± 0.07 a[a]	99.39 ± 0.07 b
	25% WHC	0.31 ± 0.03 b	99.69 ± 0.03 a
	50% WHC	0.36 ± 0.03 b	99.64 ± 0.03 a
	100% WHC	0.42 ± 0.05 b	99.58 ± 0.06 a
	175% WHC	0.34 ± 0.04 b	99.66 ± 0.04 a
Alfisol	Air-dried	0.78 ± 0.06 a	99.22 ± 0.06 c
	25% WHC	0.48 ± 0.09 b	99.52 ± 0.09 b
	50% WHC	0.35 ± 0.02 bc	99.65 ± 0.02 ab
	100% WHC	0.31 ± 0.02 bc	99.69 ± 0.02 ab
	175% WHC	0.30 ± 0.05 c	99.70 ± 0.06 a
Ultisol	Air-dried	1.43 ± 0.14 a	98.57 ± 0.14 c
	25% WHC	0.67 ± 0.03 b	99.33 ± 0.03 b
	50% WHC	0.65 ± 0.03 b	99.35 ± 0.03 b
	100% WHC	0.49 ± 0.02 bc	99.51 ± 0.02 ab
	175% WHC	0.29 ± 0.06 c	99.71 ± 0.06 a

Values are mean ± standard error (n = 3).
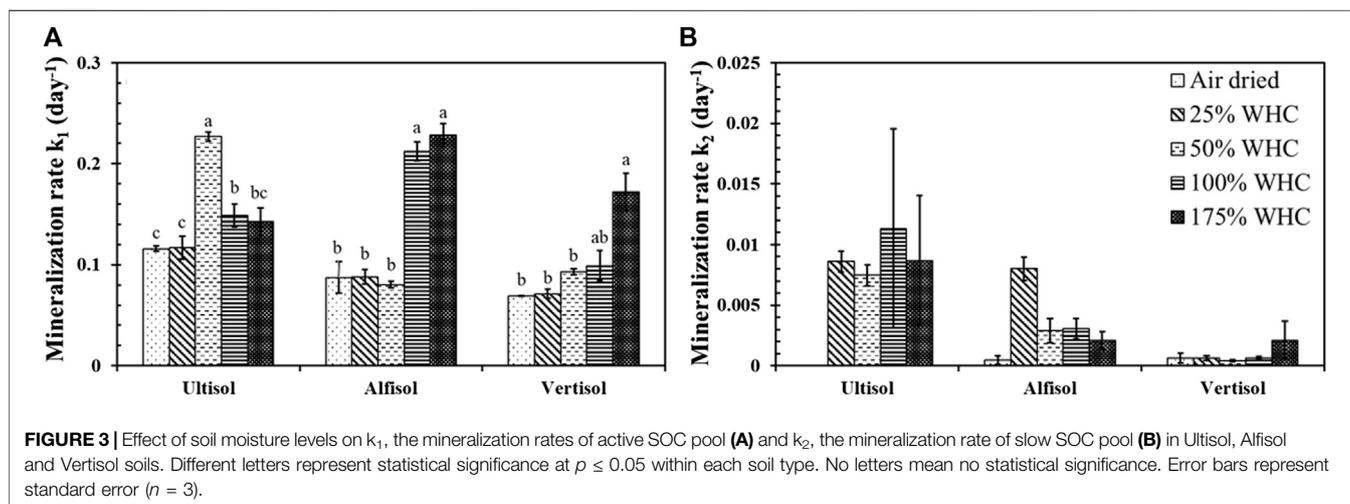[a]Letters represent statistical significance at p ≤ 0.05 within a column and soil type.

FIGURE 3 | Effect of soil moisture levels on k_1, the mineralization rates of active SOC pool **(A)** and k_2, the mineralization rate of slow SOC pool **(B)** in Ultisol, Alfisol and Vertisol soils. Different letters represent statistical significance at $p \leq 0.05$ within each soil type. No letters mean no statistical significance. Error bars represent standard error ($n = 3$).
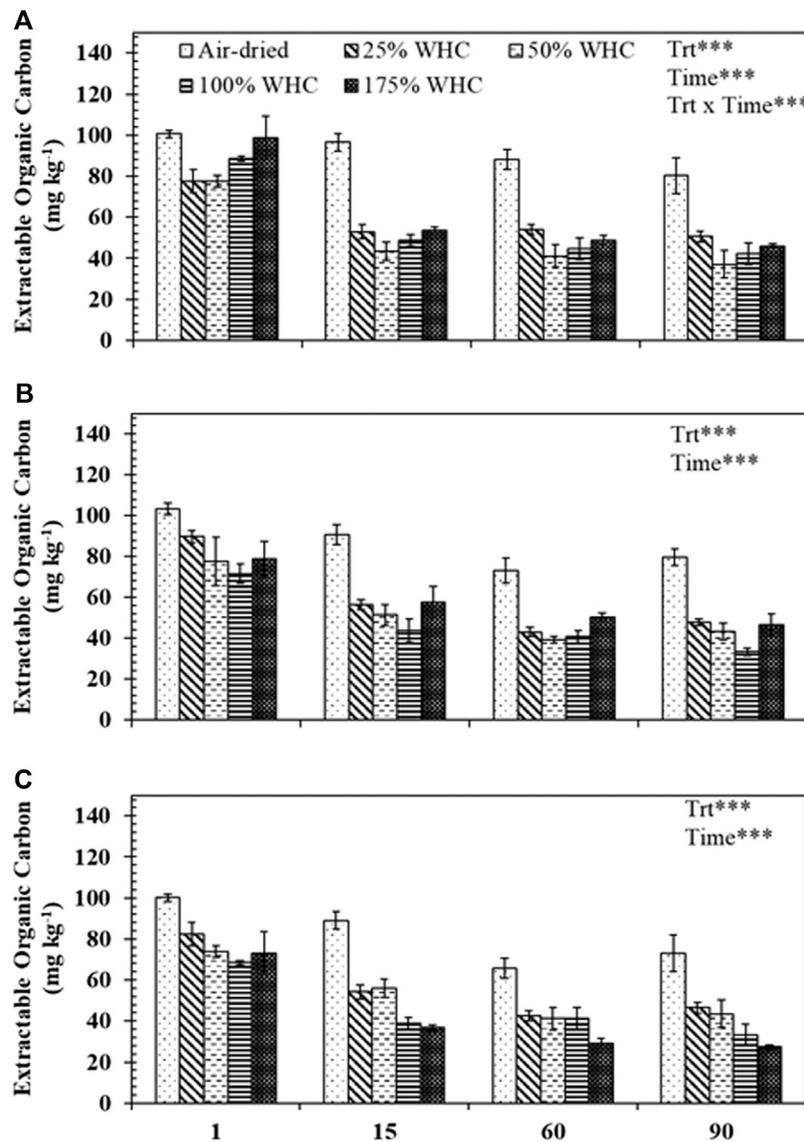
FIGURE 4 | Extractable organic carbon concentrations for different soil moisture treatments in **(A)** Ultisol, **(B)** Alfisol, and (c) Vertisol at each destructive sampling time. Error bars represent the standard errors ($n = 3$). *** denotes $p < 0.001$ for the effects of treatment (Trt), time, and treatment and time interaction (Trt x Time).

understanding that finer silt and clay sized particles tend to retain more SOC than coarser sandy particles (Hassink, 1997) due to enhanced physical and chemical protection. Nonetheless, the length of incubation is a key factor to determine the number of SOC pools with distinct turnover rates using kinetic modeling. Scharnagl et al. (2010) reported that long-term incubations with duration ranging from 600–900 days are best fit by triple-pool models (fast, intermediate, and slow SOC pools) unlike short-term incubations like ours which are best fit by double-pool models (Guntinas et al., 2013; Guo et al., 2014; Das et al., 2019).

The cumulative respiration and mineralization rate results were also supported by the EOC change across soil moistures (**Figure 4**). The EOC concentrations were the lowest at the moisture levels that resulted in the highest cumulative

respiration, suggesting the highest rate of microbial uptake of EOC (Zsolnay, 1996). No significant decrease in EOC concentrations was observed in soils on days 60 and 90 as compared to day 15 which can plausibly be explained by the addition of C by microbial turnover (Coleman and Jenkinson, 1996; Franzluebbers et al., 1999). In addition, we observed consistently higher EOC concentrations in air-dried soils compared to higher moisture levels, similar to the trend in the size of active SOC pool, again suggesting lower microbial mineralization of SOC due to moisture limitation (Schjønning et al., 2003; Or et al., 2007; Butcher et al., 2020).

In accordance with the general understanding that C acquiring extracellular enzymes are highly sensitive to changes in moisture (Steinweg et al., 2012; Zhou et al., 2013), we observed a strong
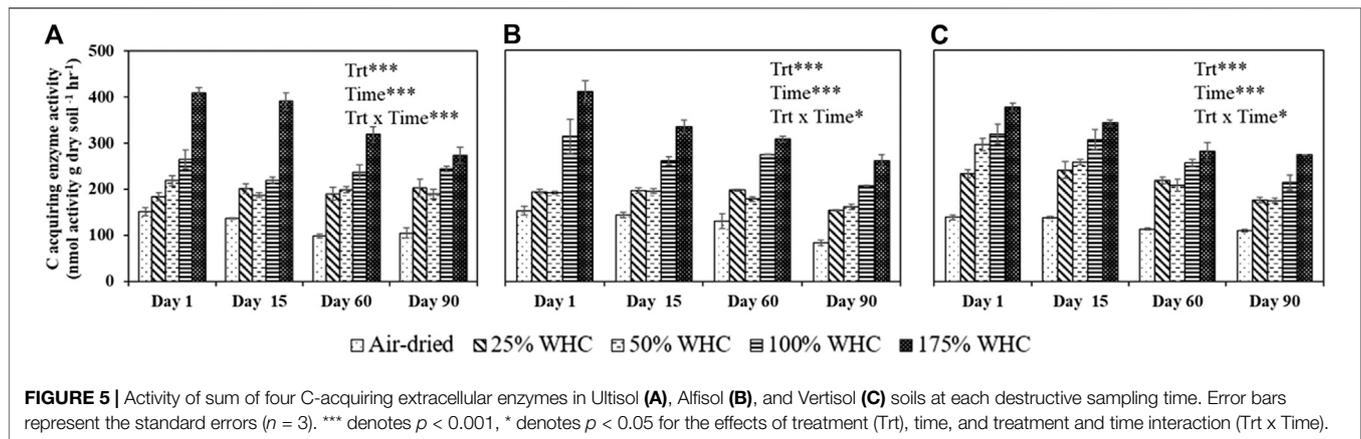
FIGURE 5 | Activity of sum of four C-acquiring extracellular enzymes in Ultisol **(A)**, Alfisol **(B)**, and Vertisol **(C)** soils at each destructive sampling time. Error bars represent the standard errors ($n = 3$). *** denotes $p < 0.001$, * denotes $p < 0.05$ for the effects of treatment (Trt), time, and treatment and time interaction (Trt x Time).

TABLE 5 | Linear regression statistics of moisture sensitivity of C acquiring extracellular enzyme assays.

Days	Soils	Slope	Intercept	R^2	p Value
1	Ultisol	2.6	158	0.99	0.0002
	Alfisol	2.7	149	0.96	0.0032
	Vertisol	2.1	179	0.83	0.0303
15	Ultisol	2.4	144	0.95	0.0043
	Alfisol	1.9	155	0.96	0.0038
	Vertisol	1.8	178	0.82	0.0342
60	Ultisol	1.9	144	0.85	0.0271
	Alfisol	1.7	152	0.84	0.0278
	Vertisol	1.4	153	0.73	0.0630
90	Ultisol	1.3	157	0.66	0.0923
	Alfisol	1.6	112	0.88	0.0182
	Vertisol	1.5	125	0.91	0.0116

Slopes and intercepts are not statistically significant.

positive effect of soil moisture on the total activity of C acquiring extracellular enzymes (BG, AG, CBH, and XYL) (**Table 4**). Unlike EOC and mineralization rates that followed the same trend as cumulative microbial respiration by showing sensitivity to different moisture optima for different soils, extracellular enzyme activity was decoupled from cumulative microbial respiration in alignment with the findings of Brangari et al. (2020). These laboratory assays were short, and therefore discerned the activity of extant enzymes pool rather than newly synthesized enzymes due to substrate addition (Steinweg et al., 2012). Enzyme activities displayed a monotonic increase with increasing moisture levels, especially in Ultisol and Alfisol soils. This trend was more visible during the early days of incubation but as the incubation progressed, the difference in enzyme activity among moist treatments reduced. For instance, for Ultisol, there was no significant difference in enzyme activity among all the moisture treatments except air-dried treatment on day 90. Nonetheless, the enzyme activity was the highest at 175% WHC regardless of the soil types and incubation time. The enzyme activity was the lowest in the air-dried soils for all three soils, and consistent with the cumulative microbial respiration results. This was probably due to decreased substrate and enzyme diffusion, reducing the direct enzyme-substrate interaction (German et al., 2011a; German et al., 2011b; Burns

et al., 2013; Ren et al., 2017) and/or increased adsorption of enzymes on soil particles (Kandeler, 1990; George et al., 2007) at lower moisture content. Also, under dry conditions, enzyme production and activity are lowered as the nutrient requirement for enzyme production exceeds the nutrient availability for microbes (Allison and Vitousek, 2005). The enzyme activities showed a consistent decrease across the length of incubation (**Figure 5**) probably due to substrate exhaustion (Acosta-Martinez et al., 2007) and lack of new production of enzymes.

Soil moisture did not influence microbial community composition determined by PLFA (**Supplementary Table S2**). Some past studies also reported no effect of soil moisture on microbial community composition (Griffiths et al., 2003; Buyer et al., 2010), while others reported the opposite (Williams, 2007; Kaisermann et al., 2015) depending on factors such as type of studies (laboratory vs field), duration of experiment, temperature, nutrient status, etc. The lack of influence of moisture on microbial community composition in our study is likely due to the short length of incubation. Nonetheless, changes in microbial community composition in laboratory microcosms may not be an accurate reflector of the activity of *in situ* microbial community. In the field, legacy field soil moisture content also contributes to shaping the microbial community (Schimel et al., 1999; Banerjee et al., 2016). The finding that microbial respiration is changing with soil moisture content, however, microbial community is not, probably implying microbial acclimation to varying moisture regimes (e.g., microbial dormancy under very low moisture). Overall, these results showed that short-term laboratory incubations at different soil moisture levels did not change microbial community composition, however, microbial activity was affected as indicated by changes in EOC, C acquiring enzyme activities and CO_2 fluxes.

CONCLUSION

Understanding the dependency of microbial respiration on soil moisture and soil texture is important for reducing the uncertainty in modeling SOC dynamics under changing climate. Results from this study showed that soil moisture effects on microbial respiration were strongly controlled by

soil texture (clay content). Consequently, different moisture optima were observed in Ultisol, Alfisol, and Vertisol soils for maximum microbial respiration, extractable organic C content, and SOC mineralization rates. Higher soil moisture supported higher enzyme activities and enhanced substrate availability, yet microbial respiration declined at higher soil moistures, but only for Ultisol and Alfisol soils. For Vertisol, respiration and enzyme activities were consistently increased with increase in soil moisture content. Our results indicate that soil moisture can potentially decouple from microbial activity in more coarsely textured soils, but such decoupling is less likely in finely textured soils.

DATA AVAILABILITY STATEMENT

The data will be made available by the authors upon request, without undue reservation.

AUTHOR CONTRIBUTIONS

SJ and SS designed the study. SS conducted the experiments, JD and PG trained SS on Phospholipid Fatty Acid analysis. SS and SK conducted data analysis. SS prepared the manuscript draft, SJ, MM, SK, JL, JW, GW, CS, JD, and PG reviewed and revised the manuscript draft, MM funded the research.

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SUPPLEMENTARY MATERIAL

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