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Microbial carbon use efficiency, biomass residence time and temperature sensitivity across ecosystems and soil depths

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ABSTRACT

Decomposition of soil organic matter by microorganisms is a fundamental mechanism driving the terrestrial carbon (C) cycle. Microbial C use efficiency (CUE), microbial biomass residence time (MRT), and soil C temperature sensitivity (Q_{10}) co-determine the fate of soil C in a changing climate. In order to reveal the effect of soil depth and varying hydrologic properties on CUE, MRT, and Q_{10} of microbial respiration, we incubated soils from three ecosystems (wetland, grassland, and forest) and soil depths (0–10, 20–30, and 50–60 cm) at two temperatures (10 and 30 °C). Microbial CUE was estimated using a substrate-independent method by incorporating ^{18}O from labeled water into microbial DNA with the simultaneous measurement of microbial respiration. CUE ranged from about 0.2 to 0.7 with a mean value of 0.5 ± 0.1, MRT ranged from 4 to 73 days with a mean value of 26 ± 19 days, and Q_{10} ranged from 1.8 to 2.9, averaging 2.3 ± 0.3 across all samples. We found that CUE increased but MRT and Q_{10} decreased along the wetland-grassland-forest hydrologic gradient; and they all increased with soil depth. Moreover, CUE and MRT were lower at 30 °C than that at 10 °C. Although there were some differences in factors regulating the variation in CUE, MRT or Q_{10} among soil depths and ecosystem types, both within individual ecosystems and depths, CUE, MRT, and Q_{10} were strongly correlated to available C:N ratios, clay content, and C quality, respectively. In conclusion, our findings emphasize the importance of stoichiometry and C quality of available substrates in predicting the variation in microbial C use efficiency and soil C temperature sensitivity in different soil depths and along a hydrologic gradient.

1. Introduction

Decomposition of soil organic matter is primarily regulated by microbial communities (Allison et al., 2010), and represents one of the most uncertain pathways in the prediction of global carbon (C) cycle and future climate (Bradford et al., 2016). Microbial C use efficiency (CUE), which is defined as the ratio of microbial growth over C uptake (Geyer et al., 2019; Qiao et al., 2019), determines the degree by which soil C is incorporated into microbial biomass promoting C sequestration, or released into the atmosphere as CO_2 (Fisk et al., 2015; Ye et al., 2019). CUE is thus a vital concept for understanding and modelling soil C storage and cycling (Sinsabaugh et al., 2013; Hararuk et al., 2015; Li et al., 2019b).

Modelling and empirical studies have demonstrated that C substrate availability, quality, and stoichiometry are primary factors regulating CUE (*e.g.*, Manzoni et al., 2012; Frey et al., 2013; Sinsabaugh et al., 2013; Mooshammer et al., 2014; Takriti et al., 2018; Qiao et al., 2019; Soares and Rousk, 2019). Usually, because low substrate availability would result in C-investment into additional enzymes to liberate limiting substrates, C substrate acquisition of soil microbes becomes more C-expensive, leading to lower CUE (Spohn et al., 2016). Organic compounds with low C substrate quality tend to be complex, with numerous types of bonds and few repeating structures, thus requiring multiple enzymatic steps for breakdown (Bosatta and Ågren, 1999). Incorporation of C from low quality compounds, which have low energy content or complex bond architecture, into microbial biomass will increase microbial investment in resource acquisition (Allison, 2014; Öquist et al., 2017); in contrast, high quality C substrates are more readily incorporated into microbial biomass and may yield a higher CUE value. Furthermore, microbial growth promotes CUE, while energy

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production reduces CUE, potentially resulting in different values of CUE (Jones et al., 2018). Finally, CUE is also expected to be greatly influenced by C:N ratios according to ecological stoichiometric theory and empirical evidence (*e.g.*, Manzoni et al., 2012; Sinsabaugh et al., 2013; Takriti et al., 2018) due to the imbalance of C:N ratios between microbial biomass and substrate (Mooshammer et al., 2014). Higher substrate C:N ratio is usually linked to lower CUE as microorganisms need to maintain their homeostatic C:N ratios through overflow respiration (Manzoni et al., 2012; Takriti et al., 2018). Despite this understanding, gradient studies have reported different trends in CUE with soil depth (Spohn et al., 2016; Takriti et al., 2018). It is reasonable to suppose, therefore, that other factors may also be important in regulating microbial CUE.

Subsoils play crucial roles in global C cycling as approximately half of the global soil organic C (SOC) was found in subsoil horizons (Jobbágy and Jackson, 2000). Soil C substrate availability and quality commonly decrease with soil depth, and therefore CUE may decrease with depth as discussed above; however, with few exceptions, C:N ratio decreases with soil depth (Rumpel and Kögel-Knabner, 2011), and so stoichiometric regulation is expected to yield a higher CUE in deeper soils. Changing soil depths also results in changes in fungal-to-bacterial ratios that exhibit crucial controls on CUE (Soares and Rousk, 2019). Thus far, the controls of CUE and whether CUE increases or decreases with depth are still under discussion (Spohn et al., 2016). In addition, since the most of SOC has been microbially processed, insights into the turnover of microbial biomass would help gain a better understanding of soil C cycling (Malik et al., 2013). Although a substantial number of studies have assessed CUE in a single ecosystem or along transects of upland ecosystems (*e.g.*, forest and grassland) (Takriti et al., 2018; Zheng et al., 2019), there is a paucity of studies that have studied CUE and microbial biomass residence time (MRT) as well as their controls simultaneously among ecosystems which span a wide hydrologic gradient (*e.g.*, from wetlands to uplands). This is an important knowledge gap because climate warming is expected to strongly influence land-cover changes along hydrological gradients, significantly affecting soil C cycling (Moomaw et al., 2018).

In addition, microbial C decomposition rates increase exponentially within a certain range of temperatures, representing important C-climate feedbacks in C cycle models (Davidson and Janssens, 2006). While temperature sensitivity of microbial respiration (Q_{10}) has been widely studied for a few decades, most previous studies focused on topsoil (*e.g.*, Fierer et al., 2006; Li et al., 2020b). Deep soil Q_{10} is considered crucial in C cycle models, and thus has drawn increasing interest recently (*e.g.*, Hicks Pries et al., 2017; Li et al., 2018; Li et al., 2020d). However, empirical studies investigating Q_{10} and its response to soil depth along hydrological gradients are largely lacking (Li et al., 2019a). In particular, as far as we know, no study has been conducted simultaneously comparing CUE and Q_{10} as well as their controls with soil depth along a wetland to upland gradient. This knowledge is important because differences in microbial CUE and Q_{10} among ecosystems and soil depths represent important uncertainties in C cycle models.

The primary objective of the present study was to assess changes in CUE, MRT, and Q_{10} as well as their controls with soil depth and along a wetland to upland gradient. We hypothesized that (i) CUE increases along the wetland-grassland-forest gradient but decreases with soil depth as CUE is usually lower under stress conditions (*e.g.*, low substrate accessibility in subsoil and wetland soil) due to the energy cost for repair and defense mechanisms (Rath et al., 2016), (ii) MRT decreases along the wetland-grassland-forest gradient but increases with soil depth as the turnover of microbial biomass-C generally slows with increases in clay (Sakamoto and Hodono, 2000), and (iii) Q_{10} decreases along the wetland-grassland-forest gradient but increases with soil depth as decomposition of low quality C should have higher temperature sensitivities (Davidson and Janssens, 2006).

To test these hypotheses, we incubated soils from three ecosystems (wetland, grassland, and forest) and soil depths (0–10, 20–30, and 50–60 cm) at two temperatures (10 and 30 °C). Microbial CUE values were determined using a substrate-independent method by incorporating ^{18}O from labeled water into microbial DNA with the simultaneous measurement of microbial respiration (Spohn et al., 2016). Compared to the widely used ^{13}C-substrate labeling method, which determines microbial substrate use efficiency rather than CUE (Sinsabaugh et al., 2013), the ^{18}O-incorporation method can directly measure actual microbial growth. To reveal factors controlling microbial CUE and Q_{10}, we considered soil clay content, substrate availability (indicated by ratios of enzyme activities to microbial biomass C (MBC) and MBC to SOC), quality (indicated by hydrolytic to oxidative enzyme activity and SOC decomposability), and stoichiometry (indicated by C:N ratios of soil, available, and microbe).

2. Materials and methods

2.1. Soil sampling

Soil samples were taken within the Cumberland Plain woodland in Richmond, NSW, Australia (33°37′S, 150°44′E). The study site is characterized by a temperate climate with a mean annual temperature of 17.7 °C (mean minimum and maximum temperature of 11.1 °C and 24.3 °C, respectively) and a mean annual precipitation of 800 mm (mean monthly minimum and maximum in September and January, respectively) (Griebel et al., 2020). The rainfall in 2018 (396 mm) was only half the long-term average. We selected a hydrological gradient of wetland-grassland-forest, along which our previous study showed significant differences in soil physicochemical properties (Li et al., 2019a).

Soils were sampled in January 2019, near the time of peak summer temperatures. In each ecosystem, three random sites/replicates were chosen to represent the vegetation communities for each ecosystem, located 100–300 m apart from each other. Samples, in areas that were representative of aboveground vegetation distribution for each ecosystem, were collected from three discontinuous mineral soil depths (*i.e.*, 0–10, 20–30, and 50–60 cm) to alleviate the dependency of topsoil and subsoil samples. The 0–10 cm layer in the wetlands was an O-horizon, but detailed classification was not conducted for this study. Soil samples were sieved (<2 mm) to remove stones and plant materials, and roots were removed; subsamples thus represented the average microbial conditions throughout each soil depth. About 50 g soil was air-dried for basic properties, and the rest was stored at 4 °C for incubation experiments.

2.2. Soil physical and chemical analyses

Soil texture was measured using the hydrometer method (Beretta et al., 2014). Soil pH, in a 1:2.5 soil and water suspension, was determined with a pH electrode (Mettler Toledo SevenEasy S20). Total C (TC) and total nitrogen (TN) was determined using a combustion analyzer (LECO Corporation TruMac Series, USA). Because these were acidic soils, we assumed SOC was equal to TC. Dissolved organic C (DOC) and nitrogen (DON) were extracted with 0.5 M K_2SO_4 and then determined using a TOC analyzer (TOC-L series, Shimadzu Corporation). Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were measured colorimetrically following the extraction with 1 M potassium chloride (Searle, 1984). Available N concentration was the sum of DON, NH_4^+, and NO_3^-; available C:N was indicated by the ratio of DOC to available N.

2.3. Incubation experiment

Soils (60 g, dry weight) were placed in 100 ml plastic cups and were adjusted to 60% water holding capacity. All soil samples were pre-incubated at 10 and 30 °C for two weeks to activate soil microbes and establish a metabolic equilibrium (Cao et al., 2019). Water loss was regularly checked and corrected for a weight basis. Soil samples were

then separated into five different subsets. For the first set of subsamples, 15 g soil was transferred into a 60 ml plastic container and put in a 500 mL glass jar for microbial respiration and Q_{10} analyses, and then afterwards for enzyme activity analyses. For the second and third set of subsamples, 10 g soil was transferred into two sets of 50 ml plastic tubes for MBC and DOC analyses. For the fourth and fifth set of subsamples, 0.3 g of soil was transferred into two 2 ml screw cap vials for CUE measurement. Before doing all the following measurements, all the sets of subsamples were incubated at the same temperature of pre-incubation for another 24 h to minimize effects of disturbance.

2.3.1. Microbial CUE

Microbial CUE was determined by incorporating ^{18}O into microbial DNA (Spohn et al., 2016; Mehnaz et al., 2019). Firstly, one-set of vials was labeled with 12.5 μl of 99 atom% ^{18}O water to reach an enrichment of 20 atom% in the final soil water content, and the other set of control vials received non-labeled Millipore water in the same amount. Secondly, the vials with open screw cap were immediately transferred into 60 ml capped cups, and were incubated for 48 h at the same temperature of pre-incubation. Thirdly, the vials were closed after 48 h of incubation, and then stored at −80 °C until DNA extraction.

DNA in the non-labeled and ^{18}O-labeled soils was extracted and quantified using a DNA extraction kit (FastDNA™ SPIN Kit for Soil, MP Biomedicals) following Mehnaz et al. (2019). Briefly, the oxygen in the DNA and ^{18}O enrichment were measured using a high temperature conversion elemental analyzer (TC/EA, PyroCube (Elementar Analysensysteme GmbH, Hanau, Germany)) coupled to an isotope ratio mass spectrometer (Isoprime VisION (Isoprime Ltd., Stockport, UK)). Microbial CUE and the residence time of microbial biomass were then calculated (Spohn et al., 2016).

$$CUE = \frac{C_{growth}}{C_{growth} + C_{respiration}} \quad (1)$$

$$Residence\ time = \frac{MBC}{C_{growth}} \quad (2)$$

where CUE is microbial C use efficiency, C_{growth} is the C allocated to microbial biomass production, $C_{respiration}$ is the C allocated to CO_2 production, and MBC is microbial biomass C. The total DNA production during the incubation was transformed into the production of MBC by using a linear regression between the concentrations of DNA and MBC ($R^2 = 0.94$, $P < 0.001$; Fig. S1). C_{growth} was then calculated by dividing the total MBC production by the duration of incubation.

2.3.2. Microbial respiration and microbial biomass C and N analyses

For the measurement of CO_2 production ($C_{respiration}$) and temperature sensitivity of microbial respiration (first set of subsamples), six blank jars were randomly distributed among those with soil samples to determine background CO_2 concentrations. 24 ml headspace gas samples were respectively collected before and after closure for 24 h at 10 °C and 6 h at 30 °C. CO_2 concentrations in gas samples were analyzed using a gas chromatograph (GC, Agilent 7890A; Agilent Corp., USA). Respiration rate was calculated on the basis of the amount of soil, the change of gas concentration over time, the headspace volume, and incubation temperature (Li et al., 2019a). The temperature sensitivity coefficient, Q_{10}, was calculated as follows:

$$Q_{10} = \left(\frac{R_{30}}{R_{10}}\right)^{\frac{10}{T_{30} - T_{10}}} \quad (3)$$

where R_{30} and R_{10} are microbial respiration rates at 30 °C (T_{30}) and 10 °C (T_{10}), respectively.

During the same 48 h period when soils were incubated with labeled ^{18}O, soil microbial C and N were measured using the fumigation-extraction method (Vance et al., 1987). Total C and N concentrations were extracted with 0.05 M K_2SO_4 (Mehnaz et al., 2019) and determined using a TOC-VCPN analyzer (Shimadzu Scientific Instruments (Oceania) Pty. Ltd.).

2.3.3. Potential enzyme activities

After microbial respiration measurement, soil samples were used for analyses of both hydrolytic and oxidative enzyme activities. Potential activities of five hydrolytic enzymes including β-glucosidase (BG), α-glucosidase (AG), β-D-cellubiosidase (CB), leucine amino peptidase (LAP), and N-acetyl-β-glucosaminidase (NAG) were measured. These hydrolytic enzyme activities were determined in 96-well microplates following a high-throughput fluorometric measurement protocol (Bell et al., 2013). Fluorescence of soil supernatants was detected at an excitation wavelength 365 nm and emission wavelength 450 nm with a POLARstar OPTIMA Multidetection Microplate Reader (BMG LABTECH, Germany). Moreover, we measured potential activities of two oxidative enzymes including phenol oxidase (POX) and peroxidase (PER) following Steinweg et al. (2013). These were performed in 96-well microplates, which were read spectrophotometrically at 460 nm using the same plate reader. We then calculated the ratio of ln(AG + BG + CB + NAG + LAP) over ln(POX + PER) (in short, hydrolytic:oxidative). The ratio of hydrolytic to oxidative enzyme activities has been used to indicate the relative availability of chemically complex, and a higher hydrolytic:oxidative enzyme ratio indicates higher substrate quality (Hill et al., 2014; Takriti et al., 2018).

2.4. Statistical analysis

One-way ANOVA was performed to reveal the differences of CUE, MRT, Q_{10}, and soil physicochemical properties among ecosystem types or soil depths. Two-way ANOVA was performed to test the effects of soil depth, ecosystem type, and their interactive effects on Q_{10} and physicochemical properties. Three-way ANOVA was performed to test the effects of ecosystem type, soil depth, incubation temperature, and their interactive effects on CUE and MRT. Data were log-transformed when necessary to reduce heteroscedasticity and to improve normal distribution. Partial correlation analysis was performed to reveal factors regulating CUE, MRT or Q_{10} when controlling all the other variables that were significantly correlated to the target factor. For example, when testing the relationship between CUE and clay, all the other factors that were significantly correlated to clay were controlled. All statistical analyses were performed using SPSS Statistics 22 (IBM) or in R statistical software (version 3.4.2).

3. Results

Ecosystem type and soil depth had significant effects on most soil physicochemical properties except for pH ($P < 0.001$, Table S1). Overall, soil clay content was higher in wetland than in upland (i.e., grassland and forest ecosystems), and it increased with soil depth (Table 1). Soil substrates (*e.g.*, SOC and TN contents) were higher in wetland than in upland, while they decreased with soil depth (Table 1). Soil C:N ratio was higher in upland than in wetland, while a higher microbial C:N ratio was found in wetland; both soil and microbial C:N ratios decreased with soil depth (Table 1).

Microbial growth and C uptake (the sum of growth and respiration) had no significant differences among ecosystems, while they decreased with soil depth (Fig. 1). CUE and MRT varied significantly among ecosystems and soil depths (Fig. 2; $P < 0.001$, Table 2). Positive relationship between CUE and MRT was observed, while they were negatively correlated to microbial growth or C uptake (Fig. S2).

Q_{10} of microbial respiration also varied significantly among ecosystems and soil depths (Fig. 3; $P < 0.001$, Table 2). Positive relationships between CUE and Q_{10} were found within each ecosystem type, while negative relationships were found within each soil depth (Fig. S3). Three-way ANOVA showed that ecosystem, soil depth, and incubation temperature all significantly affected CUE and MRT (Table 2). CUE was

Table 1
Soil physical and chemical properties among ecosystems and soil depths.

	Sand (%)	Clay (%)	pH	SOC (g kg^{-1})	TN (g kg^{-1})	Soil C:N	Microbial C:N	NH_4^+ (mg kg^{-1})	NO_3^-(mg kg^{-1})
Wetland									
0–10 cm	35.1 ± 4.5^{bA}	44.6 ± 1.5^{aB}	4.9 ± 0.2^{aA}	71.2 ± 8.2^{bB}	6.6 ± 0.8^{bB}	10.8 ± 0.2^{bA}	15.4 ± 1.7^{bB}	4.6 ± 1.3^{bB}	77.2 ± 17.4^{bB}
20–30 cm	15.0 ± 1.7^{aA}	61.0 ± 7.2^{bB}	5.2 ± 0.2^{abA}	21.3 ± 7.3^{aB}	2.9 ± 1.4^{aB}	7.9 ± 1.7^{aA}	13.8 ± 0.7^{abB}	2.0 ± 0.6^{aB}	15.2 ± 5.9^{aB}
50–60 cm	8.9 ± 2.5^{aA}	69.9 ± 4.4^{bB}	5.5 ± 0.2^{bA}	12.2 ± 3.1^{aB}	1.7 ± 0.7^{aB}	7.5 ± 1.8^{aA}	10.8 ± 2.0^{aA}	1.1 ± 0.5^{aB}	9.7 ± 2.5^{aB}
Grassland									
0–10 cm	45.0 ± 7.1^{bB}	30.6 ± 4.2^{aA}	5.0 ± 0.2^{aA}	30.4 ± 2.9^{bA}	2.7 ± 0.4^{bA}	11.6 ± 1.3^{aAB}	10.9 ± 0.3^{bA}	1.8 ± 0.6^{bA}	9.9 ± 4.6^{bA}
20–30 cm	10.1 ± 1.7^{aA}	53.8 ± 1.9^{bB}	5.3 ± 0.1^{bA}	6.6 ± 1.3^{aA}	0.7 ± 0.2^{aA}	10.2 ± 2.2^{aAB}	9.1 ± 0.8^{aA}	0.6 ± 0.2^{aA}	3.2 ± 0.9^{aA}
50–60 cm	6.8 ± 2.2^{aA}	69.2 ± 6.6^{cB}	5.5 ± 0.1^{bA}	2.9 ± 1.2^{aA}	0.4 ± 0.2^{aA}	8.6 ± 2.6^{aA}	8.5 ± 0.9^{aA}	0.3 ± 0.1^{aA}	1.8 ± 0.9^{aA}
Forest									
0–10 cm	45.6 ± 7.0^{aB}	23.3 ± 5.4^{aA}	5.1 ± 0.7^{aA}	17.0 ± 11.3^{bA}	1.3 ± 1.0^{bA}	13.4 ± 1.0^{bB}	11.5 ± 0.8^{bA}	0.6 ± 0.5^{bA}	0.5 ± 0.6^{bA}
20–30 cm	50.9 ± 4.8^{aB}	27.4 ± 4.1^{aA}	5.2 ± 0.6^{aA}	6.5 ± 4.5^{aA}	0.6 ± 0.4^{aA}	11.5 ± 1.7^{abAB}	10.9 ± 1.6^{bA}	0.3 ± 0.2^{aA}	0.2 ± 0.1^{aA}
50–60 cm	50.8 ± 5.0^{aB}	32.8 ± 6.1^{bA}	5.5 ± 0.7^{aA}	3.3 ± 2.6^{aA}	0.3 ± 0.2^{aA}	9.4 ± 0.9^{aA}	9.7 ± 1.7^{aA}	0.2 ± 0.2^{aA}	0.1 ± 0.1^{aA}

SOC, total carbon; TN, total nitrogen; C:N, carbon to nitrogen ratio; NH_4^+, ammonium; NO_3^-, nitrate.
Different lower-case letters indicate significant difference among soil depths within each ecosystem, while different upper-case letters indicate significant difference among ecosystem types within each depth at $P < 0.05$.

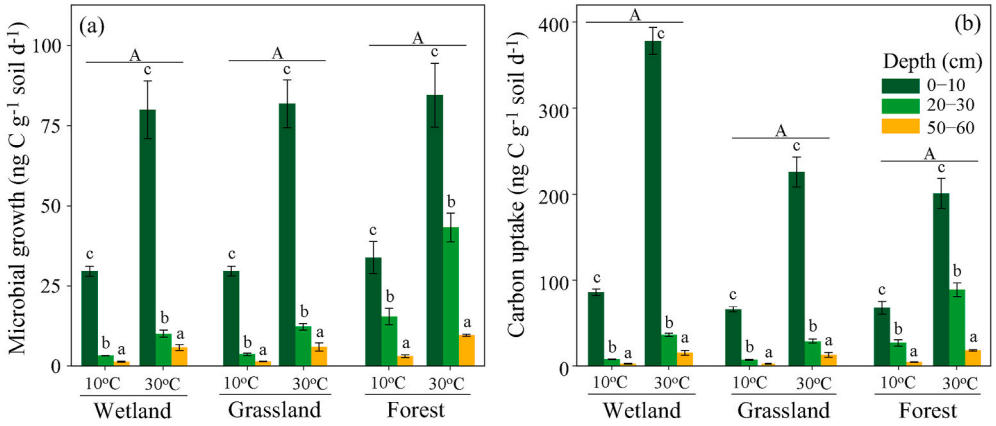

Fig. 1. Differences of soil microbial growth (a) and carbon uptake (b) among ecosystems, soil depths, and temperatures. Error bars indicate standard errors. Different lower-case letters indicate significant differences among soil depths within each ecosystem and temperature, while different upper-case letters indicate significant differences among ecosystem types ($P < 0.05$).

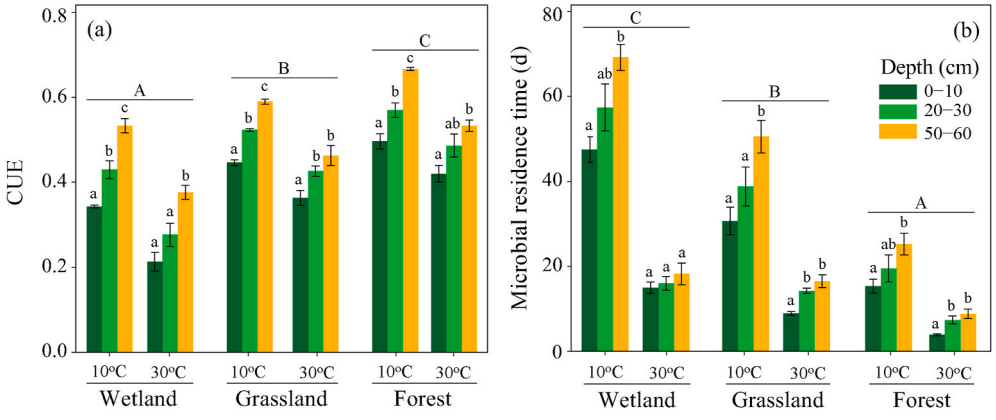

Fig. 2. Differences of soil microbial carbon use efficiency (CUE; a) and microbial biomass residence time (b) among ecosystems, soil depths, and temperatures. Error bars indicate standard errors. Different lower-case letters indicate significant differences among soil depths within each ecosystem and temperature, while different upper-case letters indicate significant differences among ecosystem types ($P < 0.05$).

highest in forest and lowest in wetland, while the opposite pattern was found for MRT; they both increased with soil depth; higher CUE and MRT were found at 10 °C than at 30 °C ($P < 0.01$, Fig. 2). Two-way ANOVA showed that Q_{10} differed significantly among ecosystems and soil depths; Q_{10} decreased across the wetland-grassland-forest gradient but increased with soil depth ($P < 0.01$, Fig. 3), and no significant interactive effect was found (Table 2).

Partial correlation analyses showed that, within each ecosystem type (across soil depths), CUE was positively correlated to available C:N, while negatively correlated to hydrolytic:oxidative ratio of enzyme activities; MRT was positively correlated to clay content; Q_{10} was positively correlated to clay content but negatively correlated to hydrolytic: oxidative and SOC decomposability (D_{soc}, indicated by SOC decomposition rate per unit SOC content) (Table 3). Within each soil depth

Table 2
ANOVA analyses of Q_{10}, CUE, and MRT for the effect of ecosystem, soil depth, incubation temperature, and their interactions.

		df	SS	MS	F	P
Q_{10}	Ecosystem	2	0.928	0.464	25.855	<0.001
	Depth	2	1.409	0.704	39.246	<0.001
	Ecosystem × Depth	4	0.010	0.002	0.133	0.968
CUE	Ecosystem	2	0.257	0.128	145.513	<0.001
	Depth	2	0.194	0.097	109.834	<0.001
	Temperature	1	0.180	0.18	204.504	<0.001
	Ecosystem × Depth	4	0.006	0.001	1.607	0.194
	Ecosystem × Temperature	2	0.007	0.003	3.731	<0.05
	Depth × Temperature	2	0.004	0.002	2.351	0.110
	Ecosystem × Depth × Temperature	4	0.001	0.000	0.239	0.914
MRT	Ecosystem	2	5140.6	25701.3	119.3	<0.001
	Depth	2	1130.9	565.5	26.2	<0.001
	Temperature	1	10026.5	10026.5	465.2	<0.001
	Ecosystem × Depth	4	68.52	17.1	0.795	0.536
	Ecosystem × Temperature	2	1808.7	904.4	42.0	<0.001
	Depth × Temperature	2	328.6	164.3	7.6	<0.01
	Ecosystem × Depth × Temperature	4	73.3	18.3	0.9	0.503

Q_{10}, temperature sensitivity of microbial respiration; CUE, carbon use efficiency; MRT, microbial residence time; SS, sum of squares; MS, mean square.

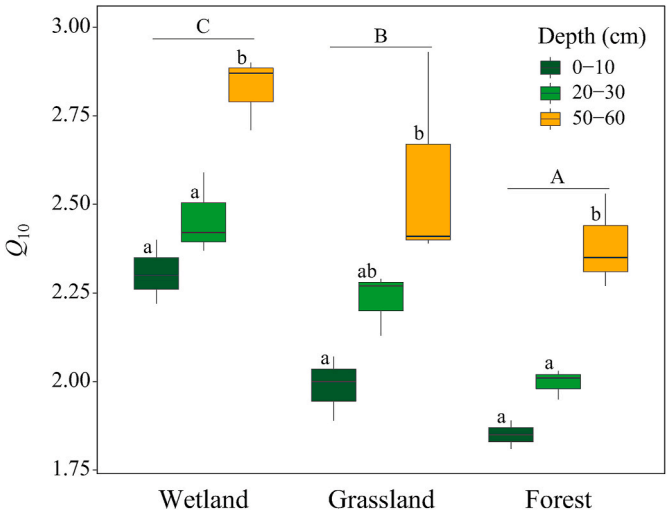

Fig. 3. Box plots of the temperature sensitivity of microbial respiration (Q_{10}) among ecosystems and soil depths. Lines within the boxes indicate medians, and upper and lower edges of the boxes represent the 25th and 75th percentiles. The top bar shows the maximum value and the bottom bar the minimum value. Different lower-case letters indicate significant differences among soil depths within each ecosystem, while different upper-case letters indicate significant differences among ecosystem types ($P < 0.05$).

(across ecosystem types), CUE was negatively correlated to clay content but positively correlated to available C:N ratio; MRT was positively correlated to clay content; Q_{10} was only negatively correlated to hydrolytic:oxidative (Table 3). Altogether, available C:N ratio was the most consistent predictor among all variables examined for CUE within individual ecosystem types and soil depths; clay was the most consistent predictor of MRT; for Q_{10}, only C quality was the most consistent predictor.

4. Discussion

Inconsistent with our hypothesis and some recent studies (*e.g.*, Spohn et al., 2016; Takriti et al., 2018), microbial CUE increased with soil depth; consistent with our hypothesis, MRT and Q_{10} increased with depth. These results demonstrate that subsoil C sequestration potential will depend on the competing effects of greater CUE and MRT leading to higher retention of C as microbial byproducts, *versus* greater temperature sensitivity that will lead to greater CO_2 losses from decomposition in coming decades. Moreover, CUE increased but Q_{10} decreased along the wetland-grassland-forest gradient, demonstrating that land-cover changes from wetland to upland may increase the potential of soil C sequestration and decrease its response to rising temperature.

Available C:N ratio, indicated by the ratio of DOC to the sum of dissolved organic and inorganic N, but not C:N ratios of soil and microbes, was important in predicting CUE (Table 3). According to the ecological stoichiometric theory supported by many studies, microbial substrate C:N ratio is expected to significantly affect CUE (*e.g.*, Manzoni et al., 2012; Mooshammer et al., 2014). However, in environmental samples, it has been reported that soil C:N ratio is not always a good predictor of CUE (Sinsabaugh et al., 2016), such as for the wide ranges of soil C:N ratios across ecosystem types or soil depths in the present study. A likely explanation is that soil C:N ratios may not represent bioavailable N since physiochemical protection can inhibit microbial decomposition of N present in organic matter (Kaiser et al., 2014). Instead, available C:N ratios reflecting bioavailability of N for microbes played the most important role in regulating microbial CUE. Indeed, CUE was positively correlated to available C:N ratios across both ecosystem types and soil depths (Table 3). These results together demonstrate that linkages between stoichiometry and microbial CUE are through effects of available C:N ratio but not C:N ratios of soil and microbes.

Soil microorganisms acquire soluble substrates for assimilation from decomposing organic substances through producing extracellular enzymes, which is strongly linked to substrate availability or quality (Loeppmann et al., 2016). Here, C quality only explained changes in CUE across soil depths (within each ecosystem), suggesting that C substrate quality might not be a good predictor for the observed variation in CUE among ecosystems. Moreover, it is generally expected that microbes decomposing low-quality compounds require more energy, leading to a lower CUE (Manzoni et al., 2012). However, in contrast to this theory, we found that the decomposition of low-quality substrate in deeper soils resulted in a higher CUE, as indicated by the negative relationship between CUE and hydrolytic:oxidative in each ecosystem (Table 3). To the best of our knowledge, the mechanisms behind this relationship are not clear, and this might be also related to differences in microbial communities at different soil depths. Directly manipulating only substrate quality or availability while controlling other variables could lead to further mechanistic insights. These results together raise caution in the use of proxies (Bailey et al., 2018) such as substrate quality to predict microbial CUE under all environmental conditions (*e.g.*, different ecosystems and soil depths).

A strong negative relationship between CUE and clay at each soil depth across the wetland to upland gradient was observed (Table 3). A plausible explanation is that clay content strongly affects substrate diffusivity and accessibility for microbes, and thus regulates microbial CUE. On the one hand, higher clay content may limit substrate diffusivity because of substrate stabilization through interactions with clay mineral surfaces (Krull et al., 2003; Li et al., 2020a). Higher clay content is usually associated with higher soil aggregation (Zeng et al., 2018), on the other hand, it can also physically protect substrate (Rabbi et al., 2016; Li et al., 2020c), and thus reduce substrate accessibility for soil biota. CUE is usually lower under "stress" conditions (*e.g.*, low substrate accessibility) due to the energy cost for repair and defense mechanisms (Rath et al., 2016) or because low substrate availability decreases the probability that an enzyme finds its substrate (Spohn et al., 2016). Thus, it is not surprising that a higher CUE was found in coarse textured forest soil than in fine textured wetland soil. In addition, strong positive relationships between MRT and clay were observed across both soil depths and ecosystem types (Table 3), suggesting greater clay mineral surfaces would lead to stronger retention of microbial C. These results may partly

Table 3
Pearson correlation coefficients of partial correlation analysis for CUE, MRT, Q_{10}, and soil parameters.

		Clay	Enzyme: MBC	MBC: SOC	Soil C:N	Available C:N	Microbial C:N	Hydrolytic: oxidative	D_{soc}
Across soil depths within each ecosystem									
CUE	Wetland	0.599	0.056	0.552	0.797	0.973*	−0.490	−0.825*	−0.764
	Grassland	0.139	0.549	0.743*	0.229	0.947*	0.010	−0.795*	−0.668
	Forest	0.335	0.296	0.788*	0.649	0. 921*	−0.446	−0.753*	−0.664
MRT	Wetland	0.891*	0.638	0.621	0.946	0.429	0.291	−0.424	0.899*
	Grassland	0.873*	0.384	0.534	0.465	0.453	0.188	0.899	0.209
	Forest	0.932*	0.242	0.339	−0.231	0.047	−0.456	−0.343	−0.217
Q_{10}	Wetland	0.967*	−0.975	0.883	0.980*	0.562	−0.579	−0.927*	−0.890*
	Grassland	0.924*	0.065	0.196	−0.482	0.842*	0.648	−0.976*	−0.883*
	Forest	0.901*	0.459	0.495	−0.454	−0.287	−0.599	−0.933*	−0.948*
Across ecosystem types within each soil depth									
CUE	0–10 cm	−0.902*	0.426	−0.607	0.758	0.974*	−0.355	0.357	−0.875*
	20–30 cm	−0.924*	−0.402	0.577	0.482	0.892*	−0.582	0.264	−0.253
	50–60 cm	−0.890*	0.339	0.304	0.392	0.918*	−0.345	−0.209	−0.344
MRT	0-10 cm	0.935*	−0.713*	−0.006	−0.414	−0.400	0.336	−0.300	0.691
	20-30 cm	0.907*	0.575	−0.692	−0.335	0.471	0.547	−0.020	0.490
	50-60 cm	0.866*	0.149	−0.409	−0.292	−0.371	0.150	−0.035	0.393
Q_{10}	0–10 cm	0.563	−0.367	−0.029	−0.694	−0.474	0.251	−0.875*	−0.812*
	20–30 cm	0.290	0.175	−0.359	−0.095	−0.093	0.522	−0.851*	0.205
	50–60 cm	0.353	0.243	−0.654	−0.394	−0.063	0.196	−0.847*	0.229

Q_{10} and CUE are estimated at 10 °C. Q_{10}, temperature sensitivity of microbial respiration; CUE, carbon use efficiency; MRT, microbial residence time; MBC, microbial biomass carbon; C:N, carbon to nitrogen ratio; available C:N, the ratio of dissolved organic carbon to the sum of ammonium, nitrate, and dissolved organic nitrogen; hydrolytic:oxidative, ratio of hydrolytic to oxidative enzyme activity; D_{soc}, SOC decomposability, indicated by SOC decomposition rate per unit SOC content. N = 9 for each correlation analysis. *$P < 0.05$.

explain the usually observed positive relationship between clay and SOC content. Altogether, these findings emphasize the importance of clay minerals in soil C sequestration potential.

Previous studies have demonstrated contradictory results for the effect of temperature on CUE, with some studies showing a decreased CUE with increased temperature (*e.g.*, Steinweg et al., 2008; Frey et al., 2013) while others have shown a minor influence of temperature on CUE (*e.g.*, Dijkstra et al., 2011; Öquist et al., 2017). Along the wetland to upland gradient and with soil depth, we found that CUE decreased with increased temperature (Fig. 2). A likely explanation is that higher temperature would enhance microbial maintenance respiration over microbial growth (Pietikäinen et al., 2005) and thus the lower CUE may contribute to greater substrate release as CO_2 at higher temperature (Pold et al., 2020). This was testable using our data showing that the ratio of microbial respiration rates at 30 °C to at 10 °C (5.34 ± 1.52 (SD)) was significantly higher than that of microbial growth rates at 30 °C to at 10 °C (3.24 ± 0.81) across ecosystems and soil depths ($t = 9.854$, $P < 0.001$). Physiologically, moreover, CUE should be lower at higher temperature if respiratory processes are more temperature sensitive compared to growth processes (Hall and Cotner, 2007).

Carbon quality regulated the increased Q_{10} with soil depth and decreased Q_{10} along the wetland-grassland-forest hydrologic gradient (Table 3). The "C-quality temperature" (CQT) hypothesis suggests that decomposition of higher quality C has lower Q_{10} than that of lower quality C (Davidson and Janssens, 2006). Consistent with this hypothesis, we found negative relationships between Q_{10} and C quality within ecosystems and soil depths (Table 3). In addition, clay content was another crucial factor regulating the depth-dependent Q_{10} (Table 3). High clay content in deep soil may effectively reduce substrate quality and availability via strong chemical bonds (Singh et al., 2018; Churchman et al., 2020), such as via cation bridging or ligand exchange, which would be much weaker in sandy soils (Kögel-Knabner et al., 2008). We suggest that these reactions would explain the observed positive clay – Q_{10} relationship along soil depth, in agreement with the CQT hypothesis. Nevertheless, more studies that directly test the effects of clay on the temperature response of microbial respiration are needed in future research.

While our study investigated CUE, MRT, and Q_{10} of microbial respiration as well as their controls along the wetland-grassland-forest hydrologic gradient, some uncertainties exist. Microbial respiration is usually determined using a very small amount of soil (*e.g.*, less than 0.5 g) in some previous studies investigating CUE using the ^{18}O-incorporation method (*e.g.*, Spohn et al., 2016; Zheng et al., 2019), which might result in uncertainties in respiration rate measurement. Thus, we used 15 g soil to more accurately determine respiration rate on a more representative subsample. Moreover, although clay particles are associated with physical protection (Hassink and Whitmore, 1997; Hemingway et al., 2019), linkages with some other direct proxies (*e.g.*, mineral associated organic matter fraction and soil aggregates) will advance our understanding of soil protection regulating microbial CUE and temperature sensitivity. Likewise, although variables of the ratio of hydrolytic to oxidative enzyme activity (Hill et al., 2014; Takriti et al., 2018) and D_{soc} (Fierer et al., 2006; Li et al., 2017) are commonly used to reflect substrate quality, direct measurement such as Fourier transform infrared spectroscopy analysis may gain further understanding on this issue (Parikh et al., 2014).

5. Conclusion

Our findings show that microbial CUE, residence time, and temperature sensitivity of microbial respiration differed significantly among ecosystems and soil depths. The C sequestration potential in subsoil is thus the result of opposing effects of greater CUE and temperature response. Land-cover changes from wetland to upland may increase microbial CUE but decrease the response to rising temperature, suggesting that uplands may have greater C sequestration potential compared to wetland soil. Moreover, our results suggest that stoichiometry of available C:N ratios, clay content, and C quality are appropriate proxies to predict microbial CUE, MRT, and soil C responses to temperature change at least along the studied wetland-grassland-forest hydrologic gradient. Therefore, further work is required to elucidate mechanisms regulating microbial CUE, MRT, and temperature sensitivity at larger geographical scales to gain further understanding on this issue.

Authors' contribution

J.L. and E.P. designed the study; J.L. conducted the overall

experiment and analyzed the data with the assistance from all the other authors; J.L. wrote the first draft, and all authors jointly revised the manuscript.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.108117.

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