Title: Phosphorus fertility regulates microbial carbon use efficiency and SOM decomposition in non-allophanic Andosols.

Authors:

Wako KOIZUMI[1], Timothy J CLOUGH[2], Soichi KOJIMA[1], Tomoyuki MAKINO[1], Soh SUGIHARA[3], Ryosuke TAJIMA[1], Yoshitaka UCHIDA[4], Toru HAMAMOTO[1]*

Address:

[1]Graduate School of Agricultural Science, Tohoku University, 468-1 Aramaki Aoba Aoba-ku Sendai Miyagi 9808572, Japan

[2]Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln, New Zealand

[3]Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu-shi, Tokyo 1838509, Japan

[4]Research Faculty of Agriculture, Hokkaido University, Kita 9 Nishi 9 Kita-Ku, Sapporo, Hokkaido, 060-8589, Japan

*Correspondence: T Hamamoto, toru.hamamoto.d1@tohoku.ac.jp

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Abstract

Phosphorus (P) availability affects soil carbon (C) cycling such as microbial carbon use efficiency (CUE) and priming effects (PEs). While non-allophanic Andosols are characterized by high organic C content and strong P retention, the effects of different soil P fertility levels on C dynamics in these soils remain poorly understood. In this study, we conducted a 20-day incubation experiment using ^{13}C-enriched glucose to investigate how different soil P levels (Truog-P: 157 mg P kg^{-1} and 12 mg P kg^{-1}) impacted microbial C dynamics in non-allophanic Andosols under different field management practices. Our results showed that soil organic matter (SOM) priming strongly depends on soil P levels, although total primed CO_2-C emissions were low in these soils. The addition of glucose alone promoted positive PEs compared to the addition of glucose with nitrogen (N). In high P soils, glucose and N addition resulted in negative PEs, whereas in low P soils, the same treatment stimulated microbial SOM mining, resulting in positive PEs. Additionally, higher CUE was found in high P soils than in low P soils after 20 days of incubation. These findings suggest that soil P levels influence both substrate-induced microbial assimilation and SOM decomposition. In P-limited soils, microbial P requirements drive SOM decomposition, which in turn regulates CUE. This study contributes to an understanding of the role of P in soil C cycling and provides insights into soil fertility management strategies for improving C sequestration in non-allophanic Andosols.

1. Introduction

Soil carbon (C) plays a central role in the global C cycle with the potential to mitigate the elevated atmospheric carbon dioxide (CO_2) concentration as soil organic matter (SOM) (Jackson et al., 2017). Soil microbes can release soil organic C (SOC) via respiration and emitting it as CO_2 (Wieder et al., 2013). Increases in the stock of SOC may occur as a result of increased microbial biomass, in response to inputs of C to the soil (Kallenbach et al., 2016; Wang et al., 2021).

Microbial C use efficiency (CUE), the ratio of C allocated to microbial biomass growth to that respired as CO□, is positively correlated with SOC levels (Feng et al., 2023). The magnitude of CUE as also been shown to depend on microbial community structure (Maynard et al., 2017), and various abiotic factors such as soil pH (Jones et al., 2019; Schroeder et al., 2024), clay content (Islam et al., 2023), nutrient availability (Manzoni et al., 2012, Spohn et al., 2016), and substrate type (Jones et al., 2018). Limited nutrient availability and unbalanced substrate additions may lower CUE by promoting excessive enzyme allocation and respiration, described as overflow metabolism (e.g., Tempest and Neijssel 1992; Russell & Cook 1995; Schimel and Weimtraub 2003; Keiblinger et al., 2010). According to Manzoni et al. (2012), CUE is also influenced by nitrogen (N) and phosphorus (P) availability, since microbes require these

nutrients to balance anabolic and catabolic processes. Phosphorus is an essential nutrient for soil microbes, as it is a key component of DNA, RNA, and has a critical role in phospholipid synthesis, and energy transfer. Depletion of P resources constrains microbial investment in biomass production, shifting the carbon allocation toward enzyme production, which can lower CUE (Zhai et al., 2022). In addition, limited P availability represents a significant agronomic challenge, as it directly constrains plant productivity and undermines soil health. Microbes compete with plants for P, thereby limiting plant activity by reducing the plant-available P in P-limited soil (Jian et al., 2024).

An imbalance of available nutrients also leads to microbial SOM decomposition and nutrient acquisition, observed as positive priming effects (PEs) (Blagodatskaya and Kuzyakov 2008; Nottingham et al., 2015; Bernard et al., 2022). For example, the addition of a high C:N substrate induces microbial N acquisition from SOM, a process known as ‘N mining’, and positive PEs are found when compared to the addition of low C:N substrates, (Craine et al., 2007; Xu et al., 2024). Nutrient mining also applies to P, with high C:P substrates inducing positive PEs to occur under P-limited soil conditions. Although there have been extensive studies on N-induced PEs, research focused on P-induced PEs has been limited yet (Qin et al., 2024). The input of P has been shown to potentially enhance CUE and negative PEs by alleviating nutrient imbalances

and increasing P availability, which may improve SOM stability (Sawada et al., 2023; Li et al., 2025). However, studies in grassland soil have also reported no effect on CUE following P fertilization (Widding et al., 2020). There remains a critical knowledge gap in understanding how P availability influences microbial CUE across different soil types and quality or amount of substrate.

Derived from volcanic ash, Andosols, mainly located on the Pacific coast (i.e., Japan, Indonesia, and New Zealand; Takahashi, 2020), are important for soil C sequestration due to their abundance of Al-organic matter complexes which contributes to high SOM stability (Nanzyo, 2002; Takahashi and Dahlgren, 2016). Andosols are classified according to their significant P retention and are mainly divided into two groups: allophanic and non-allophanic, which are distinguished by the active Al composition (Takahashi and Dahlgren, 2016; WRB, 2022). Non-allophanic Andosols, dominated by Al-organic matter complexes, account for about 30% of Japanese arable Andosols (Saigusa and Matsuyama, 1998). Non-allophanic Andosols are characterized by high acidity and high P sorption due to Al-organic matter complexes (Shoji et al., 1987). Thus, non-allophanic variants display greater Al phytotoxicity, which regulates the availability of P, consequently restricting crop growth (Ito et al., 2011). Given the dependence of soil microbial activity on P availability, it is highly expected that microbial CUE is affected by

the amount of available P. However, not only is microbial activity suppressed by low P availability it is also limited by Al toxicity, associated with low pH (Kunito et al., 2016). However, it remains unknown how CUE responds to P fertility in non-allophanic Andosols.

In this study, we conducted an incubation experiment to investigate the effect of P availability on microbial C utilization in non-allophanic Andosols. We used ^{13}C glucose to evaluate the impact of P availability on CUE and soil ^{13}C recovery. We hypothesized that: (i) in unfertilized soils, substrate additions would promote P-mining, accelerating the positive PEs; and (ii) higher CUE would be observed in P-fertilized soils, as the nutrient balance would better match microbial requirements compared to unfertilized soils.

2. Materials and methods

2.1. Soil sampling

Soil samples were collected at 0-10 cm depth in April 2023 from two contrasting fertilization fields at Field Science Center, Graduate School of Agricultural Science, Tohoku University, Japan (38°74’N, 140°75‘E, 190 m above mean sea-level). The soils were classified as non-allophanic Andosols according to the Soil Classification System of Japan (Obara et al.,

2011, Table S1). Two contrasting soils with different P content were sampled (Table 1 and S1). The high P soil (HP), which had been P-fertilized since 1987, had a high inorganic P content (157 mg P kg^{-1} soil). The amount of P input from the triple superphosphate and the fused magnesium phosphate varied depending on the cultivation years, ranging from 3.1 to 36.1 g m^{-2} yr^{-1}. Additionally, cattle manure was applied in certain years. In contrast, the low P soil (LP), cultivated since 2009 without P fertilization, had a low inorganic P content (12 mg P kg^{-1} soil). Detailed fertilization and cultivation histories are described in Table S2. The collected soils were sieved through a 2 mm sieve, and all visible roots were removed, then soils were stored at 4℃ until further use.

Table 1. Chemical and biological properties in HP and LP soils (mean (SD)).

Soil	$pH_{(CaCl2)}$		Total C		Total N		C:N	MBC		MBP		log(MBC:MBP)	DP		Truog P		HCl-P	
			(g C kg^{-1})		(g N kg^{-1})			(mg C kg^{-1})		(mg P kg^{-1})		molar basis	(mg P kg^{-1})		(mg P kg^{-1})		(g P kg^{-1})	
HP	5.29	(0.03)	88.07	(3.38)	5.23	(0.23)	16.83	1368.71	(56.55)	75.06	(18.31)	1.68	122.39	(6.31)	157.31	(5.07)	1.41	(0.029)
LP	4.49	(0.06)	91.24	(1.99)	5.36	(0.10)	17.01	1343.65	(142.09)	21.31	(11.63)	2.31	29.13	(1.33)	12.10	(0.31)	0.54	(0.063)

MBC, Microbial biomass carbon: MBP, microbial biomass phosphorus: MBC: MBP, their molar-based ratio: DP, dissolved inorganic phosphorus.

Truog P and HCl-P represent Truog-extractable P and 1M HCl-extractable P, respectively.

2.2. Experimental design

Prior to the experiment, the sieved soils were pre-incubated in the dark at 25□ for 14 days and maintained at a gravimetric moisture content of 45% (w/w). Pre-incubated soil was placed in plastic bags (each equivalent to 100 g dry weight). Soil samples received a substrate solution containing either: (1) water ("Control"), (2) ^{13}C-enriched glucose ("Glc"), or (3) ^{13}C-enriched glucose and $(NH_4)_2SO_4$ ("Glc+N"). There were 24 samples in total (two soils × three glucose levels × four replicates). The rate of added ^{13}C-enriched glucose (4 atom%, D-GLUCOSE (U−$^{13}C_6$, 99%), Cambridge, 450 mg C kg^{-1} soil) was set to be 25 to 50% of the MBC in order to increase microbial activity while avoiding exponential microbial growth and shifting microbial community structure (Blagodatskaya and Kuzyakov, 2008; Sawada et al., 2023). The concentration of added mineral N (61 mg N kg^{-1} soil) was determined according to mean microbial C:N:P ratios, 60:7:1 (Cleveland and Liptzin, 2007). The experiment was then started by wetting to 55% gravimetric soil moisture content with each substrate solution.

2.3. CO_2 emission

For measurement of soil respiration, each treatment was placed in a 450 mL airtight glass jar with a CO_2 alkali trap (5 mL of 1 M NaOH) and incubated at 25□ for 20 days. Ten mL of 0.01 M HCl solution was also placed in the glass jar to maintain the soil moisture. The NaOH

solution was collected and replaced with a fresh solution on days 1, 2, 4, 6, 8, 12, and 20, respectively. The collected NaOH solutions received 8 mL of 1 M $SrCl_2$ to form $SrCO_3$ precipitate and this was titrated using a 1 M HCl solution (Zibilske 1994; Qiao et al., 2014). After titrating, the precipitations were washed 3 times with 30mL water and then dried at 105□. The dried $SrCO_3$ was packed in tin capsules to analyze for ^{13}C enrichment on DELTA V plus isotope ratio mass spectrometer (IRMS, Thermo Fisher Scientific, Bremen, Germany). The calculation of glucose-derived CO_2-C_{glc} was adapted from Mehnaz et al., (2019) and Brant et al. (2006):

$$CO_2\text{-}C_{glc} = CO_2\text{-}C \times (^{13}C_c - {}^{13}C_{none}) / (^{13}C_{label} - {}^{13}C_{none}) \quad (1)$$

Where CO_2-C is the cumulative CO_2-C respired from the soil with glucose addition; $^{13}C_c$, $^{13}C_{none}$ and ^{13}C labels are the ^{13}C atom% values of the CO_2-C measured in the NaOH solutions with and without glucose addition and the added glucose substrate, respectively. Soil-derived CO_2-C (CO_2-C_{soil}) in glc and glcN treatments was calculated as the difference between the total (CO_2-C)and glucose-derived CO_2-C_{glc}.

2.4. Soil microbial biomass carbon and phosphorus

Soil microbial biomass carbon (MBC) was determined with a modified chloroform fumigated method (Beck et al., 1997). Briefly, two samples of 5 g dry soil of each sample with and without 24 h fumigation using chloroform were extracted with 25 mL of 0.05 M K_2SO_4 and analyzed for total organic C using a TOC-TN analyzer (TOC-V CPH/CPN, Shimadzu, Japan). MBC was calculated from the difference of their concentrations in samples with and without fumigation divided by an extraction efficiency of 0.45 (Beck et al., 1997). The ^{13}C atom% in each extract was measured on an IRMS (Thermo Fisher Scientific, USA). The ^{13}C atom% of the microbial biomass ($^{13}C_{mic}$) and the glucose-derived C in the microbial biomass ($MBC_{glucose}$) were also calculated by the following equation (Mehnaz et al., 2019):

$$^{13}C_{mic} = (C_f \times {}^{13}C_f - C_{nf} \times {}^{13}C_{nf}) / (C_f - C_{nf}) \quad (2)$$

$$MBC_{glucose} = MBC \times ({}^{13}C_{mic,c} - {}^{13}C_{mic,none}) / ({}^{13}C_{label} - {}^{13}C_{mic,none}) \quad (3)$$

where C_f and $^{13}C_f$ are the amount of C and ^{13}C atom% in the fumigated extracts, and C_{nf} and $^{13}C_{nf}$ are them in the nonfumigated extracts, respectively.

Organic carbon within K_2SO_4 extracts from non-fumigated soil was denoted as Dissolved organic carbon (DOC). The terms $^{13}C_{mic,c}$ and $^{13}C_{mic,none}$ are the $^{13}C_{mic}$ values with and without

glc addition.

Soil microbial biomass phosphorus (MBP) was measured using an anion exchange membrane method (Kouno et al., 1995) where hexanol was used as the fumigant instead of chloroform. The extracted P was determined with the malachite green colorimetric method (Ohno and Zibilske, 1991; Jeannotte et al., 2004; Song et al., 2019). The extracts were diluted appropriately to avoid interference with HCl (Angelo et al., 2001) and analyzed calorimetrically (630 nm) using a microplate reader (iMark, Bio-Rad Laboratories, USA). The MBP was calculated from the difference in extracted P concentrations between samples with and without fumigation divided by 0.40 (Brookes et al., 1982).

2.5. Inorganic phosphorus

Phosphorus within non-applied hexanol extracts for analysis of MBP was denoted as dissolved inorganic P (DP). In addition, 1M HCl extractable P described by DeLuca et al. (2015) was also measured. It represents recalcitrant inorganic P, which absorbs to Fe/Al oxides, clay particles or inorganic compounds and released by proton released by microbes or plants, thus it was denoted as HCl-P. It was measured by extracting 0.5 g of dry mass equivalent soil with 10 ml of 1 M HCl for 3 h at 200 rev min^{-1} at room temperature. After filtering, the inorganic P was

determined by the malachite green method at 630 nm using a microplate reader (iMark, Bio-Rad Laboratories, USA) as for the MBP analysis.

2.6. Carbon use efficiency and Priming effects

The calculations of carbon use efficiency (CUE) and priming effects (PEs) were adapted from Mehnaz et al., (2019). Microbial glucose CUE ($CUE_{glucose}$) of the added glucose was calculated using the following equation:

$$CUE_{glucose} = MBC_{glucose} / (MBC_{glucose} + CO_2\text{-}C_{glucose}) \quad (4)$$

The PEs were calculated as the increase in native soil organic C released after substrate application also using the following equation (Mehnaz et al., 2019):

$$PEs = CO_2\text{-}C_{soil} - CO_2\text{-}C_{ctr} \quad (5)$$

Where $CO_2\text{-}C_{soil}$ and $CO_2\text{-}C_{ctr}$ are the soil-derived CO_2-C respired from the soil with glucose addition and the cumulative CO_2-C respired from the soil without glucose addition, respectively.

2.7. Statistics

All statistical analyses were performed with R (version 4.2.2; R Core Team, 2023). We conducted a two-way analysis of variance (ANOVA) including the interaction between soils and substrates to assess their effects on the total CO_2-C, glucose-derived and soil-derived CO_2-C, and total MBC, glucose-derived and soil-derived MBC, DOC, MBP, Pi and DP using linear model. Model was validated by examining residuals using the DHARMa package (Hartig 2022). The data were further analyzed using Tukey's HSD test to find pairwise differences between soil type and substrate combinations at $\alpha = 0.05$ using multcomp package (Hothorn et al., 2008). The effects of soil P fertility on each result, N addition on PE, and time on MBP, MBC: MBP, and DP were determined by independent samples t-tests.

3. Results

3.1. CO_2 emission

The HP soil had a higher cumulative CO_2 emission (339.3 mg C kg^{-1} soil) than the LP soil (308.6 mg C kg^{-1} soil) and there were significant differences when averaged across substrate treatments during the incubation period (Fig. 1 and Fig. S1). Substrate additions significantly stimulated the CO_2 emissions from both soils at day 1 immediately after the incubation

commenced (Fig. S1). The glc treatments had significantly higher cumulative CO_2 emissions than the glcN treatments in each soil (Fig. 1).

The cumulative SOM-derived CO_2 emission was higher in the HP soil than the LP soil averaging across the substrate treatments ($p<0.05$, Fig. 1). Substrate additions increased SOM-derived CO_2 emission in each soil type 6 days after incubation ($p<0.001$). In the HP soil, the glc treatment increased the SOM-derived CO_2 emissions, while in the LP soil, glc and glcN treatments significantly increased the SOM-derived CO_2 emissions. The cumulative glucose-derived CO_2 emission showed no significant differences among soils and substrate treatments throughout the incubation period (Fig. S2). The glucose-derived CO_2 emission peaked at 161.2 mg C kg^{-1} in the HP soil and 154.5 mg C kg^{-1} in the LP soil on day 1 immediately after substrate addition when averaged across the substrate treatments (Fig. S1).

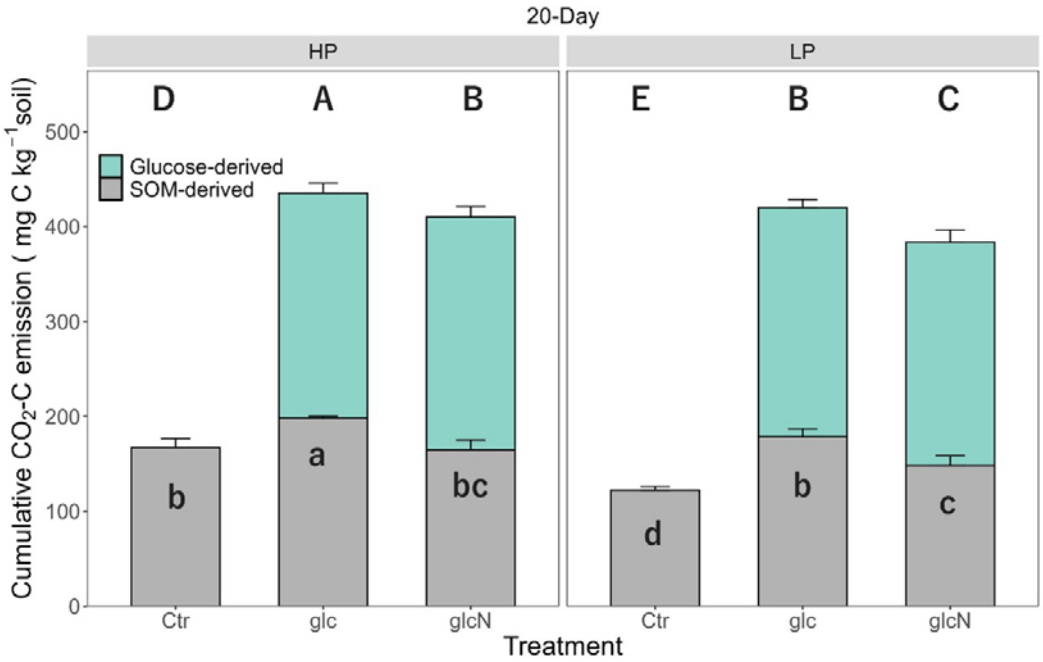

Fig. 1. Cumulative CO_2 emissions (mg C kg^{-1} soil) over 20-days incubation. Treatments include soil without any amendment (Ctr), soil with ^{13}C-enriched glucose (glc), and soil with ^{13}C-enriched glucose and nitrogen substrates (glcN). Different color bars show cumulative CO_2 emissions (mean ± SD, n=4, but n=3 in LP Ctr due to an operational error during the experiment) derived from added glucose (light green) and soil organic matter (grey). Uppercase and lowercase letters indicate significant differences ($p < 0.05$) in total and SOM-derived CO_2-C among treatments, respectively.

The PEs during incubation were lower in the HP soil than the LP across the treatments ($p<0.05$ at 1, 6, 8 days of incubation, $p<0.01$ and $p<0.001$ at 2 days and 12, 20 days of incubation, respectively) (Fig. 2 and Table S4). The glc addition had significantly higher PEs compared to the glcN addition in both soils. The addition of glc and glcN in the LP soil showed positive PEs. However, the effect of substrate treatments on PEs in the HP soil contrasted with the LP soil; the glc addition showed a positive PEs, but glcN addition showed negative PEs. The total primed CO_2-C as a % of added glucose C ranged from –0.71 to 12.88 % (Table S4).

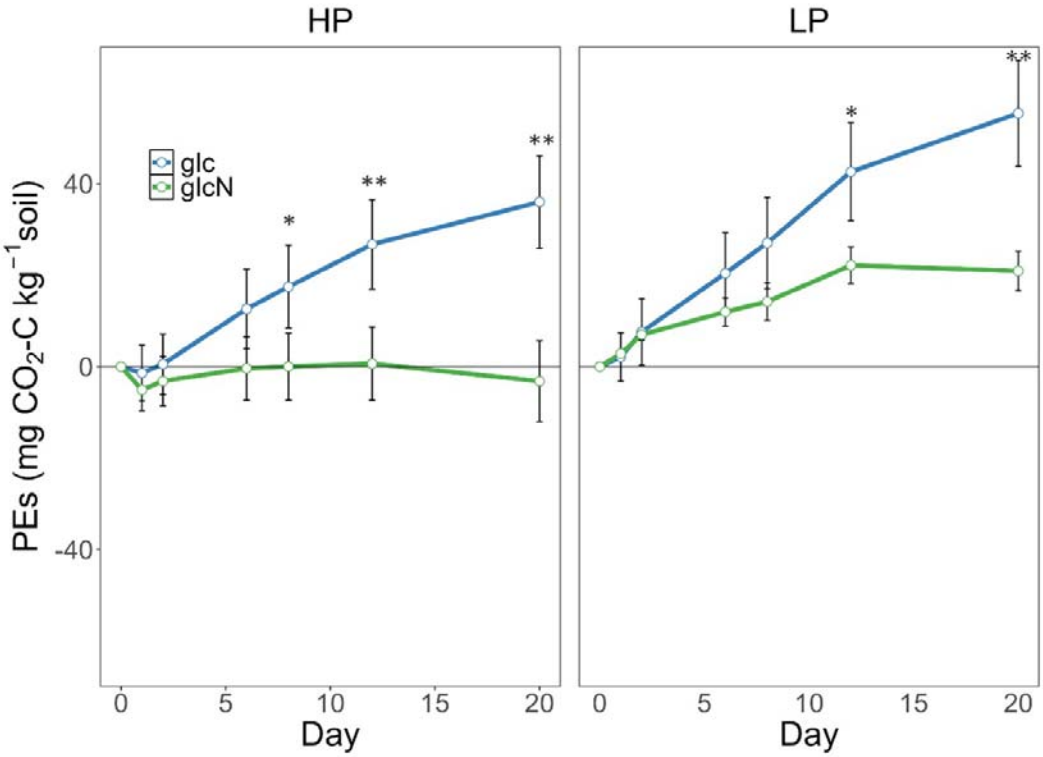

Fig. 2. The priming effects (PEs) in HP and LP soils over 20 days. Treatments include soil with ^{13}C-enriched glucose (glc) and soil with ^{13}C-enriched glucose and nitrogen substrates (glcN). Green and blue lines show the priming effect (mean ± SD, n = 4) in glc and glcN, respectively, for each soil. * ($p < 0.05$), ** ($p < 0.01$) and blank (not significant) indicate significance level of N addition effect for each soil separately.

3.2. Microbial biomass and soil chemical properties

The HP soil had significantly higher total microbial biomass carbon (MBC_{Total}) than the LP soil at 20 days of incubation, whereas no significant differences were observed after 2 days of incubation (Fig. 3 and Table S5). Substrate additions did not affect MBC_{Total} in either soil at either 2 or 20 days of incubation (Table S5). For the SOM-derived microbial biomass carbon (MBC_{SOM}), substrate additions significantly decreased MBC_{SOM} in the HP soil compared to the control at 2 days of incubation (Fig. 3 and Table S5). In contrast, the LP soil showed no differences in MBC_{SOM} among substrate treatments at 2 days of incubation. Glucose-derived microbial biomass carbon (MBC_{Glc}) showed no significant differences among soils and substrate treatments at 2 days of incubation (Fig. 3 and Table S5). However, at 20 days of incubation, MBC_{Glc} was higher in the HP soil than in the LP soil. In both soils, MBC_{Glc} was significantly lower at 20 days of incubation compared to 2 days.

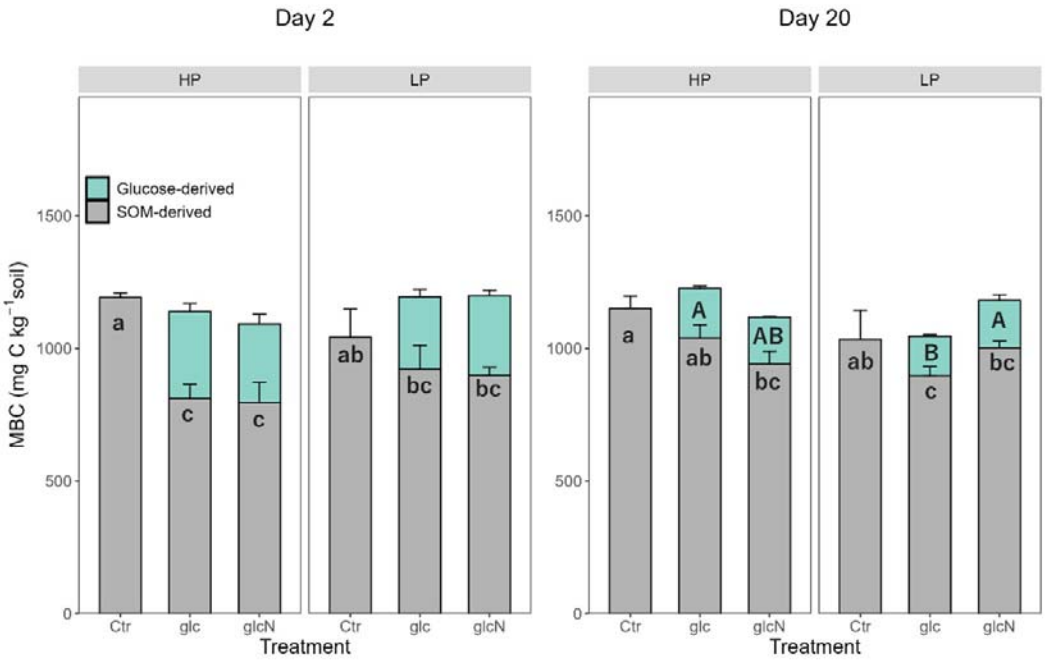

Fig. 3. Microbial biomass carbon (MBC) (mg C kg^{-1} soil) in 2 and 20-days incubation. Treatments include soil without any amendment (Control), soil with ^{13}C-enriched glucose (glc), and soil with ^{13}C-enriched glucose and nitrogen substrates (glcN). Different color bars show cumulative MBC (mean ± SD, n =4, but n =3 in LP Ctr due to an operational error during the experiment) derived from added glucose (light green) and soil organic matter (grey). Uppercase and lowercase letters indicate significant differences ($p < 0.05$) in glucose-derived MBC and SOM-derived MBC among substrate treatments and soils at each sampling time, respectively.

The HP soil had significantly higher MBP and a lower log ratio of MBC:MBP compared to the LP soil at 2 and 20 days of incubation (Table S5). However, substrate additions had no significant effect on MBP during the incubation period.

Soil pH, DP, and HCl-P were consistently higher in the HP soils than in the LP soil during the incubation period (Table S3). DP increased from 0 to 2 days of incubation in HP soil ($p < 0.001$), while there was no significant difference in LP soil ($p = 0.065$). Then DP decreased from 2 to 20 days in LP soil ($p < 0.001$), whereas no significant difference was found in HP soil ($p = 0.41$).

3.3. Microbial carbon use efficiency

Microbial carbon use efficiency (CUE) showed no significant differences between the two soils or substrate additions at 2 days after incubation (Table 2). However, at 20 days after incubation, CUE in the HP soil was higher than in the LP soil ($p < 0.05$). The HP soil with glc showed the highest CUE, while the LP soil with glc had the lowest CUE at 20 days after incubation.

Table 2. Carbon use efficiency (CUE) in the HP and LP soils at 2 and 20 days after incubation (mean (SD)). Different letters indicate significant differences ($p < 0.05$) among treatments for each soil type.

		CUE				
Soil	Substrate	2-day		20-day		
HP	glc	0.65	(0.025)	0.44	(0.015)	a
	glcN	0.61	(0.038)	0.42	(0.009)	ab
LP	glc	0.62	(0.035)	0.39	(0.012)	b
	glcN	0.63	(0.012)	0.43	(0.025)	a
ANOVA P values						
Soil		n.s		<0.05		
Substrate		n.s		n.s		
Soil × Sub		n.s		<0.01		

4. Discussion

Our results demonstrate that substrate additions stimulated soil-derived CO_2 emissions, and consequently overall microbial respiration, regardless of soil P fertility. Notably, we observed higher soil-derived CO_2 emissions in soils with glucose-only addition compared to soils with glucose+N addition (Fig. 3). This result suggests that N-mining, SOM degradation to acquire N, occurred (Li et al., 2017). The substrate addition promoted soil-derived CO_2 emission, with greater increases observed in low-P soil than in high-P soil (Fig. 3 and Table S4). This suggests that the impact of substrate addition on primed CO_2 emission depends on soil P fertility. Our study also found that the low-P soil had significantly lower DP and consistently higher MBC:P ratios compared to the high-P soil throughout the incubation period (Tables 1, S3, and S5). Under nutrient-imbalanced conditions, microbes invest abundant elements into decomposing SOM to obtain limited nutrients, thereby maximizing their productivity (Allison and Vitousek, 2005; Mooshammer et al., 2014).

Furthermore, when comparing soils treated with both glucose and N substrates, negative PEs were found in high-P soil, whereas positive priming effects were observed in low-P soil (Fig. 2 and Table S4). These results indicate that substrate-induced soil-derived CO_2 emissions from low-P soil reflect SOM degradation driven by P-mining, supporting our first hypothesis.

Moreover, MBP levels in low-P soil remained stable throughout the incubation period, possibly because positive priming induced P-mining and maintained microbial P (Spohn, 2016).

Our results showed lower primed CO_2-C as a % of added glucose C in non-allophanic Andosols compared to previous studies. In the current, values ranged from −1 to 13 % in the non-allophanic Andosols over 20 days incubation compared to values of 28 to 420 % for other soil types (Table S4) (Shahbaz et al., 2018; Mehnaz et al., 2019; Sawada et al., 2023). The values in prior research on other soil types were calculated from data between 18 to 42 days of the entire incubation period. Despite the lower PEs in this study, the size of the MBC pool was the same or larger compared to the size in these studies. These lower PEs in the non-allophanic Andosols were possibly due to the abundance of Al-organic matter complexes. The stability of SOM depends on Al-organic matter complexes and soil acidity (Miyazawa et al., 2013). The Al-organic matter complexes promote the soil aggregates formation and are also suggested to physically suppress the microbial SOM decomposition (Matsui et al., 2016). Thus, it is suggested that the low PEs shown in this study are a reflection of SOM stability found in non-allophanic Andosols.

As for assimilation, in agreement with our second hypothesis, the high-P soil had significantly higher CUE than the low-P soil after 20 days of incubation (Fig. 1, 3 and Table 2). Combined with the PE results, our findings suggest that under P-limited conditions, SOM decomposition associated with microbial P maintenance regulates CUE. The measurement of CUE in the short term (6 to 48 h) might reflect the utilization of glucose for gross production (Brant et al., 2006; Geyer et al., 2016), while measurements beyond 48 h might indicate the efficiency of the soil stability and recycling of the microbial necromass and exudates (Geyer et al., 2016; Kalu et al., 2024). After 20 days of incubation, in the low-P soil, microbes allocated more energy to catabolic pathways to acquire phosphorus, inducing lower CUE than in the high-P soil (Burnard et al., 2022).

After 2 days of incubation, CUE showed no significant differences among soils (Table 2). In this study, it is possible that the effect of soil P fertility on microbial assimilation of substrates after 2 days of incubation was offset by the chemical properties specific to non-allophanic Andosols. Soil C content is one of the regulating factors of microbial assimilation (Kamble and Bååth, 2014); Andosols have a large amount of organic C content due to Al-soil organic matter complexes (Takahashi and Dahlgren, 2016). Moreover, it has been suggested that non-allophanic Andosols have higher organic C contents than other

Andosols (Nanzyo et al., 1993). Previous studies showed that microbial growth and CUE are strongly related to C availability rather than N and P availability (Wang et al., 2021; Morris et al., 2021; Jiang et al., 2025). In this study, we found that high and low-P soils contain approximately 10% TC, this high C content may have influenced CUE after 2 days of incubation. Additionally, soil pH is also one of the controlling factors of CUE; our soils showed low $pH_{(CaCl2)}$, around 5.2 and 4.3, in high and low-P soils, respectively. Soil pH can alter the composition of microbial communities and consequently affect CUE (Manzoni et al., 2012; Zhen et al., 2019). Rousk et al. (2011) reported that fungal growth is suppressed at pH >5.5, contrasted with bacterial growth limited at pH <5.5. Keiblinger et al. (2010) reported that fungi induced higher CUE than bacteria, and fungal CUE was affected by C availability and bacterial CUE limited by N and P availability, respectively. Thus, it is possible that the microbial community composition was adapted to low pH conditions and the high C content of the soil and these factors may have contributed to the marginal effect of P fertility on CUE in the non-allophanic Andosol. Previously it was suggested that there was a relationship between CUE and pH or Al^{3+}(Jones et al., 2019), in our study, the amount of exchangeable Al was 21.30 meq kg^{-1} and 0 meq kg^{-1} in the high and low-P soil, respectively. It is possible that the lower CUE in the low P soil after 20 days of incubation was due to the toxicity of exchangeable Al, as reflected in the differing amounts of Al^{3+}. We were not able to measure

the temporal effect of Al toxicity on CUE, and this requires more research.

5. Conclusion

This study showed that substrate induced SOM decomposition in a non-allophanic Andosol depends significantly on soil P status. The combined addition of glucose with N substrate promoted positive priming effects (PEs) under low P conditions, while negative PEs occurred under high P conditions. Throughout the incubation, the priming effects in the low P soil were greater than in high P soil. Additionally, in the low P soil, substrate additions did not affect MBP and the ratio of MBC:P throughout the incubation period. These results suggest that a lack of P affected the microbial impact on SOM decomposition, possibly due to the mechanism of maintaining microbial P. Greater CUE was observed in the high P soil than in the low P soil after 20 days of incubation, with no difference due to P fertility after 2 days of incubation. This suggests that under P-limited conditions, SOM decomposition associated with microbial P maintenance regulates CUE, and that contrasting P fertilities differently affect substrate-induced microbial assimilation and SOM decomposition. Future research is needed to reveal the mechanism of microbial P acquisition coupled to SOM decomposition in non-allophanic Andosols.

Author's Contributions

W.K., T.M., and T.H. planned and designed the research. W.K. and T.H. conducted the incubation experiments. W.K. and T.H. analyzed the data, wrote the main manuscript, and prepared the figures and tables. All authors reviewed the manuscript.

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