Different root exudates C/N ratios accelerate CO2 emission from paddy soil

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Abstract

Root exudates can greatly modify microbial activity and soil organic matter (SOM) mineralization. However, the mechanism of root exudation and its stoichiometric ratio of C/N controlling upon paddy soil C mineralization are poorly understand. In this study, we used a mixture of glucose, oxalic acid, and alanine as root exudate mimics, employing three C/N stoichiometric ratios (CN6, CN10, and CN80) to explore the underlying mechanisms involved in C mineralization. The input of root exudates enhanced CO2 emission by 1.8–2.3-fold than that of the control. Artificial root exudates with low C/N ratios (CN6 and CN10) increased the metabolic quotient (qCO2) by 12% over those obtained at higher stoichiometric ratios (CN80 and C-only), suggesting a relatively high energy demand for microorganisms to acquire organic N from SOM by increasing N-hydrolase production. The stoichiometric ratios of enzymes (β-1,4-glucosidase to β-1,4-N-acetyl glucosaminidase) promoting organic C degradation compared to those involved in organic N degradation showed a significant positive correlation with qCO2; the stoichiometric ratios of microbial biomass (MBC/MBN) were positively correlated with carbon use efficiency. This suggests that root exudates with higher C/N ratios entail an undersupply of N for microorganisms, triggering the release of N-degrading extracellular enzymes. This in turn decreases SOM mineralization, implying the C/N ratio of root exudates to be a controlling factor. Our findings show that the C/N stoichiometry of root exudates controls C mineralization by the specific response of the microbial biomass through the release of C- and N-releasing extracellular enzymes to adjust for the microbial C/N ratio.

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Abstract

Root exudates can greatly modify microbial activity and soil organic matter (SOM) mineralization. However, the mechanism of root exudation and its stoichiometric ratio of C/N controlling upon paddy soil C mineralization are poorly understand. In this study, we used a mixture of glucose, oxalic acid, and alanine as root exudate mimics, employing three C/N stoichiometric ratios (CN6, CN10, and CN80) to explore the underlying mechanisms involved in C mineralization. The input of root exudates enhanced CO_2 emission by 1.8–2.3-fold than that of the control. Artificial root exudates with low C/N ratios (CN6 and CN10) increased the metabolic quotient (qCO_2) by 12% over those obtained at higher stoichiometric ratios (CN80 and C-only), suggesting a relatively high energy demand for microorganisms to acquire organic N from SOM by increasing N-hydrolase production. The stoichiometric ratios of enzymes (β-1,4-glucosidase to β-1,4-N-acetyl glucosaminidase) promoting organic C degradation compared to those involved in organic N degradation showed a significant positive correlation with qCO_2; the stoichiometric ratios of microbial biomass (MBC/MBN) were positively correlated with carbon use efficiency. This suggests that root exudates with higher C/N ratios entail an undersupply of N for microorganisms, triggering the release of N-degrading extracellular enzymes. This in turn decreases SOM mineralization, implying the C/N ratio of root exudates to be a controlling factor. Our findings show that the C/N stoichiometry of root exudates controls C mineralization by the specific response of the microbial biomass through the release of C- and N-releasing extracellular enzymes to adjust for the microbial C/N ratio.

KEYWORDS: Root exudates – Stoichiometric ratios – Microbial biomass – Extracellular enzyme – Metabolic quotients – Carbon use efficiency

INTRODUCTION

Plants can dramatically modify the soil environment through the rhizosphere, either by the release of C from plant roots (e.g., root exudates) or via the rapid uptake of their associated microorganisms by the soil (Kuzyakov, 2002; Jones et al., 2009; Fisk et al., 2015; Liu et al., 2019; Xiong et al., 2019). Across different plant species, around 1–10% of photoassimilated C is released into the soil as root exudates (Jones et al., 2004; Phillips et al., 2011; Qiao et al., 2014; Yin et al., 2014), consisting primarily of sugars, while also containing organic acids, amino acids, phenolics, and other secondary metabolites (Haichar et al., 2014; Yuan et al., 2017). In addition to acting as direct substrates for microorganisms and possess nutritional value, the stoichiometric ratio of the C and N-containing are considered to have an impact on their utilization by microorganisms (Wild et al., 2014; Liu et al., 2020). Thus, elucidating the role and underlying mechanisms of action of different exudate C/N ratios on microbial substrate utilization is of key importance for understanding soil C and N cycling, and likewise for soil C sink strength.

Several mechanisms have been proposed to explain the changes in the microbial decomposition of soil organic matter (SOM) because of root exudate addition, namely: (i) root exudates provide energy for the stimulation of SOM decomposition and change the chemical and physical properties of the soil environment (Qiao et al., 2016; Zhu et al., 2018; Mehnaz et al., 2019; Du et al., 2020); (ii) labile C promotes microbial growth, which in turn increases the N demand and likewise microbial N mining from SOM (Manzoni et al., 2010; Dijkstra et al., 2013; Qiao et al., 2016; Zhu et al., 2018); and (iii) microbial C and N demands cause community shifts that alter microbial-mediated C decomposition (Phillips et al., 2011; Wild et al., 2014; Yuan et al., 2017; Fang et al., 2020; Wei, 2020). Moreover, impacts of nutrients on stoichiometry also need to be considered (such as the addition of C substrates like glucose, accompanied by several levels of N applications), which provide a proportion of C that is incorporated into the microbial biomass at the expense of CO_2 emission, and becomes stabilized as soil organic carbon (SOC) (Creamer et al., 2014).

Recently, research on the stoichiometry of root exudate compounds combined with mineral N [e.g., $(NH_4)_2SO_4$] has shown that higher C/N ratios increase CO_2 emissions due to high microbial N demand (Du et al., 2020; Liu et al., 2020). Subsequently, resource stoichiometry alters microbial community composition to gain the required elements and increase SOM mineralization (Zhu et al., 2018; Wei, 2020). Extracellular enzyme activities, such as C- and N-acquiring enzymes, can also reflect the resource demands of microbial communities (Schimel, 2003; Hill et al., 2012). β-1,4-glucosidase (BG) and β-1,4-xylosidase (XYL) are the largest contributors to the degradation of cellulose and hemicellulose, respectively. Similarly, β-1,4-N-acetyl glucosaminidase (NAG) plays a role in the degradation of chitin and is involved in the organic N pool. The enzyme activities have similar stoichiometries, which could further relate to the elemental stoichiometry of the microbial biomass for microbial nutrient assimilation and growth (Sinsabaugh et al., 2008; Sinsabaugh and Follstad Shah, 2012). To date, the understanding of the influence of root exudates with different stoichiometric compositions on microbial growth and activities is still in its infancy, especially with respect to the interaction between microbial biomass stoichiometry and extracellular enzyme stoichiometry depending on the root exudate compositions.

In the present study, we applied different C:N stoichiometric ratios of artificial root exudates to soil to investigate how they modify microbial activities (including extracellular enzyme production and microbial biomass stoichiometric ratio) and likewise influence SOM mineralization. The stoichiometric ratios of artificial root exudates were regulated by combining different amounts of glucose, oxalic acid, and alanine, which represented low-molecular-weight organic compounds, namely, sugars, organic acids, and amino acids, respectively. We determined the activities of extracellular enzymes involved in C and N decomposition, microbial biomass C (MBC) and N (MBN), along with the associated CO_2 emissions after the addition of root exudates. We hypothesized that (1) the application of C-only artificial root exudates leads to an imbalance in resource stoichiometry, thereby inhibiting microbial activity and SOM mineralization; and (2) the inclusion of N-containing root exudates meets microbial stoichiometric requirements, thus promoting microbial growth and increasing SOM mineralization.

MATERIALS AND METHODS

2.1 Study site and soil sampling

Soil was collected from experimental rice fields located in Changsha, Hunan, China (113°19' 52" E, 28deg 33' 04" N). The site is characterized by a subtropical climate, with a mean annual temperature of 16.7 degC and mean annual precipitation of 1457 mm. Moist field soils were collected from the plow layer (0–20 cm) using a stainless-steel drill (diameter: 5 cm) in September, which is after the growing season in summer. The soil is derived from Quaternary Red Clay and classified as Haplic Acrisol according to the IUSS Working Group WRB (WRB, 2015). The soil received urea (80 kg ha^{-1} yr^{-1}) along with biological fertilizer (livestock manure) and rice plant straw residues (6000 kg ha^{-1} yr^{-1}) within a rice–rape rotation. Five soil cores were collected and thoroughly mixed; they were immediately placed in a gas-permeable plastic bag, and stored at 4 degC until analysis. A subset of soil samples was air-dried and passed through a 2-mm sieve to remove fine roots and other plant residues. The fresh soil samples were used to measure basic chemical properties such as SOC (11.27 g kg^{-1}) and total N (TN) (1.3 g kg^{-1}) using the potassium dichromate-concentrated sulfuric

acid-phenanthroline titration method (Lu, 1999). Dissolved organic C (DOC) (69.84 mg kg^{-1}) was analyzed using a Shimadzu TOC-VWP analyzer (TOC-VWP, Shimadzu, Japan). Soil pH (4.98) was measured using a Mettler Toledo 320 pH meter at a soil-to-water ratio (w:v) of 1:2.5.

2.2 Artificial root exudate experiment

The sieved and air-dried soil samples (3 kg dry weight) were respectively placed in a 50-L plastic bucket and flooded with deionized water to a depth of 2–3 cm. The soils were pre-incubated for two weeks in the dark at 25 degC. The experiment consisted of five treatments with four replicates in a completely randomized design. Afterwards, the soil was mixed well, and subsamples of 50 g (equivalent to 30 g dry soil) were placed in 500-mL incubation serum bottles. Deionized water was added to each bottle to a level of 2–3 cm above the soil surface, and the bottles were sealed using a rubber stopper to ensure anoxic conditions. To imitate root exudates, which are mainly dominated by sugars, amino acids, and organic acids (Lopez-Sangil et al., 2017; Xiong et al., 2019), we chose different proportions of glucose ($C_6H_{12}O_6$), oxalic acid ($H_2C_2O_4$), and alanine ($C_3H_7NO_2$). We adjusted three different C/N ratios (CN6-, CN10-, and CN80-treatments) by adding different amounts of alanine, along with a treatment without alanine (C-only). Treatment with only distilled H_2O was used as a control. The total amount of added C was maintained at 5% SOC, about 0.6 mg C g^{-1} soil dry weight. The relative contributions of sugar, amino acids, and organic acid-derived C were within the ranges reported in the literature (Qiao et al., 2016; Liu et al., 2020).

The solutions were prepared every five days by dissolving the abovementioned proportions of glucose, oxalic acid, and alanine in 50 mL of deionized water. The details are presented in Table 1 and Table S1. To mimic a pattern of continuous exudate excretion, we manually injected 0.5 mL of the solution into the soil samples using a 10-mL syringe daily. All samples were incubated at 25 degC in the dark for 45 days. Four bottle samples were chosen randomly and destructively harvested at days 3, 12, and 45 after initial exudate addition. Before harvesting the soil samples, four leachate water samples were collected in a 50-mL centrifuge tube and the soil samples were then mixed thoroughly; both leachate water and soil samples were stored at 4 degC for further measurements.

2.3 CO_2 efflux measurement

Gas samples (15 mL) were collected from 500-mL anaerobic bottles every day using a 20-mL syringe before exudate solutions were injected into the soil samples. Subsequently, the serum bottles were flushed with artificial air for another 10 min and then sealed using a rubber stopper. CO_2 concentration was measured using a gas chromatograph equipped with a thermal conductivity detector (Agilent 7890A, Agilent Technologies, Alto Palo, California, USA).

2.4 Soil property analysis

Soil MBC and MBN were determined using the chloroform fumigation extraction method (Wu et al., 1990; Jenkinson et al., 2004). One subsample (20 g) was directly extracted with K_2SO_4 solution (60 mL; 0.5 M); another (also 20 g) was first fumigated with ethanol-free chloroform in the dark for 24 h and then extracted with K_2SO_4solution (60 mL; 0.5 M). The extracted C in the fumigated and unfumigated samples was measured after acidification using the Shimadzu TOC-VWP analyzer (Shimadzu), and inorganic N (NO_3^- and NH_4^+) was extracted with 2 M KCl and analyzed using a continuous-flow analyzer (Fiastar 5000; Foss Tecator AB, Hoganas, Sweden). The TN of the soil samples was determined using an elemental analyzer (MACRO cube, Elementar, Germany).

MBC and MBN were calculated as the difference in C and N contents between fumigated and non-fumigated sample extracts, adjusted by a proportionality coefficient (kEC = 0.45, kEN = 0.54) to account for the extraction efficiency. The 0.5 M K_2SO_4-extractable C from the unfumigated sample was considered as DOC (Vance et al., 1987).

The leaching water samples collected for NO_3^-(NO_3^--L) and NH_4^+(NH_4^+-L) were analyzed using a continuous-flow analyzer (Fiastar 5000; Foss Tecator AB, Hoganas, Sweden). Leaching water DOC (DOC-L) was measured using the Shimadzu TOC-VWP analyzer (Shimadzu).

The metabolic quotient (qCO_2) and microbial carbon-use efficiency (CUE) were calculated using previously published methods (Anderson and Domsch, 1993; Sinsabaugh et al., 2013). qCO_2 was defined as the ratio of C in respired CO_2 and soil microbial biomass (qCO_2 = CO_2-C/MBC) and the CUE was defined as the amount of C in the microbial biomass relative to the sum of the CO_2-C and MBC [CUE = MBC/(MBC + CO_2-C)].

2.5 Enzyme activity measurement

Extracellular enzyme activity was determined using a previously established method (Marx et al., 2001). Fluorogenic methylumbelliferone-based artificial substrates were used to estimate the activity levels of BG, XYL, and NAG, which are involved in soil organic C (BG, XYL) and N (NAG) degradation (Sinsabaugh and Follstad Shah, 2012). The fresh soil (1 g) was prepared by dissolving in 125 mL of 50 mM sodium acetate buffer (pH 5) using low-energy sonication (50 J s^{-1}) for 1 min. A 200-μL aliquot of the slurry was incubated with 50 μL of the corresponding substrate at 20 °C for 4 h in the dark. Ten microliters of 1 M NaOH solution was added to each plate to terminate the reaction before measurement; the fluorescence values were then determined with an automated fluorometric plate reader (Victor3 1420–050 Multi-label Counter; PerkinElmer, Waltham, MA, USA) with an excitation wavelength of 365 nm and an emission wavelength of 450 nm. Enzyme activities were expressed as nM $g^{-1}h^{-1}$.

2.6 Calculations and statistical analysis

Statistical analysis was performed with R Studio (R version 3.5.2, CDN, Global) using one-way ANOVA, and significant differences between single treatments were analyzed using Tukey's post-hoc test ($p < 0.05$). Structural equation modeling (SEM) was performed using the Amos 17.0 software package (Smallwaters Corporation, Chicago, IL, USA) to test the significance of the hypothesized causal relationships among total C, MBC/MBN, BG/NAG, XYL/NAG, DOC/NH_4^+, DOC/NO_3^-, and CO_2emissions. The log-transformed CO_2, SOC, DOC/NH_4^+, DOC/NO_3^-, MBC/MBN, BG/NAG, and XYL/NAG were used to perform SEM on the data statistics and conceptual assumptions (Hu et al., 2014). The best-fit model was determined using a chi-square test (χ^2), P -values, goodness-of-fit index (GEI), root mean square error of approximation (RESEA), and Akaike information criteria (Chen et al., 2016).

RESULTS

3.1 SOM mineralization response to artificial root exudates

The temporal patterns of CO_2 emissions from all artificial root exudate–treated soils were similar. The CO_2 efflux was highest at the beginning of the incubation (days 1–4), and exponentially decreased until day 15. Furthermore, it reached a stable level with only minor fluctuations until the end of the chasing period (Fig. 1a). Compared with the soil without additions (Control), the input of artificial root exudates led to an increase in cumulative CO_2 emissions by 25%, 25%, 20%, and 19%, as CN6 > CN10 > CN80 > C-only, respectively (Fig 1b).

The input of root exudates with low C/N ratios (CN6 and CN10) significantly increased qCO_2 on day 3. However, the qCO_2 decreased from day 3 to day 45 in all treatments in the following order: CN6 > CN10 > CN80 > C-only > Control (Fig. 2a). In contrast, the CUE increased from day 3 to day 45 (Fig. 2b). The addition of artificial root exudates likewise decreased the CUE value in the following order: control > C-only > CN80 > CN10 > CN6.

3.2 Responses of enzymes activity and microbial biomass to artificial root exudates

Compared with the control, the activities of the three enzymes measured significantly increased. The BG and XYL enzymes increased in the order C-only > CN80 > CN10 > CN6 > Control (Fig. 3). Conversely, NAG activity decreased with decreasing N content in the simulated root exudates on day 3, following the order of CN 6 > CN10 > CN80 > C-only. Increasing the BG/NAG ratio increased qCO_2($R^2 = 0.41$, $P = 0.01$) and suppressed the CUE ($R^2 = 0.36$, $P = 0.02$) (Fig. 4a). Compared with the control, MBC and MBN remarkably increased (except in CN6 and C-only). However, there was a minor rise from day 3 to day

45 in all treatments (Table S2). C/N ratios of the microbial biomass ranged from 3.2 to 7.8 (Fig. 4c). The MBC/MBN ratio was negatively correlated with qCO_2 ($R^2 = 0.29$, $P = 0.01$) and CUE ($R^2 = 0.36$, $P = 0.02$) (Fig. 4b).

3.3 Structural equation modeling analysis on CO2 efflux

To quantify the effects of stoichiometry of root exudates on CO_2 efflux as a proxy of SOM mineralization, a SEM was constructed based on biotic parameters (microbial biomass and enzyme activity) and products of biological activity (DOC and NH_4^+). The SEM showed a reasonable fit to our hypothesized causal relationships (GEI = 0.98, Chi/DF= 1.00, RESEA < 0.01. Fig. 5) and explained 58% of the variance in CO_2 emissions using all available biotic parameters and the products of biological predictors. The increase in C/N ratios had a negative effect on CO_2 emissions (-0.29, $P< 0.05$, Fig. 6). Extracellular enzyme stoichiometry (BG/NAG and XYL/NAG ratios) exerted a positive direct effect (+0.39, $P< 0.001$), whereas the DOC/NH_4^+ ratio showed a negative direct effect (-0.30, $P < 0.001$).

DISCUSSION

4.1 Microbial response to the addition of root exudates

Artificial root exudates were designed by using three low-molecular organic compounds in four different C/N ratios, which are easily degradable and accessible by the microbial community (de Graaff et al., 2010; Bastida et al., 2013). When C and N are sufficient, microorganisms prefer to easily utilize compounds from root exudates than from native SOM (Shahbaz et al., 2017; Wei et al., 2019). We found that the addition of root exudates stimulated microbial activity, as indicated by large accumulative CO_2 emissions. These emissions responded differently to various stoichiometric ratios of root exudates with the same amount of C input, indicating that microbial activity is modified by N demand. Higher activity of the N-acquiring extracellular enzyme (NAG) was observed in C-rich soil (N limit at high C/N ratio). Thus, a continuous supply of low C/N ratio artificial root exudates increased N availability and decreased N-acquiring enzymes (Fig. 3). This is consistent with previous studies that have applied stoichiometric root exudates exacerbate CO_2 emission due to microbial N demand in paddy soils (Liu et al., 2020). However, in contrast to the observation of Liu et al. (2020), we found that a higher proportion of N in artificial root exudates stimulated more CO_2 emission than at high C/N ratios (CN6 [?] CN10 > CN80 [?] C-only) (Fig. 1a, b), and the C-acquiring enzyme activity (BG and XYL) was increased by a higher C/N ratio of artificial root exudates. These differences due to the different forms of N nutrients [$(NH_4)_2SO_4$ vs. alanine] added to paddy soils.

Furthermore, unlike labile inorganic forms of N (NH_4^+ and NO_3^-), N-containing low-molecular organic compounds (alanine in this study) can decrease microbial nutrient limitation from C/N ratios: carbohydrate hydrolase has been shown to increase with increasing C/N stoichiometric ratios of root exudates due to unbalanced nutrient addition (Allison and Vitousek, 2005; Sinsabaugh et al., 2008). On the other hand, higher N-rich artificial root exudates (CN6) showed higher qCO_2 and lower CUE than other treatments at day 3 and day 12, whereas C-only caused low CUE (Fig. 2a, b). A higher proportion of organic N-containing root exudates may have promoted microbial catabolism relative to anabolism, as there is a higher energy demand for microorganisms in acquiring organic N compared to inorganic N (Näsholm and Persson, 2001; Czaban et al., 2016). Moreover, the positive relationship between BG/NAG ratio and qCO_2 suggested that microbial catabolism of root exudates attributed to microorganisms enhancing C- and N-acquiring enzymes to obtain available C and N from both root exudates and SOM. Labile C from root exudates can be quickly utilized by microorganisms for nutrients acquiring to enhance microbial consumption of energy and released as CO_2 (Zhu et al., 2018).

Along with the increase in CO_2 emission, the continuous supply of artificial root exudates caused lower microbial CUE in all treated soils than in the control. This indicated that the addition of root exudates caused substrate and nutrient uptake by microorganisms (Blagodatskaya et al., 2014; Chen et al., 2020). Simultaneously, the higher CUE corresponded to a higher C/N stoichiometric ratio, except in C-only. This indicated that N-containing stimulates affected microbial catabolic activity, and that low N-containing formations of root exudates (i.e., with high C/N ratios) increased the CUE for SOC accumulation. A positive relationship

between the MBC/MBN and CUE (Fig. 4d) suggested that the stoichiometry of microbial biomass played a key regulator in promoted microorganism utilize input labile C source and increased the soil C accumulation (Sinsabaugh et al., 2016; Soares and Rousk, 2019). These findings thus supported our hypothesis that the addition of a higher C/N ratio with decreasing N content increased microbial biomass with lower CO_2 emissions and stimulated soil C sequestration.

4.2 Combining microbial C and N metabolism in the SEM

Root exudates are ubiquitous and quantitatively important drivers of SOM turnover in flooded paddy soil ecosystems. The stoichiometric ratios of resources that are important as energy and nutrients are dominant drivers for the biogeochemical cycles of soil C (McGroddy et al., 2004; Anderson et al., 2005).

There are two stoichiometric processes that combine microbial C and N metabolism of root exudates in paddy soils (Figs. 5, 6, and 7). First, the continuous supply of root exudates caused a C-rich condition, and thus increased microbial activity via overflow respiration of root exudates. Different C/N stoichiometric ratios of root exudates affected the elemental stoichiometry demands for microorganisms to maintain the microbial biomass C/N stoichiometric balance (Haichar et al., 2014; Liu et al., 2020; Du et al., 2020; Zhu et al., 2021). Higher energy demand for microorganisms to acquire organic N from amino acids can further increase respiration. In the present study, the stoichiometric ratios of root exudates (i.e., C-only) for microbe-regulated C mineralization did not dependent on nutrient (N-containing compounds) limitation, as the lack of N content did not affect the allocation of energy through C mineralization. Second, a combination of exogenous C and N from root exudates is required for the adaptation of microbe catabolic activity by the enhancement of C- and N-acquiring enzyme activity and by causing catabolism of soil C (Liu et al., 2020; Mori et al., 2021). This was confirmed by the results of this study, which identified a significant positive relationship between CO_2 emissions and enzyme stoichiometry. The stoichiometric ratios of root exudates provide a perspective on microbial stoichiometric requirement of nutrients and leads us to further understand the root exudate–soil–microbe interactions in the rhizosphere soil.

CONCLUSIONS

In this study, we used artificial root exudates with different C/N ratios to clarify the association of elemental stoichiometric ratios in microbial biomass and extracellular enzyme activities with soil C metabolism. We found that the addition of C-only decreased CO_2 emissions attribute to the N limitation. Simultaneously, all three different C/N ratios of root exudates (CN6, CN10, and CN80) increased microbial activities and catabolism to meet the microbial biomass ratios, thus promoting SOM mineralization. Microorganisms preferred to use easily available low-molecular compounds that lead to increased CO_2 emissions, corresponding with increased stoichiometric ratios of C- and N- hydrolases to meet microbial nutrient demands. It is, therefore, the input of root exudates with low C/N stoichiometric ratio stimulate soil C mineralization, while the inputs with high C/N stoichiometric ratio benefit soil C accumulation. These results highlight the fact that the stoichiometric of root exudates represent important derivers for understanding C cycling in plant-soil system.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Amounts of individual substrates added (mg incubation flask^{-1} day^{-1}) to the paddy soil as artificial root exudates in the different treatments.

Treatment	Glucose (C) ($C_6H_{12}O_6$)	Oxalic acid (C) ($H_2C_2O_4$)	Alanine (C) ($C_3H_7NO_2$)	Alanine (N) ($C_3H_7NO_2$)
Control	0	0	0	0
CN6	0.14	0.09	0.17	0.07
CN10	0.22	0.07	0.11	0.04
CN80	0.34	0.05	0.01	0.01
C-only	0.35	0.05	0	0

Figures

Figure 1

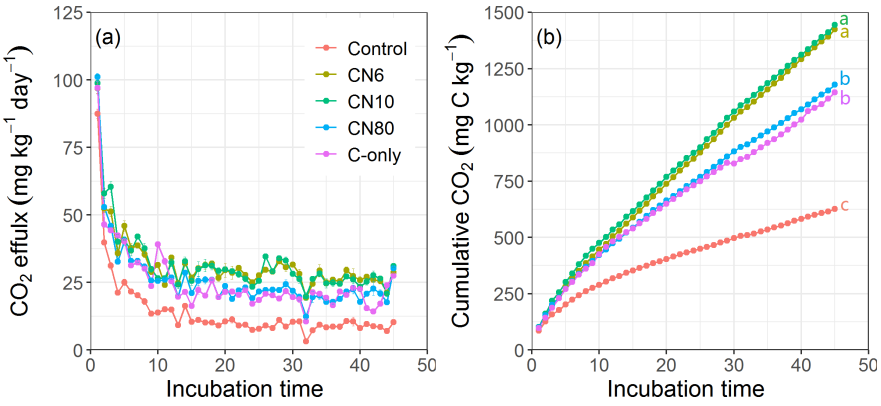


Figure 2

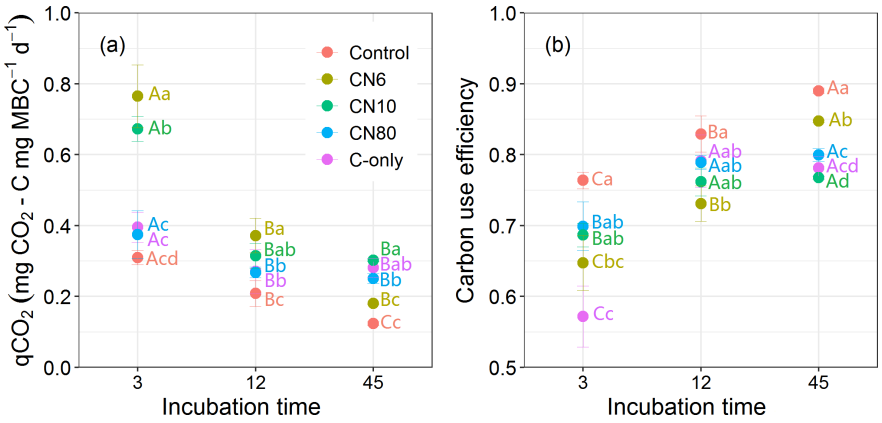
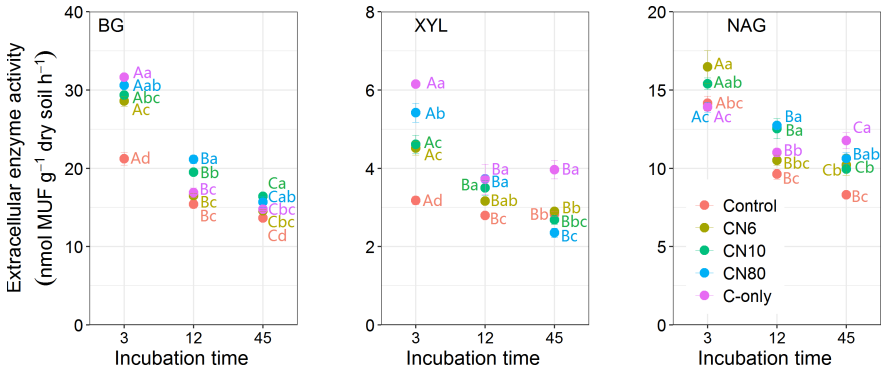


Figure 3



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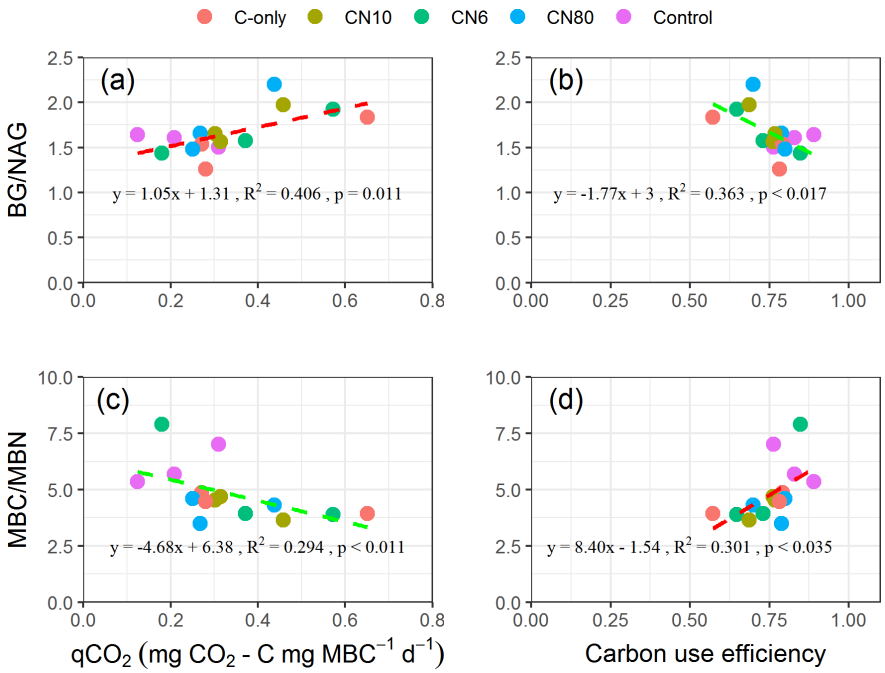

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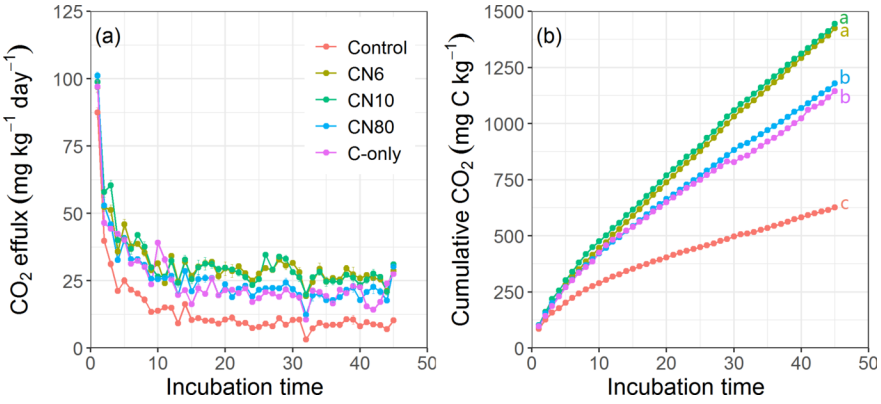

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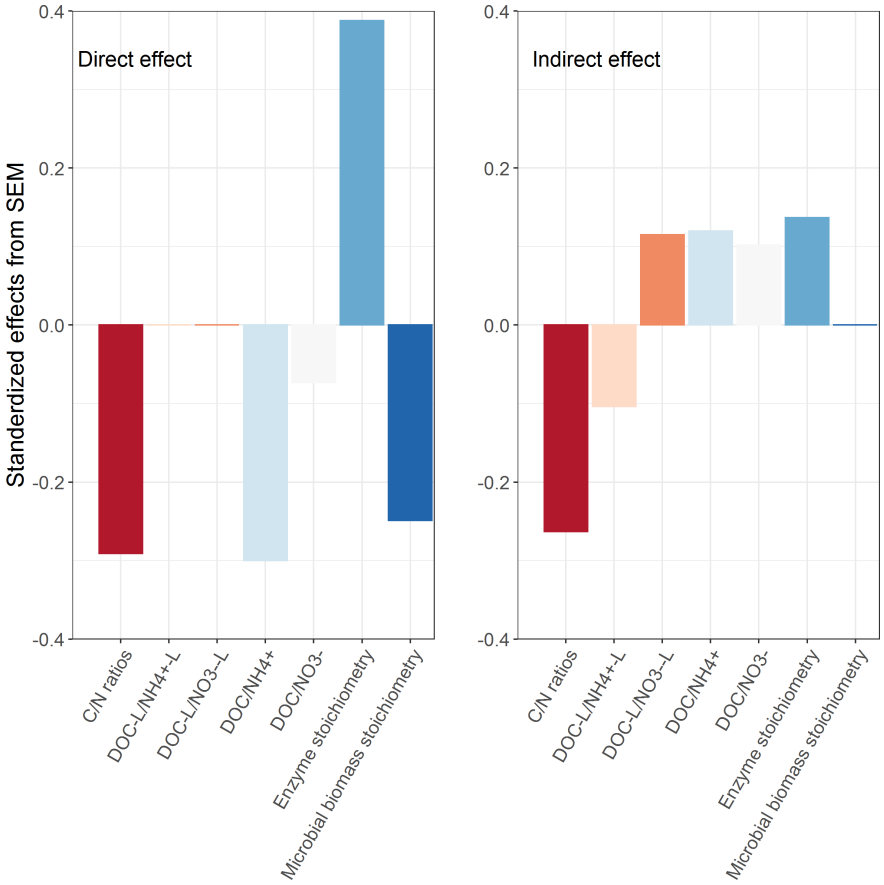

Figure 7

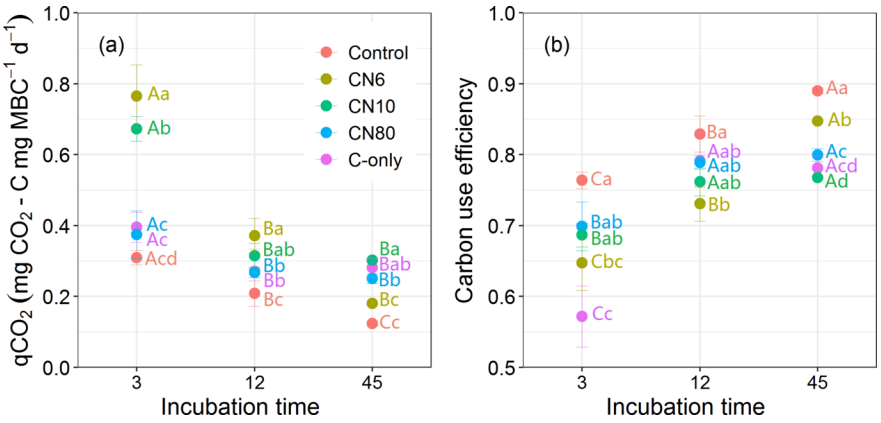

Figure legends

Fig. 1. CO_2 efflux rates (a) and cumulative CO_2 (b) over the 45-day incubation period. The control represents no addition of artificial root exudates to soil; C-only represents addition of C substrates glucose and oxalic acid only; CN6, CN10, and CN80 represent addition of C substrates glucose and oxalic acid as well as the N substrate alanine at different C/N stoichiometries of CN6, CN10, and CN80, respectively. Different letters indicate significant differences among stoichiometric ratios at the end of cumulative CO_2 emission (one-way ANOVA and LSD test;$P < 0.05$ level). Error bars show standard errors (n = 4).

Fig. 2. Metabolic quotient of soil microbial biomass (qCO_2) (a) and carbon use efficiency (CUE) (b) at days 3, 12, and 45 during the 45-day incubation period. C-only represents addition of C substrates glucose and oxalic acid only; CN6, CN10, and CN80 represent addition of C substrates glucose and oxalic acid as well as the N substrate alanine at different C/N stoichiometries of CN6, CN10, and CN80, respectively. Different letters and capital letters indicate significant differences among stoichiometric ratios and sampling periods, respectively (one-way ANOVA and LSD test; $P< 0.05$ level). Error bars show standard errors (n = 4).

Fig. 3. Activity of extracellular enzymes β-1,4-glucosidase (BG), β-1,4-xylosidase (XYL), and β-1,4-N-acetyl-glucosaminidase (NAG) at days 3, 12, and 45 during the 45-day incubation period. C-only represents addition of C substrates glucose and oxalic acid only; CN6, CN10, and CN80 represent addition of C substrates glucose and oxalic acid as well as the N substrate alanine at different C/N stoichiometries of CN6, CN10, and CN80, respectively. Different letters and capital letters indicate significant differences among stoichiometric ratios and sampling periods, respectively (one-way ANOVA and LSD test; $P< 0.05$ level). Error bars show standard errors (n = 4).

Fig. 4. Relationships between metabolic quotient (qCO_2) and soil enzyme stoichiometry (BG/NAG) (a), qCO_2 with microbial biomass stoichiometry (MBC/ MBN) (c); carbon use efficiency (CUE) with soil enzyme stoichiometry (BG/NAG) (b), CUE with microbial biomass stoichiometry (MBC/ MBN) (d). Abbreviations are BG, β-1,4-glucosidase; NAG, β-1,4-N-acetyl glucosaminidase. The dots represent data from all treatments in the 45-day incubation period.

Fig. 5. Structural equation model of the multivariate effects of C/N ratios, leaching water sample DOC-L/NO_3^--L ratios and DOC-L/NH_4^+-L ratios, soil sample DOC/NO_3^- ratios and DOC/NH_4^+ ratios, microbial biomass stoichiometry, and enzyme stoichiometry on the CO_2emission. Microbial biomass stoichiometry represents the ratios of microbial biomass C to microbial biomass N; enzyme stoichiometry represents the average ratios of the activity of β-1,4-glucosidase to β-1,4-N-acetyl glucosaminidase and the activity of β-1,4-xylosidase to β-1,4-N-acetyl glucosaminidase. The solid lines indicate positive path coefficients; dashed lines, negative. R^2 values indicate the proportion of variance explained by each variable contribution to the CO_2 emission from soil application with substrates. The numbers and the width of the arrows indicate the standardized path coefficients.

Fig. 6. Standardized effect of stoichiometric C/N ratios, leaching water sample DOC-L/NO_3^--L ratios and DOC-L/NH_4^+-L ratios, soil sample DOC/NO_3^- ratios and DOC/NH_4^+ ratios, enzyme stoichiometry, and microbial biomass stoichiometry on CO_2 emission.

Fig. 7. Schematic diagram emphasizing the importance of the stoichiometric control of plants by their release of root exudates of different N contents in the flooded rice-paddy soil system. C and N circles represent artificial root exudates; DOC-W represents dissolved organic C from water solution; SOM represents the soil organic matter; microbial biomass stoichiometry represents the C to N ratios of microbial biomass; enzyme stoichiometry represents the ratios of the activity of β-1,4-glucosidase to β-1,4-N-acetyl glucosaminidase and the activity of β-1,4-xylosidase to β-1,4-N-acetyl glucosaminidase. The solid lines represent the transportation of microbial active pathways from stoichiometric controls of root exudates.

Supporting information

Table S1. Amounts of individual substrates added (mg incubation flask-1) to the paddy soil as artificial root exudates in the different treatments.

Treatment	Glucose (C) ($C_6H_{12}O_6$)	Oxalic acid (C) ($H_2C_2O_4$)	Alanine (C) ($C_3H_7NO_2$)	Alanine (N) ($C_3H_7NO_2$)
Control	0	0	0	0
CN 6	6.17	4.11	7.71	3.00
CN 10	9.82	3.27	4.91	1.91
CN 80	15.10	2.32	0.58	0.23
C-only	15.60	2.40	0	0

Table S2 . Soil C/N ratios, microbial biomass C (MBC), microbial biomass N (MBN), DOC, and NH_4^+ at days 3, 12, and 45 during the 45-day incubation period. C-only represents addition of C substrates glucose and oxalic acid only; CN6, CN10, and CN80 represent addition of C substrates glucose and oxalic acid as well as the N substrate alanine at different C/N stoichiometries of CN6, CN10, and CN80, respectively. Different letters indicate significant differences among stoichiometric ratios at the end of cumulative CO_2 emission (one-way ANOVA and LSD test; $P< 0.05$ level). Values represent means + standard errors (n = 4).

Treatment	**3 day**	**3 day**	**3 day**	**3 day**	**3 day**
	C/N ratios	MBC (mg/kg)	MBN (mg/kg)	DOC (mg/kg)	NH_4^+ (mg/kg)
Control	$8.71+0.13^{Bab}$	$101.48+11.00^{Aab}$	$13.80+1.97^{Ac}$	$140.02+5.84^{Aa}$	$84.69+1.49^{Abc}$
CN6	$8.44+0.49^{Ad}$	$92.50+20.09^{Bb}$	$24.25+1.46^{Ab}$	$153.91+10.06^{Aa}$	$90.47+2.00^{Aa}$
CN10	$8.65+0.08^{Bc}$	$117.23+7.10^{Aa}$	$22.37+1.66^{Aa}$	$142.71+4.82^{Aa}$	$87.03+2.22^{Aab}$
CN80	$8.88+0.09^{Cab}$	$111.94+34.90^{Bab}$	$25.91+0.68^{Bb}$	$146.17+13.46^{Aa}$	$82.78+2.59^{Ac}$
C-only	$9.18+0.11^{Ba}$	$61.10+15.49^{Cc}$	$15.70+1.55^{Bc}$	$152.34+4.51^{Aa}$	$83.83+1.06^{Abc}$
Treatment	**12 day**	**12 day**	**12 day**	**12 day**	**12 day**
	C/N ratios	MBC (mg/kg)	MBN (mg/kg)	DOC (mg/kg)	NH_4^+ (mg/kg)
Control	$9.40+0.23^{Aa}$	$74.92+16.25^{Cc}$	$13.12+1.10^{Ac}$	$118.26+6.55^{Ba}$	$87.35+10.92^{Bc}$
CN6	$8.84+0.22^{Ad}$	$95.72+23.31^{Bb}$	$24.25+1.46^{Ab}$	$107.00+3.72^{Ba}$	$75.87+7.78^{Aa}$
CN10	$8.87+0.22^{Bc}$	$105.86+21.85^{Ba}$	$22.37+1.66^{Bb}$	$113.92+5.44^{Ba}$	$65.09+4.97^{Bab}$
CN80	$9.18+0.08^{Bab}$	$100.75+11.83^{Cb}$	$31.09+2.61^{Aa}$	$113.92+5.44^{Ba}$	$65.09+4.97^{Bd}$
C-only	$9.01+0.20^{Bab}$	$104.17+34.14^{Aa}$	$21.85+1.61^{Ab}$	$114.93+12.59^{Ba}$	$76.36+6.54^{Bab}$
Treatment	**45 day**	**45 day**	**45 day**	**45 day**	**45 day**
	C/N ratios	MBC (mg/kg)	MBN (mg/kg)	DOC (mg/kg)	NH_4^+ (mg/kg)
Control	$9.59+0.46^{Aa}$	$82.98+3.31^{ABd}$	$15.50+0.47^{Ad}$	$73.94+9.39^{Ca}$	$19.07+1.15^{Cd}$
CN6	$8.88+0.26^{Ab}$	$159.45+2.39^{Aa}$	$20.24+1.11^{Bc}$	$68.52+4.92^{Ca}$	$95.74+3.36^{Aa}$
CN10	$9.43+0.08^{Aa}$	$102.82+0.83^{Cc}$	$22.66+0.87^{Bb}$	$72.76+2.58^{Ca}$	$73.22+5.31^{Bb}$

CN80	9.65 ± 0.15^{Aa}	121.17 ± 11.54^{Ab}	26.41 ± 1.22^{Ba}	66.49 ± 9.79^{Ca}	36.72 ± 0.97^{Cc}
C-only	9.65 ± 0.08^{Aa}	99.66 ± 13.99^{Bc}	22.25 ± 0.61^{Ab}	74.29 ± 2.78^{Ca}	37.45 ± 1.21^{Cc}