Soil Biology and Biochemistry 168 (2022) 108636

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

The mechanism of the dose effect of straw on soil respiration: Evidence from enzymatic stoichiometry and functional genes

Shuailin Li [a,*], Yongxing Cui [b,1], Zhuqing Xia [a], Xinhui Zhang [a], Mengmeng Zhu [a], Yun Gao [a], Siyu An [a], Wantai Yu [a,**], Qiang Ma [a,***]

[a] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, 110016, China
[b] Sino-French Institute for Earth System Science, College of Urban and Environmental Sciences, Peking University, Beijing, 100871, China

ARTICLE INFO

Keywords:
Agricultural ecosystems
Soil C cycling
Ecoenzymatic stoichiometry
Microbial CUE
Microbial metabolic limitation
Functional genes

ABSTRACT

Straw return to soil is a global field practice for sequestering carbon (C) in agricultural ecosystems, and soil C mineralization depends on the soil microbial metabolic process. However, the variation patterns of microbial respiration (Rs) and associated mechanisms under long-term straw input at different levels remain unclear. Here, this study investigated the changes in Rs and microbial metabolic limitation under straw input at four levels (0, 4, 8, and 12 t ha^{-1} yr^{-1}) based on a long-term (11-year) field experiment. In addition, the C use efficiency (CUE) and C degradation genes were quantified via an enzyme-based biogeochemical-equilibrium model and high-throughput quantitative PCR-based chip technology, respectively. The results indicated that Rs significantly increased with the amount of straw addition, while its rate of increase dropped when the straw addition amount was greater than 8 t ha^{-1} yr^{-1}. Interestingly, we also observed an apparent microbial P limitation under straw addition at 0 and 4 t ha^{-1} yr^{-1} but a shift to N limitation when the straw addition rate was over 8 t ha^{-1} yr^{-1}. The shift suggested that Rs changes could be attributed to straw addition leading to soil microbes being increasingly limited by N rather than P. Moreover, straw addition significantly increased microbial biomass, reduced CUE and increased the absolute abundance of genes involved in degrading various organic polymers (e.g., starch, hemicellulose, cellulose, chitin and lignin). Partial least squares path modeling revealed that the variation in Rs was directly attributed to increased microbial biomass and C degradation genes as well as declining CUE, while C degradation genes and CUE were mediated by microbial relative C limitation and N *vs.* P limitation. This study provides insight into the mechanisms of the Rs response to straw addition by linking the Rs to microbial metabolic limitation, CUE and C degradation genes, highlighting that reducing microbial nutrient limitation by balancing metabolic demand and environmental nutrient supply potentially leads to a higher microbial CUE and lower Rs in agricultural ecosystems.

1. Introduction

Crop residue return to soils has been recommended globally in field practices to improve soil organic matter (SOM) levels (Fang et al., 2018; Cong et al., 2020). However, some field studies have shown that this technique is often not very effective in terms of increasing SOM, and the efficiency (i.e., the increase in SOM per unit of input) of crop residue incorporation decreases with the amount added (Heitkamp et al., 2012; Poeplau et al., 2015; Shahbaz et al., 2017). A lower efficiency suggests that SOM mineralization, manifested as soil microbial respiration (Rs), sharply increases as more crop residue is added. To date, the variation pattern of Rs under long-term straw return at different levels remains debatable (Paterson and Sim, 2013; Shahbaz et al., 2017; Berhane et al., 2020). For example, Guenet et al. (2010) reported that Rs with wheat straw addition is a nonlinear function that reaches saturation under the addition of 2.2 g straw kg^{-1} soil. In contrast, Poirier et al. (2013) reported an almost linear increase in Rs with the addition of up to 40 g straw-C kg^{-1} soil.

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: lishuailin@007.com (S. Li), wtyu@iae.ac.cn (W. Yu), qma@iae.ac.cn (Q. Ma).
[1] These authors contributed equally to this work and should be considered co-first authors.

https://doi.org/10.1016/j.soilbio.2022.108636
Received 22 December 2021; Received in revised form 17 March 2022; Accepted 18 March 2022
Available online 21 March 2022

Whether Rs increases nonlinearly or linearly with crop residue addition depends on the responses of microbial biomass and activity (Shahbaz et al., 2017; Fang et al., 2019). With sufficient C supply, an increasing C-to-nutrient ratio may not meet microbial stoichiometric requirements and may thus accelerate microorganism degradation of SOM to acquire nutrients (Ghimire et al., 2017; Li et al., 2019). This mechanism of microbial nutrient mining could generally explain the observed decrease in SOM stabilization with an increase in crop residue addition amount (Shahbaz et al., 2017). In addition, balancing soil nutrient stoichiometry could improve the conversion efficiency of residue carbon to soil organic carbon (SOC) (Fang et al., 2019). In contrast, the addition of N and/or P fertilizer may result in the cometabolism of straw-C and native SOM due to the stoichiometric anastomosis of microbes and resources, thus facilitating microbial activity and extracellular enzyme production (Zhu et al., 2018). Therefore, to understand the mechanism behind the variation in Rs, it is necessary to link Rs to the microbial metabolic characteristics under different straw addition amounts.

Microbial stoichiometric flexibility regulates soil C turnover by maintaining a nutrient stoichiometric balance between microbial requirements and environmental resources (Zhu et al., 2018). Most free-living soil microbial communities are limited or co-limited by energy (i.e., C) or nutrients (i.e., N or P) (Sinsabaugh and Shah, 2012; Cui et al., 2021), and they can acclimate to these limitations by reassigning more resources for nutrient acquisition than growth (Schimel et al., 2007; Cui et al., 2018). For example, even though a high level of crop residue input enhances microbial growth, microbial nutrient limitation may activate the nutrient mining mechanism and lead to overflow Rs (Sinsabaugh et al., 2013; Spohn et al., 2016). The decomposition of polymers in dead organic matter (e.g., cellulose, hemicellulose and lignin) mainly proceeds under the action of the enzymes of heterotrophic microorganisms (Waring et al., 2014; Cui et al., 2018). Moreover, the relative enzymatic activities of C *vs*. P and C *vs*. N can reflect relative resource allocation toward C, N, and P acquisition, which is also related to microbial C use efficiency (CUE) (Sinsabaugh et al., 2013). The relative microbial C limitation and nutrient (N/P) limitation can be quantified by a "vector model" that calculates clear metrics using the proportional activities of C/N/P-acquiring enzymes (Moorhead et al., 2016). For example, Ma et al. (2021b) used this method to determine that straw return at 9 t ha^{-1} could alleviate microbial P limitation by releasing available P from SOM and straw. However, there is a knowledge gap regarding the patterns of microbial metabolic limitation under different levels of crop residue input. In addition, the cascading relationships linking Rs to microbial metabolic limitation, CUE and C degradation potential remain to be illuminated.

In this study, we carried out a long-term field experiment of straw return at different levels, investigated the patterns in Rs and determined the microbial metabolic limitations and CUE using enzymatic stoichiometry and biogeochemical equilibrium models (Sinsabaugh and Shah, 2012; Moorhead et al., 2016). Furthermore, the abundances of genes related to organic matter decomposition were quantified to reflect the decomposition potential of various organic polymers. We hypothesized that (1) Rs increases linearly with the straw addition amount; (2) the direction and magnitude of microbial metabolic limitation are affected by the straw addition amount; and (3) microbial metabolic limitation influences Rs by mediating CUE and C degradation gene expression. By testing these scientific hypotheses, this study seeks to clarify the microbial mechanisms behind the change trends of Rs with increasing straw input.

2. Materials and methods

2.1. Study site and experimental design

The long-term straw return field experiment was conducted at the National Field Observation and Research Station of Shenyang Agroecosystems (41°31′ N, 123°24′ E, 41 m altitude), Chinese Academy of Sciences, Liaoning Province. The experimental station is located in southern Northeast China on the Liaohe Plain, where the topography is characterized by a temperate semihumid continental monsoon climate. The study area has four distinct seasons, with hot and rainy summers and dry and cold winters. The annual temperature is 7–8 °C, and the annual precipitation is 650–700 mm, of which more than 80% occurs from May to September. The soil contains 16.2% sand, 59.6% silt and 24.1% clay, and it is classified as Alfisol with the local name of brown soil. The physicochemical properties of the soil (0–20 cm) before straw return in 2009 were as follows: bulk density 1.06 g cm^{-3}, pH 7.21, soil organic carbon 10.89 g kg^{-1}, total N 0.98 g kg^{-1}, and Olsen-P 7.36 mg kg^{-1}. Historically, the field site was cultivated under a maize (*Zea mays* L.) system, and aboveground residues (i.e., leaves and stems) were removed from the field after harvest in late September from 1990 to 2009.

In October 2009, the straw return trial was established using a randomized block design with four replications, including four levels of the dry mass of maize straw: no straw as a control (CK), a low level of 4 t ha^{-1} $year^{-1}$ (S4), a middle level of 8 t ha^{-1} $year^{-1}$ (S8) and a high level of 12 t ha^{-1} $year^{-1}$ (S12). Each plot had an area of 7.2 m^2 (1.8 m × 4 m). The four sides of each plot were surrounded by cement walls (0.5 m in thickness and 2 m in depth). In addition, each plot was separated by a 0.5 m wide pathway to avoid cross contamination and facilitate investigation. Every October after harvest, air-dried aboveground residues (i.e., stems and leaves) were chopped to 2–3 cm using an electric crusher. Then, according to the experimental design, the chopped crop residues were manually incorporated uniformly into the soil at depths of 0–20 cm using a shovel. In the following May, maize (*Dongdan* 72 cultivar) was seeded using 25 cm intervals and 60 cm row spacing following local conventional tillage methods. N fertilizer (urea; 46% N) and P fertilizer (calcium triple superphosphate) were applied at a rate of 150 kg N ha^{-1} $year^{-1}$ and 90 kg P_2O_5 ha^{-1} $year^{-1}$, respectively, for each plot. Forty percent N fertilizer (45 kg N ha^{-1}) and 100% P fertilizer were applied as basal fertilizer by banded application before sowing and were then plowed into the 10 cm soil layer by shovel. The remaining 60% of N fertilizer (90 kg N ha^{-1}) was applied at the jointing stage using side-dressing (on either side of the stem within 5 cm). Hand weeding was performed as needed, and natural rainfall was the only water supply for each plot during the experiment (i.e., no irrigation was applied).

2.2. Soil sampling

In late September 2020 (11 years after the first straw input), bulk soil samples of the 0–20 cm layer were collected before straw return that year. In particular, visible roots did not appear in the soil samples during field sampling, which excluding the effect of root-derived C. Six cores were randomly collected in each plot using a soil auger with a 2 cm inner diameter after harvest, and then, the soil cores from one plot were mixed into one composite sample. After visible debris was removed, the samples were sieved through a 2-mm nylon sieve, and each composite sample was divided into three parts. One part of the soil was placed in an icebox containing drikold pellets, and another part was placed in an icebox containing ice bags and then transported to the laboratory. The first part was stored at −80 °C for soil DNA extraction. The second part was used to determine the soil microbial biomass, enzyme activities and soil inorganic N concentration. The remaining soil samples were dried at room temperature to measure other soil physicochemical properties.

2.3. Soil physicochemical measurements

SOC and total N were determined by an elemental analyzer (VariEL III, Elementar, Germany) after removing carbonates through pretreatment with 1 M HCl. Samples analyzed for dissolved organic C (DOC) and N (DON) were extracted with 0.5 M K_2SO_4 (soil:solution ratio, 1:4) for 1 h and filtered through 0.45 μm membranes (Jones and Willett, 2006).

Then, the extracts were measured by a LiquiTOCII analyzer (Elementar, Germany). NH_4^+ and NO_3^- were extracted with 2 M KCl and measured by flow injection analysis (TRAACS 2000 Bran and Luebbe, Germany). The total P (TP) and Olsen-P were determined by the Olsen method (Olsen and Sommers, 1982).

2.4. Quantification of microbial biomass and microbial basal respiration

The microbial biomass C, N and P (MBC, MBN and MBP) were determined by the chloroform fumigation-extraction method (Brookes et al., 1982, 1985; Vance et al., 1987), and the conversion factors of MBC, MBN and MBP were 0.45, 0.54 and 0.40, respectively (Wu et al., 1990; Joergensen and Mueller, 1996). The experimental procedure for the assessment of microbial biomass has been described in a previous study (Li et al., 2017).

After preincubation of fresh soil at 25 °C in the dark for one week, Rs was measured using 10 g soil in a 100 ml sealed serum bottle, and incubation was performed with four negative controls (without soil) for 36 h at 25 °C in the dark (Chen et al., 2020). The concentration of CO_2 was determined using a gas chromatograph (7890 A; Agilent Technologies, USA). Rs was calculated from the net accumulation of CO_2 over time and expressed as μg CO_2–C kg^{-1} h^{-1}.

2.5. Enzymatic activity assays and quantification of microbial metabolic limitation

The potential activities of three C-acquiring enzymes (β-1,4-glucosidase (BG), β-xylosidase (XYL), and β-D-cellobiosidase (CBH)), two N-acquiring enzymes (β-1,4-N-acetylglucosaminidase (NAG) and L-leucine aminopeptidase (LAP)), and one organic-P-acquiring enzyme (alkaline phosphatase (AP)) were quantified using modified versions of standard fluorometric techniques (Table S1) (Saiya-Cork et al., 2002; German et al., 2011). The experimental procedure has been described in detail (Cui et al., 2019). The activities of enzymes were expressed as nanomoles of substrate released per hour per gram of SOM (nmol g SOM^{-1} h^{-1}).

Based on the theory of enzymatic stoichiometry, the microbial metabolic limitation was obtained by the vector model using untransformed proportional activities (Moorhead et al., 2016). The vector length (unitless) represents the size of the relative C *vs.* nutrient limitation (Eq. (1)). The vector angle (°) represents the relative N *vs.* P limitation calculated by Eq. (2). Vector angles <45° represent microbial metabolism that is more limited by N than P, where the extent increases with decreasing angle; vector angles >45° represent microbial metabolism that is more limited by P than N, where the extent increases with increasing angle.

$$Vector\ length\ (unitless) = SQRT\ (x^2 + y^2) \quad (1)$$

where x denotes the relative enzymatic activities of C acquisition *vs.* P acquisition (i.e., [BG + XYL + CBH]/[BG + XYL + CBH + AP]) and y denotes the relative enzymatic activities of C acquisition *vs.* N acquisition (i.e., [BG + XYL + CBH]/[BG + XYL + CBH + NAG + LAP]).

$$Vector\ angle\ (°) = DEGREES\ (ATAN2\ (x, y)) \quad (2)$$

2.6. Quantification of microbial CUE

Microbial CUE was calculated by Eqs. (3)–(5) based on the biogeochemical equilibrium model (Sinsabaugh and Shah, 2012). The model postulates that when the microbial biomass stoichiometry matches the ratios of assimilable nutrients, microbial growth reaches the highest rate. In addition, the geometric mean of the relative supplies of N to C and P to C is proportional to the microbial growth efficiency.

$$S_{C:N} = MB_{C:N}/L_{C:N} \times 1/EEA_{C:N} \quad (3)$$

$$S_{C:P} = MB_{C:P}/L_{C:P} \times 1/EEA_{C:P} \quad (4)$$

$$CUE = CUE_{max} \times \{(S_{C:N} \times S_{C:P})/[(K_{C:N} + S_{C:N}) \times (K_{C:P} + S_{C:P})]^{0.5} \quad (5)$$

where $MB_{C:N}$ and $MB_{C:P}$ denote the molar ratios of MBC:MBN and MBC:MBP, respectively. $L_{C:N}$ and $L_{C:P}$ denote the molar ratios of labile substrate C:N and C:P, respectively. The labile substrate contents of C, N and P were quantified as dissolved C, N and P, which were extracted from nonfumigated soil samples in microbial biomass assays (Cui et al., 2020). $EEA_{C:N}$ and $EEA_{C:P}$ denote [BG + XYL + CBH]/[BG + XYL + CBH + NAG + LAP] and [BG + XYL + CBH]/[BG + XYL + CBH + AP], respectively. $K_{C:N}$ and $K_{C:P}$ denote the half-saturation constants for CUE based on the availability of C, N, and P. For all model scenarios, $K_{C:N}$ and $K_{C:P}$ were assumed to be 0.5, and CUE_{max} was assumed to be 0.6 (Sinsabaugh and Shah, 2012).

2.7. DNA extraction and C degradation gene quantification

DNA was extracted from 0.5 g of soil using the FastDNA Spin Kit for Soil (MP Biomedicals, USA) according to the manufacturer's instructions. The quality of DNA was assessed by ultraviolet absorbance (NanoDrop, Technology, Wilmington, USA). The DNA concentration was determined using the Qubit™ dsDNA HS Assay kit on a Qubit™ 3.0 fluorometer (Thermo Fisher Scientific Inc., Waltham, USA). The numbers of C degradation genes (including amyA, apu, amyX, abfA, xylA, CDH, naglu, chiA, glx, lig, and mnp) were quantified using a high-throughput quantitative PCR-based chip to assess the microbial functional potential (quantitative microbial element cycling, QMEC) (Zheng et al., 2018). Amplification was conducted on the Wafergen SmartChip Real-time PCR system (Wafergen, Fremont, CA) using a 100 nL reaction system. The detailed experimental procedure of QMEC was described by Chen et al. (2020). The primer sequences were validated by Zheng et al. (2018) and are shown in Table S2.

2.8. Definition of the incremental amounts of measured variables with increasing straw input

To clearly show the change patterns of variables with increasing straw input, we defined the increment in each variable with increasing straw input as ΔV (where V denotes the variable measured). For example, $\Delta Rs_{(S4\text{-}CK)}$, $\Delta Rs_{(S8-S4)}$ and $\Delta Rs_{(S12-S8)}$ represent the step-by-step incremental amount of Rs when the straw application rate is 4, 8 and 12 t ha^{-1}, respectively.

2.9. Statistical analysis

The effects of straw input rate on soil physicochemical properties, Rs, enzymatic activities, microbial metabolic limitation (vector length and angle), CUE and copy numbers of genes were tested by one-way analysis of variance, after which multiple comparisons were performed with Duncan's multiple range test ($P < 0.05$). The same analysis was also performed using ΔVs standardized by the zero-mean normalization method, i.e., Z score (Gong et al., 2020). These statistical analyses were performed by SPSS 20.0 (Chicago, IL, USA). Linear and third-order polynomial regression models were used to fit Rs and MBC with the amount of straw input; these models were constructed with the maximum likelihood method and compared by the "FSA" package in R. Generalized linear models were adopted to determine the relationships among all variables assessed (e.g., microbial metabolic limitation and C degradation genes). The "relaimpo" package was used to determine the relative influence of variables on explaining the variation in Rs (Gromping, 2006). Furthermore, partial least squares path modeling (PLS-PM) was performed using the package "plspm" to identify the possible pathways by which various factors control microbial metabolic limitation, C degradation gene expression and Rs (Russolillo, 2012).

These statistical analyses were performed in R version 3.6.3 (Development Core Team R, 2016).

3. Results

3.1. Effect of straw addition amount on soil nutrients

The straw addition amount significantly affected the contents of soil C, N and P and the ratios of C:N and C:P ($P < 0.05$), all of which generally increased with the amount of straw addition (Table 1). DOC and DON significantly increased with the straw addition amount ($P < 0.05$; Table 1), but Olsen-P decreased with straw addition ($P < 0.05$; Table 1).

3.2. Responses of Rs and microbial biomass to the straw addition amount

Rs and MBC significantly increased with the amount of straw addition (Fig. 1A and B). However, both ΔRs and ΔMBC showed a trend that sharply increased at S4, declined at S8, and then slightly increased at S12 (Fig. 1C and D). There were significant correlations between Rs and MBC and between ΔRs and ΔMBC (Table S3). Additionally, the smaller Akaike information criterion (AIC) and higher R^2 of the nonlinear model than the linear regression model mean that the third-order polynomial regression model is more accurate than the linear model in describing the relationship between Rs and straw input amount (Table S4).

3.3. Variations in enzymatic activities, microbial metabolism limitation and CUE

The activities of C-, N-, and P-acquiring enzymes increased with the amount of straw input ($P < 0.05$; Fig. S1). The enzymatic stoichiometry results showed that microbial metabolism in CK and S4 was limited by P over N, while that in S8 and S12 was limited by N over P (Fig. 2A and C). In addition, compared with CK, S12 exhibited significantly increased relative C limitation (Fig. 2B). CUE was significantly decreased by straw addition, and the CUE of S12 was lower than that of S8 (Fig. 2D). Furthermore, the straw addition amount significantly affected Δrelative C limitation, ΔN *vs*. P limitation and ΔCUE (Fig. 2E, F, and G). In addition, the ΔN *vs*. P limitation of S12–S8 was significantly lower than those of S8–S4 and S4-CK ($P < 0.05$; Fig. 2F).

Table 1
Effect of straw addition amount on soil nutrients.

Treatment	CK	S4	S8	S12	*F*	*P*
C	11.49 ± 0.66a	14.10 ± 0.60b	15.85 ± 0.80c	17.94 ± 0.55d	69.11	***
N	1.02 ± 0.05a	1.20 ± 0.05b	1.35 ± 0.07c	1.53 ± 0.04d	66.65	***
P	0.57 ± 0.05a	0.59 ± 0.10a	0.74 ± 0.09b	0.70 ± 0.08ab	3.84	*
C:N ratio	11.26 ± 0.17a	11.73 ± 0.19b	11.72 ± 0.16b	11.71 ± 0.10b	8.49	**
C:P ratio	20.19 ± 2.78a	24.31 ± 2.85bc	21.70 ± 2.06ab	25.90 ± 2.40c	4.06	*
N:P ratio	1.79 ± 0.22a	2.07 ± 0.26ab	1.86 ± 0.20ab	2.21 ± 0.21b	3.07	ns
DOC	37.38 ± 1.36a	45.02 ± 2.22b	51.28 ± 2.74c	72.03 ± 5.77d	74.23	***
DON	11.20 ± 1.34a	14.92 ± 0.47b	16.75 ± 2.03b	21.65 ± 1.83c	31.79	***
NO_3^-	8.57 ± 0.92a	11.30 ± 1.83b	16.83 ± 1.13d	13.46 ± 1.52c	25.08	***
NH_4^+	9.44 ± 0.97ab	8.85 ± 1.76ab	8.68 ± 0.34a	10.70 ± 1.32b	2.26	ns
Olsen-P	7.47 ± 0.94b	5.37 ± 0.94a	5.05 ± 0.68a	5.00 ± 0.73a	7.99	**
NH_4^+: Olsen-P	1.27 ± 0.05a	1.67 ± 0.32ab	1.75 ± 0.30bc	2.17 ± 0.38c	6.41	**

Note: Data are presented as means ± SD (n = 4). Different letters in the same row indicate significant differences among treatments ($P < 0.05$).
CK refers to the treatment without straw addition. S4, S8, and S12 refer to the treatments that incorporated straw into soil at 4, 8, and 12 t ha^{-1} yr^{-1}, respectively.
Different lowercase letters indicate significant differences between treatments.
DOC and DON are dissolved organic carbon and nitrogen, respectively. The units of C, N, and P are grams per kilogram.
The units of DOC, DON, NO_3^-, NH_4^+, and Olsen-P are milligrams per kilogram.
***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, $P > 0.05$.

3.4. Variations in the abundance of C degradation genes

The straw addition amount significantly increased the number of C-degrading gene copies (Fig. 3). Compared with CK, all investigated genes consistently increased with straw addition until 8 t ha^{-1}; this increase slowed for most genes when straw addition reached 12 t ha^{-1}, except for abfA, xylA and lig (Fig. 3). Additionally, the straw addition amount significantly influenced ΔC degradation genes, whose lowest value was generally observed in S12–S8 (Fig. S2).

3.5. Relationships of Rs with soil nutrients, microbial metabolism and C degradation genes

Rs was positively correlated with MBC, microbial metabolic limitation and C degradation genes but negatively correlated with CUE (Fig. 4A). The relative influence of MBC on Rs was the highest, but approximately 64% of the Rs variation could be explained by N *vs*. P limitation, relative C limitation and CUE (Fig. 4B). In addition, Rs was significantly and positively correlated with the absolute abundance of the abfA, xylA and lig genes (Fig. 4A), which had higher relative influences than other genes on Rs (Fig. 4C). Furthermore, the absolute abundance of C degradation genes was positively correlated with relative C limitation and N *vs*. P limitation (Fig. 4A). In addition, MBC, relative C limitation, N *vs*. P limitation and C degradation genes were positively correlated with the soil nutrient properties, e.g., soil C, C:N and N:P, which increased with the amount of straw addition (Fig. S3 and Table 1).

A PLS-PM model was constructed to explicitly illuminate the cascading relationships of Rs with MBC, microbial metabolic limitation, CUE and C degradation genes (Fig. 5). The results showed that the straw addition amount primarily positively affected soil C and C:N (0.969 and 0.843 direct effects, respectively) and thus positively affected MBC, relative C limitation and N *vs*. P limitation. Furthermore, microbial metabolic limitation negatively affected CUE while positively affecting the absolute abundance of hemicellulose- and lignin-degrading genes (Fig. 5B) and ultimately had a positive total effect on Rs (Fig. 5B). Overall, Rs was directly regulated by MBC, CUE and C degradation genes, while the latter two were mediated by relative C limitation and N *vs*. P limitation.

3.6. Relationships of ΔRs with Δsoil nutrients, Δmicrobial metabolic limitation and ΔC-degrading genes

ΔRs was closely correlated with ΔMBC, ΔN *vs*. P limitation, ΔCUE and ΔC degradation genes (Fig. S4), which were closely related to Δsoil nutrients (ΔDOC, ΔC:N, ΔDOC:Olsen-P, etc.) (Fig. S5). In addition, ΔC degradation genes was generally positively correlated with ΔC:N and negatively correlated with Δrelative C limitation (Fig. S4). The PLS-PM model further identified that soil ΔDOC and ΔC:N directly affected Δrelative C limitation and ΔN *vs*. P limitation and affected ΔRs by regulating ΔMBC, ΔCUE and ΔC degradation genes (Fig. S6).

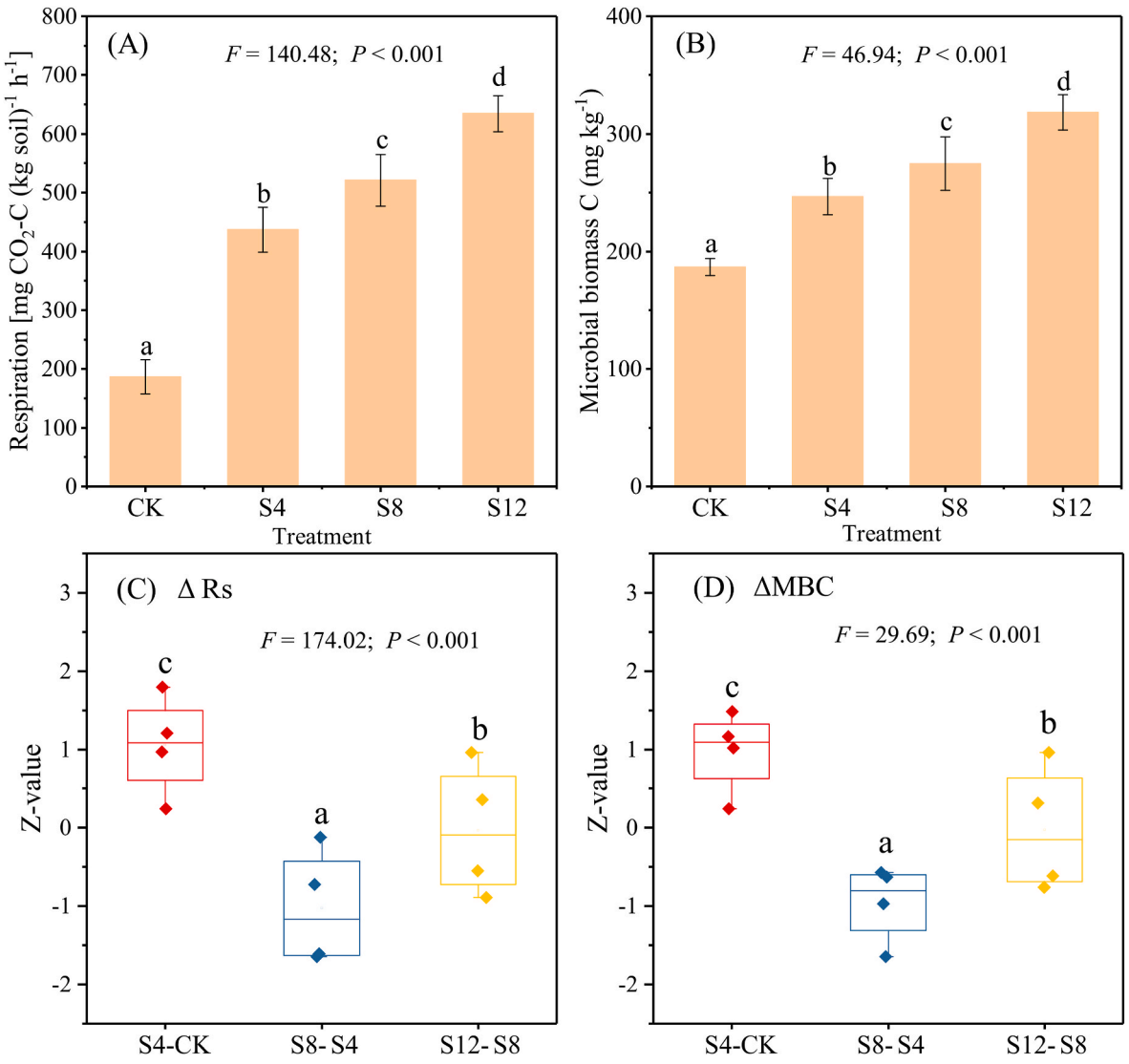

Fig. 1. Effects of straw addition amount on soil microbial respiration and microbial biomass.
Subgraphs A and B show the variation patterns of soil microbial respiration and biomass carbon under different levels of straw addition. Subgraphs C and D show the step-by-step incremental amount of Rs (ΔRs) and microbial biomass in the form of MBC (ΔMBC), respectively. CK refers to the treatment without straw addition. S4, S8, and S12 refer to the treatments that incorporated straw into the soil at 4, 8, and 12 t ha^{-1} yr^{-1}, respectively. Different lowercase letters indicate significant differences between treatments, $P < 0.05$.

4. Discussion

4.1. The responses of Rs and microbial metabolic limitation to the straw input amount

Rs significantly increased with the amount of straw input (Fig. 1), which is in line with previous studies (Shahbaz et al., 2017; Zheng and Marschner, 2017) and is usually attributed to an increase in unstable C from straw and a decrease in SOM stability (Ghimire et al., 2017; Shahbaz et al., 2017). However, Rs increased relatively slowly when the level of straw addition increased from 4 t ha^{-1} to 12 t ha^{-1} (Fig. 1). This phenomenon could be attributed to the increase rate of microbial biomass slowing down and soil microbes becoming increasingly limited by N over P as the straw input increased (Figs. 1 and 2) because Rs was closely related to and influenced by MBC and N *vs*. P limitation (Fig. 4). S8 and S12 provided relatively insufficient N for the demands of microbial metabolism and N acquisition (Fig. 2C); this insufficiency was related to the increase in Rs and may also be related to the decline in ΔRs (cf. S4-CK) (Figs. 1 and 4 and S4). The positive correlations between the C:N ratio and N *vs*. P limitation (Fig. S3) further supported that microorganisms increase their acquisition of the most limiting nutrient (N) to maintain stoichiometric homeostasis (Cleveland and Liptzin, 2007).

It is interesting that there was a transition from more P limitation to more N limitation in microbes, although soil N:P and NH_4^+:Olsen-P generally increased with an increasing rate of straw input (Table 1). Microbial nutrient demand is determined by the elemental stoichiometry of microbial biomass in relation to environmental nutrient availability (Sinsabaugh et al., 2009; Sinsabaugh and Shah, 2012). An increase in soil C can promote the growth of microorganisms with increased straw input, but an increase in C:N ratio may lead to microbial metabolism being gradually limited by N rather than P because the amount of N required for microbial growth is several to dozens of times greater than the amount of P required (Cleveland and Liptzin, 2007; Sinsabaugh et al., 2009). As a result, the increased soil N:P ratio cannot meet the sharply increased demand of microbial metabolism for N (Sinsabaugh and Shah, 2012). In fact, our results also showed that straw input influenced the stoichiometry of microbial biomass (Fig. S7), which could be attributed to microorganisms being limited by N rather than P

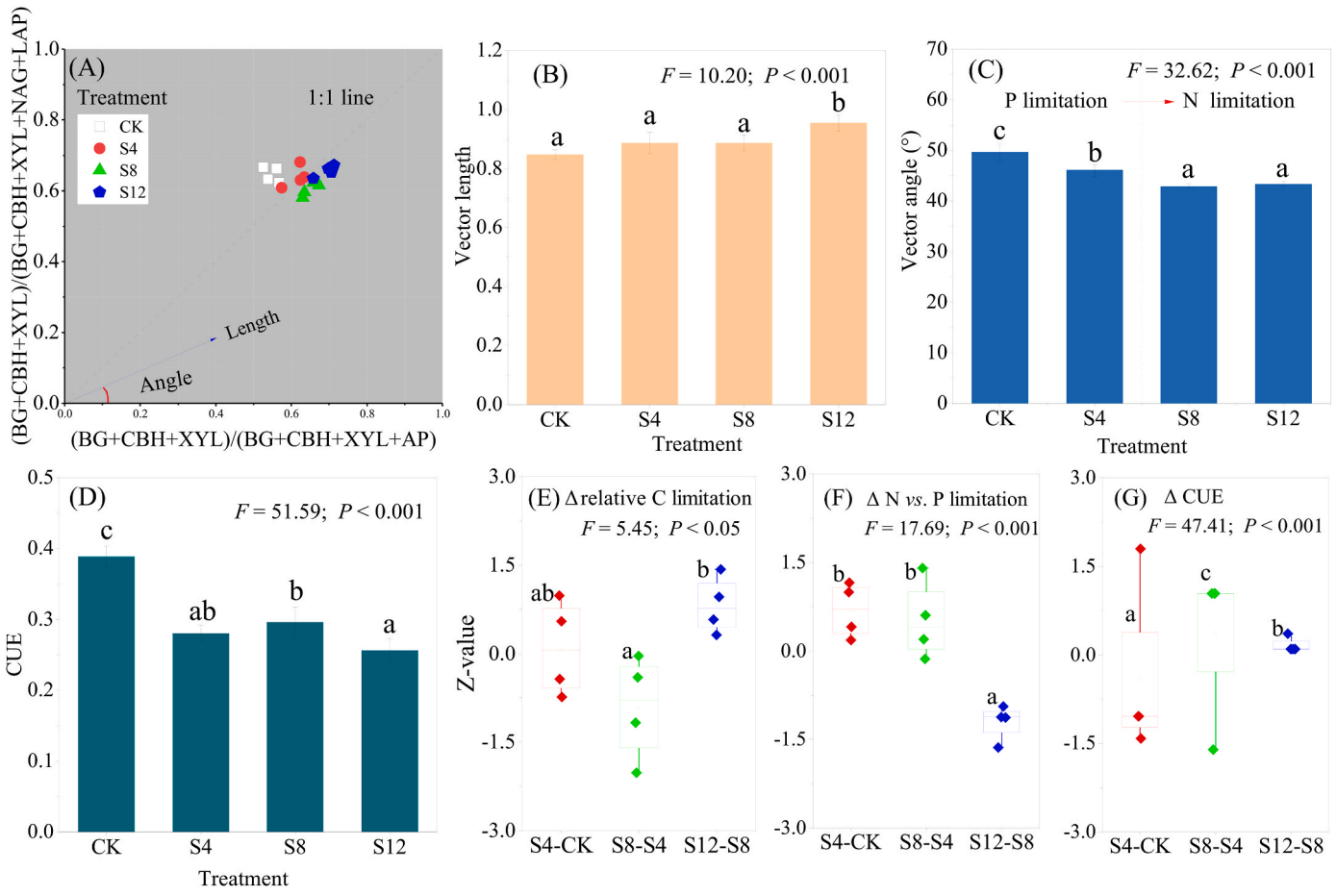

Fig. 2. Effect of straw addition amount on microbial metabolic limitation and CUE.
Subgraph A shows the extracellular enzyme stoichiometry of the relative proportions of C to N acquisition versus C to P acquisition. Abbreviations of enzymes are shown in Table S1. Subgraphs B, C and D show the variations in vector length, vector angle and microbial CUE, respectively. Subgraphs E, F and G show Δrelative C limitation, ΔN *vs.* P limitation and ΔCUE, respectively. CK refers to the treatment without straw addition. S4, S8, and S12 refer to the treatments that incorporated straw into the soil at 4, 8, and 12 t ha^{-1} yr^{-1}, respectively. Different lowercase letters indicate significant differences between treatments, $P < 0.05$.

during C assimilation. Another interesting result is that the treatment with the largest straw input (S12) had the greatest relative C limitation for microbes (Fig. 2B). This seemingly contradictory result can be simply explained by the fact that the large amount of straw-C input (S12) promoted the activities of C-acquisition enzymes without a proportional enhancement in N- or P-acquisition enzyme activities (Fig. S1). Hence, we suggest that the input of high amounts of exogenous C likely led to the decoupling of microbial nutrient acquisition potential from C acquisition potential and created a seeming relative C limitation rather than the microbes truly being more limited by C than N or P. In fact, the greatest vector length of S12 indicates that greater straw input could increase the decomposition potential of organic C (Fig. S1).

4.2. *The responses of CUE and C degradation genes to the straw input amount*

Straw addition at low to high levels (S4 to S12) significantly decreased microbial CUE (Fig. 2D), which was negatively correlated with relative C limitation and N *vs.* P limitation (Fig. 4). This result indicated that straw addition reduced the proportion of substrate C that microbes use for growth relative to respiration and enzyme secretion to acquire nutrients (Sinsabaugh et al., 2013). Furthermore, sufficient C input (S12) may increase substrate C allocation for microbial respiration and secretion of C-acquisition enzymes (cf. S4 and S8) and lead to a higher Rs and lower CUE (Fig. 2D). Our results were not consistent with van Groenigen et al. (2013), who suggested that straw incorporation does not substantially affect CUE evaluated by the ^{13}C-glucose tracing method, which may overestimate the CUE of SOM (Geyer et al., 2019). In addition, Fang et al. (2018) suggested that the CUE of straw-C (evaluated by the ^{13}C-straw tracing method) would initially increase at a high level of straw addition (20 g kg^{-1} soil) and then decrease over time as straw-C is converted to stabilized SOM. Hence, the decreased CUE in the present study may be attributed to decreases in both the CUE of SOM and the CUE of straw-C.

The absolute abundances of genes for degrading labile and recalcitrant polymers all increased with an increasing rate of straw addition until 8 t ha^{-1}, while only the genes abfA, xylA and lig continued to increase until straw addition reached 12 t ha^{-1} (Fig. 3). In addition, these three genes were closely related to Rs and had more important influences than other genes on Rs (Fig. 4A and C). This implies that abfA, xylA and lig may be the key genes for the continual increase in Rs. This result also suggested that continuous straw addition may strongly increase the contents of hemicellulose and recalcitrant lignin (Feng et al., 2019), which would drive microbial community structure succession toward a high ability to degrade hemicellulose and recalcitrant lignin (Maarastawi et al., 2018; Zhao et al., 2019). Additionally, C degradation genes were positively correlated with relative C limitation and N *vs.* P limitation (Fig. 4A), implying that the relative energy and nutrient limitations of microorganisms regulate the expression of C-degrading genes (Sinsabaugh and Shah, 2012; Geyer et al., 2019).

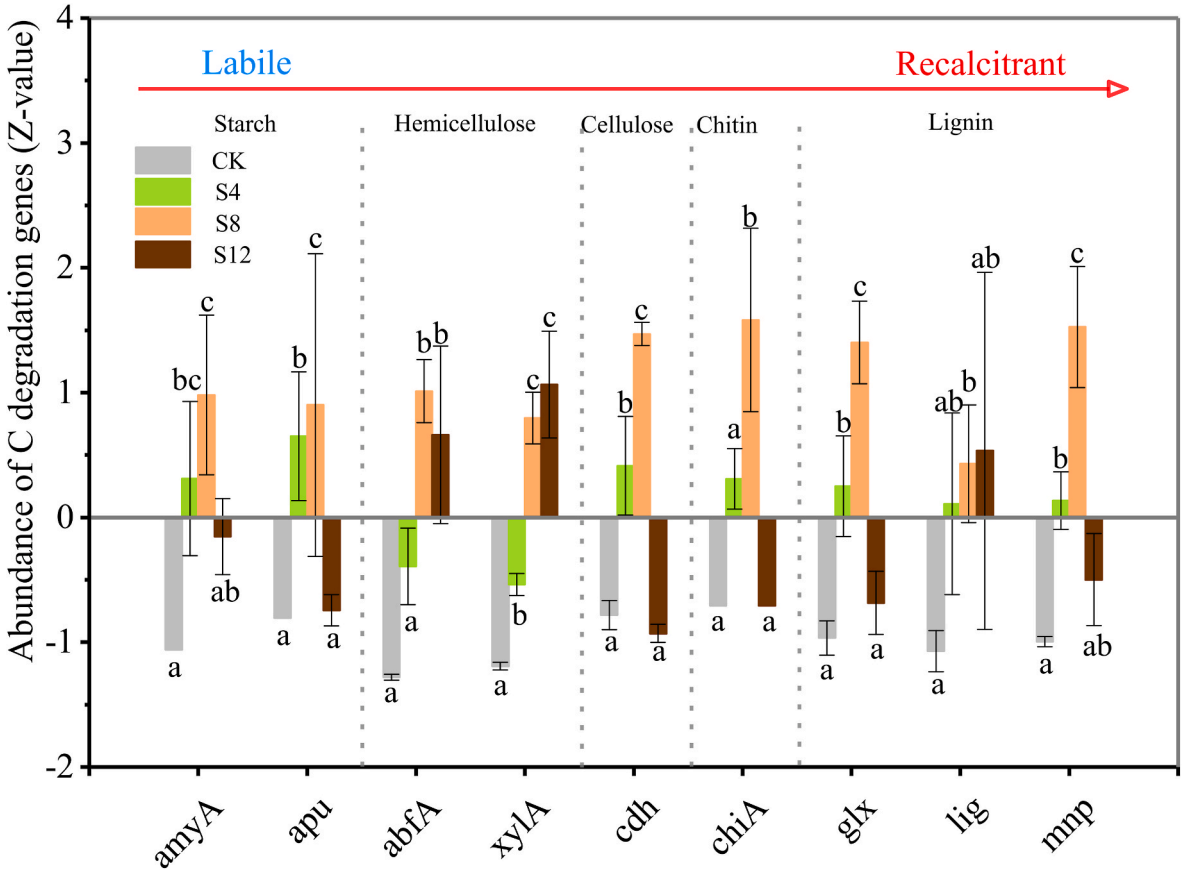

Fig. 3. Effect of straw addition amount on the absolute abundance of C degradation genes. CK refers to the treatment without straw addition. S4, S8, and S12 refer to the treatments that incorporated straw into the soil at 4, 8, and 12 t ha^{-1} yr^{-1}, respectively. Different lowercase letters indicate significant differences between treatments, $P < 0.05$.

4.3. Mechanisms of Rs variations under straw input and implications for field practice

Our results showed that the change in soil stoichiometry caused by straw input was the most fundamental cause of Rs change (Fig. 5). Increased soil C and C:N ratio values directly affected the growth of the microbial community (MBC) and microbial metabolic limitation (Fig. 5), which in turn drove microorganisms to degrade more SOM and thus acquire limiting nutrients to maintain stoichiometric homeostasis (Sinsabaugh and Shah, 2012; Cui et al., 2020; Ma et al., 2021b). Furthermore, microbial N *vs.* P limitation affected Rs by mediating microbial CUE and the degradation potential of hemicellulose and lignin (Fig. 5). In addition, ΔRs was dominated by ΔMBC and ΔN *vs.* P limitation (0.64 and 0.51 of the total effect, respectively), indicating the crucial roles of microbial biomass and microbial nutrient limitation in regulating Rs variation (Fig. S6). These results suggested that microorganisms can actively respond to the relative resource constraints caused by the continuous input of straw and therefore regulate Rs. Conservative microbial stoichiometric responses to perturbations usually constrain both individual and community responses to environmental change (Sinsabaugh and Shah, 2012; Zhu et al., 2018). However, the dramatic change in the abundance of C degradation genes implied that the microbial community shifted to respond to microbial limitation (Fig. 3). In addition, the changed microbial biomass C:N:P ratios imply that microbes can adjust their elemental stoichiometry in response to resource C:nutrient ratios to maintain stoichiometric balance (Fig. S7), which would increase microbial activity and likely lead to co-metabolism of straw and recalcitrant SOM (Mooshammer et al., 2014; Zhu et al., 2018). Therefore, microbial regulation of Rs is a feedback system between resource limitation and ecosystem responses at the microbial individual gene level and community level.

Additionally, our previous study indicated that crop productivity increased with the straw input rate (Ma et al., 2021a). Such increased productivity is generally related to enhanced root-derived C inputs, such as root biomass and rhizodeposition (Suriyagoda et al., 2014; Ma et al., 2019). These enhanced inputs may lead to a higher C concentration in the rhizosphere than in bulk soil (Maarastawi et al., 2019) and may thus intensify microbial metabolic limitation in the rhizosphere. Therefore, linking rhizosphere Rs to root-derived C inputs, CUE and microbial metabolic limitation in the future will further elucidate the mechanisms by which plant–soil–microbe interactions affect Rs.

Microbial metabolic limitation regulated Rs, which could provide some implications for field practices to improve SOM. For example, straw return at a low level (≤4 t ha^{-1}) could appropriately supplement P fertilizer or organic fertilizer rich in available P (such as pig manure) to further alleviate microbial P limitation and thus mitigate the decomposition of SOM (Ma et al., 2021b). However, when straw is added at middle or high levels (e.g., 8 or 12 t ha^{-1} in the present study), the application of extra N fertilizer could alleviate microbial N limitation and thus enhance microbial CUE and decrease C degradation potential. The mitigation of microbial metabolic limitation may promote microbial growth and activity (Zhu et al., 2018) and decrease overflow Rs (Sinsabaugh et al., 2013). The balance between environmental resources and the requirements of microbial elemental stoichiometry would accelerate the in vivo turnover and ex vivo modification of straw carbon by microorganisms (Liang et al., 2017), consequently strengthening the role of microbial anabolism in SOM improvement.

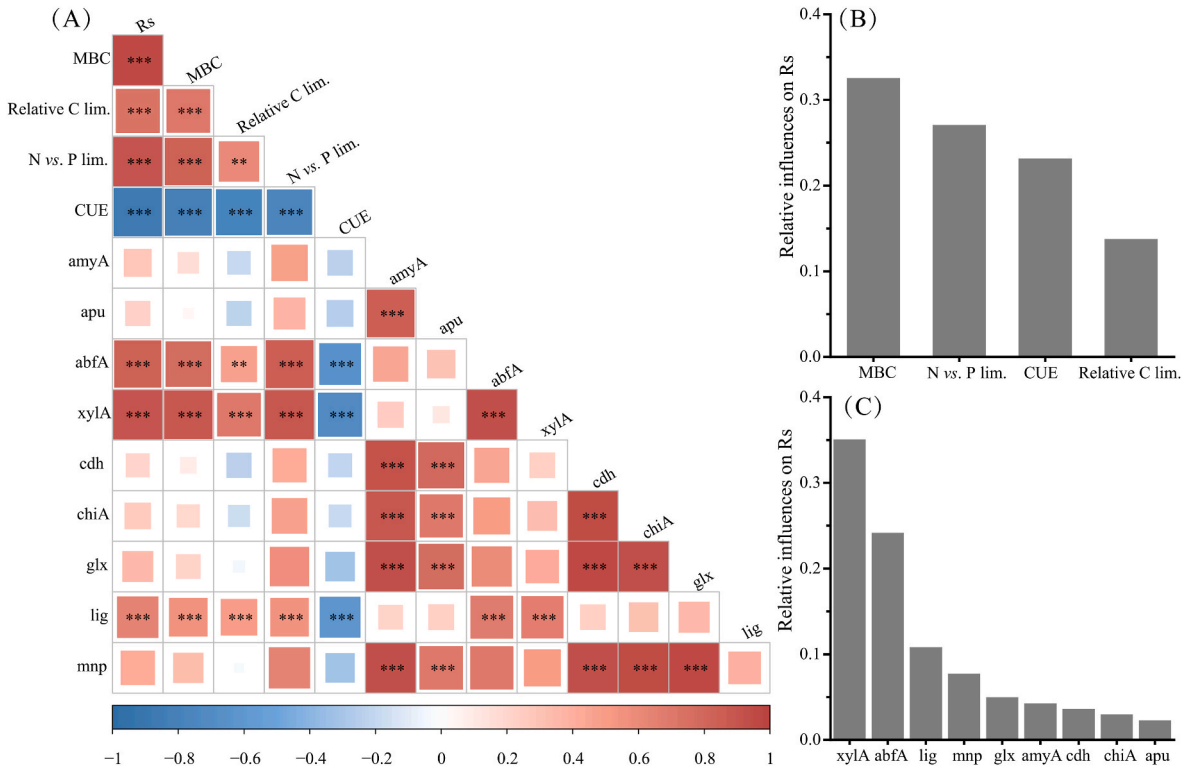

Fig. 4. Relationships of soil microbial respiration with microbial biomass, metabolic limitation, CUE and C degradation genes. Rs, MBC, relative C lim. and N *vs.* P lim. represent soil microbial respiration, microbial biomass carbon, relative C limitation and N *vs.* P limitation, respectively. Red and blue represent positive correlations and negative correlations, respectively. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

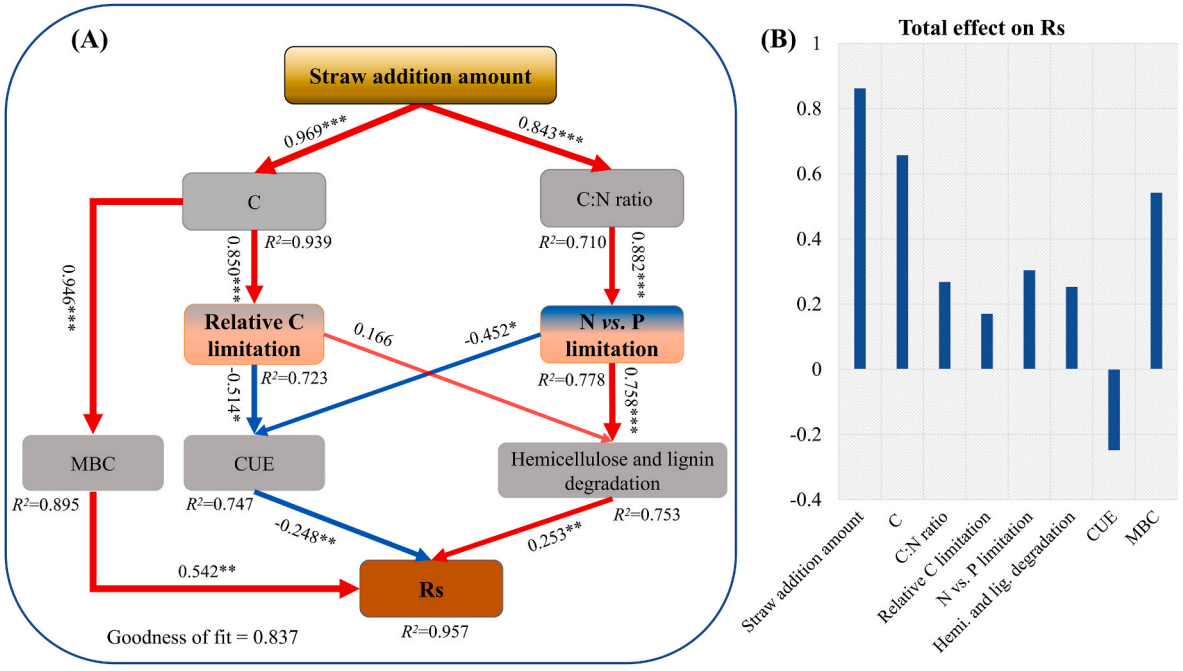

Fig. 5. Cascading relationships of soil microbial respiration with microbial metabolic limitation, CUE, C degradation genes and soil nutrient properties. Rs and MBC are soil microbial respiration and microbial biomass C, respectively. The module of hemicellulose and lignin degradation consists of the absolute abundance of abfA, xylA and lig. Hemi. and lig. degradation represent hemicellulose and lignin degradation. Red arrows represent a positive influence, and blue arrows represent a negative influence. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

5. Conclusions

Our study from an enzymatic stoichiometric perspective reveals the microbial mechanisms of Rs changes with increasing rates of straw input based on a long-term field experiment. Overall, Rs increased with the straw addition amount, while the rate of Rs increase dropped when the straw input was over 8 t ha^{-1}. This result could be attributed to the fact that straw addition increased soil C and promoted microbial growth, while the increased C:N ratio caused by straw input led to soil microbes becoming increasingly limited by N rather than P. Furthermore, microbial N *vs*. P limitation influenced Rs by mediating CUE and C degradation gene expression. These findings suggest that balancing the soil C-to-nutrient ratio to reduce microbial nutrient limitation can increase CUE and decrease the C degradation potential, thus contributing to reducing SOM mineralization. This study revealed the crucial role of microbial metabolic limitation in regulating Rs and provides insight into the mechanisms of Rs changes under straw addition.

Declaration of competing 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.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (41877107, 41877106, 32001212) and the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA28090100).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2022.108636.

References

Berhane, M., Xu, M., Liang, Z., Shi, J., Wei, G., Tian, X., 2020. Effects of long-term straw return on soil organic carbon storage and sequestration rate in North China upland crops: a meta-analysis. Global Change Biology 26 (4), 2686–2701.
Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology & Biochemistry 17, 837–842.
Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biology & Biochemistry 14, 319–329.
Chen, Q.L., Ding, J., Li, C.Y., Yan, Z.Z., He, J.Z., Hu, H.W., 2020. Microbial functional attributes, rather than taxonomic attributes, drive top soil respiration, nitrification and denitrification processes. Science of the Total Environment 734, 139479.
Cleveland, C.C., Liptzin, D., 2007. C : N : P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85, 235–252.
Cong, P., Wang, J., Li, Y., Liu, N., Dong, J., Pang, H., Zhang, L., Gao, Z., 2020. Changes in soil organic carbon and microbial community under varying straw incorporation strategies. Soil & Tillage Research 204, 104735.
Cui, Y.X., Fang, L.C., Guo, X.B., Han, F., Ju, W.L., Ye, L.P., Wang, X., Tan, W.F., Zhang, X. C., 2019. Natural grassland as the optimal pattern of vegetation restoration in arid and semi-arid regions: evidence from nutrient limitation of soil microbes. Science of the Total Environment 648, 388–397.
Cui, Y.X., Fang, L.C., Guo, X.B., Wang, X., Zhang, Y.J., Li, P.F., Zhang, X.C., 2018. Ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the northern Loess Plateau, China. Soil Biology & Biochemistry 116, 11–21.
Cui, Y.X., Moorhead, D.L., Guo, X.B., Peng, S.S., Wang, Y.Q., Zhang, X.C., Fang, L.C., 2021. Stoichiometric models of microbial metabolic limitation in soil systems. Global Ecology and Biogeography 30, 2297–2311.
Cui, Y.X., Wang, X., Zhang, X.C., Ju, W.L., Duan, C.J., Guo, X.B., Wang, Y.Q., Fang, L.C., 2020. Soil moisture mediates microbial carbon and phosphorus metabolism during vegetation succession in a semiarid region. Soil Biology & Biochemistry 147, 107814.
Fang, Y., Singh, B.P., Cowie, A., Wang, W., Arachchi, M.H., Wang, H., Tavakkoli, E., 2019. Balancing nutrient stoichiometry facilitates the fate of wheat residue-carbon in physically defined soil organic matter fractions. Geoderma 354, 113883.
Fang, Y.Y., Singh, B.P., Collins, D., Li, B.Z., Zhu, J., Tavakkoli, E., 2018. Nutrient supply enhanced wheat residue-carbon mineralization, microbial growth, and microbial carbon-use efficiency when residues were supplied at high rate in contrasting soils. Soil Biology & Biochemistry 126, 168–178.
Feng, S.Z., Su, Y.R., He, X.Y., Hu, Y.J., Zhang, Z.H., He, H.B., Kariman, K., Wu, J.S., Chen, X.B., 2019. Effects of long-term straw incorporation on lignin accumulation and its association with bacterial laccase-like genes in arable soils. Applied Microbiology and Biotechnology 103, 1961–1972.
German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology & Biochemistry 43, 1387–1397.
Geyer, K.M., Dijkstra, P., Sinsabaugh, R., Frey, S.D., 2019. Clarifying the interpretation of carbon use efficiency in soil through methods comparison. Soil Biology & Biochemistry 128, 79–88.
Ghimire, B., Ghimire, R., VanLeeuwen, D., Mesbah, A., 2017. Cover crop residue amount and quality effects on soil organic carbon mineralization. Sustainability 9 (12), 2316.
Gong, J.H., Liu, X.Y., Sun, G., Zhou, J.S., 2020. Evaluating the retest reproducibility of intrinsic connectivity network using multivariate correlation coefficient. Neural Computing & Applications 32, 14623–14638.
Gromping, U., 2006. Relative importance for linear regression in R: the package relaimpo. Journal of Statistical Software 17 (1), 1–27.
Guenet, B., Neill, C., Bardoux, G., Abbadie, L., 2010. Is there a linear relationship between priming effect intensity and the amount of organic matter input? Applied Soil Ecology 46, 436–442.
Heitkamp, F., Wendland, M., Offenberger, K., Gerold, G., 2012. Implications of input estimation, residue quality and carbon saturation on the predictive power of the Rothamsted Carbon Model. Geoderma 170, 168–175.
Joergensen, R.G., Mueller, T., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the k(EN) value. Soil Biology & Biochemistry 28, 33–37.
Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biology & Biochemistry 38, 991–999.
Li, J., Yang, H., Zhou, F., Zhang, X.C., Luo, J.F., Li, Y., Lindsey, S., Shi, Y.L., He, H.B., Zhang, X.D., 2019. Effects of maize residue return rate on nitrogen transformations and gaseous losses in an arable soil. Agricultural Water Management 211, 132–141.
Li, S., Liang, C., Shangguan, Z., 2017. Effects of apple branch biochar on soil C mineralization and nutrient cycling under two levels of N. Science of the Total Environment 607, 109–119.
Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2 (8), 17105.
Ma, L.J., Kong, F.X., Wang, Z., Luo, Y., Lv, X.B., Zhou, Z.G., Meng, Y.L., 2019. Growth and yield of cotton as affected by different straw returning modes with an equivalent carbon input. Field Crops Research 243, 10.
Ma, Q., Jiang, C.M., Li, S.L., Yu, W.T., 2021a. Maize yield and nitrogen-use characteristics were promoted as consistently improved soil fertility: 6-year straw incorporation in Northeast China. Plant Soil and Environment 67, 383–389.
Ma, Z., Zhang, X., Zheng, B., Yue, S., Zhang, X., Zhai, B., Wang, Z., Zheng, W., Li, Z., Zamanian, K., Razavi, B.S., 2021b. Effects of plastic and straw mulching on soil microbial P limitations in maize fields: dependency on soil organic carbon demonstrated by ecoenzymatic stoichiometry. Geoderma 388, 114928.
Maarastawi, S.A., Frindte, K., Bodelier, P.L.E., Knief, C., 2019. Rice straw serves as additional carbon source for rhizosphere microorganisms and reduces root exudate consumption. Soil Biology & Biochemistry 135, 235–238.
Maarastawi, S.A., Frindte, K., Geer, R., Krober, E., Knief, C., 2018. Temporal dynamics and compartment specific rice straw degradation in bulk soil and the rhizosphere of maize. Soil Biology & Biochemistry 127, 200–212.
Moorhead, D.L., Sinsabaugh, R.L., Hill, B.H., Weintraub, M.N., 2016. Vector analysis of ecoenzyme activities reveal constraints on coupled C, N and P dynamics. Soil Biology & Biochemistry 93, 1–7.
Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., 2014. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Frontiers in Microbiology 5, 22.
Olsen, S.R., Sommers, L.E., 1982. Phosphorous. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2, Chemical and Microbial Properties. American Society of Agronomy, Madison, pp. 403–430.
Paterson, E., Sim, A., 2013. Soil-specific response functions of organic matter mineralization to the availability of labile carbon. Global Change Biology 19, 1562–1571.
Poeplau, C., Kaetterer, T., Bolinder, M.A., Borjesson, G., Berti, A., Lugato, E., 2015. Low stabilization of aboveground crop residue carbon in sandy soils of Swedish long-term experiments. Geoderma 237, 246–255.
Poirier, V., Angers, D.A., Rochette, P., Whalen, J.K., 2013. Initial soil organic carbon concentration influences the short-term retention of crop-residue carbon in the fine fraction of a heavy clay soil. Biology and Fertility of Soils 49, 527–535.
Russolillo, G., 2012. Non-metric partial least squares. Electronic Journal of Statistics 6, 1641–1669.
Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology & Biochemistry 34, 1309–1315.
Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386–1394.
Shahbaz, M., Kuzyakov, Y., Heitkamp, F., 2017. Decrease of soil organic matter stabilization with increasing inputs: mechanisms and controls. Geoderma 304, 76–82.
Sinsabaugh, R.L., Hill, B.H., Shah, J.J.F., 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462, 795–798.

Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. Ecology Letters 16, 930–939.
Sinsabaugh, R.L., Shah, J.J.F., 2012. Ecoenzymatic stoichiometry and ecological theory. Annual Review of Ecology, Evolution, and Systematics 43, 313–343.
Spohn, M., Klaus, K., Wanek, W., Richter, A., 2016. Microbial carbon use efficiency and biomass turnover times depending on soil depth - implications for carbon cycling. Soil Biology & Biochemistry 96, 74–81.
Suriyagoda, L., De Costa, W., Lambers, H., 2014. Growth and phosphorus nutrition of rice when inorganic fertiliser application is partly replaced by straw under varying moisture availability in sandy and clay soils. Plant and Soil 384, 53–68.
van Groenigen, K.J., Forristal, D., Jones, M., Smyth, N., Schwartz, E., Hungate, B., Dijkstra, P., 2013. Using metabolic tracer techniques to assess the impact of tillage and straw management on microbial carbon use efficiency in soil. Soil Biology & Biochemistry 66, 139–145.
Vance, E.D., Brooks, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass. Soil Biology & Biochemistry 19, 703–707.
Waring, B.G., Weintraub, S.R., Sinsabaugh, R.L., 2014. Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. Biogeochemistry 117, 101–113.
Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. Soil Biology & Biochemistry 22, 1167–1169.
Zhao, S.C., Qiu, S.J., Xu, X.P., Ciampitti, I.A., Zhang, S.Q., He, P., 2019. Change in straw decomposition rate and soil microbial community composition after straw addition in different long-term fertilization soils. Applied Soil Ecology 138, 123–133.
Zheng, B., Marschner, P., 2017. Previous residue addition rate and C/N ratio influence nutrient availability and respiration rate after the second residue addition. Geoderma 285, 217–224.
Zheng, B.X., Zhu, Y.G., Sardans, J., Penuelas, J., Su, J.Q., 2018. QMEC: a tool for high-throughput quantitative assessment of microbial functional potential in C, N, P, and S biogeochemical cycling. Science China-Life Sciences 61, 1451–1462.
Zhu, Z.K., Ge, T.D., Luo, Y., Liu, S.L., Xu, X.L., Tong, C.L., Shibistova, O., Guggenberger, G., Wu, J.S., 2018. Microbial stoichiometric flexibility regulates rice straw mineralization and its priming effect in paddy soil. Soil Biology & Biochemistry 121, 67–76.