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Late-season rice increased the contribution of glomalin rather than amino sugar to soil organic carbon in a double-season paddy soil

Peiyue Wang[1,2,†], Xiaozhe Bao[3,†], Taotao Yang[3], Bin Zhang[3], Jing Zhang[1,*], Zhanfeng Liu[1]

1 Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China
2 College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China
3 Guangdong Key Laboratory of New Technology in Rice Breeding/Guangdong Rice Engineering Laboratory/Key Laboratory of Genetics and Breeding of High Quality Rice in Southern China (Co-construction by Ministry and Province), Ministry of Agriculture and Rural Affairs, Rice Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
* Corresponding author. E-mail: zhangj@scbg.ac.cn (J. Zhang)
† These authors contributed equally to this work.
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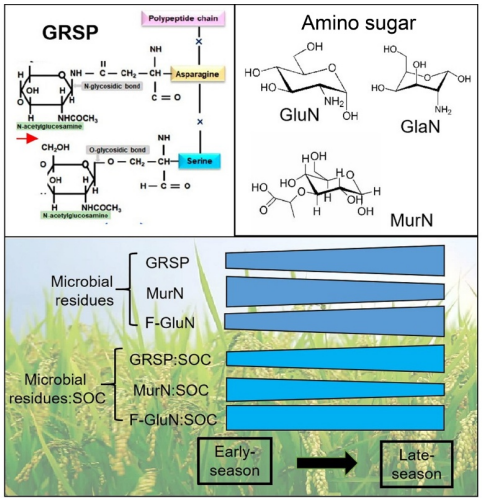
ABSTRACT

- Bacterial and fungal necromass in soil showed opposite trends with rice growth.
- The contribution of GRSP increased but ASs decreased to SOC with rice growth.
- Microbial residues were mainly influenced by living microbial biomass.



Microbial residues play an important role in soil organic carbon (SOC) sequestration. Paddy fields are important agricultural ecosystems involved in the carbon cycle; however, microbial residues change with rice growth in soil from double-season rice, and the influence of these residues on SOC sequestration is uncertain. Here, we investigated the microbial residues (amino sugars (AS) and glomalin-related soil protein (GRSP)) content and their contribution to SOC during the tillering stage (TS), heading stage (HS), and ripening stage (RS) in both early- and late-season rice in a double-cropping rice-growing area wherein the straw is returned after the early-season rice is harvested. Microbial biomass significantly increased from the early- to the late-season. In addition, the content of bacterial residues decreased (7.94%, P= 0.008), while the fungal residues increased (8.15%, P<0.001) in the late-season compared with the early-season, suggesting that bacterial residues were recycled more rapidly than fungal residues. Amino sugar content and its contribution to SOC decreased from the TS to the RS in the late-season soil, probably because of the nutrient requirements of the rapidly growing rice. The contribution of GRSP to SOC increased by 10.5%, whereas that of ASs decreased by 4.5% from the early- to the late-season. Living soil microbes rather than soil physicochemical properties were the main factors influencing microbial residue accumulation. The results of this study provide a theoretical basis from a microbial perspective which will facilitate future efforts to enhance SOC sequestration during paddy field management.

Keywords paddy soil, microbial residues, amino sugar, glomalin-related soil protein, double-season rice

1 Introduction

The soil organic carbon (SOC) pool, which is the largest carbon (C) pool in terrestrial ecosystems, exceeds the sum of the atmospheric C pool and vegetation C pool (Batjes, 2016). Therefore, even small changes in the SOC pool will have important impacts on the ecological environment and ecosystem functions (Wiesmeier et al., 2019; Wei et al., 2021). Soil organic matter is the core nutrient source in the agricultural ecosystem and therefore crucial to farmland productivity and soil fertility, and closely related to crop yield and quality (Oldfield et al., 2019). Previous studies have shown that microbial-derived C has high stability when associated with soil clay or minerals (Zhao et al., 2020) and plays an important role in SOC stock and stability (Kallenbach

et al., 2016; Ma et al., 2018). However, studies of microbial residues conducted to date have primarily focused on grasslands, forests, and other farmlands, while there have been few investigations of the changes that occur in microbial residues with rice growth in paddy fields.

Soil microbial residues, which are derived from different microbial communities, can remain in the soil long after the death of microbes (Liang et al., 2019). Different microbial residues reflect the relative contributions of different microbial communities to the turnover of SOC on a long-term scale (Liang and Balser, 2011). Amino sugars (ASs) are important biomarkers that indicate microbial necromass (Ma et al., 2018), and glomalin-related soil protein (GRSP) is the product of arbuscular mycorrhizal fungi (Wright and Upadhyaya, 1996). These compounds are two important biomarkers of microbial residues in soil. An increasing number of studies have shown that microbial residues constitute the main source of SOC (Kallenbach et al., 2016; Liang et al., 2019). Moreover, it has been reported that 28%−36% of the SOC sequestered in paddy soil in China comes from microbial residues (Chen et al., 2021). Accordingly, it is worth studying whether there is a trade-off between these stocks and the contribution of AS and (or) GRSP to SOC.

Paddy soil accounts for about 9% of the world's cropland area and is an important global SOC pool (Liu et al., 2021). Moreover, the average SOC in the cultivated layer of paddy soil in China has increased by 3.49 g kg^{-1} over the past 30 years (Li et al., 2020). Double-season rice is widely distributed in southern China. In double-rice systems, straw is typically returned to the paddy after the early-season harvest, which significantly increases the C storage in paddy soil (Han et al., 2023). To investigate the responses of microbial residues in paddy soil to the growth stage and rice-planting season, as well as the underlying mechanisms responsible for these responses, we evaluated a double-season cropping paddy soil in Southern China. We hypothesized that firstly, microbial residues and their contribution to SOC would be increased in the late-season compared with the early season due to straw return (Li et al., 2023a). Secondly, the changes in microbial residues would be mediated by both soil properties and living microbes because both decomposers and environmental factors influence the microbial residue concentration (Kallenbach et al., 2016; Mou et al., 2021; Yuan et al., 2021). Exploring the soil carbon pool will enhance grain production potential and promote sustainable development of the agricultural industry.

2 Materials and methods

2.1 Study site and experimental design

This study was conducted in the Dafeng Experimental Field of the Rice Research Institute of Guangdong Academy of Agricultural Sciences (23°09′ N, 113°22′ E). The region, which is located in the main double cropping rice area of Guangdong Province, has a subtropical monsoon climate with the mean annual temperature of 21.8 °C, the frostless period of 345 days, and an annual precipitation of 1694 mm. The soil of the experimental plot is loam, with the pH of 6.46, total nitrogen (N) content of 1.21 mg g^{-1}, and available phosphorus (P) content of 45.98 mg kg^{-1} (Table 2, see below). The rice variety grown in this experiment was Yuehesimiao (conventional *indica* rice). The experiment adopted six replicate plots with an area of 7.4 m^2 (2 m × 3.7 m). The planting time of the double-season rice was determined according to the plant species, variations, and the weather in 2021. Specifically, the sowing, transplanting, heading, and ripening dates of the early-season rice were March 16, April 6, May 29, and June 29, respectively, and July 19, August 5, September 29, and November 7 for the late-season rice. The straw was returned to the field after the early-season rice was harvested.

2.2 Sample collection and analysis methods

In 2021, a five-point sampling method was used to collect the topsoil (0−10 cm) in each plot with a shovel, after which, the five samples were mixed into one composite sample. Samples were collected before rice was planted and at the tillering stage (TS), heading stage (HS), and ripening stage (RS) of both early-season and late-season rice. Each composite sample was divided into two subsamples that were transported to the laboratory immediately. One subsample was then air-dried and the other was freeze-dried for evaluation of the soil microbial community. The air-dried subsamples were subsequently passed through a 2-mm sieve and a 53-μm sieve, and further analyzed to identify the microbial residues and soil physicochemical properties, respectively.

2.2.1 Microbial community structure

The soil microbial community was determined by the phospholipid fatty acid (PLFA) analysis method described by Bossio and Scow (1998). After extraction, separation, and purification of phospholipids in the soil, the phospholipids were analyzed by gas chromatography (7890B, Agilent Technologies). The biomass of various microbes was then calculated, and the microbial community composition was analyzed according to the internal standard methyl nonadecanoate ($CH_3(CH_2)_{17}COOCH_3$, Sigma-Aldrich (Shanghai) Trading Co. Ltd., Shanghai, China). Table 1 shows the phospholipids used to represent various microbial communities. The ratio of fungal to bacterial PLFA (F:B ratio) was

calculated based on the ratio of 18:2ω6,9c to the total bacterial indicators, as shown in Table 1.

2.2.2 Glomalin-related soil proteins

GRSP was analyzed using the methods described by Wright and Upadhyaya (1996) and Zhang et al. (2014). Easily extracted GRSP (EE-GRSP), which is mainly produced by mycelium recently, was extracted once with 20 mmol L^{-1} sodium citrate solution (pH=7.0) at 121 °C for 30 min. Total GRSP (T-GRSP) was obtained by continuous extraction with 50 mmol L^{-1} sodium citrate solution (pH=8.0) that had been sterilized at 121 °C for 60 min. The T-GRSP extraction process was performed four times for each sample. This is because T-GRSP combines closely with soil particles to form large soil aggregates. The difficult-to-extract GRSP (DE-GRSP) was determined by subtracting the EE-GRSP from T-GRSP. To determine the GRSP content, 2 mg mL^{-1} bovine serum albumin (BSA) was used as the standard. The optical density (OD) values of the BSA and GRSP extraction solution were then measured at 595 nm using an enzyme microplate reader (Thermo Multiskan FC, USA), and the values were used to calculate milligrams of GRSP per gram of dry soil.

2.2.3 Amino sugars

Amino sugars (ASs) were analyzed using the method described by Indorf et al. (2011). Briefly, 0.50 g of air-dried soil was passed through a 53 μm sieve, after which 10 mL of 6 mol L^{-1} hydrochloric acid solution was added, and the samples were vortexed and shaken evenly. Next, samples were dried in an oven at 105 °C for 6 h, after which the samples were cooled to room temperature, then 0.5 mL of the supernatant was dried with N_2 gas. Finally, 2 mL of ultrapure water was added to dissolve the residue, after which the solution was vortexed until uniform. The sample was then passed through a filter with a diameter of 13 mm and a pore diameter of 0.45 μm, after which the filtrate was stored in a liquid injection vial at −18 °C until further analysis. The extracted amino sugars were subsequently derivatized by ortho-phthaldialdehyde, the glucosamine (GluN), galactosamine (GalN), and muramic acid (MurN) contents were determined by high-performance liquid chromatography (HPLC) using a Dionex Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) with a Hypersil Gold C18 column (Acclaim120 C18; 4.6 mm × 150 mm, 3 μm; Thermo Fisher Scientific, Waltham, MA, USA). Analysis was conducted at 35 °C using an excitation of 445 emission and an excitation of 330 nm. The measurement and calculation method used to determine the contributions of different AS contents and microbial residues to SOC were conducted as described by Mou et al. (2020).

The total AS was the sum of GluN, GalN, and MurN. Because MurN exists only in the cell walls of bacteria, whereas GluN is present in both bacterial and fungal cell walls. Fungal-derived GluN (F-GluN) was calculated by subtracting the bacterial-derived GluN from the total GluN. MurN and GluN coexist in the bacterial cell wall at a ratio of 1:2 (mole percent), and bacterial-derived GluN is calculated as GluN (mol) = 2 × MurN (mol); therefore, F-GluN was calculated as follows :

F-GluN (mg kg^{-1}) = total GluN (mg kg^{-1}) − 2 × MurN (mg kg^{-1}) × (179.2/251.2)

(179.2 and 251.2 are the molecular weights of GluN and MurN, respectively)

The content of bacterial fungal necromass (MRC) was converted by F-GluN and MurN as follows:

Fungal MRC (mg kg^{-1} dry soil) = F-GluN (mg kg^{-1} dry soil) × 9

Bacterial MRC (mg kg^{-1} dry soil) = MurN (mg kg^{-1} dry soil) × 45

(9 and 45 are fungal and bacterial conversion factors, respectively, based on Appuhn and Joergensen (2006))

Total MRC was calculated as the sum of fungal MRC and bacterial MRC. The ratio of F-GluN to MurN was used to evaluate the ratio of fungal and bacterial MRC (Shao et al., 2017).

Table 1 Microbial communities represented by different phospholipids.

Microbes	Specific phospholipids	References
GP	i14:0, i15:0, a15:0, i16:0, a17:0, i17:0	Kourtev et al., 2002; Kaur et al., 2005 Frostegård and Bååth, 1996; Olsson, 1999 Stackebrandt et al., 1985
GN	16:1ω7c, cy17:0, 18:1ω7, cy19:0	
Bacteria	12:0, i14:0, 14:0, 15:0, i15:0, a15:0, i16:0, 16:1ω7c, 17:0, a17:0, i17:0, cy17:0, 18:1ω7, cy19:0	
Fungi	18:2ω6,9c	
Actinomycetes	10Me18:0, 16Mel6:0, 10Me18:0	
AMF	16:1ω5	
Other species	16:0, 16:1 2OH, 17:1ω9c, 18:0, 18:1ω9c, 18:3ω3c	

Note: GP, Gram-positive bacteria; GN, Gram-negative bacteria; AMF, arbuscular mycorrhizal fungi.

2.2.4 Soil physicochemical properties

SOC was determined by titration with a $FeSO_4$ solution following dichromate oxidation of organic carbon. The pH value was measured using a pH meter (FiveEasy Plus pH/mV, Mettler Toledo, Zurich, Switzerland) to analyze a 1:2.5 (w:v) soil:water suspension. The total N, total phosphorus (P), available N, and available P in the soil were determined according to conventional laboratory methods. Specifically, total N was determined by the semi-micro Kjeldahl method, while total P was determined using a microplate reader after samples were digested with nitric acid. Soil-available P was extracted by an acid-ammonium fluoride solution, followed by colorimetry at 700 nm. In addition, ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) extracted with 2 mol L^{-1} KCl were measured using a continuous-flowing analyzer.

2.3 Statistical analysis

All data sets were checked for normality and homogeneity of variance by the Shapiro-Wilk test and Levene test, respectively, prior to statistical analysis. Data were square-root or log-transformed whenever necessary to ensure that they met the assumptions of normality. Two-way analysis of variance (ANOVA) was used to assess the effects of the growth season, growth stage, and their interaction on the content of soil physiochemical properties, different soil microbial groups, GRSP, and ASs using SPSS 20.0 (SPSS Inc., Chicago, USA). The relationships among microbial residues (ASs and GRSP), biomass of different living microbial groups, and soil physiochemical properties were evaluated by Spearman correlation analysis using the R software (version 4.1.3). Random forest models were performed to evaluate the importance of different soil physiochemical properties and the biomass of different microbial groups using the R software with the package “ranger”. All figures were plotted with GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, USA) and R.

3 Results

3.1 Changes in soil properties with rice growth

The SOC, total P, and total N contents in early-season soil were 7.87%, 27.56%, and 25.3% (P<0.01, Table 2) lower than those in late-season soil, respectively. However, the NO_3^--N content in the early-season was 3.00 times higher than that in the late-season (P<0.001) soil. The pH value, available P, and NH_4^+-N did not change significantly with growth stage and season. The tendency of soil properties to change with growth stage differed, with SOC, pH, and NH_4^+-N, NO_3^--N increasing and total P content decreasing from the TS to RS in both the early- and late-season rice (Table 2). Total P and available P contents did not change with the rice growth stage.

3.2 Soil microbial community composition

The levels of PLFA were lower in early-season soil than in late-season soil (P<0.001, Fig. 1A−1G). Overall, during the early season, the total, fungal, bacterial, GP bacterial, GN bacterial, and AMF PLFAs showed the order TS>RS>HS. In the late-season soil, the fungal PLFA in different growth stages of rice showed a trend of TS>HS>RS, whereas the AMF PFLAs showed the opposite tendency (Fig. 1F). Moreover, the level of PLFAs was significantly increased in the late-season soil relative to the early-season soil. In addition, the F:B ratio was lower in early-season soil than in late-season soil (P<0.001, Fig. 1H), which accounted for 86.13% of late-season soil. However, the ratio of GP to GN was 1.81 times higher in early-season soil than in late-season soil

Table 2 Physiochemical properties of paddy field soil under different growth stages and growth seasons.

Growth season	Growth stage	SOC (mg g^{-1})	Total N (mg g^{-1})	Total P (mg kg^{-1})	pH	Available P (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})
UN	UN	13.2±0.7	0.99±0.15	641±34	6.92±0.06	54.8±2.7	83.6±2.0	0.83±0.07
Early-season	TS	14.5±0.1a	1.00±0.09a	669±26a	6.29±0.03b	46.9±3.9a	12.3±0.3b	1.03±0.20c
	HS	12.7±0.3b	1.09±0.07a	743±44a	6.66±0.13a	47.3±3.6a	20.5±1.8a	4.71±0.57b
	RS	14.9±0.5a	0.89±0.14a	482±61b	6.69±0.06a	52.8±1.9a	21.4±1.9a	7.90±1.25a
Late-season	TS	14.5±0.2b	1.41±0.08a	1035±129a	6.39±0.04b	48.0±2.2a	16.9±0.5b	0.84±0.11b
	HS	15.7±0.4a	1.52±0.11a	826±39a	6.53±0.05a	44.7±6.6a	26.6±1.0a	0.83±0.09b
	RS	15.5±0.2a	1.10±0.08b	753±94a	6.55±0.05a	49.5±3.3a	8.2±0.4c	2.86±0.41a
Early- vs. late-season		**	***	***	-	-	-	***

Note: Values in the table are shown as means ± SE. Different superscript letters in the same column indicate significant differences among growth stages (P<0.05). -, not significant; *, P<0.05; **, P<0.01; ***, P<0.001. UN is fallow soil before growing rice.

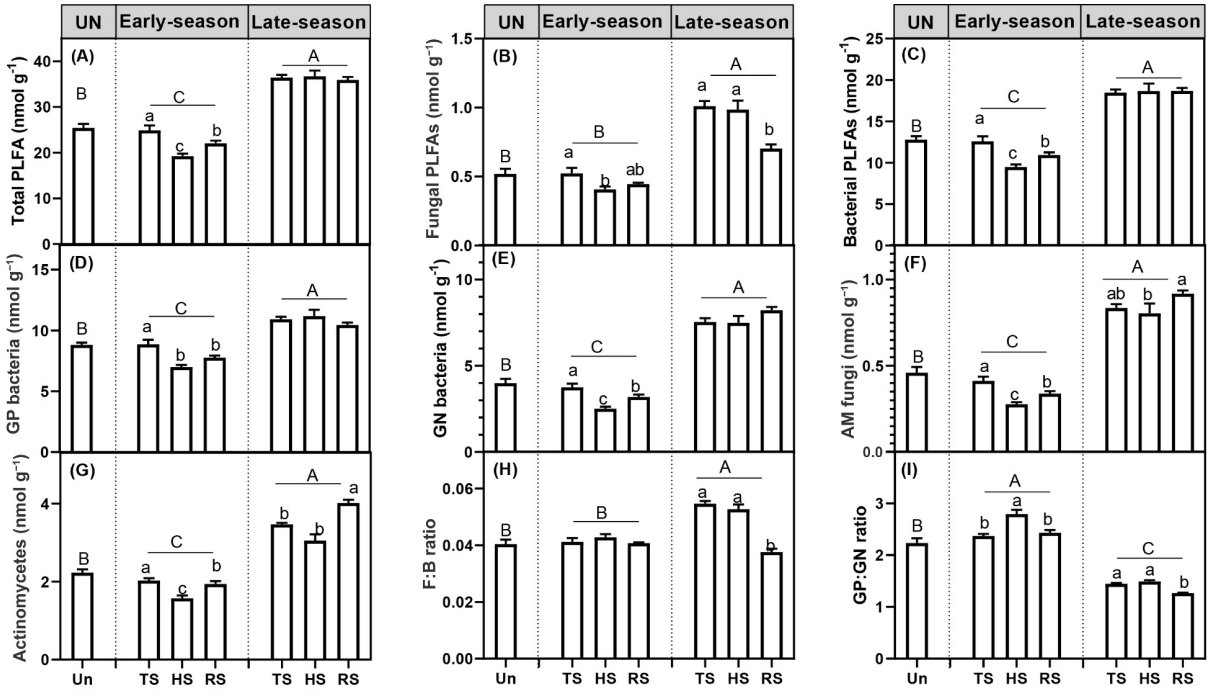


Fig. 1 Different microbial biomass in paddy soil in different growth stages and growth seasons. AM fungi, arbuscular mycorrhizal fungal PLFAs; Actinomycetes, *Actinomycetes* PLFAs; F:B ratio, the ratio of fungal: bacterial PLFA; GP: GN, the ratio of Gram-positive bacterial PLFAs to Gram-negative bacterial PLFA; UN, the fallow soil; TS, tillering stage; HS, heading stage; RS, ripening stage. Upper case letters indicate the significant differences among different growth seasons and un-growth soil, and lower case letters indicate the differences in different growth stages at $P<0.05$ level. The same below.

($P<0.001$, Fig. 1I). The ratios of F:B in the TS and HS were 1.20 and 1.23 times higher than those in the RS, respectively (Fig. 1H).

3.3 Glomalin-related soil protein content and its contribution to SOC

The growth stage had no significant effect on soil GRSP content, except for the EE-GRSP content, which increased with rice growth in the early-season soil ($P<0.05$, Fig. 2A−2C). The content of EE-GRSP, DE-GRSP, and T-GRSP in late-season soil was 1.30 times, 1.15 times, and 1.19 times higher than that in the early-season soil, respectively ($P<0.01$, Fig. 2A−2C).

There was a significant positive correlation between GRSP and SOC (Fig. 4A−4C). The C content of GRSP was set to 45% C (Schindler et al., 2007; Koide and Peoples, 2013) to estimate the contribution of GRSP to SOC. The results showed that the T-GRSP:SOC and EE-GRSP:SOC ratios were significantly influenced by the rice-growing season ($P<0.01$, Fig. 2F and 2G). The T-GRSP:SOC and EE-GRSP:SOC ratios of late-season soil were 1.09 times and 1.20 times higher than those of early-season soil, respectively. The EE-GRSP:DE-GRSP and EE-GRSP: T-GRSP ratios increased from the fallow soil to the late-season soil (Fig. 2G and 2H).

3.4 Amino sugar content and its contribution to SOC

The F-GluN content (294.60−412.93 mg kg^{-1}) was significantly higher than the MurN content (33.12−47.42 mg kg^{-1}, Fig. 3A and 3B). Specifically, the MurN content of early-season soil was 1.09 times as high as that of late-season soil, while the F-GluN content was 92.46% that of late-season soil (Fig. 3A and 3B).

The F-GluN content was positively correlated with SOC (Fig. 4E), whereas the MurN and total AS content was not significantly correlated with SOC (Fig. 4D−4F). The contribution of F-GluN to SOC did not change between the early- and late-seasons ($P>0.05$). However, the ratio of MurN:SOC and total ASs:SOC in the early-season soil was 1.18 and 1.94 times that in the late-season, respectively ($P<0.01$, Fig. 3E and 3F). Based on the conversion coefficient, microbial-derived carbon accounted for 31.96%−37.57% of the SOC by converting AS content into soil microbial residue carbon (MRC). Bacterial MRC and fungal MRC accounted for 11.82%−13.96% and 19.82%−23.62% of the SOC, respectively (Fig. 3G and 3H).

3.5 Factors affecting microbial residues and their contribution to SOC

Spearman correlation analysis of soil physiochemical properties, microbial residues, and microbial communities revealed that GRSP and ASs were significantly correlated

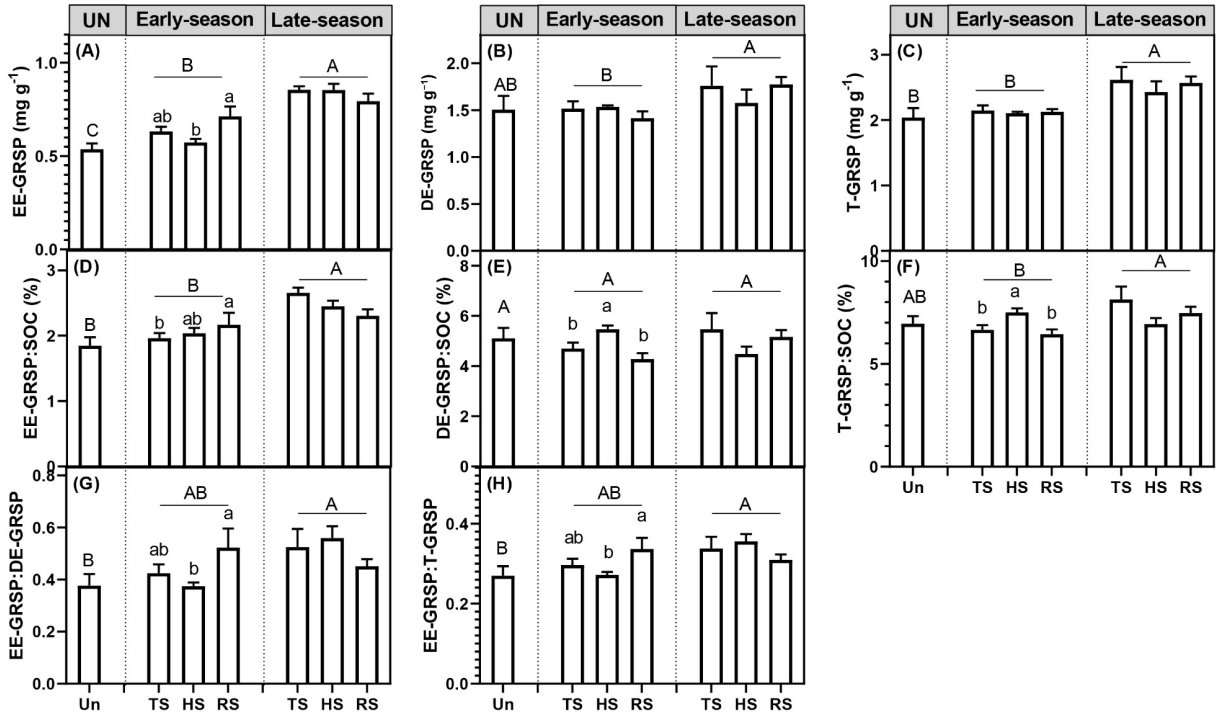


Fig. 2 Glomalin-related soil protein (GRSP) and contribution to soil organic carbon (SOC) in different growth stages and growth seasons. EE-GRSP, easily extracted GRSP; DE-GRSP, difficult extractable GRSP; T-GRSP, total GRSP. Note: a value of 45% C in GRSP (Schindler et al., 2007; Koide and Peoples, 2013) was used to estimate the contribution of GRSP to SOC in this figure.

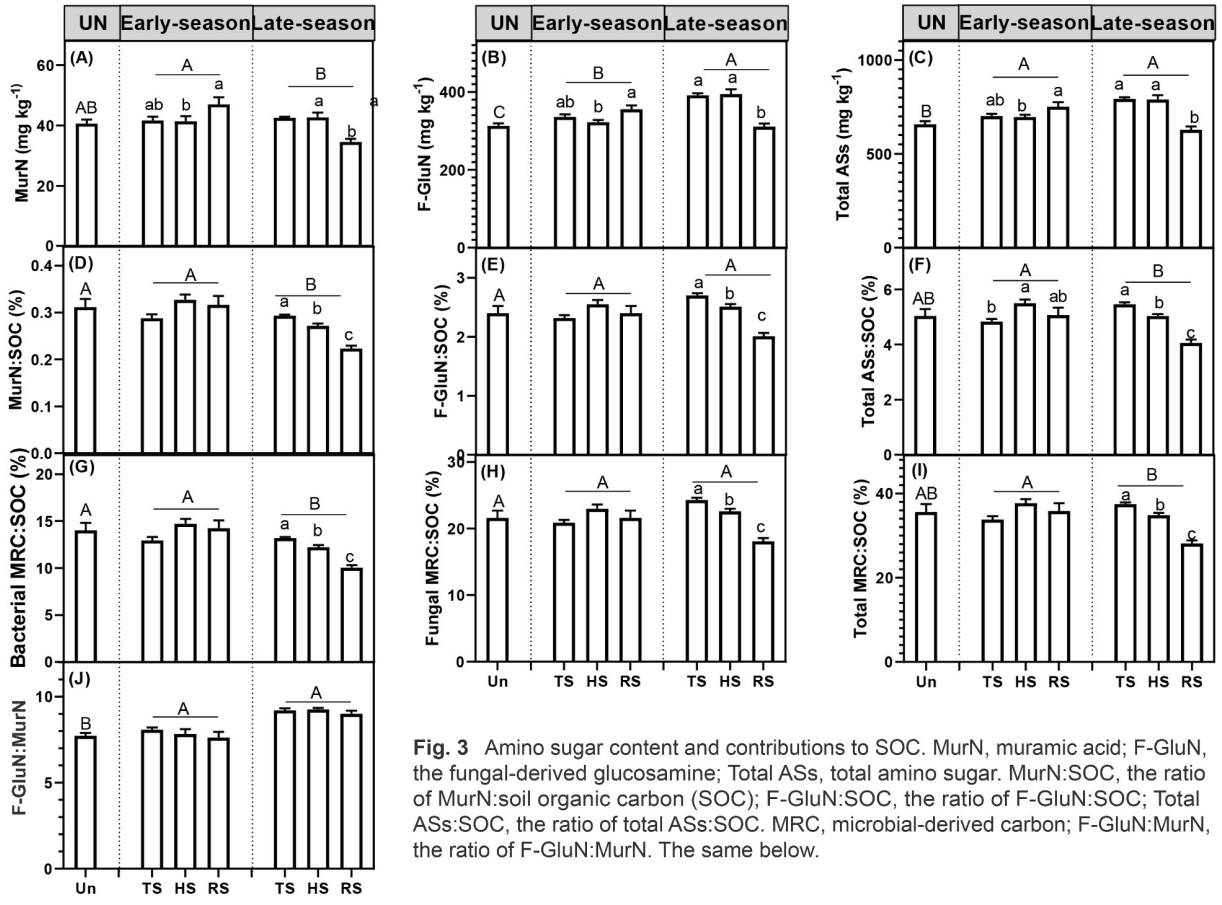


Fig. 3 Amino sugar content and contributions to SOC. MurN, muramic acid; F-GluN, the fungal-derived glucosamine; Total ASs, total amino sugar. MurN:SOC, the ratio of MurN:soil organic carbon (SOC); F-GluN:SOC, the ratio of F-GluN:SOC; Total ASs:SOC, the ratio of total ASs:SOC. MRC, microbial-derived carbon; F-GluN:MurN, the ratio of F-GluN:MurN. The same below.

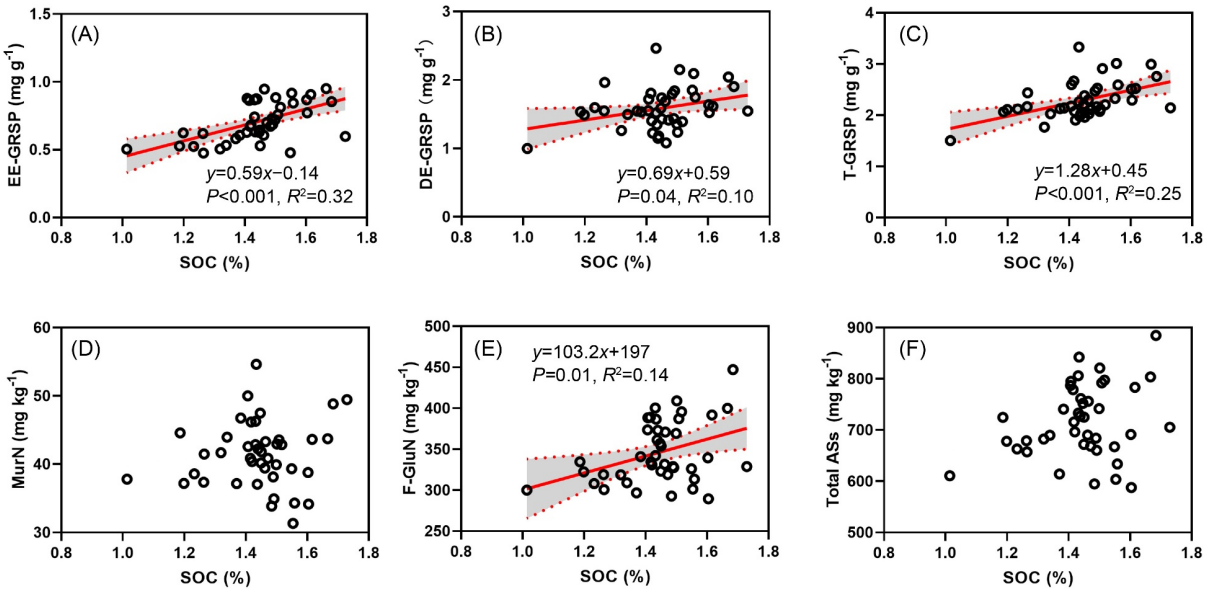


Fig. 4 Linear regressions between different soil microbial residues and SOC.

with different soil microbial groups. Specifically, there was a significant positive correlation between microbial residues and total N and total P. In addition, T-GRSP and EE-GRSP were negatively correlated with NH_4^+-N rather than with NO_3^--N. Furthermore, the ratio of T-GRSP:SOC was significantly positively correlated with total P (Fig. 5). Different microbial communities were positively correlated with total N and total P, whereas they were negatively correlated with available N (mainly NO_3^--N). Only fungal PLFA was negatively correlated with pH (P<0.05, Fig. 5).

The random forest model showed that microbial residues are mainly regulated by the structure of the living microbial community, while soil nutrients had no significant effect on residues (Fig. 6). SOC content was mainly regulated by the ratio of GP:GN, GN, AMF, and bacterial biomass. The contents of EE-GRSP and T-GRSP were influenced by GN, AMF, and bacterial biomass (Fig. 6). Different soil microbial communities were found to be the main factors influencing microbial residues. Among soil microbial residues, MurN is mainly affected by actinomycetes and the ratio of GP:GN, while F-GluN and total ASs were mainly affected by the ratio of F:B, fungal biomass, and GP (Fig. 6).

4 Discussion

4.1 Changes in microbial residues and contributions to SOC under different growth stages and growth seasons

GRSP is recalcitrant in soil (Rillig et al., 2001) and accounted for about 7% of the SOC in our study site (Fig. 2), indicating that GRSP is an important component of SOC that also maintains SOC pool stability. EE-GRSP is composed of the newly produced or readily decomposed GRSP in soil (Steinberg and Rillig, 2003). The increase in EE-GRSP from fallow to late-season rice soil suggests that labile GRSP was gradually deposited in the soil with rice planting. This increase occurred despite the AMF decreased from the fallow soil to the early-season (Fig. 1F). The discordant changes in AMF and EE-GRSP can be partly attributed to the sufficient nutrient levels provided by fertilization in the rice paddies. As a result, microbes did not need to decompose GRSP to obtain nutrients during the early-season. The content of DE-GRSP and T-GRSP only increased from the early- to the late-season (Fig. 1A), indicating that rice planting also increased the recalcitrant GRSP and facilitated the relatively stable GRSP reserve in soil.

As an important part of the soil stable carbon pool, soil microbial residues account for 30%−43% of the SOC (Khan et al., 2016), and their accumulation in the soil directly affects dynamic changes in the soil carbon pool. Our study revealed that microbial residue accounted for 31.96%−37.57% of SOC content, which was within this range (Fig. 2). Moreover, the contribution of fungal residues to SOC is much greater than that of bacterial residues (Fig. 3H and 3I), indicating that fungal residues are dominant in subtropical rice fields (Roth et al., 2011; Chen et al., 2017; Xia et al., 2021). Glaser et al. (2004) and Shao et al. (2017) found that the ratio of F-GluN to MurN is widely used to reflect the relative contribution of fungal and bacterial residues to SOC accumulation in soil. A ratio greater than 5 indicates that fungal residues dominate the accumulated microbial residues, and contribute to SOC. In this study, the ratio of F-GluN to MurN was 8.50±0.19 (Fig. 3), indicating that fungal residues play a dominant role in the SOC pool in paddy soil. This is similar to the result reported by Ning et al. (2019), who found that the ratio of F-GluN to MurN in paddy soil in Hunan province

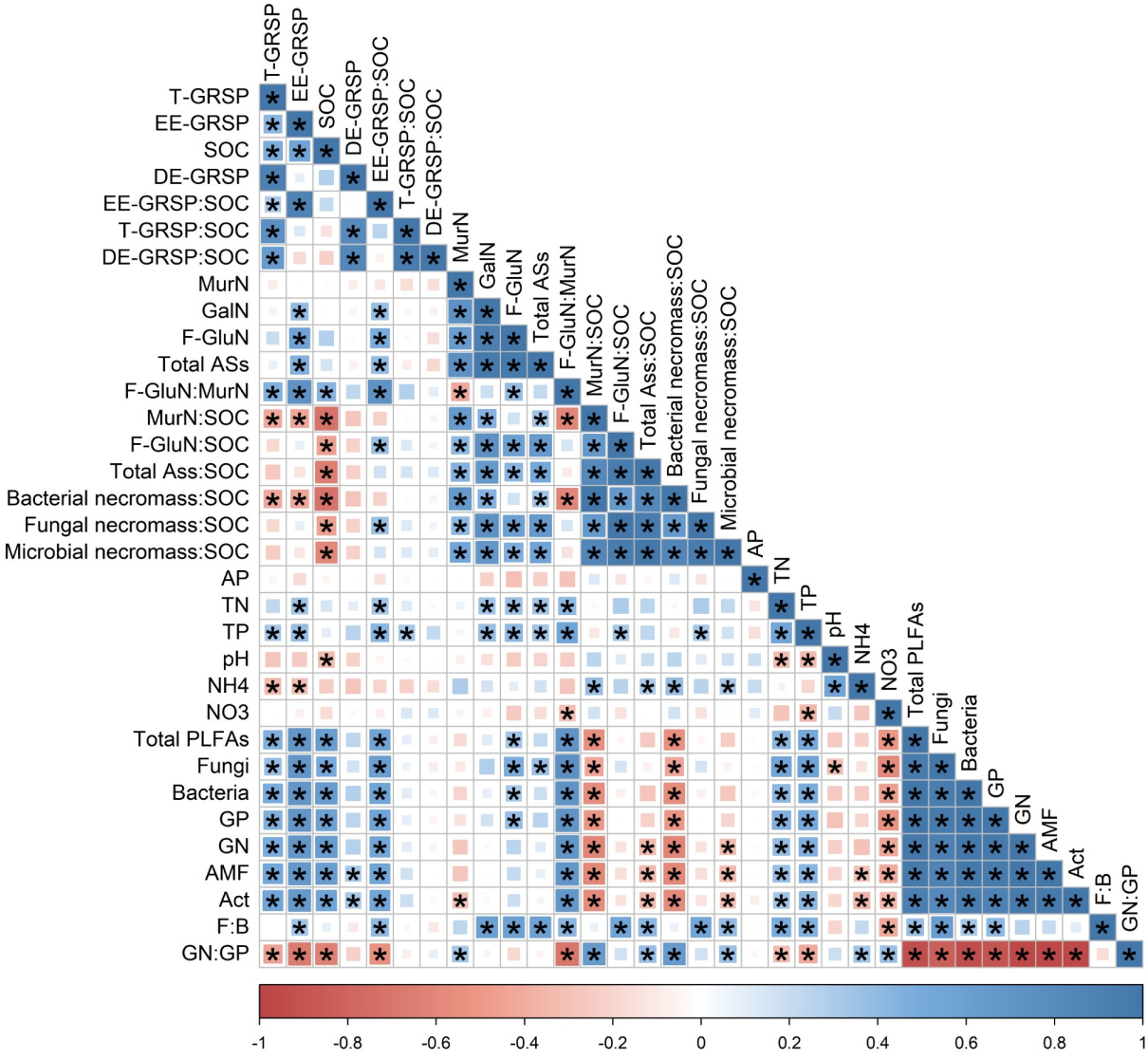


Fig. 5 Spearman correlation thermogram analysis of the relationship between different microbial indices and their contribution to SOC, soil physiochemical properties, and soil microbial community. Bacteria: bacterial PLFA; Fungi: fungal PLFA; NH4: NH_4^+-N; NO3: NO_3^--N; Act, actinomycete PLFA; Total PLFAs: total microbial PLFAs. Asterisks indicate a significant correlation (P<0.05).

was much greater than 5. Fungi have a strong degradation effect on straw after it is returned to the field (Zhang et al., 2021), which stimulates the propagation of fungi in a short period and therefore probably increases the fungal residues accordingly.

Late-season rice soil promoted the accumulation of T-GRSP (Fig. 2C) and its contribution to SOC (Fig. 2F), while simultaneously reduced the contribution of ASs to SOC (Fig. 3F). This was probably due to the greater increase in SOC compared to that of T-ASs. Specifically, the SOC content increased by 8.57% (Table 2), while the T-ASs increased by 2.93%. Therefore, the contribution of AS to SOC in late-season rice soil decreased compared with the early-season. The significant increase in SOC compared with T-ASs can likely be attributed to straw return, which resulted in the rapid conversion of a large amount of plant-derived C into the soil in a short period (Meng et al., 2024). The faster accumulation of GRSP than ASs to SOC could be explained as follows: GRSP is more stable than amino sugar (Agnihotri et al., 2022) because GRSP contains a higher proportion (>50%) of aromatic C and alkyl-C (Zhang et al., 2017; Agnihotri et al., 2022). In addition, AMF may reduce bacterial biomass and amino sugars under nutrient deficiency (He et al., 2020), which would also intensify the different accumulation rates of amino sugar and GRSP in soil. Studies conducted in forests have also shown that GRSP contributed more than T-ASs (Zhang et al., 2023; Li et al., 2023); therefore, further studies of paddy soils should be conducted to reveal the underlying mechanisms.

The bacterial biomass increased, whereas the content of

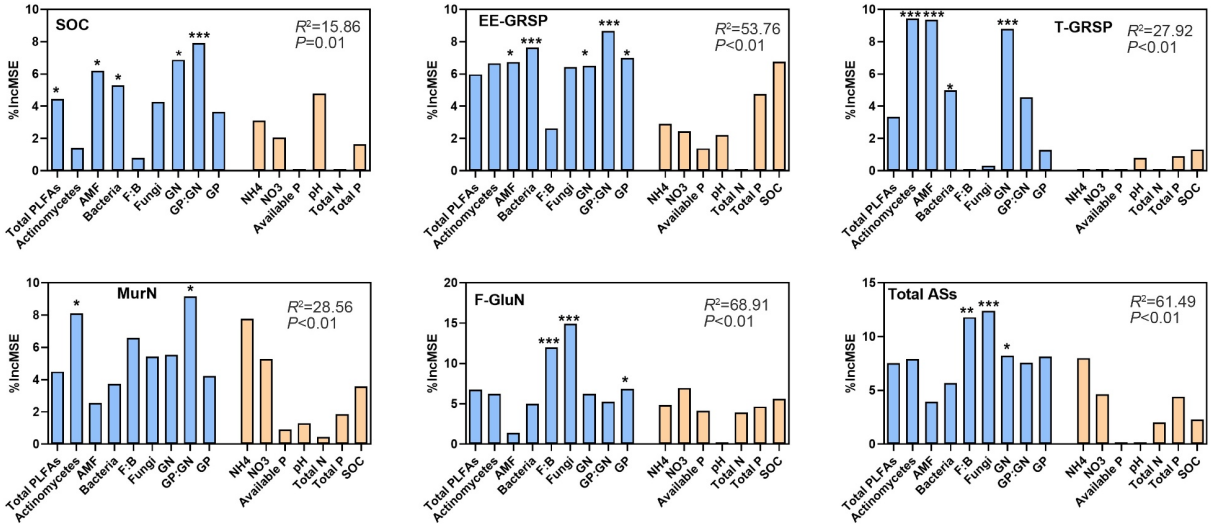


Fig. 6 The driving factors to SOC and different microbial residues by random forest model analyses. Blue columns are different living microbial groups, and yellow columns are different soil physiochemical properties. AMF, arbuscular mycorrhizal fungi; NH4, ammonium N; NO3, nitrate N; SOC, soil organic carbon.

bacterial residue decreased from the early- to the late-season (Figs. 1C and 3B). The increased bacterial biomass in the late-season was probably stimulated by the straw return before the late-season (Chen et al., 2017) because rice straw can provide energy and nutrients for microbial growth (Wang et al., 2015). However, the decreased bacterial residues from the early- to the late-season suggest high-intensity recycling of bacterial residues, which are more readily decomposed relative to fungal residues (Nakas and Klein, 1979). Thus, fluctuations in microbial residue do not always correspond to changes in living microbial biomass (Amelung et al., 2001; Turrión et al., 2002). The decreased MurN and contribution to SOC were probably caused by different rates of cell mineralization by the bacterial community. Previous studies have shown that GP bacteria contain a highly variable content of MurN that is an average of 3.8 times higher than that of GN bacteria (Appuhn and Joergensen, 2006). The increased amplitude of GN (2.46 times) relative to that of GP (1.38 times) from the early- to the late-season (Fig. 1D and 1E) and the decreased GP: GN (Fig. 1I) observed in our study site likely explain why MurN did not increase as much as the living bacteria. GP bacteria are more dependent on complex C compounds, whereas GN is more dependent on simple C compounds in organic soils (Fanin et al., 2019). The decreased GP:GN ratio indicated that returning straw before the cultivation of the late-season rice increased the simple C input. The standing content of MurN depends on its accumulation and decomposition by the living microbes. Therefore, the decreased MurN and the contribution to SOC indicated the bacterial residue accumulation rate was lower than the decomposition rate due to the changed bacterial community. The significantly positive correlation between MurN and the GP:GN ratio (Fig. 5) also supported this conclusion.

4.2 The mechanisms driving variations in microbial residues in paddy soil

Microbial residues were mainly affected by living microbes rather than soil physiochemical properties (Fig. 6), which may have been related to paddy field fertilization. The input of exogenous fertilizer affects the biomass and metabolic activities of microbes in the soil (Liu et al., 2018) and ultimately affects the total amount of microbial residues (Joergensen et al., 2010). In the case of insufficient soil nutrients, microbial residues may be related to the status of soil nutrients. This is because the stoichiometric ratios of N:P, C:N, and other elements in the soil would affect the production of microbial residues, which in turn influences the availability of microbial residues (Mooshammer et al., 2014). For example, Huang et al. (2019) demonstrated that the main factors affecting the accumulation of microbial residues are soil organic matter and living microbial biomass. However, Geyer et al. (2016) found that when nutrients are sufficient exogenous carbon can be quickly assimilated by microbes and accumulated in the soil as microbial residues. Therefore, when nutrients are sufficient, microbial residues are independent of soil nutrients; however, in some forest ecosystems with little exogenous nutrient input, microbial residues are mainly related to soil nutrients.

5 Conclusions

Straw returning after early-season planting and root residues in the soil promoted the growth of AMF and the accumulation of GRSP. In addition, the content of GRSP in late-season soil was significantly increased, as was the contribution of GRSP to SOC. Microbial biomass and

residues also changed with rice growth in one year, with the biomass of living microbes increasing more than the residues. Bacterial biomass and residue showed the opposite tendency from the early- to the late-season, probably because of a change in the bacterial community. More information on specific differences between the turnover of fungal and bacterial residues can be obtained by labeling and measuring microbial community composition and amino sugar-specific $\delta^{13}C$ in future studies.

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Authors' contributions

Jing Zhang and Xiaozhe Bao conceptualized and administered the project and acquired funding. Xiaozhe Bao and Taotao Yang collected the samples. Peiyue Wang performed the experiments. Peiyue Wang, Xiaozhe Bao, and Jing Zhang performed the data analyses and wrote the manuscript. Bin Zhang, Zhanfeng Liu, and Taotao Yang helped perform the analysis with constructive discussions. All authors contributed to the revisions during the editing process. All authors read and approved the final manuscript.

Competing interests

The authors have no financial or proprietary interests in any material discussed in this article.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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