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Title: Using milk vetch (*Astragalus sinicus* L.) to promote rice straw decomposition by regulating enzyme activity and bacterial community

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Abstract: The present study determined the dynamic changes of enzyme activity and bacterial community in rice straw (RS) and milk vetch (MV) co-decomposing process. Results showed that mixing RS and MV promoted decomposition. The mixture enhanced β-glucosidase and β-cellobiohydrolase activities relative to its monospecific residue during the mid-late stage of decomposition. The mixture enhanced *Enterobacteriaceae* (monosaccharide decomposing bacteria) abundance during the initial stage of decomposition, and the abundance of *Hydrogenispora, Bacteroides*, *Ruminiclostridium, and Acidobacteriaceae* that could hydrolyze fiber during the mid-late stage of decomposition relative to single RS and MV, respectively, which would benefit mixture decomposition. Furthermore, more interconnected and competitive relations existed between the

bacteria in the mixture. These results indicated that mixing RS and MV promoted residue decomposition by increasing hydrolytic enzyme activities and changing bacterial community. This study concluded that co-incorporating RS and MV may be recommended as a promising practice for the efficient utilization of RS resources.

Keywords: Rice straw; Milk vetch; Co-decomposition; Enzyme activity; Bacterial community

1. Introduction

Rice straw is an important renewable natural resource and is abundant in macro- and micro essential nutrient elements for crop, with a total yield of about 0.20 billion tons annually in China (Nan et al., 2020). Substantial rice straw returned directly to farmland continues to increase along with the continuous advancement of agricultural mechanization in recent years. However, rice straw has a high C/N ratio and may lead to negative effects on subsequent crop yields because of nitrogen (N) immobilization and slow decomposition (Eagle et al., 2000). There is therefore an urgent need to establish an effective method to manage rice straw in a way that alleviates negative impacts on crop yields. Previous studies have provided that converting rice straw to biochar or composting rice straw before incorporating it into soil would alleviate the negative impacts on crop yields (Qian et al., 2014; Nan et al., 2020). Yet the amount of rice straw treated by both of above method was limited. Significantly, an innovation practice, co-incorporating rice straw and leguminous green manures (e.g. milk vetch, hairy vetch, etc.), can increase rice yield in a short time in southern China (Zhou et al., 2020), and is considered to be a productive and sustainable practice for effective utilization of rice straw.

Co-decomposition occurs naturally after co-incorporation of rice straw and leguminous green

manure. Gartner and Cardon (2004) summarized and reported that the decomposition rates of most of residue mixtures were usually faster than those of individual residues due to the residue mixture effects. Hättenschwiler et al. (2005) reported that the mechanisms underlying the residue mixture effects may be the transfer of nutrient between the residues, the improvement of microclimatic conditions or habitat diversity, and the interactions across trophic levels. Meanwhile the residue mixtures increase the diversity of substrates and associated niches for microorganisms relative to a single residue (Hättenschwiler et al., 2011; Balachandar et al., 2020), both of which would stimulate the microbial population and enzyme activity, thereby accelerating residues decomposition. Previous studies have provided some evidence that microbial biomass and activity respond positively to residues mixtures (Chapman et al., 2013; Zhou et al., 2019). However, the mechanism remains unclear and there is a need to understand the effects of the residues' mixture on decomposition and changes in the microbial decomposer communities (Santonja et al., 2017).

The succession of various microbial populations is a characteristic of the decomposition process, and the availability and type of nutrients are major determinants in shifting microbial composition (Bastian et al., 2009). That is to say, microbial communities or functions in decomposing residues vary at different stages due to the changes in substrate characteristics. de Graaff et al. (2010) has also confirmed that the shift of microbial populations is one of the most important factors in regulating decomposition rates. Hence, exploring the difference in microbial populations in residues and mixtures during the decomposing process can improve understanding of the decomposition mechanisms. In addition, the network analysis of co-occurrence, focusing on the correlations between microbial species, can decipher the structure and assembly of complex microbial communities (Zhou et al., 2011; Banerjee et al., 2018). Identifying the correlations between microbial species is therefore also

important for improving understanding of the decomposition of residues mixtures.

In this study, an experiment was designed to analyze the rice straw and milk vetch mixture (consisting of carbon 405.7 g kg^{-1}, nitrogen 15.1 g kg^{-1}, dissolved organic carbon 61.2 g kg^{-1}, mineral nitrogen 1.3 g kg^{-1}) decomposition along with their associated microbial communities during the rice season. The primary objective was to answer the following questions: (1) does mixing rice straw and milk vetch cause faster decomposition than would occur in their respective monospecies? (2) if yes, does mixing rice straw and milk vetch increase the decomposition related enzyme activity and change the bacterial community? (3) and what is the microbial mechanism by which mixing rice straw and milk vetch alters decomposition?

2. Materials and methods

2.1. Experimental material preparation

Soil was obtained from a rice-rice-milk vetch system at the Gaoqiao Research Base of Hunan Academy of Agricultural Sciences in Changsha County, Hunan Province, China. The soil was derived from river alluvium deposits and is classified as Stagnic Anthrosol according to the FAO (2015). Fresh paddy soil (water content about 10%) was sieved (< 2 mm mesh) and remove stone and root for the experiment. Soil characteristics including pH (1:2.5 H_2O) 4.9, organic carbon (C) 13.3 g kg^{-1}, total nitrogen (N) 1.38 g kg^{-1}, mineral N 66.4 mg kg^{-1}, available phosphorous (P) 18.2 mg kg^{-1}, and available potassium (K) 130.5 mg kg^{-1}, C/N ratio 9.8.

The residues were collected from the same location as the soil, including rice straw (RS) and milk vetch (MV). According to the actual production status, RS was air-dried whereas MV was stayed fresh, and both of residues were cut into 5 cm pieces before experiment. The basic properties of residues were

as follows: RS, total C (TC) 39.8% (dry basis, the same below), total N (TN) 0.6%, dissolved organic C (DOC) 3.9%, mineral N (Nmin) 0.06%, C/N ratio 62.8; MV, water content 90%, TC 42.9% (dry basis, the same below), TN 3.0%, DOC 13.0%, Nmin 0.36%, C/N ratio 14.3.

2.2. Experimental design and management

A mesocosm-scale experiment was conducted in a netted enclosure out of doors. The mesocosm was designed with the following four treatments: unamended (CK), MV amendment (M), RS amendment (R), and MV and RS mixture amendment (MR). Four replicates were used for each treatment. The application rates of MV and RS were commensurate with the very usual levels observed in the field, i.e. 22500 kg fresh MV ha^{-1}, and 6750 kg dry-weight RS ha^{-1}. The same quantity of chemical fertilizer was used in each mesocosm, including 4.0 g N (Urea, 46% N), 2.0 g P_2O_5 (calcium superphosphate, 12% P_2O_5), and 2.4 g K_2O (potassium chloride, 60% K_2O). The rates of fertilizer were equivalent to 150 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1}, and 90 kg K_2O ha^{-1}, respectively.

The mesocosms were constructed in HDPE boxes with internal dimensions of 55 cm × 45 cm × 35 cm (Length × Width × Height). A total of 60 kg of soil (on dry-weight basis) was potted in each mesocosm (box) at a density of 1.20 g cm^{-3} and at a depth of 20.2 cm. The cut fresh MV and air-dried RS were placed in litterbags (20 cm × 15 cm) made of double-layer nylon mesh. The mesh size of the litterbag was 300 mesh (0.048 mm), which could allow free access for microorganisms from the soil while preventing the mixing of the residues with the surroundings. Each litterbag contained either 60 g fresh MV, or 18 g air-dried RS, or 60 g fresh MV plus 18 g air-dried RS. The litterbags were then vertically buried in soil at 20 cm depth in each mesocosm, and directly submerged with water, leaving a 3~5 cm water layer above the soil surface during the decomposition

process. Each mesocosm received 10 litterbags containing MV, RS, or MV and RS mixture, this resulted in a total of 120 litterbags (3 residue types × 4 replicates×10 sampling times). To mimic the field condition, rice was cultivated half month after the fertilizer and residues were applied (Zhou et al., 2020), with 12 seedlings planted in each mesocosm on June 3 in 2018 (see Supporting Information for the distribution of litterbags and rice plants). The rice was harvested on September 20th, 2018. The rice cultivar was Tianlong NO. 1.

2.3. Sampling and measurement

Four MV, four RS, and four MV and RS mixture litterbags were randomly sampled from mesocosms at 0, 5, 12, 27, 42, 57, 73, 88, 103, and 123 days after incorporation. On each occasion one litterbag was collected from each mesocosm. Then litterbags were transported to the laboratory where the remaining residue in each litterbag was carefully collected and weighed. Then, the remaining residue was divided into 3 parts: where one part was oven-dried (60 °C) for calculating water content and measuring total carbon (TC) and total nitrogen (TN), the second part was stored at 4 °C for determining dissolved organic carbon (DOC), mineral nitrogen (NH_4^+-N+NO_3^--N, Nmin) and enzyme activity, and another part was stored at −80 °C for DNA extraction and further microbial analysis.

Residue TC and TN were determined through an elemental analyzer (CHN, Elementar Analysensysteme GmbH, Hanau, Germany). The DOC concentration was measured with a residue extract (water to fresh residue ratio of 10:1) in a TOC/N (Multi N/C 2100, Aanalytikjena, Germany) after filtering through a 0.45 μm membrane filter (Zhang et al., 2011). The Nmin was extracted with 2 M KCl (1:100 ratio for 1 h) and measured using a continuous flow analyzer (AA3, SEAL Analytical, Norderstedt, Germany) (Shen et al., 2019). The percentage of biomass loss rate of residue was

computed using the formula (1):

$$\text{Biomass loss rate (\%)} = \frac{Mi - Mt}{Mi} \times 100 \quad (1)$$

where *Mi* is the initial biomass of residue (g), *Mt* is the biomass of residue at time t (day).

Decomposition rate of residue was calculated according to the formula (2):

$$\text{Decomposition rate (g day}^{-1}\text{)} = \frac{Mt' - Mt''}{t' - t''} \quad (2)$$

where Mt' and Mt'' are the biomass of residue at t' and t'', respectively; t' and t'' are the sampling time and the next sampling after *t* (d).

Hydrolytic enzyme activities, including β-glucosidase (BG) and β-cellobiosidase (CBH), were determined following the method of DeForest (2009). Briefly, a residue suspension was prepared by blending 1.0 g fresh residue sample in 100 ml of a 50 mM acetate buffer. The buffer, sample suspension, 200 μM substrates (4-MUB-β-D-glucoside and 4-MUB-β-D-cellobioside), and 10 μM references (4-methyl-umbelliferone) were dispensed in a 96-well black microplate. The microplates were darkly cultured at 25 °C for 4 h in an incubator, and then were measured fluorometrically with excitation at 360 nm and emission at 450 nm by using a microplate fluorometer (Scientific Fluoroskan Ascent FL, Thermo). The phenol oxidase (PHEO) activity was determined in a 96-well clear microplate by adding the substrate of L-3, 4-dihydroxyphenylalanine (Ai et al., 2012). The microplates were incubated in the dark at 25 °C for 11 h, and the activity of PHEO was calculated by measuring the absorbance at 450 nm using the microplate fluorometer.

The DNA was extracted using a FastDNA SPIN Kit for Soil (MP Biomedicals, CA, USA) following the manufacturer protocol. The DNA quality was tested by electrophoresis using 1.0% agarose gel, and its concentration was determined using a spectrophotometer (Nanodrop ND-2000, Thermo Fisher, Waltham, MA, USA). The bacterial 16S rRNA genes were amplified with the

following primers: 338F (5′-ACTCCTACGGGAGGCAGCA-3′), and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (He et al., 2018). These amplified PCR products were sequenced on the Illumina PE 300 sequencer (Illumina Inc., San Diego, CA, USA).

2.4. Bioinformatics and statistical analyses

The Quantitative Insight into Microbial Ecology (QIIME) pipeline (http://qiime.sourceforge.net/) was used to analyze the sequence data. Following quality control adopted, 6042453 high quality sequences were received from the 64 samples for bacteria. The valid sequences were clustered into operational taxonomic units (OTUs) at the 97% similarity level through the UPARSE pipeline (Edgar, 2013). The Silva database (http://www.arb-silva.de) was used to assign the taxonomic identity of each phylotype of bacteria (Quast et al., 2013).

The data measured under different treatments were subjected to one-way ANOVA and multiple comparison tests (Duncan's post hoc test, $P < 0.05$) using SAS version 8.1 software for windows. The law of biomass loss late of buried residues was fit using the power function equation (Pal and Broadbent, 1975) performed on Origin 2018 software. The STAMP (v. 2.1.3) was adopted for determining statistical differences in the OTUs abundance (relative abundance > 1%) according to the different treatments. The aggregated boosted trees (ABT) analysis was performed to reveal the effects of different factors on alpha diversity of the bacterial community using the 'gbmplus' package in R (v. 2.7.1) (Jiang et al., 2017). The principal coordinate analysis (PCoA) and partial Mantel test were performed on the 'vegan' package in R (v. 3.4.1). The relationships between sampling date, residue property, bacterial community, enzyme activity, and residue decomposition rate were analyzed using the partial least squares path modeling (PLS-PM). Microbial networks were established for bacterial

communities based on OTUs relative abundance (> 0.01%) in the decomposition process, and were performed on the Molecular Ecological Network Analyses pipeline (http://ieg2.ou.edu/MENA/) (Zhou et al., 2011).

3. Results and discussion

3.1. Biomass loss rates and chemical properties of residues

Biomass loss rates of buried residues followed a power function equation ($y = ax^b$) (Fig. 1). There were differences in the parameters '*a*' and '*b*' of the equation among the three types of residues. The value of '*a*' indicated the most easily released components of residues, and '*b*' was the growth parameter of loss rate (Pal and Broadbent, 1975). The highest values of '*a*' were in the M treatment and '*b*' were in the R treatment indicating that M had the highest decomposition rate early in the incubation and R had the fastest growth in decomposition rate (Fig. 1). The regression formulae predicted that the MR treatment would take the shortest time (576 d) for the residue to completely decompose followed by the R and M treatments which took 627 d and 975 d, respectively. Prescott (2005) reported that the early decomposition rate or initial quality of residues was not a good indicator for the whole decomposition period, mainly due to constant changes in residue quality during the decomposition process. Moreover, the mixed residues always had non-additive effects on decomposition, which would influence the decomposition rate (Hättenschwiler et al. 2005; Bonanomi et al., 2010). The present study revealed that the MR treatment shortened the decomposition time in comparison with monospecific substrates (Fig. 1), indicating that non-additive synergistic effects occurred during the decomposition process (Bonanomi et al., 2010). Similarly, previous studies documented that the loss rates of residues were often increased when residues from different species were mixed (Gartner and Cardon 2004;

Jacob et al., 2009).

During the process of decomposition, the TC concentration of residues decreased in all treatments, and declined from 428.7 to 234.7 g kg^{-1} (reduced by 45.2%), 398.0 to 247.1 g kg^{1} (reduced by 37.9%), and 405.7 to 254.9 g kg^{-1} (reduced by 37.1%) for the M, R and MR treatments, respectively (Fig. 1). However, a rapid decrease from 29.9 to 7.7 g kg^{-1} (reduced by 74.2%) in the TN concentration was found only in the M treatment across the whole incubation period (Fig. 1). Since the M treatment released N at a faster rate than C, its C/N ratio progressively increased until a threshold value was attained (Fig. 1). By contrast, the R and MR treatments released C at a faster rate than N, which caused decreases in their C/N ratios (Fig. 1). Previous studies showed that the movement direction of C/N ratio were often related to an initial C/N ratio of the residue; specifically, the initial C/N ratio of 25-30 was a threshold for an increase or decline in the C/N ratio during the decomposition period (Jacob et al., 2009). In the present study, the changes of C/N ratio were consistent with this principle.

The DOC concentration declined during the decomposition process (Fig. 1). The final DOC concentrations for M, R, and MR treatments were 1.7, 2.4, and 2.8 g kg^{-1}, respectively. Notably, after decomposing for 25 days, the order of DOC concentration was MR > R> M. Kiikkilä et al. (2012) suggested that the increase in DOC concentration in the mixed residues was due to the mixture favoring the decomposition of residues. Similarly, the Nmin concentration sharply dropped in the first two weeks (Fig. 1). The Nmin concentrations in the M and MR treatments from day 0 to day 57 were much higher than those in the R treatment, which could be explained by a higher TN concentration in the M and MR treatments during those days. It is noted that mixing residues often improves microbial activity, which can contribute to synergisms in residue decomposition (Chapman et al., 2013). Thus, the observation that the Nmin concentration was higher in MR treatment than in M and R treatments

was expected (Fig. 1).

3.2. Dynamic changes in extracellular enzyme activity during the decomposition process

The β-glucosidase (BG) and β-cellobiohydrolase (CBH) are part of the cellulase complex, which is associated with catalyzing the biodegradation and hydrolysis of cellulose present in decomposing residues; and the activities of both enzymes are synergistic (Shukla and Varma, 2010). The activities of BG and CBH ranged from high to low as follows: M > MR > R in day 5, and MR > R > M form day 27 to day 123 (besides CBH in day 123) (Fig. 2), which indicated mixing residues could enhance the two enzymes activities at the mid-late stage of decomposition. Das et al. (2013) suggested that the mixture of various residues provided essential nutrients and induced microbial growth and enzyme secretion. Moreover, the differences in activities of BG and CBH among the three treatments also could be explained by the changes in TN and Nmin concentrations (Shukla and Varma, 2010).

The activity of phenol oxidase (PHEO) in the M and MR treatments from day 5 to day 88 was much greater than that in the R treatment (Fig. 2). A possible reason was that the M and MR treatments had a higher pH value in comparison with the R treatment (Wang et al., 2012), and the activity of PHEO would increase with the increasing pH in an acidic environment (Shukla and Varma, 2010). As decomposition proceeded, the differences in pH between treatments declined, resulting in the differences of PHEO activity in the three treatments gradually decreasing (Fig. 2).

3.3. Succession of bacterial community

3.3.1 The α diversity of bacterial community

The Observed_ species, Chao1 and Shannon index values indicate the richness and diversity of

the microbial community, with higher values associated with higher richness and diversity. During the decomposition process, the richness and diversity of bacterial community changed with increasing richness and diversity over time (day 5 to day 88), before they stabilized (Table 1), indicating that a growing number of bacteria were involved. The dynamics of these indexes in residue decomposition were in good agreement with the previous findings (Dilly et al., 2004; Maarastawi et al., 2018). Furthermore, the Observed_ species, Chao1 and Shannon index values were lower at day 5 but were higher from day 27 to day 123 in the M treatment in comparison with the R and MR treatments. The aggregated boosted tree analysis revealed that the residue DOC concentration was the most important factor in influencing bacterial richness and diversity (Table 2). Therefore, the differences in bacterial richness and diversity between treatments or sampling dates could be interpreted as follows: (a) high level of available substrate such as DOC favored the enhancement of strongly competitive bacteria which released allelochemicals and then restrained the enhancement of other bacteria that assimilated the same substrates; and (b) more species or genotypes were required for decomposition or were able to grow when the residue quality was low (Dilly et al., 2004).

3.3.2 Changes in bacterial community structures during the decomposition process

Forty-one phyla were recognized based on the taxonomic analysis of the 16S rRNA sequences (Fig. 3A). *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Chloroflexi*, *Spirochaetae*, and *Actinobacteria* were considered the dominant phyla associated with residue decomposition. Collectively, they accounted for about 84% or more of all sequences (Fig. 3A). *Firmicutes* was known as the most leading bacterial phyla from day 5 to day 88, with an abundance of 62.7%, 65.4%, 52.1%, and 32.1% at day 5, day 27, day 57, and day 88, respectively. However, the relative abundance of

Firmicutes declined as decomposition progressed. This development was possibly associated to the decrease in DOC concentration of the residues. Previous studies have reported that *Firmicutes* was a fast-growing copiotrophic genus (*r* strategy) that was stimulated in environments rich in available-C substrates (Fierer et al., 2007). As the DOC concentration declined and recalcitrant substrates were enriched, the predominance of *Firmicutes* was replaced by other phyla.

Proteobacteria was another dominant bacterial phylum (Fig. 3A), and it accounted for 5.6%-39.5% of the microbes in all treatments during the decomposition period. Interestingly, the relative abundance of *Proteobacteria* firstly decreased and then increased over time, as a result of succession of *Proteobacteria* species during the decomposition process. The *Proteobacteria* are comprised of the *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria,* and *Deltaproteobacteria* classes (Bastian et al., 2009). Previous studies have shown that *Deltaproteobacteria* and *Betaproteobacteria* could be regarded as oligotrophic bacteria (*K* strategy) that could be modified to live with a slower growth rate when available substrates were insufficient (Nicolardot et al., 2007; Bastian et al., 2009); whereas *Alphaproteobacteria* and *Gammaproteobacteria* could be considered as copiotrophic populations (Bastian et al., 2009). Their different ecological niches support the results that the relative abundance of *Gammaproteobacteria* decreased while *Betaproteobacteria* and *Deltaproteobacteria* increased as decomposition progressed. However, the changes in the *Alphaproteobacteria* were contrary to expectation (decreased with time). It was possible that the *Alphaproteobacteria* could behave differently when exposed to a flooded environment as opposed to the more normal aerobiotic environment (Nemergut et al., 2010).

By contrast to the changes observed in the *Proteobacteria,* the relative abundance of *Bacteroidetes* firstly increased and then slightly decreased with over time (Fig. 3A), and its maximum

abundance was at about 15.6% (average of three treatments) at day 27. In aquatic environments, *Bacteroidetes* is commonly associated with the decomposition of cellulose (Schellenberger et al., 2010). During the decomposing process, the available C contained in DOC initially decreased sharply, and then polymeric C substrates in the residues such as cellulose were enriched, stimulating the enhancement of *Bacteroidetes,* whereas the relative abundance of *Bacteroidetes* would decrease with the cellulose decomposition and accumulation of lignin. However, the relative abundances of *Acidobacteria*, *Chloroflexi*, *Spirochaetae*, and *Actinobacteria* showed consistent changes during the decomposition process, i.e. all of them increased over time (Fig. 3A). This progression may result from their respective ecological niches or habitat preferences, and was probably linked to their capacity to decompose more complex carbon compounds. Previously, these four phyla have generally been classified as oligotrophic or *K*-selected groups, with the capability of decomposing lignocellulose and lignin (Fierer et al., 2007; Pandit et al., 2016).

3.3.3 Difference in bacterial community structure between the mixed residues and monospecific residue

The PCoA plot exhibited that there were obvious differences in bacterial community structure between sampling dates (Fig. 3B). This finding suggested that the succession of bacterial community had occurred, which was possibly attributed to the shifts in the supply of nutrients during the decomposition period (Ma et al., 2018). Furthermore, the bacterial community structure in the MR treatment was distinctly different from that in the M and R treatments at days 5, 27 and 88 (Fig. 3B). This suggested that there was a different bacterial community structure involved in the decomposition of the MR treatment from that in the M and R treatments, especially in the initial period. However,

there was insignificant difference in bacterial community structure between the MR and R treatments at day 57 and day 123 (Fig. 3B), suggesting that the bacterial community structure was convergent in the MR and R treatments over time. The results could be explained by the changes in the quality of residues (Qiu et al., 2019), and the partial Mantel test clearly confirmed that the bacterial community structure was shaped by the Nmin concentration and C/N ratio of residues (Table 3).

Specifically, the MR treatment increased the relative abundance of members of *Enterobacteriaceae* relative to the R treatment at the early stage of decomposition; and enhanced the relative abundance of members of *Hydrogenispora, Bacteroides*, *Ruminiclostridium,* and *Acidobacteriaceae* when compared with the M treatment, especially at the mid to late stage of decomposition (Fig. 4). Degelmann et al. (2009) identified *Enterobacteriaceae* as the primary phylotype that could assimilate monosaccharides under anoxic conditions, and the phylotypes belonging to *Hydrogenispora, Bacteroides*, *Ruminiclostridium,* and *Acidobacteriaceae* might be favored by nitrogen-rich environments. This was confirmed by the results that showed that available C (e.g. DOC) and N levels (e.g. TN) in the MR treatment were higher than those of the R and M treatments at the initial stage and mid-late stages, respectively (Fig. 1). Moreover, the ability of *Enterobacteriaceae* (facultative aerobes) to decompose monosaccharides outcompetes the obligate anaerobes, and the members of *Hydrogenispora, Bacteroides*, *Ruminiclostridium,* and *Acidobacteriaceae* which can produce large amounts of cellulase and hemicellulase to hydrolyze fiber and wood fiber (Fig. 2; Degelmann et al., 2009; Schellenberger et al., 2010). Hence, these differences in bacterial community structure could be responsible for higher rates of decomposition in the MR treatment than was observed in the R and M treatments at the initial and mid-late stages, respectively (Fig. 1).

3.3.4 Difference in ecological co-occurrence networks between the mixed residues and monospecific residue

Soil microorganisms may not live in isolation yet communicate through positive, negative, or neutral ecological relationships with those in nature (Faust and Raes, 2012). A microbial co-occurrence network provides a valuable method for investigating these numerous types of interactions in microorganisms (Banerjee et al., 2018). To reveal the differences in bacterial assemblages involved in the mixed and monospecific residues during the decomposition process, three networks were constructed through random matrix theory (RMT; Fig. 5A). The values of degree distribution for co-occurrence networks were 0.87 to 0.94 and followed power-law distributions (Table 4), indicating their non-random co-occurrence patterns and scale-free features (Zhang et al., 2018). The average path distance (GD), average clustering coefficient (*avgCC*), and the empirical networks modularity were larger than those of corresponding random networks (Table 4), indicating the observed networks had 'small world' properties (Zhang et al., 2018). Moreover, the calculated modularity index values were higher than the random networks and greater than 0.4 (Table 4), indicating typical module structures (Chen et al., 2020).

In the three treatments, the bacterial assemblages involved in the M treatment formed a more complex network, according to the total links, average connectivity (*avgK*), and graph density, which were higher than those in the R and MR treatments (Table 4, Zhou et al., 2011; Chen et al., 2020). This finding could be supported by the observation that the M treatment had the highest change in nutrient availability or properties such as C/N ratio during its decomposition which promoted various taxonomic co-occurrences (Fig. 1). The partial Mantel test results also clearly confirmed that the C/N

ratio was significantly correlated with the M network (Table 5). However, there were a greater number of negatively correlated links between OTUs in the MR network (Table 4, Fig. 5A), suggesting that more inhibitive or competitive connections in bacterial communities were involved in the MR network. A more stable nutrient indicator such as the C/N ratio in the MR treatment may result in the bacteria feeding on a narrow range of substrates (Table 5), which stimulated competition for the same food among microbial populations. To a certain extent, the most intense competition between bacterial species could explain the slight decrease in the diversity and richness of communities in response to the MR treatment (Table 1, Deng et al., 2016).

Microbial communities can harbor keystone populations, whose removal has been shown to cause a drastic shift in the composition and functioning of a microbiome (Banerjee et al., 2018). Keystone populations in a network analysis can be computationally identified as hubs with a high among-module connectivity ($Pi > 0.62$) or high within-module connectivity ($Zi > 2.5$) (Zhou et al., 2011; Chen et al., 2020). In the present study, the proportions of module hubs ($Pi < 0.62$, $Zi > 2.5$) in both R and MR treatments were larger than it in the M treatment, whereas the MR treatment increased the proportion of connector nodes ($Pi > 0.62$, $Zi < 2.5$) in comparison with monospecific residue (Fig. 5B). As a whole, this suggested that the bacterial community in mixed residues was more interconnected than that in monospecific residues (Fan et al., 2018), which would promote communication between bacteria, and allow a more rapid response to environmental change. Moreover, it was important to note that most of OTUs identified as keystone populations had a very low-abundance (< 0.1%) in this study, indicating that some low-abundance microorganisms could play more critical roles than some abundant species in maintaining ecological functions.

3.4. The relationships among residue property, bacterial community, enzyme activity, and decomposition rate

To investigate the driving mechanism underlying residue decomposition, a PLS-PM was conducted to identify the potential indirect and direct effects of the residue property, bacterial community structure, and enzyme activity on decomposition (Fig. 6). In this study, although residue property negatively impacted bacterial diversity, it positively modulated the enzyme activity and bacterial community structure. Moreover, the enzyme activity and bacterial community structure were directly and positively related to decomposition rate, suggesting that the increase in enzyme activity and shifts in bacterial community structure benefited residue decomposition. This finding could be supported by the results from Chen et al. (2020) who showed that microbial community reconstruction benefitted from nutrient mineralization.

Furthermore, the partial Mantel test results revealed that the connectivity of the M network correlated well with the activities of BG and PHEO, and that of the R network was significantly correlated with the CBH activity, whereas that of the MR network was significantly related to the activities of BG and CBH (Table 5). This finding suggested that different enzyme systems caused by different bacterial network structures catalyzed decomposition of different residues, and specially indicated that the bacteria from the MR network decomposed the residues mixture probably by increasing the activities of BG and CBH. Additionally, the bacterial networks of M and MR treatments correlated well with their individual decomposition rates of residues, respectively (Table 5). In the present study, combined with the real results of decomposition rate, the bacterial network of M treatment may favor the decomposition of residues at the initial stage, whereas the MR bacterial network may benefit the decomposition of residues at the mid-late stage.

Bonanomi et al. (2010) suggested that increased decomposition with higher residue diversity could be related to more efficient nutrient transfer and a greater habitat complexity which sustained higher activities of the decomposer community. In the present study, mixing rice straw and milk vetch enhanced the enzyme activity at the mid-late stage of decomposition (Fig. 2), and created a specialized bacterial community or network structure (Fig. 3B, Fig. 4, and Fig. 5A) in comparison with monospecific residues, which favored residue decomposition and resulted in more complete and efficient utilization of resources.

4. Conclusion

This study provides an important mechanistic explanation and theoretical support for the observation that residue mixtures decompose more rapidly and achieve more efficient resource utilization than single residue components. Brefiely, mixing rice straw and milk vetch favored the decomposition of residues by enhancing hydrolytic enzyme activity and regulating bacterial community. More complete decomposition of rice straw can release plant nutrients which will be available to subsequent crops. The practice of mixing crop residues following rice cultivation should therefore be encouraged in order to support more sustainable agricultural practices in the rice growing areas.

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Appendix A. Supplementary data

E-supplementary data for this work can be found in e-version of this paper online.

Reference

1. Ai, C., Liang, G., Sun, J., Wang, X., Zhou, W., 2012. Responses of extracellular enzyme activities and microbial community in both the rhizosphere and bulk soil to long-term fertilization practices in a fluvo-aquic soil. Geoderma 173, 330-338.
2. Balachandar, R., Baskaran, L., Yuvaraj, A., Thangaraj, R., Subbaiya, R., Ravindran, B., Chang, S.W., Karmegam, N., 2020 Enriched pressmud vermicompost production with green manure plants using Eudrilus eugeniae, Bioresour. Technol. 299, 122578.
3. Banerjee, S., Schlaeppi, K., van der Heijden, M.G.A., 2018. Keystone taxa as drivers of microbiome structure and functioning. Nat. Rev. Microbiol. 16, 567-576.
4. Bastian, F., Bouziri, L., Nicolardot, B., Ranjard, L., 2009. Impact of wheat straw decomposition on successional patterns of soil microbial community structure. Soil Biol. Biochem. 41, 262-275.
5. Bonanomi, G., Incerti, G., Antignani, V., Capodilupo, M., Mazzoleni, S., 2010. Decomposition and nutrient dynamics in mixed litter of Mediterranean species. Plant Soil 331, 481-496.
6. Chapman, S.K., Newman, G.S., Hart, S.C., Schweitzer, J.A., Koch, G.W., Hauke, S., 2013. Leaf litter mixtures alter microbial community development: mechanisms for non-additive effects in litter decomposition. Plos One 84, e62671.
7. Chen, Y., Bonkowski, M., Shen, Y., Griffiths, B.S., Jiang, Y., Wang, X., Sun, B., 2020. Root

ethylene mediates rhizosphere microbial community reconstruction when chemically detecting cyanide produced by neighbouring plants. Microbiome 8, 4.

8. Das, A., Paul, T., Halder, S. K., Jana, A., Maity, C., Das Mohapatra, P.K., Pati, B.R., Mondal, K. C., 2013. Production of cellulolytic enzymes by Aspergillus fumigatus ABK9 in wheat bran-rice straw mixed substrate and use of cocktail enzymes for deinking of waste office paper pulp. Bioresour. Technol. 128, 290-296.

9. Claus, H., 2004. Laccases: structure, reactions, distribution. Micron. 35, 93-96.

10. DeForest, J., 2009. The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and L-DOPA. Soil Biol. Biochem. 41, 1180-1186.

11. de Graaff, M.A., Classen, A.T., Castro, H.F., Schadt, C.W., 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. New Phytol. 188, 1055-1064.

12. Deng, Y., Zhang, P., Qin, Y., Tu, Q., Yang, Y., He, Z., Schadt, C.W., Zhou, J., 2015. Network succession reveals the importance of competition in response to emulsified vegetable oil amendment for uranium bioremediation. Environ. Microbiol. 18, 205-218.

13. Degelmann, D.M., Steffen, K., Marc, D., Colin, M.J., Drake, H.L., 2009. Enterobacteriaceae facilitate the anaerobic degradation of glucose by a forest soil. FEMS Microbiol. Ecol. 68, 312-319.

14. Dilly, O., Bloem, J., Vos, A., Munch, J.C., 2004. Bacterial diversity in agricultural soils during litter decomposition. Appl. Environ. Microb. 70, 468-474.

15. Eagle, A.J., Bird, J.A., Horwath, W.R., Linquist, B.A., Brouder, S.M., Hill, J.E., van Kessel, C.,

2000. Rice yield and nitrogen utilization efficiency under alternative straw management practices. Agron. J. 92, 1096-1103.

16. Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996-998.

17. Fan, K., Weisenhorn, P., Gilbert, J.A., Shi, Y., Bai, Y., Chu, H., 2018. Soil pH correlates with the co-occurrence and assemblage process of diazotrophic communities in rhizosphere and bulk soils of wheat fields. Soil Biol. Biochem. 121, 185-192.

18. FAO, 2015. World Reference Base for Soil Resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome.

19. Faust, K., Raes, J., 2012. Microbial interactions: from networks to models. Nat. Rev. Microbiol. 10, 538-550.

20. Fierer, N., Bradford, M., Jackson, R., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354-1364.

21. Gartner, T.B., Cardon, Z.G., 2004. Decomposition dynamics in mixed-species leaf litter. Oikos 104, 230-246.

22. Hättenschwiler, S., Fromin, N., Barantal, S., 2011. Functional diversity of terrestrial microbial decomposers and their substrates. CR Biol. 334, 393-402.

23. Hättenschwiler, S, Tiunov, A.V., Scheu, S., 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annu. Rev. Ecol. Evol. Syst. 36, 191-218.

24. He, Q., Chen, L., Zhang, S., Chen, R., Wang, H., Zhang, W., Song, J., 2018. Natural sunlight induced rapid formation of water-born algal-bacterial granules in an aerobic bacterial granular

photo-sequencing batch reactor. J. Hazard. Mater. 359, 222-230.

25. Jacob, M., Weland, N., Platner, C., Schaefer, M., Leuschner, C., Thomas, F.M., 2009. Nutrient release from decomposing leaf litter of temperate deciduous forest trees along a gradient of increasing tree species diversity. Soil Biol. Biochem. 41, 2122-2130.

26. Jiang, Y., Liu, M., Zhang, J., Chen, Y., Chen, X., Chen, L., Li, H., Zhang, X., Sun, B., 2017. Nematode grazing promotes bacterial community dynamics in soil at the aggregate level. ISME J. 11, 2705-2717.

27. Kiikkilä, O., Kitunen, V., Spetz, P., Smolander, A., 2012. Characterization of dissolved organic matter in decomposing Norway spruce and silver birch litter. Eur. J. Soil Sci. 63, 476-486.

28. Ma, S., Fang, C., Sun, X., Han, L., He, X., Huang, G., 2018. Bacterial community succession during pig manure and wheat straw aerobic composting covered with a semi-permeable membrane under slight positive pressure. Bioresour. Technol. 259, 221-227.

29. Maarastawi, S.A., Frindte, K., Geer, R., Kröber, E., Knief, C., 2018. Temporal dynamics and compartment specific rice straw degradation in bulk soil and the rhizosphere of maize. Soil Biol. Biochem. 127, 200-212.

30. Nan, Q., Wang, C., Wang, H., Yi., Q., Liang, B., Xu, J., Wu, W., 2020. Biochar drives microbially-mediated rice production by increasing soil carbon. J. Hazard. Mater. 387, 121680.

31. Nemergut, D.R., Cleveland, C.C., Wieder, W.R., Washenberger, C.L., Townsend, A.R., 2010. Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. Soil Biol. Biochem. 42, 2153-2160.

32. Nicolardot, B., Bouziri, L., Bastian, F., Ranjard, L., 2007. A microcosm experiment to evaluate the influence of location and quality of plant residues on residue decomposition and genetic

structure of soil microbial communities. Soil Biol. Biochem. 39, 1631-1644.

33. Pal, D., Broadbent, F.E., 1975. Influence of moisture on rice straw decomposition in soils. Soil Sci. Soc. Am. J. 39, 59-63.

34. Pandit, P.D., Gulhane, M.K., Khardenavis, A.A., Purohit, H.J., 2016. Mining of hemicellulose and lignin degrading genes from differentially enriched methane producing microbial community. Bioresour. Technol. 216, 923-930.

35. Prescott, C.E., 2005. Do rates of litter decomposition tell us anything we really need to know? Forest Ecol. Manag. 220, 66-74.

36. Qian, X., Shen, G., Wang, Z., Guo, C., Liu, Y., Lei, Z, Zhang, Z., 2014. Co-composting of livestock manure with rice straw: characterization and establishment of maturity evaluation system. Waste Manage. 34, 530-535.

37. Qiu, X., Zhou, G., Zhang, J., Wang, W., 2019. Microbial community responses to biochar addition when a green waste and manure mix are composted: a molecular ecological network analysis. Bioresour. Technol. 273, 666-671.

38. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl. Acids Res. 41, 590-596.

39. Santonja, M., Rancon, A., Fromin, N., Baldy, V., HäTtenschwiler, S., Fernandez, C., Montès, N., Mirleau, P., 2017. Plant litter diversity increases microbial abundance, fungal diversity, and carbon and nitrogen cycling in a Mediterranean shrubland. Soil Biol. Biochem. 111, 124-134.

40. Schellenberger, S., Kolb, S., Drake, H.L., 2010. Metabolic responses of novel cellulolytic and saccharolytic agricultural soil bacteria to oxygen. Environ. Microbiol. 12, 845-861.

41. Shen, Q., Sun, H., Yao, X., Wu, Y., Wang, X., Chen, Y., Tang, J., 2019. A comparative study of pig manure with different waste straws in an ectopic fermentation system with thermophilic bacteria during the aerobic process: performance and microbial community dynamics. Bioresour. Technol. 281, 202-208.

42. Shukla, G., Varma, A., 2010. Soil enzymology (Vol. 22). Springer Science & Business Media.

43. Wang, Y., Tang, C., Wu, J., Liu, X., Xu, J., 2012. Impact of organic matter addition on pH change of paddy soils. J. Soil Sediment. 13, 12-23.

44. Zhang, B., Zhang, J., Liu, Y., Shi, P., Wei, G., 2018. Co-occurrence patterns of soybean rhizosphere microbiome at a continental scale. Soil Biol. Biochem. 118, 178-186.

45. Zhang. J., Zeng. G., Chen. Y., Yu. M., Yu. Z., Li. H., Yu. Y., Huang. H., 2011. Effects of physico-chemical parameters on the bacterial and fungal communities during agricultural waste composting. Bioresour. Technol. 102, 2950-2956.

46. Zhou, G., Cao, W., Bai, J., Xu, C., Zeng, N., Gao, S., Rees, R.M., 2019. Non-additive responses of soil C and N to rice straw and hairy vetch (*Vicia villosa Roth* L.) mixtures in a paddy soil. Plant Soil 436, 229-244.

47. Zhou, G., Gao, S., Lu, Y., Liao, Y., Nie, J., Cao, W., 2020. Co-incorporation of green manure and rice straw improves rice production, soil chemical, biochemical and microbiological properties in a typical paddy field in southern China. Soil Till. Res. 197, 104499.

48. Zhou, J., Deng, Y., Luo, F., He, Z., Yang, Y., 2011. Phylogenetic molecular ecological network of soil microbial communities in response to elevated CO_2. mBio 2, e00122-11.

Figure legend

Fig. 1. Biomass loss rate of residues and dynamic changes in residue properties under different treatments. Data refer to mean ± standard deviation of 4 replicates for each residue.

Fig. 2. Dynamic changes in extracellular enzyme activities during the decomposition process. Data refer to mean ± standard deviation (n = 4), different lower-case letters in each sampling date suggest statistically significant differences among treatments (Duncan test, $P < 0.05$).

Fig. 3. Bacterial community composition at the phylum level during the decomposition process (A); principle coordination analysis (PCoA) of the bacterial community (based on OTU level) by Bray-Curtis distances (B). Data refer to mean ± standard deviation (n = 4).

Fig. 4. Statistically significant differences in the relative abundance of OTU (representatives with relative abundance > 1%) among three treatments during the decomposing process. The graphic shows only the OTU with statistical differences with a confidence interval of 95%, and the OTU numbers are noted in Supporting Information.

Fig. 5. An overview of the bacterial network interactions in different treatments (A); *Zi*-*Pi* plot revealing the OTUs distribution based on certain topological roles (B). The most abundance OTUs (> 0.01%) was originally employed for network construction through the RMT-based network approach. The topological role of each OTU was defined by the scatter plot of among-module connectivity (*Pi*) and within-module connectivity (*Zi*). The module hubs, network hubs, and connectors are labeled with OTU numbers, and the OTU numbers are noted in Supporting Information.

Fig. 6. Path analysis diagrams showing the relationships among sampling date, residue properties, bacterial community structure, bacterial diversity, enzyme activity, and residue decomposition rate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Highlights:

1. Mixing milk vetch and rice straw (MR) shortened the decomposition process
2. MR increased hydrolytic enzyme activity compared with its monospecific residues
3. MR enhanced the monosaccharide decomposing bacteria abundance relative to rice straw
4. MR enhanced the fiber decomposing bacteria abundance relative to milk vetch
5. More interconnected and competitive relations existed between the bacteria in MR

Table 1. Dynamic changes in bacterial α-diversity index during the decomposition process.

Treatment		*Observed_species*	Chao1	Shannon
5 day	M	422.95 c D	657.11 c C	5.63 c D
	R	778.93 a D	1071.83 a D	6.69 a E
	MR	592.95 b D	824.31 b D	6.14 b D
27 day	M	1126.30 a C	1604.65 a B	7.31 a C
	R	1022.05 ab C	1427.64 ab C	7.06 ab D
	MR	956.73 b C	1371.96 b C	6.65 b C
57 day	M	1436.28 a B	2000.17 a A	8.04 a B
	R	1314.10 b B	1865.29 ab B	7.44 b C
	MR	1268.43 b B	1803.21 b B	7.21 b B
88 day	M	1606.13 a A	2143.15 a A	8.47 a A
	R	1487.53 ab A	2075.59 ab A	8.06 b B
	MR	1427.10 b A	1905.55 b AB	8.13 ab A
123 day	M	1639.78 a A	2104.84 a A	8.58 a A
	R	1543.25 ab A	2070.94 a A	8.61 a A

	MR	1481.55 b A	2016.44 a A	8.47 a A
Soil		1315.10	1665.08	8.47

Note: Different lower-case letters and capital letters suggest statistically significant differences between treatments at the same sampling date and between sampling date of the same treatment, respectively (Duncan test, $P < 0.05$).

Table 2. The relative influence of the driving factors for bacterial α-diversity as determined by aggregated boosted tree (ABT) analysis.

Driving factors	Relative influence (%)		
	Observed_species	Chao1	Shannon
DOC	38.0	34.9	54.6
TC	29.8	26.2	19.1
Sampling date	19.7	25.2	12.0
Nmin	8.43	9.61	10.5
TN	2.40	2.45	2.43
C/N ratio	1.67	1.65	1.36

Table 3. The relationship between the bacterial community structure and different environmental variables analyzed using the partial Mantel test.

Variable	Pearson correlation coefficient (*r*)	*P* value
Sampling date	0.058	**0.046**
TC	0.043	0.078
TN	-0.022	0.717
DOC	0.013	0.338
Nmin	0.090	**0.025**
C/N ratio	0.081	**0.014**

Note: Permutations 999 (using the most abundances of OTU (> 0.01%) as input in analysis).

Table 4. Topological properties of the empirical functional molecular ecological networks (fMENs) during the decomposing process in comparison with the random networks

Network metrics	Treatments		
	M	R	MR
Empirical networks			
Number of original OTUs [a]	927	936	892
Similarity threshold	0.95	0.98	0.98
R^2 of power-law	0.92	0.94	0.87
Total nodes [b]	335	238	268
Total links	552	321	416
Positive links	467 (84.6%)	230 (71.7%)	269 (64.7%)
Negative links	85 (15.4%)	91 (28.3%)	147 (35.3%)
Average clustering coefficient (*avgCC*)	0.18	0.10	0.06
Average connectivity (*avgK*)	3.30	2.70	3.10
Average path distance (GD)	5.12	5.58	4.41
Density	0.012	0.010	0.011
Modularity (Number of modules)	0.74 (20)	0.76 (23)	0.65 (18)
Random networks			
avgCC ± SD	0.015 ± 0.005	0.016 ± 0.007	0.037 ± 0.010
GD ± SD	4.388 ± 0.059	4.641 ± 0.106	3.929 ± 0.067
Modularity ± SD	0.467 ± 0.017	0.649 ± 0.009	0.577 ± 0.008

[a] The number of OTUs (most abundance OTUs > 0.01%) was originally used for network construction by the RMT-based network approach.

[b] The number of OTUs (i.e. nodes) in a network.

Table 5. The partial Mantel test on the relationship between connectivity of networks, residue property, enzyme activity, and decomposition rate.

Parameter	M network		R network		MR network	
	r	p	r	p	r	p
TC	0.0987	**0.004**	0.1382	**0.002**	-0.0004	0.452
TN	-0.0061	0.566	0.0113	0.333	0.0328	0.135
DOC	0.0126	0.253	0.1803	**0.001**	-0.0114	0.622
Nmin	-0.0412	0.945	0.0344	0.100	0.0232	0.199
C/N ratio	0.0869	**0.001**	0.0449	0.077	0.1501	**0.001**
BG	0.0467	**0.038**	-0.0323	0.866	0.0708	**0.015**

CBH	0.0210	0.142	0.0622	**0.021**	0.1192	**0.001**
PHEO	0.0969	**0.003**	-0.0775	0.996	0.0052	0.377
Decomposition rate	0.0628	**0.023**	0.0256	0.054	0.0577	**0.025**

Note: significant differences at $p < 0.05$ are indicated in bold.