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1 Title

2 Effects of green manure application on soil microbial communities and activities in the
3 decontaminated sandy soil paddy field in Fukushima, Japan

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For review

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23 Abstract

24 On March 11, 2011, Japan experienced an unprecedented earthquake off the Pacific
25 coast of Tohoku, and suffered the direct and long-term effects of the earthquake and
26 tsunami in the area. In Fukushima prefecture, agricultural land contaminated with
27 radioactive Cesium from the Fukushima Daiichi Nuclear Power Plant. Therefore,
28 surface soil were removed for decontamination, and low fertility sandy soil was covered.
29 Organic matter input is necessary to increase soil organic matter and green manure
30 application is an effective method to improve soil fertility in the paddy field. Soil
31 microbes and enzyme activities are sensitively responded to organic matter addition, but
32 their dynamics on the dressed field are not well investigated. In this study, we focused
33 on changing the microbial community, diversity and enzyme activities along with the
34 green manure decomposition process in the sandy soil dressed paddy field in Japan. The
35 green manure of hairy vetch and oat were harvested and incorporated in May 2020 and
36 their decomposition process as cellulose and hemicellulose contents were determined.
37 Soil bacterial communities were analyzed using 16S amplicon sequencing. The green
38 manure was rapidly decomposed within the first 13 days, and they did not remain 50
39 days after green manure incorporation. Soil microbial biomass carbon was higher in the
40 M treatment after GM treatment, but was not significant between treatments after 50

41 days. Dehydrogenase and β -glucosidase activities changed during the harvesting period,
42 but did not correlate with GM decomposition. Microbial diversity (OTU numbers and
43 Shannon index) also changed with GM application, but they were not associated with
44 GM decomposition. Soil prokaryotic communities and some bacteria (*Bacilli* and
45 *Chloroflexi*) are significantly influenced by GM treatment. However, *Clostrida* was not
46 affected by GM. Mixed green manure treatment showed significantly rapid
47 hemicellulose decomposition than other treatments. In this process, *Anaerolineae* were
48 negatively correlated with the decreasing of hemicellulose in this treatment. These
49 results showed that GM treatment affected microbial communities, and their response
50 was active during the decomposition process.

52 Key words

53 16S rRNA, Enzyme activity, Decontaminated soil, Mixing effect

55 Introduction

56 The Great East Japan Earthquake and tsunami on 11 March 2011, caused damage to
57 the Fukushima Daiichi Nuclear Power Plant (NPP), resulting in serious radioactive
58 pollution throughout Eastern Japan. The radioactive fallout extensively polluted
59 agricultural lands, including paddy fields, with radioactive Cs (MEXT 2011). The Cs

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7 60 contaminated agricultural soil were removed for depth 10cm, and the sandy soils with
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10 61 low soil carbon and nitrogen content (total carbon is 8.29 g kg soil⁻¹ and total nitrogen is
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12 62 0.74 g kg soil⁻¹) were covered as decontamination. The decline of soil organic carbon
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15 63 negatively impacts crop productivity and sustainability of agriculture (Lal 2004;
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18 64 Agegnehu et al. 2016). Organic amendments can provide available nutrients for plants,
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21 65 and the coupling of carbon and nutrient transformation during organic matter
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24 66 decomposition strongly interacts with plant nutrient uptake (Kaye and Hart, 1997). The
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27 67 application of organic materials to rice fields for yield increase has a long history in
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30 68 Asian countries. Recent studies have focused on re-considering traditional fertilization
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33 69 practices to enhance soil organic input by amendments of crop residues, green manure,
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36 70 and farmyard manure (Liu et al. 2009). The most useful organic matter in the paddy
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39 71 field is rice straw. However, the rice yields in this decontaminated paddy field in
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42 72 Fukushima are less than half the average yield in Japan, therefore, not enough rice straw
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45 73 can be applied to increase soil fertility. Livestock wastes are another important organic
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48 74 amendment; however, the stock rising was not restarted yet in this area. The application
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51 75 of green manure (GM) to paddy fields is considered a good management practice
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54 76 (Zhang et al. 2017). GM application has been reported to increase soil organic matter,
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and fertility, and nutrient retention, reducing the occurrence of plant disease and long-term green manure incorporation increases rice yields (Gao et al. 2013; Li et al. 2019).

Soil microbes play an important role in maintaining soil fertility and productivity and drive most soil processes, e.g. decomposition of organic materials, nutrient availability and retention, and soil organic matter sequestration (Coleman et al., 2004). Soils with high fertility generally possess larger microbial biomass, higher enzyme activities, and better soil structure than those with low fertility (Fontaine et al. 2011; Lang et al. 2012) could provide a suitable environment for substrate utilization by microbes. However, microbial communities, abundance, and their activities that respond to plant residue decomposition in the sandy soil are still limited. For recovering SOM in the covered sandy paddy field after decontamination in Fukushima, it is essential to investigate microbial response to SOM decomposition. It was reported that the mixing of different species of GM can effectively improve soil fertility than a single GM application (Fageria et al., 2005; Tosti et al., 2014). The mixtures of plant residue at rates faster than expected from the average of the decomposition rates of the plant types component. This phenomenon is termed the "mixing effect" and the hypotheses proposed to explain it include physical, chemical, and biological mechanisms. The aim of this study is to investigate the effects of GM application on soil enzyme activity and

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95 microbial community during the GM decomposition process in sandy soil

96 decontaminated paddy fields in Fukushima Prefecture.

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98 **Material and Methods**

99 **Sampling fields**

100 The paddy field was located in Tomioka town, Fukushima Prefecture, Japan (37°20'N,
101 140°60'E). The fields are about 12km away from Fukushima 1st NPP. The soil was
102 sandy loam soil (Fluvaquents) in the top 0.5 m, with 78.7 % sand, 17.7 % silt and 3.6 %
103 clay. Soil physicochemical properties in the field were shown in Table 1. Two species
104 of green manures were used, i.e., oat (A: *Avena strigosa* cv. Hayoats) and hairy vetch
105 (V: *Vicia villosa* cv. Fujiemon). These species were sown as pure crops at an ordinary
106 sowing rate (4 kg of hairy vetch and 15 kg oat each in 10a⁻¹) and as a mixture with the
107 same quantity (M: 4 kg of hairy vetch and 15 kg of oat 10a⁻¹). Non-green manure
108 treatment plots (control: C) were included in the experiments. The experimental design
109 was a randomized complete block with three replicates. Each plot size was 225 m² (15
110 m×15m). GM was cultivated from 1 November 2018 to 5 May 2019.

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112 **Litterbag experiments**

Each green manure was harvested from randomly selected 2 points (0.5m × 0.5m) in each treatment on 5 May. The yields and chemical properties of each GM were shown in Table 1. The GM dried at 70 °C and powdered. The litterbags (1mm mesh size) were filled with 30g of air-dried paddy field soil mixed with each GM. The amount of each GM input was equal to the amount of input carbon rate in the field (0.05 % of O and V treatment and 0.11% of M treatment) (Table 2). A total of 72 litterbags (4 treatment × 6 sampling times × 3 replicants) were prepared. All litterbags were incorporated in each control plot of the paddy field on 30 May 2020. Within each plot, 24 litterbags (4 treatments and 6 were incorporated into the soil by burying them at 15cm depth. Each litterbag from each plot was removed chronologically from 12 June (13 days), 3 July (34 days), 30 July (50 days), 21 August (72 days), and 4 October (116 days). One gram of soil in the litterbags were transported to the laboratory using an icebox and processed immediately after their removal from the field.

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127 **Soil chemical properties**

128 Total carbon and nitrogen contents were measured with a NC analyzer (SUMIGRASH
129 NC-80, Sumitomo Chemical Co. Ltd., Tokyo, Japan). Cellulose and hemicellulose
130 content were determined with colormetric anthrone-sulfuric acid method (Koehler
131 1952) after hydrololysis of component sugars by Oades et al. (1970). The cellulose and

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132 hemicellulose content of the control at that time was subtracted from each treatment
133 section to obtain the cellulose and hemicellulose content at that time. The content at the
134 time of treatment was set to 100, and the degradation rate was determined.

135 **Soil biological properties**

136 Soil enzyme activity was determined as below. Dehydrogenase activity was
137 determined with the reduction of iodonitrotetrazolium chloride (INT) by Von Mersi and
138 Schinner (1991). β -glucosidase activities were assayed on the basis of p-Nitrophenyl- β -
139 D-glucopyranoside (PNG) hydrolysis after cleavage of enzyme-specific synthetic
140 substrates by Hayano (1973). Microbial biomass carbon and nitrogen were determined
141 by chloroform fumigation-extraction method with 0.5 M K_2SO_4 at 1:4 soil to extraction
142 ratio (Moore et al. 2000)

143 **DNA extraction and microbial community analysis**

144 DNA was extracted from 0.5 g of soil using the ISOIL for bead beating kit (Nippon
145 Gene Co., Ltd., Tokyo, Japan), according to the manufacturer's instructions. DNA
146 quantification and integrity were measured using a NanoDrop spectrophotometer
147 (Thermo Fisher Scientific, Waltham, MA, USA) and gel visualization (0.8% agarose in
148 Tris/acetic acid/ethylenediaminetetraacetic acid buffer), respectively. The V4 region of

the 16S rRNA gene of each sample was amplified by PCR using the bacterial and archaeal universal primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGAC-TACVSGGGTATCTAA-3') (Caporaso *et al.* 2011). A library was prepared by adaptor ligation with the PCR primer pairs using the TruSeq Nano DNA Library Prep Kit (Illumina, Inc., San Diego, CA, USA). When two or more bands were detected using 1.5%-agarose gel electrophoresis, PCR products of approximately 300 bp in length were excised from the gel, non-specific amplicons were removed, and the products were purified using a MonoFas DNA purification kit for prokaryotes (GL Sciences, Inc., Tokyo, Japan). Each PCR amplicon was cleaned twice to remove the primers and short DNA fragments using the Agencourt AMPure XP system (Beckman Coulter, Inc., Brea, CA, USA) and quantified using a Qubit Fluorometer (Invitrogen Corporation, Carlsbad, CA, USA). The PCR products were adjusted to equimolar concentrations and subjected to unidirectional pyrosequencing, which was performed by Bioengineering Lab. Co., Ltd. (Kanagawa, Japan) using a MiSeq instrument (Illumina, Inc.). Overall, 3,521,651 sequences were obtained from the 72 samples (Supplemental Table 1). Sequencing data were deposited in the DNA Database of Japan Sequence Read Archive under the accession number DRA006673.

Statistical analysis

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6 168 Illumina sequence data were sorted based on unique barcodes and quality-controlled
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9 169 using the Quantitative Insights Into Microbial Ecology Qiime2 (version 2017.8,
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12 170 <https://docs.qiime2.org/2017.8/>) with plugins demux (<https://github.com/qiime2/q2->
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15 171 demux), dada2 (Callahan et al., 2016) and feature-table (McDonald et al., 2012). Alpha
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18 172 and beta diversity analyses were performed by using plugins alignment (Kato and
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21 173 Standley, 2013), phylogeny (Price et al., 2010), diversity (<https://github.com/qiime2/q2->
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24 174 diversity), and emperor (Vazquez-Baeza et al., 2013). A pre-trained Naive Bayes
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27 175 classifier based on the Greengenes 13_8 99% OTUs database (<http://greengenes>.
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30 176 secondgenome.com/), which has been trimmed to include the v4 re- gion of 16S rRNA
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33 177 gene, bound by the 515F/806R primer pair, was applied to paired-end sequence reads to
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36 178 generate taxonomy tables. Taxonomic and compositional analyses were conducted by
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39 179 using plu- gins feature-classifier (<https://github.com/qiime2/q2-feature-> classifier), taxa
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42 180 (<https://github.com/qiime2/q2-taxa>) and composition (Mandal et al., 2015). The
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45 181 significant difference among each treatment was analyzed by Tukey-Kramer HSD
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48 182 method using R. Beta diversity was measured according to Bray–Curtis distances which
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51 183 were calculated by R, and displayed using Principal Coordinate Analysis (PCoA). The
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54 184 significance of grouping in the PCoA plot was tested by analysis of similarity
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57 185 (ANOSIM) in R with 999 permutations. The interacted factor between cellulose,
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hemicellulose, and carbon decomposition and related microbes were analyzed using Spearman's rank correlation. The correlation coefficients (Rho value) less than -0.4 were defined as negatively correlated microbes and more than 0.4 were defined as positively correlated microbes.

Results

The dynamics of GM content during the harvesting period

GM, which mainly consists of cellulose and hemicellulose, was mainly degraded in 31 days after GM treatment. At the 13 days, 56-72% of cellulose was decomposed, and the degradation rate did not differ among the treatments (Fig. 1). After 31 days, 5 to 14% of cellulose remained in all treatments and they remained unchanged through the harvesting period. Hemicellulose contents also decreased significantly over 13 days, but they differed among treatments: M treatment, a mixture of V and A treatment, decreased significantly (99%) compared to the other treatments (50% in V and 23% in A). Thereafter, the residues remained below 10% until day 116. In the V treatment, the rate of residues reached 33% on day 72, but they did not significantly differ from the other treatments. Total carbon and total nitrogen contents were gradually decreased in all treatments and the highest in M on 34 days after GM treatment (Figure 1). After 50

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204 days, TC and TN contents were similar among each GM treatment. Therefore, GM
205 might be completely decomposed until this time. Total carbon content in each field was
206 also changed during the harvesting stage, though they were not different between the
207 treatments (Supplemental figure 1). After the rice harvesting, soil carbon and nitrogen
208 content, cellulose, and hemicellulose were not different among the treatment (Table 3).

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210 **Dynamics of microbial biomass, community structure, diversity, and enzyme** 211 **activities response to GM application**

212 Soil microbial biomass carbon was significantly increased in M treatment followed by
213 A, V, and C treatment on 13 and 34 days after GM treatment (Figure 2). They
214 significantly decreased and were not significantly different among the treatments after
215 50 days. Dehydrogenase activities were increased 34 days after treatment, and they in A
216 treatment showed the highest activities than other treatments. After 50days, the
217 activities decreased and were not significantly different among the treatment. Beta-
218 glucosidase activities were drastically changed during the harvesting stage; however,
219 they were not significantly different among the treatments. Soil microbial diversity,
220 OTU numbers and Shannon index, did not differ among treatments on days 13 and 34.
221 They were the highest in M treatment compared to other treatments on 50 days and then

decreased. On day 72 and 116, they in V treatment were higher than A treatment and M treatment, respectively.

The main prokaryotic phyla in the field were Firmicutes and Proteobacteria (Figure 4). They were occupied more than 50% in all treatments and sampling times. In class level, Bacilli were the most dominant bacteria in all treatments followed by Clostridia, Alphaproteobacteria, and unidentified Proteobacteria (Figure 5). The relative abundance of *Bacilli* was decreased on day 31 compared with that on 13 days, then was again high in the C and A treatments at day 50. Chloroflexi was higher in the M treatment than in the V treatment at day 50; the relative abundance of *Clostridia* did not differ among treatments (Figure 6). Soil prokaryotic communities were affected by GM treatment (anosim $p < 0.05$). PCoA analysis based on Bray-curtis analysis showed that prokaryotic communities were roughly clustered by sampling times (Figure 7). Prokaryotes sampled in 13 and 31 days were clustered by sampling times. But their communities were not clustered in sampling time after 50 days. We analyzed the microbes correlated between total carbon, hemicellulose, and cellulose contents (Table 4). *Lachnospiraceae* and *Clostridiales* belonged to Clostridia positively correlated to cellulose and hemicellulose content, and *Bacillus* correlated to total carbon content. Cellulose contents were negatively correlated ($\rho < -0.4$) *Anaerolineae* SJA15 and

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240 unidentified Chloflexi belonged to Chloflexi, and *Rhizobiales* belonged to
241 Alphaproteobacteria. Hemicellulose contents were negatively correlated to
242 *Anaerolineae* SJA15 and unidentified Chloflexi belonged to Chloflexi, unidentified
243 betaproteobacteria, *Pedospaera* belonged to Verrcomicrobia, *Chrolobi*, and
244 *Methanomicrobia* belonged to Euryarchaeota. Total carbon was negatively correlated
245 with *Ktedonobacteria* and unidentified Chloroflexi belonged to Chloroflexi, *Rhizobiales*
246 belonged to Alphaproteobacteria, and *Chlorobi* BSV26. We compared the correlation
247 between hemicellulose content and each microbe among the GM treatments (Table 5).
248 *Bacillus* were positively correlated to hemicellulose content in all treatments, and
249 *Lachnospiraceae* belonging to Clostridia positively correlated with E and M treatment.
250 On the other hand, *Anaerolineae* were negatively correlated in A and E treatment,
251 respectively, but not in M treatment. Some methanogen (*Methenomicrobia* and
252 *Methanogulaceae*) were negatively correlated with hemicellulose content only in M
253 treatment.

255 Discussion

256 Microbial communities and activities during GM decomposition process

257 GM is used as a useful organic matter in the paddy field and it increases soil organic
258 carbon and nitrogen (Lee et al. 2010; Hwang et al. 2015). Gao et al. (2016) reported that

about 70% of wheat residue was mineralized under the anaerobic condition as well as aerobic condition in a Cambisol after 12-month in a field experiment. Zech et al. (1997) concluded that 30-45 % of plant residue carbon was accumulated in the soil after 1 year treated with various SOM conditions. In Japanese field, 67 to 79 % of carbon from rice residues was decomposed during one year (Shiga et al. 1985). On the other hand, in our fields, cellulose and hemicellulose were decomposed within 50 days and soil carbon was not accumulated after the rice harvesting period. The aboveground biomass of green manure (barley and hairy vetch) in past related studies ranges from 3.66 to 11 t ha⁻¹ and is incorporated into the field (Tosti et al. 2014; Jeon et al. 2008, Hwang et al. 2015). However, in our field, only 1.24-2.55 t ha⁻¹ of GM was harvested due to the cold climate of Fukushima and low soil fertility of the covered sandy soil (Table 1). Another possibility of the rapid GM decomposition is the sandy textured soil property. Most sandy soils have low soil organic matter content and show low water holding capacity and high permeability due to their coarse texture (Rutkowska and Piłkuła, 2013; Šimanský and Bajčan, 2014). Some studies showed that organic amendments generally decompose rapidly in sandy soils due to high temperatures and aeration (Glaser et al., 2002). Xu et al. (2019) showed that mineralization of residue carbon after 360 days of incubation was higher in low fertility soils than in high fertility soils. Low fertility soils

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277 have a higher C/N ratio than high fertility soils. In this case, soil microorganisms that
278 are deficient in nitrogen nutrients will use nitrogen derived from the residue pool, which
279 is more easily decomposed, and will preferentially decompose plant residues to meet
280 their nitrogen requirements. These two factors might contribute to the rapid degradation
281 of GM.

282 Dehydrogenase is an intracellular enzyme that participates in oxidative
283 phosphorylation in microorganisms and basically depends on the metabolic state of the
284 soil microbes (Tabatabai et al. 1994; Insam, 2001). β -glucosidase is an extracellular
285 enzyme that contributes to the degradation of cellulose and other β -1,4 glucans to
286 glucose and is considered to be one of the proximate agents of organic matter
287 decomposition (Sinsabaugh et al., 2008). Soil organic matter is the substrate for these
288 soil enzymes, therefore, dehydrogenase and beta-glucosidase are positively correlated
289 with soil organic matter (Bhattacharyya et al. 2012). However, in our study, each
290 enzyme activity was not correlated with soil carbon, cellulose, and hemicellulose
291 contents. Moreover, no microbes were correlated with each enzyme activity. The soil
292 microbial biomass carbon in this study (13.4 to 87.4 mg C kg soil⁻¹) was much lower
293 than that in the previous study (more than 200 mg C kg soil⁻¹) (Lu et al. 2002;
294 Bhattacharyya et al. 2012; Zheng et al. 2016). Because the amount of green manure

input was low, the small amount of enzymes may be secreted, and no difference was observed between the treatment. Moreover, β -glucosidase is an extracellular enzyme; therefore, it was leached from the outside of the litter bag in the flooded paddy field and contaminated each treatment.

Clostridia, organic matter degradation bacteria under anaerobic conditions, is known to increase during the degradation process under flooded conditions (Weber and Conrad 2001; Shrestha *et al.* 2011). Lee *et al.* (2017) showed that dried rice callus cells, a model of easily degradable plant residues, are mainly degraded by *Clostridia* under anaerobic conditions. In contrast, the abundance *Clostridia* did not change during the harvest period and was positively correlated with cellulose and hemicellulose content. *Bacillus*, the dominant aerobic bacteria in the field, was also significantly reduced during the harvest period. Soil condition might be anaerobic and it is favorable for *Clostridia* increasing. *Clostridia* and *Bacilli* are known for rapidly increasing bacteria response to organic matter addition (Bao *et al.* 2019). Therefore, it was suggested that they might be involved in the fast decomposition of GM and then they dominated. Thereafter, they could not grow because of low substrates. Compared with their decreasing, bacteria belonging to *Chloroflexi*, *Rhizobiales*, *Betaproteobacteria*, and *Chlorobi* BSV26 negatively correlated to soil carbon and plant cell wall content. They

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313 are well-known oligotrophic bacteria (Fierer *et al.* 2007; Tian et al. 2015). They can
314 grow in the low nutrient soil as oligotrophs, nitrogen-fixing, sulfate-reduction, and
315 photosynthesis. In this study, there was no effect of GM treatment on carbon
316 accumulation in soil. On the other hand, we revealed that the soil microbial community
317 was affected by GM treatment. The microbial community associated with carbon from
318 GM accumulation was also increased. In the future, the microbes involved in carbon
319 sequestration and link it to the implementation of soil improvement and rice production
320 in the covered field.

322 **Mixing effect on GM decomposition and microbial properties**

323 The types of GM also affected their decomposition rate. In general, plant residue
324 decomposition is correlated with C/N ratio of each plant (). The combination of barley
325 and hairy vetch optimizes the C/N ratio, which can favor the mineralization of organic
326 substrates in soil (USDA, 2011). In this study, C/N ratio of V was 12 and that of E was
327 61, therefore, we hypothesized the decomposition rate was $V > M > E$. Cellulose
328 decomposition rates were not different among the treatment, while M treatment showed
329 the highest hemicellulose decomposition rates compared with other treatments.
330 Chapman and Koch et al. (2007) showed mixtures of plant litter decomposed up to 50%

331 faster than expected decomposition speed and single litter types. Cuchietti et al (2014)

332 explained that the fast-slow mixture decomposition rate is greater than expected due to

333 the fast decomposing GM. In chemical aspects, the carbon limitation for decomposing

334 fast decomposing plant residue and the slow decomposing plant residue it contains high

335 CN ratio, will supply the carbon for carbon decomposition, therefore, decomposition

336 will be accelerated. They indicated that fast-decomposing species would transfer

337 nutrients to slow decomposing species, thereby increasing the decomposition rate of the

338 slow decomposition species. Microbial communities also influence the mixing effect.

339 Our results showed that microbes affected by hemicellulose decomposition in single and

340 mixed treatment showed differently. Methanogens negatively correlated with

341 hemicellulose content only in M treatment. They are faculty anaerobic, use H_2/CO_2 and

342 formate as a substrate for methanogenesis, and acetate is required for growth. The

343 microbes involved in the mixing effect are not well studied. Our study provides some

344 evidence that can shed light on some mechanisms of in the sandy soil environemnt, but

345 on microbial and to the mechanisms proposed is critical.

346

347 **Acknolwgement**

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For review

534 **Figure legends**

535

536 **Figure 1. Dynamics of cellulose, hemicellulose, total carbon and nitrogen contents in** 537 **each treatment during the harvesting period**

538 Error bars indicate standard deviations. Statistically significant treatments in each

539 sampling days are indicated by alphabetic labels (Tukey HSD analysis, $p < 0.05$).

540

541 **Figure 2. Dynamics of microbial biomass, and each enzyme activity during the** 542 **harvesting period**

543 Error bars indicate standard deviations. Statistically significant treatments in each

544 sampling days are indicated by alphabetic labels (Tukey HSD analysis, $p < 0.05$).

545

546 **Figure 3. Dynamics of microbial diversity and richness during the harvesting** 547 **period**

548 Error bars indicate standard deviations. Error bars Statistically significant treatments in

549 each sampling days are indicated by alphabetic labels (Tukey HSD analysis, $p < 0.05$).

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551 **Figure 4. Changing the relative abundance of prokayoric communities during the**
552 **harvesting period in genus level**

553 The date indicated the sampling days and the alphabet at the bottom indicated each
554 treatment.

556 **Figure 5. Changing the relative abundance of prokayoric communities during the**
557 **harvesting period in order level**

558 The date indicated the sampling days and the alphabet at the bottom indicated each
559 treatment.

561 **Figure 6. Dynamics of each microbes during the harvesting period**

562 Error bars indicate standard deviations. Statistically significant treatments in each
563 sampling days are indicated by alphabetic labels (Tukey HSD analysis, $p < 0.05$).

565 **Figure 7. Beta diversity: principal coordinate analysis (PCoA) of prokaryotic**
566 **community structure based on Buray–Curtis distances for each treatments during**
567 **the harvesting period.**

568 Sample names were assigned x/y-z in which x, y and z indicate the date of sampling and

569 replication number, respectively (ex, 6/12-1 was sampled at 12 June and the replication 1).

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For review

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Table 1. Soil physicochemical properties of the paddy field

pH(H ₂ O)	EC (mS m ⁻¹)	TC (g kg ⁻¹)	TN	C/N	Soil texture (%)		
					sand	silt	clay
5.72	2.59	8.29	0.74	11.24	77.5	18.8	3.6

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Table 2. Yields and chemical properties of each GM

	Yields	TC	TN	CN ratio
	t/ha	t/ha	t/ha	
V	1.24	0.53	0.045	11.8
A	1.31	0.61	0.01	61.0
M	2.55	0.9	0.03	30.0

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Table 3. Inputs of GM in the litter bag

	Input amount	Input carbon	Input nitrogen
	mg litter bag ⁻¹	contents	contents
		(mg kg ⁻¹)	(mg kg ⁻¹)
C	0	0	0
V	37.2	500	42.3
A	39.3	500	8.3
M	76.5	1000	50.7

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Table 4. Soil chemical and microbial properties after the harvesting stage

	TC	TN	Cellulose	Hemicellulose	Microbial biomass C	beta-glucosidase	Dehydrogenase	OTU number	Shannon index
C	5.52±0.39	0.43±0.03	238±17	391±18	27.3±16.5	0.09±0.02	0.27±0.17	1271±323	6.39±0.45
V	5.84±0.36	0.48±0.04	226±32	397±6	28.1±14.5	0.11±0.08	0.35±0.01	1893±389	7.03±0.25
A	5.61±0.88	0.49±0.06	222±49	370±38	25.5±17.8	0.16±0.04	0.41±0.15	1334±375	6.37±0.31
M	6.66±0.66	0.56±0.06	282±51	422±30	24.9±4.4	0.15±0.04	0.48±0.2	838±125*	5.69±0.19*

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Table 5. Correlations between each microbes and cellulose, hemicellulose, and soil carbon content

Phylum	Closest relatives	Cellulose	Hemicellulose	Total Carbon
Firmicutes	<i>Lachnospiraceae</i>	0.606	0.679	0.242
	<i>Clostridiales</i>	0.46	0.401	0.203
	<i>Bacillus</i>	0.261	0.285	0.759
<i>Chloroflexi</i>	<i>Anaerolineae SJA15</i>	-0.474	-0.551	-0.347
	<i>Ktedonobacteria</i>	-0.205	-0.053	-0.438
	<i>Unidentified Chloroflexi</i>	-0.41	-0.406	-0.417
Proteobacteria	<i>Rhizobiales</i>	-0.457	-0.292	-0.757
	<i>Unidentified Betaproteobacteria</i>	-0.394	-0.45	-0.173
Verrucomicrobia	<i>Pedospaerales</i>	-0.373	-0.404	-0.088
Chlorobi	<i>BSV26</i>	-0.277	-0.496	-0.401
<i>Euryarchaeota</i>	<i>Methanomicrobia</i>	-0.097	-0.435	-0.219

Table 6. Correlations between each microbes and, hemicellulose contents in each treatment

Pylum	Closest relatives	A	E	M
Firmicutes	<i>Bacillus</i>	0.46	0.50	0.45
	<i>Lachnospiraceae</i>	-0.08	0.45	0.41
	<i>Ruminococcaceae</i>	0.32	-0.43	0.24
Chloroflexi	<i>Anaerolineae</i>	-0.41	-0.42	-0.01
	Unidntified Chloroflexi	-0.49	0.05	-0.37
Proteobacteria	<i>Desulfobulbaceae</i>	0.01	-0.44	-0.10
	<i>Betaproteobacteria</i>	0.06	-0.42	-0.25
Chlorobi	BSCV6	0.22	-0.49	-0.21
Euryarchaeota	<i>Methanomicrobia</i>	0.05	-0.23	-0.41
	<i>Methanoregulaceae</i>	-0.02	-0.05	-0.45

OTU number

Shannon index

Supplemental Figure 1. Changing the soil carbon content in the paddy field