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SHORT COMMUNICATION

Effect of weed residue application on rice-straw decomposition and soil fungi-to-bacteria ratios

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Abstract

Purpose Undecomposed rice straw incorporated into the soil can negatively impact rice growth, but it is also an important source of soil carbon. The objective of this study was to evaluate whether the addition of weeds that naturally grow in rice paddy ecosystems could accelerate the decomposition rate of rice straw. The microbes that contribute to the decomposition process were also investigated.

Method *Trifolium pratense* (clover) and *Rumex obtusifolius* (bitter dock) both alone and in combination were decomposed along with rice-straw litterbags in rice paddy soils. The rice-straw decomposition rate was measured using the weight changes of the litterbags. The rice-straw carbon-to-nitrogen ratio and microbial abundance (fungi and bacteria) were also measured, as well as the soil respiration rate every seven days.

Results The addition of weed residues increased the soil respiration rates, but it did not influence the rice-straw decomposition rate. However, the carbon-to-nitrogen ratio of the remaining rice-straw and the fungi-to-bacteria ratio in the soil were both affected by the presence of the weeds, and the magnitudes of the effects were dependent on the carbon-to-nitrogen ratio of the added weeds.

Conclusion Given that the addition of weeds altered the quality of the remaining rice straw and the soil microbial community composition, longer term studies are required to determine whether the addition of weed residues primes the rice straw for the later stage of decomposition.

Keywords Weed residues, Rice-straw decomposition, Carbon-to-nitrogen ratio, CO_2-C respiration, Fungi-to-bacteria ratio

Introduction

Returning rice straw to the soil is a common practice used to recycle agricultural residues and maintain soil organic matter. Rice straw contains the nutrients necessary for crop growth, e.g., nitrogen, phosphorus, potassium, and sulfate; thus, the long-term incorporation of rice straw into soil reduces the need for chemical fertilizers and enhances microbial abundance in the soil (Ponnamperuma 1984; Zhang et al. 2016; Carricondo Anton et al. 2020). However, slow rice-straw decomposition rates can negatively affect rice farming, e.g., through microbial nitrogen immobilization and the production of rice root-harming chemicals such as hydrogen sulfide (Liou 2001). Thus, studies on the acceleration of rice-straw decomposition are needed to ensure nutrients are effectively recycled and carbon (C) is sequestered into soils.

Plant-derived phytochemicals released during the decomposition of plant residues can have positive or negative effects on bacterial and fungal activities (Kong et al. 2008; Dong et al. 2014; Nornasuha and Ismail 2017); thus, several approaches have been developed to accurately quantify the soil microbial activity during the decomposition of rice straw. Real time quantitative PCR is a molecular approach used to evaluate microbi-

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al copy number; using different primers, bacterial and fungal abundances can be separately quantified (Smith and Osburn 2009). Soil microbial biomass C can be measured using a chloroform fumigation method that indicates the amount of C stored in microbial bodies (Fierer et al. 2009; Liang et al. 2011). Soil respiration rates are related to the loss of decomposed plant residues as gases, but they also indicate the activity of microbes (Bentham et al. 1992; Phillips and Nickerson 2015). By combining these approaches, the fate of C added to the soils can be better understood, particularly the efficiency of adding C to increase soil organic C.

In addition to microbes, the carbon-to-nitrogen (CN) ratio in soils and residues is important for the decomposition of agricultural residues. In general, the CN ratio of microorganisms is approximately 10. Therefore, if the CN ratio of the organic material is >30 (e.g., rice straw), nitrogen (N) levels become insufficient for microbial proliferation, which results in immobilization (Green and Blackmer 1995; Moritsuka et al. 2004). Wang et al. (2013) suggested that when the CN ratio of the incorporated organic material was 10–20, this would induce soil microbial activity while also maintaining the available N in the soil for crop uptake.

With the aim of accelerating the decomposition of agricultural residues with a high CN ratio, including rice straw, "priming effect" theory has been evidenced in previous studies (Kuzyakov et al. 2000; Chapman and Koch 2007). This theory suggests that the incorporation of higher quality organic matter (with an easily available source of C) will activate microbes and thereby accelerate the decomposition of recalcitrant and low-quality organic matter. For example, Shahbaz et al. (2018) reported that combined materials (glucose + plant residues) or sole plant residues promoted the decomposition of relatively more recalcitrant soil organic matter. Therefore, the addition of plant residues other than rice straw as C and N resources may have positive effects on the rice-straw decomposition rate.

The use of previously wasted biomass to accelerate rice straw decomposition in the soil is increasingly receiving attention from researchers. For example, weeds that naturally grow in the ecosystems around rice paddies can be added to the soils to promote the decomposition of rice straw, but the efficiency of this approach is not well understood. Thus, we investigated whether the addition of different types of weed residues could improve soil microbial activities in relation to the decomposition of rice straw. We hypothesized that the incorporated weeds would increase the abundance and activity of bacteria and fungi in the soil (priming effects), leading to an increased rate of rice straw decomposition. We also hypothesized that the use of weeds with a relatively low CN ratio would accelerate the effects of rice-straw decomposition.

Materials and methods

Soil and weed residue sampling

Soil samples were taken from a naturally farmed rice paddy in Hokkaido University, Field Science Center for Northern Biosphere, Experimental Farm, in Sapporo, Hokkaido, Japan (N43°04′39"151, E141°20′03"634) (Appendix: Fig. A1). The soil was classified as gray lowland soil (Gleysol; FAO/UNESCO); it was collected from the surface to a depth of 10 cm prior to transplanting in 2019. The water retention capacity (%) of the sampled soil was 65.1% ± 5.0% (standard deviation; n = 3). The pH of the soil was 6.01 ± 0.07 (n = 3), and the ammonium and nitrate levels were 4.22 ± 0.3 mg kg^{-1} and 3.14 ± 0.63 mg kg^{-1}, respectively. To measure soil pH, 5 g of dry soil was first mixed with 25 ml of Milli Q water and shaken for 30 min; pH was then measured using a pH meter (AS800; AS ONE Corporation, Osaka, Japan). The method used for measuring the water retention capacity of the sampled soil was previously described by Kneifel and Seiler (1993) and Brischke and Wegener (2019). A 50-g dried sampled soil was placed in a funnel that was itself placed in a paper filter (circle 110 mm; ADVANTEC). A 100 ml measuring cylinder was placed under the funnel and 100 ml of water was poured slowly into the funnel. Parafilm was placed over the top of the funnel to prevent evaporation. A few holes were made on the surface of Parafilm to maintain atmospheric pressure on the soil, which was drained overnight. The water retention capacity was calculated using the following equation: [Water held in soil (mL) ÷ Initial weight (g)] × 100. The soil ammonium and nitrate content of the soil samples were extracted using 2 mol L^{-1} KCl (w/v; soil:KCl = 1:5) and detected using a flow injection analyzer (AQLA-700; Aqualab, Tokyo, Japan) as described by Hamamoto and Uchida (2015). In terms of the reagents used to measure ammonium and nitrate, ammonium was reacted with R1 solution [phenol and sodium pentacyanonitrosylferrate(III) dehydrate] and R2 solution (sodium hydroxide and sodium hypochlorite) to form an indophenol blue

derivative; potassium chloride was used as the carrier solution. Nitrate was initially reduced to nitrite through a cadmium column installed in the FIA instrument and then nitrite was reacted with R1 solution (sulfanilamide coupled with N-1-naphthylethylenediamine dihydrochloride) to form a magenta-colored azo dye; EDTA-4NA and ammonium chloride were mixed and used as the carrier solution.

After harvesting in early October, 2019, the dominant weed species found at the experimental sites were red clover, *Trifolium pratense* (hereafter, "clover"), and bitter dock, *Rumex obtusifolius* (hereafter, "*Rumex*"). Consequently, clover and *Rumex* were used as the plant residues in this experiment. Each plant, including the leaves, stem, and roots, were sampled around the naturally farmed rice paddy in Hokkaido University, Field Science Center for Northern Biosphere, Experimental Farm where soil was sampled in early October 2019 (Appendix Fig. A2). The collected plants were dried at 60°C for 2 days and then cut into pieces of <1 cm in length; the rice straws were collected immediately after harvesting and cut into 1-cm lengths after which they were dried at 60°C for 2 days. Both the plant residues and rice straws were immediately stored at 4°C in preparation for inorganic N and CN ratio analysis or at −40°C in preparation for molecular analysis. The C and N content of each plant residue was measured using a CN analyzer (EA 2400 Series; Perkin Elmer, Foster City, CA, USA). The properties of the weed and rice-straw residues are shown in Table 1.

Table 1 The properties of weed and rice-straw residues

Plant residue treatment	Carbon (%)	Hydrogen (%)	Nitrogen (%)	CN ratio
Clover	39.17 (4.45)	6.04 (0.63)	2.89 (0.98)	14.69 (6.55)
Rumex	38.72 (1.87)	6.37 (0.13)	2.23 (0.13)	17.46 (1.90)
Rice straw	35.59 (0.71)	4.70 (0.11)	0.83 (0.06)	42.83 (2.60)

Values are based on the air-dried weights and are shown as means (± standard deviations; n = 3).

Incubation setup

To produce soil cores, 745-g samples of wet soil (equivalent to 514 g of dried soil; hereafter, units of "kg soil" or "g soil" refer to oven-dried soils) were placed in glass bottles (9.5 cm in diameter and 18 cm in depth). Milli Q water was applied to the soils so that their water-filled pore space was 60%, which represents the optimal condition under which microorganisms are fully functional (Groffman and Tiedje 1991). The density of the soils was adjusted to 1 g cm^{3-1}. The glass bottles were covered with metal lids containing two holes (made with a pin) to avoid contamination and excess evaporation. Three replicates were prepared for each treatment. In total, 12 cores, representing four treatments (control, *Rumex*, clover, and a weed mix) each with three replicates, were arranged. These cores were preincubated for one week at room temperature (22°C ± 3°C).

A litter-bag method was used in the straw decomposition experiment. The rice-straw mass was calculated based on the weight of the litterbags sampled from the incubation bottles. After the preincubation period ended, 48 litter bags were prepared for the incubation experiment. The amount of rice straw was adjusted to 3,000 mg−C kg dry $soil^{-1}$, which is 1.088 g, and then placed into one litter bag (4.7 cm in length and 10 cm in width).

After preincubation, plant residues for clover, *Rumex,* and the mix were applied to the soils, whereas the control treatment received no residues. The amount of applied plant residue was adjusted to 3,000 mg−C kg dry $soil^{-1}$ and the rice straw-to-plant residue ratio was adjusted to 1.0–1.5. The weight of clover and *Rumex* was 26.8 mg and 11.7 mg, respectively. The mix treatment (30% clover and 70% *Rumex*) was adjusted to have the same amount of C, i.e., 16.25 mg. During residue application, the soils and residues were mixed well with a stainless-steel spoon. Four litter bags were separately buried in a vertical position in each glass bottle. Four bags were collected from each replicate after 7, 14, 21, and 28 days.

Analysis of rice-straw CN ratio and the microbial abundance

The carbon content of the rice-straw residues in each collected litter bag was determined using combustion

and an elemental analyzer (EA 2400 Series; Perkin Elmer, Foster City, CA, USA). The microbial abundance of *16S* and *ITS* rRNA genes was assessed using quantitative polymerase chain reaction (qPCR) conducted with a CFX96 (Bio-Rad) PCR System. On each sampling day, straw DNA was extracted from 0.2 g of fresh straw with a NucleoSpin Soil kit (TAKARA BIO INC., Shiga, Japan) according to the manufacturer's instructions. The extracted DNA was purified with Agencourt AMPure XP (Beckman Coulter) using the predetermined protocol. The concentration of the purified DNA was measured with Qubit dsDNA HS Assay Kit according to the manufacturer's instructions (Invitrogen, USA). The purified DNA was then diluted 50 times with nuclease-free water in preparation for qPCR. To measure the *16S* gene copy number, F515 and R806 primers were used. A standard curve was prepared with serial dilutions of amplified *Escherichia coli* (10^{-3}–10^{-9} ng/µl). The initial denaturation temperature was 95°C with an annealing temperature of 95°C, and extension was conducted for 1 min at 58°C for 30 cycles. The final extension was completed at 72°C for 1 min. The threshold line was calculated using R and the result was represented as the log copy number of the *16S* rRNA gene g $soil^{-1}$. To measure the *ITS* gene copy number, ITS1 and ITS2 primers were used. A standard curve was prepared using serial dilutions of the highest raw DNA concentration (10^{-3}–10^{-9} ng/µl). The initial denaturation temperature was 95°C with an annealing temperature of 95°C, and the extension was conducted for 1 min at 55°C for 35 cycles. The final extension was performed at 72°C for 1 min. The threshold line was calculated using R and the result was represented as the log copy number of the *ITS* rRNA gene g $soil^{-1}$.

Analysis of soil respiration and microbial biomass carbon

Soil respiration (CO_2 emissions) was measured at 7, 14, 21, and 28 days from the first day of incubation; thus, there were four measuring times in total. Each soil pot was sealed with a screw lid. At 1, 6, and 11 min after closing the lid, a 250-ml gas sample was collected from the septa. The collected gas sample was injected into a Carbon Dioxide Isotope Analyzer (CCIA-38-EP, Ros Gatos Research, CA, USA) to measure the CO_2 concentration. Data showing a correlation index >0.7 were used to calculate soil respiration. The soil respiration rate was calculated based on the increase in CO_2 concentration during the 11 min and the headspace within the bottle. Results are presented as mg CO_2–C kg $soil^{-1}$ day^{-1}.

Microbial biomass–C was determined using a chloroform fumigation–extraction method (Hasebe et al. 1985). For each treatment, three replications of 5-g fresh soil samples were taken from the soil cores after 28 days of incubation. The 48 soil samples were placed in 6.4 × 1.7-cm glass Petri dishes and fumigated with chloroform for 24 h. The fumigated and non-fumigated soils were shaken with 0.5-M K_2SO_4 for 30 minutes through a filter (Grade 5C, <5 mm; Advantec, Tokyo, Japan). The extract was stored at −30°C until the measurement. The carbon concentration in the extract was measured using a combustion catalytic oxidation method with TOC (TOC-5000A, Shimadzu, Kyoto, Japan). The amount of microbial biomass–C was calculated as microbial biomass–C = Ec / Kec, where Ec is the carbon content in the fumigated soil minus the carbon content in the non-fumigated soil and Kec is 0.45 (Vance et al. 1987). The microbial biomass–C analysis results are represented as mg kg $soil^{-1}$.

Statistical analysis

For pH, inorganic N, microbial biomass–C, and soil respiration data, two-way ANOVA was performed to determine the effects of treatment and sampling date. For the microbial abundance data, two-way ANOVA and Tukey's test were performed to determine the effect of treatment. Statistical analysis was performed using R ver. 4.0.1 (R Core Team 2018).

Results and discussion

The soil respiration rates (Fig. 1) from the clover, *Rumex*, and mix treatments were relatively higher than those of the control throughout the incubation period, but the rates of the clover treatment were the highest. The control treatment had the lowest cumulative CO_2–C respiration rate (52 ± 7 mg C/28 days), whereas the clover treatment had the highest (219 ± 21 C/28 days). The cumulative CO_2–C respiration rates for the clover, *Rumex*, and mix treatments were significantly higher than those of the control. The amount of respired CO_2–C emitted from the soils (Fig. 2) was negatively correlated with the CN ratio of the added

residues (Table 1). This suggests that soil microbial activities were enhanced by the additional input of high-quality organic matter, i.e., the readily decomposable weed residues. The results are in agreement with previous studies reporting that soil respiration rates were greater when relatively lower CN ratio residues were added (Lützow et al. 2006; Marschner et al. 2015; Truong and Marschner 2018; Partey et al. 2018).

In contrast, there were no effects of plant type on the amount of decomposed rice straw-C, as calculated based on the weight changes of the litterbags, which were 304−332 mg (approximately 30% of the total weight) (Fig. 2). The decomposition rates of the rice straw in the current study (Fig. 3) were similar to those in previous studies (Guo et al. 2018; Chen et al. 2018; Guan et al. 2020). However, a "priming effect," or the acceleration of rice-straw decomposition caused by weed residue application, was not observed in the current study, which was in contrast to previous reports (Kuzyakov et al. 2000; Chapman and Koch 2007). This difference may have been due to the quality of the weeds used in our study, i.e., they may not have been of sufficient quality to trigger the priming effect, or perhaps the relatively short (28-day) experimental period. The current investigation focused on the decomposition of the less recalcitrant parts of the rice straw and their decomposition could not be "primed" by the addition of weed residues.

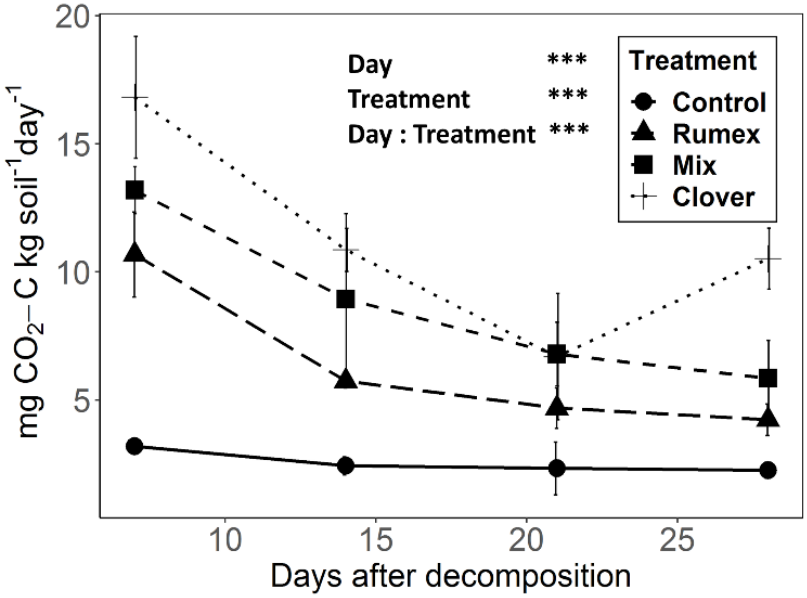

Fig. 1 Soil respiration at 7, 14, 21, and 28 days in the control, *Rumex*, mix, and clover treatments
The level of significance was determined by two-way ANOVA. "Day:Treatment" shows the interaction between the day and treatment effects. ***, $p < 0.001$. Error bars represent standard deviations of mean values (n = 3).

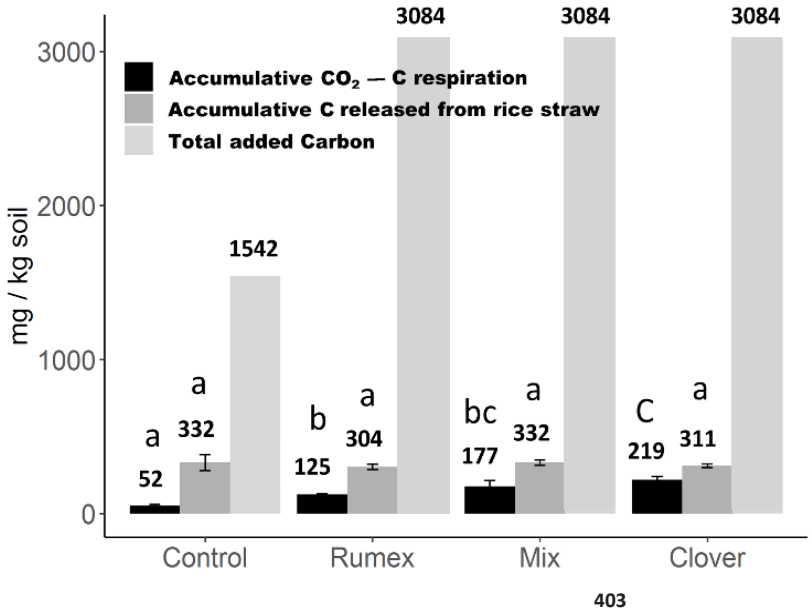

Fig. 2 Amount of C emitted as CO_2–C and the C released from the rice straw (based on litter-bag weight changes) after 28 days of incubation with the control, *Rumex*, mix, and clover treatments
Level of significance was determined by two-way ANOVA. Different lowercase letters indicate significant differences between the treatments ($p < 0.05$). Error bars represent standard deviations of mean values (n = 3).

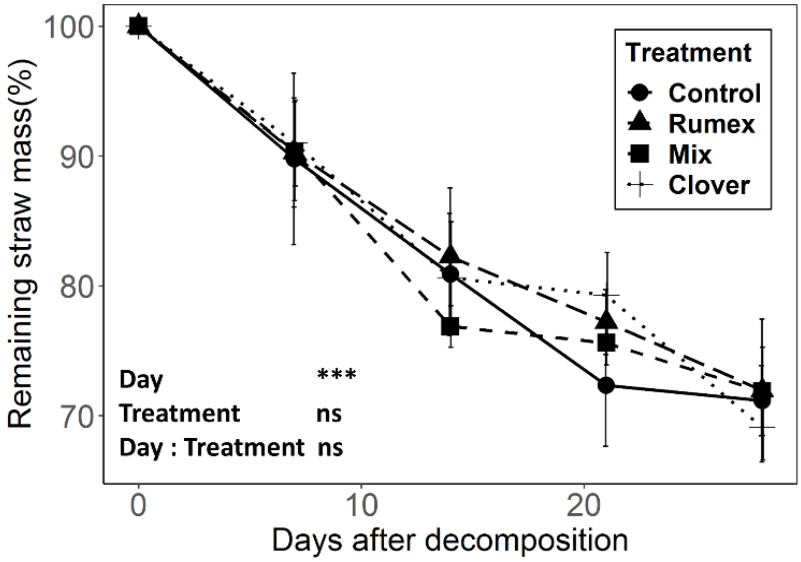

Fig. 3 Rice straw decomposition rate at 7, 14, 21, and 28 days under the control, *Rumex*, clover, and mix treatments

Significance was determined using two-way ANOVA. "Day: Treatment" shows the interaction between the day and treatment effects. ***, $p < 0.001$; ns, not significant. Error bars represent standard deviations of mean values (n = 3).

However, plant treatment significantly affected the CN ratio of rice straw during its decomposition ($p < 0.001$; Fig. 4). With the clover treatment, rice straw had the lowest CN ratio (26 ± 3) across the incubation period, whereas rice straw under the control treatment had a CN ratio that was relatively steady and remained relatively high toward the end of the experiment compared with the CN ratios under the other treatments. This suggests that the remaining rice straw residues could be relatively less recalcitrant with the addition of weeds when compared to the treatment with rice straw only. Although we did not clearly observe the effects of adding weed residue on the decomposition rates of the rice straw (Fig. 3), the results show that weed residue addition did influence the quality (CN ratio) of the remaining rice-straw residue, which could have potentially affected the later stages of decomposition. Further studies will be required to verify this.

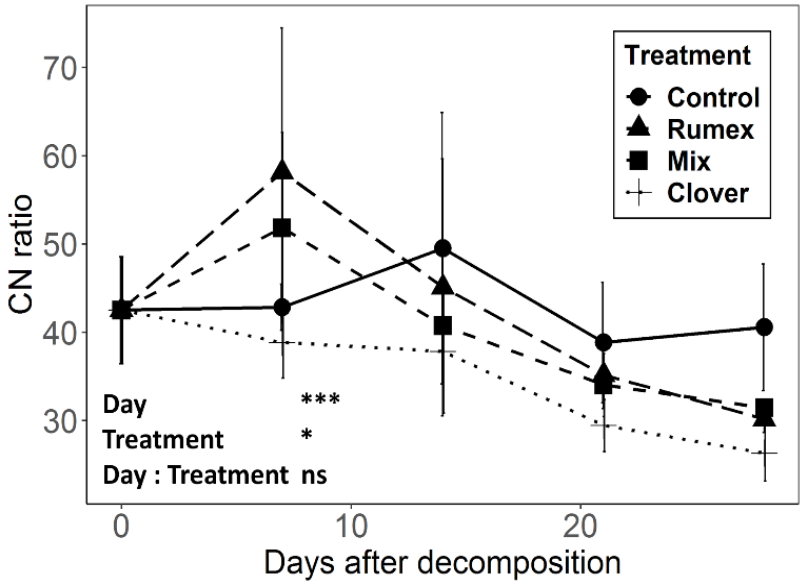

Fig. 4 Changes in the rice straw CN ratio at 7, 14, 21, and 28 days under the control, *Rumex*, mix, and clover treatments

Significance was determined using two-way ANOVA. "Day: Treatment" shows the interaction between the day and treatment effects. *, $p < 0.05$; ***, $p < 0.001$; ns, not significant. Error bars represent standard deviations of mean values (n = 3).

Our results also showed that the fungi-to-bacteria ratio (F:B ratio) increased with the addition of *Rumex* residues (Fig. 5B and 5C). The microbial copy number in the control treatment decreased for bacteria and increased for fungi and the F:B ratio (Fig. 5A and 5C). Contrastingly, the clover treatment had a lower F:B ratio, indicating that its dynamics changed depending on the type of weed residue applied ($p < 0.01$). Zhou et al.

(2019) indicated that the composition of clover phytochemicals, i.e., polysaccharides, lignins, lipids, and nitrogenous compounds, significantly stimulated soil bacterial and fungal reproduction. Nonetheless, the higher potassium, zinc, magnesium, and tannin levels of *Rumex* (Merfield 2018) seemed to be more favorable to bacterial and fungal growth compared with the clover and control treatments. The influence of plant residue phytochemicals on soil microorganisms during decomposition should be further studied to determine potential correlations. The concentrations of the extracted raw DNA from the control treatment (Fig. 5D) corresponded with the bacterial copy number, which showed a decreasing trend over time (Fig. 5B). Previous studies have found that, in general, bacteria play important roles in the early stages of rice-straw decomposition because of the presence of readily decomposable C, whereas fungi dominate the later stages due to the presence of recalcitrant C, such as the lignin, that remains in the decaying rice straw (Wang et al. 2004; de Boer et al. 2005). Generally, the range of the F:B ratio in rice paddy, grassland, and forests is from 0.2–0.6 (de Vries et al. 2006; Ge et al. 2017; Wang et al. 2019), and this is in accordance with the values observed in the current study (Fig. 5C). Further research is required, however, as other studies have suggested that the phytochemical composition of the incorporated plant residues could activate different microbes with different functional genes (Bai et al. 2015; Jacoby et al. 2017); therefore, the co-occurring decomposition of rice straw with other plant residues could influence soil microbial functions (e.g., the cycling of nutrients such as nitrogen and phosphorus).

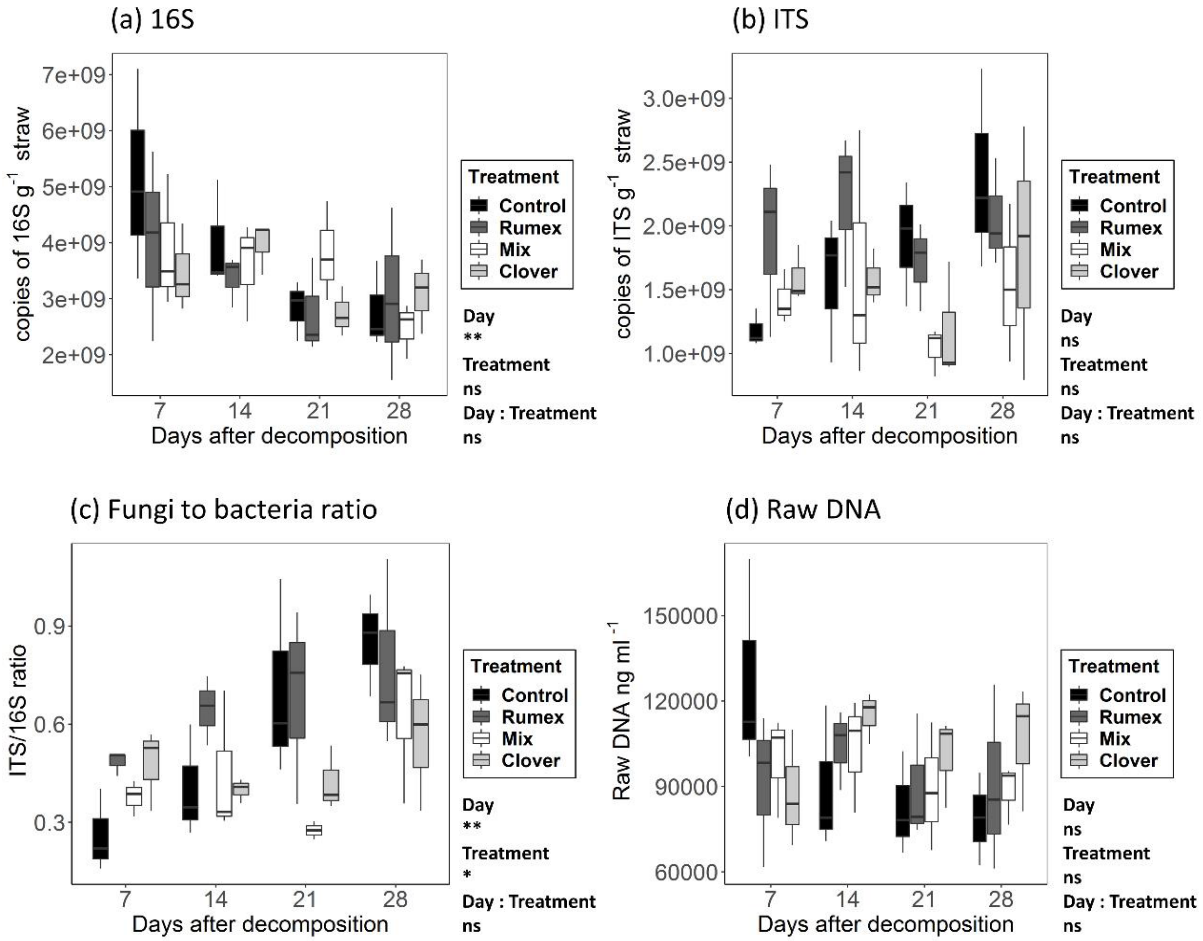

Fig. 5 *16S* bacterial abundance (a), *ITS* fungal abundance (b), fungi-to-bacteria ratio (c), and raw DNA abundance (d) at 7, 14, 21, and 28 days under the control, *Rumex*, mix, and clover treatments

The level of significance was determined using two-way ANOVA. "Day: Treatment" shows the interaction between the day and treatment effects. *, $p < 0.05$; **, $p < 0.01$; ns, not significant. Error bars represent standard deviations of mean values (n = 3).

Despite the effects of the added weed residues on the soil respiration rates and F:B ratio, the soil microbial biomass-C was not affected. The microbial biomass-C in the soil at the end of the incubation period was ~2,500 mg C kg^{-1} soil on average, yet there was no significant difference among the treatments (Fig. 6). This may have been because the added C masked the fluctuations of the soil microbial biomass−C. Previous studies have indicated the limitations of the chloroform fumigation method when used to quantify soil microbial biomass−C, especially when the soil systems are disturbed by factors such as residue decomposition (Gaillard et al. 1999; Nicolardot et al. 2007; Li et al. 2018). Furthermore, the soil microbial biomass−C measured in the present study was relatively large compared with the values obtained from previous studies of Japanese rice paddy soils (48−1327 mg C kg^{-1} soil; Hasebe et al. 1984, 1985; Inubushi et al. 2002; Kyaw et al. 2005; Yamashita et al. 2014). Thus, in our investigation, soil microbes utilized C to maintain their biomass−C (i.e., through respiration) rather than increase their biomass. Consequently, the addition of weed residues influences soil microbial compositions (e.g., F:B ratio) but not the amount of biomass. Further investigation is required to determine whether changes in composition influence the decomposition of rice straw in the later stages of the process.

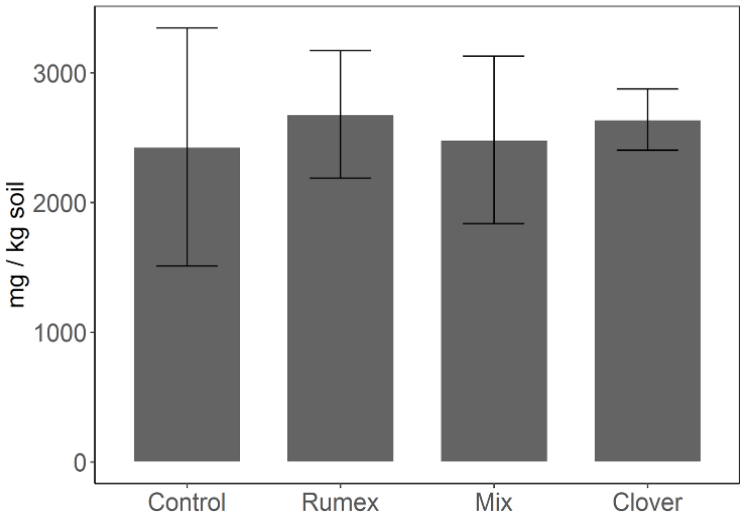

Fig. 6 Microbial biomass−C in the control, *Rumex*, mix, and clover treatments at 28 days of incubation
Level of significance was determined using one-way ANOVA. There were no significant differences among treatments ($p > 0.05$). Error bars represent standard deviations of mean values ($n = 3$).

Conclusion

We added low-CN-ratio residues from clover, *Rumex*, and a mixture of the two to provide an extra N source and observed whether the incorporated rice-straw decomposition rate was accelerated. Consequently, our findings were as follows:

- The co-occurrence of rice straw and weed residue decomposition did not accelerate rice straw decomposition rates during the early (28-day) stage of rice straw decomposition.
- The quality of the remaining rice-straw residues (i.e., the CN ratio) and the microbial composition associated with decomposition (i.e., F:B ratio) were influenced by the addition of weed residues.
- Microbial biomass−C was not influenced by the added weed residues, suggesting that they were mostly used as a source of respired-C.

Additional studies should focus on the changes to soil microbial function due to the presence of weed residues, given that the changes in F:B ratio could affect these functions. Longer term studies are also needed to investigate whether the added residues can prime the rice straw for decomposition in the later stages of the process, as we only observed the decomposition of approximately 30% of the rice straw (on the basis of weight).

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

Appendix

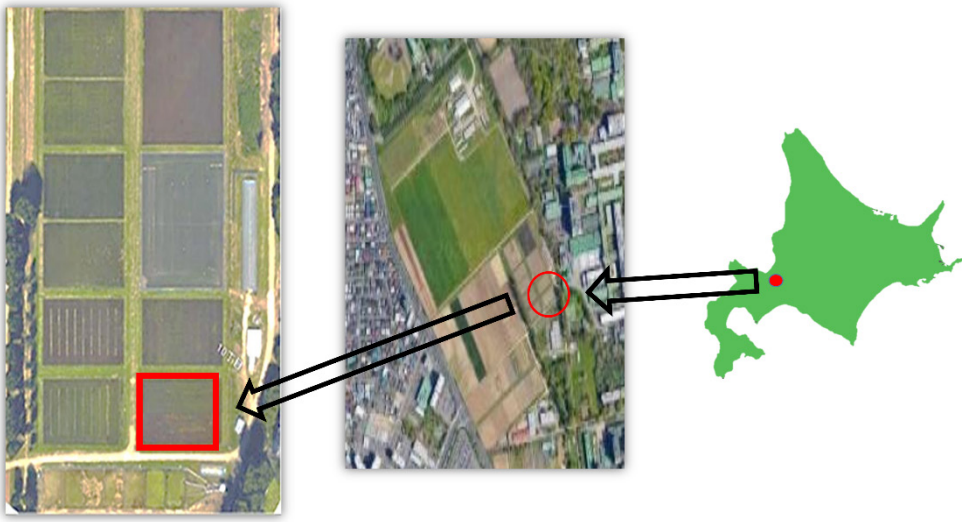

Fig. A1 Soil samples were collected in the "natural farming" rice paddy located in the Field Science Centre of Hokkaido University, Sapporo, Japan (N43°04′39"151, E141°20′03"634)

Fig. A2 Weeds were sampled around the "natural farming" rice paddy located in the Field Science Centre of Hokkaido University (N43°04′39"151, E141°20′03"634)

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