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ABSTRACT

Rice is the third most important crop worldwide. Unfortunately, in most rice-producing countries, crop residues are burned, increasing emissions of greenhouse gases and toxic compounds. Incorporation of rice straw (RS) into the soil, either or not accompanied by a microbial inoculum, may offer a viable alternative. However, the effects of such treatments on soil, including the microbial community structure and function in the next crop cycle, still remain largely unknown. Here, we studied the effect of four different RS management strategies (leaving RS as mulch with or without a microbial inoculum, incorporation of RS into the soil with microbial inoculum, and RS burning) on rice growth and flowering-stage rhizosphere microbiomes. The relevant microbiomes were examined by amplicon sequencing based on the 16S rRNA and ITS1 gene regions. In comparison to the zero situation, all four treatments tended to increase the soil organic carbon content, albeit without significant differences. Furthermore, none of the treatments had major effects on either (rice) crop yield or phytopathogen incidence in the next cycle. However, leaving RS as a mulch incited a decrease in soil pH, and showed a trend of reducing yield by up to 1 ton·ha^{-1}. Moreover, the different RS treatments affected the structures and predicted functions of the bacteriomes and fungomes in the rice rhizosphere. The mulching treatment was associated with an enhanced abundance of *Acidobacteria*, particularly *Bryobacter* spp. In contrast, the non-mulch treatments incited raised abundances of *GammaProteobacteria*, *Bacteroidia* and *Campylobacteria*. The rice rhizosphere fungomes, consisting mostly of *Ascomycota*, were less affected by the treatments, although the microbial inoculum was shown to drive the respective fungome structures.

1. Introduction

Rice is the staple food for over half the world's population. In 2018, the world rice production was close to 760 million tons (FAO, 2018), yielding an estimated 760–1140 million tons of rice straw (RS) in rice production areas. Traditionally, burning has been considered to constitute the 'best' practice for rapidly "cleaning" fields from RS (Romasanta et al., 2017). Such removal is important in tropical countries that practice double or triple rice cropping during the year (Romasanta et al., 2017). However, RS burning has clear disadvantages, like the loss of most of the nitrogen, 25% of the phosphorus, 20% of the potassium and 5–60% of the sulfur (Dobermann and Fairhurst, 2002). The losses of carbon due to burning may amount to 352–384 g C·kg dry straw^{-1} (Arai et al., 2015). RS burning in the field is intrinsically "uncontrolled", and incomplete combustion, yielding CO, may occur. In the RS burning process, hazardous compounds are emitted, including greenhouse gases (i.e. CO_2, CH_4 and N_2O) (Arai et al., 2015). Additionally, burning can affect soil health/quality (Gupta et al., 2004), negatively affecting microbial abundance in the topsoil (Tung et al., 2016). Consequently, there is a trend in rice-producing countries to increasingly ban RS burning, although such practices still persist (Chivenge et al., 2020).

Aside from burning, there are emerging efforts to incorporate RS into the soil or use it as a mulch (Yuan et al., 2014). Clearly, such incorporation and mulching practices can exert effects on the subsequent crop, for instance wheat (Rahman et al., 2005) and Chinese milk vetch

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(*Astragalus sinicus* L.), by virtue of modulations of soil microbial activity and diversity (Tang et al., 2020). Moreover, the treatments require mechanization and often water and additional fertilizer are added to enhance decomposition processes (Kalkhajeh et al., 2021). Furthermore, in flooded soils, RS decomposition may substantially increase methane emissions (El-Sobky, 2017). To mitigate this effect, it has been proposed to leave RS on the field for at least 30 days after harvest before planting the next crop, so as to facilitate decomposition under aerobic conditions (Knoblauch et al., 2014).

Rice straw constitutes a rich source of carbon, containing 32–47% cellulose, 19–27% hemicellulose, 5–12% lignin and 3.3–5.7% protein. Its incorporation into soil increases organic carbon and nitrogen concentrations and modulates the quality and quantity of the soil organic matter (SOM) by increasing the labile, particulate and dissolved organic carbon, and improving physicochemical soil properties, having a crucial influence on the soil microbial biomass and microbial soil activity (Luo et al., 2016; Tang et al., 2020; Yan et al., 2020). Thus, returning rice straw to soil may contribute with 31–42 kg N ha^{-1}, 8 kg P ha^{-1}, 34–61 kg K ha^{-1} and 2.1–2.2 ton C h^{-1} per crop cycle (Hung et al., 2019). Shallow tillage (at 5–10 cm depths) in rice-rice systems is used for residue incorporation, as it enhances soil aeration. This treatment may favor the soil beneficial microbes, thus improving soil health (Dobermann and Fairhurst, 2002; Nie et al., 2018). However, RS incorporation may also negatively affect rice cropping, as it may hamper effective tillage which decreases seedling emergence (Knoblauch et al., 2014). Moreover, it may even foster the spread of phytopathogens to the new crop (Dobermann and Fairhurst, 2002).

Microbial inoculants that enhance RS degradation in soil and/or antagonize phytopathogens may mitigate some of the aforementioned problems. The use of inoculants in rice cropping has been evaluated in soil microcosm studies. Goyal and Sindhu (2011) studied the effect of a consortium of the fungi *Aspergillus awamorii*, *Paecilomices fasisporus* and *Trichoderma viride* on the composting of RS incubated for 90 days, and found a decrease in OM of 17.4%, and of C:N ratio from 73.7 to 16.6. This resulted in a significant increase in enzyme activity in relation to an uninoculated control. Similarly, Stella and Emmyrafedziawati (2015) showed that a bacterial consortium of 30 different strains was able to boost the degradation of RS, significantly reducing the cellulose and hemicellulose fractions. In another study, Cruz-Ramirez et al. (2017) showed that the use of a commercial mix of *Trichoderma* spp. (*T. harzianum*, *T. koningi*, and *T. viridae*) plus *Bacillus pumilus* (strain IBUN 02717) contributed to the degradation of RS in soil.

Soil microbiomes play crucial roles in agroecosystems, as they affect soil health and plant development (Sessitsch et al., 2012). Moreover, they are the key players in most biogeochemical cycles, including the transformations of crop residues, thus affecting SOM status (van Elsas et al., 2019). Soil bacteria like *Bacillus*, *Paenibacillus*, *Sphingobacterium*, *Klebsiella*, *Flavobacterium*, *Streptomyces*, *Kitasatospora* and others are main players in the degradation of cellulose, hemicellulose, starch, pectin and other plant compounds (Jiménez et al., 2014; Guo et al., 2020). Also, soil fungi, in particular those belonging to the *Ascomycota* (e.g. members of *Trichoderma* and *Penicillium*), have been reported as key degraders of plant remains, transforming in particular the cellulose moieties of these (Guo et al., 2020).

So far, few studies have examined the effects of RS management on the soil microbiome, in particular with respect to the next crop's rhizosphere. Interestingly, Maarastawi et al. (2018) described a successional pattern during RS degradation in soil, using ^{13}C-labeled RS and amplicon sequencing of the 16S rRNA gene and ITS1 region. These authors found that mostly aerobic bacterial taxa belonging to the *Alpha-*, *Beta-*, and *Gamma-Proteobacteria* and *Bacilli* were initially stimulated, while fungal taxa (mostly *Sordariomycetes* and *Agaricomycetes*) became involved in the later stages of degradation (Maarastawi et al., 2018). Similarly, Zhan et al. (2018), in a 26-year field experiment, found a strong effect of soil fertilization (chemical vs organic amendments) on RS degrading microbiomes selected in an anaerobic microcosm setup with ^{13}C-labeled RS. On the other hand, Edwards et al. (2015), in conventional (no RS incorporation) field and greenhouse studies, showed that rice root microbiomes of six-week-old plants vary across the rhizocompartments, being the rhizosphere microbiome similar to the bulk soil but different from the endosphere microbiome. The microbiomes also varied with the rice developmental stage, being members of the *Burkholderiales* early colonizers, while *Rhodocyclales* and *SBla 14* were typical late colonizers (Edwards et al., 2018). Similarly, the microbiomes were found to be influenced by soil type and rice genotype, as well as cultivation practices (Edwards et al., 2015).

Notwithstanding these emerging studies, there still is a paucity of knowledge on the implications of highly practice-related in-field RS management strategies, for the quality of, and microbiomes associated with, the next (rice) crop. Here, we hypothesized that the differential use of RS will affect (1) the performance of a subsequent rice crop in terms of yield, soil organic carbon and disease incidence, and (2) the structure and function of the rhizosphere microbiomes of the next crop (at flowering stage). To test these hypotheses, an incomplete randomized block design was set up in the field. To analyze the flowering-stage rhizosphere microbiomes, soil DNA-based methods were employed.

2. Materials and methods

2.1. Area of study

The experimental area was located at Las Lagunas Research Station (3°54′56.4 N, 74°58′57E) of Federación Nacional de Arroceros (Fedearroz), Saldaña - Tolima, Colombia. The research station is located in a tropical region, with an annual rainfall of 210 mm and an annual average temperature of 28 °C. Before any treatment, the physical and chemical soil parameters at the experimental site were evaluated. The soil was characterized as a loamy clay, with pH 5.24; N 1 g·kg^{-1} of soil; organic carbon 4 g·kg^{-1} of soil; P 40.3 mg·kg^{-1}; Ca 4.8 m-equivalents (mEq) ·100 g^{-1}; Mg 0.8 mEq·100 g^{-1}; Na 0.3 mEq·100 g^{-1}, and Al 0.4 mEq·100 g^{-1}.

2.2. Biological material

The rice variety used in this study was Fedearroz 68 (F-68). This variety is tolerant to the fungi *Pyricularia grisea* and *Rhizoctonia solani.* These are the common phytopathogens causing rice blast and sheath blight diseases, respectively, in rice. F-68 has a vegetative cycle of 105 days, i.e. 20 days less than most commercial varieties, at high potential yield (13 t·ha^{-1}).

To accelerate the degradation of RS and reduce the incidence of rice phytopathogens, a microbial consortium (Fitotripen® Natural Control, Ltda) was applied in two of the four treatments (see below). Fitotripen contains the fungi *Trichoderma harzianum*, *T. koningii*, and *T. viride* and the bacterium *Bacillus pumilus* (strain IBUN 02717; Genes and Strain Collection, Biotechnology Institute, Universidad Nacional de Colombia, Bogota). It was selected on the basis of its dual capacity as biological control agent and as an accelerator of RS degradation due to its cellulolytic and proteolytic activities (Cruz-Ramirez et al., 2017).

To prepare the bacterial suspension, *B. pumilus* IBUN 02717 was grown in 2-L flasks containing 200 mL of sporulation medium (Cruz-Ramirez et al., 2017) at 28 °C (shaken at 175 rpm during 10 days). Following growth, the cells were washed three times with sterile saline (0.85% NaCl) solution (SSS) by spinning down (10 min at 7258*g*) at room temperature, and resuspending the pellet in 200 mL SSS. Then, the spore concentration was estimated by plating on LB agar by using the microdrop technique. The suspension was stored at 4 °C until application in the field. The fungal inoculum was prepared by diluting the commercial powder containing the three strains in SSS to reach the propagule concentrations needed. Thus, two liters of the microbial suspensions, at 1.7×10^7 bacterial spores·mL^{-1} and of fungal suspension at 1×10^6 conidia·mL^{-1} were applied to the approximately 34 Kg RS per

plot, by using a manual garden spray pump for each suspension.

2.3. Field experiment

The experimental field was set up in an incompletely randomized block design. It was designed to evaluate the effect of the four treatments for the management of RS in a standard rice crop system under flooding. Common practice in this region is to drain all the water from the field before applying fertilizers and then to re-establish the water layer after fertilizer application, with an estimated production of RS of around 10 ton·ha^{-1}. The composition of the used RS was: crude protein 4.4%, 9.3% lignin, 43.8% cellulose, and 19.4% hemicellulose.

The treatments were:

(1) RS incorporated into the soil, with microbial inoculants (RS-IMO): RS was treated with a brush cutter in order to decrease the straw size, and then approximately 34 kg of RS per plot was inoculated with 2 L of microbial inoculum (1.7×10^7 bacterial spores · mL^{-1} and 1×10^6 fungal conidia · mL^{-1}). The inoculated RS was incorporated into the top soil layer (20 cm) with a disc plowing machine at day10 after the start of the experiment. The soil was then left for 20 days until preparation for the next crop cycle; (2) RS as mulch, with microorganisms (RS-MMO): approximately 34 kg of RS per plot was inoculated and treated with 2 L (per plot) of the microbial inoculum applied as before. The inoculated RS was left on the field for 30 days as mulch, until soil preparation for the next crop cycle; (3) RS as mulch (without inoculum; RS-M): approximately 34 kg of RS was applied to the field as mulch and soil was kept for 30 days, as above; (4) RS (approximately 34 kg per plot) was added to the soil surface, followed by burning (RS-B): burning took place in the field and this was followed by incorporation of the ashes into the soil (20 cm) with a disc plowing machine at day 10 after the start of the experiment. Then, soil was left for 20 days until the next crop cycle. This is the most used procedure in the region for the management of RS, and for this reason we used it as the baseline to which we compared the other treatments.

On the basis of the clear clustering of the microbiome data (see Results section), the RS-IMO and RS-B treatments were joined in a (statistical) group coined *Early Incorporation* (*EI*) treatments. Treatments RS-M and RS-MMO (being still visibly present in the field until soil preparation for the next crop - day 30 following the start of the experiment), were coined the *Mulching* (*M*) treatment group.

The experimental setup consisted of four replicate 33.75-m^2 plots, for each of the four treatments, in a field of 550 m^2 with a history of continuous rice cropping for more than 10 years. The experiment was assessed after a total of 180 days. Rice seeds were applied randomly in each plot at a ratio of 175 kg·ha^{-1}. During the experiment, the daily mean temperature was 29.5 °C (range 20.2 °C to 38.3 °C). The mean solar radiation, relative humidity and precipitation were 458.5 cal·cm^{-2}·d^{-1}, 73.6% and 2.6 mm respectively. All treatments were fertilized following the recommendations of the Fedearroz Research Station for the region as follows: 229.1 kg N·ha^{-1}, 28.6 kg P·ha^{-1}, 85.5 kg K·ha^{-1}, 47.4 kg S·ha^{-1} and 3.0 kg Zn·ha^{-1} (Online Resource 1).

The rice rhizosphere soil samples were collected at the flowering stage of the rice panicles (26th August; 128 days after initiation of the assay, within the rainy season).

2.4. Vegetative, reproductive and phytosanitary parameters of the rice crop

At harvest, ten plants were taken at random from each experimental plot to determine the plant height (cm). We also determined the grain yield (ton·ha^{-1}) at a water content of 14%, from a sampling area of 4m^2 within each plot. Panicles obtained within a plastic frame of 0.25 m^2 were harvested and the number of panicles and filled grains (%) recorded. The crop phytosanitary status was determined following standard procedures by field rice experts (Fedearroz Las Lagunas Research Station), by assessment of symptoms/presence of the following phytopathogens: *Helmintosporium* sp., *Rhizoctonia* sp., *Pyricularia* sp., *Gaeumannomyces* sp. and *Burkholderia glumae*, 1 week before harvest. The phytopathogen incidence was recorded by determining the number of symptomatic plants over the total number of plants present in an area of 50 × 50 cm evaluated in each replicate of every treatment.

2.5. Soil physicochemical analyses

We analyzed the physicochemical properties of the soil at harvest time, following standard procedures at the Water and Soils Analysis laboratory of the Faculty of Agricultural Sciences, Universidad Nacional de Colombia. The pH was determined by potentiometry in water. Exchangeable Ca, K, Mg and Na were extracted with ammonium acetate and measured by atomic absorption. Al was extracted with KCl and volumetrically titrated. Effective cation exchange capacity (ECEC) was estimated by the sum of exchangeable cations. Organic carbon (OC) was determined by the Walkley-Black method. Total nitrogen (N) was assayed by the Kjeldahl method and total P by the Bray II method. Finally, soil texture was determined by the Bouyoucos method (Pansu and Gautheyrou, 2006).

2.6. Rhizosphere soil sampling, DNA extraction and sequencing

Five plants per plot were removed, from separated points, for rhizosphere sampling. Each plant was shaken by hand to remove large soil aggregates and loosely-adhering soil. The soil remaining on the roots was considered to constitute the rhizosphere soil and collected using the protocol from Lundberg et al. (2012) with some modifications. Briefly, roots (10 g fresh weight), with associated rhizosphere soil, were placed into 50 mL sterile tubes, and treated with 30 mL of 1× phosphate buffer plus Tween 20® 2% (*v*/v); then, each tube was shaken during 10 min in a Multi-wrist® shaker. After that, the suspensions were centrifuged for 5 min at 7258*g* and the pellets conserved for a second round of washing as before. At the end, rhizosphere soil was collected and immediately processed for DNA extraction.

Total soil DNA was extracted from 250 mg of initial material using the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA), following the manufacturer instructions. DNA preparations were electrophoresed over agarose gels in order to assess DNA purity and quality (average size). Extracted DNA samples were quantified using Nanodrop (ThermoFisher Scientific, Waltham, Massachusetts, USA).

The V3–V4 16S rRNA gene regions were amplified using the universal primer set 341F and 805R. The fungal ITS1-5.8S-ITS2 region was amplified using the ITS1F-ITS4R primer set. Libraries were then constructed following the Illumina Paired-End Metagenomic Prep Kit protocol, and sequenced at the University of Wisconsin-Madison Biotechnology Center using a MiSeq platform (Illumina, San Diego CA). Biological resource access permit No 01023 from the National Environmental Licensing Authority of Colombia.

2.7. DNA sequence analysis

2.7.1. 16S rRNA gene sequence analysis

16S rRNA raw sequence data were processed using QIIME2 v 2019.7 (https://qiime2.org) (Bolyen et al., 2019). Denoising, dereplication of the sequences and filtering chimeras with consensus method were performed using the pipeline of DADA2 (Callahan et al., 2016) inside QIIME2. Forward reads were truncated at 271 bp to eliminate low quality sequences (Q 20), and singletons were discarded as part of the data process inside the DADA2. The taxonomic assignment of Amplicon Sequence Variants (ASVs) (97% sequence identity), was made using the "fit-classifier-naïve-Bayes" plugin, against the SILVA 132 reference database. ASVs were analyzed when these were present in minimum two samples for at least one treatment.

2.7.2. ITS sequence analysis

ITS sequences were processed with PIPITS (Gweon et al., 2015) using only forward reads. The highly variable ITS amplicon length precludes paired-end assembly, therefore, only ITS1 was used. Briefly, the non-truncated sequences were quality filtered (Q20), then the specified ITS1 region was extracted. Subsequently, a dereplication step was made for the removal of redundant sequences and short (<100 bp) unique sequences, and the detection and removal of chimeras was performed using UNITE v7.2 (Abarenkov et al., 2010), and UCHIME reference data set. Finally, PIPITS uses VSEARCH for clustering sequences in OTUs (97% sequence identity), and it chooses the representative sequence of each cluster for its taxonomical assignation using the RDP Classifier, against the UNITE v7.2 fungal ITS reference data set.

The DNA raw sequences for this project were deposited at the National Center for Biotechnology Information (NCBI) under Bioproject PRJNA528984, with accession numbers SRR8785128 through SRR8785139 for 16S rRNA gene sequences, and SRR8785186 through SRR8785197 for ITS region sequences. The datasets generated and analyzed during the current study are available in the FigShare repository.

2.8. Functional guilds

PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), hosted by GitHub (https://github.com/picrust/picrust2/wiki) was used for predicting the functional capabilities of microbial communities based on reference genomes and gene families. FunGuild v1.1.py database hosted by GitHub (https://github.com/UMNFuN/FUNGuild), was used to predict ecological functions, or trophic modes (pathotroph, symbiotroph and saprotroph), of each fungal OTU found within this study (Nguyen et al., 2016).

2.9. Statistical analyses

Statistical analysis of vegetative, reproductive and phytosanitary parameters of the rice crop were performed in quadruplicate and subjected to analysis of variance (ANOVA) and Kruskal-Wallis tests, in dependency of compliance with the assumptions and in accordance with the experimental design using R® (R Core Team, 2013). Differences between treatments were compared by post-hoc Tukey (HSD) for ANOVA and Dunn for Kruskal-Wallis respectively, the significant differences were defined as $P \leq 0.1$.

16S rRNA gene based α-diversity was estimated based on a rarefied ASV (19.424 reads per sample), which included observed ASV richness and Shannon diversity indices. ITS region α-diversity estimation was based on rarefied OTUs (48.806 reads per sample), and included the same index mentioned before. Sampling effort was estimated using Good's coverage index; using ASV or OTU ≥ 2 counts. To test for significant differences in bacterial and fungal α-diversity between group samples, a non-parametric Kruskal-Wallis test was performed, with the Bonferroni correction method at $P \leq 0.05$ in R. The R packages installed were FSA, lattice, psych, multcompView, and rcompanion (R Core Team, 2013). The visualization of the underlying driving forces of the microbial community variation between the samples was accomplished by a principal coordinate analysis (PCoA), in combination with a permutational multivariate analysis of variance (PERMANOVA); both were based upon Bray-Curtis dissimilarities matrices. Hierarchical cluster analysis of bacterial and fungal communities was done using the Bray-Curtis dissimilarity matrix with Ward's method. A method to test for differential expression by use of negative binomial generalized linear models, DESeq2, was applied for identifying differential abundance in ASVs (bacteria) or OTUs (fungi), using the MicrobiomeAnalyst web-based platform for processing and statistical analysis of microbiome data (https://www.microbiomeanalyst.ca/; Dhariwal et al., 2017). The identification of the core microbial community at 0.1% of relative abundance, with the rarefaction mentioned before (19.424 and 48.806 reads for bacterial and fungal community respectively), was used identifying the commonalities between treatments through a Venn diagram of the composition type, using a relative abundance of 0.5 for bacterial and 0.3 for fungal community. Finally, Euclidean distance measure and Ward clustering algorithm was used to generate hierarchical clustering heatmaps of the core microorganism using the web platform MetaCoMet (https://probes.pw.usda.gov/MetaCoMET/).

STAMP bioinformatics software was used for analyzing the differential abundance of KO genes in each treatment (Parks et al., 2014) and between the resultant groups of the hierarchical clustering applying Kruskal-Wallis test with the Bonferroni correction method at $P \leq 0.05$.

3. Results

3.1. Vegetative, reproductive and phytosanitary parameters of the rice crop

Rice crop yield was relatively high in all treatments, i.e. above 12 t·ha^{-1}. Remarkably, there were no significant differences in yield across the RS management strategies ($P \geq 0.05$). However, although not significant, the RS-M treatment stood out, as it showed 1 ton less rice production per ha, on average, than that in all other treatments. The other agronomic variables (plant height, filled grain %, number of panicles·ha^{-1} and 1000-grain weight in g), did not show significant differences between the treatments ($P \geq 0.05$), with one exception: plant height revealed higher values in the RS-B than in the RS-M treatments ($P \leq 0.05$; Table 1). With respect to the rice phytosanitary status at harvest, *Gaeumannomyces graminis* var. *graminis* showed high incidences across all treatments (~91.9%), without statistical differences ($P = 0.75$) between the treatments. The incidence of *Pyricularia oryzae* was, on average, 34.2%, also not showing significant differences between the treatments ($P = 0.26$). No *Burkholderia glumae* - associated symptoms were found in the plants at harvest (Table 1). In contrast, *Helmintosporium* sp. revealed a significantly higher incidence ($P = 0.07$) in the RS-M (33.0%) and RS-MMO (40.2%) treatments than in the RS-IMO (11.4%)

Table 1
Effect of rice straw management on agronomical variables and incidence of pathogens.

		Treatments			
		RS-MMO	RS-IMO	RS-B	RS-M
Agronomical variables	Plant heigh (cm)	99.2 ± 6.73ab	100.7 ± 4.31ab	101.5 ± 6.27a	98.1 ± 4.03b
	Yield (Kg/ha)	13.1 ± 1.65a	13.4 ± 1.96a	13.1 ± 3.56a	12.1 ± 2.96a
	Empty grain (%)	11.5 ± 3.33a	14.2 ± 4.15a	15.7 ± 5.79 a	11.2 ± 2.34a
	1000 grains weight	25,51 ± 215a	25,54 ± 361a	24,52 ± 292a	27,34 ± 391a
	Panicles (#panicles/m^2)	146 ± 17a	147 ± 14a	150 ± 22a	138 ± 30a
Pathogens (% incidence)	*Burkholderia*	ND	ND	ND	ND
	Rhizoctonia	6.7 ± 3.29b	14.9 ± 6.79a	6.7 ± 3.51b	10.9 ± 3.67ab
	Gaeumannomyces	98.1 ± 3.85a	80.5 ± 39.1a	89.0 ± 21.95a	100 ± 0a
	Pyricularia	28.5 ± 17.95a	57.5 ± 28.80a	32.9 ± 22.39a	17.8 ± 10.60a
	Helmitosporium	40.2 ± 40.2a	11.4 ± 3.63b	17.8 ± 10.95 ab	33.0 ± 6.75a

ND: Not detected.
Means with the same letter do not differ significantly (Tukey's HSD) $P \leq 0.05$ $n = 4$.
RS-M: Rice straw as mulch; RS-MMO: Rice straw as mulch plus microbial inoculum; RS-IMO: Rice straw incorporated plus microbial inoculum; RS-B: Rice straw burning.

treatment. *Rhizoctonia solani*, as expected due to the tolerance of rice cv F68 to this fungus, presented a low fungal disease incidence in all treatments; however, the RS-IMO treatment revealed a significantly higher *R. solani* incidence (14.9%), than the RS-B (6.7%) and RS-MMO (6.7%) treatments ($P = 0.07$) (Table 1).

3.2. Soil physicochemical factors

The soil pH showed a decrease at the end of cropping for all four treatments. It went from an initial value of 5.24 to, respectively, 5.06, 4.98, 4.91 and 4.69, in the RS-B, RS-MMO, RS-IMO and RS-M treatments, with significant differences between RS-B and RS-M ($P = 0.02$). For the other soil chemical properties, i.e. the levels of K, Na, Al, P and N, no significant differences between the treatments were observed during the entire experiment ($P \geq 0.05$; Table 2). In particular the Ca, Mg, Na and CEC levels showed decreases, from the initial values, at harvest. In contrast, the Al levels increased in all treatments. The P levels increased over time in the RS-IMO and RS-B treatments, but remained at the initial value in all other treatments. Similarly, the SOC values revealed increases at harvest (in relation to time zero), of 54.5%, 61.4%, 61.4 and 56.8% for the RS-MMO, RS-M, RS-IMO and RS-B treatments, respectively, without statistical differences between treatments ($P \geq 0.05$).

3.3. Effect of RS management on the rhizosphere bacteriomes at rice flowering time

Across all 12 flowering-stage rhizosphere samples, totals of 803.226 partial 16S rRNA gene sequences were obtained. After quality filtering, 408.009 good-quality reads were obtained, with a mean of 25.789 reads per sample. After filtration, a total of 1.122 different amplicon sequence variants (ASVs) was identified among all samples.

Rarefaction analysis revealed that the sequencing effort was sufficient to capture most of the bacterial diversity in the different treatments (Online resource 3). Although the bacterial communities were diverse, there were no statistically significant differences in alpha diversities in the rice rhizospheres between the treatments ($P > 0.05$; Table 3). However, the β-diversity values diverged into two groups as a function of the first component, as shown in the PCoA (Fig. 1a): (1) the mulching (*M*) treatments RS-MMO and RS-M, and (2) the early incorporation (*EI*) treatments RS-IMO and RS-B. In subsequent analyses the *M* treatments were compared to the *EI* ones, as they revealed significant differences (as found in the PCoA using PERMANOVA - $R^2 = 0.15$; $F = 1.78$; $P = 0.007$) (Fig. 1b). Then, a hierarchical cluster analysis based on all 16S rRNA gene ASVs across all treatments confirmed the presence of the two separate clusters, *M* and *EI* (Online Resource 4).

Across all treatments, the rice rhizosphere bacteriomes showed the presence of 18 bacterial phyla. *Proteobacteria* (42% relative abundance, on average, range 40.3–45.0%), was the dominant phylum, followed by *Bacteroidetes*, *Firmicutes*, *Cyanobacteria*, *Epsilonbacteraeota* and *Acidobacteria* (at average abundances of 9, 8, 8, 6 and 6%, respectively). In total, more than 79% of all ASVs was associated with these six phyla (Online Resource 5). The relative abundances (RAs) of these phyla revealed no significant differences between the treatments. However, after separate clustering, the *M* and, on the other hand, the *EI* treatments revealed significant differences in the *Proteobacteria* ($P = 0.024$), *Epsilonbacteraeota* ($P = 0.024$), *Bacteroidetes* ($P = 7.036E\text{-}4$), and *Acidobacteria* ($P = 0.007$), as detailed hereafter (Fig. 2). Within the *Proteobacteria*, *Gamma-Proteobacteria* was the most abundant class in all treatments, with 32.1% RA across treatments (Online Resource 6). This class was higher in the *EI* treatments ($P = 0.019$, average 35.2% RA between RS-IMO and RS-B) than in the *M* treatments (average 28.9% RA between RS-MMO and RS-M). *Bacteroidia* ($P = 0.0022$) and *Campylobacteria* ($P = 0.051$) were also more abundant in the *EI* treatments (average RA 11.0% and 8.3% for *Bacteroidia* and *Campylobacteria*, respectively), than in the *M* ones (average RA 7.7% and 5.5% for *Bacteroidia* and *Campylobacteria*, respectively). The *Acidobacteria* class had a higher ($P \leq 0.01$) RA in the *M* treatments (average RA 7.1%) than in the *EI* ones (average RA 3.7%) (Online Resource 7).

At the family level, *Burkholderiaceae* was the dominant taxon across treatments, at an average 12.4% RA (range 10.9–15.0%). This was followed by *Sulfuricellaceae*, *Sulfurospirillaceae*, *Chitinophagaceae* and *Aeromonadaceae*, with average RA values of 7.6, 6.2, 4.0 and 2.9%, respectively (Online Resource 8). However, no significant differences were found between the treatments. On the other hand, *Tannerellaceae* had a significantly higher ($P = 0.002$) RA in the *EI* (average RA 1.06%) than in the *M* treatments (average RA 0.19%) (Online Resorce 9).

At the genus level, *Ferritrophicum*, *Sulfurospirillum*, *Comamonas*, *Polaromonas*, *Aeromonas*, *Bacillus* and *Bradyrhizobium* were the most abundant groups, with average RA values of 7.6, 6.2, 4.1, 2.9, 2.9, 2.2 and 2.0% respectively, without significant differences between the treatments. Remarkably, *Bryobacter* ($P = 0.0025$) and *Micromonospora* ($P = 0.036$) showed significantly higher RA values in the *M* (1.7 and 1.3% respectively) than in the *EI* treatments (0.8 and 0.8%, respectively) (Fig. 3). Additionally, *Aeromonas* revealed a higher RA in RS-B (5.5%) than in all other treatments (mean RA 2.1%), but without significant differences. Similarly, *Polaromonas* had an RA in RS-IMO of 4.5%, being higher than that in all other treatments (mean RA 2.4%), again without significant differences (Online Resource 10).

3.4. Effects of RS management on the "core" bacteriome at flowering time

The minimum community of ASVs that was shared by all four treatments, coined the 'core bacteriome', was then explored by using the program MetaCoMET. This core contained 170 ASVs and corresponded to 15% of the overall bacteriome. At each phylogenetic level, the existence of an *M* versus *EI* group dichotomy was confirmed, as there were differences in the RAs of the dominant taxa (Online Resource 11). At phylum level, the RA values of *Actinobacteria* and *Proteobacteria* in the *M* (3.3% and 4.1% respectively) exceeded those in the *EI* treatments (2.4% and 3.8% respectively). Conversely, *Epsilonbactereaota* RA values were higher in the *EI* (2.1%) than in the *M* treatments (1.5%). At order level, *Campylobacteriales* RA's were higher in the *EI* (2.1%) than in the *M* (1.5%) treatments, while the RA velues of the *Micromonosporales*, *Chloroflexales* and *Rhizobiales* were higher in the *M* (1.8%, 3.3% and 8.5%, respectively) than in the *EI* treatments (1.2%, 2.7% and 6.6%, respectively). *Sulfurospirillum* was by far the most common genus of the core, with 17% RA across all four treatments. However, *Comamonas* (with 7% RA in average), *Bradyrhizobium*, and *Flavisolibacter* (with 5%

Table 2
Physicochemical characteristics of soil at initial and at final stage of experimental field. Means with the same letter do not differ significantly $P \leq 0.1$ n = 4. Statistical test ANOVA - Tukey's HSD.

	Treatments	pH		Ca	K	Mg	Na	Al	CICE	CO	N	P	NH_{4+}	NO_{3-}
				Meq/100 g						%		mg/kg		
Initial time		5.24		4.8	0.19	0.84	0.27	0.36	6.5	0.44	0.05	40.3	2.2	16.0
Final time	RS-MMO	4.98	A	2.91	0.17	0.55	0.11	0.71	4.45	0.68	0.08	39.03	ND	ND
	RS-IMO	4.91	AB	2.79	0.19	0.56	0.10	0.73	4.36	0.71	0.04	48.45	ND	ND
	RS-B	5.05	A	3.02	0.18	0.54	0.09	0.72	4.54	0.69	0.02	47.08	ND	ND
	RS-M	4.69	B	2.81	0.18	0.47	0.09	0.84	4.37	0.71	0.00	45.60	ND	ND

Table 3
Richness and diversity of soil bacterial and fungal communities. Kruskal–Wallis with Bonferroni correction with a $P \leq 0.05$ was used for statistical analysis. Mean and standard deviation of $n = 3$ plots are presented for each treatment. RS-M: Rice straw as mulch; RS-MMO: Rice straw as mulch plus microbial inoculum; RS-IMO: Rice straw incorporated plus microbial inoculum; RS-B: Rice straw burning.

Treatments	Bacterial community			Fungal community		
	Richness	Shannon	Good's coverage	Richness	Shannon	Good's coverage
RS-M	155 ± 13.6	4.19 ± 0.0	99.9 ± 0.0	69.3 ± 4.5	1.91 ± 0.2	99.9 ± 0.0
RS-MMO	149 ± 4.2	4.00 ± 0.2	99.9 ± 0.0	70.3 ± 3.5	1.85 ± 0.1	99.9 ± 0.0
RS-IMO	160 ± 16.0	4.11 ± 0.1	99.9 ± 0.0	68 ± 6.6	1.88 ± 0.1	99.9 ± 0.0
RS-B	159 ± 2.1	4.22 ± 0.0	99.9 ± 0.0	68.3 ± 3.1	1.82 ± 0.2	99.9 ± 0.0

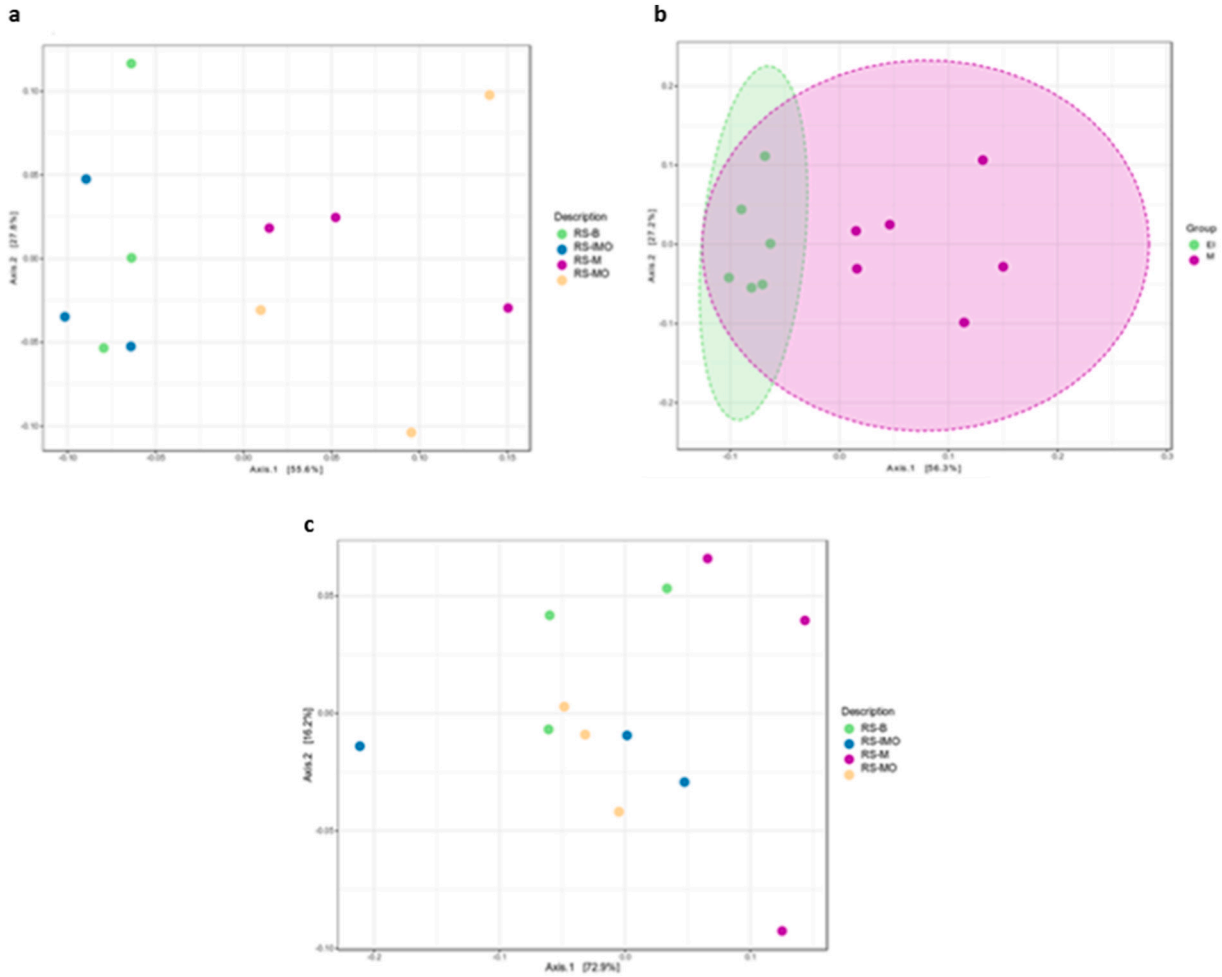

Fig. 1. Principal coordinate analysis (PCoA) ordination of a Bray-Curtis dissimilarity matrix. Plots illustrate distances between communities of each treatments for: total bacterial community ($R^2 = 0.32$, F = 1.28, $P = 0.061$) (**a**); bacterial community grouped by mulching (*M*) and early incorporation (*EI*) treatments ($R^2 = 0.15$, F = 1.78, $P = 0.007$) (**b**); and fungal communities ($R^2 = 0.29$; F value = 1.09; $P = 0.34$) (**c**) in rice rhizosphere soil. All replicates of each treatment are present ($n = 3$). RS-MMO: Rice straw applied as mulch plus addition of microorganisms (MO); RS-IMO: Rice straw early incorporated to the soil with previous addition of MO to RS; RS-B: Common practice in field, burning of RS with early incorporation of ashes; RS-M: RS applied as mulch.

RA each) and *Burkholderia-Caballeronia-Paraburkholderia* (with 4% RA), also had an important contribution to the core. In detail, *Sulfurospirillum* revealed 17.9% RA in the *EI* treatments versus 13.9% in the *M* ones. *Micromonospora* was more abundant in the *M* (1.7% RA on average) than in the *EI* treatments (with 1.2%).

3.5. Prediction of bacterial function using PICRUSt

Using PICRUSt2, we found significant differences ($P \leq 0.057$) in some predicted biosynthetic and degradation processes between the *EI* and *M* treatments. In detail, the numbers of predicted biosynthetic processes were higher in the *M* (10) than in the *EI* treatments (6), whereas the numbers of different degradation processes were higher in the *EI* (5) than in the *M* treatments (3). Most of the predicted biosynthetic processes referred to the biosynthesis of amino acids (lysine, threonine, methionine, glycine and aspartate, among others) and of DNA, next to energy production. The degradation processes included the degradation of simple (sucrose, glucose) as well as complex (catechol) carbon sources, or phosphorus-containing (myo-inositol) compounds (Online Resource 12).

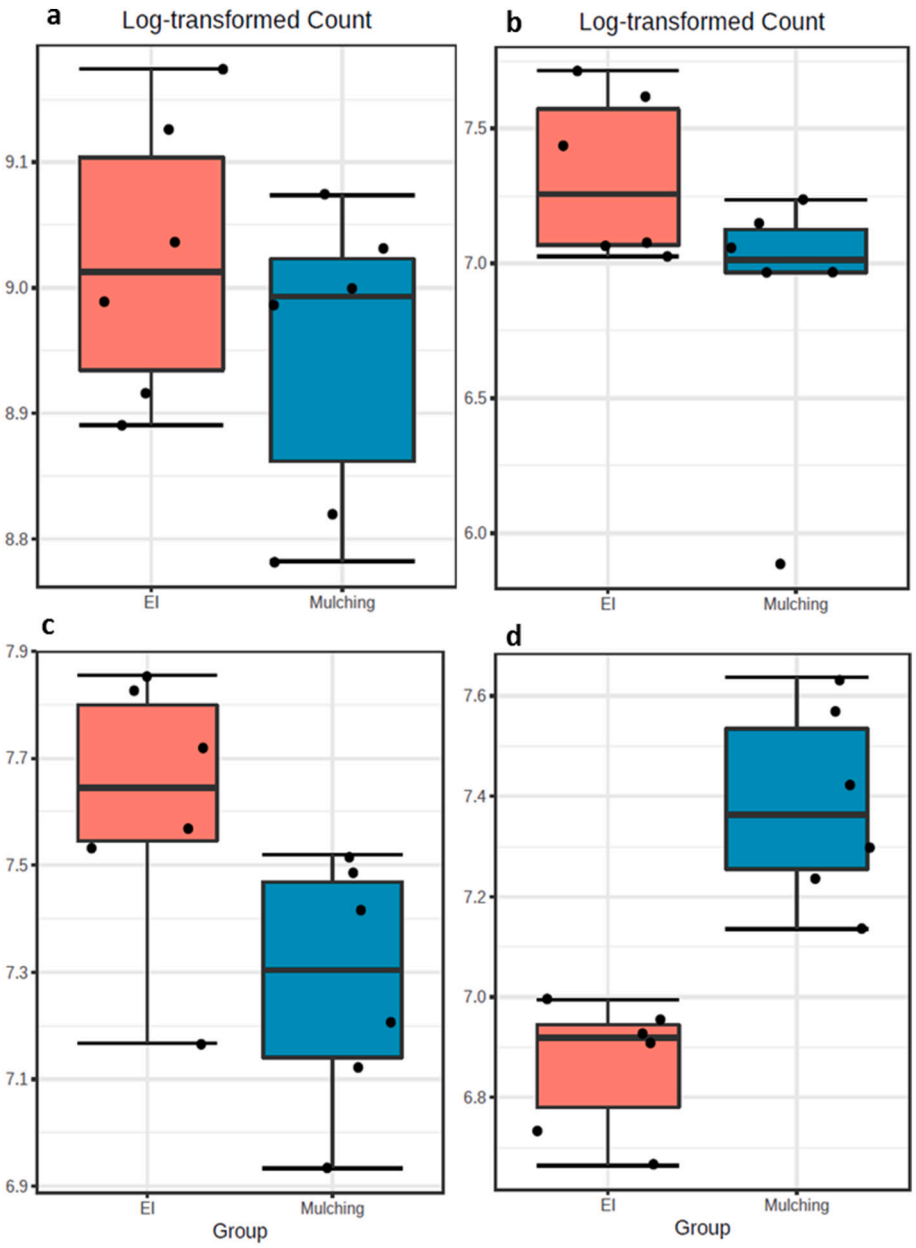

Fig. 2. Differential abundance between *Early Incorporation* (*EI*) treatments and *Mulching* (*M*) treatments at phylum level at $P \leq 0.05$ based on Log transformed count. *Proteobacteria* ($P = 0.024$) (a), *Epsilonbactereaota* ($P = 0.024$) (b), *Bacteroidetes* ($P = 7.036E4$) (c), and *Acidobacteria* ($P = 0.007$) (d).

3.6. Effect of RS management on the rhizosphere fungomes at flowering time

Across the 12 rhizosphere samples, a total of 1.363.746 raw ITS sequences was obtained. After quality filtering, 1.177.977 good-quality reads were obtained, with a mean of 67.324 reads per sample. Finally, 932 operational taxonomic units (OTUs), falling into 132 phylotypes, were identified. Rarefaction analysis indicated that the sequencing effort was sufficient to capture most of the fungal diversity (Online Resource 3). The fungal communities had lower alpha-diversities than the bacterial communities. No statistically significant differences were observed in the α or β diversities between the treatments (Table 3; Fig. 1c)); the fungal β -diversities between the treatments were examined by PERMANOVA ($R^2 = 0.29$; F value = 1.09; $P = 0.34$).

With respect to the phyla found, *Ascomycota* was the most abundant phylum (52.1% of the total reads, range (between treatments) 47.6–56.0%). This was followed by *Chytridiomycota* (0.37%, range 0.02–0.85%), *Mortierellomycota* (0.23%, range 0.03–0.48%), *Rozellomycota* (0.29%, range 0.13–0.40%) and *Basidiomycota* (0.23%, range 0.15–0.28%). Approximately 44.5% of the fungal reads could not be assigned to any specific taxonomic group (Online Resource 13).

Twenty-seven taxa were present at the order level. *Sordariales* (average 23.5% of the total reads, range 13.9–28.5%), *Pleosporales* (3.4%, range 3.1–3.7%), *Hypocreales* (1.2%, range 0.9–1.3%) and *Eurotiales* (0.78%, range 0.5–1.1%) were the most abundant orders across all treatments (Online Resource 14). *Sordariales* and *Hypocreales* showed lower RA values in the RS-B (1.4% and 0.9%, respectively) than in the other treatments (2.6% and 1.2% on average, respectively). On the other hand, *Eurotiales* was higher in RS-B (1.1%) than in the other treatments (0.6% on average).

Across all treatments, the most abundant fungal genera were: *Penicillium* (average 0.3%, range 0.13–0.69%), *Edenia* (0.27%, range 0.11–0.58%), *Aspergillus* (0.23%, range 0.17–0.33%), *Lagena* (0.22%, range 0.13–0.28%) and *Talaromyces* (0.19%, range 0.14–0.30%).

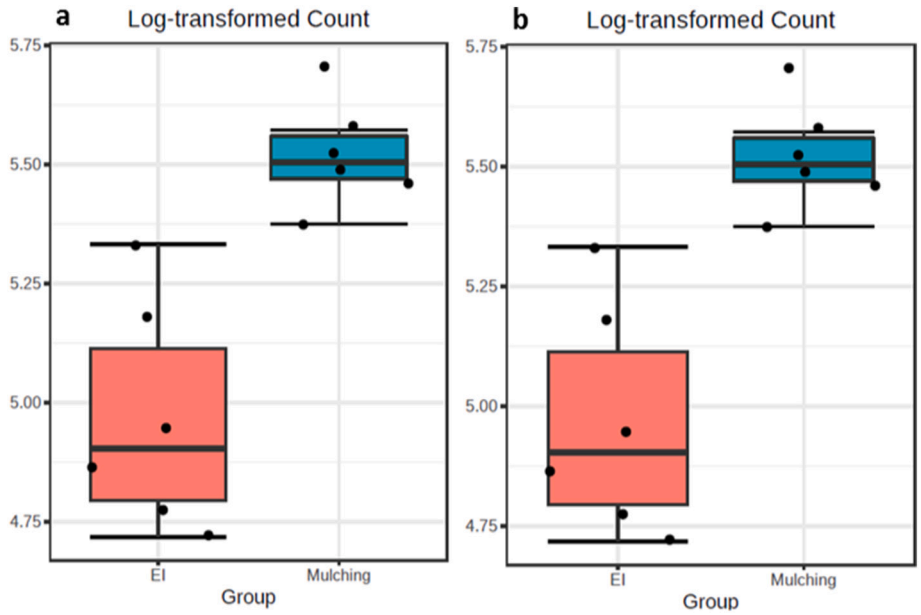

Fig. 3. Differential abundance between *Early Incorporation* treatments (*EI*) and *Mulching* (*M*) treatments at genus level at $P \leq 0.05$ based on Log transformed count. *Bryobacter* ($P = 0.0025$) (a) and *Micromonospora* ($P = 0.036$) (b).

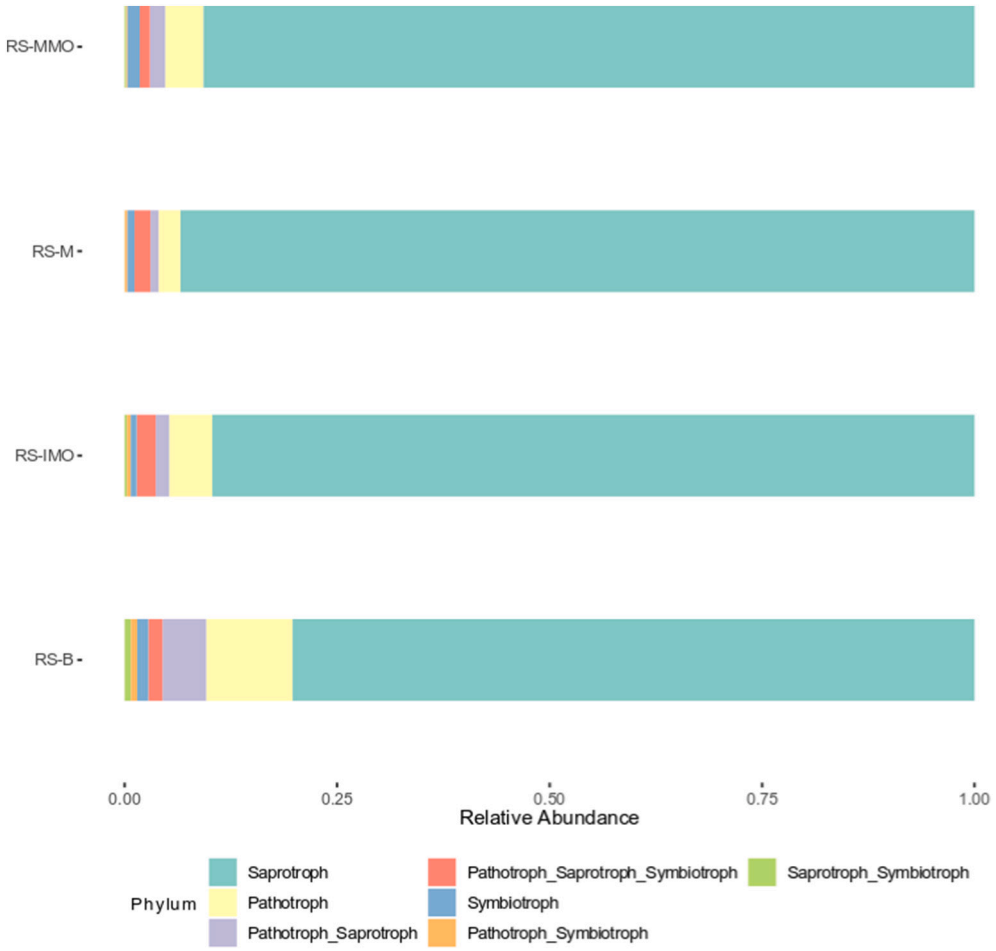

Fig. 4. Functional guilds composition of fungal community. Bar plots show the average abundance of three replicates per sample (n = 3). RS-MMO: Rice straw applied as mulch plus addition of microorganisms (MO); RS-IMO: Rice straw early incorporated to the soil with previous addition of MO to RS; RS-B: Common practice in field, burning of RS with early incorporation of ashes; RS-M: RS applied as mulch.

Despite the absence of significant differences between the treatments, it is important to highlight that *Penicillium* and *Edenia* presented higher RA values in RS-B (0.69% and 0.58% respectively) than in the other treatments (0.17% and 0.17%, respectively). Interestingly, *Sarocladium* had a significantly higher RA in the RS-MMO than in the RS-B treatment (0.29% versus 0.02% for RS-MMO and RS-B respectively; $P = 0.0018$) (Online Resource 15). Unfortunately, the majority (92.5%) of the fungal OTUs could not be assigned at genus level (Online Resource 16).

3.7. Effect of RS management on the core fungome of the rhizosphere at flowering time

The minimum community of fungal OTUS shared by all four treatments, was analyzed using MetaCoMET in order to explore the 'core' fungome. The core contained 204 OTUs, corresponding to 21.9% of the whole fungome. Remarkably, 42% of the sequences fell into unassigned fungi. *Ascomycota* was the most dominant phylum, with 57% of relative abundance. At order level, *Sordariales* and *Pleosporales* dominated the core, with 30% and 4% RA across treatments, respectively. A heatmap of the core was produced, clearly indicating that all non-burn RS treatments clustered together at order level (Online Resource 17), thus coining the burn (RS-B) treatment as a unique cluster. Besides, the inoculated treatments formed a specific cluster (RS-MMO and RS-IMO) (Online Resource 17), based on the relative abundance of some of the dominant taxa. For instance, the RA values of the *Sordariales* were higher in these two treatments (34.9%) than in RS-M and RS-B (31.0% and 19.8%, respectively), whereas the *Sebacinales* presented lower RA (0.087%) in the treatments with microorganisms (RS-MMO and RS IMO) than in RS-M and RS-B (having 0.05 and 0.01%, respectively).

3.8. Prediction of fungal function using FUNGuild

The application of FUNGuild showed that only 10.7% of the total fungal OTUs could be associated with the key trophic modes pathotroph, saprotroph and symbiotroph, and their combinations (Fig. 4). Totals of 22 functional guilds were identified, most of them affiliated with undefined saprotrophic (74.8% of relative abundance), dung-saprotroph-plant-saprotroph (12.5%) and plant pathogenic (5.4%) forms. The other predicted functional guilds occurred at abundances below 1%. These were dung saprotroph, animal pathogen, orchid mycorrhiza, ectomycorrhiza, endophyte, wood saprotroph, endomycorrhiza, bryophyte and epiphyte functional guilds, among others. No statistically significant differences between the treatments were found for any of the aforementioned functional guilds (data not shown).

4. Discussion

RS incorporated into soil can establish a hot spot of microbial activity. Another such hot spot of microbial activity is provided by the rhizosphere, as a consequence of the carbon allocation into the soil through rhizodeposition. Here, we analyzed the effects of contrasting rice straw based soil pretreatments, including RS-MMO, RS-IMO, RS-M and RS-B, on rice crop performance, soil status and the structures and predicted functions of the rice-associated bacterial and fungal microbiomes at flowering stage.

With respect to the effects on the soil, RS is known to contain nutrients that can be beneficial to soil fertility and crop yield (Zhao et al., 2019). Here, the increase in SOM, of around 58% on average, across the board, indicates a high input of carbon irrespective of RS treatment. The lack of differences of the mulching and RS-IMO treatments with the RS burning treatment was surprising. Clearly, the incorporation of ashes and partially-burned materials (RS-B treatment) gave a contribution to the organic carbon in the soil comparable to that of the incorporation treatments (Chivenge et al., 2020). Weighed against the SOM that was already present in the field soil, as a consequence of continuous rice production practices, the addition of the RS by either means had a very low impact on the differences of SOM between treatments. In previous work, Zhu et al. (2015), found that incorporation of the whole RS into the soil (taken from a previous field with a production of around 9 ton of $RS{\cdot}ha^{-1}$) does not produce a significant increase of total SOC, dissolved OC, or microbial carbon, as compared to no incorporation of RS (Zhu et al., 2015). In contrast, the incorporation of 50% of RS gave a significant increase in all variables mentioned above Zhu et al. (2015). Thus, RS return at 100% may interfere with microbial decomposition activity by blocking the interchange of gases (Zhu et al., 2015). Besides, the enhancement of SOC across all treatments may be consistent with an expected low degradation rate of the RS (~13.9% per month) as a result of its high C:N ratio (~35–61) (Chivenge et al., 2020).

The lack of significant differences in rice crop yield between the RS treatments, as found in this study, was consistent with results obtained by Zhu et al. (2015). They evaluated the annual yields over 2 years in a rice-wheat rotation system, with a straw return rate of 100% (contrary to a yield increase at 50% straw return rate). However, our finding of the one ton yield reduction in the RS-M treatment, as compared to the other treatments, was striking. Rice grain yield is highly dependent on the number of panicle-bearing tillers produced per plant, and clearly the RS-M treatment showed the lowest number of panicles. The effect may be related to the fact that the RS-M treatment had the lowest soil pH, the lowest Ca and the highest Al levels. According to Alam, (1981), soil pH has a significant effect on the growth of rice. Also, at low Ca levels, ion transport may be impaired, increasing root membrane damage, and promoting the loss of nutrients. Besides, in soils with $pH < 5.0$ toxic forms of Al are released, causing both damage and growth inhibition of the roots (Liang et al., 2013; Panhwar et al., 2014). Clearly, leaving RS as mulch, at least under the experimental conditions evaluated in this study, can pose a problem to rice production, associated with a potentially problematic drop in soil pH.

Burkholderia glumae can cause bacterial panicle blight in rice, which may reduce crop yield up to 75%. Interestingly, this pathogen was not detected in the rice crop across the treatments, in spite of the fact it was the basis of the burn treatment before field preparation for next crop. Possibly, our cropping in the middle of the year, under climatic conditions not favorable for *B. glumae*, was the underlying reason for this lack of *B. glumae* detection. In contrast, the finding that *Helmintosporium* was significantly higher in the *M* treatments irrespective of the presence of the microbial inoculum, suggests that RS mulching favors the presence of this fungus. Contrary, the lower incidence of *Helmintosporium* in the RS-IMO treatment suggested that the action of the microbial inoculum used in this treatment was favored by the incorporation of RS in the soil. RS-IMO showed even better results than burning in terms of disease incidence reduction. On the downside, it incited a higher incidence of *R. solani* in comparison to the RS-B and RS-MMO treatments, suggesting that incorporation of RS favor the incidence of this fungus in rice plants. Overall, the lack of distinctive patterns in phytosanitary status of the rice crop across treatments, in particular the RS incorporation versus burning, suggested the absence of a predicted 'cleaning' effect of burning. Lanoiselet et al. (2005), in a lab experiment, demonstrated that RS burning can kill *Rhizoctonia* ($\geq$121 °C) or inhibit its development ($\leq$ 110 °C). Possibly, the burning treatment reduced the pathogen inoculum level in the soil in the form of dormant sclerotia, but did not completely eradicate it. Moreover, the effect of burning is unpredictable, as it is dependent on intensity, being influenced by the amount, moisture content and quality of the RS, next to wind velocity and burn duration and temperature (Lanoiselet et al., 2005). On the other hand, consolidation of pathogen inoculum from RS to the next crop cycle also depends on the concentration of the pathogen (Zhu et al., 2014).

Our field data showed, remarkably, that both the rice-associated bacteriomes and fungomes at rice flowering stage were – to variable extents – affected by the soil treatments, even though rather similar bacteriome and fungome alpha diversities were found across treatments. The latter is consistent with the overriding effect that the rhizosphere exerts on the local microbial communities, as compared to the

corresponding bulk soil (Edwards et al., 2018). This rhizosphere effect is generated by the strong effects of compounds in root exudates, which are characteristic of each plant type across the cultivation stage, acting to recruit specific bacteria and fungi. A representative microbiome for each plant/condition may ensue, reflecting direct effects of the soil next to direct and indirect ones of the plants. Clearly, an overall rich and similar diversity of niches was offered in the different treatments, resulting in similar overall α-diversities. As, across treatments, only one rice cultivar was used and sampling was similar across treatments, differentiating factors like different rhizocompartments (rhizosphere, rhizoplane, endosphere) (Edwards et al., 2015), cultivar, fertilization regime (Zhan et al., 2018) and plant growth stage (Edwards et al., 2018), were not examined in this study.

The β-diversity (as assessed by either PCoA (Fig. 1a), hierarchical clustering (using Bray-Curtis dissimilarity matrix; Online Resource 4), and core microbiome analyses (Online Resource 17), consistently revealed a dichotomy across treatments into two groups, i.e. (1) the *mulching* (*M*) and (2) the *early incorporation* (*EI*) treatments. This suggests that the strategy of application of RS as either a mulch, or via early incorporation, is a major factor contributing to the assembly of bacteriomes in the rhizosphere of subsequently grown rice. Probably, one major driver of this difference is the soil pH, which showed a drop (mainly in the M group) that tends to favor acidophilic bacteria (e.g the Acidobacterium *Bryobacter* in the *M* group). This finding is important, as the effect was observed at the rice flowering stage, thus suggesting that the composition of the rice-associated bacteriome is 'set' by agronomical measures taken several weeks before. Whether or not such changes are beneficial for the plant is difficult to say at this stage, as no significant differences were observed in terms of rice productivity or soil physicochemical attributes, between the two treatment groups. However, given the effect of treatment on soil pH that selected particular *Acidobacteria*, and the decreased rice productivity, leaving RS as a mulch may not be the best option to process RS. Our findings indicate that more fine-tuned measures that counterac`t the production losses may be necessary in the quest for better and more productive cropping practices (Chivenge et al., 2020). Thus, nutrients that accumulated should become more available over time. Similarly, effects on soil structure, organic matter, enzymatic and soil biological activity, in addition to the soil conditioner, take time to become manifest in rice yields (Zhao et al., 2019).

The dominance of particular bacterial phyla as found in our study (i.e. *Proteobacteria*, *Bacteroidetes* and *Firmicutes*; Online Resource 5) is consistent with that found in previous studies, in rice and other plants. Such phyla, since long, are considered to be common inhabitants of soil (Edwards et al., 2015, 2018; Guo et al., 2020). The clear dichotomy between the *M* and *EI* treatments was a major finding; it was supported by the differential abundances of *Proteobacteria*, *Epsilonbacteraeota* and *Bacteroidetes* (more abundant in the *EI* group) and *Acidobacteria* (most abundant in the *M* group) (Fig. 2). Possibly, the former effect is connected to the higher overall degradation activity, as predicted by PICRUSt2 in the *EI* treatments. However, this method for prediction of bacteriome functions requires caution in the analysis of the results generated, because it depends on the availability of annotated reference genomes in a particular database, and can be strongly and differentially affected by horizontal gene transfers across the genomes of the members of a microbial community (Jiménez et al., 2014). Despite such considerations, in particular *Bacteroidetes* spp. have been associated with lignocellulose degradation (Jiménez et al., 2014), their genomes having high levels of genes encoding enzymes for cellulose and hemicellulose bioconversion, suggesting a role of this group in the biodegradation process in the *EI* group. However, further analyses should be done to identify those kinds of correlations.

GammaProteobacteria was the most abundant proteobacterial class found across all treatments. The abundance of this group is often related to (increases of) the carbon-to-nitrogen (C:N) ratio in soil (Hermans et al., 2017). A possible connection to the increase of low-molecular-weight (MW) carbon, found in SOM decomposition and in root exudates, has been presumed (Cleveland et al., 2007). Similarly, many *Bacteroidetes* and *Bacillus* types are fast-growing organisms involved in polysaccharide degradations (Cleveland et al., 2007; van der Lelie et al., 2012).

It has been shown (Cleveland et al., 2007; Fierer et al., 2007), that (oligotrophic) *Acidobacteria* dominate in nutrient-scarce environments, whereas (copiotrophic) *GammaProteobacteria* dominate in systems with a high abundance of low-MW compounds. Thus the amount of labile carbon present in a system may be a key driver of the abundance of oligotrophic versus copiotrophic bacteria (Fierer et al., 2007). In this context, the significantly higher abundances of *GammaProteobacteria* and *Bacteroidetes* in the *EI* versus the *M* treatments, in contrast with the significantly higher abundance of *Acidobacteria* in the *M* treatments, indicate a differential availability of low-MW carbonaceous compounds across these treatment groups, thus indicating differences in the soil carbon metabolisms. Such differences may be driven either by a differential effect of the treatments on SOM decomposition or plant rhizodeposition or soil physical chemical status. For instance, several authors reported strong negative correlations between soil pH and the abundance of *Acidobacteria* subgroup 1 in soil, with optimum pH values between 4.0 and 5.5. This is consistent with our observation in the RS-M treatment (revealing the lowest soil pH at the end of the crop cycle), with the highest abundance of this subgroup.

Bryobacter has previously been shown to be a common inhabitant of rice paddy soils (Chen et al., 2015). In our study, it showed significantly higher abundances in the *M* than in the *EI* treatments. Hence, it may be one of the acidobacterial genera that is sensitive to the application of RS to the soil. On the other hand, *Micromonospora* is an Actinobacterium involved in the degradation of various recalcitrant materials. In association with plant roots, it stands out with functional traits such as nitrogen fixation and production of bioactive compounds (Trujillo et al., 2015). The higher abundances in the *M* treatments of these two genera indicate a particular role in the turnover of SOM at flowering, probably due to its later incorporation to the soil in comparison to *EI* treatments.

With respect to the fungomes, lower diversity levels were found across the treatments in relation to bacteria (Table 3), which is consistent with findings by (Guo et al., 2020). Fungi may be less competitive than bacteria in wet soils, and hence the inundation may have limited the fungal diversity values (Tian et al., 2013). On the other hand, there was a high percentage of unidentified OTUs in the core fungome, which is consistent with the fact that low percentages (<5% up to 2007) of fungi have as-yet been described (Mueller and Schmit, 2007). The finding of *Ascomycota* as the most abundant phylum across treatments confirmed results by Nie et al. (2018) and Guo et al. (2020) in paddy soils. However, contrary to these studies, we did not find an increase in *Basidiomycota* as a response to the incorporation of RS into the soil. Several members of the *Ascomycota* are recognized by their capabilities of cellulose and hemicellulose degradation, enabling them to assimilate carbon from RS, particularly at late decomposition stages (Guo et al., 2020). Included in the core fungome were orders such as *Sordariales*, considered to be primary decomposers of RS (Alberto et al., 2015) and *Pleosporales,* which was previously reported to increase at rice flowering stage due to capabilities to use carbon sources from the roots. Also, both taxa have been reported as producers of cellobiohydrolases as a response of high input of fresh carbon. Other fungi included in the core were *Sebacinales* that encompass mainly ecto- and endomycorrhizae and endophytes, and are recognized as plant growth promoters and inducers of stress tolerance.

The hierarchical cluster analysis performed on the core fungomes (Online Resource 17), led us to hypothesize that the presence of both the microbial inoculum and RS contributes to the shaping of the structure of the fungomes associated with roots of the subsequent rice crop. This may be due to either an acceleration of the breakdown of the lignocellulosic material, favoring other filamentous fungi or bacteria that may be the main decomposers in the system (Anasontzis et al., 2017), or to an effect on microbial antagonists that inhibit the establishment of a beneficial

fungal or bacterial community. This leaves aside the effect of the burning treatment, that may kill heat-sensitive fungi altering the structure of fungome.

At genus level, the commonly beneficial fungal genus *Trichoderma* was not found to be abundant, even in the treatments where three *Trichoderma* spp. were applied in the inoculant mix. This suggests that, despite its effect on shaping the fungal community, the inoculum levels decreased over time, to low levels, possibly below the detection limit of the DNA-based approach used in this study.

Remarkably, by using FunGuild, only a fraction (14.2%) of the fungal community could be placed within a particular trophic mode, leaving about 85% of the sequence reads unassigned. In contrast, Nie et al. (2018) showed around 74% of FunGuild-based functional assignments in paddy soils with different fertilization regimes. We surmised that a high number of diverse and as-yet-unassigned fungal taxa was present in the paddy soils under study. Most assigned OTUs belonged to saprotrophic fungi in all treatments, being slightly raised in the mulch treatments. Saprotrophic fungi contribute to carbon mineralization in soil, as they are effective decomposers of organic matter and soil carbon mineralizers (Tung et al., 2016).

5. Conclusions

Overall, our findings clearly reveal that the mode of prior RS incorporation into rice paddy soil governs the assembly of microbiomes of the rice rhizosphere at flowering stage. The *M* treatments had a strong effect on the rice-associated bacteriome, which was linked to the soil pH. On the other hand, the rice-associated fungome was affected by both the addition of RS and application of the microbial inoculum. The lack of significant differences between the RS treatments, in terms of crop yield and soil carbon, is consistent with the tenet that a too high return rate of RS to a highly productive rice field (9 t·ha^{-1} or above, as described by Zhu et al. (2015)), may be detrimental even in the incorporation of partially burned straw and ashes of the RS-B treatment, as it interferes with microbial activity and therefore the availability of C. Besides, the effects of incorporation of RS into soil may take time to emerge, potentially being more prominent in long-term applications.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Raw data are in NCBI repository.

Code availability

The software applied here were: R®, Microbiomeanalyst and MetaCoMet.

Authors' contribution

All authors contributed to the study conception and design. Material preparation, and data collection was performed by V O-J; analysis of the data was performed by VO-J and DU-V. The first draft of the manuscript was written by V O-J and all authors contributed to the editing of the manuscript. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare there are no competing interests.

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