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Combined application of N-fixing PGPB and rice straw mulch compensates N immobilization by straw, improving crop growth

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Abstract

Background Rice straw, often treated as waste, provides many benefits to crops when used as a mulch. However, straw degradation promotes nitrogen immobilisation due to its high C:N ratio, causing N competition between soil microorganisms and the crop. Currently, sustainable practices to remedy nitrogen immobilisation are hardly being implemented. In microcosm conditions we assessed whether the inoculation with N-fixers could offset the transient nitrogen deficiency caused by straw mulch, thereby harnessing the benefits of straw while mitigating its negative impact on nitrogen depletion and exerting a synergistic effect on crop growth.

Results Inoculation with N-fixers increased the nitrogen content in the soil (the increase ranged from 14% up to 90% for NH_4^+ and from 20% to 60% for NO_3^-) and, in most cases, also the nitrogen content in the plant (ranging from 10% to 15% increase), compared to the non-inoculated control. Therefore, inoculation would compensate for the lack of nitrogen caused by nitrogen immobilisation, and this resulted in an increased biomass production by the crop compared with the uninoculated control (the increase ranged from 25% to 85%). In addition, inoculation with N-fixers did not lead to a permanent change in the bacterial community composition, whereas straw addition increased the biodiversity of the soil microbiome.

Conclusions The results obtained in microcosm conditions are a first indication that complementing straw mulching with the inoculation of N-fixers could avoid the transient N immobilisation produced during straw degradation. Thus, the benefits of the combination would be a yield increase, while improving the biodiversity of the soil microbiome, stabilising soil temperatures and increasing water soil content.

Keywords Plant growth-promoting bacteria, Sustainable fertilisation, Bacterial biodiversity, *Azotobacter chroococcum*, *Azotobacter salinestris*, *Azospirillum brasilense*, Soil DNA sequencing

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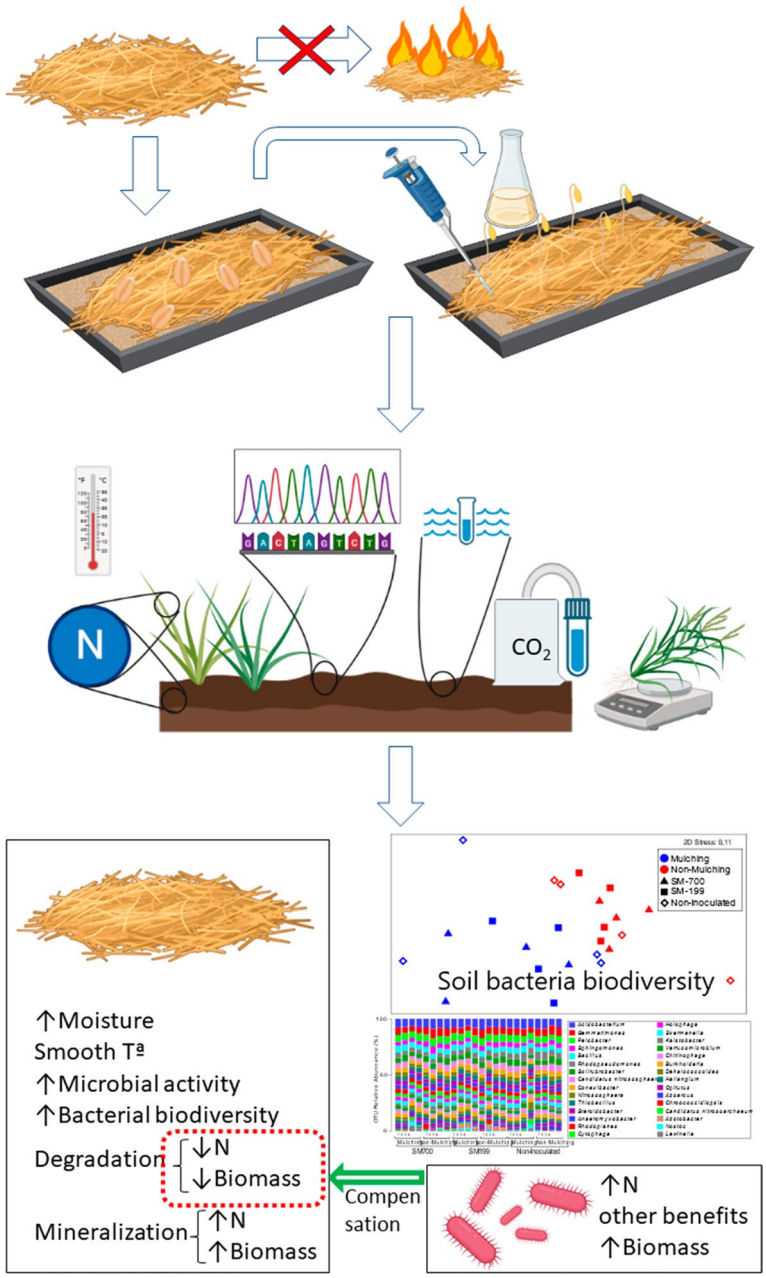
Graphical Abstract



Background

Soil erosion and drought are major environmental and agricultural threats worldwide, especially in the Mediterranean climate region [1, 2]. An efficient agronomic practice to reduce soil erosion and increase water availability is the use of mulch in crops. Mulching decreases runoff rates and increases water uptake and storage by improving infiltration capacity and reducing incident solar radiation and water evaporation from the soil. In addition, mulch increases crop performance, regulates soil temperature, enhances soil structure and organic content, and reduces weed infestation [3–5]. Among the different types of mulch, straw mulch is one of the

most beneficial and adds nutrients to the soil when decomposed [4, 6, 7].

The use of straw as a mulch offers an attractive and environmentally friendly option in cereal-producing areas [5]. In addition, straw is often treated as a residue and burned,thus, the use of straw as a mulch avoids its burning and the consequent emission of aerosols, greenhouse gases and loss of nutrients [8, 9]. A collateral effect of the use of mulching straw is the temporary immobilisation of nitrogen (N) during its decomposition due to its low N content [10]. In the long term, this immobilisation is beneficial because it provides a reserve of N and prevents the loss of excess N. However, excessive N immobilisation leads to competition for this nutrient with plants and deficiencies in the crop, requiring an external N supply [11, 12].

The use of plant growth-promoting bacteria (PGPB) in agriculture has increased in recent years, partially replacing chemical fertilisers. PGPB acts fixing N_2 from atmosphere, solubilising or mineralising phosphate, potassium or iron, enhancing nutrient uptake or producing numerous plant growth regulators [13, 14]. In addition, PGPB protects plants from pathogens and can multiply and participate in nutrient cycling [13, 15]. A concern regarding the use of bacterial inoculants is the possible impact that they can exert on the original soil microbial community, although this has not been sufficiently studied [16].

The effects of combining straw mulch and PGPB on crops have been little explored until now. The starting hypothesis of this study is that the combination of two components: (i) rice straw mulch and (ii) N-fixing PGPB strains compensate for the transient lack of N available due to N immobilisation caused by the straw decomposition process, while improving the agronomic and environmental performance as a consequence of the individual and combined effects of both components. With this purpose, in this study we combined PGPBs isolated from citrus crop in the Valencian Community (eastern Spain) with rice straw mulch also from the Valencian community. The agronomic objective of this work was to evaluate the effect in the performance of rye grass in microcosm conditions and to unravel the role of the inoculated N-fixers in the soil N content and in the N assimilation by the crop. The environmental objective was to evaluate the effect in the soil microbiome including microbial activity, biodiversity and composition. The parameters evaluated in the microcosm trial were the crop growth, the nutrients content in the soil and in the crop biomass, the impact on the soil water content and temperature, and on the microbial activity and native soil microbial community composition over a period of 1 year.

Methods

Bacterial strains used, characterisation of the novel strains and bacterial culture media

The strains selected for this work were as follows: *Azotobacter chroococcum* strains SM199, SM232 and SM700 and *Azotobacter salinestris* strain SM662, which were isolated from citrus crop soils located in the Valencian Community (eastern Spain) and cited in this work for the first time; and *Azospirillum brasilense* strain Az39 provided by the structure, dynamics and function of Rhizobacterial Genomes group of the Estación Experimental del Zaidín (EEZ-CSIC). Strains SM199, SM232, SM700 and SM662 belonged to the IQUIMAB bacterial collection (University of León, Spain).

The Az39 strain was previously characterised (Accession no. MT212725, GenBank; [17]), while the *Azotobacter* strains were selected from among other isolates based on the best results in terms of N-fixation and siderophore and indole-3-acetic acid (IAA) production. For the taxonomic identification of *Azotobacter* strains, amplification and sequencing of the 16S rRNA gene was performed by Macrogen (The Netherlands) using the primers and conditions previously described by Marcano et al. [18]. The obtained sequences were processed and deposited at GenBank Benson et al. [19] (accession no. OR354397, *Azotobacter chroococcum* SM199; OR354395, *Azotobacter chroococcum* SM232; OR354403, *Azotobacter salinestris* SM662; OR354396, *Azotobacter chroococcum* SM700) and compared with those from the EzTaxon-e server, which contains the type strains of all described bacterial species Kim et al. [20].

For the isolation and maintenance of the *Azotobacter* and *Azospirillum* strains, Ashby-mannitol and Congo Red media were used, respectively. The Ashby media (1 L water) contained mannitol (5 g), K2HPO4 (0.2 g), $MgSO_4.7H_2O$ (0.2 g), CaSO4 (0.1 g), NaCl (0.2 g) and $CaCO_3$ (0.1 g). The Congo Red media (1 L water) contained DL-malic acid (5 g), KOH (4.8 g), K_2HPO_4 (0.5 g), $MgSO_4.7H_2O$ (0.2 g), NaCl (0.1 g), yeast extract (0.5 g), and $FeCl_3.6\ H_2O$ (0.015 g) and Red Congo solution (0.25%; 15 mL).

For the inoculum production used in the microcosm assay, the culture media consisted of a 2.3% (v:v) molasses medium to recycle a waste product. The characteristics of the molasses, provided by Azucarera Española Ebro (Toro, Zamora, Spain), are shown in Additional file 1: Table SX. Fermentation conditions were: 30 °C, pH 7, 120 rpm, aeration of 3 L min^{-1} and addition of 0.5% (v:v) of primary inoculum.

Determination of PGP traits in vitro

Determination of in vitro N-fixation capacity

The N-fixation capacity of the selected strains was determined by the acetylene reduction assay (ARA) following Habibi et al. [21]. The concentration of ethylene in the vial was determined using an HP-5890 gas chromatograph (Hewlett Packard, Wilmington, DE) equipped with a Poropak R 80/100 mesh column and an FID detector.

Indole-3-acetic acid (IAA) production

The different strains were grown in Burk medium supplemented with 0.2 g L^{-1} of l-tryptophan, and the IAA production rate was determined as described in Mulas [22].

Siderophore production

Siderophore production was estimated following Alexander and Zuberer [23] and as described by Marcano et al. [18]. The formation of an orange zone around the bacterial colony indicated that the isolate produced siderophores. The siderophore production capacity of each isolate was evaluated by measuring the size of the halozone.

Microcosm assay

To evaluate the effect of the combination of rice straw mulch inoculated with the different strains, a microcosm assay was conducted in 54 × 39 cm trays. The assays were carried out in a completely randomised design with three replicates and two factors. One factor was mulching with two alternatives: soil with rice straw and naked soil control. The other factor was the inoculation with six options: inoculation with strains SM199, SM232, SM662 or SM700 of *Azotobacter*, inoculation with Az39 of *A. brasilense*, or non-inoculated control (Additional file 1: Table S1).

The microcosm assay was initiated in September 2020 (Fig. 1). The trays were filled with soil (loam texture, pH 8.0, M.O. 5.5%, assimilable P (Olsen) low < 8 mg kg^{-1}, K 0.22 cmol(+) kg^{-1}) mixed with vermiculite in a 3:1 ratio. Ryegrass (*Lolium multiflorum*) was planted in the trays, and 50 g of rice straw was spread on the soil surface in each tray of the mulched treatments (Fig. 1). When the ryegrass germinated, trays were inoculated at two different sites with 41 mL of inoculant containing 2×10^7 CFU/mL (Fig. 1).

Plants were grown in a greenhouse under natural temperature and light conditions, and the straw was replenished as it decomposed. Sampling was conducted in different months of 2021. The dependent variables analysed were as follows.

Crop biomass

Ryegrass plants growing within a radius of 7.5 cm from the inoculation point of each treatment were harvested at ground level in February, April and September 2021 (Fig. 1). Fresh weight and dry weight after drying at 70 °C for 3 days were measured.

Soil N content

The concentrations of NH_4^+ and NO_3^- in the soil were measured in April and June 2021 (Fig. 1). The NO_3^- content was determined in an extract obtained with a saturated $CaSO_4$ solution from the second derivative in the absorbance obtained at a wavelength between 219 and 225 nm using a UV–visible spectrophotometer (BECKMAN SU 640), according to the method described by Sempere et al. [24]. NH_4^+ was extracted by adding 2 M KCl (1:10 wt soil:vol KCl), followed by shaking for 30 min and filtering. The NH_4^+ of the filtrate was determined using a 781 pH/Ion Meter (Metrohm) consisting of an ion-selective electrode.

Plant N content

The N content in the aerial biomass of the ryegrass was determined using the Kjeldahl method (%) from the dry aerial part of the plant. The N plant content was measured in plants harvested during the September sampling (Fig. 1).

N derived from atmospheric N (Ndfa %)

To assess the N fixed by the N-fixing bacteria that were inoculated, the technique based on the natural abundance of ^{15}N from Unkovich and Baldock [25], as described by Pastor-Bueis et al. [26], was used. The non-inoculated control was used as a blank to exclude natural fixation due to pre-existing microbiota in the soil. Ndfa (%) was measured in plants harvested during the September sampling (Fig. 1).

Soil respiration

In the area where plant sampling was carried out, a soil respiration test was performed immediately after harvesting in April and September 2021 (Fig. 1). For this purpose, the static absorption method [27] modified by Alef [28] was used. Cylinders with a basal surface of 0.011 m^2 and a height of 23 cm were placed at a depth of 2 cm in the soil. Carbon dioxide efflux was collected in a beaker for reaction with 20 mL of 1 M NaOH for 24 h to avoid diurnal changes. Blanks consisted of a sealed chamber of the same volume, enclosing a beaker of 1 M NaOH. The sodium hydroxide solution was then precipitated by saturated $BaCl_2$ solution. The amount of CO_2 absorbed in 2 M NaOH was determined

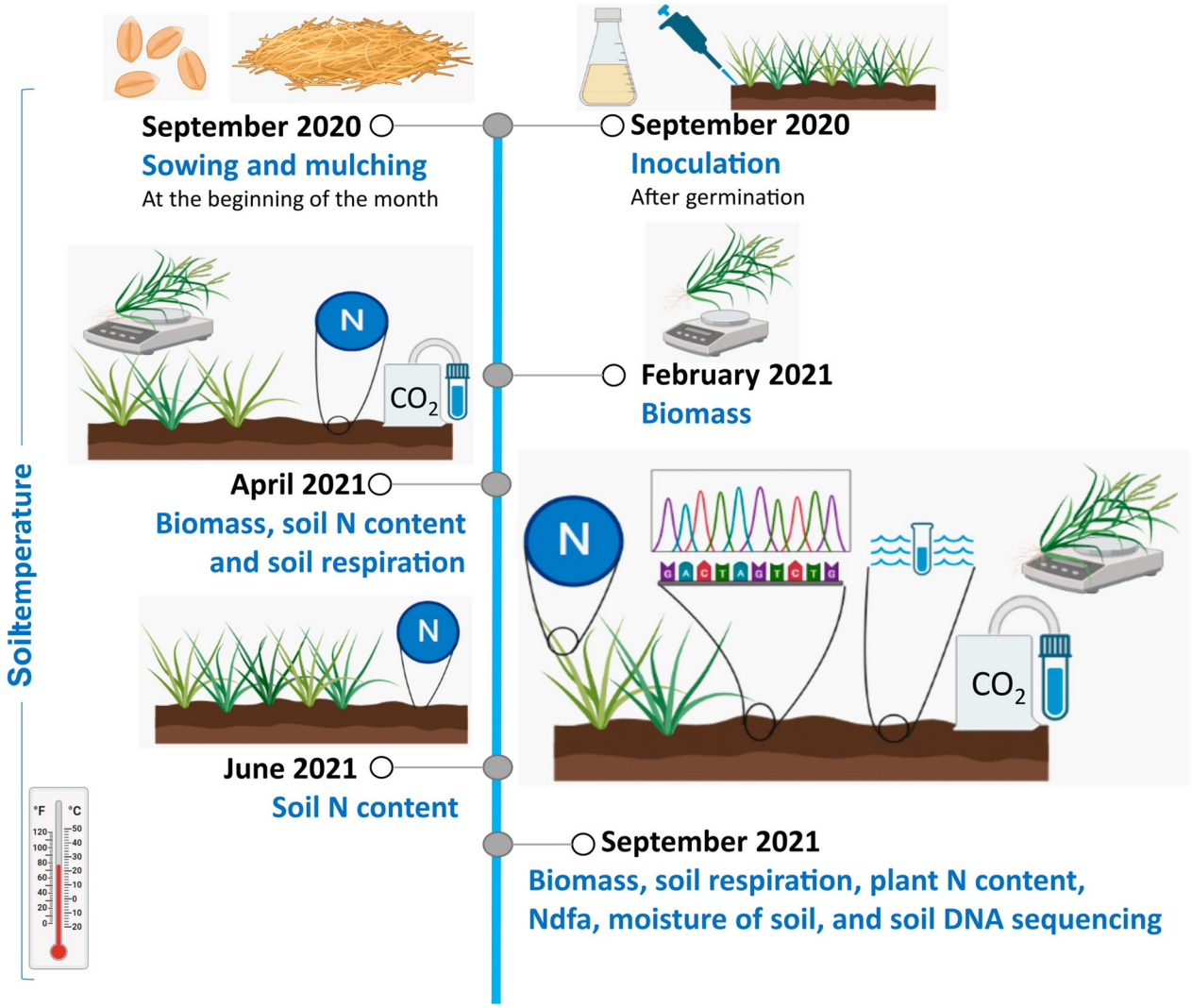


Fig. 1 Timeline summarising the different events performed in the microcosm assay. Ndfa, nitrogen derived from atmospheric

titrimetrically with a 0.5 M HCl solution using a phenolphthalein indicator. After titration, CO_2 efflux rates were calculated as CO_2–C with the following formula:

$$\mathrm{CCO_2}\left(\mathrm{mg\ C/m^{--2}day^{--1}}\right) = \frac{(\mathrm{B}-\mathrm{V})\cdot \mathrm{N}\cdot \mathrm{E}}{\mathrm{A}},$$

where B is the volume of HCl needed to titrate the NaOH solution from the control (Blank), V is the volume of HCl needed to titrate the NaOH solution in the beakers exposed to the soil atmosphere, $N = 1.0$ (molarity of HCl), E is the equivalent weight (6 for C; 22 for CO_2) and A is the area (m^2) of the chamber base.

Soil temperature

To assess the ability of mulch to regulate soil temperature, it was recorded from September 2020 to September 2021 (Fig. 1) in two replicates with mulch and two non-mulched treatments with a Testo 176 data logger thermometer. Mean, maximum and minimum daily temperatures were extracted and analysed.

Soil moisture

In September 2021 (Fig. 1), 10 g of soil from each replicate of the different treatments was collected and dried at 70 °C for 2 days. Then, each sample was reweighed, and the water content was calculated.

Statistical analysis

Analysis of variance (ANOVA) appropriate to a complete randomised design was performed. For the dependent variables temperature and soil moisture a one-way ANOVA was performed with the factor mulch as independent variable. For the dependent variable increase in

atmospheric N-fixation (ΔNdfa) the one-way ANOVA was performed with the factor inoculation as independent variable. For the rest dependent variables, a two-way ANOVA was performed with mulch and inoculation as factors. The software SPSS Statistics v.26.0 was used for all the analysis using the univariate or multivariate procedure as corresponded. The normality of standardised residuals was checked with Kolmogorov–Smirnov's test and homoscedasticity with Levene's test. The mean values of the dependent variables for the inoculated treatments were compared with the Dunnett test using the non-inoculated control treatment as a reference for comparison.

Bacterial community assessment

The effects of inoculation and mulching on different parameters of the soil bacterial community were evaluated. To evaluate the effect of inoculation and mulching on different parameters of the soil bacterial community, a massive sequencing of soil DNA was carried out for mulched and non-mulched treatments and for each of these treatments, those inoculated with SM700, SM199 and the non-inoculated control. The two strains were selected as representatives of the inoculated treatments, because SM700 and SM199 were the best nitrogen fixers according to the results of the ARA assay (Table 1). For each treatment combination, four replicates were sequenced. Soil samples for sequencing were taken at the end of the experiment in September 2021.

For this purpose, bacterial DNA was extracted using the DNeasy Power Soil kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. With the extracted DNA, paired-end amplicon sequencing of the 16S rRNA gene was then performed using the Illumina MiSeq high-throughput sequencing platform at Molecular Research DNA (MR DNA) (www.mrdnalab.com; Shallowater, TX, USA) (accessed April 5, 2022). The primer set used was 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGGGTWTCTAAT-3′), which are specific to the V4 region of 16S rRNA. Sequence data were processed using the MR DNA pipeline (MR DNA, Shallowater, TX, USA). Primers, short sequences < 150 bp and sequences with questionable base calls were removed. The remaining sequences quality filtered at Q25 quality with a maximum error threshold of 1.0, and subsequently dereplicated and denatured. A sequence elimination procedure was performed to remove sequences with PCR point errors, chimeric sequences and singletons to obtain denoised sequences or amplicon sequence variants (ASV). Taxonomy was then assigned using BLASTn against a database derived from the Ribosomal Database Project II (RDPII, http://rdp.cme.msu.edu, accessed 6 April 2022) and the National Centre for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov, accessed 6 April 2022) [29]. The raw data obtained from this analysis were deposited in the Sequence Read Archive (SRA) of the NCBI under nucleotide sequence accession number PRJNA1023497.

Once the relative abundances of the different ASVs were obtained, the analysis was performed using PRIMER version 7 software Clarke et al. [30]. A dissimilarity matrix was constructed using the Bray–Curtis index. From this matrix, a permutational multivariate analysis of variance (PERMANOVA) for 999 permutations was performed, with the mulching and inoculation treatments as fixed factors. To check whether the results observed in the PERMANOVA were due to differences in centroid location or dispersion, a PERMDISP analysis was performed for each factor. To graphically visualise the changes in the microbial community due to mulching and inoculation, nonmetric multidimensional scaling (NMDS) was performed.

The alpha diversity was measured using the Shannon index and the beta diversity of each treatment was estimated by extracting the dissimilarity values of the Bray–Curtis index between pairs of samples and computing the mean dissimilarity of the pairs of samples belonging to the same treatment [31]. The relative abundances of the *Azotobacter* genus were calculated for each treatment to check the permanence of the inoculum. The effect of

Table 1 Details of isolate, closest relative and physiological characteristics

Isolate name	Closest relative based on 16S rRNA gene sequence (percentage similarity)	ARA[a]	Siderophore production[b]	IAA production (μg ml^{-1})
SM199	*Azotobacter chroococcum* (100)	14,993	2.13	10.36
SM232	*Azotobacter chroococcum* (99.7)	6278	2.35	6.22
SM700	*Azotobacter chroococcum* (99.7)	13,571	2.75	12.88
SM662	*Azotobacter salinestris* (99.6)	3027	2.40	0.63

[a] Acetylene reduction assay (ARA). Values represent activity expressed as nmol ethylene h^{-1}

[b] Siderophore production. Units represent the size of the orange area (in mm) caused by the presence of siderophores

mulching and inoculation on alpha and beta diversity and *Azotobacter* relative abundance was assessed by PERMANOVA using 999 random permutations on the basis of Euclidean distances.

A Venn diagram was constructed to show the richness and exclusive genera present at each level of the analysed factors: mulching and inoculation. For this purpose, the genera present in more than one replicate of each treatment were extracted and the Venn diagram was generated with the web-based tool InteractiVenn [32].

Results

Genetic characterisation and physiological properties of selected isolates

Four isolates were selected for the 16S rRNA sequence analysis. The isolates were selected based on the ARA, siderophore production activity and IAA production activity. SM199 and SM700 showed the highest N-fixing activity values. All strains showed high siderophore production values, with SM700 being the major producer. As for IAA production activity, the highest values were again presented by SM199 and SM700 (Table 1). The closest relative based on its 16S rRNA sequence was *Azotobacter chroococcum* for isolates SM199, SM232 and SM700 and *Azotobacter salinestris* for isolate SM662 (Table 1).

Effect of inoculation and mulching on crop biomass

To determine the effect of inoculation and mulching on crop growth, ryegrass biomass production was evaluated using a microcosm assay at different seasons of the year. The two independent variables analysed, i.e. inoculation and mulch, produced significant changes in the dependent variables of biomass (Additional file 1: Table S2). However, there was no significant interaction between the factors inoculation and mulching (Additional file 1: Table S2), which indicates that the increase in crop biomass production as a result of inoculation occurs both in the presence and absence of mulch. Inoculation with the bacterial strains increased ryegrass biomass production with respect to the non-inoculated control in all seasons of the year, with the most significant increases in the February and April samplings (Table 2). *Azotobacter chroococcum* strain SM232 produced the greatest increase in ryegrass biomass (Table 2). For the mulching factor, the treatments with naked soil showed higher biomass than the mulched treatments in the samples collected in February and April. The mulched treatments showed a higher biomass in the last sampling carried out in September (Table 2).

N-fixers compensate for temporary N blocking

To evaluate the effect of mulching on temporary N immobilisation and the capacity of N-fixers to compensate for the possible lack of N in plants, the NH_4^+ and NO_3^- content in soil, the total N content in plants and increase in atmospheric N-fixation (ΔNdfa) were evaluated. In the first sampling carried out in April 2021, in general, in the treatments with mulch, the N concentration in the soil was lower, since although NH_4^+ content was slightly higher, such an increment did not compensate for the major reduction in NO_3^- content. However, in the June sampling, the presence of mulch did not alter the NH_4^+ content but strongly increased the NO_3^- content (Table 3). However, inoculation with the different strains increased the NH_4^+ and NO_3^- content of the soil in both samples (Table 3). Additionally, all strains showed a positive atmospheric N-fixation rate compared to the non-inoculated control, resulting in an increase in plant N content in all cases except for SM700 (Table 3).

Table 2 Mean values of the biomass ± SE produced by ryegrass plants grown in microcosm conditions at the indicated sampling dates for the factors inoculation and the presence of mulching

Factor	Treatment	February sampling		April sampling		September sampling	
		Fresh (g)	Dry (g)	Fresh (g)	Dry (g)	Fresh (g)	Dry (g)
Inoculation	Non-inoculated control	7.21 ± 0.43	2.32 ± 0.17	11.23 ± 0.52	3.47 ± 0.16	6.14 ± 0.33	1.7 ± 0.08
	Azotobacter chroococcum SM199	10.54 ± 0.36***	3.49 ± 0.14***	15.42 ± 1.09***	4.73 ± 0.33**	9.19 ± 0.7**	2.49 ± 0.22**
	Azotobacter chroococcum SM232	10.42 ± 0.65***	3.39 ± 0.18***	20.98 ± 0.87***	6.48 ± 0.28***	9.41 ± 0.73**	2.80 ± 0.2***
	Azotobacter salinestris SM662	9.13 ± 0.38**	2.91 ± 0.19 ns	18.40 ± 0.41***	5.71 ± 0.11***	7.8 ± 0.19 ns	2.05 ± 0.09 ns
	Azotobacter chroococcum SM700	9.14 ± 0.38**	2.97 ± 0.09*	14.32 ± 0.90**	4.44 ± 0.27**	7.94 ± 0.44 ns	2.26 ± 0.11**
	Azospirillum brasilense Az39	9.80 ± 0.82***	3.15 ± 0.29**	18.60 ± 0.60***	5.78 ± 0.17***	7.52 ± 1.2 ns	2.08 ± 0.26 ns
Mulch	Non-mulching control	10.13 ± 0.37	3.26 ± 0.12	17.21 ± 0.74	5.32 ± 0.23	7.18 ± 0.24	1.98 ± 0.09
	Mulching	8.62 ± 0.33***	2.81 ± 0.13**	15.77 ± 0.97**	4.88 ± 0.30*	8.82 ± 0.53**	2.47 ± 0.13***

Asterisks indicate the level of significance of the difference between the mean values of each treatment and its corresponding control, according to Dunnett's test. Significance levels: ***$p < 0.001$; **$0.001 < p < 0.01$; *$0.01 < p < 0.05$; ns, not significant.

Table 3 Mean values ± SE of the soil nitrogen content, ryegrass plant nitrogen content and increase in atmospheric N-fixation (ΔNdfa) obtained in microcosm conditions at the indicated sampling dates for the factors inoculation and the presence of mulching (in the case of ΔNdfa only for the factor inoculation)

Factor	Treatment	Soil nitrogen content				Plant nitrogen content		ΔNdfa	
		April sampling		June sampling		September sampling		September sampling	
		mg NH_4^+/g^{-1}	mg NO_3^-/g^{-1}	mg NH_4^+/g^{-1}	mg NO^{3-}/g^{-1}	(%)	(mg/plant)	(%)	
Inoculation	Non-inoculated control	0.0171 ± < 0.01	0.1487 ± 0.02	0.0149 ± < 0.01	0.1415 ± < 0.01	1.02 ± 0.11	17.34 ± 1.12	–	–
	Azotobacter chroococcum SM199	0.0211 ± < 0.01 ns	0.2176 ± 0.03***	0.0288 ± < 0.01***	0.2215 ± 0.01***	1.14 ± 0.14*	28.38 ± 1.38**	8.58	± 2.43 ns
	Azotobacter chroococcum SM232	0.0265 ± < 0.01***	0.1908 ± 0.02*	0.0276 ± < 0.01***	0.2146 ± 0.02***	1.18 ± 0.07**	33.04 ± 0.72***	7.03	± 3.86 ns
	Azotobacter salinestris SM662	0.0262 ± < 0.01***	0.2433 ± 0.02***	0.0254 ± < 0.01***	0.2296 ± 0.03***	0.84 ± 0.07**	17.22 ± 0.69 ns	2.33	± 0.80 ns
	Azotobacter chroococcum SM700	0.0196 ± < 0.01 ns	0.2090 ± 0.04**	0.0198 ± < 0.01*	0.1783 ± 0.02*	1.01 ± 0.11 ns	22.82 ± 1.13*	2.18	± 0.68 ns
	Azospirillum brasilense Az39	0.0242 ± < 0.01***	0.1778 ± 0.03 ns	0.0198 ± < 0.01*	0.1415 ± 0.01 ns	1.13 ± 0.18*	23.50 ± 1.86*	3.45	± 1.01 ns
Mulch	Non-mulching control	0.0214 ± < 0.01	0.2525 ± 0.01	0.0226 ± < 0.01	0.1548 ± < 0.01	1.30 ± 0.05	25.74 ± 0.53		
	Mulching	0.0234 ± < 0.01*	0.1432 ± 0.01***	0.0228 ± < 0.01 ns	0.2208 ± 0.01***	0.81 ± 0.03***	20 ± 0.32**		

Asterisks indicate the level of significance of the difference between the mean values of each treatment and its corresponding control, according to Dunnett's test. Significance levels: ***$p < 0.001$; **$0.001 < p < 0.01$; *$0.01 < p < 0.05$; ns, not significant.

Effects on soil respiration, temperature and moisture

The soil respiration rate was measured as an indicator of microbial activity to assess the effect of mulching and inoculation on soil microbial load. Both inoculation with each of the strains and mulching produced an increase in the soil respiration rate, which was statistically significant in most cases (Table 4).

The recording of average, maximum and minimum daily temperatures during different seasons of the year showed that mulching reduced maximum temperatures, especially during winter and spring. In addition, there was a tendency for higher minimum temperatures with mulching, but it was not significant; finally, there was no effect on the average daily temperature (Table 4; Fig. 2).

Furthermore, based on the soil water content data, the mulched soil showed a higher soil moisture retention capacity than the naked soil (Table 4).

Bacterial community composition in mulched and inoculated soil compared with controls

NMDS revealed clear differences in the bacterial community composition between the mulched and non-mulched treatments (Fig. 3a). However, NMDS showed no differences between the treatments inoculated with strains SM700 and SM199 and the non-inoculated control (Fig. 3a). The major community change by mulching and the lack of change by inoculation were confirmed by PERMANOVA analysis (PERMANOVA: $F_{Mulching} = 3.41$. $p_{Mulching} = 0.035$; $F_{Inoculation} = 1.10$; $p_{Inoculation} = 0.36$). In addition, the dispersion between samples was not significant for either factor (PERMDISP: $F_{Mulching} = 2.25$; $p_{Mulching} = 0.18$; $F_{Inoculation} = 1.48$; $p_{Inoculation} = 0.37$); therefore, the modification shown via PERMANOVA was due to the distance between centroids. However, most of the major genera were found in all treatments, with *Acidobacterium*, *Gemmatimona* and *Pelobacter* being the most abundant genera (Fig. 3b).

Alpha and beta diversity differed between treatments. Alpha diversity was higher for treatments inoculated with strains SM199 and SM700 compared to non-inoculated treatments and in mulched treatments compared to non-mulched treatments (Table 5; Fig. 4a). In addition, mulching increased beta biodiversity, while inoculation decreased it (Table 5; Fig. 4b). The increase in beta diversity with the addition of mulch was also evident in the increased dispersion of the mulched samples in

Table 4 Mean values ± SE of the respiration rate, temperature and soil water content obtained in microcosm conditions at the indicated sampling dates for the factors inoculation and the presence of mulching (in the case of temperature and soil water content only for the factor mulching)

Factor	Treatment	Soil respiration rate (mg $CO2\ m^{-2}\ h^{-1}$)		Temperature			Water content (%)
				Average	Maximum	Minimum	
		April sampling	September sampling	September2020	September 2021	Sampling	September sampling
Inoculation	Non-inoculated control	259.1 ± 15.1	181.28 ± 4.43				
	Azotobacter chroococcum SM199	298.6 ± 11.7 ns	196.12 ± 3.35 ns				
	Azotobacter chroococcum SM232	312.5 ± 10.8**	181.92 ± 9.92 ns				
	Azotobacter salinestris SM662	319.4 ± 4.8**	202.57 ± 9.87***				
	Azotobacter chroococcum SM700	309.7 ± 5.9*	192.25 ± 5.63 ns				
	Azospirillum brasilense Az39	306.9 ± 18.7*	189.02 ± 7.66 ns				
Mulch	Non-mulching control	291.4 ± 8.8	178.48 ± 3.31	14.82 ± 0.24	24.42 ± 0.34	8.40 ± 0.25	26.29 ± 0.22
	Mulching	310.7 ± 6.7*	202.57 ± 3.12***	14.87 ± 0.23 ns	22.51 ± 0.30***	8.95 ± 0.24 ns	24.89 ± 0.62*

Asterisks indicate the level of significance of the difference between the mean values of each treatment and its corresponding control, according to Dunnett's test. Significance levels: ***$p < 0.001$; **$0.001 < p < 0.01$; *$0.01 < p < 0.05$; ns, not significant.

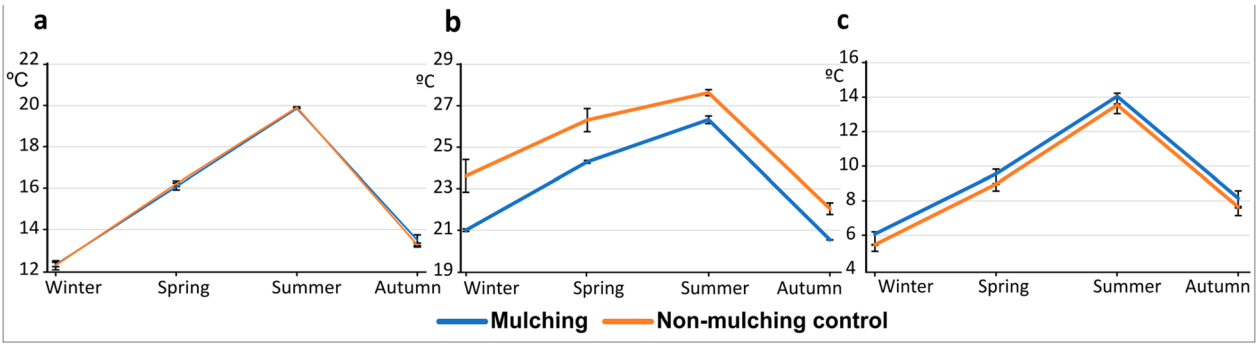


Fig. 2 Soil temperature of the microcosm assay at different seasons of the year, measured between September 2020 and September 2021. (**a**) Average daily mean temperatures. (**b**) Average daily maximum temperatures. (**c**) Average daily minimum temperatures

the NMDS (Fig. 3a). Genera richness, represented by the number of genera present in more than one replicate within each treatment, showed a pattern similar to alpha diversity. The inoculated and mulched treatments showed higher richness than the non-inoculated control and the non-mulched control, respectively (Fig. 4c). In addition, the SM700-inoculated treatments showed 16 exclusive genera, and the mulched treatments showed 38 genera (Fig. 4c).

Furthermore, the relative abundance of *Azotobacter* genus at the time of sampling, 8 months after inoculation, remained higher for the treatments inoculated with strain SM199, with 1.05% relative abundance, compared to the non-inoculated control (Table 5; Fig. 3b). However, it did not vary among treatments inoculated with strain SM700 with respect to the non-inoculated control or among mulched and non-mulched treatments (Table 5; Fig. 3b).

Discussion

The use of rice straw as mulch provides several advantages for crops since it reduces soil erosion, regulates soil temperature, retains moisture, allowing for reduced water use, improves soil structure, reduces weeds, and increases soil organic matter and nutrients [5, 33, 34]. However, the high C/N ratio of straw leads to the

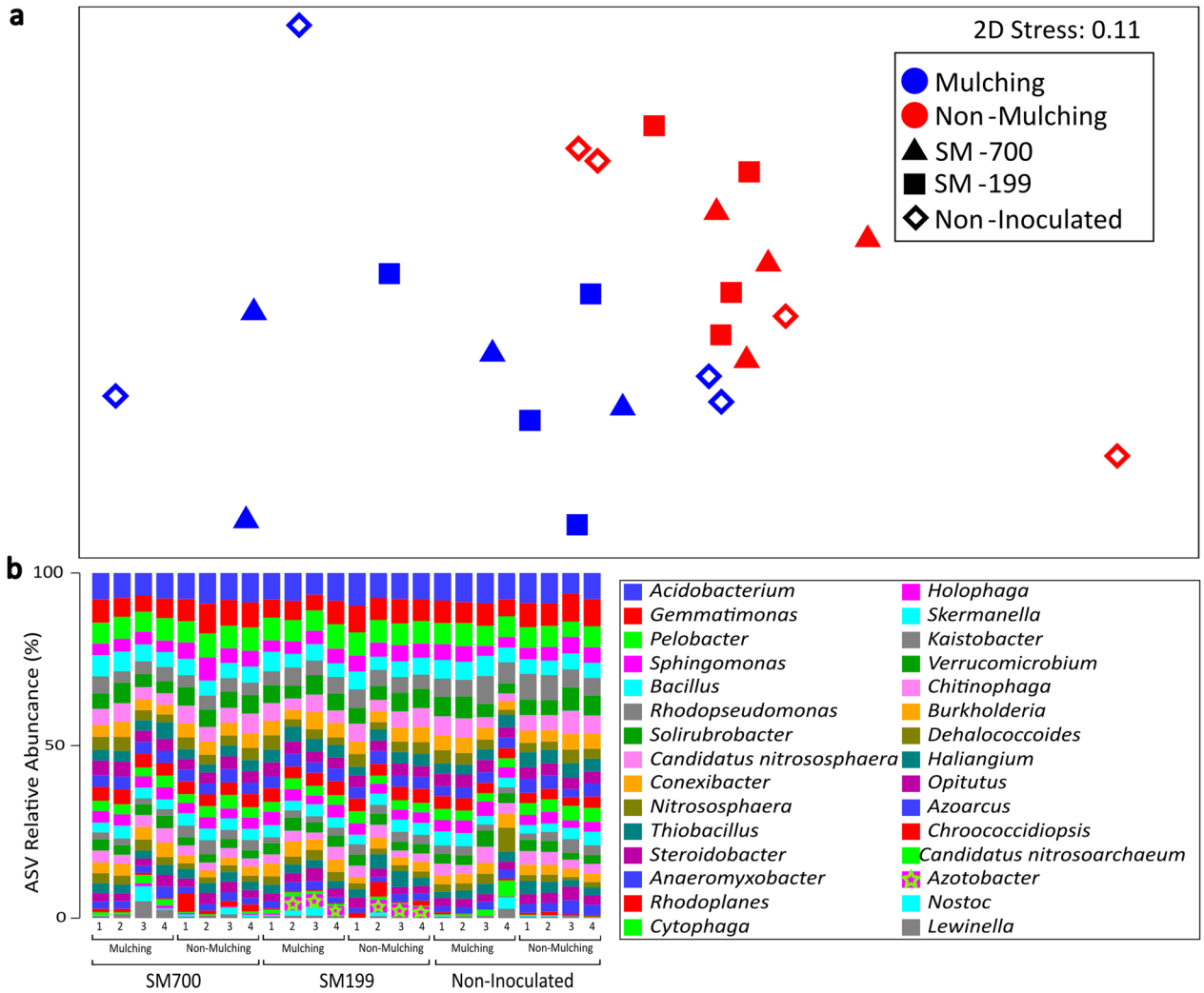


Fig. 3 Effect of mulching and inoculation on soil bacterial community. The soil sampling used for the analysis was carried out at the end of the experiment in September 2021. (**a**) Non-metric multidimensional scaling (NMDS) of bacterial community composition. (**b**) Relative abundance of the top 30 bacterial genera. The star marks the genus *Azotobacter* inoculated in the treatments. Mulching, mulched treatments; non-mulching, non-mulched control treatments; SM700 and SM199, treatments inoculated with *Azotobacter* strains SM700 and SM199, respectively; non-inoculated, non-inoculated control treatments

temporary immobilisation of N during its degradation [12]. We observed that the lack of N caused by immobilisation can be compensated for by inoculation with N-fixers, avoiding the need to supply chemical fertilisers. Moreover, inoculum production was based on molasses, a residue from the sugar production industry. Thus, the sustainable agricultural practice proposed in this work focuses on a circular economy model in which the benefits of mulching and inoculation with PGPB are exploited while valorising wastes, such as straw and molasses and reducing the use of chemical fertilisers.

In the first two harvests of February and April, mulching caused a reduction in ryegrass biomass production. These two harvests were conducted four and six months after straw mulching; thus, the decrease in biomass observed may have been caused by N immobilisation due to straw degradation [12]. The higher soil respiration in the treatments with mulch that we observed reflects the increased activity and growth of microorganisms that are performing straw degradation [35]. Straw degradation with a high amount of C due to its high C/N ratio promotes an increase in soil microbial biomass, which is typically limited by the lack of C [10, 36]. However, the lower soil N content in the mulched treatments noted in the April sampling showed N immobilisation in the early stages of straw degradation. Other research also reported

Table 5 Average alpha and beta diversity and relative abundance of *Azotobacter* genus in soil samples of microcosm assay and results of permutational multivariate analysis of variance (PERMANOVA)

Treatment		Alpha diversity Shannon	Beta diversity	Nº bacterial genera	Relative abundance Azotobacter
Non-inoculated control		4.59	18.64	443	4.93E-4
Azotobacter chroococcum SM199		4.71*	14.63*	451	1.05*
Azotobacter chroococcum SM700		4.77*	15.18*	472*	0.03
Non-mulching control		4.63	14.85	445	0.35
Mulching		4.75*	17.45*	474*	0.36
PERMANOVA/ANOVA (pseudo-F/F values and signif. level)		D.F			
Inoculation	2	9.63*	5.34*	9.48**	13.39***
Mulching	1	13.21*	5.76*	14.21***	4.16E-3 ns
Inoculation x mulching	6	1.84 ns	0.32 ns	0.60 ns	5.12E-3 ns

The soil sampling used for the analysis was carried out at the end of the experiment in September 2021. Treatments correspond to inoculations with the indicated strains and the mulching application. Asterisks indicate the level of significance between the different levels of the independent variable. For the independent variable inoculation; asterisks indicate the level of significance of the differences between each treatment and the non-inoculated control using a PERMANOVA pairwise test. Significance levels: ***$p < 0.001$; **$0.001 < p < 0.01$; *$0.01 < p < 0.05$; ns, not significant; D.F., degrees of freedom.

the highest N immobilisation in straw mulch soil in the first 4 months after straw application [12, 35]. Due to N immobilisation, there is N competition between microorganisms and plants, causing a loss of crop yield, as described by Kuzyakov and Xu [37].

However, in the September sampling of crop biomass, mulching had a positive effect on crop yield. The increase in biomass observed may be due to the mulch effect on temperature and moisture retention, as well as N mineralisation, which is achieved. In such a way, we have observed that mulch softens temperatures, especially maximum temperatures, by reducing direct radiation on the soil, as has already been described by Ramakrishna et al. [5]. Mulch from plant material reduces daytime temperatures and increases nighttime temperatures better than mulch from other materials, and this could explain the observed increase in crop biomass, as previously for crop yield by Noor et al. [34]. Additionally, we confirmed the ability of mulch to increase soil moisture retention, as observed by Ramakrishna et al. [5], and this could also improve crop growth. Thus, mulching, via the softening of temperatures and increased moisture retention, leads to improved crop yields during the summer months, as evidenced by the September harvest biomass. The biomass increase is also due to the more advanced decomposition phase of straw, whereby N immobilisation is overcome by mineralisation [12, 35]. In the June soil N content analysis after the first two harvests and prior to the September harvest, an increase in soil N content was observed, which could be provided by degraded straw. N from microbial biomass contributes to the primary source of potentially mineralisable N in the soil and in limited N because soil microorganisms produce certain depolymerising extracellular enzymes to obtain N from soil organic matter [36, 37]. Thus, the N immobilised by microorganisms during the first months of straw degradation is later mineralised and becomes available for the crop [35].

Therefore, with the application of straw mulch, the microbiota is used as an N reservoir by N immobilisation in the early stages of straw decomposition; later, this N will be available to the plant through mineralisation [35, 36]. N immobilisation avoids N loss in the soil and the consequent contamination of the hydrosphere with NO_3^- or the atmosphere with nitrous oxide (NO_2) [38]. However, excessive N immobilisation can lead to a decrease in crop yield, as observed in the first months of straw decomposition [11, 12]. When straw decomposition is more advanced, the immobilised N is mineralised, promoting an increase in crop yield,thus, an adequate straw mulch supply is necessary to promote a balance between immobilisation and mineralisation [34, 39]. In this experiment, the rate of straw replenishment was adequate since after the first stage of N immobilisation, the N input from the mineralisation of the older degraded straw was higher than the N immobilisation caused by the degradation of fresh straw.

Inoculation with either N-fixing strain tested in this study compensated for the crop yield loss caused by N immobilisation. According to Araujo et al. [40], the observed improvement in crop yield could mostly be driven by the increase in atmospheric N-fixation (ΔNdfa) observed for all inoculated strains. N-fixation led to an NH_4^+ and NO_3^- increase in the soil with either strain and increased plant N content in the strains with the highest ΔNdfa values (SM199, SM232 and Az39). Only

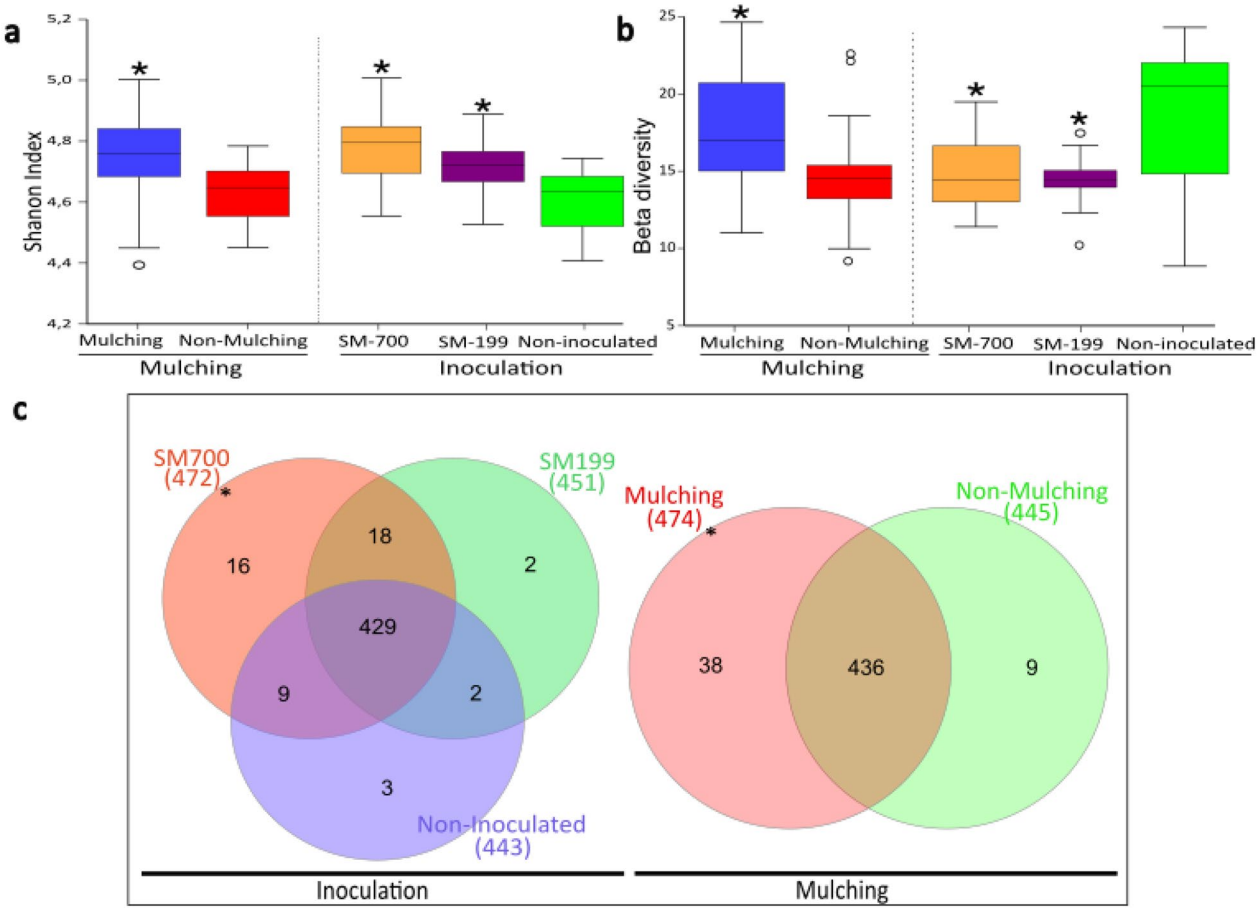


Fig. 4 Effect of mulching and inoculation on the bacterial biodiversity of soil. The soil sampling used for the analysis was carried out at the end of the experiment in September 2021. (**a**) Bacterial alpha diversity estimated by Shannon's diversity index. (**b**) Bacterial beta diversity was estimated by calculating the mean Bray–Curtis dissimilarity index between pairs of samples within each treatment. (**c**) Venn diagram showing the number of bacterial genera present in more than one sample in each treatment. The crossover zone indicates the bacterial genera crossed in different datasets. Mulching, mulched treatments; non-mulching, non-mulched control treatments; SM700 and SM199, treatments inoculated with *Azotobacter* strains SM700 and SM199, respectively; non-inoculated, non-inoculated control treatments. Asterisk indicates significant differences for $p < 0.05$ with respect to the corresponding control (non-mulching control or the non-inoculated control) according to the PERMANOVA test or the ANOVA pair-wise test

inoculation with strain SM662 decreased the plant N content despite being the strain that most increases soil NO_3^-; however, it was also the strain that most increased microbial activity, which could result in competition for N assimilation between the crop and other non-N-fixing bacteria enhanced by inoculation with SM662 [37].

Nevertheless, inoculation with any strain, including SM662, led to an increase in crop yield, partly because the strains tested had other PGPB characteristics, such as siderophore or IAA production, that further promoted crop production [41]. The use of N-fixers reduces the need for N fertilisers by up to 50%, because improves the nutrients use efficiency by the crop [13]. In addition, the genera *Azotobacter* and *Azospirillum* produce plant hormones that stimulate and facilitate plant growth and siderophores that chelate iron, molybdenum or vanadium and can be phosphate solubilisers. These genera are also associated with the suppression of pathogenic plant diseases [42, 43].

A disadvantage of inoculation with PGPB is the possible disruption of the pre-existing microbial community [44]. Microbial community composition is a key factor for correct soil performance, as microbial communities control nutrient cycling. However, metagenomic analysis performed one year after inoculation in treatments inoculated with SM700 and SM199 strains showed that the microbial community composition of the soil was similar to the non-inoculated control; therefore, the possible modification that these strains caused in the soil bacterial community was not permanent. The resilience

of the soil microbial community is governed by the physicochemical structure of the soil; thus, in a specific soil, the original bacterial structure and composition tend to recover, and the inoculated strains must compete with the pre-existing strains to remain in the soil [44, 45]. However, the immediate alteration after inoculation leads to an increase in alpha diversity, as minor taxa increase their relative abundance [44]. In addition, in the inoculated treatments, genera not detected in the non-inoculated treatments appeared, possibly due to the increase in N derived from atmospheric fixation conducted by the inoculated strains. This effect of N concentration on microbial diversity has been previously described [46, 47].

Straw mulch produced a prolonged modification in the structure and composition of the original bacterial community. This is partly because straw mulch modifies soil parameters, such as temperature, moisture and organic matter content, increasing biomass and microbial activity [34, 48, 49]. However, the modification is positive since it promotes an increase in alpha and beta biodiversity, mainly due to the increase in soil organic carbon, as straw provides carbon sources for soil microorganisms, thus increasing soil microbial diversity [49–51]. Furthermore, straw increases the variability of soil properties and composition, depending on the depth, which in turn produces a greater diversity of ecological niches available for microorganisms with different requirements [48]. In addition, straw contains new bacterial genera that are introduced into the soil, which would explain the higher richness of bacterial genera in the mulched treatments.

Finally, the relative abundance of *Azotobacter* remained high eight months after inoculation in treatments inoculated with strain SM199 but not in the SM700-inoculated treatments. However, after the initial stage of straw decomposition, N immobilisation decreases, making the beneficial effects of inoculated strains less necessary [12, 35].

Conclusions

Rice straw mulching benefits crops by retaining moisture, regulating temperature, and increasing microbial activity and biodiversity. However, during the early stages of straw degradation, N immobilisation is excessively high, causing a decrease in crop growth. Inoculation with N-fixers compensates for the lack of N during the immobilisation period and has other benefits, leading to an increase in crop yield without permanent modification of the original microbial community composition and structure and with an increase in alpha diversity. Therefore, the results obtained in microcosm condition are a first indication for beneficial effects of combined application of rice straw mulching with microbial N-fixers, although these effects need to be validated in field experiments in order to recommend this practice for improving sustainable crop production.

Abbreviations

N	Nitrogen
PGPB	Plant growth-promoting bacteria
IAA	Indole-3-acetic acid
ARA	Acetylene reduction assay
Ndfa(%)	N derived from atmospheric N
ANOVA	Analysis of variance
SRA	Sequence read archive
ASVs	Amplicon sequence variants
PERMANOVA	Permutational multivariate analysis of variance
NMDS	Nonmetric multidimensional scaling
NO_2	Nitrous oxide
ΔNdfa	Increase in atmospheric N-fixation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00555-3.

Additional file 1. **Supplementary tables.**

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Author contributions
IMA: data curation, investigation, writing—original draft, writing—review and editing, formal analysis; SS: data curation, investigation; JHC: data curation, investigation; NOL: data curation, investigation; PLB: conceptualization, funding acquisition, visualization; FGA: conceptualization, investigation, visualization, writing—review and editing, validation, formal analysis, methodology, conceptualization, funding acquisition. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated and/or analysed during the current study are available in the Sequence Read Archive (SRA) of the NCBI repository, PRJNA1023497.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that there are no competing interests.

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References

1. Borrelli P, Robinson DA, Panagos DP, Lugato E, Yang JE, Alewell C, Wuepper D, Montanarella L, Ballabio C. Land use and climate change impacts on global soil erosion by water (2015–2070). 2020; https://doi.org/10.1073/pnas.2001403117/-/DCSupplemental
2. Tramblay Y, Koutroulis A, Samaniego L, Vicente-Serrano SM, Volaire F, Boone A, et al. Challenges for drought assessment in the Mediterranean region under future climate scenarios. Earth Sci Rev. 2020. https://doi.org/10.1016/j.earscirev.2020.103348.
3. del Pozo A, Brunel-Saldias N, Engler A, Ortega-Farias S, Acevedo-Opazo C, Lobos GA, Jara-Rojas R, Molina-Montenegro MA. Climate change impacts and adaptation strategies of agriculture in Mediterranean-climate regions (MCRs). Sustainability. 2019. https://doi.org/10.3390/su11102769.
4. Prosdocimi M, Jordán A, Tarolli P, Keesstra S, Novara A, Cerdà A. The immediate effectiveness of barley straw mulch in reducing soil erodibility and surface runoff generation in Mediterranean vineyards. Sci Total Environ. 2016. https://doi.org/10.1016/j.scitotenv.2015.12.076.
5. Ramakrishna A, Tam HM, Wani SP, Long TD. Effect of mulch on soil temperature, moisture, weed infestation and yield of groundnut in northern Vietnam. Field Crop Res. 2006. https://doi.org/10.1016/J.FCR.2005.01.030.
6. Gomez-Ramirez LF, Uribe-Velez D. Phosphorus solubilizing and mineralizing *Bacillus* spp. Contribute to rice growth promotion using soil amended with rice straw. Curr Microbiol. 2021; https://doi.org/10.1007/s00284-021-02354-7
7. Jourgholami M, Fathi K, Labelle ER. Effects of litter and straw mulch amendments on compacted soil properties and Caucasian alder (*Alnus subcordata*) growth. New For. 2020. https://doi.org/10.1007/s11056-019-09738-5.
8. Dobermann A, Fairhurst TH. Rice straw management. Better Crops Int. 2002;16:7–11.
9. Mittal SK, Singh N, Agarwal R, Awasthi A, Gupta PK. Ambient air quality during wheat and rice crop stubble burning episodes in Patiala. Atmos Environ. 2009. https://doi.org/10.1016/j.atmosenv.2008.09.068.
10. Williams WA, Mikkelsen DS, Mueller KE, Ruckman JE. Nitrogen immobilization by rice straw incorporated in lowland rice production. Plant Soil. 1968;28:49–60.
11. Bird JA, Horwath WR, Eagle AJ, van Kessel C. Immobilization of fertilizer nitrogen in rice. Soil Sci Soc Am J. 2001. https://doi.org/10.2136/sssaj2001.6541143x.
12. Chesire MV, Bedrock CN, Williams BL, Chapman SJ, Solntseva I, Thomsen I. The immobilization of nitrogen by straw decomposing in soil. Eur J Soil Sci. 1999;50:329–41.
13. Pastor-Bueis R, Mulas R, Gómez X, González-Andrés F. Innovative liquid formulation of digestates for producing a biofertilizer based on *Bacillus siamensis*: field testing on sweet pepper. J Plant Nutr Soil Sci. 2017. https://doi.org/10.1002/JPLN.201700200.
14. Prasad M, Srinivasan R, Chaudhary M, Choudhary M, Jat LK. Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture. PGPR Amelioration Sustain Agricult. 2019. https://doi.org/10.1016/b978-0-12-815879-1.00007-0.
15. Basu A, Prasad P, Das SN, Kalam S, Sayyed RZ, Reddy MS, Enshasy H. Plant growth promoting rhizobacteria (Pgpr) as green bioinoculants: recent developments, constraints, and prospects. Sustainability. 2021. https://doi.org/10.3390/su13031140.
16. Ambrosini A, de Souza R, Passaglia LMP. Ecological role of bacterial inoculants and their potential impact on soil microbial diversity. Plant Soil. 2016. https://doi.org/10.1007/s11104-015-2727-7.
17. Rivera D, Revale S, Molina R, Gualpa J, Puente M, Maroniche G, et al. Complete genome sequence of the model rhizosphere strain *Azospirillum brasilense* Az39, successfully applied in agriculture. Genome Announc. 2014. https://doi.org/10.1128/GENOMEA.00683-14/FORMAT/EPUB.
18. Marcano IE, Díaz-Alcántara CA, Urbano B, González-Andrés F. Assessment of bacterial populations associated with banana tree roots and development of successful plant probiotics for banana crop. Soil Biol Biochem. 2016. https://doi.org/10.1016/J.SOILBIO.2016.04.013.
19. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Ostell J, Pruitt KD, Sayers EW. GenBank. Nucleic acids research. 2018.
20. Kim OS, Ch, YJ, Lee K, Yoon SH, Kim M, Na H, et al. Introducing EzTaxone: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. International journal of systematic and evolutionary microbiology. 2012; 62: 716-721. https://doi.org/10.1099/ijs.0.038075-0
21. Habibi S, Djedidi S, Prongjunthuek K, Firoz Mortuza M, Ohkama-Ohtsu N, Sekimoto H, et al. Physiological and genetic characterization of rice nitrogen fixer PGPR isolated from rhizosphere soils of different crops. Plant Soil. 2014. https://doi.org/10.1007/s11104-014-2035-7.
22. Mulas García R. Análisis del valor agronómico de los analysis of agricultural value based on rhizobia biofertilizers, in cereal crops tesis doctoral. 2015
23. Alexander B, Zuberer DA. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol Fertil Soils. 1991;12:39–45.
24. Sempere A, Oliver J, Ramos C. Simple determination of nitrate in soils by second-derivative spectroscopy. J Soil Sci. 1993;44:633–9.
25. Unkovich M, Baldock J. Measurement of asymbiotic N2 fixation in Australian agriculture. Soil Biol Biochem. 2008. https://doi.org/10.1016/J.SOILBIO.2008.08.021.
26. Pastor-Bueis R, Jiménez-Gómez A, Barquero M, Mateos PF, González-Andrés F. Yield response of common bean to co-inoculation with Rhizobium and Pseudomonas endophytes and microscopic evidence of different colonised spaces inside the nodule. Eur J Agron. 2021. https://doi.org/10.1016/J.EJA.2020.126187.
27. Anderson JPE. Soil respiration. Methods of soil analysis: Part 2 chemical and microbiological properties. 1982; https://doi.org/10.2134/AGRONMONOGR9.2.2ED.C41
28. Alef K. Enrichment, isolation and counting of soil microorganisms. Methods Appl Soil Microbiol Biochem. 1995. https://doi.org/10.1016/B978-012513840-6/50019-7.
29. Ortiz-Liébana N, Zotti M, Barquero M, González-Andrés F. An organic fertilizer 'doped' with a bacillus strain improves melon and pepper yield, modifying the rhizosphere microbiome with negligible changes in the bulk soil microbiome. Agronomy. 2022. https://doi.org/10.3390/agronomy12112620.
30. Clarke KR, Gorley RN. Getting started with PRIMER v7. PRIMER-E: Plymouth, Plymouth Marine Laboratory. 2015.https://scholar.google.com/scholar_lookup?title=Getting+Started+with+PRIMER+V7&author=Clarke,+K.R.&author=Gorley,+R.N.&publication_year=2015
31. Schroeder PJ, Jenkins DG. How robust are popular beta diversity indices to sampling error. Ecosphere. 2018. https://doi.org/10.1002/ecs2.2100.
32. Heberle H, Meirelles VG, da Silva FR, Telles GP, Minghim R. InteractiVenn: A web-based tool for the analysis of sets through Venn diagrams. BMC Bioinformatics. 2015. https://doi.org/10.1186/S12859-015-0611-3/FIGURES/4.
33. Abrantes JRCB, Prats SA, Keizer JJ, de Lima JLMP. Effectiveness of the application of rice straw mulching strips in reducing runoff and soil loss: laboratory soil flume experiments under simulated rainfall. Soil Tillage Res. 2001. https://doi.org/10.1016/J.STILL.2018.03.015.
34. Noor MA, Nawaz MM, Ma W, Zhao M. Wheat straw mulch improves summer maize productivity and soil properties. Ital J Agron. 2021. https://doi.org/10.4081/IJA.2020.1623.
35. Cao Y, Sun H, Zhang J, Chen G, Zhu H, Zhou S, Xiao H. Effects of wheat straw addition on dynamics and fate of nitrogen applied to paddy soils. Soil Tillage Res. 2018. https://doi.org/10.1016/J.STILL.2017.12.023.
36. Tu C, Ristaino JB, Hu S. Soil microbial biomass and activity in organic tomato farming systems: effects of organic inputs and straw mulching. Soil Biol Biochem. 2006. https://doi.org/10.1016/J.SOILBIO.2005.05.002.
37. Kuzyakov Y, Xu X. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytol. 2013. https://doi.org/10.1111/nph.12235.
38. Reichel R, Wei J, Islam MS, Schmid C, Wissel H, Schröder P, Schloter M, Brüggemann N. Potential of wheat straw, spruce sawdust, and lignin as high organic carbon soil amendments to improve agricultural nitrogen retention capacity: an incubation study. Front Plant Sci. 2018. https://doi.org/10.3389/fpls.2018.00900.

39. Huang S, Zeng Y, Wu J, Shi Q, Pan X. Effect of crop residue retention on rice yield in China: a meta-analysis. Field Crop Res. 2013. https://doi.org/10.1016/j.fcr.2013.08.013.
40. Araujo J, Díaz-Alcántara CA, Urbano B, González-Andrés F. Inoculation with native Bradyrhizobium strains formulated with biochar as carrier improves the performance of pigeonpea (*Cajanus cajan* L.). Eur J Agron. 2020. https://doi.org/10.1016/J.EJA.2019.125985
41. Omar AF, Abdelmageed AHA, Al-Turki A, Abdelhameid NM, Sayyed RZ, Rehan M. Exploring the plant growth-promotion of four streptomyces strains from rhizosphere soil to enhance cucumber growth and yield. Plants. 2022. https://doi.org/10.3390/plants11233316.
42. Bashan Y, de-Bashan LE. How the plant growth-promoting bacterium azospirillum promotes plant growth-a critical assessment. Adv Agronomy. 2010. https://doi.org/10.1016/S0065-2113(10)08002-8
43. Kumar-Sethi S, Prasad-Adhikary S. Azotobacter: a plant growth-promoting rhizobacteria used as biofertilizer. Dyn Biochem Process Biotechnol Mol Biol. 2012;6:68–74.
44. Gadhave KR, Devlin PF, Ebertz A, Ross A, Gange AC. Soil inoculation with Bacillus spp. modifies root endophytic bacterial diversity, evenness, and community composition in a context-specific manner. Microb Ecol. 2018. https://doi.org/10.1007/s00248-018-1160-x
45. Griffiths BS, Hallett PD, Kuan HL, Gregory AS, Watts CW, Whitmore AP. Functional resilience of soil microbial communities depends on both soil structure and microbial community composition. Biol Fertil Soils. 2008. https://doi.org/10.1007/s00374-007-0257-z.
46. Chen S, Xiang X, Ma H, Penttinen P, Zhao J, Li H, Gao R, Zheng T, Fan G. Straw mulching and nitrogen fertilization affect diazotroph communities in wheat rhizosphere. Front Microbiol. 2021. https://doi.org/10.3389/fmicb.2021.658668.
47. Zhao Y, Mao X, Zhang M, Yang W, Di HJ, Ma L, Liu W, Li B. The application of Bacillus Megaterium alters soil microbial community composition, bioavailability of soil phosphorus and potassium, and cucumber growth in the plastic shed system of North China. Agricult Ecosyst Environ. 2021. https://doi.org/10.1016/J.AGEE.2020.107236
48. Siczek A, Wielbo J, Kidaj D. Benefits of flavonoids and straw mulch application on soil microbial activity in pea rhizosphere. Int J Environ Sci Technol. 2018. https://doi.org/10.1007/s13762-017-1434-8.
49. Tang Z, Zhang L, He N, Gong D, Gao H, Ma Z, et al. Soil bacterial community as impacted by addition of rice straw and biochar. Sci Rep. 2021. https://doi.org/10.1038/s41598-021-99001-9.
50. Chen Z, Wang H, Liu X, Zhao X, Lu D, Zhou J, Li C. Changes in soil microbial community and organic carbon fractions under short-term straw return in a rice–wheat cropping system. Soil Tillage Res. 2017. https://doi.org/10.1016/J.STILL.2016.07.018.
51. Wu L, Ma H, Zhao Q, Zhang S, Wei W, Ding X. Changes in soil bacterial community and enzyme activity under five years straw returning in paddy soil. Eur J Soil Biol. 2020. https://doi.org/10.1016/J.EJSOBI.2020.103215.

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