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Fully Organic Agroforestry Practices in Rice Cultivation: Effects on Soil Bacterial Microbiota, Soil Health and Grain Quality

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ABSTRACT

Introduction: Sustainable practices are increasingly recognised for benefiting soil biodiversity, health, and overall grain quality. This study examined a unique rice agroecosystem adopting fully organic practices and agroforestry through a seasonal characterisation of soil bacterial microbiota and physicochemical parameters. Furthermore, the first nutritional characterisation of an heirloom rice variety was provided, explicitly detailing how the soil biodiversity and parameters influence the grain quality.

Materials and Methods: Following the rice growth cycle, soil bacterial microbiota were studied through a metabarcoding approach on environmental DNA. Soil physicochemical properties were determined with standardised protocols and an FT-IR analysis on the labile and recalcitrant carbon. Besides, the grain nutritional profile was obtained using standardised kits and protocols, while grain protein quantification was performed by combining SDS-PAGE assay and MALDI-TOF mass spectrometry.

Results: Microbiota profiling highlighted the cover crops field's role in hosting functionally important bacteria, in support of the subsequent rice cultivation. Taxonomical and functional diversity significantly increased across the rice growing season, peaking at flowering, a delicate physiological phase directly impacting crop yields. Soil ROC, OM and flooding conditions primarily drove the bacteria community dissimilarity inter-stages ($R^2 = 0.637$). The sustainable practices employed for rice cultivation (i.e., green mulching, zero external inputs) led to a stabilisation of TOC and OM, increased available labile C, and to a carbon sequestration at the end of the growing season (7%), besides soil N and P conservation. Correlation analysis showed that CEC, C/N and bacterial diversity mainly influenced the carbohydrate fraction of the rice grain, with an indirect effect on the protein content.

Conclusion: Through a multidisciplinary approach, this study highlights that sustainable rice cultivation provides vital ecosystem services by positively affecting bacterial biodiversity, nutrient cycling, and grain quality. These sustainable practices represent an evidence-based strategy to achieve global Sustainable Development Goals and to redesign rice agriculture.

1 | Introduction

Agroecosystems cover more than 40% of Earth's surface, and since the Green Revolution, crop production has steadily increased to meet the demands of a growing population (Pellegrini and Fernández 2018; FAO 2024). This intensification led to the adoption of deleterious practices (e.g., mechanical operations, utilisation of fertilisers and phytosanitary products) to boost production, with direct environmental effects on the agroecosystem's sustainability (Abdo et al. 2025). On the other hand, organic practices are known to enhance agroecosystem resilience (Gamage et al. 2023; Gallardo 2024). This is particularly true for the soil matrix. Soil is a complex environmental matrix, rich in different microhabitats, and able to support over half of the global biodiversity (Anthony et al. 2023). The importance of soil biodiversity lies in the ecosystem services (ES) provisioning, a critical asset for food security and agroecosystem functioning (Aksoy et al. 2017). The soil bacterial microbiota drives nutrient cycling, soil fertility and structure as well as crops' overall health and productivity (Hartmann and Six 2022; Michl et al. 2023; Nasuelli et al. 2023). Thus, soil bacterial microbiota is fundamental for the One Health framework, and the transition toward more sustainable farming systems is a central strategy to safeguard both environmental and human health (Yan et al. 2022).

Sustainable agricultural practices have proved to be beneficial for the bacterial community and their inherent functionality within soils compared to conventional managements (Tahat et al. 2020; Barros-Rodríguez et al. 2021). In rice agroecosystems, the bacterial communities are subject to periodical flooding that causes dynamic redox shifts, supporting consortia of aerobic, anaerobic, and facultative anaerobic taxa (Varghese et al. 2025). Practices such as crop rotation and reduced tillage proved to enhance microbial diversity and activity (Wang et al. 2023; Qi et al. 2024), contemporarily to soil health improvement and greenhouse gas emissions mitigation (Dash et al. 2025). Sustainable agriculture supports the utilisation of ancient crop varieties, which also serve as a vital reservoir of functional genes for plant resilience (Pathirana and Carimi 2022; Salgotra and Chauhan 2023). Besides, rice grains provide a nutritionally valuable and hypoallergenic protein source, with a favourable essential amino acid profile (Jayaprakash et al. 2022). Within the broad spectrum of sustainable practices, agroforestry has gained a reputation as a key long-term solution to ensure agroecosystem sustainability. Agroforestry offers a potential solution to the simplified landscape of intensive agricultural systems, by integrating woody vegetation within or between crop fields, thereby enhancing spatial heterogeneity in agroecosystems (Wilson and Lovell 2016). Such systems provide multiple ES, directly supporting several Sustainable Development Goals (SDG 2, SDG 13 and SDG 15) (Goparaju et al. 2020). When integrated with organic farming, agroforestry offers a strategy aligned with agroecological principles (Rosati et al. 2021). In Europe, the adoption of agroforestry systems remains limited and faces structural and policy-related constraints (Sollen-Norrlin et al. 2020), whereas such systems are more widely established in Asia (Wangpakapattanawong et al. 2017) and Africa (Rodenburg et al. 2022). Agroforestry in rice fields, combined with other organic practices, leads to an increased bacterial diversity associated with greater soil organic carbon stocks (Kumar et al. 2025), nutrient availability (Wang et al. 2022) and soil biological quality (Nayak et al. 2024). Besides, rice agroforestry systems can reduce input costs and improve resource efficiency compared to traditional ones (Wu and Liu 2024).

This study investigated a rare example of a rice farm managed under a fully organic agroforestry system in Italy, and to the best of our knowledge, in Europe (Santoro et al. 2020; Monaco et al. 2022). Starting from the hypothesis that such a regime can enhance soil bacterial diversity and improve soil parameters, a multidisciplinary approach involving environmental DNA metabarcoding and soil physicochemical characterisation was used. Additionally, the first characterisation of the grain nutritional profile of an ancient Italian rice variety was provided. The aims of the study conducted in this peculiar context were: i) to characterise, considering rice phenological stages, the soil bacterial biodiversity; ii) to determine possible shifts in soil chemical parameters during the cultivation period; iii) to provide a baseline characterisation of the nutritional profile for the *Oryza sativa* var. Chinese Originario, for which the farm is the holder of the germplasm, and the influence of soil bacteria and physicochemical parameters on grain macronutrient accumulation.

2 | Materials and Methods

2.1 | Study Area

Soils were sampled in an organically managed rice field (R1) located in Rovasenda (Vercelli, north-west Italy, 45.5390° N, 8.3146° E), operated in compliance with European Regulation (EU) 2018/848 and integrated with agroforestry. Sustainable practices included annual crop rotation, cover crops later used as green mulch (i.e., *Vicia villosa*, *Lolium multiflorum*, *Secale cereale* and *Avena sativa*) to contrast weed growth (Vitalini et al. 2020), minimum tillage, and the absence of external inputs. Sampling was conducted during season 2022 (Supporting Information S1: Table S2), across the rice growth cycle at four timepoints (Figure 1): basal (R1.0, right after rice broadcast seeding, cover crops crimping and a short flooding period to guarantee rice rooting), vegetative (R1.1, flooded four-leaf stage), flowering (R1.2, flooded), and dry harvest-maturity stage (R1.3).

GPS coordinates were used to relocate the same points across phases (Supporting Information S1: Table S2). Soil was collected to a depth of 15 cm using sterilised shovels, targeting the rice rhizosphere. At each point, three cores spaced 20–30 cm apart were combined, transported to the laboratory in a cooler with ice in a few hours, and stored at −20°C. Ten sampling points were selected per phase, with an additional 10 samples from a resting cover crop field (E1), which in this farm represents the source of the green mulching used in the rice fields, yielding 50 samples in total.

The field was cultivated with a parental variety of *Oryza sativa* var. Chinese Originario, an heirloom genetic lineage likely originating in Japan and introduced into Italian rice cultivation early in the 1900s (Sansoni et al. 2024). At the maturation phase (R1.3), rice grains (R1A-L) were harvested concurrently with soil sampling.

2.2 | Library Preparation and Sequencing

Environmental DNA extraction was performed from each sample starting from 0.25 g of soil using the DNeasy PowerSoil

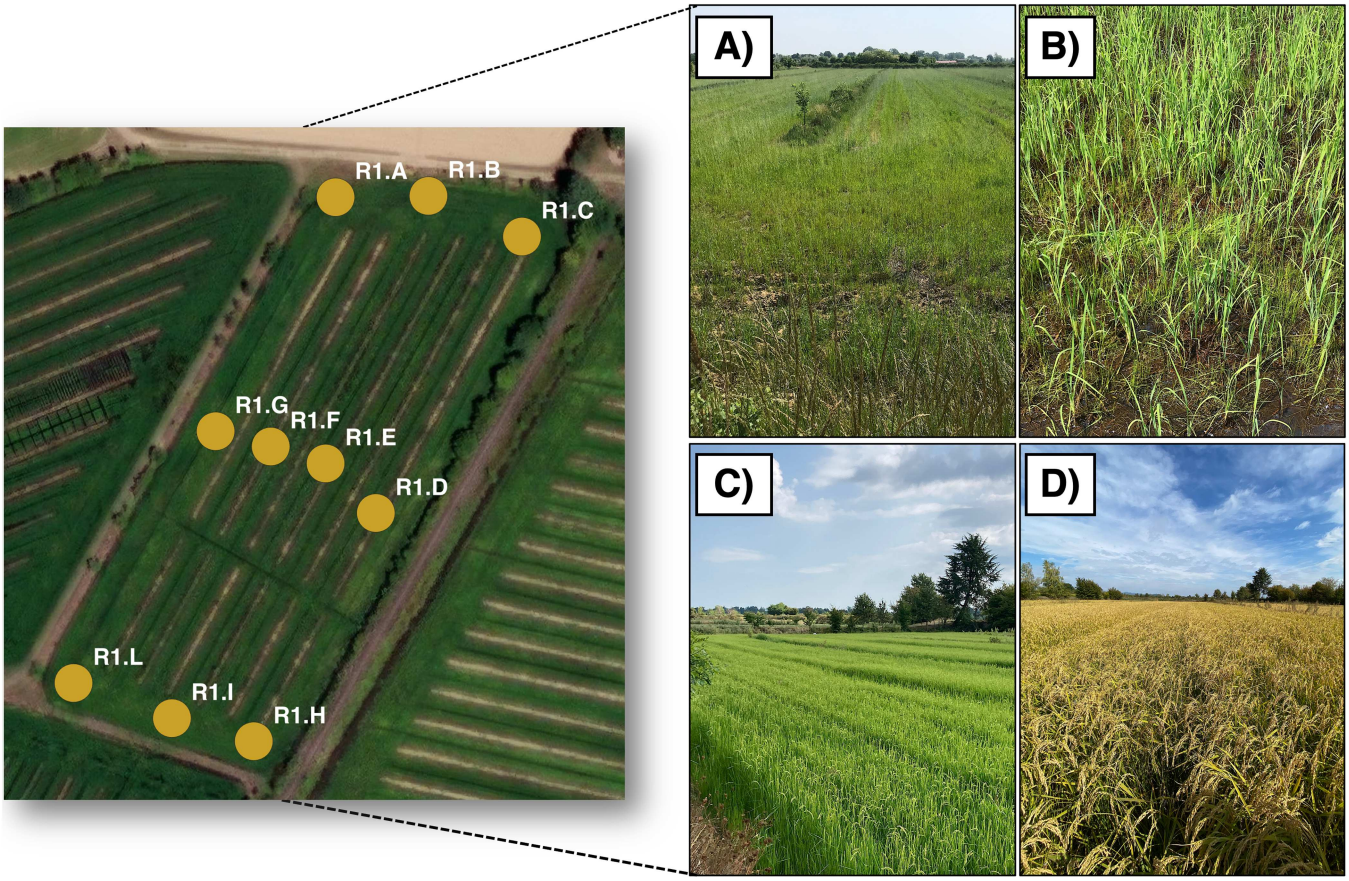

FIGURE 1 | The four different phenological stages sampled in the R1 rice field. (A) Basal (R1.0, right after rice broadcast seeding, cover crops crimping and a short flooding period to guarantee rice rooting), (B) vegetative (R1.1, flooded four-leaf stage), (C) flowering (R1.2, flooded), and (D) dry harvest-maturity stage (R1.3).

Kit (Qiagen, Milan, Italy), following the manufacturer's instructions. Extracted DNA was quantified using Qubit 1X dsDNA HS Assay Kit on the Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, USA). After DNA dilution to equimolar concentration, library construction was performed following the protocol provided by Microbiota Solution B Kit (Arrow Diagnostics s.r.l., Genoa, Italy), targeting the hypervariable region V3–V6 of the 16S rRNA bacterial gene. Each library was then quantified and pooled together at equimolar concentration. The final solution (6 pM) was prepared and sequenced using the Illumina MiSeq Reagent (Illumina, San Diego, United States), v2 chemistry allowing 500 cycles PE, with a 20% Phix spike.

2.3 | Bioinformatic and Ecological Analyses

Raw fastq reads were processed in MicrobAT Suite (SmartSeq s.r.l., Alessandria, Italy), as described in other publications (Bona et al. 2021; Novello et al. 2024). Biodiversity analyses of bacterial communities were conducted in R version 4.5 (R Core Team 2025) within RStudio (Posit team 2025). Sequencing data were imported into *microeco* (Liu et al. 2021), filtering out unclassified features and those occurring fewer than 10 times. Feature abundances were normalised using scaling with ranked subsampling (SRS) (Beule and Karlovsky 2020) to ensure comparability of communities (McKnight et al. 2019).

Relative abundances in percentage of bacterial phyla were calculated across rice phenological phases and according to water presence. The non-parametric Wilcoxon rank-sum test was employed to assess the statistical significance of abundance variations. Within-sample diversity was estimated using the observed number of features, Shannon and Simpson indices, considering rice phases as discriminant. Statistical significance of alpha-diversity metrics was assessed with ANOVA and Duncan's post hoc test. Shared and unique features across phases were visualised with a Venn diagram.

Linear Discriminant Analysis Effect Size (LEfSe) was used to identify phase-specific signatures applying an abundance threshold of 0.001 and a minimum LDA score of 3. Functional diversity analysis was conducted by comparing the identified features with the FAPROTAX database (Louca et al. 2016) to estimate the functional redundancy (FR) of the bacterial communities within each rice growth stage and in the cover crops resting field. Statistical significance was assessed with ANOVA and Duncan's post hoc test. In addition, the signature features identified by the LEfSe analysis were matched with the corresponding functional role based on the entries in the FAPROTAX database. Differences in community composition were examined using Principal Coordinates Analysis (PCoA) based on Bray–Curtis dissimilarities, with significance tested via PERMANOVA and via a beta dispersion analysis, to evaluate within-group variability. To test the contribution of soil

parameters to the bacteria community differentiation, a Redundancy Analysis (RDA) was performed with forward selection to identify the most influential drivers and to exclude collinearity.

2.4 | Soil Physicochemical Properties Characterisation

Soil samples were air-dried and sieved to 2 mm. Soil pH, soil texture, cation exchange capacity (CEC), total organic carbon (TOC) and total nitrogen (TKN) were determined as reported in Tambone and Adani (2017). All the analyses were carried out in triplicate. Dissolved organic carbon (DOC) and recalcitrant organic carbon (ROC) were extracted from all soil samples as reported in Chiaffarelli et al. (2024). Lyophilised extracts of DOC and ROC were subjected to FT-IR analysis. For this purpose, a Shimadzu IRAffinity-1S, equipped with a Miracle Pike ATR device (Shimadzu Italia s.r.l., Milano, Italy), was used. The FT-IR spectra were acquired in total reflectance mode (ATR) (wavenumber range of 4000–500 cm^{-1} and resolution of 2 cm^{-1}, 64 scans for each acquisition). All statistical analyses were carried out using SPSS statistical software (vers. 16) (SPSS, Chicago, IL).

2.5 | Grain Nutritional Characterisation

2.5.1 | Nutritional Profile

Seeds were ground to a meal with a coffee grinder and then sieved through a 60-mesh sieve. The water content of the samples was quantified by weighing the sample and leaving it to dry at 110°C overnight or until a constant weight was reached.

Total starch content was quantified using the Megazyme kit "Total Starch (α-amylase/amyloglucosidase) code K-TSTA-100A, strictly in accordance with the manufacturer's instructions analysing 100 mg of each sample per replica. The absorbance of each sample was measured at 510 nm using a Perkin-Elmer Lambda II spectrophotometer, and the glucose concentration was calculated based on a standard curve. The starch content was calculated with the Megazyme Mega-Calc spreadsheet. Oligosaccharides content was determined using the "Raffinose/Sucrose/Glucose" Megazyme kit (code K-RAFGL), strictly following the manufacturer's instructions. The content of insoluble fibre was determined using the "Total Dietary Fibre" Megazyme kit (K-TDFR-100A), strictly under the manufacturer's instructions, analysing 1 g of sample for each replica. To assess the purity of the isolated dietary fibre, residual proteins using the Kjeldahl method, and ash content by incineration overnight at 525°C, were carried out. The number of calories (kcal) present in the samples was calculated with the following equation:

$$(\text{g of total carbohydrates} \times 4\ \text{kcal}) + (\text{g of total proteins} \times 4\ \text{kcal}) + (\text{g of total lipids} \times 9\ \text{kcal}).$$

The carbohydrate fraction comprises starch, glucose, raffinose, and raffinose series oligosaccharides. The calculation was made considering 100 g of the sample.

The lipid quantification was determined gravimetrically, and samples were previously dried overnight at 110°C. A sample of 0.150 g was weighed, and 1.5 mL of pentane was added (ratio 1:10). Then, the samples were stirred for 1 h at room temperature. After that, they were centrifuged at 1300 rpm for 5 min. The supernatant was discarded, and another 1.5 mL of pentane was added. This procedure was repeated three times. The amount of proteins was determined by the Kjeldahl method according to ISO (2013). The catalyst for mineralisation was from Merck (Cat. No. 1.15348). A conversion factor of 6.25 was used.

2.6 | Protein Quantification and Characterisation

Seeds collected from the 10 sampling points were pooled and dehulled prior to processing. The resulting grains were milled into flour using a laboratory blender in four 30-s pulses, allowing brief cooling intervals between cycles to minimise heat-induced degradation of biomolecules. Sequential extraction of seed storage proteins (SSPs) was carried out according to Givonetti and Cavaletto (2026). The protein content of each fraction was determined using a Bradford assay adapted for microplate readings. Bovine serum albumin (BSA; Quick Start Bovine Serum Albumin Standard #5000206, Bio-Rad, USA) was employed as the calibration standard. All measurements were carried out in technical triplicate. SDS-PAGE analyses were performed by first combining 10 μg of each protein sample with one-half volume of 4× Laemmli reducing buffer and denaturing at 95°C for 5 min. Samples were loaded onto 4%–20% precast polyacrylamide gels and resolved at 120 V using a Mini-PROTEAN electrophoresis system (Bio-Rad) with a Precision Plus Protein Dual Colour molecular weight standard. Gels were subsequently stained with the Blue Silver method, destained, and scanned using a GS-900 densitometer (Bio-Rad, USA). Matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) analysis was performed as follows: 1.5 μL of each protein sample was mixed with 10 μL of sinapinic acid matrix (10 mg/mL in 30:70 ACN/0.1% TFA, v/v) as reported in Cattaneo and coworkers (Cattaneo et al. 2023). A 1.0 μL aliquot of the resulting mixture was deposited in triplicate onto a ground-steel MTP 384 target plate (Bruker Daltonics) and air-dried overnight at room temperature. Spectra were acquired on an UltrafleXtreme mass spectrometer (Bruker Daltonics) equipped with a SmartBeam II Nd:YAG laser (355 nm), controlled via FlexControl 3.3 software. Data were collected in linear positive-ion mode over an m/z range of 2000–20,000, accumulating 6000 laser shots per spot with the random-walk and partial-sample modes enabled. External calibration was performed using the "Protein Calibration Standard I" (Bruker Daltonics) within FlexAnalysis 3.3.

2.7 | Correlation Analysis

To investigate the interactions between soil microbial alpha diversity (by mean of Shannon index), soil properties and rice grain quality, a bivariate correlation analysis was conducted. Firstly, the samples from the E1 field were excluded from the analysis as the plot was not the same as the rice field. Subsequently, all time-series data (i.e., bacterial diversity and soil physicochemical parameters) were averaged per rice growth stage prior to the analysis. Spearman's rank correlation

coefficients were calculated on the aggregated dataset and conducted utilising the *Hmisc* package for correlation matrix computation (Harrell 2026). Statistical significance was evaluated at a threshold of $p < 0.05$, while notable trends ($0.05 \leq p < 0.10$) were also reported.

3 | Results

3.1 | Bacteria Community Composition Analyses

After sequencing, a total of 2,696,966 raw reads were obtained. The genomic sequences were included in the BioProject PRJNA1160448 available in the NCBI database and contained 50 BioSamples. After bioinformatic analysis, 1,837,315 reads were retained, and 36.84% of them resulted in unidentified and, therefore, were removed before downstream analyses. The remaining reads were assigned to 4360 features, with a mean sequence coverage of 23,209 per sample. The frequency filtration step led to a final dataset of 1,140,011 reads clustered into 1191 features.

The estimation of bacterial abundances across rice growth phases and the cover crops field showed that the community was mainly composed of five phyla: Pseudomonadota, Actinomycetota, Chloroflexota, Acidobacteriota, and Bacillota (Figure 2A).

The most pronounced differences concerned Actinomycetota, more abundant in the cover crop field (27%), then decreasing in the R1.0 basal timepoint (11%, $p < 0.001$), followed by a slight increase in the vegetative and flowering stages (14.4% and 17.7%, respectively, $p < 0.01$), and an abundance reduction in the maturation timepoint (11.6%, $p < 0.001$). No significant effect of flooding was detected (Figure 2B). Chloroflexota showed a similar pattern, being more abundant in the cover crops field (17.6%) than in the basal timepoint (8.17%, $p < 0.01$). Significant increase was found in the vegetative stage (15.1%, $p < 0.01$) but not in the flowering timepoint (11.5%, $p > 0.05$). At the end of the growing season, Chloroflexota significantly increased compared to the flowering stage (13.9%, $p < 0.05$). Even in this case, the flooding condition did not influence the phylum abundance (Figure 2B). Acidobacteriota abundance significantly increased solely between the cover crop field and the basal timepoint of the rice paddy (from 9.79% to 11.8%, $p < 0.01$), and was subject to a variation led by water (from 11.5% to 12.7%, $p < 0.05$, Figure 2B). Bacillota significantly increase between the basal R1.0 and vegetative R1.1 stages (from 3.38% to 11.4%, $p < 0.01$), then preserved similar values in the flowering timepoint. At maturation R1.3, a strong decrease was detected (4.43%, $p < 0.001$). The effect of the water presence in the vegetative and flowering stages greatly influenced the Bacillota community (4.25% in the absence of water and 10% under flooding conditions, $p < 0.001$, Figure 2B). Pseudomonadota showed significant but minor variations throughout the rice cultivation season. Starting from 24.3% of abundance in the cover crop field, an increase was found in the basal timepoint R1.0 (34.3%, $p < 0.01$), followed by a decrease in the vegetative and flowering stages (24.7% and 29.3%, respectively, $p < 0.001$)

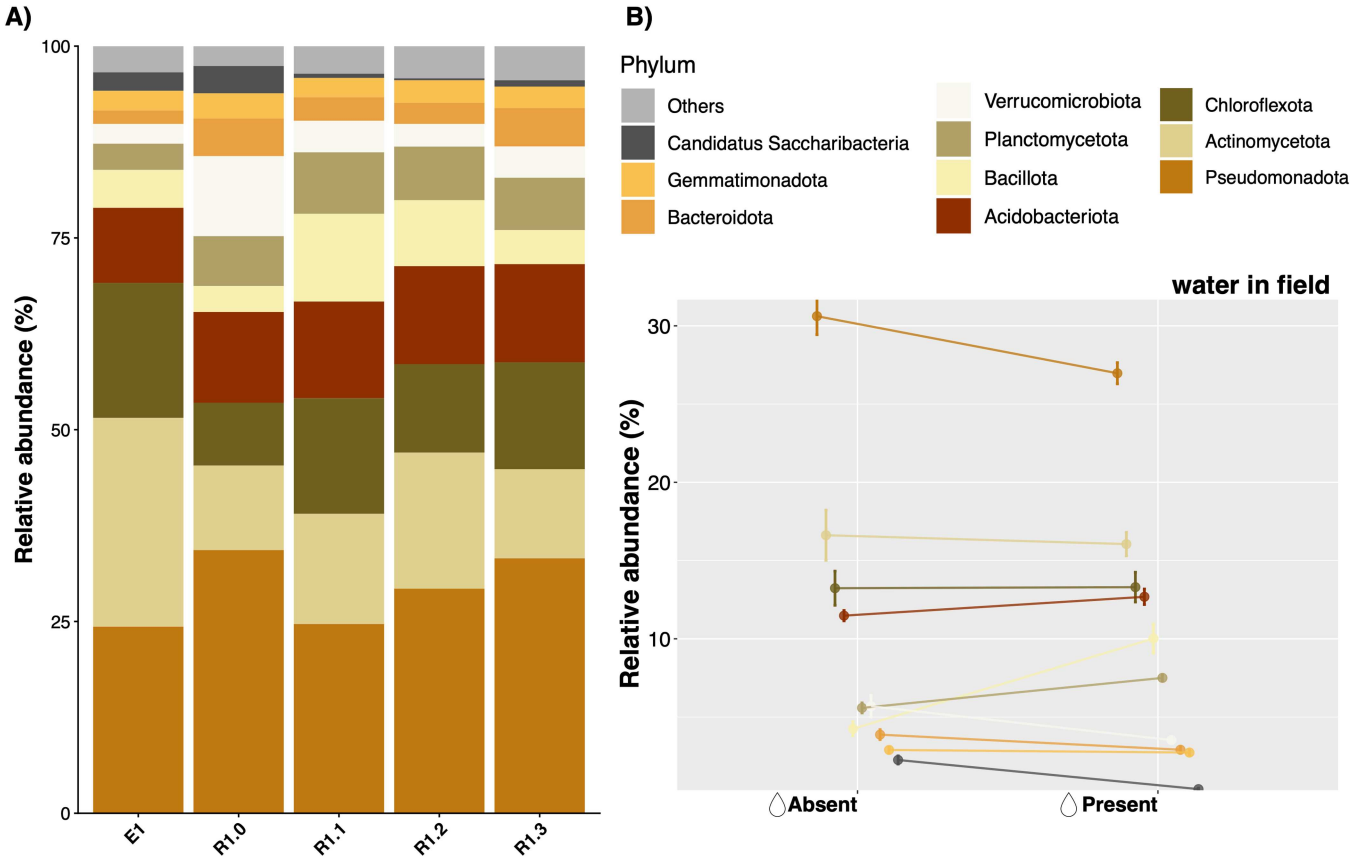

FIGURE 2 | (A) Barplot representing the percentage differences in the phyla abundances found in the different rice growth stages and in the cover crops field. (B) Line plot highlighting the changes in the phyla abundances as a consequence of the flooding conditions. The non-parametric Wilcoxon rank-sum test was employed to assess the statistical significance of abundance variations ($p < 0.05$).

and a final increase at rice maturation (33.2%, $p < 0.01$). A significant decrease was assessed in relation to the water presence (from 30.6% in the absence of water to 27% under flooding conditions, $p < 0.01$, Figure 2B).

Figure 3A–C show alpha diversity estimates across the studied phases. Both observed features and Shannon index values were significantly lower at the basal timepoint (R1.0). Taxonomic diversity (Figure 3A) increased in the cover crops field and during the rice growing season, reaching the highest values at R1.2 and R1.3. For the Shannon index (Figure 3B), significant differences were detected between the basal phase (R1.0) and all other phases, as well as between the vegetative (R1.1) and flowering (R1.2) phases. In contrast, Simpson's diversity index (Figure 3C) showed fewer differences, with comparable values across phases and significant variation only between the cover crops field (E1) and the flowering phase (R1.2). Venn diagram (Figure 3D) indicated that 438 features were shared among phases, while stage-specific features contributed to community differentiation. The cover crops field (E1) showed the highest number of unique features (52), whereas the rice flowering phase (R1.2) was the most diverse rice stage, with 27 exclusive features.

Linear discriminant analysis effect size (LEfSe—Supporting Information S1: Table S1) identified 16 features in the cover crops field (E1), belonging to Actinomycetota (i.e., *Arthrobacter* and *Mycobacterium*) and Pseudomonadota (i.e., *Sphingomonas* and *Pseudolabrys*). The basal timepoint (R1.0) was characterised by several Acidobacteriota and Pseudomonadota, as well as taxa typical of moist environments, such as *Paludibacter propionicigenes* str. WB4 Type (Bacteroidota) and *Tepidisphaera mucosa* str. 2842 Type (Planctomycetota). During the vegetative phase, four signature features were detected (e.g., *Clostridium saccharoperbutylacetonicum* str. N1-4 Type and *Dictyobacter aurantiacus* str. S-27 Type), whereas the flowering phase was characterised by signatures from Pseudomonadota (e.g., *Bradyrhizobium* and *Usitatibacter rugosus*) and Bacillota (genus *Priestia*). The maturation phase was characterised by two strains of *Sideroxydans lithotrophicus*, together with 2 methanotrophic strains (*Methylocystis* and *Methylobacter*).

The FR of the bacterial community (Figure 4) showed a strong shift from aerobic to anaerobic pathways. Aerobic chemoheterotrophy (Figure 4A) was significantly dominant in the cover crops field E1 but showed a progressive and significant decline starting from the basal timepoint of the rice field as the cultivation season advanced. Conversely, the anaerobic chemoheterotrophic pathway (Figure 4B) presented the lower values in the E1 field (0.062) and an increase in the rice field following the growth stages. A similar trend was observed for the fermentation pathway (Figure 4C). Methanotrophy (Figure 4F) and methylotrophy (Figure 4G) both reached their maximum potential at R1.3 (0.029 and 0.031, respectively), showing a

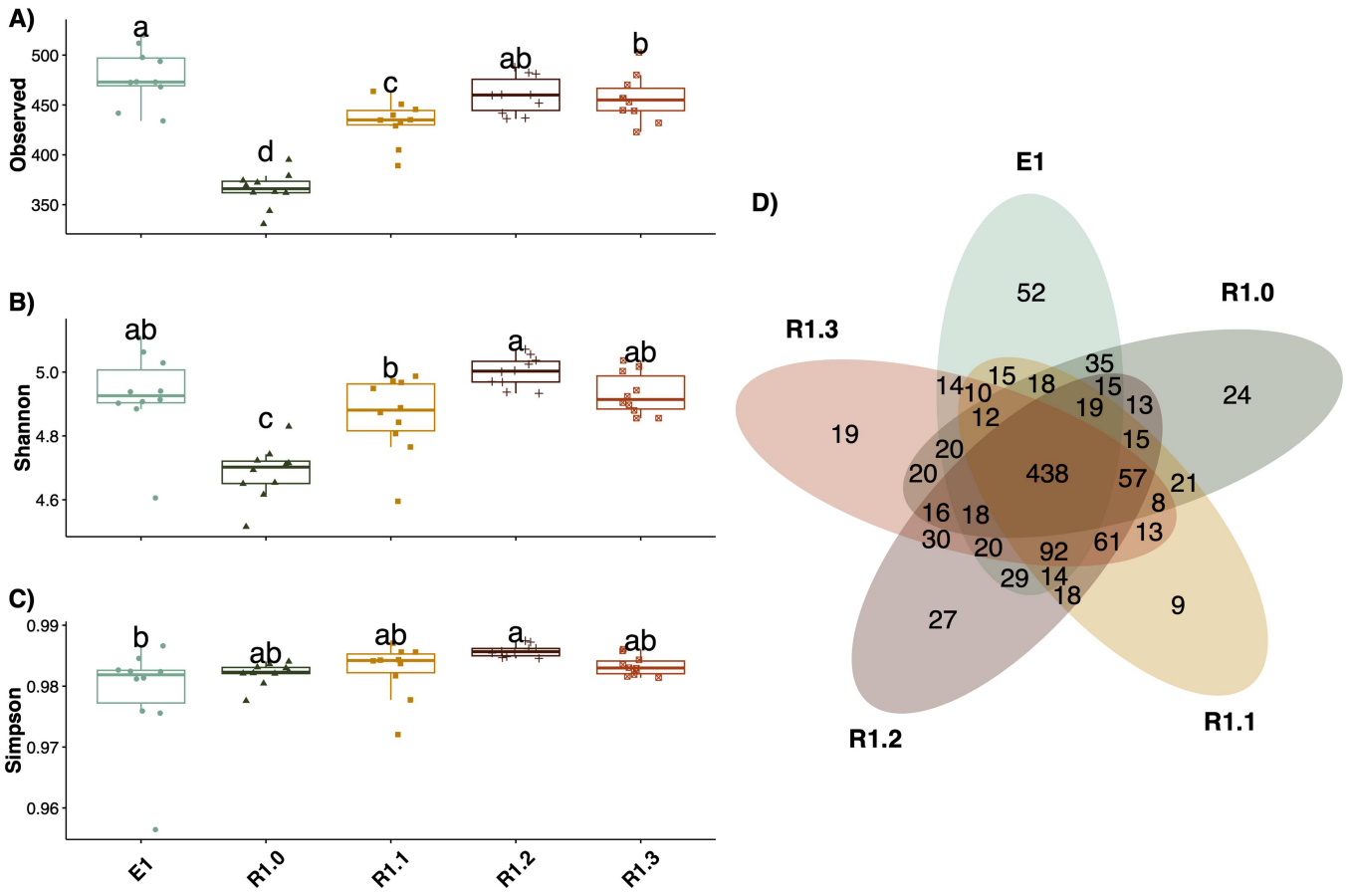

FIGURE 3 | Bacterial taxonomical diversity. Alpha diversity estimates considering the bacterial diversity found in the different rice growing phases and in the cover crops field. (A) Observed diversity, (B) Shannon index, (C) Simpson's diversity index. Letters above the boxplots indicate Duncan's post hoc test estimations. (D) Venn diagram highlighting the number of exclusive or shared features within the tested communities.

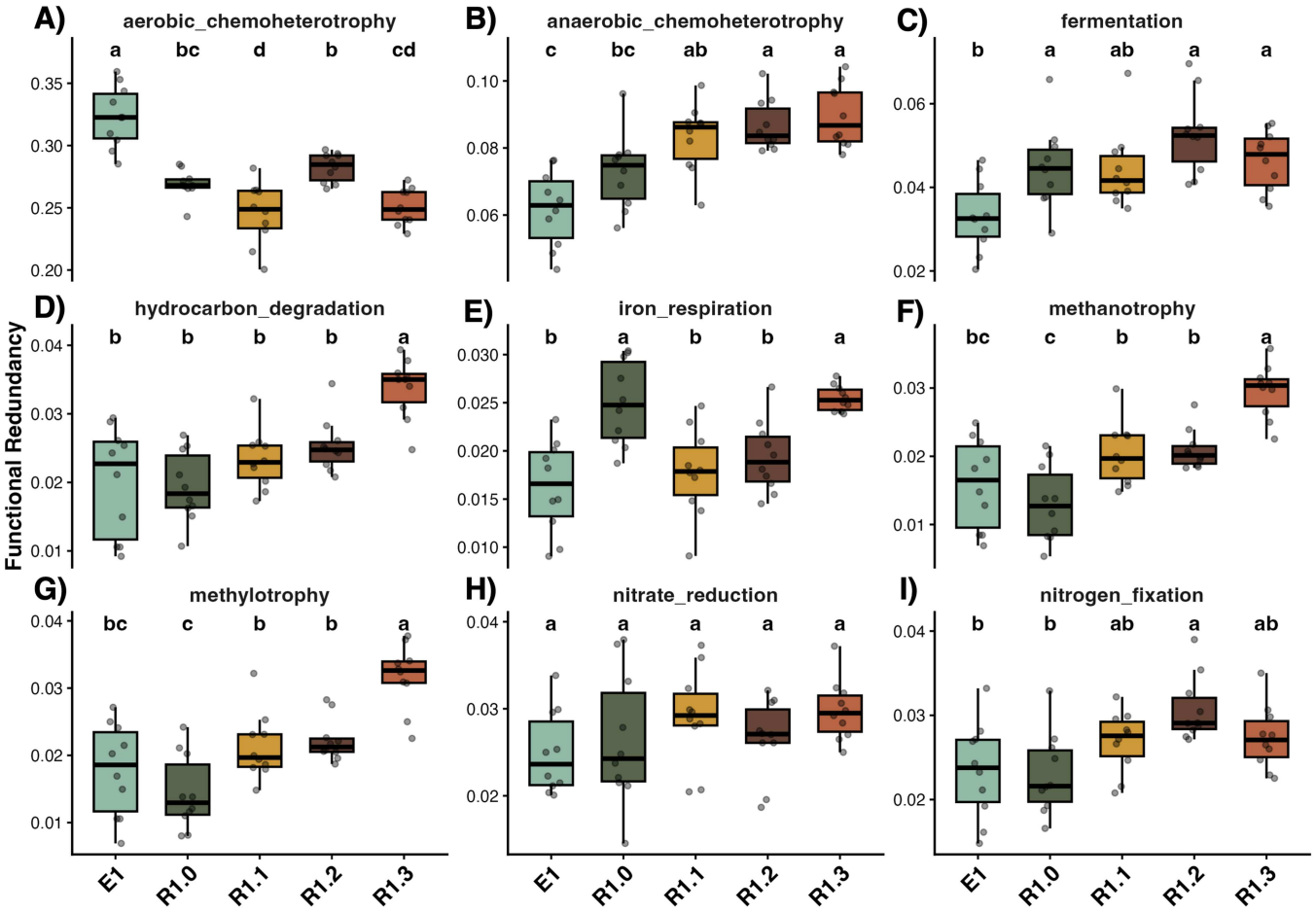

FIGURE 4 | Functional redundancy analysis considering the ecological role of the bacteria identified in the cover crops field (E1) and in the rice paddy along the cultivation season (R1.0–R1.3). The tested metabolic pathways were: (A) aerobic chemoheterotrophy; (B) anaerobic chemoheterotrophy; (C) fermentation; (D) hydrocarbon degradation; (E) iron respiration; (F) methanotrophy; (G) methylotrophy; (H) nitrate reduction and (I) nitrogen fixation. Letters above the boxplots indicate Duncan's post hoc test estimations.

twofold increase compared to the R1.0 stage (methanotrophy R1.0 = 0.013, methylotrophy R1.0 = 0.014). Furthermore, hydrocarbon degradation (Figure 4D) showed a significant spike specifically at the R1.3 stage (0.033), while remaining significantly lower and stable in all previous phases. Iron respiration (Figure 4E) showed a dual pattern, with significantly higher potential during the initial flooding (R1.0 = 0.025) and the final maturation stage (R1.3 = 0.025), while decreasing during the intermediate phases (R1.1 and R1.2). Nitrogen fixation (Figure 4I) showed a significant increase during the active rice growth stage, peaking at R1.2 (0.030). In contrast, nitrate reduction potential (Figure 4H) remained stable across all experimental stages, with no significant differences observed.

Beta diversity analysis (Figure 5) showed a marked dissimilarity between bacterial communities in the cover crops field and those associated with the different rice growth phases (PERMANOVA $F_{1,4} = 18.88$, $p < 0.001$). Within the rice phases, a clear compositional shift was observed (PERMANOVA $F_{1,3} = 19.20$, $p < 0.001$), with the basal timepoint (R1.0) hosting a distinct community ($R^2 = 0.58$–0.66, mean between-distance 0.440), while communities associated with rice plants were more similar to each other but remained clearly differentiated, sharing limited overlap (Figure 5; $R^2 = 0.25$–0.34, mean between-distance 0.283). Beta dispersion analysis revealed significant differences in dispersion among treatments ($F_{1,4} = 5.79$, $p = 0.003$), driven primarily by the cover crop field, whereas rice phenological stages did not differ significantly in dispersion.

The RDA (Figure 6) revealed that soil physicochemical parameters explained 63.7% of the total variance of the bacterial community (adjusted $R^2 = 0.605$, $p = 0.002$). The forward selection identified soil organic matter (OM), water presence (H20), pH and ROC as the main drivers of the differentiation. Specifically, pH resulted as the major driver of soil bacteria within the basal timepoint R1.0, while OM, H20 and ROC were more influential in the differentiation of the communities found in the active rice growth stages.

3.2 | Soil Characterisation

3.2.1 | *Soil Chemical and Physical Characteristics*

The main soil characteristics are reported in Supporting Information S1: Table S3. Soil chemical properties showed clear differences between the resting field (E1) and the samples collected throughout the rice growth cycle (R1.0–R1.3). Soil pH ranged from 5.26 to 6.55, with the highest value observed at

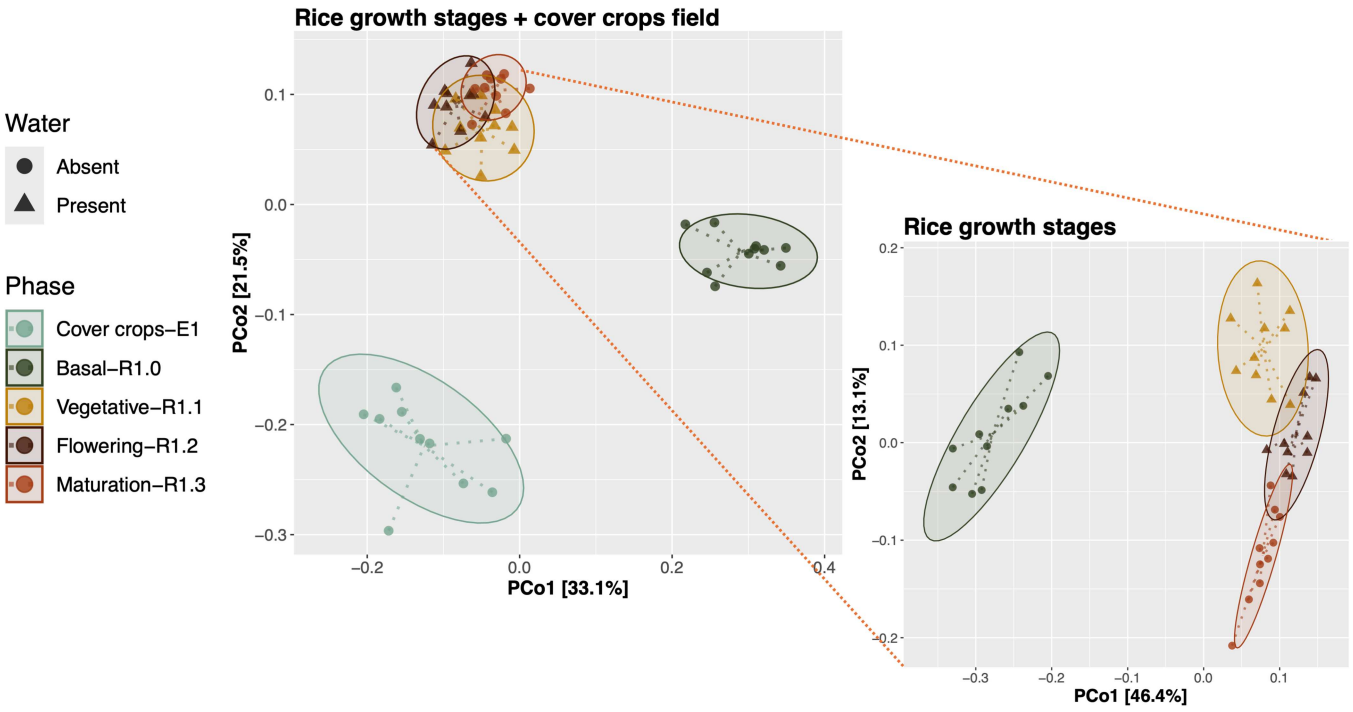

FIGURE 5 | Principal coordinates analysis (PCoA) showing the estimation of the beta diversity as dissimilarity between the bacteria communities found within the tested rice growth stages and the cover crops field.

the basal stage (R1.0) and a progressive acidification toward maturation (R1.3—$p < 0.05$). CEC varied between 22.2 and 27.4 $cmol^+ kg^{-1}$ and did not differ significantly from the cover crops resting field. Total Kjeldahl nitrogen (TKN) remained relatively stable across stages (1.34–1.47 $g\,kg^{-1}$), with slightly lower values in the resting soil. Similarly, TOC showed no statistically significant variation (11.5–12.7 $g\,kg^{-1}$), resulting in a consistent C/N ratio (7.96–9.32) across all growth stages. ROC peaked at the vegetative stage (R1.1), where values reached 11,102 $mg\,kg^{-1}$, significantly higher than both the basal stage and the resting field ($p < 0.05$). DOC displayed the strongest variability, decreasing sharply from 75.3 $mg\,kg^{-1}$ in E1 to 16.9 $mg\,kg^{-1}$ in R1.0 after flooding, followed by a gradual increase until flowering (37.2 $mg\,kg^{-1}$ in R1.2). Available phosphorus (P_2O_5) showed minimum values at the basal stage (178 $mg\,kg^{-1}$) and increased progressively up to the maturation phase (210 $mg\,kg^{-1}$). Soil texture remained stable across the sampling points and was classified as silt loam.

Qualitative FT-IR spectra of DOC and ROC are presented in Supporting Information S1: Figure S1. DOC spectra (Supporting Information S1: Figure S1A) were characterised by a carbohydrate-related C–O stretching peak around 1000 cm^{-1} (Lammers et al. 2009), which intensified and shifted from 995 to 1041 cm^{-1} between R1.0 and R1.3. A stable band at 1620–1690 cm^{-1} was assigned to amide C═O and aromatic C═C stretching (Guo et al. 2012). Aliphatic groups (–CH, $–CH_2$, $–CH_3$) at 2875 cm^{-1} remained weak throughout the season (absorbance 0.20–0.30; Fu et al. 2016). In contrast, the broad band around 3400 cm^{-1} (N–H and phenolic O–H stretching; Barber et al. 2001) declined from R1.0 to R1.3, suggesting a reduction in nitrogenous or phenolic components. ROC spectra (Supporting Information S1: Figure S1B) exhibited increasing complexity in the polysaccharide region (945–1056 cm^{-1}), progressing from a single peak at 993 cm^{-1} (R1.0) to a triplet (around 1100, 1028 and 985 cm^{-1}) at maturity (Thai et al. 2021). Bands associated with primary and secondary amides, aromatics, and quinones (1569–1691 cm^{-1}) became more pronounced in later phases. Similarly, signals for aliphatic and aromatic acids, as well as humic substances (1680–1800 cm^{-1}), intensified relative to R1.0. The persistence of peaks at 2875 and 1489 cm^{-1} (Kaiser et al. 1997; Fu et al. 2016) confirmed the presence of plant-derived aliphatics, specifically cutin and suberin.

3.3 | Grain Nutritional Characterisation

3.3.1 | *Nutritional Profile*

The compositional analyses of grain samples are shown in Supporting Information S1: Table S4. The analyses revealed, as expected, starch as the dominant component, ranging from 70.51% (R1C) to 89.20% (R1I), while protein content varied between 5.22% (R1H) and 8.30% (R1F). Lipid content was generally low (< 2%), with non-detectable levels in R1I and the highest value observed in R1G (1.94%). Fibre content ranged from 1.62% (R1D) to 3.72% (R1C). Soluble sugars were present at low concentrations, with glucose ranging from 0.03% to 0.32% and sucrose from 0.00% to 0.74%, showing sample-specific variability. Moisture content showed limited variability across samples, with values between 14.10% (R1F) and 17.85% (R1C).

3.4 | Protein Quantification and Characterisation

The SSPs of Chinese Originario represent 59.3% of the total protein content. The relative distribution of reserve proteins extracted shows that glutelins constitute the predominant

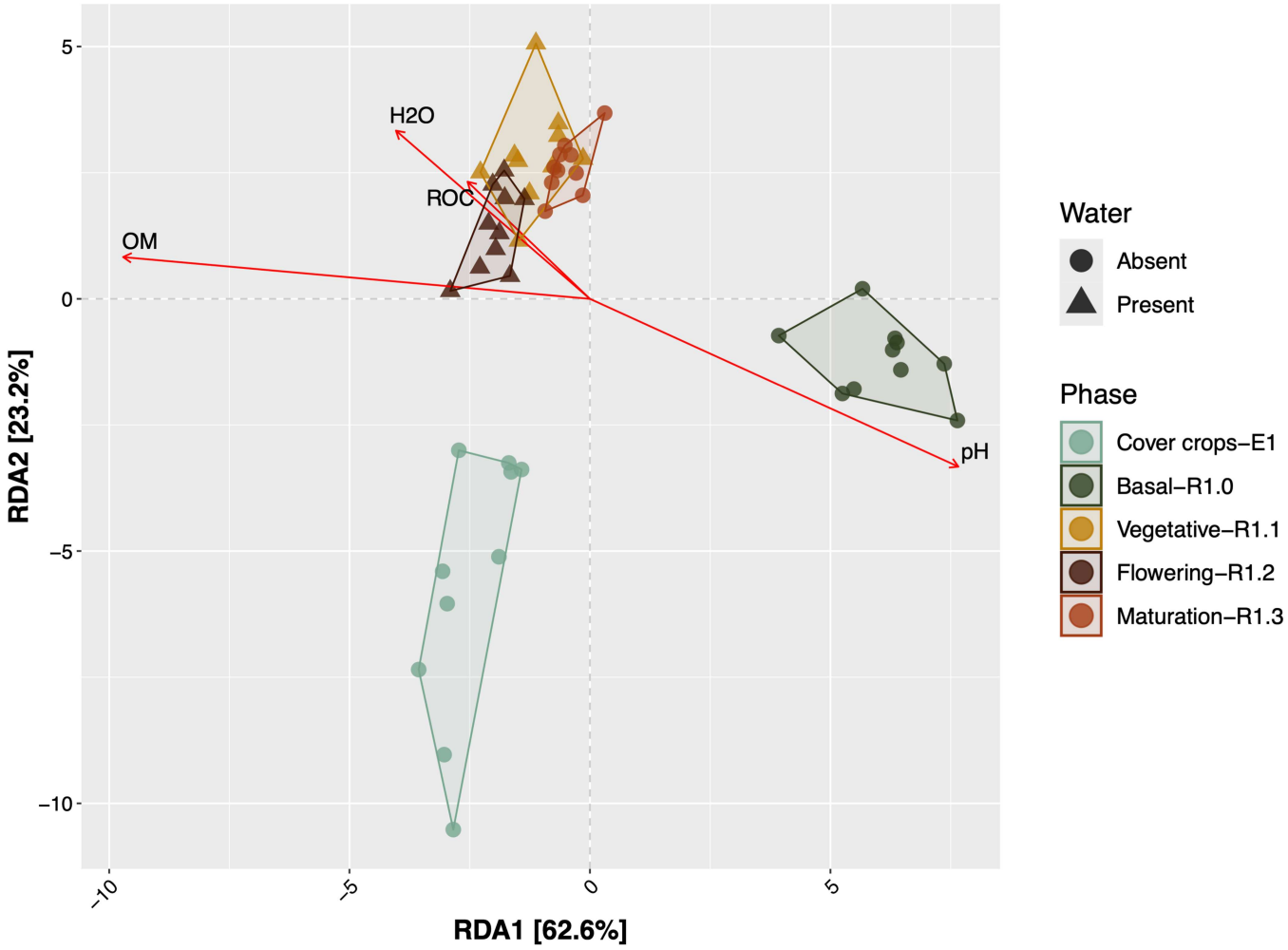

FIGURE 6 | Redundancy analysis (RDA) highlighting the effect of soil physical-chemical parameters on the bacterial community differentiation along the different stages of the rice growth tested. Significant soil parameters were estimated with a forward selection.

fraction, accounting for 75.15%, followed by albumins at 12.94%, globulins at 9.55%, and prolamins at 2.36% ($N = 3$) (Supporting Information S1: Table S5). The protein profiles of the four fractions are shown in Supporting Information S1: Figure S2. The albumin fraction displays a broad range of molecular weights, from 10 to 100 kDa, with the most prominent bands occurring at 50 kDa, between 37 and 25 kDa, and between 15 and 10 kDa. In the globulin fraction, notable bands appear at 50 and 20 kDa, and two bands between 15 and 10 kDa. The glutelin fraction is characterised by three clear bands: between 37 and 25 kDa, around 20 kDa, and between 15 and 10 kDa. Finally, the prolamine fraction shows a band at 20 kDa, another just below it, and two very intense bands between 15 and 10 kDa. The MALDI-TOF spectra of the fractions are shown in Supporting Information S1: Figure S3, with a notable distribution observed between 20 and 2 kDa. All four analysed fractions feature molecules with a mass under 10 kDa. Additionally, the globulin fraction displays a peak at 18.89 kDa and a protein population between 13 and 16 kDa. In the glutelin fraction, low-intensity peaks are visible between 14 and 15 kDa. For prolamins, a peak at 18.89 kDa is present, along with a peak population between 13 and 16 kDa.

3.5 | Correlation Analysis Between Microbial Diversity, Soil Parameters and Grain Quality

3.5.1 | *Soil Parameters Effects on Grain Quality*

The correlation analysis revealed that soil physical-chemical parameters are strong determinants of grain quality (Supporting Information S1: Table S6). CEC resulted as negatively correlated with the glucose content in the rice grains (rho = −0.69, $p = 0.038$), in addition to the C to N ratio, which emerged as a regulatory driver for the resource allocation in rice grains. A strong positive correlation was found between C/N ratio and grain fibre (rho = 0.82, $p = 0.007$), on the contrary to a pronounced negative correlation with grain starch (rho = −0.73, $p = 0.024$). Although purely a trend ($p = 0.08$), a positive correlation between the C/N ratio and protein and sucrose level in the grains was detected (rho = 0.60).

3.5.2 | *Soil Bacterial Diversity Role in Grain Resource Allocation*

The Shannon index showed a significant negative correlation with the concentration of glucose estimated in the rice grains (rho = −0.69, $p = 0.03$) (Supporting Information S1: Table S6), suggesting a possible role of the soil bacterial microbiota in

improving the metabolic efficiency of the rice crops. However, physiological constraints emerge in the correlation analysis between starch and fibre content, bound by a strong negative correlation ($\text{rho} = -0.92$, $p < 0.001$). Additionally, a strong positive correlation between protein and sucrose was found ($\text{rho} = 0.70$, $p = 0.03$).

4 | Discussion

4.1 | Soil Bacterial Microbiota and Health

This study presents the first longitudinal assessment of soil bacterial microbiota and health in a rare fully organic agro-forestry rice system in northwest Italy, a unique case in Europe. Within its sustainable farming approach, the farm preserves and cultivates heirloom varieties, including the paternal line of *Oryza sativa* var. Chinese Originario. Regarding this specific variety, the study provides the first description of the grain nutritional profile and its associated bacterial microbiota.

Considering the bacterial communities, this work confirmed that phyla Pseudomonadota, Actinomycetota, Chloroflexota, Acidobacteriota and Bacillota were the most common bacteria constituents of the rice soils, as also found in previous studies focused on rice fields from other geographical regions (Jiao et al. 2019; Zhou et al. 2020). These phyla were similarly dominant in the cover crops of field E1. Following the farm's rotation plan, cover crops fields are always transitioned to rice cultivation in the subsequent season, thus representing its baseline. As expected, the bacterial microbiota in this field was highly dissimilar from the one characterised in the active rice field. Nevertheless, in the E1 field, several important bacterial signatures were identified. For example, *Sphingomonas* sp. and *Pseudolabrys* sp. have been reported in other agricultural grasslands. *Sphingomonas* sp. was found to be a bioindicator of sustainable practices (Shi et al. 2021) while *Pseudolabrys* sp. is associated with nitrogen fixation and phosphate solubilisation (Sapkota et al. 2023). *Arthrobacter humicola*, originally isolated from rice paddy soils (Kageyama et al. 2008), has been reported to produce indole-3-acetic acid (IAA) and siderophores under comparable conditions (pasture fields; Özdoğan et al. 2022). Besides, the presence of *Mycobacterium* sp. has been reported to benefit rice growth and health (Karmakar et al. 2021), coherently with a recent meta-analysis highlighting the positive effect of cover crops on main crop productions (Peng et al. 2024). Although rice yield estimations were out of the scope of this study, the agricultural practices adopted in the present work have proved to ensure higher yields under harsh climatic conditions while maintaining economic stability and resilience (Chiaffarelli and Vagge 2025). The bacteria microbiota characterisation of the cover crops field provided in this study showed its positive effect on the soil bacteria diversity, being able to support the subsequent rice growth and guarantee the overall sustainability of the farming systems (Garland et al. 2021; Zecchin et al. 2026).

The rice bacterial microbiota varied across the studied period, reflecting the well-known sensitivity of soil bacteria to multiple factors. Likely, the combined effects of slight mechanical operations and flooding-induced environmental changes drove the bacterial community differentiation at the basal timepoint R1.0. The green mulching technique adopted in the studied farm involved cover crop crimping and seed broadcasting on a minumum- tilled soil, later flooded for a short period of time. At this stage, lower alpha diversity and marked differences in community composition were observed, likely because soil disturbance, even when conservatively applied, and temporary changes in oxygen availability, can strongly affect soil microbial communities (Khmelevtsova et al. 2022; Lu et al. 2025). In contrast, the subsequent rice growth stages (i.e. vegetative and flowering) were characterised by uninterrupted flooding, which promoted higher environmental stability and a bacterial microbiota shift (Das et al. 2025). This directly exerted an influence on the subsequent unflooded maturation stage. In fact, soil bacterial diversity exhibited a progressive increase starting from the vegetative stage, reaching the highest values of observed feature and Shannon index at flowering and stabilising at maturation. Beta diversity patterns of these communities confirmed significant divergence from the basal timepoint as well as partial inter-stage overlap, suggesting a gradual microbial succession. The RDA analysis highlighted the effect of flooding conditions on the bacterial community in rice fields. Besides, these diversity changes throughout the rice growth stages might be related to the rhizosphere effect (Edwards et al. 2015). The exudation type and rates change during rice development, where it increases at the vegetative and flowering stages and declines at maturation (Aulakh et al. 2001). The root exudation can drive the dynamic shifts in rhizosphere communities and recruiting bacteria able to support nutrient cycling, stress tolerance, and grain development (Zhang et al. 2018; Aminurrasyid et al. 2025). Unclear remains the role of the rice genotype in shaping the bacterial microbiota found, as no body of evidence is reported in the literature for *O. sativa* var. Chinese Originario.

Coherently with the dynamics of the bacterial diversity and community composition, the functional redundancy greatly varied across the rice growth stages. Anoxic flooding conditions led to a significant reduction in bacteria with aerobic chemoheterotropy, enhancing anaerobic chemoheterotropic taxa and fermentation pathways, especially in the active growth stages, a known effect in flooded rice paddies (Ding et al. 2019). In these environmental conditions, shifts in redox potential drive the sorption–desorption equilibria and modify the temporal variability of DOC and ROC (Said-Pullicino et al. 2016; Schwarz et al. 2024). DOC peaked concurrently with the vegetative stage (R1.1) and decreased at the flowering stage, later stabilising until rice maturation. The peaks are coherent with the significant establishment of rice roots exudation (Lu et al. 2004) as observed in the intensification of the 995–1041 cm^{-1} carbohydrate bands in DOC DRIFT spectra, indicating rising availability of labile substrates under prolonged reducing conditions that favour fermentative metabolisms. In fact, the progressive consumption might be related to an increased bacteria diversity and functional activity found at flowering, as bacteria are the main consumers of labile C in soils (Wang and Kuzyakov 2024). The progressive DOC stabilisation is shown by the intensification of the 1690–1560 cm^{-1} bands in the spectra. Likewise, the ROC concentration was higher at the vegetative stage, suggesting transient accumulation of more stable carbon forms at the beginning of the flooding season. This is consistent with the well-established resilience of soil organic carbon stocks in

flooded systems, where anaerobic conditions slow decomposition processes (Chen et al. 2019; Schwarz et al. 2024). ROC spectra showed an increase in complexity related to the strengthening of amide, aromatic/quinone, and lignin-related peaks (1691–1569 cm^{-1}) in the later rice growth stages, resulting in higher values of this fraction at the end of the rice growth cycle compared to the basal timepoint. This might suggest a promotion of long-term carbon stabilisation, measured in this study to be around 7%. Past studies have shown that rice farms implementing cover crops, no-tillage or minimum-tillage, agroforestry and with balanced nutrient cycling (i.e., nitrogen fixation) can improve the ROC stabilisation and increase overall carbon sequestration (Pandey et al. 2014; Valenzuela-Balcázar et al. 2021; Monaco et al. 2022; Hu et al. 2023). RDA showed a significant influence of ROC and OM in driving the bacterial community composition of the rice stages, which serve as labile and more stable sources of nutrients for soil bacteria (Liesack et al. 2000; Chen et al. 2019). Relative stability of TOC and C/N ratio was observed across stages (R1.0–R1.3), suggesting that the soil organic matter pool was resistant to short-term fluctuations associated with rice phenology. Interestingly, changes in labile and recalcitrant carbon stocks at maturation might also be related to the significantly higher levels of taxa capable of methanotrophy, methylotrophy and hydrocarbon degradation (Ma et al. 2013; Malyan et al. 2021). The LEfSe analysis identified *Usitatibacter rugosus*, a recognised hydrocarbon consumer taxon (Bariya et al. 2025). Besides, two aerobic methanotrophic strains (i.e., *Methylocystis echinoides* str. IMET 10491 Type, *Methylobacter tundripaludum* str. SV96 Type) were signatures of the maturation stage. The activity of these bacteria is enhanced in the oxic-anoxic interfaces in paddy soils, and they can balance the CH_4 production and help mitigate this greenhouse gas emission, as well as acting as PGPB (Mohite et al. 2023; Ume et al. 2025). It has been reported that aerobic diazotrophic methanotrophs can be found in rice paddies, and they can strongly contribute to soil TKN (Cui et al. 2022). In fact, TKN showed higher values in the cover crops field, which included Fabaceae species (i.e., *Vicia villosa*), and at the end of the rice growing season (R1.3). This is of particular interest considering the absence of external fertiliser inputs and the importance of nitrogen sources for the rice crops' growth and grain development (Tirol-Padre et al. 1996). The positive impacts of cover crops with leguminouses on soil nitrogen availability in rice paddies have been highlighted in previous studies, which reported an increase in N concentration throughout the growing season (Li et al. 2021; Sugai et al. 2024). Although cover crops are known to increase the abundance of diazotrophic bacteria activity (Blesh 2018), nitrogen fixation resulted lower in the E1 field and in the basal timepoint and increased starting from the vegetative stage (R1.1), reaching the highest value in the flowering stage. Notably, at the flowering timepoint R1.2, the LEfSe analysis identified several strains of *Bradyrhizobium*, an important endophytic plant-growth-promoting bacteria (PGPB) of rice, capable of nitrogen fixation (Ding et al. 2019). On the contrary, nitrate respiration remained stable throughout the entire rice season.

In addition, iron respiration increased at the maturation stage. Although ferrous ions accumulate during flooding, spikes in unflooded fields have been described. Specifically, other studies have reported an establishment of cyclic iron oxidation and reduction in rice fields (Brune et al. 2000; Weber et al. 2006) led by redox potential changes as consequence of the flooding conditions and by the activity of specific taxa in different soil pores (Liesack et al. 2000; Ratering and Schnell 2000; Chen et al. 2022) Two strains of the microaerophilic bacteria *Sideroxydans lithotrophicus* were identified at the maturation phase, a ferrous ions oxidising bacteria, able to create a terminal electron acceptor for iron-reducing bacteria that are generally present in more anoxic microhabitat in the soil matrix (Scheid et al. 2004; Naruse et al. 2019). Iron respiration might also be involved in nutrient cycling, enhancing the nitrogen-fixing activity of methanotrophic bacteria under limited oxygen availability (Yu et al. 2024) and increasing the concentration of phosphorus in the soils. In this study, phosphorus concentration significantly increased during the rice cultivation season and reached the highest values at maturation, contrary to what was expected, as phosphorus is a fundamental element for rice growth (Gao et al. 2023). Experimental evidence shows that labile C inputs can stimulate Fe reduction and, under certain conditions, increase P solubilisation in flooded soils. Although this mechanism was not directly measured here, the cooccurrence of high DOC and increased P_2O_5 in active rice stages suggests that carbon-iron-phosphorus interactions may be contributing to the observed patterns (Lynn and Htwe 2021). Literature shows that in paddy soils, redox-active Fe oxides promote organo-mineral associations that stabilise organic C and modulate anaerobic microbial pathways; this can explain the observed enrichment of recalcitrant signatures in ROC as the season progresses (Gao et al. 2025). Considering the agricultural practices, most of the organic carbon substrate is expected to derive from the green mulching utilised in the rice fields. In addition, a meta-analysis (Kim et al. 2020) highlighted the positive direct effect of cover crop residues in enhancing the bacteria phosphatase activity, thus increasing P availability in soil. In fact, several taxa with known phosphatase enzymes were recognised, especially in the flowering stage, where rice roots exudation aims to enhance nutrient uptake to guarantee a successful growth and development of the grains (Aminurrasyid et al. 2025). These taxa included *Priestia* and *Bradyrhizobium* taxa, which are recognised as phosphate-solubilising bacteria (PSB) (Estrada et al. 2013; Phringpaen et al. 2023), and *Rhodoblastus acidophilus*, whose strains can alleviate the metal toxicity for rice and increase P availability through the release of siderophores (Khuong et al. 2017).

4.2 | Rice Grain Quality

Correlation analysis of the results showed that soil parameters serve as signalling mechanisms that regulate nutritional balance and resource distribution in rice grain. Proximate analysis indicates a starch-dominant profile, typically ranging from 70.5% to 75.5%. However, sample R1I is a significant outlier, with starch accumulation and undetectable lipid levels, suggesting a localised metabolic diversion toward carbohydrate storage. According to our results, the literature reported that a C/N ratio in favour of N leads to the accumulation of storage carbohydrates like starch, which synthesis requires a high level of nitrogen. On the contrary, structural carbohydrates are synthesised in C-rich soils (Iqbal et al. 2021; Yue et al. 2022). Besides, secondary pathways for starch allocation in grain have

been reported, where the conversion of starch to sucrose in crop stems was found in low external input of N. This mechanism moves the storage carbohydrates from other crop organs to the grain (Li et al. 2018). The correlation analysis highlighted a significant negative correlation between the fibre and starch, revealing a notable trade-off in the bran-to-endosperm ratio.

Low concentrations of glucose across all samples confirm advanced physiological maturity and efficient starch biosynthesis. In fact, this is underpinned by the negative correlation found between glucose and both CEC and bacterial Shannon index. This might reflect the positive effect of CEC and the related cations, where high CEC values are associated with an increased nutrient transport within the phloem and thus their allocation in the grains (Yang et al. 2022). In addition, higher grain quality is known to be correlated with a rich bacterial microbiota and their functional roles, as confirmed in Feng et al. (2024).

Protein content remained relatively stable, with peak values in R1F, potentially reflecting higher nitrogen availability at that site. The correlation analysis underlines a positive relation between sucrose and protein contents, since sucrose provides carbon skeletons for the biosynthesis of protein, especially SSPs (Yamagata et al. 1982; Stein and Granot 2019).

Regarding protein characterisation, the combined SDS-PAGE and MALDI-TOF analyses verified the diverse molecular weight distribution of SSP fractions. The albumin fraction displayed a broad range of proteins between 10 and 100 kDa on SDS-PAGE, with prominent bands near 50 kDa, 37–25 kDa and 15–10 kDa. MALDI-TOF showed a concentration of peaks below 10 kDa, consistent with the 10–200 kDa range reported by Jayaprakash et al. (2022) and the 24–96 kDa subunits described for Basmati rice by Chandi and Sogi (2007). The globulin fraction exhibited major SDS-PAGE bands at 50 kDa, 20 kDa, and 15–10 kDa, with MALDI-TOF detecting a peak at 18.89 kDa and a range between 13 and 16 kDa, matching the 20–110 kDa range and the 23–27 kDa α-globulin subunits reported by Ghanghas et al. (2022). The prolamin fraction presented a distinct band around 20 kDa and intense bands between 15 and 10 kDa, aligning with the MALDI-TOF peak at 18.89 kDa and the 13–16 kDa prolamin components reported by Ghanghas et al. (2022). Lastly, the glutelin fraction revealed three strong SDS-PAGE bands (37–25 kDa, approximately 20 kDa, and 15–10 kDa) along with MALDI-TOF peaks between 14 and 15 kDa, consistent with the 22.28 kDa subunit described by Chandi and Sogi (2007) and the α (30–35 kDa) and β (19–25 kDa) polypeptides identified by Jayaprakash et al. (2022). These findings confirm that glutelins remain the dominant storage proteins in rice, with albumins, globulins, and prolamins contributing to the complex molecular profile of the seed proteome. The observed compositional variability highlights the plastic response of *O. sativa* var. Chinese Originario provides a baseline for understanding how environmental factors modulate grain quality and nutritional density.

5 | Conclusion

This study aimed to characterise the dynamics of the soil bacterial diversity and soil physicochemical parameters throughout the rice cultivation period with a multidisciplinary approach. This assessment was conducted within a unique rice agroecosystem represented by the studied farm, which implements strictly organic cultivation practices in agroforested rice fields. Distinct bacterial community shifts were observed between the cover crop and rice cultivation phases, driven primarily by flooding, rhizodeposition, and key soil physicochemical properties (i.e., ROC and OM). Alpha and functional diversity constantly increased and peaked during rice flowering, directly supporting crop development, along with the presence of stage-specific taxa with known plant growth-promoting capabilities. The sustainable practices implemented in the farm successfully promoted soil carbon sequestration, maintaining stable TOC and OM, whilst essential macronutrients (i.e., N and P) were conserved at the end of the growing cycle.

Furthermore, this study establishes a baseline nutritional profile for *Oryza sativa* var. *Chinese Originario* and showed that soil parameters (i.e., CEC and C/N), in addition to bacterial diversity, dictate grain carbohydrate allocation and indirectly influence protein content.

These findings underscore that adopting sustainable practices in rice cultivation can benefit soil bacterial biodiversity and their functional roles, while also contributing to the maintenance of soil nutrients, with a direct effect on the grain quality. This is of fundamental importance, particularly in the current environmental context, as they address the requirements outlined by European and global strategies for soil conservation (e.g., Soil Monitoring Law), agroecosystem management and the Sustainable Development Goals. The findings of this study should be interpreted considering some limitations. First, the characterisation of soil bacterial microbiota and physicochemical parameters was conducted within a single rice field over one cultivation season. Due to the specific agricultural model adopted by the farm, it was not possible to identify a suitable comparative site within the same study area (i.e., Rovasenda) or one that shared identical pedoclimatic conditions and the same rice variety (*O. sativa* var. Chinese Originario). Consequently, a direct comparison between different management practices was not feasible within this experimental setup. Future research should focus on assessing the direct impact of the agroforested levees within the rice fields on the soil bacterial microbiota. Besides, metagenomic or transcriptomics approaches, leveraging on the results reported in this study, could further shed light on the functional profile of bacteria communities in strictly organic agroecosystems. Additionally, expanding this research to a larger scale is necessary. This would involve selecting multiple farms with diverse agricultural practices and different pedoclimatic conditions, while maintaining the same rice varieties, to achieve a more comprehensive understanding of the relationships between soil bacterial biodiversity, soil health, and their effect on grain quality.

Author Contributions

Martina Nasuelli: conceptualisation, samples collection, methodology, formal analysis, data curation, investigation, writing – original draft preparation. **Irene Pellegrino:** conceptualisation, samples collection, writing – review and editing. **Maria Cavaletto:** methodology, formal analysis, data curation, investigation, writing – review and editing. **Giulia Ceravolo:** methodology, formal analysis, data curation, investigation, writing – review and editing. **Annalisa Givonetti:** methodology, formal analysis, data curation, investigation, writing –

original draft preparation. **Alex Golinelli:** methodology, formal analysis, data curation, investigation, writing – review and editing. **Alessio Scarafoni:** methodology, formal analysis, data curation, investigation, writing – original draft preparation. **Fulvia Tambone:** methodology, formal analysis, data curation, investigation, writing – original draft preparation. **Elisa Bona:** supervision, project administration, conceptualisation, sample collection, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in BioDivRice_R1 at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1160448/, reference number PRJNA1160448. Raw sequences contained in fastq files are available in the Sequence Read Archive (SRA) of NCBI.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.
Supporting File 1: sae270175-sup-0001-supplementary_information_1.xlsx.
Supporting File 2: sae270175-sup-0002-supplementary_information_2.docx.