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Host selection has a stronger impact on leaf microbiome assembly compared to land-management practices

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Abstract

Introduction: Plant microbiomes contribute directly to plant health and productivity, but mechanisms that underpin plant microbiome assembly in different compartments (e.g., root, leaf) are not fully understood. Identifying environmental and management factors that affect plant microbiome assembly is important to advance understanding of fundamental ecological processes, and to harness microbiome for improved primary productivity and environmental sustainability. Irrigation and fertilization are two common management practices in Australian tree plantations, but little is known about the effects of these treatments on soil, plant host and their microbiome. Here, we investigated the impact of decade-long irrigation, fertilization and their combined application on soil, plant traits and microbiome of a *Eucalyptus saligna* plantation.

Materials and Methods: Microbial profiling of bulk soil, rhizosphere, root and leaves was performed using amplicon sequencing 16S ribosomal DNA and internal transcribed spacer (ITS) markers for bacteria and fungi, respectively, along with measurements of soil properties and plant traits.

Results: The results indicated that both management practices significantly affected soil properties and soil and root microbiomes. Irrigation increased but fertilizer treatment reduced microbial alpha diversity. However, neither irrigation nor fertilizer treatment impacted the leaf microbiome. Our findings suggest that management practices impact soil edaphic factors, which in turn influence the below-ground microbiome (soil and root), but the leaf microbiome remains unaffected. In addition, the leaf microbiome was distinct from soil and root microbiomes, and a source tracker analysis suggested that root and bulk soils only contributed to 53% and 10% operational taxonomic units of the leaf bacterial community, suggesting strong and sequential host selection of the leaf microbiome. In addition, management practices had a limited impact on leaf traits and, consequently, the leaf microbiome maintained its distinct composition.

Conclusion: These findings provide mechanistic evidence for ecological processes that drive plant microbiome assembly and indicate that host selection plays a more important role than management practices in the leaf microbiome assembly.

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KEYWORDS
fertilizer, irrigation, plant microbiome, plant traits, soil properties

1 | INTRODUCTION

The plant microbiome is considered the extended part of a plant's genome and plays a pivotal role in regulating plant fitness and functions. For example, the microbiome provides resilience against biotic and abiotic stresses in exchange for energy and habitats provided by plant hosts (Trivedi et al., 2020). This association is mutually beneficial and has coevolved over millennia (Heckman et al., 2001). The current paradigm suggests that the microbiome assembly in plants is regulated by a plethora of biotic and abiotic factors, ranging from soil-associated variables that impact soil microbiomes, such as soil pH, moisture and nutrient availability (Compant et al., 2019, Qi et al., 2018; Turner et al., 2013), to host-associated factors such as host genotype, physiology and plant compartment (Hamonts et al., 2018; Xiong et al., 2021). It is proposed that the host plant employs a filtering mechanism to select microbial endophytes (Xiong et al., 2020). In general, the host plant releases chemicals and root exudates into the soil, which serve as a signal to initiate the recruitment of specific microbes in the rhizosphere, which subsequently enter inside the plant through the root and are then transported across the plant compartments through the shoot into leaves, seeds, flowers and so forth (Liu et al., 2017; Miransari & Smith, 2014; Shade et al., 2017). The plant microbiome assembly requires mutual recognition where microbes recognize chemical signals produced by the plant to respond, while the plant immune system ensures that only recognized microbes can colonize (Fitzpatrick et al., 2020). However, relative contributions of different ecological processes (e.g., roles of host vs. environmental vs. management factors) that govern plant microbiome assembly remain poorly understood.

Environmental factors such as management practices, the presence of pathogens and climate are also known to play roles in the plant microbiome assembly (Liu et al., 2017; Ware et al., 2021). For example, land management practices such as irrigation, tillage and use of fertilizer and pesticide can influence plant growth and survival. These management practices are also known to affect soil and rhizosphere microbiome assembly (Bazany et al., 2022; Eisenhauer et al., 2022; Hartman et al., 2018; Kavamura et al., 2019; Sengupta et al., 2020; Toju et al., 2018). For example, nitrogen fertilizers were reported to reduce bacterial diversity (Yang, Zhang, et al., 2020; Zhang et al., 2018). On the other hand, fertilizer application has been observed to improve both above- and below-ground plant fungal diversity (Fornara & Tilman, 2012; Li et al., 2015). Irrigation, on the other hand, increases the soil moisture content and soil microbial diversity (Colombo et al., 2016; Zheng et al., 2017). Recent studies have demonstrated increased bacterial and fungal diversity under irrigated sites, but not under fertilization treatments (Colombo et al., 2016; Zheng et al., 2017), which was linked to an increased rate of soil functions (Colombo et al., 2016). However, the response of plant (e.g., root, leaf) microbiome to land management practices is not known. This is a major knowledge gap given the soil microbiome is considered as the main source of plant microbiome, which ultimately affects plant health and productivity (Singh et al., 2020; Trivedi et al., 2020). Such knowledge is needed to develop microbial solutions to increase plant productivity in an environmentally sustainable way (Li et al., 2022; Trivedi et al., 2022).

In this work, we aimed to examine the impact of management practices (irrigation and fertilizer) on *Eucalyptus saligna* (commonly known as Sydney Blue Gum and widely used by the plantation industry) microbiome assembly. We hypothesized that (i) management practices will affect the soil edaphic properties and soil microbiome assembly, which, in turn, can influence the diversity and structure of endophytic (e.g., root and leaf) communities; (ii) leaf endophytes will be less impacted by management practices compared to root microbiome due to host specificity and sequential host filtering. To test these hypotheses, we analysed the bacterial and fungal composition of four different niches, bulk soil, rhizosphere soil, roots and leaves of *E. saligna* under irrigation, fertilizer and irrigation plus fertilizer treatments. Further, we measured leaf traits to account for management-induced changes on traits and their impact on the leaf microbiome. To understand the implication of host-associated factors and their effect on plant microbiome assembly, we built a plant microbiome source prediction model and analysed the impact of soil and leaf-associated factors on the community assembly.

2 | MATERIALS AND METHODS

2.1 | Sampling site and sampling strategy

The field site is located at the Hawkesbury Forest Experiment (33°36′40″ S, 150°44′26.5″ E), at Western Sydney University, Hawkesbury Campus, Richmond, New South Wales. It was converted from natural to improved pastures in 1997. The site is made up of alluvial formation of sandy loam soil classified as a Chromosol, with low soil organic matter content (0.7%) and low water-holding capacity (Barton et al., 2010; Hu et al., 2015). *E. saligna* were planted in 2007 at a density of 1000 trees per hectare. The site has a total of 16 plantation plots. The mean annual temperature of the site is 16.8°C and annual precipitation is about 870 mm $year^{-1}$ (https://en.climate-data.org/oceania/australia/new-south-wales/richmond-14607/).

The experiment consisted of four different treatments: an unaltered condition, that is, without application of irrigation and fertilizers (Control-C); a fertilizer treatment (F); an irrigation treatment (I); a fertilizer × irrigation (IF). Sixteen plots were arranged in a randomized block design with four blocks each containing four treatment plots and each measuring 38.5 × 41.6 m^2. Trees were planted in 10 rows for each plot. At the time of planting, each plant was treated with an application of 50 g of diammonium phosphate blend (nitrogen [N] 15.3%, phosphorus [P] 8.0%, potassium [K] 16.0%, sulphur [S] 7.7% and calcium [Ca] 0.3%) to promote growth.

Control plots received no further irrigation or fertilizer application. For both F and IF treatments, the first application of solid N fertilizer took place in January 2008 (N 20.6%, P 3.0%, K 7.5%, S 3.8% and Ca 4.4%) at an amount equivalent to 25 kg N ha^{-1} $year^{-1}$. From October 2008 onwards, solid N fertilizer (N 21.6%, P 8.1%, K 12.0% and S 0.6%) application was initiated for F treatment at a rate of 150 kg N ha^{-1} $year^{-1}$, and IF plots were treated with liquid fertilizer 150 kg N ha^{-1} $year^{-1}$ (Nutrifeed19 and Liquid N; Amgrow Fertilizers). Both I and IF treatments received water at a rate of 1000 mm $year^{-1}$ in addition to rainfall (Zheng et al., 2017).

Sampling was conducted twice to check for temporal variations, the first in the Autumn (February 2020) and repeated in the following Spring (October 2021). In 2020, three healthy trees from each of the 16 plots were selected from the middle of the plot to avoid any edge effect. Around each tree, three soil cores (2.5 cm wide and 15 cm deep) were taken. The replicate soil samples for each tree were pooled and *E. saligna* roots were separated from the soil. Soil samples were subsampled. For soil physicochemical properties, samples were sieved through a 2-mm mesh and stored at 4°C, while other soil and root samples were stored at −20°C for DNA extraction.

Using a cherry picker, young green and healthy leaves from each of the selected trees were collected for leaf trait and nutrient analysis as well as for DNA extraction from a uniform height of 15 m. Leaves for DNA isolation were stored at −20°C.

2.2 | Soil physicochemical properties

Soil pH was determined by shaking 2.5 ± 0.01 g of soil with 12.5 mL of MilliQ water at 180 rpm (Lab Companion SK-71 Shaker) for 1 h at room temperature, after which pH was measured using a Delta pH meter (Mettler-Toledo Instruments). Soil moisture was determined by mass loss after drying at 105°C for 24 h. Soil moisture content was determined as a percentage of water per gram of dry weight of soil.

Extractable inorganic N was determined on fresh bulk soil by shaking soil with 2 M KCl (1:10 w/v) for 1 h at 180 rpm. Extracts were filtered (Whatman 42) and analysed colorimetrically for the determination of nitrate (NO_3) and ammonia (NH_4) (Keeney & Nelson, 1982) using a SEAL AQ2 Analyser (SEAL Analytical). Extractable inorganic P was determined on fresh bulk soil by shaking with Bray's reagent (0.03 M ammonium fluoride—NH_4F and 0.025 M hydrochloric acid—HCl) (Bray & Kurtz, 1945) for 1 min at 180 rpm. Extracts were filtered (Whatman 42) and analysed colorimetrically using a SEAL AQ2 Analyser (SEAL Analytical). Oven-dried soil samples were ground using a Mixer Mill MM400 grinder (Retsch), before determination of total C and N using a LECO macro-CN analyser (LECO).

2.3 | Measurement of leaf functional and chemical traits

Leaf dry weight was determined following drying at 40°C for 48 h. Leaf dry matter content (mg g^{-1}) was determined using the following formula:

LDMC = oven dried weight /fresh weight of leaves. Formula (1)

Specific leaf area (SLA; mm^2 mg^{-1}) was determined using five dried leaves for each tree and was calculated using the following formula (Pérez-Harguindeguy et al., 2013):

SLA = area /dry mass. (Formula (2).

Total C and N were obtained from oven-dried leaf samples similar to the method described for total C and N for soil.

2.4 | Surface sterilization, DNA isolation and amplicon sequencing

Rhizosphere soil was collected by washing roots with sterile Milli-Q water. Root and leaf samples were surface sterilized with 70% ethanol for 2 min, followed by 2% sodium hypochlorite for 3 min, and again with 70% ethanol for 1 min. The samples were washed twice with sterile distilled water to remove any remnants of sterilizing agents. After surface sterilization, the leaf and root samples were cut into small pieces. DNA extraction was performed on 0.25 gm of leaf, root, bulk soil and rhizosphere soil samples using the PowerSoil® DNA Isolation kit (Qiagen Inc.), following the manufacturer's instructions. The quality of DNA was evaluated using a NanoDrop spectrophotometer (NanoDrop Technologies). Microbial community profiling was performed using amplicon-based sequencing methods. For bacteria, the V5–V6 region of the 16S ribosomal RNA gene was amplified using 799F and 1193R (Kembel et al., 2014) set of primers, whereas for fungi, ITS2 region was amplified using fITS7 and ITS4 primers (Ihrmark et al., 2012) at the Next-Generation Sequencing Facility at Western Sydney University (Richmond, New South Wales, Australia) using Illumina MiSeq sequencing platform.

2.5 | Bioinformatic analysis and data processing

Sequenced raw reads were initially processed using FASTQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) for quality control, and high-quality reads having a quality score (Q) > 30 were processed using Mothur V1.46.0 (Schloss et al., 2009, 2020). Unique sequences were selected for processing and chimeric reads were identified using VSEARCH and eliminated from the analysis. Non-chimeric reads were then classified using Mothur's Bayesian classifier for taxonomic classification. Unite (V8.2) and Silva (V138.1) databases were used for fungi and bacteria, respectively. The sequences were picked at 97% similarity. All mitochondrial and chloroplast-associated classifiers were removed, and the remaining reads were classified into operational taxonomic units (OTUs). All singletons were removed from the analysis following the OTU assignment to get rid of errors due to sequencing artifacts. Altogether, 3.4 million reads for bacteria and 8.5 million reads for fungi were obtained. The processed files were then used for statistical analysis. Samples were rarefied at 400 reads for

analysis (for leaf) as a minimum library size for both fungi and bacteria. Samples were then rarefied for individual plant compartments, 1000 reads per sample for root and samples were again subset at 10,000 reads for both bulk and rhizosphere soil. Though no significant differences were observed when samples were rarefied for individual compartments (Supporting Information: Figures 1–4). Amplicon sequencing reads have been submitted to NCBI (https://www.ncbi.nlm.nih.gov/sra) and can found at Sequence Read Archive (SRA) store under Bioproject PRJNA953161.

2.6 | Statistical analysis

Files generated from Mothur were imported into R and processed using the phyloseq package (McMurdie & Holmes, 2013). Samples were subset niche-wise (bulk soil, rhizosphere, root and leaf) and analysed. The alpha-diversity index (Shannon index) was calculated using the VEGAN package (Oksanen et al., 2019), and differences in alpha diversity across treatments were visualized as box plots using ggplot2 (Wickham, 2016). A negative binomial generalized linear mix-model analysis was performed to identify major drivers of bacterial and fungal alpha diversity using the R package (R Core Team, 2021) MASS (Venables & Ripley, 2002) for each niche (Ripley et al., 2019). The beta diversity of both bacterial and fungal communities was assessed using the Bray–Curtis distance matrix and further ordinated using principal coordinate analysis (PCoA) using Vegan and ggplot2 packages. The relative contributions of variables such as treatment, time point and soil- and leaf-associated factors were evaluated with permutational multivariate analysis of variance (PERMANOVA) for every niche at 999 permutations using a Vegan package. Additionally, Spearman's correlation analysis was performed to explore the relationship and impact of management practices, soil- and leaf-associated traits and factors and microbial diversity. The result was represented in the form of a correlogram. One-way analysis of variance (ANOVA) and Tukey's post hoc test were performed to assess the effects of management practices over soil measures and leaf traits. For ANOVA, management practices were used as predictors.

Differential abundance analysis was performed using the edgeR package (Robinson et al., 2010). Samples were first divided into subsets according to plant niches—leaf, root, rhizosphere soil and bulk soil. Then, OTUs prevalent in more than 50% of samples were selected for further analysis. Subsequently, the samples were analysed across all treatments, and only significantly enriched OTUs ($p > 0.05$) were retained (Nearing et al., 2022).

The Source Tracker (Knights et al., 2011) tool from QIIME (V.1.9.0) (Caporaso et al., 2010) was used to build a source prediction model. The OTU table and associated metadata files were imported into QIIME for this analysis. This tool uses the source and sink approach to detect the possible source of OTUs based on metadata description. The following niches were selected as a source in different instances (Table 1). Using the information from Source Tracker analysis, plant microbiome source prediction models were designed for both fungi and bacteria.

TABLE 1 Description of different niches used as sources for different sink niches.

Serial no.	Niche as sink	Niche as source
1	Rhizosphere	Bulk soil
2	Root	Rhizosphere and bulk soil
3	Leaf	Root, rhizosphere and bulk soil

3 | RESULTS

3.1 | General description of the data set

We found that the phylum-level composition of the plant microbiome differed substantially between different plant compartments (Supporting Information: Figures 5 and 6). The bacterial community was represented by a total of 22,784 OTUs, divided among the leaf (3628 OTUs), the root (8021 OTUs), the rhizosphere soil (13,625 OTUs) and the bulk soil (16,384 OTUs). The bulk soil was dominated by Proteobacteria (39%), followed by Actinobacteria (24%), Firmicutes (13%) and Acidobacteria (6%). In the rhizosphere, Actinobacteria accounted for nearly 35% of the bacterial community, followed closely by Proteobacteria (31%), Firmicutes (8%) and Acidobacteria (5%). This trend was different in root endophytes, where Proteobacteria accounted for 45% of the bacterial community, followed by Actinobacteria (38%) and Acidobacteria (4%). Proteobacteria was more abundant in the leaf compared to other phyla, accounting for >62% of the bacterial community, followed by Actinobacteria (21%) and Firmicutes (8%).

The fungal microbiome, or mycobiome, had a total of 18,717 OTUs. Compartment-wise leaf had 2397 OTUS, the root had 4163, the rhizosphere had 9947 OTUs and the bulk soil had 12,487 OTUs. Contrary to the bacterial community, the phylum-level composition did not change substantially in terms of dominance across the compartments (Supporting Information: Figure 6). The leaf mycobiome was dominated by Ascomycota (80%), Zygomycota (7%) and Basidiomycota (2%). Root had a similar trend in terms of dominance, but the composition varied here, Ascomycota (71%), Zygomycota (22%) and Basidiomycota (2%). In the rhizosphere, Ascomycota accounted for nearly 78% of the fungal population, followed by Zygomycota (15%) and Basidiomycota (2%), whereas in the bulk soil, Ascomycota was 66%, followed by Zygomycota 28% and Basidiomycota (3%).

3.2 | Host selection pressure, niche specification and plant microbiome transmission

From Source Tracker analysis, plant microbiome source prediction models were developed for both bacteria and fungi. For bacteria, bulk soil provided 87% of the rhizosphere microbial community. For root endophytes, rhizosphere and bulk soils served as a source of 52% and 30% of the bacterial community, while 18% came from an unknown source. Similarly in the leaf, root accounted for 53%, rhizosphere

14% and bulk soil 10% of the bacterial community, while 23% of the source could not be determined (Figure 1a).

For fungi, similar trends of transmission were observed. Bulk soil served as a source for 84% of rhizosphere fungi. For the root microbiome, 59% of members were from bulk soil and 27% from the rhizosphere, while 14% of fungi were unique to the root. In the case of leaf, root contributed 60%, rhizosphere 19% and bulk soil provide 10% of the fungal community, while the source for 11% of leaf mycobiome remains unaccounted (Figure 1).

3.3 | Effect of land management practices on *E. saligna* microbiome

Irrigation and fertilization treatments had significant impacts on the alpha diversity of the bulk soil, rhizosphere and root microbiome of bacteria. Fertilizer treatment reduced the diversity of all compartments except for leaves (Supporting Information: Figure 7). For fungi, the alpha diversity of the rhizosphere and the bulk soil were significantly impacted by the fertilizer and irrigation treatment (Supporting Information: Figure 8). Like bacteria, fertilizer decreased alpha diversity, while irrigation increased it. PCoA and PERMANOVA analyses showed that plant compartments had a greater effect on microbial community structure than management practices, for both bacteria (Figure 1c) and fungi (Figure 1d). The leaf microbiome formed a separate cluster for both bacteria and fungi and was not significantly affected by management practice, whereas the other niches responded significantly to land management practice. The effect of the niche was comparatively higher for the fungal community ($R^2 = 0.23834$, $p < 0.001$) compared to bacteria ($R^2 = 0.20988$, $p < 0.001$) (Supporting Information: Table 1). Management practices (fertilizers and irrigation) induced a clear separation in the root, bulk soil and rhizosphere microbiome for both bacteria and fungi. However, the effect of management practice was stronger for the bacterial community ($R^2 = 0.18294$, $p < 0.001$) than the fungi community ($R^2 = 0.1132$, $p < 0.001$)) (Supporting Information: Table 1). There was no significant effect of sampling time on the microbial community ($R^2 = 0.0093$, $p > 0.5$).

3.4 | Effect of land management practices on soil measurement and leaf traits

One-way ANOVA analysis revealed that management practices significantly influenced soil physiochemical properties including soil pH and moisture along with extractable nitrate (Table 2). Fertilizer treatment reduced pH (4.78125 ± 0.23), while irrigation increased pH

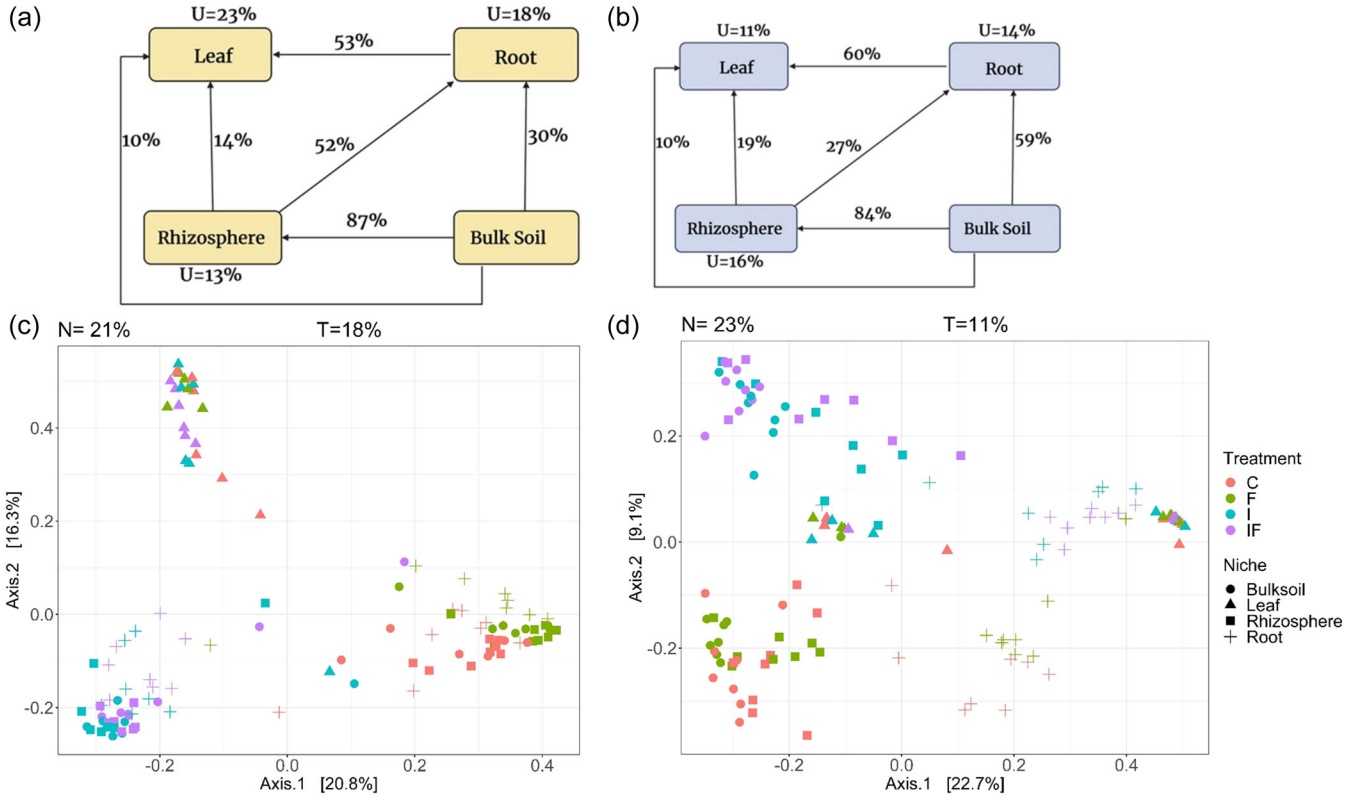


FIGURE 1 Plant source prediction model based on Source Tracker analysis for (a) bacteria and (b) fungi. U represents an unknown source or source other than the defined plant compartment. Principal coordinate analysis (PCoA) plot represents the effect of treatment (land management practices) on (c) bacterial community and (d) fungal community for different niches. C, control; F, fertilizer; I, irrigation; IF, irrigation and fertilizer. Letters on the top, niche (N) and treatment (T), are based on permutational multivariate analysis of variance (PERMANOVA) analysis (Supporting Information: Table 1).

(6.51 ± 0.31), as compared to control plots. Irrigation treatment also increased the soil moisture content (8.89 ± 0.83%) as expected as compared to control plots.

Among leaf traits, the SLA and total C content of leaves were significantly affected by treatments. Soil total C and N contents were not significantly impacted by any of the management practices, while fertilizer and irrigation–fertilizer treatment increased extractable nitrate and ammonia along with extractable phosphate content (Table 3).

TABLE 2 Results from one-way ANOVA, soil pH, soil moisture, extractable nitrate, SLA and leaf total C were significantly affected by land management practices.

	F value	*p* value
Soil measures		
Soil pH	60.6	1.10E−08***
Soil moisture (%)	141.7	6.85E−13***
Extractable nitrate (mg N L^{-1})	16.91	0.000281***
Extractable ammonia (mg N L^{-1})	2.217	0.147
Extractable phosphorus (mg P L^{-1})	0.054	0.818
Total C (%)	0.419	0.522
Total N (%)	0.21	0.91
Leaf measures		
LDMC (mg g^{-1})	1.117	0.299
SLA (mm^2 mg^{-1})	4.505	0.0422*
Total C (%)	6.073	0.0204*
Total N (%)	1.938	0.174

Abbreviations: ANOVA, analysis of variance; LDMC, Leaf dry matter content; SLA, specific leaf area; total C, total carbon; total N, total nitrogen.

The last row of the table denotes the significance level. Significance codes: ***$p < 0.001$; *$p < 0.05$.

3.5 | Interactive impact of management practices, soil and leaf properties and microbial diversity

The generalized mixed model revealed a relationship between management practice, soil and leaf properties and microbial diversity. When the effect of all factors was assessed together, the microbial diversity (Chao index) of all niches responded differentially (Table 4). For bacteria, fertilizer treatment along with soil pH and soil nitrate concentrations significantly impacted the bacterial diversity of the bulk soil negatively. However, the rhizosphere bacterial community responded to soil pH, soil moisture and irrigation treatment. Rhizosphere bacterial diversity negatively correlated with soil total N and soil ammonia. Soil total C along with irrigation and fertilizer treatment impacted the root bacterial diversity significantly. Root bacterial diversity positively correlated with soil moisture, irrigation–fertilizer and negatively with soil ammonia. Conversely, the bacterial diversity of leaves was neither significantly impacted by management practices nor by measured leaf traits.

For fungi, bulk soil diversity was correlated positively with extractable nitrate and soil total C. Similarly, many soil-associated factors such as soil moisture, extractable nitrate, ammonia and phosphorus, and soil total had significant influence over fungal diversity. Whereas root fungi were impacted by soil pH only. Unlike the bacterial community, leaf fungal diversity was significantly positively correlated with SLA. The relationship between microbial diversity and soil- and leaf-associated

TABLE 3 Mean values of various soil and leaf parameters studied across all four management practices.

Soil factors	C	F	I	IF
Soil pH	5.02 ± 0.10(a)	4.78 ± 0.23(a)	6.63 ± 0.31(b)	6.56 ± 0.14(b)
Soil moisture (%)	5.68 ± 0.79(a)	6.03 ± 0.51(a)	8.89 ± 0.83(b)	8.52 ± 0.68(b)
Extractable nitrate (mg N L^{-1})	0.173 ± 0.086(a)	0.25 ± 0.070(a)	0.3100 ± 0.16(b)	0.44 ± 0.19(ab)
Extractable ammonia (mg N L^{-1})	0.21 ± 0.065(ab)	0.422 ± 0.078(a)	0.158 ± 0.026(b)	0.197 ± 0.084(ab)
Extractable phosphorus (mg P L^{-1})	1.64 ± 0.42(c)	14.28 ± 1.25(a)	2.39 ± 0.029(c)	6.266 ± 0.96(b)
Total C (%)	0.74 ± 0.17(a)	0.87 ± 0.16(a)	0.79 ± 0.24(a)	0.89 ± 0.31(a)
Total N (%)	0.07 ± 0.011(a)	0.16 ± 0.06(a)	0.08 ± 0.03(a)	0.14 ± 0.03(a)
Leaf measures				
LDMC (mg g^{-1})	247.51 ± 25.92(a)	261.00 ± 39.42(a)	252.28 ± 25.01(a)	273.75 ± 26.40(a)
SLA (mm^2 mg^{-1})	12.84 ± 1.98(a)	13.36 ± 2.02(a)	13.52 ± 1.88(a)	14.84 ± 1.51(a)
Total C (%)	50.8 ± 084(a)	50.33 ± 0.76(a)	50.21 ± 1.03(a)	49.83 ± 0.6(a)
Total N (%)	2.45 ± 0.50(a)	2.57 ± 0.51(a)	2.61 ± 0.56(a)	2.92 ± 0.42(a)

Note: Letters in brackets denote the results obtained from Tukey's post hoc test. Plus or minus indicates standard error.

Abbreviations: C, control; F, fertilizer; I, irrigation; IF, irrigation and fertilizer; LDMC, leaf dry matter content; SLA, specific leaf area; Total C, total carbon; Total N, total nitrogen.

TABLE 4 Results from a generalized linear mixed model representing the effect of management practices, soil measures and leaf traits on microbial alpha diversity.

Niche	Soil_pH	Soil_Moisture	$Soil_NO_3$	$Soil_NH_4$	$Soil_PO_4$	Soil_Total N	Soil_Total C	I	F	IF	Leaf_N	Leaf_C	SLA	LDMC
Bacteria														
Bulk soil	0.020936*	0.139053	4.14E−14***	0.020684*	0.464565	0.312437	9.97E−11***	0.010042*	0.208256	0.000342***	NA	NA	NA	NA
Rhizosphere	0.30602	0.010085*	0.000237***	0.004879**	0.032615*	0.019301*	0.135678	0.135375	0.007368**	0.310692	NA	NA	NA	NA
Root	0.0198*	0.2114	0.6849	0.0155*	0.5621	0.1146	0.3373	0.01*	0.3093	0.024*	NA	NA	NA	NA
Leaf	NA	NA	NA	NA	NA	NA	NA	0.84196	0.48549	0.23027	0.48184	0.4578	0.6298	0.5348
Fungi														
Bulk soil	0.032*	0.472	0.046*	0.339	0.124	0.751	0.562	0.431	0.0373*	0.602	NA	NA	NA	NA
Rhizosphere	0.0363*	0.0404*	0.3855	0.5579	0.6157	0.7351	0.171	0.0466*	0.5511	0.4009	NA	NA	NA	NA
Root	0.15126	0.25365	0.68088	0.05005*	0.21853	0.88805	0.00476**	0.00708**	0.41711	0.00781**	NA	NA	NA	NA
Leaf	NA	NA	NA	NA	NA	NA	NA	0.666	0.628	0.313	0.96	0.518	0.833	0.164

Abbreviations: C, control; F, fertilizer; I, irrigation; IF, irrigation and fertilizer; LDMC, leaf dry matter content; NA, not available; NH_4, ammonia; NO_3, nitrate; PO_4, phosphate; SLA, specific leaf area; Total C, total carbon; Total N, total nitrogen.

Significance codes: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

factors were further evaluated using Spearman's correlations (Figure 2), which confirmed the above results.

3.6 | Fungal OTUs are more responsive to land management practices than bacterial OTUs at the community level

Results from differential abundance analysis revealed different trends among bacteria and fungi across various niches (Figure 3a,b). Fungal OTUs were more responsive toward land management practices. Sixteen differentially abundant bacterial OTUs (DABs) were identified in the leaf, 20 in the root, 25 in the rhizosphere and 28 in bulk soil. Two DABs were common between and root, one between leaf–rhizosphere and leaf–bulk soil and one between leaf–rhizosphere and bulk soil. The number of shared DABs between bulk soil and rhizosphere was comparatively higher, amounting to a total of 11, whereas root and rhizosphere shared 6 DABs among them (Figure 3a).

In leaves, bacterial OTUs belonging to Alcaligenaceae were comparatively more abundant in the fertilizer-treated plots, whereas OTUs from *Micromonoporineae* and *Bacillus* were more abundant in irrigation and irrigation–fertilizer-treated plots. (Supporting Information: Figure 9). In the root, OTUS from the family Acetobacteraceae was less abundant in irrigated plots as compared to fertilized and control plots. Fertilized plots also had a lesser abundance of OTUs belonging to the family Enterobacteriaceae. While on the other hand, OTUs from *Xanthomonas*, *Streptomycinae* and unclassified *Rhizobiales* were common in irrigation fertilizer combinations and irrigated plots for root, rhizosphere and bulk soil (Supporting Information: Figures 10–12). In addition, OTUs from the genus *Acidobacterium* were much more abundant in control and fertilized control plots across both rhizosphere and bulk soil. Fertilized plots were characterized by an increased OTU abundance from Acetobacteraceae and Corneybacteraceae, while irrigation led to an increased abundance of OTUs belonging to *Bacillus*.

Fungi had a higher number of differentially abundant fungal OTUs (DAFs) as compared to bacteria. In the leaf, a total of 6 DAFs were identified, for the root, there were a total of 28 DAFs, while at the same time, rhizosphere and bulk soil had 39 and 32 DAFs, respectively. Leaf had all exclusive DAFs, whereas four DAFs were shared between root–rhizosphere, root–bulk soil shared one DAF, rhizosphere–bulk soil shared seven DAFs and all three below-ground compartments shared four DAFs among them. (Details of the family-level distribution of DAFs are in Supporting Information: Materials.)

4 | DISCUSSIONS

4.1 | Land management practices significantly affect below-ground microbiomes but not the leaf microbiome

Our findings suggest that while management practices strongly influenced bulk soil and rhizosphere microbial communities, plant

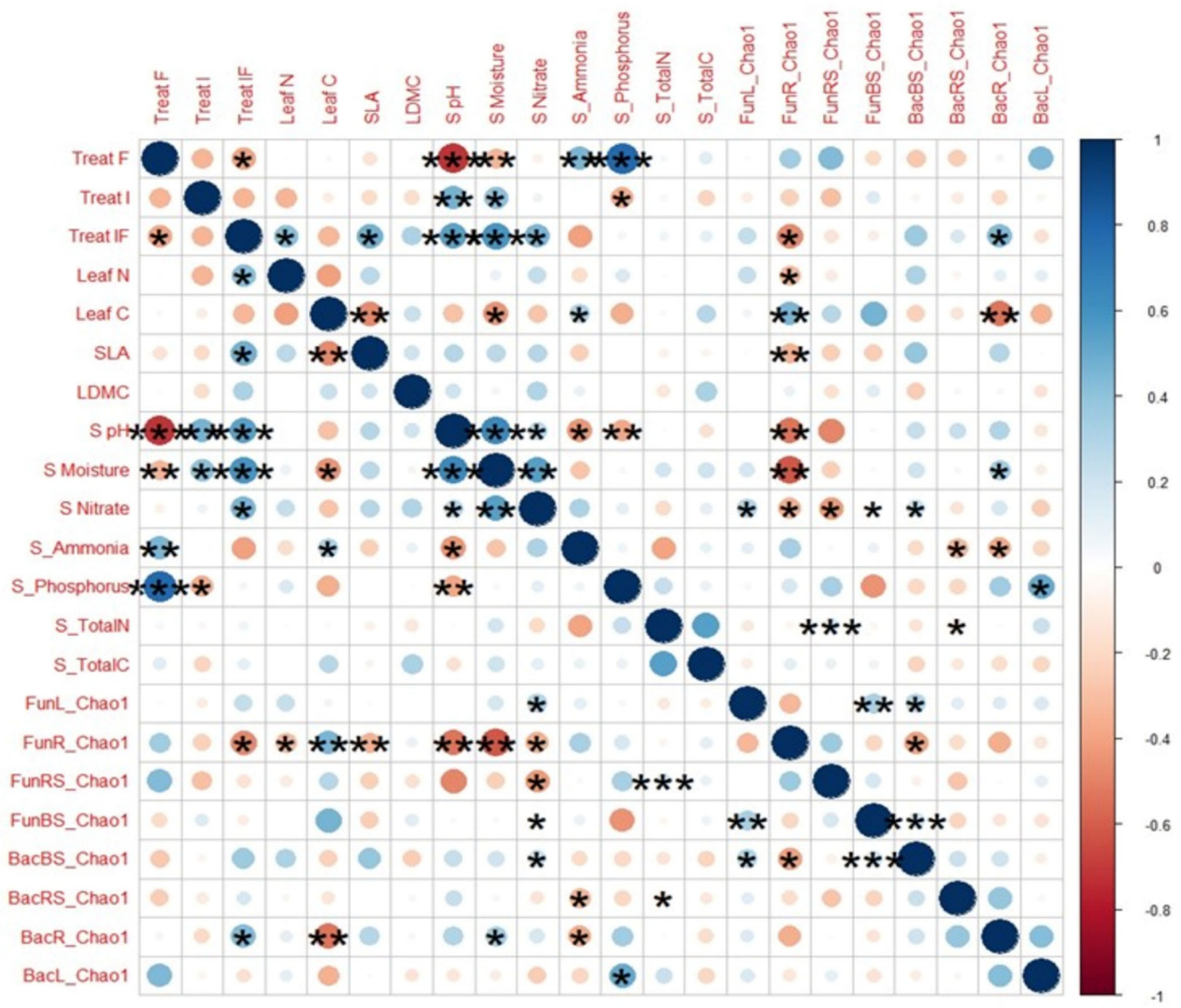


FIGURE 2 Correlogram representing Spearman's correlation among the variables and microbial diversities of different niches. Red colour indicates a negative correlation, whereas blue represents a positive correlation. Significance codes: ***$p < 0$ 0.001; **$p < 0.01$; *$p < 0.05$.

endophytic microbiome, particularly leaf microbiome, were less impacted by management practices and soil properties. This suggests a strong role in host selection. Our finding is supported by previous reports that host-associated factors generally dominate leaf microbiome assembly compared to environmental factors (Copeland & Schulze-Lefert, 2020; Gao et al., 2020; Morella et al., 2020, Sun et al., 2021; Wagner et al., 2016; Xiong et al., 2021). However, the soil serves as the main microbiome bank for the plants, and the source prediction model supported this for both root and leaf microbiome. Nevertheless, some leaf endophytes also originate through dust deposition and spore dispersal in the atmosphere. This may be a contributing factor to unknown sources in our plant microbiome source prediction model. The distinct insignificant response of leaf microbiome to management practices can be attributed to a unique environment and the hosts exert a stronger host selection mechanism (i.e., sequential filtration through rhizosphere, root and stems to reach to leaf) that potentially allow only specialized and plant recognized microbes to colonize the leaf (Compant et al., 2019; Trivedi et al., 2020). Leaf microbiome assembly is governed by host genetics, physiology and environment and their interactions. The host's immune system and filtering mechanism allow only a certain category of microbes to colonize the leaf, while soil acts as a reservoir of competent microbes that can colonize plant tissues. The internal environment of the leaves provides a unique set of conditions for microbes, and nutrient content, leaf chemistry and ecophysiological traits add up additional constraints for microbe filtration (Compant et al., 2019; Trivedi et al., 2020). For example, leaf endophytes are less exposed to daily fluctuation in temperature and external inputs compared to soil/rhizosphere microbiomes. We observed that though management practices led to changes in the total C of leaves and SLA, these changes did not alter the diversity (alpha and beta) and composition of the leaf microbiome. Additionally, we found that endophyte colonization in leaves was

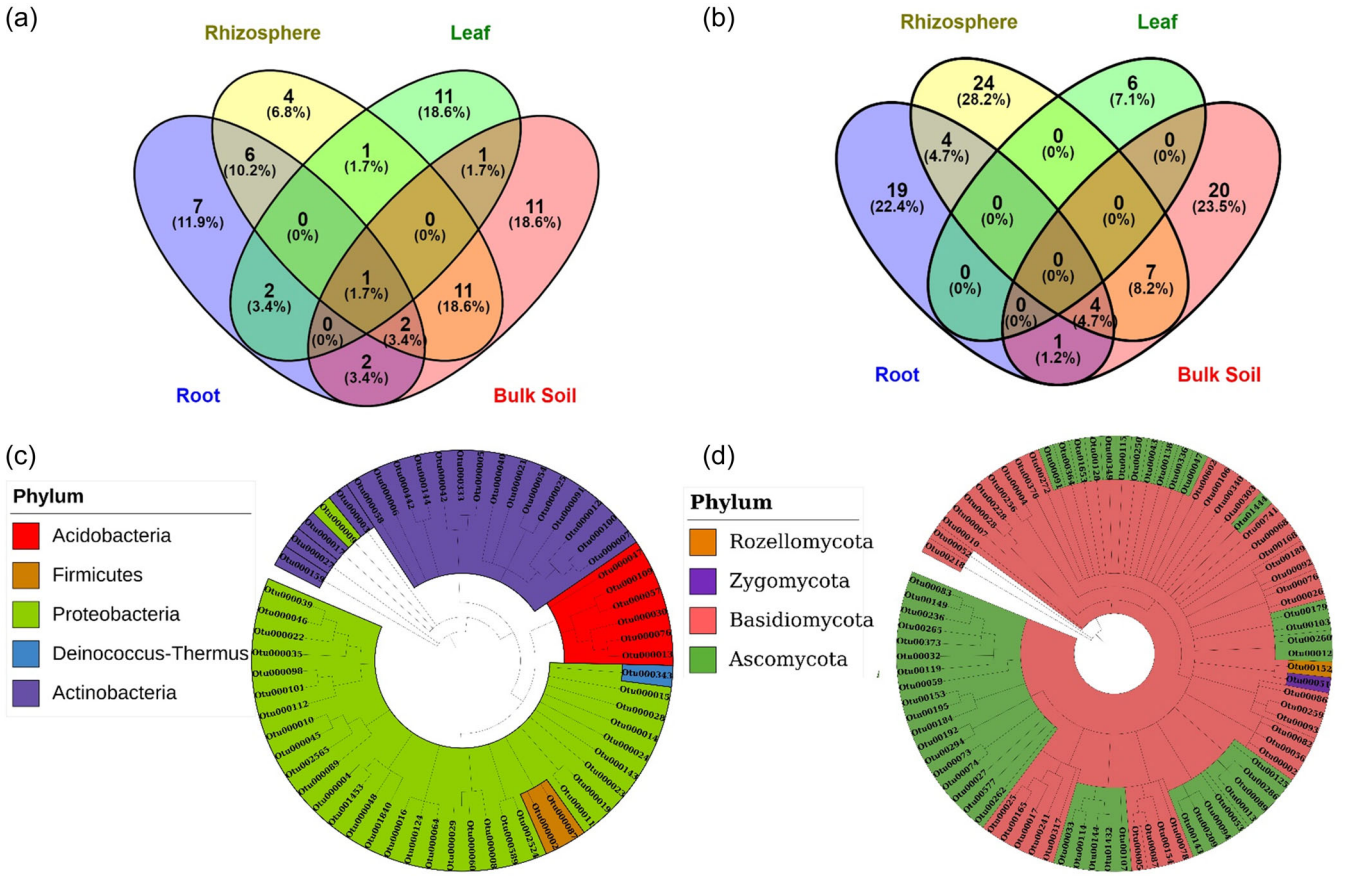


FIGURE 3 Venn diagrams representing shared differentially abundant operational taxonomic units (OTUs) across a different niche in (a) bacteria and (b) fungi. Phylogenetic representing taxonomic affiliations of differentially abundant OTUs in (c) bacteria and (d) fungi.

nonresponsive to temporal effect. This suggests that plant endophytic microbiomes are subject to stronger host selection pressure, which leads to a resilience toward regular fluctuation in the soil properties and hence the management effect is diluted as endophytic microbes move from root to leaf (Xiong et al., 2020). In other words, soil properties affect soil microbiome more than root and leaf endophytes (Brown et al., 2020; Xiong et al., 2020). Our findings suggest that leaf endophytes are less susceptible to management practices, whereas root endophytic microbes are still affected by land management practices, though the effect is much lower as compared to bulk and rhizosphere microbiomes, supporting host sequential filtration theory.

The above discussion is supported by our Source Tracker analysis that indicated that the bulk soil serves as a major source of endophyte microbes of *E. saligna*. This is supported by results from previous studies with crop species (Xiong et al., 2020, 2021). Our study provides evidence of the sequential host filtering of the microbiome in the following pattern:

Bulk soil → Rhizosphere → Root → Stem → Leaf.

Our results indicate that the host's filtering mechanism becomes stronger as it moves from soil to leaf. This explains lower microbial diversity in the root and leaf compared to bulk and rhizosphere soils. This finding is supported by previous studies which reported that the root has lower microbial diversity than the soil, while the leaf has much lower microbial diversity than the root (De Souza et al., 2016; Qian et al., 2019; Xiong et al., 2020, 2021). Further, our results suggest that while management practices have a strong impact on bulk soil microbial communities, plant endophytic microbiomes, particularly leaf microbiomes are less influenced by management practices, and soil properties suggest a strong role in host selection. These findings are supported by the findings of Wipf et al. (2021), where management practices affect the soil microbiome more than the root microbiome of *Sorghum bicolor*. Similar findings were reported for drought-affected rhizosphere more than the root microbiome (Fitzpatrick et al. 2018). Overall, our findings provide evidence that the host selection is the main driver of endophyte assembly rather than management practices (Xiong et al., 2020), where leaf endophyte assembly was least affected by treatment as compared to bulk and rhizosphere soil microbiomes of wheat, maize and barley crops.

On the other hand, a significantly high number of differentially abundant OTUs were observed for bacteria and fungi in response to management practices, especially in below-ground compartments. The shared differentially abundant OTUs among different niches were quite low. For example, leaf fungi did not share any DAFs with

other compartments, and similarly, shared bacterial DABs between the leaf and other compartments were also low, supporting the critical role of host selection in leaf colonization (Hamonts et al., 2018; Trivedi et al., 2020; Xiong et al., 2021). The dominance of genera such as *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Pantoea*, *Propionibacterium* and *Streptomyces* in the leaf supports the argument that host selection and microbes with specific abilities can colonize the leaf. Bacteria from some of these genera are known to colonize the leaves of various plant species, such as rice (Yang, Cheng, et al., 2020), maple trees (Wemheuer et al., 2019), wheat and faba beans (Granzow et al., 2017). Our study advances this understanding by providing evidence from a long-term study that leaf microbiome assembly is comparatively more resistant to management practices as compared to soil and root microbiome.

The below-ground microbiome (i.e., bulk soil, rhizosphere and root) responded significantly to land management practices. Soil-associated factors are known to serve as the major driving factor of soil microbiome assembly (Hossain & Sugiyama, 2011; Xue et al., 2018) and rhizosphere microbiome likely due to direct contact with bulk soils (Xiong et al., 2020, 2021). Soil fertilization and irrigation impact soil edaphic properties, nutrient availability and microbial diversity by enhancing microbial activities and host productivity, which in turn leads to increased rhizodeposition and litter deposition. Though the rhizosphere effect plays an important role in shaping the rhizosphere microbiome (Compant et al., 2019; Li et al., 2022), these niches are more susceptible to change in the management practices than the above-ground compartments, as they are exposed to treatments and associated physicochemical changes. Our results also support that in this environment (dryland), the microbiome assembly is driven by water availability than nutrient enrichment through fertilizer treatment (Colombo et al., 2016; Hu et al., 2015).

4.2 | Application of fertilizer has a negative impact on microbial diversity in the soil and roots, while irrigation has the opposite effect

The bacterial community (both diversity and composition) responded more strongly to management practices than the fungal communities. The opposite was found for the effect of niche over microbiome assembly, as the effect of the niche was higher for the fungal community compared to the bacterial community. Fungal communities exhibited more resilience toward the management practices as they have a complex genome and cellular structure as compared to bacteria. For example, fungi can acquire nutrients through mycelia, which enables them to sustain in any temporary alteration in the environment, whereas most bacteria have comparatively rapid life cycles, which makes alteration in bacterial composition an easy task as compared to fungi. Similar observations were reported by De Vries et al. (2018), in which they found that bacterial networks were less stable than fungal networks under drought conditions. Similar findings were reported by Jiao et al. (2021), where soil bacterial communities and networks were more affected than their fungal counterparts. Fungi have been reported to fluctuate in soil moisture and nutrient content as they can acquire nutrients through multiple sources (Hodge et al., 2001; Rousk et al., 2009).

Among the management treatments, the fertilizer treatment led to decreased soil and root bacterial diversity, whereas this trend was not evident for the fungal community. Conversely, irrigated plots had higher microbial diversity compared to the controlled plots. This trend supports previous studies from the same site (Colombo et al., 2016; Zheng et al., 2017) and from other regions (Frene et al., 2022; Hartman et al., 2018). Interestingly, the clusters from PCoA analysis and ANOVA results on the Shannon diversity index suggested that across all below-ground microbiomes, irrigation alone and irrigation–fertilizer treatments had similar profiles, suggesting a stronger impact of irrigation on microbial community compared to fertilization treatments (Li et al., 2021). This can be explained by the fact that fertilizer treatment and irrigation have opposite effects on the soil edaphic properties as well soil nutrients. Increased soil pH and soil moisture due to irrigation has provided a comparatively better niche for microbes, hence the diversity was comparatively higher in these plots. Water availability has been reported to have a positive impact on biological processes and microbial functions in arid regions and particularly at this site by previous studies supporting the results (Colombo et al., 2016; Hu et al., 2015: Martins et al., 2015). The relative abundance of copiotrophs belonging to families such as Bacillaceae, Hyphomicrobiaceae and Caulobacteraceae increased in the irrigated plots, supporting that this ecosystem is limited by water and less so for nutrient availability.

Fertilizer treatment interferes with soil microbiome assembly in multiple ways. Fertilizers typically contain high levels of nitrogen, phosphorus and potassium that can lead to an increase in the growth of certain bacteria and fungi that proliferate in high nutrients environments (Alori et al., 2017), while suppressing the growth of other microbes that are sensitive to high nutrient conditions. In addition to the decreased pH, fertilizer treatment led to low soil moisture content and comparatively very high amounts of extractable ammonia and phosphate. This explains why there was reduced microbial diversity given that it promoted the growth of acidophiles. For example, in bacteria, fertilizer treatment was associated with an increased abundance of oligotrophs belonging to families such as Acidobacterium, Acetobacteraceae, Catenulisporineae and Edaphobacter in both soil (bulk and rhizosphere) and root microbiome families. The abundance of these families has been reported to negatively correlate with soil pH (Hegyi et al., 2021; Lauber et al., 2009; Rousk et al., 2010).

In the fungal community, the fertilizer treatment led to an enrichment of saprotrophs such as *Helicoma* sp., *Mortierell* sp. and *Trechispora* sp., along with ectomycorrhizal fungi like *Cenococcum* sp. This supports the finding of Zheng et al. (2017), who reported that management practices enriched saprotrophic and mycorrhizal fungi. Especially *Mortrierella* sp. has been reported to thrive in fertilizer-treated low-pH soil as it can produce acid during metabolic processes (Han et al., 2010; Wen et al., 2020).

Overall, our study demonstrates that leaf endophyte diversity is largely resilient to management practices, such as irrigation and

fertilization, with only minor changes in community composition observed. In contrast, the diversity and community structure of below-ground microbiomes were significantly impacted by management practices. These results suggest that the leaf microbiome is primarily shaped by host selection and is protected from external fluctuations in physicochemical properties, while bulk and rhizosphere soils are strongly responsive to management practices. Therefore, future studies on microbial response to environmental factors should explicitly consider compartment niches, as microbiomes of different compartments can have a differential impact on host health and functions.

AUTHOR CONTRIBUTIONS

Pankaj K. Singh, Eleonora Egidi, Catriona A. Macdonald and Brajesh K. Singh conceived the idea. Pankaj K. Singh carried out experiments and data analyses with help from all and wrote the first draft of the manuscript, with everybody contributing to improving it.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

DNA sequence data are available from NCBI (https://www.ncbi.nlm.nih.gov/sra) Sequence Read Archive (SRA) under Bioproject PRJNA953161. Other data are available upon request.

ETHICS STATEMENT

Ethics statement is not applicable to this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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