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Reduced Tillage Intercropping With *Thymus hyemalis* Reshapes the Rare Soil Microbiome and Co-Occurrence Networks in a Semi-Arid Almond Orchard

Jessica Cuartero¹ | Beat Frey¹ | Margarita Ros² | Jose Antonio Pascual² | Virginia Sánchez-Navarro^{3,4} | Loredana Canfora⁵ | Onurcan Özbolat^{3,4} | Marcos Egea-Cortines^{3,4} | Antonella Lamontanara⁵ | Carolina Boix-Fayos² | Maria Martinez-Mena² | Maria Almagro⁶ | Elvira Diaz-Pereira² | Raul Zornoza^{3,4}

¹Rhizosphere Processes Group, Swiss Federal Institute for Forest, Snow, and Landscape Research WSL, Birmensdorf, Switzerland | ²Departamento de Conservación de Suelos y Agua, Centro de Edafología y Biología Aplicada del Segura – Consejo Superior de Investigaciones Científicas, Murcia, Spain | ³Department of Agricultural Science, Universidad Politécnica de Cartagena, Paseo Alfonso XIII 48, Cartagena, Spain | ⁴Instituto de Biotecnología Vegetal (IBV), Campus Muralla del Mar, Edificio I + D + I, Universidad Politécnica de Cartagena, Cartagena, Spain | ⁵Research Centre for Genomics & Bioinformatics, Council for Agricultural Research and Economics (CREA), Fiorenzuola d'Arda (PC), Italy | ⁶IFAPA, Andalusian Institute of Agricultural and Fisheries Research and Training, Granada, Spain

Correspondence: Jessica Cuartero (Jessica.cuartero@wsl.ch)

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ABSTRACT

Environmentally friendly farming practices are gaining interest, and intercropping is a promising option to protect soil health, yet its effects on orchard microbiomes and functions remain underexplored. In this study, we assessed a 3-year intercropping assay of *Capparis spinosa* (D1) and *Thymus hyemalis* (D2) with almond and reduced tillage in a semi-arid Mediterranean orchard. Soil bacterial and fungal communities were profiled by metabarcoding (16S rRNA gene for bacteria/archaea and ITS region for fungi), functional potential was inferred from gene-abundance proxies and co-occurrence networks were constructed to evaluate alterations in microbial connectivity. Both intercropping treatments along with reduced tillage altered the soil microbial composition and enriched plant growth-promoting genera (e.g., *Amycolatopsis*, *Bosea*, *Dyadobacter*, *Janthinobacterium*, *Leifsonia*) and AMF (*Glomeromycota*), but effects were stronger in D2. Reduced tillage and intercropping with *T. hyemalis* increased the richness of less abundant bacteria, increased the abundance of functional potential linked to nitrogen fixation, cellulolysis, and fermentation. It also reconfigured co-occurrence networks toward higher connectivity with more module hubs – patterns consistent with greater functional stability. Overall, *T. hyemalis* intercropping with reduced tillage enhanced beneficial soil microbiota and higher functional potential than *C. spinosa*. Long-term monitoring is essential to ensure that the beneficial effects of intercropping persist and translate into real agronomic gains. For farmers, intercropping with thyme offers a valuable strategy to improve soil health and potential functionality while also generating additional income through products like essential oils—all without compromising almond orchard productivity. This dual benefit supports more sustainable and profitable farming in semi-arid regions.

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1 | Introduction

Intensive farming methods, characterised by single-crop cultivation, the overuse of chemical inputs, and high tillage intensity, have led to extensive soil deterioration. These intensive practices have decreased soil health, resulting in a decrease of productivity, diminished soil microbial biodiversity, and heightened susceptibility to pests and diseases (Bedolla-Rivera et al. 2023; Cuartero et al. 2025; Gržinić et al. 2023). Furthermore, these intensive practices have altered the soil's physical and chemical properties (e.g., reducing organic matter; Lal 2020) and increased greenhouse gas emissions (e.g., CO₂ emissions; Chataut et al. 2023), thereby contributing to climate change.

Almond thrives in a wide range of soils and environments (De Boni et al. 2022), including rainfed conditions where other crops may underperform, and is recognised for its drought resilience, making it popular in dryland regions, which are characterised by low rainfall, high temperatures, and occasional extreme events like cold-drop storms. Conventionally, almond is cultivated as a monoculture with tillage – (Martínez-Mena et al. 2013) – practices that can accelerate soil degradation and reduce soil resilience. This loss of resilience is especially concerning because almonds are also vulnerable to extreme weather, water shortages, and episodic events, pressures expected to intensify with climate change in the coming years (Freitas et al. 2023). This highlights the urgent need to develop innovative agricultural systems that enhance crop resilience, improve water use efficiency, and safeguard long-term soil health in the face of increasingly challenging environmental conditions.

Intercropping, an agricultural practice involving the simultaneous cultivation of two or more crops, is a promising solution to restore soil health and improving agricultural sustainability (Weih et al. 2022). By increasing plant diversity, intercropping can significantly enhance soil structure, suppress weeds, improve nutrient cycling and even crop yield (Cuartero et al. 2022). A key mechanism behind these benefits is the stimulation and diversification of soil microbial communities (Dang et al. 2024), which drive carbon and nitrogen cycling through organic-matter decomposition, releasing plant-available nutrients, and ultimately modifying soil physico-chemical properties such as the phylum acidobacteria (Özbolet et al. 2023). However, achieving these positive shifts is more challenging in dryland soils because of their inherent instability – low organic matter, poor water-holding capacity, and high susceptibility to erosion (Srinivasarao et al. 2023). Therefore, the choice of alternative crops is nontrivial under dryland conditions; scarce water and nutrient availability heighten interspecific competition, potentially depressing the principal crop, exacerbating nutrient depletion, and elevating pest pressure (Stott et al. 2023). In this context, thyme (*Thymus hyemalis*) and caper (*Capparis spinosa*) are promising intercrops for almond orchards because they are native to the Mediterranean, tolerate semi-arid conditions with low water and nutrient availability, and provide additional products – essential oils from thyme and edible buds from caper—that can increase farm income (De Martino et al. 2015).

Changes in soil physical and chemical properties are slow and highly variable, unfolding over months to, in some cases,

hundreds of years. In this context, microorganisms offer a rapid window into soil change: their small size and short generation times make them far more sensitive to environmental shifts than bulk soil properties. Changes in microbial populations and activities may therefore function as an excellent indicator of change in soil health (Joos et al. 2023), and in some instances these changes can even precede detectable shifts in soil physical and chemical properties. Although microbial communities are often studied as a whole, including all microorganisms within the same environment, some studies have demonstrated that the abundance of specific taxa is closely linked to their functional role in soils (Zhou et al. 2022). The most abundant microorganisms are typically associated with biomass production and energy flow, while the less abundant – often referred to as “rare” – contribute to species richness and enhance ecosystem resilience (Zhou et al. 2022). Also, different studies have reported that certain microbial members can act as key organisms within modules and across the entire community, indicating their fundamental role in maintaining ecosystem stability (Coyte et al. 2015; Cuartero et al. 2024). These organisms often serve as hubs in microbial networks, orchestrating interactions among diverse microbial groups. This suggests that to fully understand the effects of intercropping on soil microbial communities, it is necessary to study them in depth. This involves considering not only significant changes in taxa abundance but also examining their roles within the community and their functional contributions.

Advances in molecular techniques such as DNA metabarcoding enable comprehensive analyses of microbial diversity, taxonomy, and functional potential, facilitating detailed assessments of soil microbial communities. In this study, we evaluated for the first time the intercropping of thyme and caper with almond trees under Mediterranean climate conditions by analysing soil microbial communities, their potential functional roles, and microbial co-occurrence. To achieve this, we set three primary objectives: (1) to evaluate the impact of reduced tillage and intercropping on the diversity and relative abundance of soil bacterial and fungal communities; (2) to assess shifts in the functional potential of soil microbiomes and identify microbial taxa indicators associated with each treatment; and (3) to analyse changes in microbial co-occurrence networks and identify keystone taxa for each treatment.

2 | Materials and Methods

2.1 | Study Site and Experimental Design

The experiment was set up in a commercial almond orchard (*Prunus dulcis* [Miller] D. A. Webb) in Murcia, SE Spain, with 540 trees in the 2.63-hectare *P. dulcis* orchard. They were planted in 1950 at a spacing of 7 m by 7 m. Comprehensive information about the experimental site is thoroughly described by Sánchez-Navarro et al. (2022). The average annual temperature is 18°C, and the average annual precipitation is 280 mm, indicating a semiarid Mediterranean environment. The potential evapotranspiration rate is 1300 mm year⁻¹. The soil is classified as a Calcaric Eutric Regosol (IUSS Working Group WRB 2014). It has a silt loam texture (containing 9%, 65% and 26% of sand, silt, and clay, respectively), 59% CaCO₃, a pH of 8.4, a bulk density of 1.30 g cm⁻³, 3.86 g kg⁻¹ of total organic

carbon, 0.60 g kg⁻¹ of total nitrogen, and a cation exchange capacity of 14.5 cmol⁺ kg⁻¹ at a depth of 0–30 cm Ap Horizon (arable layer).

A randomised block design was implemented with three different treatments, each treatment replicated three times ($n = 3$). Experimental plots measured 210 m² and consisted of rows containing five trees. The treatments were: (1) almond monoculture with conventional tillage in all plot surface (chisel ploughing 2 times yr⁻¹ at 20 cm depth) (MC); (2) almond plantation with reduced tillage (rototiller (Lander 180, Spain) 2 times yr⁻¹ at 20 cm only 1.5 m around each tree trunk), with no till in the rest of the alley, and diversified with *Capparis spinosa* L. (caper bush) as alley crop (D1); and (3) almond plantation with reduced tillage as explained in D1 and diversified with the aromatic species *Thymus hyemalis* L. (winter thyme) as alley crop (D2). On January 10, 2018, *C. spinosa* seedlings that were purchased from a nearby nursery were manually planted at 3.5 m x 3.5 m. On May 11, 2018, *T. hyemalis* seedlings, purchased from a nearby nursery, were manually planted throughout the alley, except for the area tilled surrounding each tree, at a spacing of 1 m x 0.5 m. It should be noted that these two plants grow spontaneously and are part of the local flora in the study area, indicating that they are well adapted to the specific local conditions. Additionally, their selection was informed by participatory systems involving farmers, co-operatives, and scientists to identify diversification options in almond orchards that provide economic benefits, with the ultimate goal of achieving diversified agriculture with low inputs, both in terms of organic matter and irrigation. All the plots were maintained in rainfed conditions. No herbicides were applied in the MC treatment, weed control was carried out through tillage. In treatments D1 and D2, no tillage was performed in the alley. As a result, the D1 and D2 plots were primarily colonised by *Artemisia herba-alba* Asso, *Piptatherum miliaceum* (L.) Coss, *Dittrichia viscosa* (L.) Greuter, *Phagnalon saxatile* (L.) Cass., *Sonchus tenerrimus* L., and *Diplotaxis erucoides* DC. However, due to the low weed density – reflected in an average invasive-to-caper-thyme ratio of 3:2 (see Supporting Information Figures S1–S3) – no negative impact on alley crop growth was observed.

2.2 | Soil Sampling and Soil Physicochemical Methods

On March 2021, after 3 years of the implementation of intercropping, soil was sampled using an auger at a depth of 0–10 cm. The layer where management-induced changes are most intense, as reported in previous studies on tillage, cover crops and green manures in Mediterranean and semi-arid agroecosystems (Özbolat et al. 2023). To minimise field variability, three composite soil samples were collected from the alley of each plot. Each composite comprised five randomly selected subsamples from within the plot, yielding nine composite samples per treatment on which measurements were performed. Bulk soil samples were collected in the alleys between the trees, 2 m from the tree trunks. In D1 and D2, soil was taken between 2 plants of *T. hyemalis* (0.30–0.50 m from each plant) and between 2 plants of *C. spinosa* (1.00–1.50 m from each plant), to avoid the direct influence of the rhizosphere. The bulk soil samples were divided into two aliquots,

one of which was stored in a cool box with ice for biological analyses, nitrate, and ammonium, and the other was kept at room temperature for physicochemical analyses. The samples were brought to the lab immediately. For physicochemical analyses, the soil was allowed to air dry for a week. To determine the aggregate size distribution, one fraction of the dried soil was sieved at < 8 mm, and the remaining analyses used a second fraction that was sieved at < 2 mm. The soil was sieved for microbial community analyses, nitrate, and ammonium at a size of less than 2 mm once in the laboratory and kept at –20°C. Soil pH was measured in deionized water at a 1:2.5 (wt/vol) ratio. Total nitrogen (TN) and total organic carbon (TOC) contents (g kg⁻¹) were determined using an N/C elemental analyser (Flash 1112 EA, Thermo Finnigan, Bremen, Germany) after the removal of carbonates with 1 M HCl. The remaining analyses – including electrical conductivity, available phosphorus, particulate organic matter, cation exchange capacity water content, and micro- and macronutrient concentrations – were performed following established protocols (Almagro et al. 2023; Sánchez-Navarro et al. 2025; Sansupa et al. 2021). For genes involved in nitrification and denitrification, we targeted *amoA*, *nirK*, and *narG* using the following primer sets: forward *amoA*1F, *nirK*876F, *narG*1960m2F; reverse *amoA*2R, *nirK*1040R, *narG*250m2R. Detailed information about PCR details and primer sequences can be found in (Sánchez-Navarro et al. 2025). Detailed information on the effects of intercropping and tillage on soil properties and crop yield can be found in Almagro et al. (2023) and Sánchez-Navarro et al. (2022).

2.3 | DNA Extraction, PCR Analysis and Pathogen Detection

Soil DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, Germany). The quality of the extracted DNA was assessed by calculating the A260/A280 and A260/A230 ratios using a NanoDrop One (ThermoFisher Scientific, USA). DNA concentration was measured using a Qubit fluorometer (ThermoFisher Scientific, USA) as previously described in (Cuartero et al. 2021; Fritze et al. 2024). The bacterial community composition was studied through next-generation sequencing of the 16S rRNA hypervariable regions using the Ion Torrent Personal Genome Machine (PGM, London, UK). Sequencing required a minimum DNA input of 18 µL at a concentration of ≥ 1.5 ng/µL, meeting spectrophotometric quality standards (A260/A280 = 1.8–1.9 and A260/A230 ≥ 1.9). The 16S rRNA gene hypervariable regions were amplified using the Ion 16S Metagenomics Kit (Thermo Fisher Scientific), which includes two primer pools targeting the V2–4–8 and V3–6,7–9 regions. According to the manufacturer, the combination of the two primer pools allows for sequence-based identification of a broad range of bacteria. Each 30 µL PCR reaction contained 15 µL of 2X Environmental master mix, 3 µL of primer pool (10X), and 2–12 µL of DNA template. Thermal cycling conditions consisted of an initial denaturation at 95°C for 10 min, followed by 18–25 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 20 s, with a final extension step at 72°C for 7 min. The amplified products were processed using the Ion Xpress Plus Fragment Library Kit and barcoded with the Ion Xpress Barcode Adaptor 1–96 Kit (Thermo Fisher Scientific). Purification steps between

incubation and amplification reactions during library preparation were performed using DynaMag magnetic racks (Thermo Fisher Scientific) in conjunction with an AMPure XP Purification Kit (Beckman Coulter). The size and concentration of the final libraries were assessed using an Agilent 2100 Bioanalyzer system with the Agilent High Sensitivity DNA kit (Agilent, Santa Clara, CA). Sequencing templates were prepared using an Ion OneTouch System and an Ion PGM Hi-Q View OT2 Kit (Thermo Fisher Scientific). Sequencing was carried out on the Ion Torrent PGM platform using the Ion PGM Hi-Q View Sequencing Kit, following previously an established protocol (Cuartero et al. 2021; Fritze et al. 2024).

For the preparation of fungal ITS1 sequencing libraries, a modified version of the method proposed by Smith and Peay (2014) was used. PCR amplification was performed with locus-specific primers (ITS1f-ITS2) attached to Illumina adaptors to generate sequencing libraries. The primers used for fungal ITS region amplification was as follows: Forward Primer: 5' GGAAGTAAAAGTCGTAACAAGG, Reverse Primer: 5' GCT GCGTTCTTCATCGATGC. The reverse primers were barcoded for multiplexing using 12-base Golay barcodes (Caporaso et al. 2012). The PCR amplification reaction was conducted in a final volume of 30 µl, which included 3 µl of 10X buffer, 0.7 µl of each primer (10 mM), 0.9 µl of 50 mM MgSO₄, 0.6 µl of 10 mM dNTP, and 0.12 µl of Invitrogen Platinum Taq DNA polymerase High Fidelity (Cat N° 11,304-011). The thermal cycling conditions consisted of an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 1 min, and extension at 72°C for 1 min. A final extension was carried out at 72°C for 10 min. Prior to sequencing, the amplicon libraries were combined with 10% PhiX control library to enhance sequence diversity. Fungal libraries were sequenced on the Illumina MiSeq platform, producing paired-end reads of 300 bp. Fungal pathogen detection by qPCR was performed on soil DNA using a 7500 Fast Real-Time PCR System with hydrolysis probes (15 µL reactions: 1× TaqMan Universal Master Mix II no UNG, 0.3 µM primers, 0.1 µM probe, 0.1 mg mL⁻¹ BSA, 3 µL template; cycling: 95°C 10 min; 40×[95°C 10 s, 60°C 40 s]; final 50°C 2 min), in triplicate per sample. Targets were SBP panels for *Xanthomonas arboricola* pv. *pruni*, *Agrobacterium tumefaciens*, *Xylella fastidiosa*, and *Monilinia laxa* using the following kits: SCP0059, SCP0088, SCP0060, SCP0020 respectively from MICROGAIA PHYTO-DIAGNOSTICS. The raw sequencing data (16S and ITS) are available from the European Nucleotide Archive (ENA) under the project accession number PRJEB82303.

2.4 | Bioinformatic Data Analysis

For bacteria, barcodes, adaptors, and primers were removed from raw sequences using the BaseCaller application. Single-end sequences from Basecaller were denoised with ACACIA (Bragg et al. 2012). For fungal, raw reads were processed with Trimmomatic (Bolger et al. 2014) to eliminate adaptors and low-quality reads using a sliding window of 20 bp and a quality cutoff of 20. Paired-end reads were merged using Pear (Zhang et al. 2014). Then, sequences were imported into QIIME2 v2019.1.0 (Bolyen et al. 2019). The DADA2 algorithm was then applied to denoise the sequences, truncating them at an average quality score of Q > 25 (Callahan et al. 2016). Using VSEARCH

(Rognes et al. 2016), sequences were clustered into Operational Taxonomic Units (OTUs) with a similarity threshold of 97%. Low-confidence OTUs were excluded, and the remaining sequences were classified using the 'classify-consensus-vsearch' command with the SILVA 132 database as a reference for bacteria and UNITE database version 8.2 dynamic release (Nilsson et al. 2019) for fungi. To normalise the sampling depth and make the treatments comparable, we rarefied the sequence datasets to match the soil sample with the lowest number of reads after quality filtering, which consisted of 19,093 reads for bacteria and 4039 reads for fungi. The sampling depths are illustrated in Supporting Information Figure S4.

2.5 | Statistical Analysis

Before the statistical analysis and after rarefaction, the OTU tables for bacteria and fungi were split into two different databases: one containing the most abundant bacteria (MAB) and fungi (MAF). Based on previous research that reported the rare biosphere as taxa with relative abundances below 0.1% or 0.01% (Pedrós-Alió 2012) and findings by Wu et al. (2021), who observed similar results and conclusions using both thresholds in soil samples, we decided to use a threshold of 0.1% to classify the most abundant taxa (Carrascosa-Robles et al. 2024; Cuartero et al. 2022; Qiu et al. 2024), while the remaining taxa were considered less abundant bacteria (LAB) and less abundant fungi (LAF). Only OTUs that appeared in 40% of the samples were retained using the 'filter_taxa' function from *microeco* package. All the statistical analysis were performed using the R version 4.2.3 (R Core Team 2023) and most of the functions were executed through *microeco* v.0.16.0 (Liu et al. 2021) package. Due to the low number of field replicates ($n = 3$), we employed the non-parametric Kruskal-Wallis test to assess significant differences between the treatments, followed by pairwise comparisons using the Dunn's test, performed with the functions 'kruskal_test' and 'dunn_test' from the *rstatix* v0.7.2 (Kassambara 2023) package. These non-parametric methods are particularly well-suited for small datasets, as they do not rely on assumptions of normality or homogeneity of variance, thus providing a statistically robust approach under conditions of limited sample size. To ensure the reliability of our findings, p -values were adjusted for multiple comparisons using the Benjamini-Hochberg correction.

Alpha and beta diversity were calculated using the functions 'trans_alpha' and 'trans_beta' from the *microeco* v.0.16.0 package. After testing for homoscedasticity, statistical differences in beta diversity were assessed using PERMANOVA through the 'adonis' function from the *vegan* v2.6.4 (Oksanen et al. 2022) package, with the Bray-Curtis distance metric and with 999 permutations. The relationship between the most and less abundant microorganisms (both bacteria and fungi) and soil physicochemical properties was assessed using Redundancy Analysis (RDA) performed using the 'rda' function from the *vegan* package. The significance of soil physicochemical properties effects was tested using both forward and backward selection methods through the 'ordiR2step' function from the *vegan* package. The RDA model included the variables obtained through 'ordiR2step' as explanatory variables and the Hellinger-transformed microbial community matrix as the response

variable. Statistical significance of the constrained axes was assessed using ANOVA on the RDA object with 999 permutations. To identify distinct ASV classified at genera level between treatments, a Venn diagram was created. The diagram was generated using 'ggVennDiagram' function from *ggVennDiagram* package v1.5.2 (Gao et al. 2024). Microbial indicators by treatment were identified via indicator species analysis using *multipatt* function from *indicspecies* package v1.7.15 (Cáceres and Legendre 2009).

To evaluate the effects of intercropping on co-occurrence networks, we created three microbial networks (combining both bacteria and fungi) and analysed the differences using the 'trans_network' function from the *microeco* package (Cuartero et al. 2024). Correlations between taxa were calculated using Pearson's correlation. The correlation threshold was optimised using Random Matrix Theory (RMT). Only significant correlations ($p < 0.05$, adjusted using FDR) were included in the network, and modules were identified using the fast greedy clustering algorithm. The differences were tested using the *meconetcomp* v0.2.0 (Liu et al. 2021) package with three replicates per treatment. To identify connectors, we followed the classification criteria described by Olesen et al. (2007). Then, microbial role was categorised into peripherals ($Z_i < 2.5$; $P_i < 0.62$), connectors ($Z_i < 2.5$; $P_i \geq 0.62$), module hubs ($Z_i \geq 2.5$; $P_i < 0.62$) and network hubs ($Z_i \geq 2.5$; $P_i \geq 0.62$). Fungal guilds (symbiotrophs, saprotrophs, and pathogens) were classified using the FUNGuild database (Nguyen et al. 2016), accessed on August 20th, 2024, while the potential functionality of bacteria was determined using FAPROTAX v1.2.10 (Louca et al. 2016) which has been tested as a reliable tool for fast-functional screening (Sansupa et al. 2021) and is commonly used in soil studies (e.g., Carrascosa-Robles et al. 2026).

3 | Results

3.1 | Effects of Reduced Tillage and Intercropping on Almond Yield, Soil Physicochemical Properties and Nitrogen Cycle

Almond yield showed high variability with no significant differences among treatments (Supporting Information Table S1), ranging from 64 kg ha⁻¹ (D2, lowest) to 187 kg ha⁻¹ (MC, highest), while both intercropping systems produced caper or essential oil crops in parallel. The measurements of soil physicochemical properties (such as pH, electrical conductivity or particulate organic carbon), water status (such as field moisture, water content at field capacity), nutrients (such as NH₄⁺, NO₃⁻), and texture (such as sand, silt) showed no significant differences among treatments (Supporting Information Table S2). However, the abundance of the *amoA* gene, involved in nitrification, was significantly higher in D2 than in MC ($\chi^2 = 6.5$; $p = 0.04$; Supporting Information Table S3).

3.2 | Effects of Intercropping and Reduced Tillage on Soil Microbial Diversity, Community, and Composition

The number of 16S and ITS sequences can be found in Supporting Information Table S4. After rarefaction, for LAB, richness was significantly higher in D2 compared to MC, while no significant differences were detected in the Shannon index. No significant differences were found among treatments for either MAB, MAF and LAF richness or Shannon index (Table 1). Regarding beta-diversity, D2 treatment was mostly separated from the other groups in

TABLE 1 | Effects of reduced tillage and intercropping on (A) Bacterial diversity indexes (richness and Shannon index) in most abundant bacteria (MAB) and less abundant bacteria (LAB) and (B) fungal diversity indexes (richness and Shannon index) in most abundant fungi (MAF) and less abundant fungi (LAF). Values represent means $\pm$ standard deviation ($n = 3$). Statistical test was performed using non-parametric Kruskal Wallis. Post hoc comparisons were conducted using Dunn's test with Benjamini-Hochberg correction, and the letters indicate significant differences between groups ($p < 0.05$), with different letters representing statistically distinct groups. MC: Almond monoculture; D1: Almond with *Capparis spinosa* intercropping; D2: Almond with *Thymus hyemalis* intercropping. Significant p -values are highlighting in bold.

(A)				
Treatment	MAB		LAB	
	Richness	Shannon index	Richness	Shannon index
MC	193 $\pm$ 3	5.04 $\pm$ 0.01	864 $\pm$ 17 ^b	6.57 $\pm$ 0.06
D1	195 $\pm$ 3	5.03 $\pm$ 0.02	1068 $\pm$ 68 ^{ab}	6.68 $\pm$ 0.06
D2	198 $\pm$ 3	5.00 $\pm$ 0.02	1395 $\pm$ 160 ^a	6.75 $\pm$ 0.20
Kruskal wallis test	$\chi^2 = 1.77$; $p = 0.41$	$\chi^2 = 2.22$; $p = 0.33$	$\chi^2 = \mathbf{7.20}$; $P = \mathbf{0.027}$	$\chi^2 = 1.16$; $p = 0.56$
(B)				
Treatment	MAF		LAF	
	Richness	Shannon index	Richness	Shannon index
MC	60 $\pm$ 1	2.66 $\pm$ 0.20	65 $\pm$ 5	3.61 $\pm$ 0.18
D1	57 $\pm$ 3	2.95 $\pm$ 0.18	66 $\pm$ 1	3.32 $\pm$ 0.26
D2	59 $\pm$ 1	2.98 $\pm$ 0.02	50 $\pm$ 10	3.09 $\pm$ 0.07
Kruskal wallis test	$\chi^2 = 0.80$; $p = 0.67$	$\chi^2 = 2.40$; $p = 0.30$	$\chi^2 = 3.29$; $p = 0.19$	$\chi^2 = 3.20$; $p = 0.20$

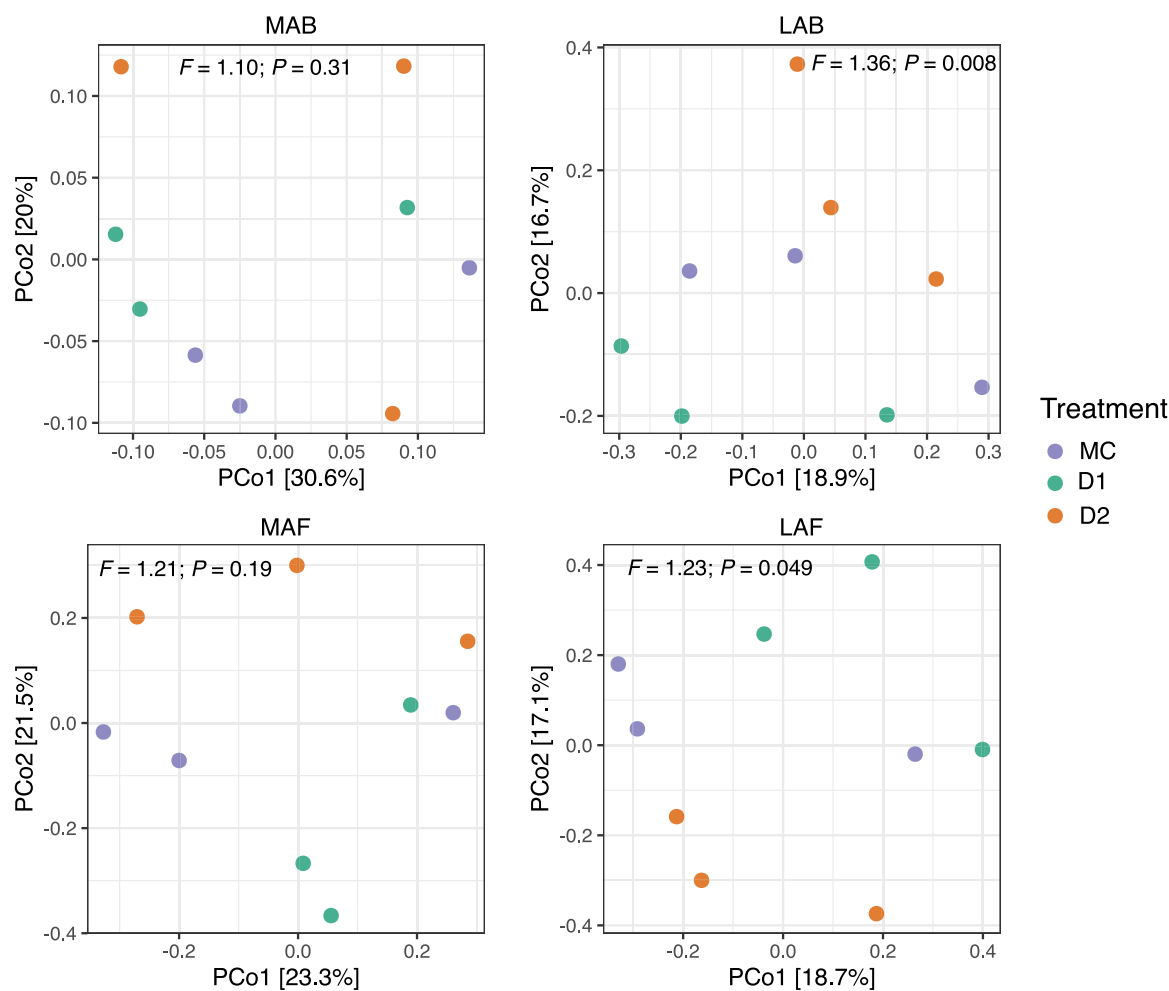


FIGURE 1 | Principal coordinate analysis (PCO) showing beta diversity based on Bray-Curtis distances for the most abundant bacteria (MAB), less abundant bacteria (LAB), most abundant fungi (MAF), and less abundant fungi (LAF). Statistical significance was assessed using permutational multivariate analysis of variance (PERMANOVA). MC, Almond monoculture; D1, Almond with *Capparis spinosa* intercropping; D2, Almond with *Thymus hyemalis* intercropping. Significant *p*-values are highlighting in bold.

MAB and MAF (Figure 1), although these differences were not significant. Significant differences were found in LAB ($F = 1.36$; $p = 0.008$) and LAF ($F = 1.23$; $p = 0.049$). After removing collinearity among predictors, the remaining variables – bulk density (BD), soil water content at wilting point (SWW), total nitrogen (TN), electrical conductivity (EC), exchangeable Mg, cation exchange capacity (CEC), pH, total organic carbon (TOC), available K, sand content (SA), and available P (Pav) – indicated that only BD and SWW were significant drivers of the most abundant microorganisms (MAB + MAF), whereas only BD explained the less abundant microorganisms (LAB + LAF) (Figure 2; Supporting Information Table S5). For bacteria, the relative abundance of the dominant taxa showed no significant differences at the phylum or genus level (Figure 3A,C; Supporting Information Table S6). For fungi, at phylum level (Figure 3B; Supporting Information Table S7A), we found that the relative abundance Glomeromycota significantly increased in D1. At genera level (Figure 3D; Supporting Information Table S7B), we found that the *Tricharina* significantly increased in D1 while *Humicola* significantly increased in D2 compared to MC.

3.3 | Reduced Tillage and Intercropping Altered the Composition of Microbial Less Abundant Taxa and its Functional Potential

The Venn diagrams for MAB and MAF reveal that the treatments share more than 90% of OTUs (Supporting Information Figures S5A and S6A), indicating almost no differences in their composition. However, in LAB and LAF, this overlap decreases significantly to approximately 20%, indicating that most microorganisms are specific to each treatment (Supporting Information Figures S5B and S6B). The genus-level Venn diagram revealed specific taxa unique to each treatment in LAB and LAF (Supporting Information Figures S5B and S6B). In D2, we identified 17 distinct LAB OTUs, including *Polycyclovorans*, *Arcticibacter*, *Amycolatopsis*, *Bosea*, *Dyadobacter*, *Janthinobacterium*, *Leifsonia* and *Cytophaga* (Figure 4; Supporting Information Table S8A). In D1, we found *Tumebacillus*, *Glycomyces*, *Sorangium*, and *Pantoea*, while in MC, the genera identified were *Xanthomonas*, *Nibribacter*, *Steroidobacter*, and *Frankia* (Figure 4; Supporting Information Table S8A). Within LAF, we identified the symbiotrophic genera *Glomus* and *Rhizophagus*, along with the saprotrophic genera *Dioszegia*,

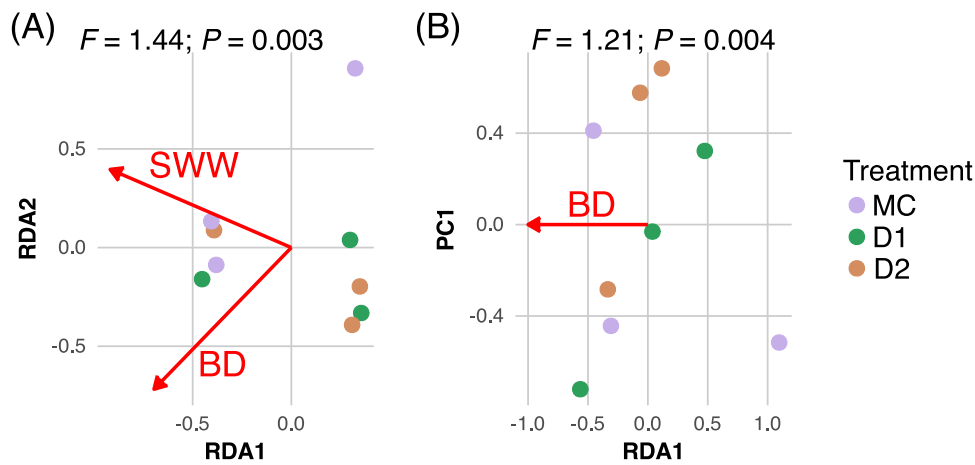


FIGURE 2 | Redundancy analysis (RDA) biplot showing (A) the most abundant microorganisms (both bacteria and fungi) and (B) the less abundant microorganisms (both bacteria and fungi), along with the physicochemical soil properties: soil water content at wilting point (SWW), and bulk density (BD). MC, Almond monoculture; D1, Almond with *Capparis spinosa* intercropping; D2, Almond with *Thymus hyemalis* intercropping. Significant *p*-values are highlighting in bold.

Iodophanus, and *Wallemia*, as well as the potential plant pathogens *Olpidium*, *Torula*, and *Periconia* in D2 (Figure 4; Supporting Information Table S8B). In D1, we identified the symbiotic genera *Pisolithus*, *Septoglosum* and *Suillus*, as well as the saprotrophic genera *Conocybe*, *Hamigera*, and *Lophiostoma*. Additionally, we found several pathogenic-saprotrophic genera, including *Ceratobasidium*, *Aplosporella*, *Coprinus* and *Peniophora* (Figure 4; Supporting Information Table S8B). Finally, in MC, we identified the saprotrophic genera *Peziza* and *Kamunisia*, the symbiotrophic genus *Atla*, and the pathogenic genera *Chrysosporium* and *Macrophomina*. The shared OTUs among treatments can be found in Supporting Information Table S9. No significant differences were observed in the functional potential of MAB (Table 2A). In LAB, we observed a higher abundance in D1 and D2 for aerobic ammonia oxidation (AAO), nitrification and fermentation functional potential (Table 2B).

3.4 | Effects of Intercropping and Reduced Tillage in Co-Occurrence Microbial Networks and Microbial Keystone Taxa

Significant differences were observed in the topology of the co-occurrence networks (Table 3). In D2, we observed a significant increase in the number of links (representing the correlation between microorganisms), and a larger network diameter. Furthermore, among these links, the number of negative links was significantly higher in D2 compared to MC, which had the lowest values (Table 3). No significant differences were observed in D1 compared to MC or D2. In the network representation (Figure 5). These differences are clearly displayed between MC (Figure 5A) and D2 (Figure 5C), but not for D1 (Figure 5B). The three networks shared the same three main modules, although the composition of their nodes (microbial members) varied. Furthermore, we found that the number of keystone taxa (module hubs; microorganisms that connect many members within their own module) differed across the networks, with the highest number observed in D2

(4: *Craurococcus*, *Massilia*, *Microclunatus*, and *Elsterales*). In contrast, both D1 and MC had only 2 module hubs each: *Bradyrhizobium* and *Geodermatophilus* in D1, and *MND1* and *Xanthobacteraceae* in MC (Figure 5). Physicochemical soil properties had a significant effect on microbial modules. Focusing only on D2 and MC treatments (due to minimal differences with D1), we observed that changes in pH had a significant negative correlation with M25 and M35, while TOC negatively correlated with M23, M39, M45 and M48 in D2. Conversely, the correlation was positive for M12 and TN, while M6 exhibited a negative correlation. In MC, all correlations were positive between pH and M49 and M37, TOC with M1, M52 and M44, and TN with M1 and M52 (Supporting Information Table S10).

3.5 | Soil Microbial Taxa Indicators in Almond Orchard

Species indicator analysis based on soil DNA abundance identified *Parviterribacter* as an indicator for D1, and *Kocuria* and *Flavobacterium* for D2; no indicator taxa were unique to MC, although *Friedmanniella*, *Pseudomonas*, *Pedobacter*, and *Novosphingobium* were shared indicators for D2 and MC (Supporting Information Table S11). No fungal indicator taxa were detected.

4 | Discussion

4.1 | Effects of Reduced Tillage and Intercropping on Crop Yield, Soil Physicochemical Properties and Microbial Diversity

Our results showed no significant differences in almond yield among treatments, although we observed a decrease in D2 compared to MC. Given the large natural variability among almond trees, it is necessary to continue comparisons over time; however, in the previous 2 years, there were also no significant differences for D1 or D2 relative to MC (Almagro et al. 2023), indicating that reduced tillage and intercropping with

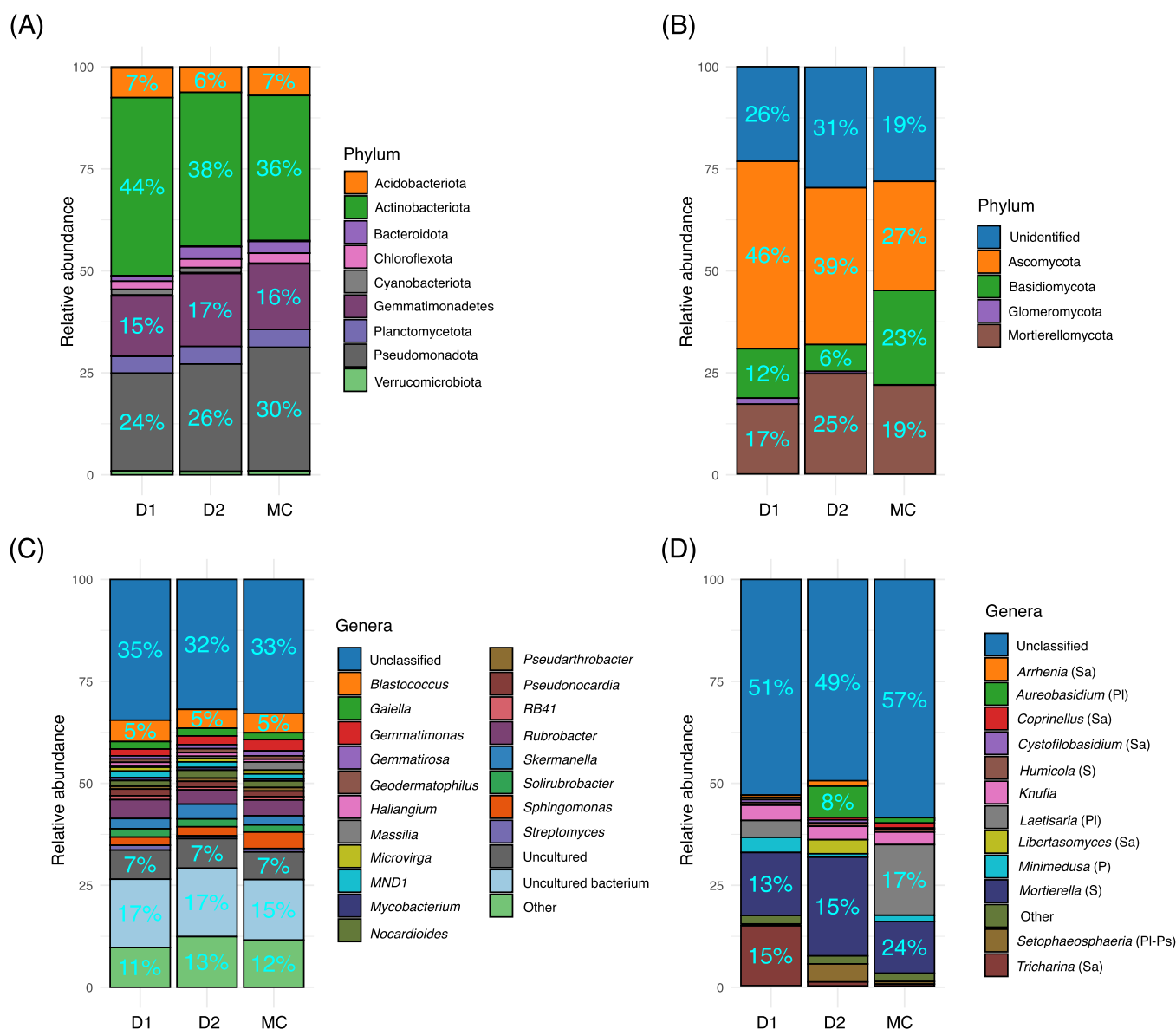


FIGURE 3 | Relative abundance of the most abundant phylum in (A) bacterial and (B) fungal communities, and the most abundant genera in (C) bacterial and (D) fungal communities. MC, Almond monoculture; D1, Almond with *Capparis spinosa* intercropping; D2, Almond with *Thymus hyemalis* intercropping. Significant *p*-values are highlighting in bold. P, non-plant pathogen; S, Symbiotroph; PI, plant pathogen; Ps, plant sapotroph; Sa, sapotroph. Fungal guilds (symbiotrophs, saprotrophs and pathogens) were classified using the FUNGuild.

T. hyemalis and *C. spinosa* does not negatively affect almond production. No significant differences were detected in soil physicochemical properties. Nonetheless, shifting these attributes through management is inherently difficult – especially in dryland systems characterised by persistent stress. Our results also revealed that reduced tillage and intercropping did not significantly affect alpha-diversity metrics for MAB, MAF or LAF; however, it significantly increased LAB microbial richness in D2. In semi-arid soils, microbial communities tend to display resilience to environmental perturbations (Pérez-Guzmán et al. 2020), which often dampens short-term changes in diversity metrics. The division among abundant and less abundant bacteria could reveal niche creation processes. The most abundant bacteria, characterised by competitive dominance and broad ecological niches, cannot readily adapt to newly created microhabitats. Conversely, less abundant bacteria

with their narrow niche specialisation – can exploit specific niches promoted by increased vegetation heterogeneity in the alley (He et al. 2023). Previous long-term studies (> 10 years) in rainfed almond orchards show that the initial yield decline under reduced tillage systems combined with cover crops tends to disappear as soil fertility improves and microbial functions, particularly nutrient mineralisation, become fully developed (Martínez-Mena et al. 2021; Almagro et al. 2021).

Regarding the relative abundance of microbial taxa at the phylum level, we observed an increase in relative abundance of *Glomeromycota* in D1 and D2. This group is well-known for comprising arbuscular mycorrhizal fungi (AMF) that promote plant growth (McConnaughey 2014), suggesting that both intercropping along reduced tillage practices can enhance AMF abundance in the soil (Wilkes et al. 2021). AMF play a vital

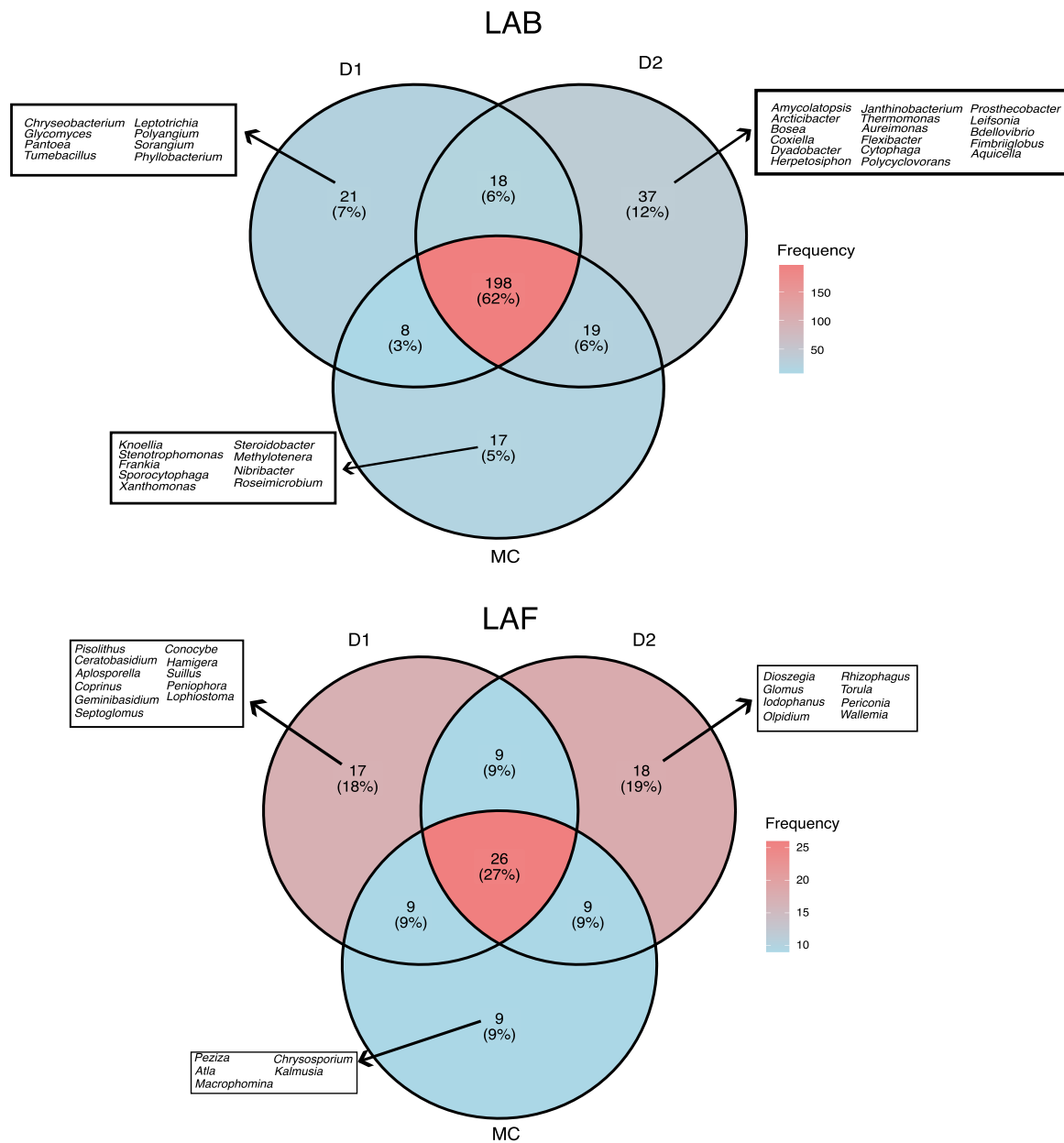


FIGURE 4 | Venn diagram showing the shared OTUs, classified at genus level, between less abundant bacteria (LAB) and less abundant fungi (LAF). The diagram includes labels for OTUs that have been classified at the genus level. The percentage represents the proportion of OTUs shared between treatments or unique to each treatment. MC, Almond monoculture; D1, Almond with *Capparis spinosa* intercropping; D2, Almond with *Thymus hyemalis* intercropping. Significant *p*-values are highlighting in bold. Frequency represents the number of OTUs present in each section of the Venn diagram.

role in maintaining soil health through the production of glomalin, a glycoprotein that improves soil structure, increases organic carbon content, and stabilises soil moisture (Agnihotri et al. 2022). Thus, conserving AMF populations supports higher glomalin levels, which in turn enhances soil aggregation and water retention, contributing to overall soil stability and resilience in intercropping systems (Almagro et al. 2023). At the genus level, in treatment D2, we found a significant increase in the plant growth-promoting genus *Humicola* (Mahmoud and Narisawa 2013). This genus is of particular interest due to its industrial potential, as it produces high-quality and abundant secondary metabolites (Ibrahim et al. 2021), including antifungal compounds, which

contribute to disease suppression and plant health (Gomez et al. 2023). However, future research (long-term) should include multiple sampling times across seasons and a greater depth resolution (additional layers below 10 cm) to better capture the temporal dynamics and vertical distribution of soil microbial communities under reduced tillage and intercropping systems. And that incorporating plant functional traits – such as specific root length (SRL), root diameter, litter C/N ratio, and root exudation profiles – would strengthen the mechanistic links between plant strategies, soil properties, and microbial community responses. Furthermore, moving beyond DNA metabarcoding towards shotgun metagenomics and metatranscriptomics would be pivotal to distinguish between

TABLE 2 | Effects of reduced tillage and intercropping on potential prokaryotic functionality (FAPROTAX) in (A) most abundant bacteria and (B) less abundant bacteria after three years of intercropping adoption. Values represent means $\pm$ standard deviation ($n = 3$). Statistical test was performed using non-parametric Kruskal Wallis. Post hoc comparisons were conducted using Dunn's test, and the letters indicate significant differences between groups ($p < 0.05$), with different letters representing statistically distinct groups. MC, Almond monoculture; D1, Almond with *Capparis spinosa* intercropping; D2, Almond with *Thymus hyemalis* intercropping. AAO, aerobic ammonia oxidation; AC, aerobic chemoheterotrophy; ACD, aromatic compound degradation. Significant p -values are highlighting in bold.

(A)				
	MC	D1	D2	Kruskal wallis test
AAO	1.69 $\pm$ 0.71	2.37 $\pm$ 0.52	2.40 $\pm$ 1.06	$\chi^2 = 1.69$; $p = 0.43$
Nitrification	1.69 $\pm$ 0.71	2.37 $\pm$ 0.52	2.40 $\pm$ 1.06	$\chi^2 = 1.69$; $p = 0.43$
Nitrogen fixation	1.45 $\pm$ 0.07	1.42 $\pm$ 0.17	1.32 $\pm$ 0.62	$\chi^2 = 1.83$; $p = 0.40$
Fermentation	3.92 $\pm$ 0.23	4.40 $\pm$ 0.41	6.18 $\pm$ 3.67	$\chi^2 = 1.68$; $p = 0.43$
AC	41.39 $\pm$ 0.26	39.93 $\pm$ 0.43	39.93 $\pm$ 3.83	$\chi^2 = 0.64$; $p = 0.72$
ACD	2.03 $\pm$ 0.33	1.67 $\pm$ 0.09	2.15 $\pm$ 0.09	$\chi^2 = 3.47$; $p = 0.18$
Nitrate reduction	5.90 $\pm$ 0.56	7.38 $\pm$ 1.42	5.36 $\pm$ 0.85	$\chi^2 = 2.76$; $p = 0.25$
(B)				
	MC	D1	D2	Kruskal wallis test
AAO	2.25 $\pm$ 0.34	1.55 $\pm$ 0.76	1.53 $\pm$ 0.48	$\chi^2 = 2.98$; $p = 0.23$
Nitrification	2.13 $\pm$ 0.79	1.74 $\pm$ 0.81	1.61 $\pm$ 0.84	$\chi^2 = 1.16$; $p = 0.56$
Nitrogen fixation	0.94 $\pm$ 0.03 ^b	1.58 $\pm$ 0.21 ^{ab}	1.96 $\pm$ 0.15 ^a	$\chi^2 = \mathbf{6.88}$; $p = \mathbf{0.032}$
Cellulolysis	0.09 $\pm$ 0.02 ^b	0.11 $\pm$ 0.02 ^{ab}	0.49 $\pm$ 0.19 ^a	$\chi^2 = \mathbf{6.31}$; $p = \mathbf{0.043}$
Fermentation	0.95 $\pm$ 0.15 ^b	1.53 $\pm$ 0.37 ^{ab}	2.36 $\pm$ 0.17 ^a	$\chi^2 = \mathbf{7.20}$; $p = \mathbf{0.027}$
AC	32.31 $\pm$ 2.49	24.77 $\pm$ 1.00	30.17 $\pm$ 3.53	$\chi^2 = 5.42$; $p = 0.06$
ACD	1.23 $\pm$ 0.53	1.23 $\pm$ 0.45	1.77 $\pm$ 0.53	$\chi^2 = 1.69$; $p = 0.43$
Nitrate reduction	2.74 $\pm$ 0.19	2.94 $\pm$ 0.51	2.63 $\pm$ 0.28	$\chi^2 = 0.62$; $p = 0.73$

TABLE 3 | Effects of reduced tillage and intercropping on network attributes based on the three different replicates per treatment after three years of experiment. Values represent means $\pm$ standard deviation ($n = 3$). Statistical test was performed using non-parametric Kruskal Wallis. Post hoc comparisons were conducted using Dunn's test, and the letters indicate significant differences between groups ($p < 0.05$), with different letters representing statistically distinct groups. MC: Almond monoculture; D1: Almond with *Capparis spinosa* intercropping; D2: Almond with *Thymus hyemalis* intercropping. The number of peripherals, connectors, module hubs, and network hubs was calculated using the global network. Significant p -values are highlighting in bold.

Network attribute	MC	D1	D2	Kruskal wallis test
Nodes	1248 $\pm$ 120	1390 $\pm$ 126	1690 $\pm$ 241	$\chi^2 = 0.62$; $p = 0.73$
Links	85,081 $\pm$ 8800 ^b	126,440 $\pm$ 30,279 ^{ab}	161,499 $\pm$ 45,194 ^a	$\chi^2 = \mathbf{6.01}$; $p = \mathbf{0.049}$
Average degree	161 $\pm$ 27	180 $\pm$ 27	254 $\pm$ 178	$\chi^2 = 0.27$; $p = 0.88$
Average path length	1.18 $\pm$ 0.13	1.05 $\pm$ 0.01	1.26 $\pm$ 0.09	$\chi^2 = 3.82$; $p = 0.15$
Network diameter	17 $\pm$ 1 ^b	14 $\pm$ 1 ^{ab}	33 $\pm$ 1 ^a	$\chi^2 = \mathbf{06.02}$; $p = \mathbf{0.045}$
Clustering coefficient	0.99 $\pm$ 0.01	0.99 $\pm$ 0.01	0.99 $\pm$ 0.01	$\chi^2 = 0.80$; $p = 0.67$
Density	0.13 $\pm$ 0.02	0.13 $\pm$ 0.01	0.16 $\pm$ 0.06	$\chi^2 = 0.0$; $p = 1.00$
Heterogeneity	1.28 $\pm$ 0.05	1.28 $\pm$ 0.03	1.20 $\pm$ 0.17	$\chi^2 = 0.88$; $p = 0.96$
Modularity	0.08 $\pm$ 0.02	0.07 $\pm$ 0.01	0.07 $\pm$ 0.03	$\chi^2 = 0.0$; $p = 1.00$
Positives	94,664 $\pm$ 23,714	119,377 $\pm$ 27,562	97,066 $\pm$ 14,	$\chi^2 = 1.07$; $p = 0.59$
Negatives	5310 $\pm$ 154 ^b	7062 $\pm$ 2,739 ^{ab}	10,620 $\pm$ 870 ^a	$\chi^2 = \mathbf{6.48}$; $p = \mathbf{0.039}$
Peripherals	1100	617	1307	
Connectors	0	0	0	
Module hubs	2	2	4	
Network hubs	0	0	0	

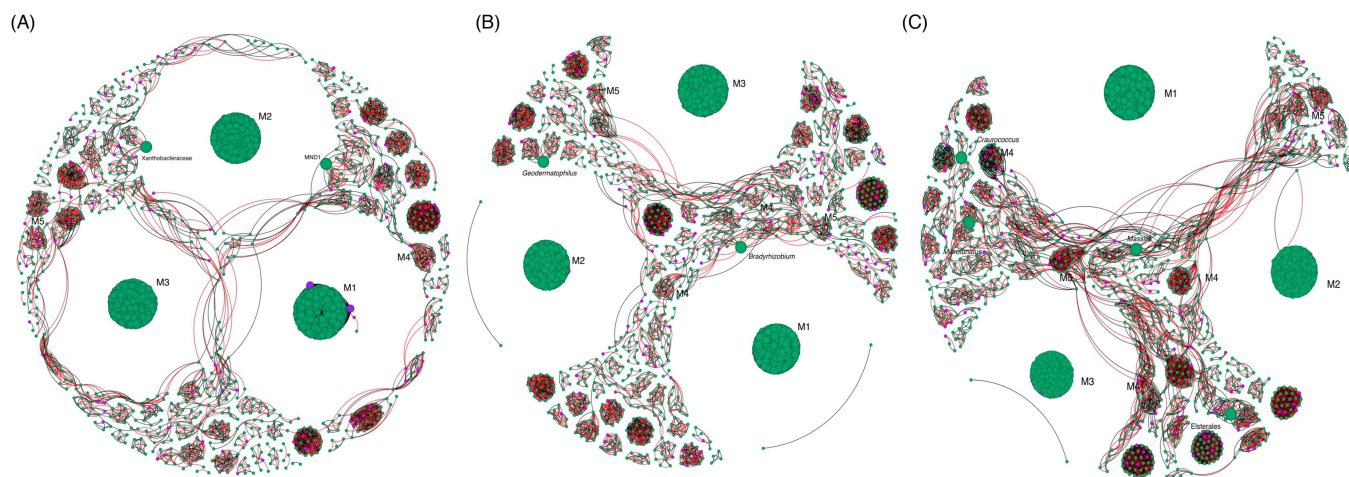


FIGURE 5 | Bacterial and fungal co-occurrence networks for (A) almond monoculture, (B) almond with *Capparis spinosa* intercropping, and (C) almond with *Thymus hyemalis* intercropping. In the network, nodes are colour-coded: green represents bacterial members, and purple represents fungal members. The size of each node reflects its degree, with the largest nodes corresponding to the module hubs identified in the network. Only the first five modules are highlighted in the plot which are labelled as M1, M2, M3, M4 and M5.

the standing genetic potential and the actual metabolic activity of the soil microbiome.

4.2 | *T. hyemalis* and Reduced Tillage Altered the Set of Unique Genera and the Soil Functional Potential

Within LAB, in D2, we detected unique OTUs belonging to the genera *Amycolatopsis*, *Bosea*, *Dyadobacter*, *Janthinobacterium* and *Leifsonia*, all of which are described as plant growth promoters (Gandham et al. 2024; Kang et al. 2017; Kumar et al. 2018), or can act as plant protectors against pathogens (Haack et al. 2016; Khoury et al. 2021). Similar results were observed in D1, with the increase of the plant promoters such as *Chryseobacterium*, *Pantoea* (Mengstie et al. 2023; Nascimento et al. 2020) or plant protectors such as *Glycomyces*, *Leptotrichia*, *Polyangium* (Balode 2012; Das and Dhal 2022; Kim and Yun 2011; Liao et al. 2018). While in D1 we found some bacteria plant pathogens such as *Stenotrophomonas* or *Xanthomonas* (Abbott et al. 2011; Timilsina et al. 2020). Within LAF, we identified noteworthy OTUs in D1 and D2, including symbiotrophs (e.g., *Glomus*, *Rhizophagus* or *Suillus*), saprotrophs and, even plant pathogens (e.g., *Ceratobasidium*, *Peniophora* or *Olpidium*). While the detection of potential plant pathogens such as *Olpidium* and *Periconia* in the soil microbiome does not pose a significant threat to almond trees, *Torula* species can potentially cause trunk cankers, root rot, or crown rot in susceptible hosts. However, no such disease symptoms were observed in any of the sampled almond trees, indicating that despite their detection, these fungal taxa remained metabolically inactive or non-pathogenic under the prevailing soil and host conditions. To confirm the absence of the major quarantine pathogens commonly associated with almond production in Mediterranean regions, we employed quantitative PCR targeting *Xanthomonas arboricola* pv. *pruni*, *Xylella fastidiosa*, and *Monilinia laxa*. None of these pathogens were detected in any samples, indicating that the intercropping management system did not facilitate the establishment or proliferation of these almond pathogens.

Regarding functional potential, in D2, there was a significant increase in nitrogen fixation, cellulolysis, and fermentation functional potential compared to MC. These findings suggest that *T. hyemalis* significantly increased the relative abundance of cellulolysis and fermentation functional potential that may be attributed to the increased organic matter input, likely due to the growth and subsequent decomposition of the plants (Larionova et al. 2017; Talbot et al. 2012; Yarwood 2018). In our plots, the planting density of *T. hyemalis* was higher than that of *C. spinosa*. Moreover, *C. spinosa* is a deciduous shrub that sprouts in April and loses all aerial biomass by November, providing substantial litter in autumn but leaving the soil exposed during winter. These differences in phenology and biomass cover likely explain why intercropping with *T. hyemalis* can increase total organic carbon – as reported by Almagro et al. (2023) – and thereby enhance soil functionality more effectively than intercropping with *C. spinosa*. In addition, the evergreen habit and sustained root activity of *T. hyemalis* supply a continuous flow of organic compounds that support beneficial microbes such as *Humicola*, whereas the seasonal soil exposure under *C. spinosa* may limit such microbial enrichment during the dormant months. Also, this enrichment of organic matter can stimulate microbial processes involved in breaking down complex plant residues, further enhancing soil fertility and structure. The highest difference between D2 and MC compared to D1 may be related, as explained before, to the fact that *T. hyemalis* is an evergreen species allowing a continuous soil cover along the entire year, while *C. spinosa* loses all the shoots in winter. Moreover, the quality of litter inputs is different between both intercropped species, and vegetal residues of *T. hyemalis* are more resistant to decomposition in short-term owing to high C/N ratio than those of *C. spinosa* (Sánchez-Navarro et al. 2022). It is important to highlight that, although Faprotax is a valuable tool for understanding the functional potential of prokaryotes and is trustable (Sansupa et al. 2021), it has limitations as it relies on curated databases and cultured taxa, which may not fully represent the overall functional diversity of uncultured or poorly studied microorganisms.

4.3 | Effects of Intercropping and Reduced Tillage on Microbial Co-Occurrence Network

Significant changes in the microbial co-occurrence network occurred in D2, but not in D1, compared with MC. Specifically, we found a significant increase in the network diameter in D1. A larger network diameter, combined with a low number of nodes, suggests lower network compactness (Yarwood 2018), which may indicate less efficient communication between nodes (microorganisms). However, from a community resistance perspective, this increased distance between nodes can also act as a buffer, slowing the propagation of environmental perturbations across the entire network (Stouffer and Bascompte 2011). Despite there being no significant differences in the number of nodes, we found a significant increase in the number of links and, specially, negative links. These findings suggest that while the overall network may be less compact, the level of microbial interaction and complexity was higher in D2 compared to MC. This combination of higher connectivity and larger diameter suggest a modular robustness a complex system where interactions are strong but compartmentalised, as previously reported in other different intercropping systems (Jiang et al. 2024; Zhang et al. 2023). Moreover, the significant increase in negative correlations could indicate greater regulation and control among network members, contributing to higher network stability and preventing the dominance of specific taxa under stress (Coyte et al. 2015; Cuartero et al. 2022). Supporting this, we also found that the number of module hubs (microorganisms with a high number of correlations within their own group) was higher in D2 compared to D1 and MC. These generalists could help their respective groups respond more rapidly to environmental disruptions, thereby increasing the community's resistance to environmental disturbances (Cuartero et al. 2022). In fact, the importance of these module hubs in microbial networks is underscored by studies suggesting that they could serve as key points of vulnerability for future plant-targeted control strategies (Agler et al. 2016). However, despite the valuable insights that co-occurrence networks can provide, it is important to acknowledge their potential limitations, as they are based on statistical correlations that may include indirect or spurious relationships. To address these challenges, we carefully adjusted the thresholds to minimise noise and enhance the robustness of our findings, and we recommend working with and interpreting such networks with caution.

4.4 | Indicator Species Analysis

The identification of distinct indicator taxa across treatments reveals how plant identity and reduced tillage shape the microbial community. Indicator species are statistically significant taxa whose abundance patterns reflect specific management conditions, serving as proxies for soil health and functional potential (Shen et al. 2022). D1 enrichment of *Parviterribacter*, known for stress tolerance and organic matter degradation, contrasted with D2 enrichment of *Kocuria* and *Flavobacterium* – the latter a plant growth-promoting bacterium with pathogen-suppressive capacity (Seo et al. 2024). *Nodosilinea*, a nitrogen-fixing cyanobacterium, emerged as a shared indicator in both intercropping treatments, suggesting that intercropping itself – independent of plant identity – selects for

nitrogen-fixing microorganisms, reflecting enhanced ecosystem functionality. The higher abundance of *Pseudomonas*, *Friedmanniella*, *Pedobacter* and *Novosphingobium* in D2 and MC, but their absence in D1, suggests that D2 intercropping maintains ecological and functional continuity with the baseline soil microbiome despite management shifts. These taxa possess broad functional versatility and ecological niches, enabling them to persist across management intensities. Their retention likely reflects the essential nature of their functions – lignocellulose degradation (*Pedobacter*; Zhou et al. 2019) and nutrient cycling (*Pseudomonas*) – which remain necessary in D2 soils despite altered plant inputs (Liu et al. 2021). These findings provide mechanistic evidence that management practices restructure soil microbiomes in functionally meaningful ways, with distinct indicator taxa validating the capacity of reduced tillage intercropping to enhance microbial diversity and soil multifunctionality.

5 | Conclusions

Our results demonstrate that reduced tillage along with intercropping with almond can modify soil microbial composition even within a relatively short time frame and under semi-arid conditions. Although both *T. hyemalis* and *C. spinosa* showed certain benefits, *T. hyemalis* had the strongest impact on the community. Specifically, it increased the richness of less abundant bacterial taxa and favoured the growth of plant growth-promoting genera such as *Amycolatopsis*, *Bosea*, *Dyadobacter*, *Janthinobacterium* and *Leifsonia*. In addition, reduced tillage and intercropping with *T. hyemalis* altered the functional potential of the microbial community, enhancing functional potential such as nitrogen fixation, cellulolysis, and fermentation. However, no significant changes in functional potential were observed in soils with reduced tillage and intercropped with *C. spinosa*. Moreover, microbial network analysis revealed that almond intercropping with *T. hyemalis* and reduced tillage had a more highly connected structure, with a greater number of module hubs, suggesting greater resilience to environmental changes. Overall, these findings highlight *T. hyemalis* as a promising intercrop for almond orchards with reduced tillage, with potential to improve soil health, benefit farmers, and enhance the soil sustainability without comprising almond yield.

Author Contributions

Maria Almagro, Maria Martinez-Mena, Elvira Diaz-Pereira designed the experiment and organised the field work. Marcos Egea-Cortines, Onurcan Özbolat, Maria Almagro, and Loredana Canfora conducted the molecular analyses. Raul Zornoza, Virginia Sánchez-Navarro conducted the physicochemical analysis. Jessica Cuartero and Maria Almagro performed the bioinformatic analysis. Jessica Cuartero performed the statistical analyses. Jessica Cuartero draughted the first version of the article, and Margarita Ros, Beat Frey, Jose Antonio Pascual and Raul Zornoza, contributed to the writing. All authors have reviewed the final article.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The sequences can be accessed in the ENA repository PRJEB82303.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Figure S1: Experimental layout of the almond monoculture under conventional tillage. **Figure S2:** Intercropping layout of almond with *Capparis spinosa* at blossom in June under reduced tillage. **Figure S3:** Intercropping layout of almond with *Thymus hyemalis* under reduced tillage. **Figure S4:** Rarefaction curves for bacteria and fungi showing sequencing depth (number of reads) and observed OTUs ($n = 3$). **Figure S5:** Venn diagram displaying the shared OTUs between (A) the most abundant bacteria (MAB) and (B) the less abundant bacteria (LAB). **Figure S6:** Venn diagram illustrating the shared OTUs between (A) the most abundant fungi (MAF) and (B) the less abundant fungi (LAF). **Table S1:** Effects of reduced tillage and intercropping on almond crop yield ($n = 3$). **Table S2:** Effects of reduced tillage and intercropping on soil physicochemical properties, soil nutrients and soil texture. **Table S3:** Effects of reduced tillage and intercropping on soil nitrogen genes and DNA. Metrics are shown as means $\pm$ standard deviation ($n = 3$). **Table S4:** Number of sequences obtained for bacteria and fungi for each sample ($n = 3$). **Table S5:** Forward and backward stepwise

RDA results showing F -statistics and P -values (999 permutations) for environmental variables explaining microbial community composition. **Table S6:** Effects of reduced tillage and intercropping on relative abundance of the most abundant (> 0.1% relative abundance) bacteria at (A) phylum level and (B) genus level. **Table S7:** Effects of reduced tillage and intercropping on relative abundance of the most abundant fungi (> 0.1% relative abundance) at (A) phylum level and (B) genus level. **Table S8:** Effects of reduced tillage and intercropping on relative abundance of (A) distinct less abundant (< 0.1% relative abundance) bacteria (LAB) and (B) distinct less abundant fungi (LAF), as determined by the Venn diagram (Figure 4). **Table S9:** Shared OTUs among treatments for (A) less abundant (< 0.1% relative abundance) bacteria (LAB) and (B) less abundant fungi (LAF), taxonomically classified at the genus level. **Table S10:** Correlations between module eigengenes and soil properties, with brief taxonomic composition of each module. **Table S11:** Indicator species analysis identifying taxa associated with each treatment.