



Optimizing purslane cultivation through legume intercropping and crop rotation: a study on yield and rhizosphere bacterial communities

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Abstract

Aims Mixed cropping systems such as intercropping and crop rotation have been proven to be sustainable agronomic tools that provide agro-ecological services and improve crop yield through soil physical, chemical and biological changes in the soil. In this study, we aimed to assess the impact of different mixed cropping systems on a crop well-adapted to high temperatures and low precipitation, like purslane (*Portulaca oleracea* L.) and to study the underlying microbial mechanisms involved.

Methods A field experiment in a semiarid region of southern Spain was conducted to study the short-term effects of crop rotation (R) and intercropping (I) with peas or cowpeas, as well as a combination of both (IR) on purslane yield and soil quality parameters such as microbial enzymatic activity, bacterial diversity, microbial composition and functionality.

Results All the tested treatments increased purslane yield without significant differences among each other, but only the R and IR treatments affected the rhizospheric soil properties, through the increase of the enzymatic activities and the modification of the bacterial composition and functionality, and promoted organic matter degrading bacteria such as *Bacillaceae*, *Myxococcaceae*, and *Planococcaceae* and nitrogen-fixing bacteria, mainly *Rhizobiaceae* and *Beijerinckiaceae*.

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Conclusion This study demonstrates how sustainable cropping practices may improve the yield of a low maintenance crop like purslane under low-maintenance conditions by improving soil fertility in semi-arid areas and also provides insights into the biological mechanisms responsible for the recorded effects.

Keywords *Portulaca oleracea* · Cropping systems · Bacterial community · Enzyme activities

Introduction

Agriculture in semiarid areas, such as the south Mediterranean agro-ecosystems faces significant challenges, experiencing soil degradation and exacerbated by the effects of climate change including the increased intensity and frequency of droughts and the rise of temperature, posing further challenges to traditional farming systems (Jacobsen et al. 2012; Trambay et al. 2020; Zandalinas et al. 2021). Additionally, there is a need to reduce the use of chemical fertilizers whose excessive use leads to the contamination of surface and groundwater, as well as surrounding water bodies (Jang and Chen 2015; Cui et al. 2020). These conditions necessitate the development of new cropping systems being less dependent on agrochemical inputs. Such systems should not only be profitable and sustainable in the long term but also effective in facilitating the recovery of degraded soils, as suggested by Gomiero et al. (2011).

A wide variety of endemic plants, known as Wild Edible Plants (WEPs), usually adapted to drought and high temperatures are found in the Mediterranean ecosystems and they are highly valued due to their taste and nutritional properties (Ceccanti et al. 2018; Karkanis et al. 2018; Pereira et al. 2020). Purslane (*Portulaca oleracea* L.) is a worldwide distributed WEP, well adapted to adverse conditions such as high temperatures, drought, or moderate salinity stress (Jin et al. 2016; Petropoulos et al. 2018; Pereira et al. 2020) with relatively low nutritional requirements, thus being able to achieve high yields with low fertilization rates (Carrascosa et al. 2023a, b). Purslane is also notable for its nutritional profile, which includes high levels of omega-3 fatty acids (α -linolenic acid) and antioxidants (Montoya-García et al. 2023; Srivastava et al. 2023). Thus, the species is an interesting and promising crop, especially in semi-arid regions

of the Mediterranean. Several sustainable farming methods are under study, including precision agriculture practices, reduced tillage, and the use of organic fertilizers among others (Lal et al. 2011; Chen et al. 2018b; Syed Ab Rahman et al. 2018; Sandén et al. 2018), to meet the requirements of increased agricultural production, it remains crucial to improve the yield of agricultural systems within the scenario of climate change, land degradation, and the increase of global population (Lal et al. 2011).

The use of multispecies systems, such as crop rotation or intercropping systems, have been known and used in the farming sector for a long time, while recently they have been pointed out as an eco-friendly alternative approach to mono-cropping and conventional farming (Martin-Guay et al. 2018; Han et al. 2023; Angon et al. 2023). Several studies suggest that mixed cropping systems may improve crop production through the enhancement of soil quality by improving microbial diversity or activity (Duchene et al. 2017; Ortiz-Cornejo et al. 2017; Chamkhi et al. 2022; Yang et al. 2024). Most mixed cropping systems incorporate legume crops since they can establish symbiotic relationships with nitrogen-fixing bacteria from the *Rhizobium* genus and thus, fix otherwise unavailable atmospheric nitrogen through biological nitrogen fixation (BNF) and further reduce the need for mineral nitrogen fertilizers inputs (Gopalakrishnan et al. 2015; Jensen et al. 2020; Kebede 2021). Moreover, it has been estimated that the rhizobia–legume BNF can contribute to an average of 115 kg N ha⁻¹ annually thus significantly contributing to agrochemical inputs reduction (Herridge et al. 2008). During crop rotation—especially when accompanied by the incorporation of plant residues into the soil as soil mulching—fixed nitrogen could be incorporated into the soil, making it quickly available for the subsequent crop (Thilakarathna et al. 2016; Murphy-Bokern et al. 2017; Radicetti et al. 2017). On the other hand, in intercropping systems the nitrogen fixed by BNF from the donor plant (legume) can be immediately assimilated by the associated crop used in the system (Duchene et al. 2017).

Crop rotation and intercropping not only directly enhance soil fertility through the recruitment of nitrogen-fixing bacteria but also selectively modify the structure and diversity of rhizospheric soil microbial communities via exudate production or organic matter mineralization (Duchene et al. 2017; Schaedel

et al. 2021; Lai et al. 2022). These functions facilitate the proliferation of symbiotic plant growth-promoting bacteria (PGPBs) that participate in nitrogen cycle, mineralization of organic matter, increase of P and K availability or the production of phytohormones and overall, they enhance soil fertility and crop development (Chen et al. 2018a; Chamkhi et al. 2022).

Thus, the use of new crops with low nutritional requirements and better adapted to regional environmental conditions, such as purslane for the semi-arid regions of southern Europe, in combination with sustainable farming systems not dependent on agrochemical inputs that can be of great interest to agriculture, allowing for crop production in challenging regions affected by climate change or soil degradation, and improving the resilience of these soils by increasing their fertility and health. This helps meet global food demands while maintaining and improving the quality of agricultural soils. (Baveye et al. 2016; Karlen et al. 2019; Lehmann et al. 2020; Carrascosa et al. 2023b).

Considering that around 25% of the world's agricultural soils are degraded (Food and Agriculture Organization of the United Nations (FAO) 2022), a better understanding of the effects of sustainable cropping systems integrating a WEP such as purslane and legumes through intercropping with cowpea (*Vigna unguiculata* L.) and crop rotation with pea (*Pisum sativum* L.) on soil quality will allow to introduce them in the Mediterranean small-scale farming systems.

Therefore, the main objective of this study was to analyse how a low-maintenance crop such as purslane, could provide ecosystem services through crop rotation and intercropping with legumes in semi-arid Mediterranean conditions, focusing on the changes in the structure and functionality of soil bacterial communities. Furthermore, it is hypothesized that the cultivation of purslane combined with legumes could conduct ecosystem services and offer farmers the optimization of land use in marginal areas of the Mediterranean basin under low N application regimes.

Materials and methods

Experimental design and sample collection

An experiment of intercropping and crop rotation cultivation system using purslane and legumes was

conducted at the CEBAS – CSIC Experimental Farm (Santomera, Murcia, Spain 38° 06' 14'' N 1° 01' 59'' W) under organic conditions from January to August 2022. The experimental conditions correspond to a semi-arid Mediterranean climate with an annual temperature of 19.2 °C, 300 mm of annual rainfall and a high potential evapotranspiration of 1000 mm y⁻¹ (Carrascosa et al. 2023a). The field experiments were conducted in a homogeneous orchard, that had been used for lemon trees cultivation for 15 years. The trees were uprooted in 2020, and the land remained fallow until it was tilled prior to the start of our experiment. The soil is classified as lithic xeric haploxeroll with a clay-loam texture (clay fraction: 41% illite, 17% smectite and 30% palygorskite). The soil chemical properties were similar along the orchard and are presented in Table 1.

The experimental design included four treatments: (i) Purslane (*Portulaca oleracea* L.) mono-crop grown in the summer (Control); (ii) Purslane – Cowpea (*Vigna unguiculata* L.) intercropping system, where purslane and cowpea were cultivated at the same time in alternative rows in the summer (I); (iii) Purslane – Pea (*Pisum sativum* L.) crop rotation system, where peas were cultivated in the winter followed reintroducing the plant residue after harvest into the soil prior the purslane cultivation in the summer (R); and (iv) a mixed system of Purslane – Pea – Cowpea intercropping and crop rotation, consisted on the combination of R and I treatments, meaning peas were cultivated in the winter followed by cowpeas and purslane grown simultaneously in the summer (IR). A schematic representation of the experimental design along with the dates and periods can be found in the Fig. S1. This first-year experiment was conducted to determine the short-term effects of intercropping and rotation treatments on the purslane cropping system similar to other researchers who

Table 1 Chemical and physico-chemical properties of soil prior to the beginning of the experiment

pH	EC (μS cm ⁻¹)	TN (g kg ⁻¹)	TOC (g kg ⁻¹)	C/N	AP (g kg ⁻¹)
8.1	1000	5.7	7.3	1.3	0.6

EC Electrical conductivity, TN Soil total nitrogen, TOC Soil total organic carbon, C/N Total organic carbon and total nitrogen ratio, AP Soil available phosphorous

have also obtained short-term results (Cuartero et al. 2022b, a; Shen et al. 2024).

The experiment followed the completely randomized design with six plots per treatment corresponding to a total of 14 plants per plot -2 rows and 7 plants per row- (Control and R treatments had 14 purslane plants and I and IR treatments had 7 purslane plants and 7 cowpeas plants, grown in the summer period; while in R and IR treatments, 14 plants of peas were cultivated in the winter period). Each pooled sample consisted of the 5-central purslane plants for each plot ($n=6$). All plants were irrigated once a week and no fertilizers or pesticides were applied. Eight weeks after sowing, the five purslane plants were harvested for plant yield determination and dried at 60 °C until constant weight to determine the dry weight. Rhizospheric soil (0 – 10 cm depth) was sampled at the end of the experiment, sieved through a 2 mm mesh and separated into two portions; one portion was stored at -20 °C for DNA sequencing, while the remaining soil was stored at 4 °C for chemical and biochemical analyses.

Chemical properties and enzyme activities of rhizospheric soil

The soil pH and EC were measured in deionized water at 1:2.5 and 1:5 soil/water ratios, respectively, after shaking at 230 rpm for 30 min and centrifuging at 2500 rpm for 10 min. Total Organic carbon (TOC) was determined by dichromate oxidation (Walkley and Black 1934). Total nitrogen (TN) and total carbon (TC) were determined by a Dumas combustion gas chromatography in an elemental CHNS-O analyser (EA- 1108, Carlo Erba, Barcelona, Spain), briefly 0.15 g of grounded dry soil is introduced into the purge chamber prior to the complete combustion in oxygen, the resulting CO₂ and NO_x gases are filtered and measured using infrared cells for CO₂ and using thermal conductivity for NO_x after its reaction to N₂. The C/N ratio was calculated by dividing the TOC by the TN. Total phosphorous (TP) and total potassium (TK) were analysed using ICP-OES (ICAP 6500 DUO, Thermo Fisher Scientific, Hayward, California, USA) as it is described by Nicolas-Espinosa et al. (2024). TN, TC, TP and TK were conducted at the Ionomics Laboratory of the CEBAS (CSIC), Murcia, Spain. Available phosphorous was extracted according to Olsen et al. (1954) and determined colorimetrically

by using molybdate-reactive P according to Murphy and Riley (1962). β -Glucosidase activity (GLC) was determined as described by Eivazi' and Tabatabai (1988); Alkaline phosphomonoesterase activity (PHO) was determined at pH 8 according to Tabatabai and Bremner (1969); Dehydrogenase activity (DHA) was measured according to Trevors et al. (1982) and modified by García et al. (1993); and urease activity (URE) was determined using urea as substrate according to the protocol of Kandeler et al. (1999).

Soil DNA extraction and Illumina sequencing

Soil DNA was extracted from 0.5 g of soil from each sample using the DNeasy PowerSoil DNA Isolation kit (Qiagen, Hilden, Germany) following the manufacturer protocol. DNA yield and quality were checked by electrophoresis in 0.8% (w/v) agarose gels stained with GelRed and using a Qubit 3.0 fluorometer (Life Technologies, Grand Island, NY) prior to sequencing on an Illumina MiSeq platform at the genomics service of the Institute of Parasitology and Biomedicine “López Neyra” (CSIC), Granada, Spain. The bacterial libraries were constructed using the Nextera XT DNA Library Preparation kit (Illumina Inc., CA, USA) and the amplifying the hyper-variable regions (V3 – V4) of the prokaryotic 16S rRNA gene was performed using the primer pair 341F (5'-CCTACGGGNNBGCASCAG-3') and 806R (5'-GACTACNVGGGTATCTAATCC-3') (Takahashi et al. 2014). The resulting amplicons were tagged to be attached to PNA PCR clamps to reduce plastid and mitochondrial DNA amplification (Lundberg et al. 2013). The runs were sequenced using a paired-end 2×275p (PE 275) strategy.

Sequencing data processing

Raw sequence data were processed in QIIME2 v 2022.2 (Caporaso et al. 2010). Prior to this step, sequence quality analysis was performed using FastQC program v 0.12.1 (Andrews 2023). Then, sequences were imported into QIIME2 and primers and the sequences with less than 250 pb length were removed using cutadapt (Martin 2011). The Amplicon Sequence Variants (ASV) were generated by DADA2, truncating to an average length of Q>30 and singletons were removed. The resulting ASVs were taxonomically classified using “classify-sklearn” algorithm. The taxonomic assignments were made against the prokaryotic

16S/18S SILVA v.138 database (Quast et al. 2012) released in November 2020. The potential predicted bacterial functionality was carried out using the functional annotation of prokaryotic taxa (FAPROTAX) database (Louca et al. 2016), selecting the predicted functions associated with C and N cycling.

Statistical analyses

All statistical analyses were performed using R v 4.1.2 (R Development Core Team 2021). Data curation, manipulation and plotting were performed using the tidyverse packages (Wickham et al. 2019).

The normality of plant biomass and nutrients, soil chemical and biological variables, alpha diversity indices, phylum, family and gene expression relative abundances data was first tested using a Shapiro – Wilk test, using the stats v 4.1.2 (R Development Core Team 2021) package. If normality assumption was met, then the Bartlett's test was performed to test the homocedasticity among groups using the stats package. When both normality and homoscedasticity were confirmed, one-way analysis of variance (ANOVA) was performed for means comparisons followed by a post hoc Tukey's HSD test with agricolae v 1.3–6 package (de Mendiburu 2023). Otherwise, a non-parametric Kruskal–Wallis test was performed using the stats package, followed by a post hoc Dunn's test with Bonferroni correction using the FSA v0.9.5 package (H. Ogle et al. 2023). When data were heterocedastic, a Welch's ANOVA followed by a Games – Howell's post hoc test using the rstatix v 0.7.2 package (Kassambara 2023) was performed. For reproducibility purposes, a random seed was settled using the command set.seed(123) prior to the analysis when needed. Rarefaction analysis was conducted to enable comparisons between samples, standardized to the lowest sequencing depth found within a sample (30,702 reads).

The rarefied ASV table was used for further analysis. Any sequences corresponding to Eukaryotes, Mitochondria, Chloroplasts, and Archaea were eliminated from the data, as well as ASVs that could not be assigned to a known bacterial phylum. The microeco package v 1.1.0 (Liu et al. 2021a, b) was used to analyse the microbiome data: the alpha Shannon and Chao1 indices; a principal coordinate analysis (PCoA) that was performed to visualize bacterial structure (beta diversity) using a Bray – Curtis distance obtained with

the absolute bacterial abundances to visualize communities' structure; a Redundancy Analysis (RDA) that was performed to visualize the relationship between soil chemical properties, enzymatic activity, plant biomass and the bacterial community at family level using the same Bray – Curtis matrix; and a Permutational Multivariate Analysis of Variance (perMANOVA) that was carried out on the same Bray – Curtis matrix with 999 permutation with false discovery rate (FDR) corrections for multiple comparisons (Storey 2002) using the cal_betadisper() and cal_manova() functions. To identify biomarkers, a Linear Discriminant Analysis (LDA) Effect Size (LEfSe) (Segata et al. 2011) was performed (Kruskal Wallis $\alpha=0.05$, using FDR for adjusting p-value and no LDA threshold applied) to estimate the effect size between abundances of all taxonomic levels; a non-parametric Kruskal–Wallis test was conducted to identify significant differences in the abundances of each taxonomic group among treatments, followed by an LDA test to estimate the effect size for each group; A cladogram was plotted with the top 40 biomarkers, using the ggtree v3.8.2 package (Yu et al. 2017) on a trans_diff environment from microeco package and a bar chart representing the LDA scores obtained for each taxonomic group.

Results

Growth of purslane and soil chemical properties

All treatments increased purslane yield significantly; the I and IR treatments showed approximately 54% more yield, while the R treatment showed about 34% more, all compared to the Control. Comparing the different cropping systems, they did not show significant differences between them (Fig. 1).

The pH, EC, total nitrogen (TN), total organic carbon (TOC), C/N, total phosphorus (TP) and available phosphorous (AP) were not affected significantly by the cropping systems (Table 2). The R and IR treatments significantly increased alkaline phosphomonoesterase (PHO), urease (URE) and dehydrogenase (DHA) activities compared to the Control and the I treatment, but only the IR treatment showed a significant increase on β -glucosidase (GLC) activity compared to the Control. No differences were observed between R and IR treatments, while the I treatment did not show any differences compared to the Control (Table 2).

Fig. 1 Total dry weight of purslane plants cultivated under different cropping systems ($n=6$). Vertical bars represent standard deviation. Different letters correspond to significant difference ($p<0.05$) according to Tukey's HSD test

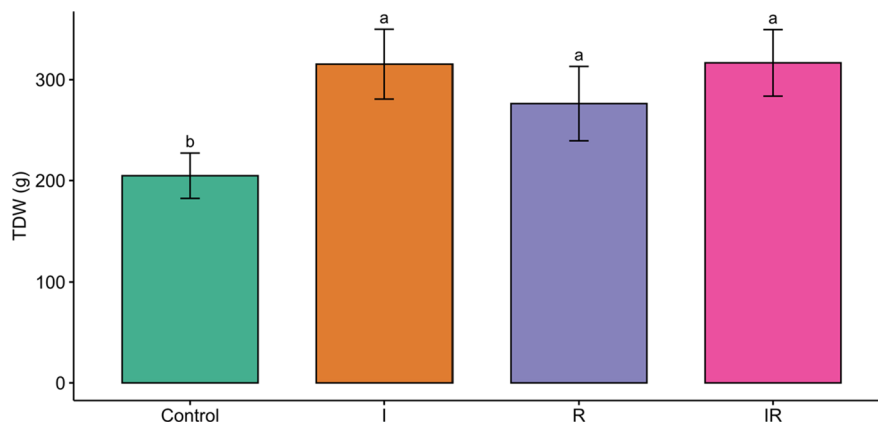


Table 2 Chemical, physico—chemical and biological properties of the rhizosphere of purslane plants

Soil chemical properties	Control	I	R	IR	ANOVA
pH	7.9 ± 0.5	8.1 ± 0.4	8.1 ± 0.1	8.1 ± 0.0	ns
EC (μS cm ⁻¹)	616 ± 20	597 ± 22	624 ± 24	622 ± 25	ns
TN (g Kg ⁻¹)	2.1 ± 0.9	1.6 ± 0.6	2.3 ± 0.4	1.9 ± 0.4	ns
TOC (g Kg ⁻¹)	24.9 ± 3.9	23.0 ± 6.2	25.5 ± 2.0	25.5 ± 5.1	ns
C/N	12.0 ± 3.2	14.5 ± 1.8	11.0 ± 2.7	13.8 ± 2.8	ns
TP (g Kg ⁻¹)	0.87 ± 0.07	0.86 ± 0.06	0.89 ± 0.08	0.92 ± 0.15	ns
AP (g Kg ⁻¹)	0.28 ± 0.05	0.31 ± 0.08	0.32 ± 0.06	0.33 ± 0.07	ns
GLC (μmol PNF * g ⁻¹ h ⁻¹)	0.968 ± 0.142 b	1.028 ± 0.109 ab	1.207 ± 0.264 ab	1.309 ± 0.12 a	**
PHO (μmol PNF * g ⁻¹ h ⁻¹)	2.382 ± 0.194 b	2.415 ± 0.174 b	3.329 ± 0.796 a	3.217 ± 0.355 a	**
DHA (μmol TPF * g ⁻¹ h ⁻¹)	3.503 ± 0.204 b	3.086 ± 0.545 b	4.27 ± 0.385 a	4.524 ± 0.088 a	***
URE (μmol N-NH ₄ * g ⁻¹ h ⁻¹)	4.521 ± 0.551 b	4.404 ± 0.497 b	5.799 ± 0.471 a	5.964 ± 0.724 a	***

Values (mean ± sd, $n=6$). Different lowercase letters correspond to different statistical groups ($p<0.05$) as determined by the Tukey's HSD test

EC Electrical conductivity, TN Soil total nitrogen, TOC Soil total organic carbon, C/N Total organic carbon and total nitrogen ratio, TP Soil total phosphorous, AP Soil available phosphorous, GLC β – glucosidase, PHO alkaline phosphomonoesterase, URE urease, DHA Dehydrogenase, ANOVA analysis of variance

** $p<0.01$, *** $p<0.001$, ns non-significant

The bacterial community composition and relationship with soil properties

After quality sequencing, a total of 859,255 paired end reads were obtained, ranging from 30,742 to 40,347 reads per sample (mean = 35,802 reads). For downstream analysis, the data were rarefied to 30,742 sequences, which had 7,446 ASV. The rarefaction curves showed that all samples reached a well-defined plateau of the curves, indicating adequate capture of the bacterial community present in these samples (Fig. S2).

Shannon or Chao1 indices did not show significant differences between treatments (Table S1). However,

the bacterial community composition was influenced by the cropping systems as confirmed by the per-MANOVA analysis ($F=3.181$; $p=0.001$), showing significant differences between two distinct groups. One group was formed by the IR and R treatments and the other by Control and I treatments, as represented in the PCoA plot (Fig. 2A) where axes 1 and 2 explained 30.3% and 9.2% of the variance, respectively.

The RDA axes 1 and 2 explained 77% and 12.8% of the bacterial community variation at the family level in response to environmental variables, respectively (Fig. 2B; $R^2=0.63$). The RDA representation showed that most soil properties influenced the microbial community of the R and IR treatments, in which GLC,

PHO, URE and DHA with longer arrows, showed a significant correlation ($p=0.001$, 0.044 , 0.024 and 0.014 respectively), followed by AP, pH, TDW and TOC with smaller arrows. These soil properties had a positive effect on the families *Xanthomonadaceae*, *Rhizobiaceae*, *Bevibacillaceae*, *Myxococcaceae*, *Beijerinckiaceae*, *Planococcaceae*, *Bacillaceae* but a negative effect on the families *Rubrobacteriidae*, *Vicinamibacteraceae*, and 67–14 (an unclassified family within the Actinomycetota phylum).

The most abundant phyla under different treatments were Actinomycetota (~26%), Pseudomonadota (~22%), Bacillota (~16%) and Acidobacteriota (~9.4%) followed by Chloroflexota, Planctomycetota, Myxococcota, Bacteriodota, Verrucomicrobiota, Gemmatimonadota and Nitrospirota. The relative abundances of Pseudomonadota, Bacillota, Myxococcota, and Bacteriodota were significantly higher under the treatments R and IR, while those of Actinomycetota, Acidobacteriota, and Gemmatimonadota were significantly higher in the Control and I treatments (Table 3).

The most abundant family was *Bacillaceae* (~11.19%) followed by 67–14 (unclassified family), *Nocardioideae*, *Micrococcaceae*, *Sphingomonadaceae*, *Vicinamibacteraceae*, JG30-KF-CM45 and *Beijerinckiaceae*, all together accounting for 18.67%. The relative abundance of *Bacillaceae* increased significantly in the R treatment, whereas the relative abundance of *Micrococcaceae*, *Beijerinckiaceae* and *Planococcaceae* were significantly higher under the treatments R and IR compared to the Control and I treatments. It is also notable that the relative abundances of nitrogen fixing related bacteria families like *Rhizobiaceae* (1.32%), *Xanthomonadaceae* (1%), *Azospirillaceae* (0.98%) and *Pseudomonadaceae* (0.24%) were also significantly higher in both crop rotation (the R and IR) treatments (Table 4).

The first 40 bacterial biomarkers identified through LEfSe were selected, representing 6 different phyla that were significantly influenced by the treatments ($p<0.05$; Fig. 3A). The observed biomarkers were *Blastocatellia* class and the phylum Actinomycetota; along with the unclassified family 67–14 for Control treatment; the phylum Acidobacteriota and the family *Vicinamibacteraceae* for the I treatment; the phylum Bacillota along with the family *Planococcaceae* and the phylum Myxococcota with the family *Myxococcaceae* for the R treatment; and the phylum Pseudomonadota, featuring the families *Xanthomonadaceae*,

Beijerinckiaceae and *Rhizobiaceae* and the phylum Bacteriodota with the families *Brevibacillaceae* and *Paenibacillaceae* for the IR treatment (Fig. 3B).

Predicted bacteria functional community

The functional analysis using FAPROTAX showed significant differences among functional groups related with C and N cycling (Table 5). The analysis of functional groups related to the C cycle exhibited a significantly higher relative abundance of chemoheterotrophy, cellulolysis, chitinolysis and xylanolysis in the R and IR treatments compared to the Control and I treatment. Among the functional groups related to the N cycle, only the nitrogen fixation and the ureolysis showed significant differences among treatments, being significantly higher in the R and IR treatments (Table 5).

Discussion

Legume-based cropping systems are increasingly valued within the context of sustainable agriculture, due to their ability to enhance crop yield while reducing fertilizer inputs (Murphy-Bokern et al. 2017; Schaedel et al. 2021; Kebede 2021). This is particularly important in regions where soil degradation, groundwater contamination, and the general loss of soil fertility in agricultural lands are more pronounced, such as in semi-arid Mediterranean areas (Jacobsen et al. 2012; Lagacherie et al. 2018; Ferreira et al. 2022). In this context, evaluating the potential of crops better adapted to Mediterranean conditions such as purslane, alongside the use of sustainable farming systems, may be crucial for the sustainability of agriculture in small-scale farms across the Mediterranean region.

This study provides evidence that crop rotation (peas – purslane) as well as intercropping (cowpea – purslane) and the combination of both practices (peas – cowpea – purslane) improved purslane yield in semi-arid Mediterranean conditions. All treatments positively impacted the growth of purslane compared to the Control as expected, considering this plant's pronounced response to soil nitrogen even at low concentrations (Carrascosa et al. 2023a, b). Comparing the different treatments, although no significant differences were found the results showed a slightly

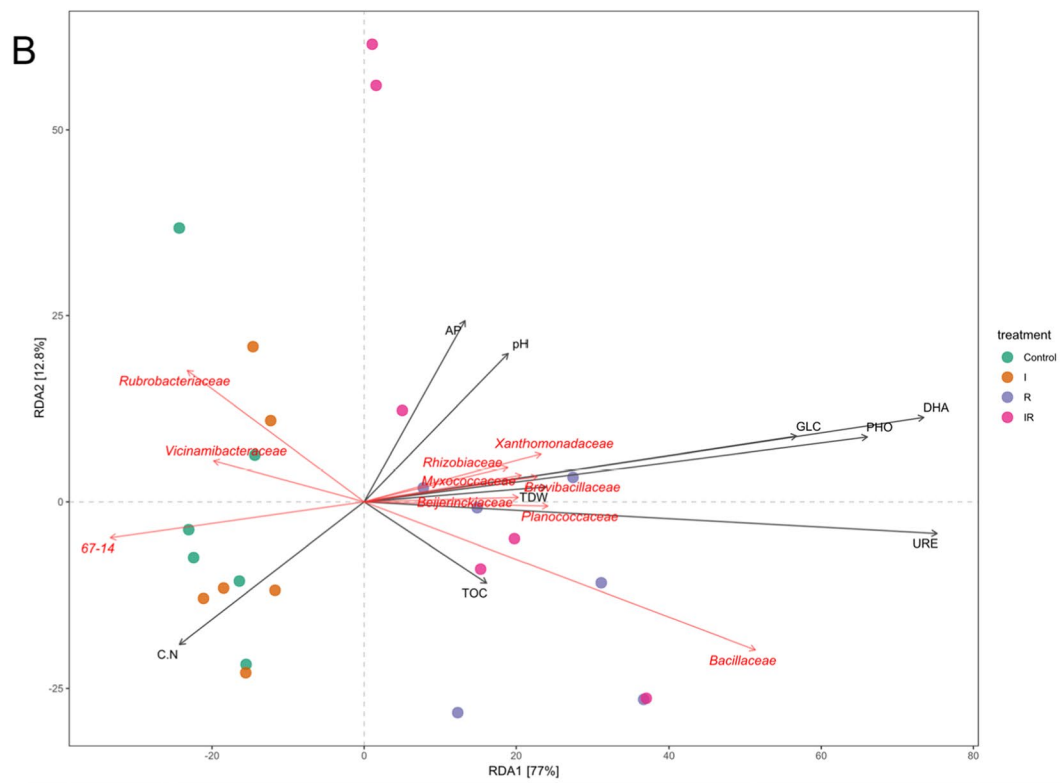
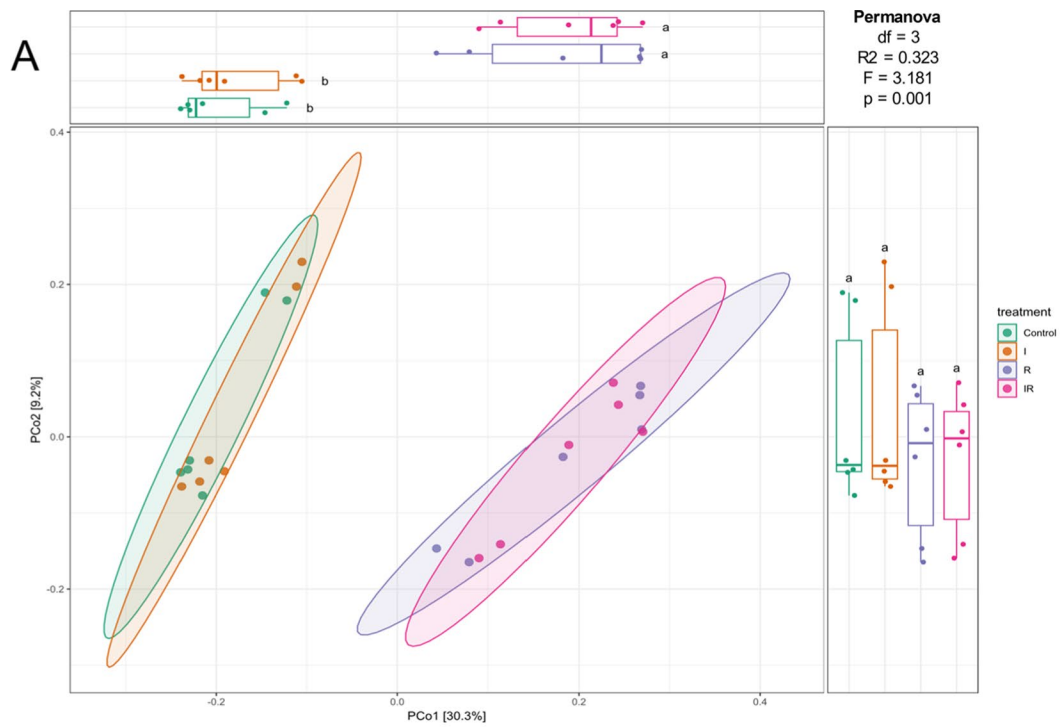


Fig. 2 **A** Principal coordinate analysis (PCoA) and PERMANOVA for bacterial communities of purslane rhizosphere under different cropping systems ($n=6$). The boxplots above and at the right side of the PCoA plot represent the sample values on PCo1 and PCo2 axes, respectively. Different letters in each chart denote significant differences between groups (Dunn's test, $p < 0.05$). **B** Redundancy Analysis (RDA) between bacterial communities at family level and environmental factors

higher increment in treatments I and IR (both with an approximate increase of 54% compared to the Control), while the R treatment had an increase of 34% compared to the Control. This could be due to the nitrogen fixed by the cowpea and peas stimulating the growth of purslane plants (Angon et al. 2023; Han et al. 2023). In particular, the slight difference in yield could be attributed to nitrogen fixation by the legume in the I and IR treatments which was probably immediately assimilated by purslane plants due to its roots could reach the vicinity of the nodules where BNF occur (Duchene et al. 2017). Moreover, the nitrogen predominantly found in the legume residue applied as green manure to the soil in the R treatment, could be first mineralised before assimilation by the following crop (Thilakarathna et al. 2016). Thus, purslane responded positively to fertilization during rotation and intercropping caused by the nitrogen fixation, achieving significant results without the need for extra fertilization. This is likely due to the plant's low nutritional requirements, allowing it to significantly increase its productivity even with minimal fertilization inputs under sustainable practices (Carrascosa et al. 2023b). These results are of great interest, as they highlight purslane as a potential crop for the Mediterranean region, capable of significantly increasing its yield under sustainable agricultural systems such as crop rotation and intercropping, while significantly reducing the use of agrochemical inputs in agroecosystems.

Both crop rotation and intercropping had a positive impact on organic carbon accumulation in the soil, stimulating soil microbial activity through root exudate production or plant residue accumulation, thereby promoting microbial growth and enzymatic activity (Curtright and Tiemann 2021; Li et al. 2024). Soil enzymatic activity serves as an indirect indicator of soil microbial activity and fertility, being correlated with nutrient cycling such as P and N, and organic matter degradation (Rao et al.

2014; Mencil et al. 2022). It has also been demonstrated that the enzymatic activity can increase in crop rotation systems along with the organic carbon accumulation in the soil in long term studies (Yang et al. 2024). However, similarly to Pittarello et al. (2021) our data indeed showed an increase in microbial enzymatic activity but without a significant accumulation of soil organic carbon. This may be because the enzymatic processes of mineralisation took place rapidly before the increase of organic carbon in the soil was detected and therefore slight changes in soil quality were recorded (Pascual et al. 2000; Bastida et al. 2006).

As expected, an increase was observed in enzymatic activities, namely PHO, URE, and DHA, in the crop rotation treatments (R and IR), as well as in GLC activity in the IR treatment that might stem from a stimulation of microbial activity caused by the peas root exudates and mostly by the incorporated legume residues as green manure after pea cultivation. On the other hand, no effect was detected by the I treatment on purslane rhizosphere enzymatic activity. The increase in DHA activity is associated with intracellular respiration processes and serves as an indirect indicator of microbial activity and organic matter degradation (Nannipieri et al. 2012) and similarly to our results it has been linked to short-term crop rotation studies, even with modest increases in soil organic matter (López-Garrido et al. 2014). The GLC activity is associated with cellulose degradation in legume residues, as observed in other studies (Jain et al. 2018; Carlos et al. 2022). However, significant effects in GLC activity were only found in the IR treatment, suggesting that the legume residue input alone (e.g., R treatment) is not sufficient to stimulate GLC activity in our conditions although it can be benefited from the association with the intercropping system (Cuartero et al. 2022b). The URE enzyme, involved in urea hydrolysis, is considered an indicator of organic N mineralization in the soil due to its relationship with organic C (Klose and Tabatabai 2000; Nannipieri et al. 2012). Its increase could be linked to organic matter accumulation in crop rotation systems (Jain et al. 2018; Qaswar et al. 2019; Klose and Tabatabai 2000). The PHO enzyme plays a crucial role in the soil phosphorus cycle by hydrolysing organic compounds to release assimilable P for organisms. Its activity is related to increased organic matter and nitrogen availability (Jain et al. 2018; Margalef et al.

Table 3 Relative abundance (%) of the top~99% bacterial phyla in the rhizosphere of purslane plants under different cropping systems

Phylum	Control	I	R	IR	ANOVA	KW
Actinomycetota	31.9±2.1 a	30.7±1.2 a	21.9±2.0 b	21.7±2.2 b	-	***
Pseudomonadota	19.5±0.7 b	19.5±1.0 b	23.0±1.5 a	24.9±1.8 a	***	-
Bacillota	10.9±1.6 b	12.1±0.8 b	20.7±3.8 a	18.6±4.2 a	-	***
Acidobacteriota	11.3±0.6 a	11.5±0.5 a	7.5±1.0 b	7.5±0.7 b	-	***
Chloroflexota	8.6±0.8	8.4±0.8	7.5±1.1	8.2±1.2	-	ns
Planctomycetota	5.3±0.5	5.3±0.7	4.7±0.9	4.7±0.3	ns	-
Myxococcota	3.0±0.3 b	2.9±0.3 b	4.2±0.6 a	4.1±0.6 a	***	-
Bacteroidota	2.6±0.3 b	2.7±0.3 b	4.3±0.7 a	4.2±0.5 a	-	***
Verrucomicrobiota	2.0±0.3 a	1.8±0.4	2.2±0.3	2.1±0.1	ns	-
Gemmatimonadota	2.2±0.2 a	2.3±0.3 a	1.5±0.4 b	1.6±0.2 b	***	-
Nitrospirota	0.6±0.1 a	0.6±0.1 a	0.4±0.1 b	0.5±0.1 b	*	-

Values (mean ± sd, $n=6$). Different lowercase letters correspond to different statistical groups ($p<0.05$) as determined by the Tukey's HSD test for ANOVA and by Dunn's Kruskal Wallis multiple comparison test

ANOVA Analysis of variance, KW Kruskal Wallis analysis of variance

* $p<0.05$, *** $p<0.001$, ns non-significant

2021), and its increased activity has been reported in various crop rotation studies similar to our findings (Jain et al. 2018; Nath et al. 2023).

Our data shows that the I treatment itself did not show any differences with respect to the soil properties compared to the Control, which may suggest that the differences found in the IR treatment would correspond only to the effect of the crop rotation. However, there might be a slight intercropping effect as observed in the case of GLC activity, which only increased in respect to the Control in the case of the IR treatment. In contrast to our results, other studies have also shown how intercropping with leguminous plants can also influence the enzymatic activity of the rhizosphere of the associated crop in intercropping systems (Eichler-Löbermann et al. 2021; Singh et al. 2021; Curtright and Tiemann 2021; Liao et al. 2022). However, it has also been pointed out that the effect of intercropping systems on enzymatic activities may depend on various factors, including the length of the study, sampling depth, planting framework or the residue quality (Curtright and Tiemann 2021; Cuartero et al. 2022b). Therefore, the absence of increased enzymatic activity in purslane rhizosphere in the I treatment, probably could be due to the short period of the experiment and the sowing distance between cowpea and purslane plants which were not close enough to facilitate such effects. Despite the absence of increased enzymatic activity, purslane

yield increased in the I treatment, and that could be attributed to the capacity of the purslane root system to extend into the cowpea rhizosphere and assimilate the nitrogen fixed by its nodules where the BNF is taking place.

Overall, crop rotations and intercropping systems are capable of increasing the diversity of microorganisms (Duchene et al. 2017; Yang et al. 2024), enhancing soil ecosystem functionality which has been linked to both soil and plant health (Brussaard et al. 2007; Berendsen et al. 2012). However, the effect of microbial diversity in cropping systems is heterogeneous among studies (Venter et al. 2016). Our results did not show any significant effect on the bacterial diversity in any of the I, R or IR treatments compared to the Control, similarly to other researchers who also did not report any changes in bacterial diversity in crop rotation (Town et al. 2023; Liu et al. 2023a) or in intercropping systems (Pivato et al. 2021; Xie et al. 2022; Cuartero et al. 2022b). On the other hand, remarkable differences were found related to the bacterial community composition as expected, where treatments involving rotation (R and IR) were grouped and differentiated from the treatments without rotation (Control and I). These results are consistent with the findings of other researchers who have reported that rotation systems, of both leguminous and non-leguminous species, can modify the structure of microbial communities (Jiang et al. 2016;

Table 4 Relative abundance (%) of the top ~50% bacterial families in the rhizosphere of purslane plants under different cropping systems

Family	Control	I	R	IR	ANOVA	W-ANOVA	KW
<i>Bacillaceae</i>	9.1 ± 1.3 b	9.9 ± 0.6 ab	14.4 ± 2.4 a	11.4 ± 4.0 ab	-	**	-
<i>67–14</i>	5.4 ± 0.5 a	5.1 ± 0.5 a	2.8 ± 0.5 b	2.7 ± 0.7 b	***	-	-
<i>Nocardioidaceae</i>	2.8 ± 0.6	2.7 ± 0.4	2.6 ± 0.5	2.8 ± 0.5	-	-	ns
<i>Micrococcaceae</i>	2.2 ± 0.2 b	2.3 ± 0.2 ab	2.9 ± 0.4 a	2.4 ± 0.5 a	*	-	-
<i>Sphingomonadaceae</i>	2.2 ± 0.4	2.3 ± 0.5	2.5 ± 0.4	2.9 ± 0.5	ns	-	-
<i>Beijerinckiaceae</i>	1.7 ± 0.2 b	1.8 ± 0.3 b	2.9 ± 0.4 a	3.0 ± 0.3 a	***	-	-
<i>Vicinamibacteraceae</i>	2.9 ± 0.2 a	2.9 ± 0.3 a	1.8 ± 0.3 b	1.8 ± 0.2 b	-	-	***
<i>JG30-KF-CM45</i>	2.4 ± 0.1	2.2 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	-	-	ns
<i>Rubrobacteriaceae</i>	2.7 ± 0.7 a	2.8 ± 1.0 a	1.2 ± 0.7 b	1.1 ± 0.5 b	-	-	**
<i>Gemmatimonadaceae</i>	2.0 ± 0.2 a	2.0 ± 0.4 a	1.3 ± 0.3 b	1.4 ± 0.2 b	***	-	-
<i>Chitinophagaceae</i>	1.2 ± 0.2 b	1.2 ± 0.2 b	2 ± 0.3 a	2.2 ± 0.2 a	-	-	***
<i>Planococcaceae</i>	0.8 ± 0.3 b	0.9 ± 0.3 b	2.6 ± 0.9 a	2.1 ± 0.9 a	-	**	-
<i>Geminicoccaceae</i>	2.0 ± 0.3 a	1.8 ± 0.5 ab	1.2 ± 0.4 c	1.1 ± 0.3 bc	**	-	-
<i>WD2101_soil_group</i>	1.7 ± 0.3 a	1.6 ± 0.3 ab	1.2 ± 0.3 b	1.3 ± 0.1 b	**	-	-
<i>Gemmataceae</i>	1.5 ± 0.2	1.5 ± 0.2	1.4 ± 0.3	1.4 ± 0.2	ns	-	-
<i>Myxococcaceae</i>	0.7 ± 0.2 bc	0.7 ± 0.1 c	2.1 ± 0.4 a	2.0 ± 0.3 ab	-	-	***
<i>Rhizobiaceae</i>	0.8 ± 0.2 b	0.9 ± 0.1 b	1.8 ± 0.4 a	2.0 ± 0.3 a	***	-	-
<i>Pirellulaceae</i>	1.5 ± 0.3	1.6 ± 0.4	1.2 ± 0.3	1.2 ± 0.2	ns	-	-
<i>Nitrosomonadaceae</i>	1.7 ± 0.1 a	1.6 ± 0.2 a	1.0 ± 0.2 b	1.0 ± 0.2 b	***	-	-
<i>Steroidobacteraceae</i>	1.1 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.4 ± 0.3	-	-	ns
<i>Streptomycetaceae</i>	1.4 ± 0.3 a	1.6 ± 0.2 a	0.8 ± 0.2 b	0.8 ± 0.1 b	***	-	-
<i>Blastocatellaceae</i>	1.1 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	ns	-	-

Values (mean ± sd, $n=6$). Different lowercase letters correspond to different statistical groups ($p < 0.05$) as determined by the Tukey's HSD test for ANOVA, Games – Howell's test for Welch's ANOVA and by Dunn's Kruskal Wallis multiple comparison test ANOVA Analysis of variance, W—ANOVA Welch's analysis of variance, KW Kruskal Wallis analysis of variance

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns non-significant

Zhou et al. 2017; Woo et al. 2022). However, contrary to our hypothesis we did not observe any structural changes in the bacterial community in the I treatment compared to the Control or the IR treatment compared to the R treatment. Supporting this observation, Pivato et al. (2021) reported that pea—wheat intercropping, resulted in no alterations in the bacterial community of the wheat rhizosphere. Moreover, other researchers have observed impacts on community structure due to intercropping (Liu et al. 2021b) and some studies have even identified differences in bacterial community structure under intercropping conditions with varying cropping frameworks (Cuartero et al. 2022b), or through the use of meshes to limit interactions between the root systems of the crops (Xie et al. 2022).

Regarding the taxonomy, our results showed that at phylum level the most abundant taxa were

Actinomycetota, Pseudomonadota, Bacillota, Acidobacteriota, Chloroflexota, Planctomycetes, Myxococcota, Bacteroidetes, Verrucomicrobia, Gemmatimonadetes, and Nitrospirota, a finding which is in accordance with the results reported by Bastida et al. (2017) in the same semiarid area. The composition of bacterial phyla in the rotation treatments (R and IR) were similar and showed a significant increase in the relative abundance of Pseudomonadota, Myxococcota, Bacteroidetes and Bacillota phylum, being the first four phyla associated with organic matter degradation (Hernández-Lara et al. 2022; Matheri et al. 2023). By contrast, the Actinomycetota, Acidobacteriota, Gemmatimonadetes, and Nitrospirota phylum decreased, following a similar trend as previously observed in other studies where Pseudomonadota increased and Actinomycetota decreased (Xie et al. 2022; Cuartero et al. 2022b; Bai et al. 2022). Also,

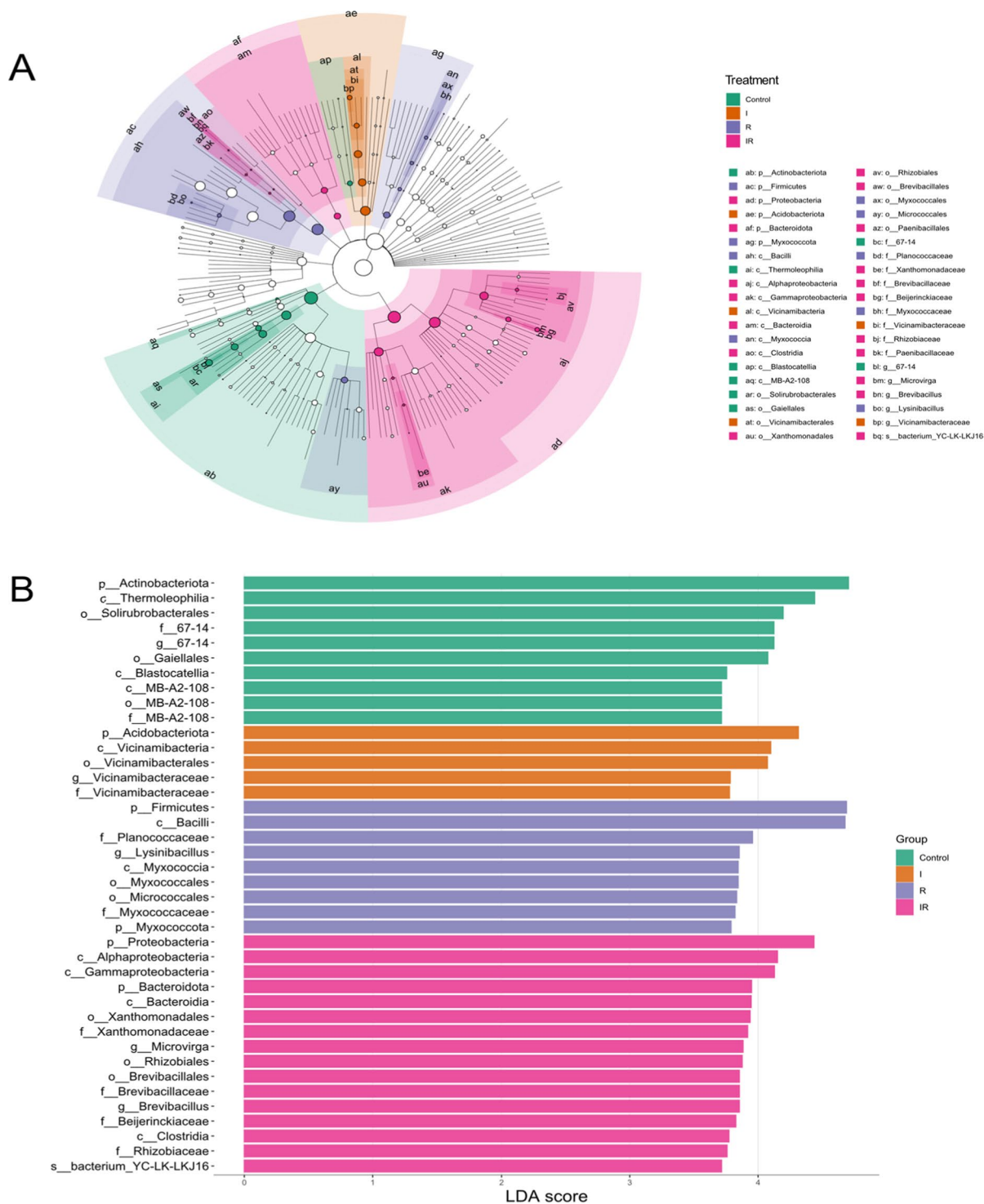


Table 5 Relative abundance of predicted pathways by FAPROTAX in purslane rhizosphere bacterial communities under different cropping systems

Pathway	Control	I	R	IR	ANOVA	W-ANOVA	KW
Chemoheterotrophy	22.35 ± 0.9 b	22.09 ± 1.14 b	27.05 ± 2.16 a	27.46 ± 1.76 a	-	***	-
Fermentation	0.71 ± 0.12 c	0.80 ± 0.13 bc	1.19 ± 0.45 a	1.33 ± 0.17 ab	-	-	**
Cellulolysis	0.29 ± 0.08 b	0.30 ± 0.19 b	0.92 ± 0.22 a	0.85 ± 0.29 a	***	-	-
Chitinolysis	0.29 ± 0.11 b	0.28 ± 0.12 b	0.91 ± 0.37 a	0.86 ± 0.23 a	-	-	***
Xylanolysis	0.01 ± 0.03 b	0.04 ± 0.06 ab	0.19 ± 0.12 a	0.13 ± 0.12 a	-	-	*
Nitrate reduction	2.30 ± 0.30	2.15 ± 0.27	2.17 ± 0.16	2.20 ± 0.34	ns	-	-
Nitrogen fixation	0.45 ± 0.12 b	0.61 ± 0.17 b	0.87 ± 0.20 a	1.08 ± 0.11 a	***	-	-
Ureolysis	0.47 ± 0.06 b	0.51 ± 0.11 b	0.92 ± 0.12 a	0.89 ± 0.11 a	***	-	-
Denitrification	0.14 ± 0.06	0.18 ± 0.08	0.23 ± 0.07	0.23 ± 0.12	ns	-	-
Nitrification	0.03 ± 0.04	0.04 ± 0.05	0.01 ± 0.03	0.04 ± 0.04	-	-	ns

Values (mean ± SD, $n=6$). Different lowercase letters correspond to different statistical groups ($p<0.05$) as determined by the Tukey's HSD test for ANOVA and Games – Howell test for Welch—ANOVA and by Dunn's Kruskal Wallis multiple comparison test

ANOVA Analysis of variance, W—ANOVA Welch's analysis of variance, KW Kruskal Wallis analysis of variance

* $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns non-significant

the lower relative abundance of Actinomycetota phylum under the R and IR treatments may be influenced by the preference of Actinomycetota for less fertile soils (Bao et al. 2021). These results are aligned with those of Ortiz-Cornejo et al. (2017), who also observed a higher relative abundance of Actinomycetota in the conventional cultivation system compared to the application of legume residues.

Within the Pseudomonadota phylum, notable families such as *Rhizobiaceae* and *Beijerinckiaceae* stood out, playing a pivotal role in the nitrogen cycle due to their ability to fix atmospheric nitrogen (Carareto Alves et al. 2014; Marín and Ruiz Arahal 2014), as we observed a significant increase in their relative abundance under the R and IR treatments. Members of the *Rhizobiaceae* family typically form symbiotic relationships with legumes forming nodules on their roots for atmospheric nitrogen fixation being one of the key motivations for crop associations involving legumes (Zahran 1999; Kebede 2021), whereas members of the *Beijerinckiaceae* family are free-living, non-symbiotic nitrogen-fixers that can utilize available soil organic matter for the energy required for nitrogen fixation (Marín and Ruiz Arahal 2014; Yoneyama et al. 2019). Although no significant differences were recorded, a slight increase of *Rhizobiaceae* and *Beijerinckiaceae* families was observed under the I treatment compared to the Control, and under the IR treatment compared to the R treatment. This might suggest

that despite intercropping did not significantly affect the overall purslane rhizosphere community composition, due to the separation between crops or the short duration of the experiments, the presence of cowpea plants might have slightly increased the abundance of N fixing bacteria (Curtright and Tiemann 2021; Cuartero et al. 2022b), thus explaining the increase of purslane yield under the I treatment.

Within the Bacillota phylum, the *Bacillaceae* family was the most abundant family in all treatments, accounting for between 9 and 14% of relative abundance, and along with the *Planococcaceae* and *Micrococcaceae* families they were significantly more abundant in both rotation treatments (R and IR) compared to the Control. These families comprise members associated with organic matter degradation, cellulolytic properties and denitrification, characterized by high microbial activity (Ishikawa et al. 2013; Ortiz-Cornejo et al. 2017; Silva et al. 2022; Cao et al. 2023). Therefore, the incorporation of legume residue may be the cause of the increase in the relative abundance of those families which may be responsible for the overall observed increase in soil enzymatic activity, related to organic matter degradation, as Ortiz-Cornejo et al. (2017) also observed when they used bean plant residues compared to conventional cropping.

The *Myxococcaceae* family, within the Myxococcota phylum, also increased its relative abundance under the

R and IR treatments, being capable of degrading complex carbon compounds, and through predation, they can contribute to community structure (Liu et al. 2022; Ibrahim et al. 2023). *Myxococcaceae* family has been suggested as an indicator of soil fatigue (Wolińska et al. 2018), thus, the increase of *Myxococcaceae* relative abundance in the treatments involving rotation (R and IR) might be an indicator of the improvement of soil quality caused by the rotation and the incorporation of legumes residues to the soil. Also, Liang et al. (2023) reported a positive association, similar to our results, between the *Myxococcales* order and the nodulation of *Rhizobacteria* in peanut plants.

Biomarkers revealed through the LEfSe analysis showed which taxa were responsible for explaining differences in bacterial communities among treatments. Bacterial biomarkers for the IR treatment included the *Rhizobiaceae* and *Beijerinckaceae* families among the *Rhizobiales* order. This reinforces our hypothesis that cowpea cultivation, while not capable of resulting in significant changes on purslane rhizosphere, it could reinforce the nitrogen fixing families especially those established previously by the rotation system. However, it is noteworthy that only the *Vicinamibacteraceae* family within the Acidobacteriota phylum was assigned as a biomarker under the intercropping treatment. This gives us an indication of the limited influence this treatment may have on the purslane rhizosphere by itself.

The changes in the bacterial community observed under the R and IR treatments seem to be linked to positive changes observed in enzymatic activities (GLO, PHO, DHA, and URE), soil organic carbon, and purslane yield. This was reflected in the RDA analysis, where families such as *Bacillaceae*, *Planococcaceae*, or *Myxococcaceae* were positively associated with the R and IR treatments, and could play a key role in the mineralization of legume residues. Thus, they could be accountable for the increase in microbial activity and the enhancement of nutrient availability (Ishikawa et al. 2013; Silva et al. 2022; Liu et al. 2022). Families like *Rhizobiaceae* and *Beijerinckiaceae* were also positively linked to the R and IR treatments and could be responsible for atmospheric N fixation in the soil (Carareto Alves et al. 2014; Marín and Ruiz Arahal 2014). These alterations in the bacterial community and soil characteristics seem to indicate that the stimulation of purslane growth could be caused by two factors: The N fixation (BNF) produced by the N-fixing bacteria recruited by

pea plants during rotation on one hand; and the degradation of organic matter, releasing nutrients into the environment on the other hand.

In addition to the taxonomic composition of soil bacterial communities, studying their potential functionality and their role in carbon and nitrogen cycles is crucial for understanding their connection to soil fertility. In general, higher potential functionality was found under the R and IR treatments in the carbon and nitrogen functional groups. Regarding C related functional groups, the increase of potential chemoheterotrophy could be associated with a general increase of the bacterial metabolism and activity, since it could be related to the utilization of a range of substrates (sugars, cellulose, lignin etc.), that could be related to the higher DHA and GLC enzymatic activity found in the R and IR treatments. Moreover, the increase of potential cellulolytic and xylanolytic activity, could be directly related to the higher degradation activity of the legume residues applied on the R and IR treatments which are rich in cellulose (26%–38%) and hemicellulose (5% – 12%) (Yetimwork et al. 2014). The Pseudomonadota and Bacillota phyla, specifically the Bacillaceae and Planococcaceae families, might account for the enhanced carbon molecule degradation rates recorded in our experiment, considering their substantial abundance, a finding which is in accordance with the findings from other studies (Francioli et al. 2016; Pang et al. 2017; Silva et al. 2022; Li et al. 2022; Cao et al. 2023). Related to processes associated with the N cycle, an increase only in the nitrogen fixation and ureolysis potential activity was found. As expected, potential ureolytic activity increased in the R and IR treatments similarly to the increase of URE enzymatic activity, since microorganisms have the capacity to mobilize N concealed in organic forms, and due to the supply of N from the legume residues (Craine et al. 2007). Additionally, as expected, an increase of potential N fixation activity was found in the R and IR treatments that could be directly attributed to the increase of N fixing bacteria such as *Rhizobiaceae* due to the prior cultivation of pea, that could have recruited them and consequently benefit purslane plants. However, it was unexpected that no other significant differences were observed in other N related functions such as denitrification or nitrogen assimilation. Some authors have noted that the addition of residues of legumes or maize may increase denitrification compared to monoculture

(Guo et al. 2011; Li et al. 2019). However, other authors observed differences in nitrogen fixation but not on nitrification, denitrification, or assimilation processes (Samaddar et al. 2021; Liu et al. 2023b). Thus, as an increase in the soil N was not observed, this could indicate that the fixed N is being rapidly assimilated by purslane roots, stimulating its growth but without increasing the total N in soil that could stimulate other N related functions. However, it is noteworthy to acknowledge that, even when the FAPROTAX database has been tested as a valuable test to predict the potential functionality of bacterial communities in soil (Sansupa et al. 2021), it is a limited tool based on culturable microbes (Louca et al. 2016) and therefore, other gene-based analysis such as functional gene amplicon sequencing or metagenomic sequencing would be more helpful to better understanding soil functionality (Li et al. 2019).

Both farming systems appear advantageous for purslane cultivation under semi-arid Mediterranean conditions. In essence, our results demonstrate that both crop rotation and intercropping have a beneficial effect on purslane growth despite that the rhizospheric mechanisms responsible for the growth increase were only observed during crop rotation. Nevertheless, as this is a first-year agricultural system, further research is needed to extrapolate the results to larger areas and assess their consistency over time.

Conclusions

To the best of our knowledge, this is the first study examining how sustainable cultivation systems based on legume association such as crop rotation and intercropping may affect purslane cultivation in semiarid areas and their impact on the composition, structure, and functionality of rhizosphere bacterial communities. Purslane responded positively to all the treatments studied, due to its high responsiveness to very low fertilization rates, however, changes in bacterial activity, composition, and functionality were only observed in the R and IR treatments. These changes could be attributed to an increase of organic matter degradation and nutrient mineralisation mediated by bacteria such as *Bacillaceae*, *Myxococcaceae*, and *Planococcaceae*, as well as to the incorporation of legume residues and the increase of nitrogen-fixing activity specifically by bacterial groups such

as *Rhizobiaceae* and *Beijerinckiaceae*, established during the preceding legume cultivation. Moreover, although I treatment did not influence the purslane rhizosphere, it did enhance purslane growth, as observed in the other tested treatments. Consequently, our study introduces novel perspectives for evaluating mixed cropping systems involving low-maintenance crops like purslane in semiarid regions under agrochemical free conditions, by linking the increase of crop production to changes in the rhizosphere and ecosystem services. Our findings could be useful in integrating wild edible species with low input requirements and notable tolerant to climate change conditions in small-scale farming systems of the Mediterranean basin.

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Data availability The bacterial sequences analysed in this study were submitted to the NCBI Sequence Read Archive repository (<https://www.ncbi.nlm.nih.gov/sra>). The sequences are available through the BioProject PRJNA1066122.

Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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