

The effect of sustainable management practices on the bacterial community in different European croplands

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ABSTRACT

Currently, monitoring Europe's soils is crucial since over 60 % of these soils are experiencing different types of degradation, which can potentially affect food production. Recently, a shift toward sustainable soil management has occurred. This shift away from conventional management is postulated to increase soil microbial diversity. However, it is unclear whether sustainable management can shift bacterial community across Europe. The Diversifarming project uses case studies to explore how diversified cropping systems with low-input practices can increase soil fertility, sequester carbon, and increase microbial diversity under differing climate conditions. To explore this, we employed metabarcoding sequencing to amplify the 16S rRNA region and soil chemical properties to assess the effects of organic amendment, rotation/intercropping and diversification with reduced tillage compared to conventional systems in different case studies. We observed that richness and Shannon index were mainly affected by climate and soil chemical properties but not by diversification. However, diversification changed the microbial community and enhanced potential microbial functionality, especially diversification of organic amendments, which also increase total organic carbon and nitrogen. We identified specific bacterial taxa associated with diversification, such as *Rubrobacter*, *MND1*, *Pontibacter* and *Sphingomonas*, highlighting the potential benefits of some species of these genera in diversification management ecosystems.

1. Introduction

Soil is a non-renewable resource, primarily recognized for supporting food production. It is estimated that 95 % of food is directly or indirectly produced on soil (FAO, 2015). However, due to factors such as erosion, pollution, and unsustainable practices (e.g., monocropping, the use of mineral fertilizers, or overexploitation), around 60–70 % of European soils are currently affected by one or more degradation processes (Commission et al., 2020). This degradation implies a decline in physical, chemical, and biological soil properties, affecting soil structure, functionality, and soil quality. The European Union (EU) has included concrete actions to achieve healthy soils by 2050, with specific actions

planned for 2030 to guarantee soil biodiversity that will maintain its functioning and its role as a provider of various ecosystem services (Delgado-Baquerizo et al., 2020).

The microbial community plays a key role in several soil processes, such as the carbon, nitrogen, and phosphorus cycles (Hu et al., 2021; Osburn et al., 2023; Saccá et al., 2017). Therefore, microbial community diversity, composition, and functionality can be good indicators of biodiversity (Schloter et al., 2018). Nevertheless, a common framework and suitable actions to protect and recover soil health are still lacking, highlighting the need for a new strategy based on the latest scientific evidence. The use of sustainable cropping systems increases soil microbial diversity, which can contribute to enhancing agricultural

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productivity (Koehler-Cole et al., 2020), carbon sequestration (Kay et al., 2019), and preventing soil erosion (Panagos et al., 2020). The objective is to preserve the soil for future generations, by maximizing biodiversity to improve soil health (Daunoras et al., 2024; Yang et al., 2021). However, there are many different sustainable cropping systems, such as incorporating organic amendments instead of mineral fertilizers (Cuartero et al., 2022a), reducing tillage to avoid soil structure disruption (Özbolat et al., 2023), or performing rotations and intercropping (Cuartero et al., 2022b; Fritze et al., 2024). These sustainable practices are localized, and it is still unclear whether using these sustainable cropping systems can increase microbial abundance, diversity, and functionality in locations with different conditions. Therefore, investigating whether soil bacterial communities respond predictably to sustainable agricultural management across a wide variety of climate conditions will reveal their potential as indicators of soil quality and health (Astudillo-García et al., 2019). These indicators can help managers design strategies for monitoring microbial diversity and soil function for end users and policymakers (Ranjard et al., 2010).

In this study carried out as part of the Diverfarming project (H2020), our hypothesis is that different climatic and sustainable management zones change soil bacterial composition, diversity, and potential function related to C, N, and P cycles. To test this hypothesis, soil samples from different long-term experiments (more than ten years) under sustainable management (non-tillage, organic amendment, and rotation/intercropping) and conventional cropping experiments were analyzed. Soil bacterial diversity indexes, taxonomy, and potential bacterial functionality analyzed by the Phylogenetic Investigation of

Communities by Reconstruction of Unobserved States (PICRUSt2), as well as soil physicochemical and chemical properties were measured. Therefore, the aims of this study were: (1) to identify potential bacterial indicators to characterize and monitor different types of sustainable soil management across various climate zones, (2) to detect changes in soil physicochemical properties through sustainable management in different climatic zones; and (3) evaluate which types of sustainable management improve soil bacterial structure and functional communities, regardless of the climatic zone.

2. Materials and methods

2.1. Experimental design and sampling

Soil sampling from long-term (LTs) experiments in several European countries, including Spain, Italy, Finland, and the Netherlands, covered a range of climate zones (Table 1). The long-term (LTs) experiments encompassed three types of sustainable management practices: i) zero- or reduced-tillage, ii) organic amendments, and iii) rotation or intercropping. These practices are collectively referred to as “diversification” throughout this manuscript. As controls, we also sampled their respective conventional cropping systems (Table 1). Each management in each LT was established in triplicate plots in the field. The soil was sampled between 2018 and 2019 at 0–10 cm depth using an auger (10 cm diameter). Two composite random samples derived from five subsamples were collected in each replicated plot, trying to cover the entire plot surface, avoiding the edge effect. The soil samples were separated

Table 1
Characteristics of the long-term experimental designs.

LT	Country	Pedoclimatic conditions	Type of crop	Main crop	Type of soil	Time of management	Management
LT1	Spain (Cartagena, Murcia region)	MS	Annual	Multiple vegetables	Haplic Calcisol (Loamic, Hypercalcic)	From 1995	Conventional with mineral fertilizers (LT1_C) Organic with manure (LT1_D1) Organic with compost and compost tea (LT1_D2)
LT2	Italy (Foggia, Apulia region)	MN	Annual	Wheat	–	From 1995	Monocrop tillage (LT2_C1) Monocrop non-tillage (LT2_C2) Rotation tillage (LT2_D1) Rotation non-tillage (LT2_D2)
LT4	Spain (Huesca, Aragon region)	MN	Annual	Barley	–	From 2010	Monocrop tillage (LT4_C) Monocrop non-tillage (LT4_D1)
LT7	Finland (Toholampi, Ostrobothnia region)	Boreal	Annual	Cereal grass	Gleyic Podzol	From 1997 and 2001	Conventional cereal with mineral fertilizers (LT7_C2) Organic cereal with manure (LT7_D2) Conventional grass with mineral fertilizers (LT7_C1) Organic grass with manure (LT7_D1)
LT8	Netherlands (Groningen)	Atlantic	Annual	Grass clover	–	>30 years	Conventional grass-maize-grass (LT8_C1) Rotation 1 (Wheat-Peas-Grass-Maize) (LT8_D1) Rotation 2 (Grass-clover-col-oat-potato) (LT8_D2) – 10 years Rotation 3 (Grass-clover-col-oat-potato) -20 years (LT8_D3) Conv-DSV mensegel with mycorrhizas (LT8_C4) Conv-DSV mensegel without mycorrhizas (LT8_D4)
Pedoclimatic conditions		Geographical coordinates		Mean annual temperature	Mean annual precipitation	Annual potential evapotranspiration	
Mediterranean south (MS) (LT1)		35°57'31"N, 0°56'17"O 37°51'59"N, 1°43'11"W		17.5 °C	280 mm	1288 mm	
Mediterranean north (MN) (LT4)		41°54'12"N, 0°30'15"W		13.4 °C	327 mm	1197 mm	
Mediterranean north (MN) (LT2)		41°27'57.3"N, 15°30'19.8"E		15.8 °C	529 mm	734 mm	
Boreal (B)(LT7)		63°46'17.8"N 24°14'52.1"E		3.0 °C	570 mm	105 mm	
Atlantic (A) (LT8)		53°21'32.8"N 6°20'01.0"E		9.4 °C	11.5 mm	Not available	

into two aliquots, one kept at room temperature for physicochemical analyses and the other stored in a cool box with ice for molecular analyses. The samples were immediately taken to the laboratory. The soil was air-dried for physicochemical analyses. The soil for molecular analyses was sieved at <2 mm once in the lab and stored at -20°C . For consistency with the project, we have maintained the numbering according to the Diverfarming project, which can be found on its website: <http://www.diverfarming.eu/index.php/en/diverfarmingpractices-2/repository-3>.

2.2. Measurement of physicochemical and chemical soil properties

Different soil physicochemical properties were measured, including pH and Electrical Conductivity (EC), as well as chemical properties such as Total Organic Carbon (TOC), Total Nitrogen (TN), Available Phosphorus (P), Exchangeable Calcium (Ca), Magnesium (Mg), and Potassium (K), were measured. Bulk Density (BD) was also measured. All these measurements were performed following the standardized protocols of the Diverfarming handbook (Alvaro-fuentes et al., 2019).

2.3. Soil DNA extraction, PCR amplification, and sequencing

Soil DNA was extracted from 1 g of soil (wet weight) using the DNeasy Power Soil Kit (Qiagen). Subsequently, the quantity of the DNA were assessed using a Qubit 3.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, USA) and the purity of the obtained DNA were determined through 260/280 nm absorbance measures using the NanoDrop spectrophotometer 2000 (Thermo Fisher Scientific, Waltham, MA, USA). The 16S hypervariable region was amplified using the Ion Torrent™ Personal Genome Machine™ with the Ion Xpress™ Plus Fragment Library Kit and Ion Xpress™ Barcode adapters (Thermo Fisher Scientific). Detailed information about the measurements can be found in the Diverfarming handbook (Alvaro-fuentes et al., 2019).

2.4. Metabarcoding data processing

The sequences obtained were denoised using ACACIA (Bragg et al., 2012) and subsequently imported into QIIME2 v2020.2 (Bolyen et al., 2019). In QIIME2, the sequences were denoised using the DADA2 algorithm, with sequences truncated at the quality threshold of $Q > 30$ (Bolyen et al., 2019). Amplicon Sequence Variants (ASV) generated by DADA2 were clustered and taxonomically classified using the SILVA 132 database (Quast et al., 2012). Following taxonomic classification, PICRUST2 was employed to predict the potential functional profiles based on the 16 rRNA sequences (Douglas et al., 2020). Since the data used in this experiment originated from specific small experimental projects, the sequences can be accessed in their respective ENA projects e.g., LT1: PRJEB38121; LT7: PRJEB60421. The sequences from the other LTs will be provided upon request.

2.5. Statistical analysis

LT1, LT7, and LT8 were analyzed using organic treatments, LT2 and LT4 employed reduced or zero-tillage, and LT2 and LT8 with rotation or intercropping (Table 1). The comparison among the sustainable management techniques were relativized through Response Ratio (RR):

$$RR = \ln \frac{\text{Diversified}}{\text{Conventional}}$$

It should be mentioned that the RR was calculated based on the number of diversified or conventional treatments at each experiment site. For example, in LT1, the two sustainable cropping systems (LT1_D1 and LT1_D2) were compared with the conventional system (LT1_C) (Table 1). In the experiments with two types of conventional management, the RR was calculated for each one: LT2_D1 vs. monocrop tillage (LT2_C1) and LT2_D2 vs. rotation tillage (LT2_C2). Positive and negative

lnRR values reflect the increased and reduced effect of diversification on soil, respectively.

Differences among climate zones and management practices were tested using one-way statistical analysis. Normality and homoscedasticity of variance were evaluated using the Shapiro-Wilk and Levene tests. When assumptions were met, a one-way ANOVA was performed, followed by Tukey's significant difference test. If assumptions were not met, a non-parametric Kruskal-Wallis test was conducted. Alpha diversity metrics (richness (number of species) and Shannon index) were calculated for each site. Bacterial structure (beta diversity) was studied by calculating the distance between replicates using the Bray-Curtis distance and represented through 3-D. Non-metric multidimensional scaling (NMDS) was used to display climate zones and management using the vegan v2.6-4 package (Oksanen et al., 2022). To evaluate the impact of climate zones (using the principal coordinates of the neighborhood matrix on geographical coordinates), management practices, and soil physicochemical properties (pH, TOC, EC, Pav, and NT) on bacterial diversity (richness (number of species) and Shannon index (with Hellinger-transformed communities)), a variance partitioning analysis using the varpart function from the vegan package was conducted (Plassart et al., 2019). Prior to the analysis, pairwise correlations among environmental variables were examined, and none exceeded $|0.8|$; therefore, multicollinearity was considered negligible.

Bacterial indicators were identified using Indicator Species Analysis (ISA). To associate indicators with climate zone and management conditions, sites were clustered according to soil physicochemical properties using the k-means method (Hartigan and Wong, 1979; Plassart et al., 2019), implemented with the k-means function from the stats v4.3.3 package (R Core Team, 2024). Subsequently, soil types were identified based on the cluster centers, and bacterial indicators specific to each soil type were identified using the multipatt function from the indispesies v1.7.17 package (De Cáceres and Legendre, 2009). All the analyses were performed using R v4.3.3, and the plotting was done using the ggplot2 v3.5.0 package (Wickham, 2016).

3. Results

3.1. Changes in bacterial diversity due to sustainable cropping systems

The variation in the bacterial Shannon diversity index was explained by climate zones, but not by diversification (Table 2). The effects of climate zones on richness and the Shannon index increased more in climate zone A than in the other climate zones and decreased in all the diversifications (Table 2). The representation of bacterial community (ASVs) by NMDS (Stress < 0.1) (Fig. 1) showed differences by climate zone, but did not show great differences among diversification. The

Table 2

The Response Ratio (RR) of Richness and Shannon index classified by climate zone and diversification.

(A, Atlantic; B, Boreal; MN, Mediterranean north; MS, Mediterranean south).

	Richness	Shannon index
Climate zone		
A	0.02 ± 0.18	0.03 ± 0.03 ^a
B	-0.23 ± 0.17	-0.05 ± 0.04 ^b
MN	-0.17 ± 0.32	-0.05 ± 0.07 ^b
MS	-0.03 ± 0.25	-0.01 ± 0.05 ^{ab}
<i>p-Value</i>	0.202	0.040
Diversification		
Organic	-0.13 ± 0.15	-0.03 ± 0.03
Rotation/Intercropping	-0.07 ± 0.20	-0.01 ± 0.04
Reduced Tillage	-0.10 ± 0.23	-0.03 ± 0.07
<i>p-Value</i>	0.41	0.44

Lower case indicated significant differences between climate zones, and diversifications ($p < 0.05$).

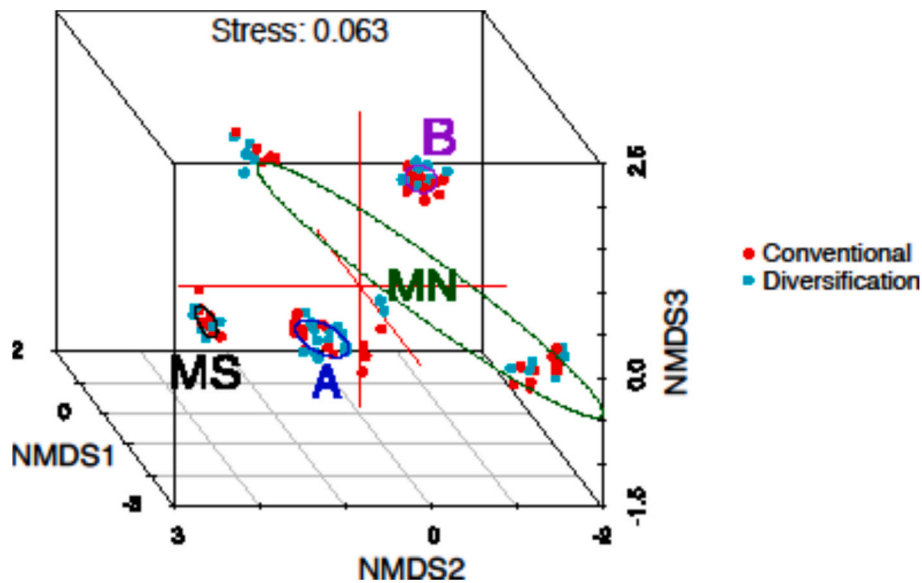


Fig. 1. Non-metric multidimensional scaling (NMDS) showing the bacterial community of all ASVs. (The points correspond to each plot site classified as conventional or diversified management, the circle represents the confidence at 60 %, A, Atlantic (LT8); B, Boreal (LT7); MN, Mediterranean north (LT2, LT4); MS, Mediterranean south (LT1, LT3); The stress < 0.1 indicates a good ordination).

variance partitioning analysis showed that richness and the Shannon index were more influenced by climate zone (15 %–12 %) (Fig. 2). Soil chemical properties explained between 10 %–11 % of the variance, while diversification only explained between 1 %–3 % (Fig. 2). The interaction between soil physicochemical and climatic zone showed the higher influence in richness and Shannon index (37 %–50 %) (Fig. 2).

3.2. Changes in bacterial guilds due to sustainable cropping systems

The study of potential bacterial functionality revealed differences in functional guilds for each climate zone and diversification in the carbon (Table 3), nitrogen (Table 4), and phosphorous cycles (Table 5). By climate zone, genes associated with C cycling increased in climate zone A and, in general, decreased in the other climate zones (Table 3). Rotation/intercropping increased genes related to C degradation (hemicellulose (FUCA), cellulose (malZ), and lignin (Catalase and gpx)) and C fixation (ppc). Organic management showed more genes related to C degradation (hemicellulose (FUCA) and Lignin (Catalase)), and C fixation (ppc), while reduced tillage showed higher C fixation (ppc), and C degradation (hemicellulose (FUCA), cellulose (malZ), and lignin (gpx))

(Table 3).

The potential abundance of *amoA* and *amoB* (nitrification) increased in MS and decreased in other climate zones. The genes *nifH*, *nifD*, and *nifK* (N fixation) increased in MS and B, while *hao*, *nosZ*, and *nirK* (denitrification) decreased in B (Table 4). In general, organic systems increased the potential abundance of genes involved in nitrification and nitrogen fixation, and some involving denitrification pathways. However, rotation/intercropping and reduced tillage decreased the potential abundance of nitrogen fixation and nitrification and increased the denitrification process (Table 4).

The potential abundance of polyphosphate kinase (*ppk*) and phosphonoacetaldehyde hydrolase (*phnX*) increased in climate zones A and B, and with rotation/intercropping, while only *phnX* increased in organic systems, and the abundance of *ppk* increased in reduced tillage (Table 5).

3.3. Changes in soil chemistry due to sustainable cropping systems

The effect of climate zones and diversified treatments changed the physicochemical and chemical properties of the soil (Table 6). Across

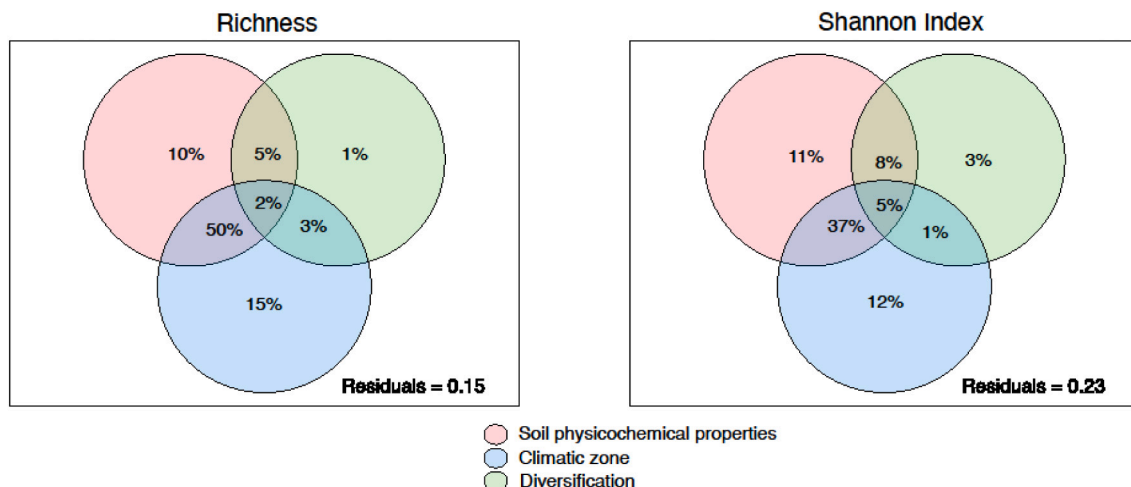


Fig. 2. Venn diagram showing the contributions of soil physicochemical properties, climatic zone, and diversification to richness and Shannon index.

Table 3
The Response Ratio (RR) of selected genes involved in the Carbon cycle by climate zone and diversification.

	<i>malZ</i>	<i>catalase</i>	<i>gpx</i>	<i>fuca</i>	<i>ppc</i>
Climate zone					
A	0.08 ± 0.11	0.10 ± 0.04 ^a	0.06 ± 0.07	0.05 ± 0.10 ^b	0.01 ± 0.02
B	-0.09 ± 0.15	0.07 ± 0.19 ^a	-0.04 ± 0.09 ^b	0.11 ± 0.28 ^{ab}	0.04 ± 0.08
MN	0.02 ± 0.07	-0.02 ± 0.06 ^b	-0.02 ± 0.08	0.21 ± 0.14 ^a	0.01 ± 0.10
MS	-0.09 ± 0.11	-0.03 ± 0.11 ^b	-0.08 ± 0.11	-0.05 ± 0.10 ^b	0.04 ± 0.04
<i>p-Value</i>	0.059	0.009	0.131	0.028	0.443
Diversification					
Organic	-0.09 ± 0.08 ^b	0.02 ± 0.10 ^{ab}	-0.06 ± 0.06 ^b	0.03 ± 0.14	0.04 ± 0.04
Rotation/ Intercropping	0.07 ± 0.07 ^a	0.07 ± 0.04 ^a	0.05 ± 0.05 ^a	0.11 ± 0.08	0.02 ± 0.02
Reduced Tillage	0.07 ± 0.15 ^{ab}	-0.08 ± 0.10 ^b	0.110 ± 0.28 ^{ab}	0.21 ± 0.05	1.06 ± 0.35
<i>p-Value</i>	0.005	0.012	0.009	0.340	0.075

Lower case indicated significant differences between climate zones and diversification ($p < 0.05$). *malZ*: Endo-1,4-β-mannosidase; *catalase*: Catalase; *gpx*: Glutathione peroxidase; *FUCA*: Alpha-L-fucosidase; *ppc*: Phosphoenolpyruvate carboxylase.

different climate zones in B and MN, the content of TOC, TN, Caex, and Mgex increased, while in zone A, TOC, TN, Mgex, and BD decreased, and pH and Pav increased. Regarding diversification, the organic cropping system increased pH, TOC, TN, and BD levels. In contrast, the rotation/intercropping systems reduced TOC, BD and TN, while increasing pH. Reduced tillage increased TOC but reduced TN and pH, among others (Table 6).

3.4. Climate zone, soil physicochemical and chemical properties, and management-affected bacterial composition

Three clusters were established based on the elbow method (Table S1; Fig. S1). Based on the Z scores, soils were clustered and classified as follows: 1) samples with positive values of pH and TN (common soils), 2) samples with high EC and Pav and with low TN content (rich in phosphorous), and 3) samples with low pH and Pav and

Table 4
The Response Ratio (RR) of selected genes involved in the Nitrogen cycle by climate zone and diversification.

	<i>amoA</i>	<i>amoB</i>	<i>hao</i>	<i>narG</i>	<i>nosZ</i>	<i>norB</i>	<i>nirK</i>	<i>nifH</i>	<i>nifD</i>	<i>nifK</i>
Climate zone										
A	-0.2 ± 0.4	-0.2 ± 0.4	-0.1 ± 0.3	0.1 ± 0.1	0.3 ± 0.2 ^a	0.2 ± 0.1	-0.2 ± 0.1 ^b	-0.5 ± 0.3 ^b	-0.5 ± 0.3 ^b	-0.54 ± 0.27 ^b
B	0.1 ± 0.9	-0.1 ± 0.9	-0.1 ± 0.8	0.1 ± 0.2	-0.1 ± 0.2 ^b	0.2 ± 0.2	-0.1 ± 0.3 ^b	0.3 ± 0.4 ^a	0.3 ± 0.5 ^a	0.27 ± 0.44 ^a
MN	-0.3 ± 0.5	-0.2 ± 0.5	-0.1 ± 0.4	0.2 ± 0.1	0.1 ± 0.2 ^{ab}	0.2 ± 0.2	0.2 ± 0.2 ^a	0.1 ± 0.2 ^a	-0.1 ± 0.1 ^a	-0.01 ± 0.14 ^a
MS	0.1 ± 0.3	0.2 ± 0.3	0.5 ± 0.3	-0.1 ± 0.1	0.1 ± 0.1 ^{ab}	0.1 ± 0.1	-0.1 ± 0.2 ^b	0.1 ± 0.2 ^a	0.1 ± 0.2 ^a	0.10 ± 0.23 ^a
<i>p-Value</i>	0.654	0.618	0.276	0.058	0.021	0.44	< 0.001	0.003	0.004	0.002
Diversification										
Organic	0.1 ± 0.4	0.1 ± 0.4	0.3 ± 0.4	-0.1 ± 0.1 ^b	-0.1 ± 0.1 ^b	0.1 ± 0.1	-0.1 ± 0.2	0.2 ± 0.2 ^a	0.2 ± 0.2 ^a	0.19 ± 0.23 ^a
Rotation/ Intercropping	-0.3 ± 0.4	-0.3 ± 0.4	-0.1 ± 0.3	0.1 ± 0.1 ^b	0.2 ± 0.1 ^a	0.2 ± 0.1	-0.1 ± 0.1	-0.3 ± 0.2 ^b	-0.3 ± 0.2 ^b	-0.33 ± 0.20 ^b
Reduced Tillage	-0.1 ± 0.9	-0.1 ± 1.0	-0.1 ± 0.7	0.3 ± 0.3 ^a	0.2 ± 0.7 ^{ab}	0.3 ± 0.6	0.3 ± 0.5	0.1 ± 0.4 ^{ab}	-0.1 ± 0.4 ^{ab}	-0.04 ± 0.38 ^{ab}
<i>p-Value</i>	0.538	0.625	0.276	0.006	0.036	0.863	0.071	0.003	0.004	0.003

Lower case indicated significant differences between climate zones and diversification ($p < 0.05$); *amoA*, *amoB*: Subunits of ammonia monooxygenase; *hao*: Hydroxylamine oxidoreductase; *narG*: Nitrate reductase catalytic subunit; *nosZ*: Nitrous oxide reductase; *norB*: Nitric oxide reductase subunit; *nirK*: Copper-containing nitrite reductase; *nifH*, *nifD*, *nifK*: Structural subunits of nitrogenase.

high TOC and TN content (rich in organic carbon and nitrogen).

One hundred and twelve bacterial indicators ($P < 0.05$) were identified. The five most significant were *Rubrobacter*, *Blastococcus*, *MND1*, *Sphingomonas*, and *Pontibacter* for cluster 1 (common soils); *Ilumatobacter*, *Thermomonas*, *Terrimonas*, *Cellulomonas*, and *Terruginibacter* for cluster 2 (rich in phosphorous); and *Oryzihumus*, *Bradyrhizobium*, *Dokdonella*, *Candidatus Solibacter*, and *Hyphomicrobium* for cluster 3 (rich in carbon and nitrogen) (Table 7). Among these microorganisms, the majority were mainly influenced by soil physicochemical properties and climate zones (Table 7). However, *Rubrobacter*, *Blastococcus*, *MND1*, *Sphingomonas*, and *Pontibacter* (cluster 1) were also affected by diversification, showing similar and even slightly higher abundances than those in the conventional system (Table 7).

4. Discussion

Measuring and monitoring soil biodiversity is essential for preserving soil quality and the ecosystem services it provides (Bardgett and van der Putten, 2014). Therefore, identifying the structure and function of the soil microbial community is necessary because microorganisms play a vital role in the nitrogen, phosphorous, sulfur, and carbon cycles (Van

Table 5
The Response Ratio (RR) of selected genes involved in the Phosphorous cycle by climate zone and diversification.

	<i>phnX</i>	<i>ppk</i>
Climate zone		
A	0.52 ± 0.26 ^a	0.03 ± 0.04 ^a
B	0.15 ± 0.59 ^{ab}	0.02 ± 0.05 ^a
MN	-0.23 ± 0.24 ^b	0.04 ± 0.06 ^a
MS	0.16 ± 0.38 ^{ab}	-0.06 ± 0.04 ^b
<i>p-Value</i>	0.002	0.008
Diversification		
Organic	0.15 ± 0.32	-0.02 ± 0.03
Rotation/Intercropping	0.28 ± 0.21	0.03 ± 0.02
Reduced Tillage	-0.50 ± 0.77	0.06 ± 0.23
<i>p-Value</i>	0.060	0.069

Lower case indicated significant differences between climate zones and diversification ($p < 0.05$). *phnX*: Phosphonoacetaldehyde hydrolase; *ppk*: Polyphosphate kinase;

Table 6

The response ratio (RR) of soil physicochemical and chemical properties classified by climatic zone and diversification.

	BD	pH	EC	TOC	TN	Pav	Caex	Mgex	Kex
Climate zone									
A	-0.04 ± 0.04	0.13 ± 0.03 ^a	-0.03 ± 0.25	-0.35 ± 0.19 ^b	-0.088 ± 0.32 ^b	0.31 ± 0.20 ^a	0.67 ± 0.23 ^a	-1.10 ± 0.35 ^b	-0.02 ± 0.30
B	0.02 ± 0.12	0.02 ± 0.02 ^b	-0.03 ± 0.06	0.10 ± 0.09 ^a	0.10 ± 0.10 ^a	-0.51 ± 0.13 ^b	0.01 ± 0.10 ^b	0.16 ± 0.21 ^a	0.11 ± 0.18
MN	-0.04 ± 0.07	-0.02 ± 0.0 ^b	0.55 ± 0.53	0.14 ± 0.18 ^a	0.04 ± 0.13 ^a	0.32 ± 0.21 ^a	0.20 ± 0.15 ^b	0.40 ± 0.36 ^a	-0.01 ± 0.14
MS	0.05 ± 0.06	0.02 ± 0.02 ^b	-0.17 ± 0.28	-0.01 ± 0.3 ^a	0.06 ± 0.30 ^a	-0.45 ± 0.49 ^b	-0.01 ± 0.22 ^b	0.03 ± 0.20 ^a	0.29 ± 0.20
<i>p-Value</i>	0.204	<0.001	0.179	0.002	<0.001	<0.001	<0.001	<0.001	0.209
Diversification									
Organic	0.03 ± 0.05 ^a	0.019 ± 0.013 ^b	-0.10 ± 0.13 ^b	0.04 ± 0.15 ^a	0.08 ± 0.14 ^a	-0.48 ± 0.23 ^b	-0.01 ± 0.11 ^b	0.09 ± 0.13 ^b	0.20 ± 0.13
Rotation/ Intercropping	-0.02 ± 0.04 ^{ab}	0.081 ± 0.08 ^a	-0.04 ± 0.15 ^b	-0.21 ± 0.13 ^b	-0.51 ± 0.27 ^b	0.27 ± 0.14 ^a	0.43 ± 0.19 ^a	-0.66 ± 0.31 ^c	-0.04 ± 0.18
Reduced Tillage	-0.14 ± 0.05 ^b	-0.069 ± 0.03 ^c	1.79 ± 0.63 ^a	0.36 ± 0.62 ^a	-0.03 ± 0.38 ^{ab}	0.56 ± 0.46 ^a	0.49 ± 0.15 ^a	1.11 ± 0.92 ^a	0.16 ± 0.37
<i>p-Value</i>	0.010	<0.001	0.001	0.001	0.011	<0.001	0.001	<0.001	0.982

Bulk density (BD), Electrical conductivity (EC), Total Nitrogen (TN), Exchangeable Ca, K, and Mg, Total Organic Carbon (TOC), Available Phosphorus (Pav). MS (Mediterranean South), MN (Mediterranean North), A (Atlantic), B (Boreal). (Average + Margin). Letters represent significant differences between climatic zone and diversification ($p < 0.05$).

Table 7

The most significant bacterial indicators based on the number of clusters using soil physicochemical properties. Number of species identified: Cluster 1 = 43, Cluster 2 = 41 and Cluster 3 = 28.

Genus (cluster)	Relative abundance (%)		Variance partitioning (%)		
	Diversified	Conventional	Soil properties	Diversification	Climatic zone
<i>Rubrobacter</i> (1)	0.7	0.6	<0.1	<0.1	<0.1
<i>Blastococcus</i> (1)	1.9	2.0		1.4	16.0
<i>MNDI</i> (1)	2.6	2.5	0.2	3.1	6.1
<i>Sphingomonas</i> (1)	7.8	8.0	14.1	4.4	34.0
<i>Pontibacter</i> (1)	1.3	1.1	0.3	0.6	14.4
<i>Ilumatobacter</i> (2)	0.3	0.3	35.1		7.8
<i>Thermomonas</i> (2)	0.2	0.1	24.9		
<i>Terrimonas</i> (2)	0.4	0.3	45.3		4.6
<i>Cellulomonas</i> (2)	0.3	0.2	20.0		1.8
<i>Ferruginibacter</i> (2)	0.5	0.2	29.4		2.0
<i>Oryzihumus</i> (3)	1.5	1.8	5.1		3.9
<i>Bradyrhizobium</i> (3)	3.5	5.7	5.9		2.5
<i>Dokdonella</i> (3)	0.1	0.1	0.9		
<i>Candidatus.Solibacter</i> (3)	0.2	0.3	2.1		1.1
<i>Hyphomicrobium</i> (3)	0.4	0.3			4.8

Der Heijden et al., 2008). Microbial communities are influenced by various factors, including location, management practices, soil physicochemical properties, and climate conditions (Drenovsky et al., 2010; Hopkins et al., 2023; Madegwa and Uchida, 2021; Bahram et al., 2018). In our study, diversity did not increase in the diversification treatments compared to the control (Table 2). Similar to observed by Özbolat et al. (2023) with reduced tillage and green manure amendments. Furthermore, soil physicochemical properties and climate zones were the main drivers of microbial diversity (Fig. 2), consistent with findings by Bahram et al. (2018) and Finn et al. (2021). Our limited understanding of microbial diversity does not align with its essential roles in ecosystem functions and is insufficient to address threats such as climate change and human disturbances (Bodelier, 2011; Guerra et al., 2020; Zhou and Ning, 2017).

In our study, the overriding effects of climate zones were higher than those on diversification of bacterial community and diversity (Figs. 1 and 2). Climate is one of the five soil forming factors and has a significant influence on soil properties. The Atlantic climatic zone (A) showed an increase in diversity considering that temperature and high humidity greatly contribute to above-and below-ground diversity patterns and community composition (Huang et al., 2023). Noll et al. (2022)

highlight the important role of soil geochemistry when estimating microbial nutrient cycling at continental to global scales and they demonstrate that, at this scale, soil parent material and climate override the effects of land use on soil organic N transformations, considering that soil fertility and plant productivity are globally constrained by N availability.

Our study demonstrated that rotation/intercropping diversification increases potential genes related to C degradation (lignine, hemicellulose and cellulose) (Table 2), probably due to promotion of diverse microbial communities that play a crucial role in breaking down organic matter and releasing nutrients (Czaban et al., 2018; Li et al., 2023). This increase in C degradation was not correlated with a rise in TOC (Table 6). The possibly induce potential loss of C accumulation in the soil can be due to the enhanced potential abilities of microbial catabolism. These are likely responsible for accelerated decomposition of SOC components with slow turnover rates to the acceleration of organic matter break down (Chen et al., 2020). Soil OC storage in agricultural systems is a balance between C inputs from crop residues and organic amendments, and C losses, mainly as CO₂ released into the atmosphere during the decomposition of organic matter (OM) (Bird et al., 2002). Organic amendment diversification increase of genes associated with C

fixation (ppc) and hemicellulose degradation (FUCA) (Table 2) and was linked to increased TOC, probably due to the composition of the organic amendments (compost, manure or plant residues). The increased carbon input as organic amendment, is mineralized by soil microbes into dissolved organic carbon, that is then assimilated into microbial biomass or sorbed to mineral surfaces (Mattila and Vihanto, 2024). Microbes also utilize carbon uptake in synthesizing residues like exopolymeric substances, which are involved in carbon sequestration. Therefore, the more carbon assimilated into microbial biomass, the better the carbon sequestration/stabilization (Kallenbach et al., 2016). Reduced tillage diversification also increased potential genes related to C fixation (ppc) (Table 2). Shi et al. (2024) observed that over 70 % of the tillage-responding KEGG orthologs (KOs) associated with C fixation (primarily in the reductive citric acid cycle) were more abundant under zero-tillage compared to conventional tillage.

The temperature varied across the climate zones (B < A < MN < MS), affecting decomposition rates more than primary production, and the net result should be a decrease in soil C content (Batjes and S, 1997). However, the situation is probably more complex, because, if increasing temperature is accompanied by low water availability, the decomposition rate should decrease. Therefore, water availability becomes the main conditioning factor (Bottner et al., 2000).

Organic amendment diversification increased the total nitrogen in the soil and also increased N fixation and nitrification processes (Table 3). The increased nitrogen fixation in organic amendment diversification is probably due to the increased soil pH and high organic carbon input that facilitate the growth of diazotrophs, and thus enhance the nitrogenase activity and nifH gene abundance (Shi et al., 2021). The fixed nitrogen that is provided by biological nitrogen fixation is less prone to leaching and volatilization as it is utilized in situ, and therefore the biological process contributes an important and sustainable input into agriculture (Dixon and Kahn, 2004). Vadakattu and Paterson (2006) observed the importance of these bacteria; where an intensive wheat rotation farming system demonstrated that free-living microorganisms contributed 20 kg per hectare per year to the long-term nitrogen requirements of this cropping system (30–50 % of the total needs). Organic amendments contain nitrogen in varying concentrations, with some forms more readily available than others (Lashermes et al., 2010). Xiao et al. (2019) also concluded that adding organic N fertilizers promotes a higher ammonia-oxidizing abundance than inorganic N fertilizers. In this study, manure was the organic amendment most widely used. It is considered an excellent source of nitrogen also in the organic amendments due to the increase of labile C and the growth of the heterotrophic or mixotrophic microorganisms (Rahav et al., 2016; Zheng et al., 2023). However, intercropping/rotation showed lower nitrogen fixation and nitrification, contrary to Liu et al. (2021) who showed that intercropping was associated with the enrichment of nitrogen-fixing bacteria and higher soil nitrogen utilization efficiency. This could be due to the level of nitrogen applied. Ruibo et al. (2021) determined that as levels of applied nitrogen fertilizer rose, nitrogen-fixing bacterial abundance decreased. Also, the right combination of crops can influence the improvement of soil properties (Giacometti et al., 2021). An increase in some denitrification genes with soil diversification (Table 4) could indicate higher soil N₂O emissions (Shaw et al., 2006), probably due to an excess in inorganic nitrogen. However, the increase in nosZ in reduced tillage and rotation/intercropping could indicate greater N₂O–N₂ conversion and lower greenhouse gas production (Krause et al., 2017). Cuartero et al. (2022b) observed similar results in an intercropping system.

The principle of nitrogen-phosphorus ratio stoichiometry states that increases in nitrogen enhance microbial inorganic phosphorous demands (Xiao et al., 2018). This was observed in our study of the organic amendment, which increased the abundance of phosphorous cycling genes. The most abundant phosphorous cycling gene present in the climatic zones A, B, and MN and in rotation/intercropping and reduced tillage was ppK (Table 5), which catalyzed the polymerization of

phosphorous monomers to generate polyphosphate molecules. Polyphosphate molecules serve as energy reservoirs in microbes for biochemical processes involving the phosphorylation of biomolecules such as sugars, nucleic acids, and proteins and enhance their survival and growth in the environment (Holden, 2015; Rao et al., 2009). The *phnX* gene product phosphonoacetaldehyde hydrolase cleaved the C–P bond from its unique substrate to yield acetaldehyde and Pi (Dumora et al., 1989; La Nauze et al., 1977) for the crop in the organic amendment and rotation/intercropping.

Results suggested that the type of diversification did not significantly affect all the taxa (Table S1). They were more influenced by soil physicochemical properties, which is consistent with previous studies (Mhete et al., 2020; Sui et al., 2021; Wakelin et al., 2016). The genera affected by diversification practices were (*Rubrobacter*, *MND1*, *Blastococcus*, *Sphingomonas* and *Pontibacter*) associated to soils with positive values of pH and TN. *MND1*, belonging to the Nitrosomonadaceae family represents taxonomic units comprising ammonia-oxidizing bacteria (Kong et al., 2016). A recent study suggested that *Sphingomonas* possesses multifaceted functions ranging from reducing environmental pollution to producing highly beneficial phytohormones, e.g., gibberellins and indole acetic acid, which promote plant growth (Asaf et al., 2020). *Pontibacter* (Bacteroidetes) and *Sphingomonas* (Pseudomonadota) are also annual soil indicators affected by management that have been related to plant growth (Dastager et al., 2011; Mazoyon et al., 2023). *Blastococcus* exhibit heavy metal tolerance and advantages in stress resistance, indicating promising potential for mitigating polluted soil ecosystems (Hou et al., 2021; Rakita et al., 2020). Alcalá-Herrera et al. (2023) showed that differences between conventional management and sustainable agriculture can probably be attributed to families and genera ascribed to Actinobacteria (*Arthrobacter* and *Rubrobacter*). Certain *Rubrobacter* species are involved in microbial ammonium uptake, suggesting that they play a role in nitrogen assimilation within the diversification practices (Andrade et al., 2005) and also in organic matter decomposition and mineralization (Adeleke et al., 2019; Raimi et al., 2017). These results suggest that diversification influences beneficial microorganisms for plant and soil, regardless of their location.

5. Conclusion

Our results reveal that soil bacterial community is more influenced by soil physicochemical properties and climate zones than by diversification practices. The potential abundance of genes involved in carbon, nitrogen, and phosphorus cycles indicates that soils, especially those with organic management, could increase their potential functionality, as well as TOC and TN. Based on soil physicochemical properties, we identified potential bacterial indicators where some species are beneficial to plant and soil genera, and were strongly affected by diversification (*Rubrobacter*, *MND1*, *Pontibacter* and *Sphingomonas*).

CRedit authorship contribution statement

J. Cuartero: Writing – review & editing, Writing – original draft, Software, Formal analysis, Conceptualization. **B. Frey:** Writing – review & editing, **R. Zornoza:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **V. Sánchez-Navarro:** Writing – review & editing, Formal analysis. **L. Canfora:** Writing – review & editing, Methodology, Formal analysis. **O. Özbolat:** Writing – review & editing. **M. Egea-Cortines:** Writing – review & editing, Formal analysis. **R. Farina:** Writing – review & editing. **H. Fritze:** Writing – review & editing, Methodology. **T. Tuomivirta:** Writing – review & editing, Resources. **K. Lång:** Writing – review & editing, Resources, Formal analysis. **R. Lemola:** Writing – review & editing, Resources, Formal analysis. **J. Álvaro-Fuentes:** Writing – review & editing, Resources, Data curation. **E. Huerta-Lwanga:** Writing – review & editing, Resources, Data curation. **J.A. Pascual:** Writing – review & editing, Validation, Supervision, Conceptualization. **M. Ros:** Writing – review &

editing, Writing – original draft, Validation, Supervision, Conceptualization.

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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2025.106456>.

Data availability

Since the data used in this experiment originated from specific small experimental projects, the sequences can be accessed in their respective ENA projects e.g., LT1: PRJEB38121; LT7: PRJEB60421. The sequences from the other LTs will be provided upon request.

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