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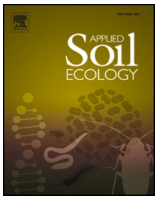

Research paper

Metataxonomic analysis reveals that the farming system has minimal effect on soil microbiome variation in cereal fields

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ABSTRACT

The soil microbiota, particularly mycotoxin-producing fungal communities, has garnered increasing attention owing to its implications for human and animal health. Mycotoxins commonly contaminate cereal crops and their by-products, with their associated health risks intensified by climate change. The growing demand for organic food, driven by perceptions of enhanced safety, contrasts with the inconclusive research on mycotoxin contamination in organic versus conventional cereals. Here, metagenomic techniques such as amplicon sequencing offer valuable tools for elucidating this issue. In this study, we employed metataxonomics to investigate potential variations in fungal and bacterial community diversity between organic and conventional farming systems in cereal fields, with a focus on toxigenic fungi. Our analysis revealed that geographic location of the samples, rather than farming practice (organic/conventional), was the primary driver of diversity differences. Furthermore, we found no significant evidence of differential abundance across toxigenic fungal groups between the two systems. To validate taxonomic assignments, we constructed a phylogenetic tree using sequences classified under the genus *Fusarium*. Additionally, we assessed the potential activity of biocontrol agents from certain bacterial genera against toxigenic fungi by analysing their co-occurrence patterns in both conventional and organic soils. Most correlations were weak, although a few significant associations involved *Bacillus*, *Streptomyces*, and *Pseudarthrobacter*. Notably, we detected mycotoxigenic species of concern (e.g., potential producers of aflatoxins or trichothecenes) in cereal fields irrespective of the farming system.

1. Introduction

The study of soil microbiota, including bacterial and fungal communities, is of growing interest owing to the biodiversity and functional importance of the soil ecosystem. Soil microorganisms are essential for many biogeochemical processes; their interactions with aboveground ecosystems help regulate plant health, and consequently, human health (Peddle et al., 2024). However, soil is also a reservoir for harmful microorganisms, such as mycotoxin-producing filamentous fungi. Mycotoxins are small, stable, and toxic secondary metabolites whose presence in cereals and cereal-based foods represents a major threat to human and animal health (Palumbo et al., 2020; Yu and Pedroso, 2023). The most prevalent mycotoxins in cereal crops and their processed food products include aflatoxins; fumonisins; ochratoxin A; trichothecenes such as deoxynivalenol, T-2, and HT-2 toxins; citrinin; patulin; and zearalenone (Malachová et al., 2018; Wan et al., 2020). The inherent prevalence of mycotoxins and toxigenic fungi in cereals is further exacerbated by the effects of climate change. These effects, including high carbon dioxide levels, increasing temperatures, and the combination of extreme droughts with extreme precipitation, are known to impact fungal growth and thus the occurrence of mycotoxins (Zingales et al., 2022). From a geographical perspective, studies focused on mycotoxigenic species in developed countries with temperate climates reveal a shift in *Fusarium* and *Aspergillus* abundance across Europe (Kos et al., 2023). These findings support the growing evidence that mycotoxins represent an increasing health concern.

Biocontrol is a promising strategy for mitigating mycotoxin contamination in soil, involving the use of naturally occurring microorganisms to reduce crop damage. These biocontrol agents (BCAs) can act either directly (e.g. through antibiosis) or indirectly (by inducing plant-mediated responses) (Raymaekers et al., 2020). BCA application provides the advantage of reducing the dose of fungicides, which are unsafe for both the environment and human health (Ons et al., 2020). Using BCAs to control the presence of toxigenic fungi in soils can help reduce the production of mycotoxins, which typically spread from soil to plants. In this regard, certain bacteria normally present in soils may have

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the ability to reduce the growth of mycotoxigenic species. Bacterial diversity in soils is therefore an important factor to consider when addressing the problem of mycotoxins.

In addition to climate change, mycotoxin contamination in cereals is influenced by various factors including weather conditions during growth and harvest, crop protection strategies, rotation practices, and storage conditions (Alizadeh et al., 2021; Bernhoft et al., 2022; Mielniczuk and Skwaryło-Bednarz, 2020; Powell and Vujanovic, 2021). In recent years, consumer demand for organic food has increased owing to perceived advantages over conventionally grown food, including enhanced biodiversity, sustainability, food quality, and safety (Wang et al., 2024). Due to this, the production system (organic or conventional) has emerged as another possible differential factor in the occurrence of toxigenic fungi and mycotoxins in crops and food products.

Organic farming regulations limit the use of certain products, including mineral fertilizers, pesticides, and other additives (Rempelos et al., 2021). Weed, pest, and disease control are typically based on preventive and non-chemical protection strategies, including crop rotation, use of resistant varieties, and mechanical weeding. Furthermore, organic farming standards allow the implementation of biological as well as mineral-, plant extract-, and microbial extract-based control methods, provided they are utilised only as a last resort (Rempelos et al., 2023). However, this condition is not consistently satisfied (Burandt et al., 2024; Tamm et al., 2022). Organic farming regulations also recommend the use of organic fertilizers such as manure and compost, along with leguminous crops in rotation, to boost nitrogen levels and maintain a balanced nitrogen-to-phosphorus ratio in the soil (Rempelos et al., 2021). In most countries, these standards are defined by government laws and regulations (European Commission, 2016; USDA, 2024).

The persisting controversy regarding the relative risk of mycotoxin exposure associated with the consumption of organic and conventional cereals was recently addressed by Wang et al. (2024) in a comprehensive literature review. This review sought to clarify whether contamination levels are significantly different between organic and conventional cereals intended for human consumption. The results validated the notion that contamination levels are comparable in both farming systems, given that some studies report higher levels in conventional fields (Bakutis et al., 2006; Błajet-Kosicka et al., 2014; Munger et al., 2014), others detect them in organic grains (Kirinčič et al., 2015; Perkowski et al., 2007; Twarużek et al., 2013), and some describe no significant differences (Mäder et al., 2007; Váňová et al., 2008).

To determine whether the farming system affects mycotoxin contamination levels, one possible line of action is to account for differences in fungal diversity between conventional and organic fields, with a focus on toxigenic fungi. Similar diversity profiles would indicate that the farming system does not alter the mycobiome, whereas differences in fungal diversity would demonstrate the role of farming systems in the differential shaping of fungal communities. In this regard, genomics and next-generation sequencing methods emerge as powerful and promising tools that allow the unravelling or prediction of the presence of undesirable fungi in a microbial community, even allowing the characterisation of unculturable or difficult-to-isolate mycobiota (Gil-Serna et al., 2024).

In this work, we present what we believe to be the first next-generation sequencing approach aimed to elucidate the potential diversity differences between organic and conventional cereal fields. Amplicon metagenomics (also known as metataxonomics or metabarcoding) was used to characterise the mycobiome of organic and conventional cereal fields. A ploughed field—pastureland converted to tilled soil for agricultural use at the time of sampling—was used as control. Simultaneously, bacterial communities were analysed to assess whether the farming system affects the presence of beneficial bacteria (potential BCAs) in charge of controlling toxigenic fungi. Diversity and differential abundance analyses were performed to determine differences attributable to the farming system, if any. Sequences taxonomically assigned to the mycotoxin-producer genus *Fusarium* were phylogenetically grouped with other *Fusarium*-curated sequences to confirm their taxonomic assignment.

2. Methods

2.1. Sample collection and DNA sequencing

Soil samples, provided by the Agricultural Technological Institute of Castilla and Leon (ITACyL), were collected from cereal fields in Castilla y León (Spain) at harvest time in 2022. Samples were collected from the Zamadueñas land (ZAM), Fuentelapeña (FUE), and Riofrío (RIO) municipalities. The sampled cereal fields were subject to different farming systems: conventional, organic, and ploughed. Particularly, three conventional samples (ZAM CON1, ZAM CON2, and FUE CON1), two organic samples (FUE ORG1 and FUE ORG2), and one ploughed sample (RIO PLO) were collected. The organic samples were derived from fields that have been managed under this system for 20 years. Sampling points and coordinates are provided in Fig. S1 and Table S1.

First, the soil samples were air-dried at room temperature for 36 h and filtered through a 200 μm pore-size sieve. Air-drying leads to an exponential reduction in moisture content but does not cause substantial changes in the composition of the fungal or bacterial community in soil samples (Wang et al., 2021). DNA extraction was conducted using the DNeasy PowerSoil Kit (QIAgen, Düsseldorf, Germany), starting from 250 mg of powdered soil. For each sample, six DNA extractions were conducted. The extracted DNA was subsequently mixed in pairs and concentrated to a volume of 50 μL using a vacuum concentrator (Concentrator plus, Eppendorf, Hamburg, Germany). Library construction and sequencing were performed in FISABIO facilities (Valencia, Spain). The libraries were amplified using ITS3_KYO2/ITS4_KYO1 primers (GATGAAGAACGYAGYRAA/TCCTCCGCTTWTTGWTWTGC) (Toju et al., 2012) for the analysis of internal transcribed spacer 2 (ITS2) and Bakt_341F/Bakt_805R primers (CCTACGGGNGGCWGCAG/GACTACHVGGGTATCTAATCC) (Klindworth et al., 2013) for the V3–V4 region of 16S rRNA. Amplicon libraries were prepared using the KAPA HiFi HotStart ReadyMix (Roche Molecular Systems Inc., Pleasanton, CA, USA). llumina sequencing adaptors and dual-index barcodes were added to the amplicon using the Nextera XT index kit v2 (Illumina, Foster City, CA, USA). Libraries were normalised and pooled prior to sequencing. The pool containing indexed amplicons was loaded onto the MiSeq reagent cartridge v3 (Illumina, Foster City, CA, USA), spiked with 10 % PhiX control to improve base calling. Sequencing was performed using a paired-end 2 × 300 bp cycle on an Illumina MiSeq sequencing system.

2.2. Metataxonomic analysis

2.2.1. Quality control

An initial quality assessment of the demultiplexed FASTQ files was conducted using FastQC (v0.12.1) (Andrews, 2010). During 16S sequencing analysis, the primer sequences were trimmed using Cutadapt (v4.6) (Martin, 2011). After trimming, a second round of quality assessment was conducted using FastQC. Cutadapt was not applied for ITS2 (see Section 2.2.2). MultiQC (v1.21) was used to summarise the FastQC and Cutadapt output (Ewels et al., 2016). Finally, the sequences were imported into QIIME 2 (v2024.2.0) (Bolyen et al., 2019) for the downstream analysis.

2.2.2. ITS2 extraction

For fungal sequences, the ITS2 region was extracted using ITSxpress via the q2-itsxpress plugin (Rivers et al., 2018). Briefly, hidden Markov models were used to locate the boundaries of the ITS2 region and remove the conserved flanking regions (5.8S and 28S rRNA). The ITS2 primers are situated within these conserved regions; therefore, allowing them to remain in the sequences aided their identification. An indirect consequence of extracting the ITS2 region was the removal of low-quality positions located at the terminal ends of the sequences.

2.2.3. ASV count

Reads were denoised using the DADA2 algorithm (v1.26.0) (Callahan et al., 2016) via the q2-dada2 plugin. For 16S, forward reads were trimmed to 250 nucleotides and reverse reads to 200 nucleotides. For ITS2, no additional trimming was required as ITSxpress already conducted trimming accordingly. The amplicon sequence variants (ASVs) from the resulting feature tables were taxonomically annotated using naive Bayes machine-learning classifiers via the q2-feature-classifier plugin (Bokulich et al., 2018). Classifiers were trained against the Silva 138 99 % OTUs full-length sequences database (Quast et al., 2013; Robeson et al., 2021) for 16S, and against the UNITE 10 99 % identity, all eukaryotes, singletons as RefS database (Abarenkov et al., 2024) for ITS2. A confidence threshold of 0.7 was set to limit taxonomic inaccuracies. The feature tables were filtered to exclude any ASV lacking phylum-level annotation or not belonging to the expected taxa (bacteria for 16S and fungi for ITS2).

2.2.4. Diversity analysis

Diversity metrics were calculated using the q2-diversity QIIME 2 plugin. For within-sample (alpha) diversity, the computed metrics included Chao1 (Chao, 1984), Shannon (Shannon, 1948), and Simpson (Simpson, 1949) indices. For between-sample (beta) diversity, binary Jaccard distance (Jaccard, 1908), Bray–Curtis dissimilarity (Sørensen, 1948), and Aitchison distance (Aitchison et al., 2000) were measured. All metrics were computed on a feature table rarefied to the shallowest sample, except for Aitchison distance, which was computed on the unrarefied table adding a pseudocount of 1 (Gloor et al., 2017). Beta diversity metrics were visualised using principal coordinate analysis (PCoA) (Gower, 1966), a multidimensional scaling method that represents dissimilarity in a low-dimensional, Euclidean space.

2.2.5. Differential abundance

Differential abundance analysis was performed using the Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) method (Lin and Peddada, 2020) via the q2-composition plugin. ANCOM-BC adjusts for compositional effects and accounts for sampling variability in microbiome data by estimating absolute abundances using a bias-correction model. The following comparisons were tested: ploughed versus organic, ploughed versus conventional, and conventional versus organic.

2.3. Phylogenetic inference

ASVs taxonomically classified as genus *Fusarium* were selected from among the ASVs differentially abundant between conventional and organic soil. Eight ASVs were combined in a dataset along with 60 other *Fusarium* ITS2 sequences and one *Cephalosporium gramineum* outgroup sequence, retrieved from the NCBI Nucleotide database (NCBI, 2022) for a previous work (Gil-Serna et al., 2022).

The sequences were aligned using the MAFFT algorithm (v7.525) (Katoh and Standley, 2013) with the iterative refinement method L-INS-i. Subsequent minor adjustments in the resulting alignment were manually performed using Jalview (v2.11.4.0) (Waterhouse et al., 2009). Gap stripping was intentionally avoided to preserve the evolutionary information inherent to ITS2 indels. An evolutionary substitution model was fitted for the whole ITS2 region via ModelTest-NG (v0.1.7) (Darriba et al., 2020). A selected template was applied to ensure that the candidate model set contained only models available in the MrBayes tool. For each candidate model, a Maximum Likelihood optimised topology with optimised branch lengths was used for testing. A fixed random seed (1234) was used to support reproducibility. The best-fit molecular evolutionary model, selected using the corrected Akaike Information Criterion, was JC + G4 (Jukes–Cantor with a gamma distribution, shape parameter = 0.333), a simple four-by-four nucleotide model suitable for non-coding sequences. The primary reason for selecting the corrected Akaike Information Criterion is that it mitigates the tendency to favour more complex models when the sample size, that is, the number of positions in the alignment, is smaller than the number of parameters in the model (Posada and Buckley, 2004).

Phylogeny was reconstructed using Bayesian inference in MrBayes (v3.2.7) (Altekar et al., 2004; Ronquist et al., 2012). This method was selected over other methods such as Maximum Likelihood owing to its robust framework for assessing uncertainty in tree topology. Briefly, two independent runs of four Markov Chain Monte Carlo chains were performed for a total of 2×10^6 generations, with tree sampled every 100 generations. Convergence was assessed with Tracer (v1.7.2) (Rambaut et al., 2018). The initial 20 % of the sampled trees (400,000 generations; 4000 trees) were discarded as burn-in. Topological congruence and stability of the sampled trees was assessed using DensiTree (v3.1.0) (Bouckaert and Heled, 2014). The consensus tree was visualised and the nodes were annotated with posterior probabilities in iTOL (v1.7) (Letunic and Bork, 2024).

2.4. Cross-domain correlations

ASVs classified as toxigenic fungal genera (*Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria*) and potential BCA bacterial genera (*Bacillus*, *Arthrobacter*, *Pseudarthrobacter*, and *Streptomyces*) were selected to evaluate the effectiveness of bacterial BCAs against toxigenic fungi. Two sets of bacteria–fungi correlation values (one for conventional samples and one for organic samples) were analysed using the Sparse Cross-Correlations between Compositional data method implemented in the R package CompoCor (v1.0.0) (Jensen et al., 2024).

2.5. Statistical analysis

Alpha diversity comparisons were performed using the non-parametric Kruskal–Wallis test (Kruskal and Wallis, 1952). The significance threshold was set at $P \leq 0.05$. For beta diversity, permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) was conducted to test for significant differences across groups. By contrast, permutational analysis of multivariate dispersion (PERMDISP) (Anderson, 2006) was performed to assess homogeneity of group dispersions to determine whether the observed group differences were due to variation in dispersion or centroid differences (Anderson and Walsh, 2013). For both tests, *p*-values were computed as the observed probability of finding a more extreme rearrangement of data, based on 999 permutations. Ellipses representing 95 % confidence intervals were plotted around the group centroids in the PCoA ordination to visually assess differences between the groups.

Differential abundance was assessed using thresholds of log2 fold-change ($|\text{log2FC}| \geq 2$) and corrected *P*-value ($Q \leq 0.05$). Additionally, phylogenetic tree nodes were underpinned by posterior probabilities, which determine the statistical support for each clade based on the observed data and evolutionary model. For cross-domain correlation analysis, p-values were computed as the observed probability of finding a more extreme rearrangement of correlation distributions, based on 10,000 permutations.

2.6. Computational details

This work was performed using computational resources (Brigit HPC server) provided by the Complutense University of Madrid. Files in QIIME 2 format were imported into R (v4.2.3) (R Core Team, 2023) with the R package *qiime2R* (v0.99.6) (Bisanz, 2024). Data used for the cross-domain correlations were arranged using the R package *phyloseq* (v1.50.0) (McMurdie and Holmes, 2013). Plots were created with the R packages *ggplot2* (v3.5.0) (Wickham, 2016), *ggpubr* (v0.6.0) (Kassambara, 2023), *microeco* (v1.8.0) (Liu et al., 2021), *EnhancedVolcano* (v1.16.0) (Blighe et al., 2022), and *pheatmap* (v1.0.12) (Kolde, 2018). The sample location map was plotted using the R packages *sf* (v1.0.6) (Pebesma, 2018) and *mapSpain* (v0.9.1) (Hernangómez, 2024). R package *patchwork* (v1.2.0) was used to assemble the plots (Pedersen, 2024). Computational method parameters that were not explicitly stated were maintained at their default values.

3. Results

We sampled soil from cereal fields in Spain corresponding to two farming systems: conventional and organic. Samples from a ploughed cereal field were used as control. We then extracted environmental DNA from the samples and sequenced two marker genes to profile the

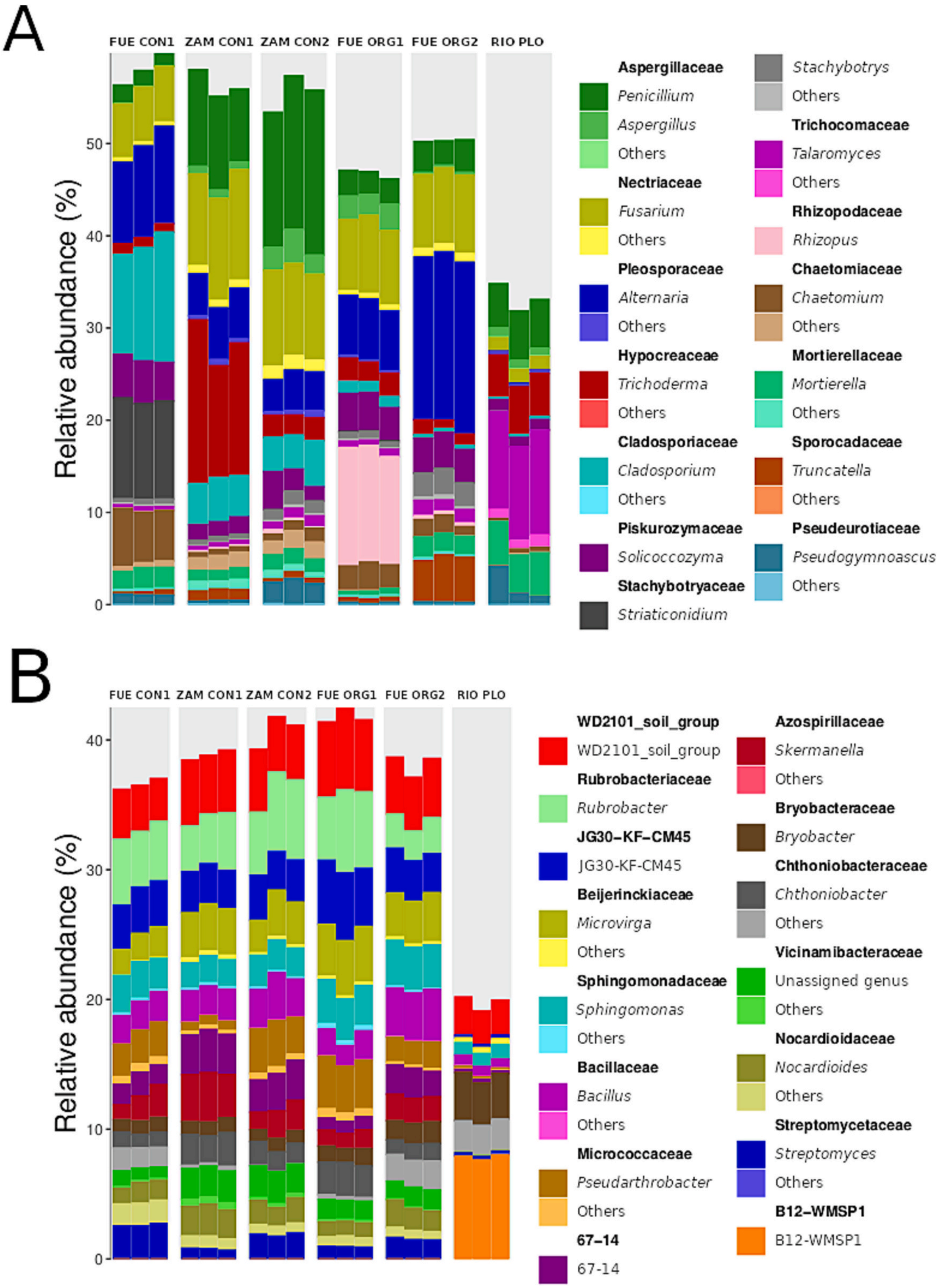

Fig. 1. Overview of taxonomic classification. **(A)** Bar plots of relative abundance of fungal genera. **(B)** Bar plots of relative abundance of bacterial genera. Only ASVs with at least genus-level annotation are represented.

inherent fungal and bacterial communities. Next, we constructed ASVs, measured the sample diversity, and compared it across the different farming systems and locations. A phylogenetic tree was generated using differentially abundant sequences corresponding to *Fusarium* spp. to ensure their correct taxonomic classification. Finally, we analysed the potential BCA activity of bacteria against toxigenic fungi in conventional and organic fields (See Section 2. for details of the analysis).

3.1. *Taxonomic classification*

Fig. 1A shows the result of taxonomic classification of fungal ASVs at the genus level in terms of relative abundance. In general, the presence or absence of fungal genera was consistent within the sample replicates, and variations were mostly found across samples. Regarding toxigenic fungi, *Penicillium* and *Fusarium* genera were detected across all samples. *Aspergillus* spp. was also observed in all samples, except in one conventional sample (FUE CON1). Similarly, *Alternaria* spp. was present with a high relative abundance in all samples, except in the ploughed sample (RIO PLO). Table 1 shows the toxigenic species from these genera that were detected in both conventional and organic samples. From a broader perspective, genera including *Trichoderma* and *Solicoccozyma* were present across all samples. Conversely, the soil genus *Rhizopus* was relatively abundant in one organic sample (FUE ORG1) and was also present, albeit with a small relative abundance, in three other samples from different locations and farming systems. A similar phenomenon was observed in the fungal genus *Cladosporium*, which was notably abundant (but not restricted to) one conventional sample (FUE CON1). We also found a relatively high abundance of fungi from the genus *Talaromyces* in the ploughed sample, along with the absence of genera such as *Truncatella* and other non-*Trichoderma* genera from the Hypocreaceae family.

Fig. 1B presents the taxonomic classification of bacterial ASVs at the genus level in terms of relative abundance. Bacterial presence was consistent across all samples, except the ploughed sample. Regarding potential BCA genera, we detected the presence of *Bacillus*, *Pseudoarthrobacter*, and *Streptomyces* across all samples. The WD2101 soil group was present with a high relative abundance in all samples. Similarly, genera such as *Rubrobacter*, *Microvirga*, and *Skermanella*, as well as the Chloroflexi clade JG30-KF-CM45, were observed in all conventional and organic samples. The ploughed sample was the only one in which members of the clade B12-WMSP1 were present.

3.2. *Diversity analysis*

3.2.1. *Within-sample (alpha) diversity*

Fig. 2A shows three different alpha fungal diversity indices aggregated by farming system and location. In general, all alpha diversity indices were high for each farming system and location, indicating that the fungal communities were rich, uniform (high Chao1 and Shannon indices), and diverse (high inverse Simpson indices). Regarding the farming system, no significant differences were found between the organic and conventional systems in any of the three indices. Contrastingly, all indices corresponding to the ploughed sample were significantly higher than those corresponding to the conventional and organic samples. Regarding location, all indices were significantly different in all cases.

Table 1
Mycotoxigenic species detected in both conventional and organic samples. % CON: Percentage of conventional samples in which the species was detected. % ORG: Percentage of organic samples in which the species was detected.

Species	% CON	% ORG
Aspergillus parasiticus	50	44.44
Aspergillus flavus	83.33	22.22
Fusarium oxysporum	100	100
Fusarium tricinctum	100	33.33
Fusarium algeriense	66.67	100

Fig. 3A presents the alpha bacterial diversity indices aggregated by farming system and location. Similar to fungal alpha indices, the bacterial alpha indices indicated rich, uniform, and diverse communities across all samples. Chao1 and Shannon indices of the ploughed sample were significantly lower than those of the conventional and organic samples. However, the inverse Simpson indices of ploughed samples were significantly lower than those of the conventional samples, whereas no significant differences were found between the ploughed and organic samples. This could be attributed to the high variance of inverse Simpson indices in certain samples. A similar trend was observed in bacterial alpha indices aggregated by location. For Chao1 and Shannon indices, no significant differences were found between the FUE and ZAM samples, whereas the inverse Simpson indices significantly differed only between the RIO and ZAM samples.

3.2.2. *Between-sample (beta) diversity*

Fig. 2B shows the PCoA ordinations of fungal Aitchison distances aggregated by farming system and location. Regarding the farming system, both PERMANOVA ($P = 0.001$) and PERMDISP ($P = 0.001$) were significant under 999 permutations (see Section 2.5). Confidence intervals corresponding to the conventional and organic samples overlapped in the ordination. This overlap indicated that the centroids were not statistically different. Regarding location, PERMANOVA ($P = 0.001$) and PERMDISP ($P = 0.039$) were again significant; however, no overlap could be observed between the confidence intervals of any of the locations. This implies that the centroids, and thus fungal diversity, differ significantly across locations. Similar results were obtained for Jaccard distances and Bray–Curtis dissimilarity (Fig. S2).

Fig. 3B presents the PCoA ordinations of bacterial Aitchison distances aggregated by farming system and location. Consistent with the aforementioned results, both farming system (PERMANOVA $P = 0.001$, PERMDISP $P = 0.01$) and location (PERMANOVA $P = 0.001$, PERMDISP $P = 0.001$) aggregations showed significant p-values under 999 permutations. Confidence ellipses around the group centroids were plotted to assess whether the differences were due to diversity or data dispersion. No overlap was detected in the ordination by location, whereas the confidence ellipses for conventional and organic systems clearly overlapped. This indicates that bacterial diversity differs significantly by location rather than by farming system. Jaccard distances and Bray–Curtis dissimilarity ordinations showed similar results (Fig. S3).

3.3. *Differential abundance*

Fig. 2C shows volcano plots of differentially abundant fungal ASVs between farming systems. The number of differentially abundant ASVs between conventional and organic samples (111 and 84, respectively) was substantially lower than that between ploughed and organic samples (415 and 262), as well as between ploughed and conventional samples (514 and 153) (Table S2).

Fig. 3C presents volcano plots of differentially abundant bacterial ASVs between farming systems. Again, the number of differentially abundant ASVs between conventional and organic samples (328 and 458) was considerably lower than that between ploughed and organic samples (1315 and 1372), as well as between ploughed and conventional samples (1307 and 1021) (Table S3).

To investigate whether differentially abundant fungal ASVs between conventional and organic samples were toxigenic, we retrieved all ASVs with a species-level taxonomic classification within known mycotoxin-producing genera (*Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria*). Five ASVs were found to be differentially abundant in the conventional samples, whereas four were differentially abundant in the organic samples. The complete list is shown in Table 2.

Regarding bacteria, owing to the high conservation of the 16S rRNA gene in closely related species, its resolution was insufficient for

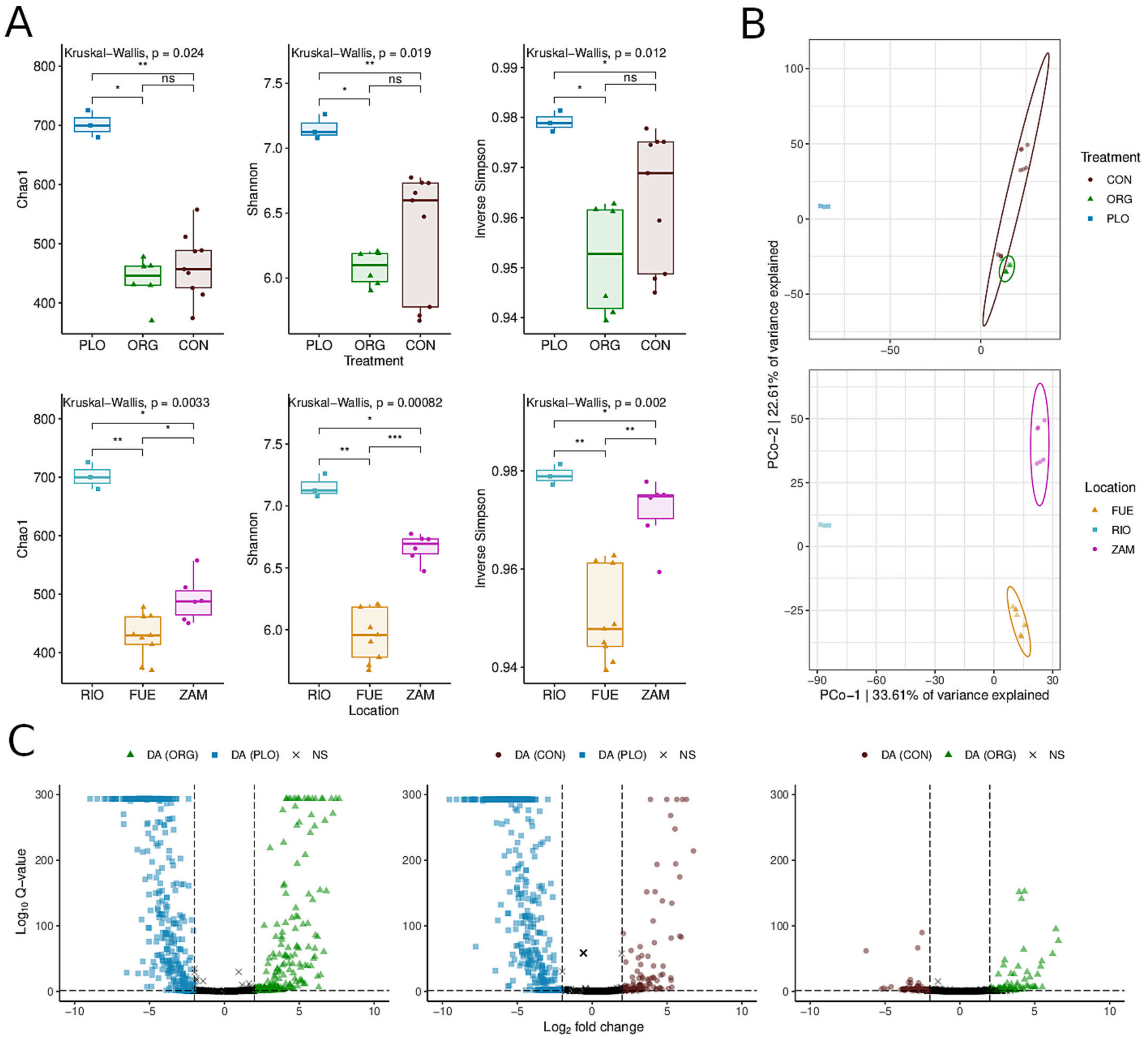

Fig. 2. Fungal diversity is not associated with the farming system. **(A)** Alpha diversity indices are not dependent on the farming system. Box plots of Chao1 (left), Shannon (center) and inverse Simpson (right) indices aggregated by farming system (above) and location (below). **(B)** Beta diversity indices are not dependent on the farming system. Aitchison distances represented in a Principal Coordinates Analysis (PCoA) ordination aggregated by farming system (left) and location (right). **(C)** Volcano plots of differential abundance analysis comparing ploughed and organic (left), ploughed and conventional (center) and conventional and organic farming systems (right). DA, differentially abundant; NS, not significant. Farming systems: CON, conventional; ORG, organic; PLO, ploughed. Locations: RIO, Riofrío; FUE, Fuentelapeña; ZAM, Zamadueñas.

classifying most differentially abundant bacterial ASVs at the species level. Therefore, we retrieved all ASVs with a genus-level taxonomic classification within potential BCA bacterial genera (*Bacillus*, *Arthrobacter*, *Pseudarthrobacter*, and *Streptomyces*). The ASVs are listed in Table 3. With the exception of *Bacillus nealsonii*, which was differentially abundant in organic samples, all differentially abundant ASVs lacked a species-level taxonomic classification.

3.4. Phylogenetic inference

We collected all *Fusarium* spp. sequences from the differentially abundant ASVs listed in Table 2, along with differentially abundant ASVs classified as *Fusarium* sp. Next, we constructed a Bayesian Inference phylogenetic tree together with 60 curated *Fusarium* ITS2 sequences retrieved from the NCBI Nucleotide database. Fig. 4 shows the results of the phylogenetic inference process.

Table 4 presents the taxonomic assignment before and after phylogenetic inference. While some ASVs were assigned to a specific *Fusarium* species, others could only be assigned to a species complex. Among the ASVs assigned to a *Fusarium* species, we found *F. domesticum* and *F. solani* to be differentially abundant in the conventional samples. Meanwhile, *F. brachygibbosum* and a member of the *Fusarium tricinctum* species complex were differentially abundant in the organic samples.

3.5. Cross-domain correlations

Abundance correlation values of toxigenic fungal and potential BCA bacterial ASVs were estimated for both the conventional and organic

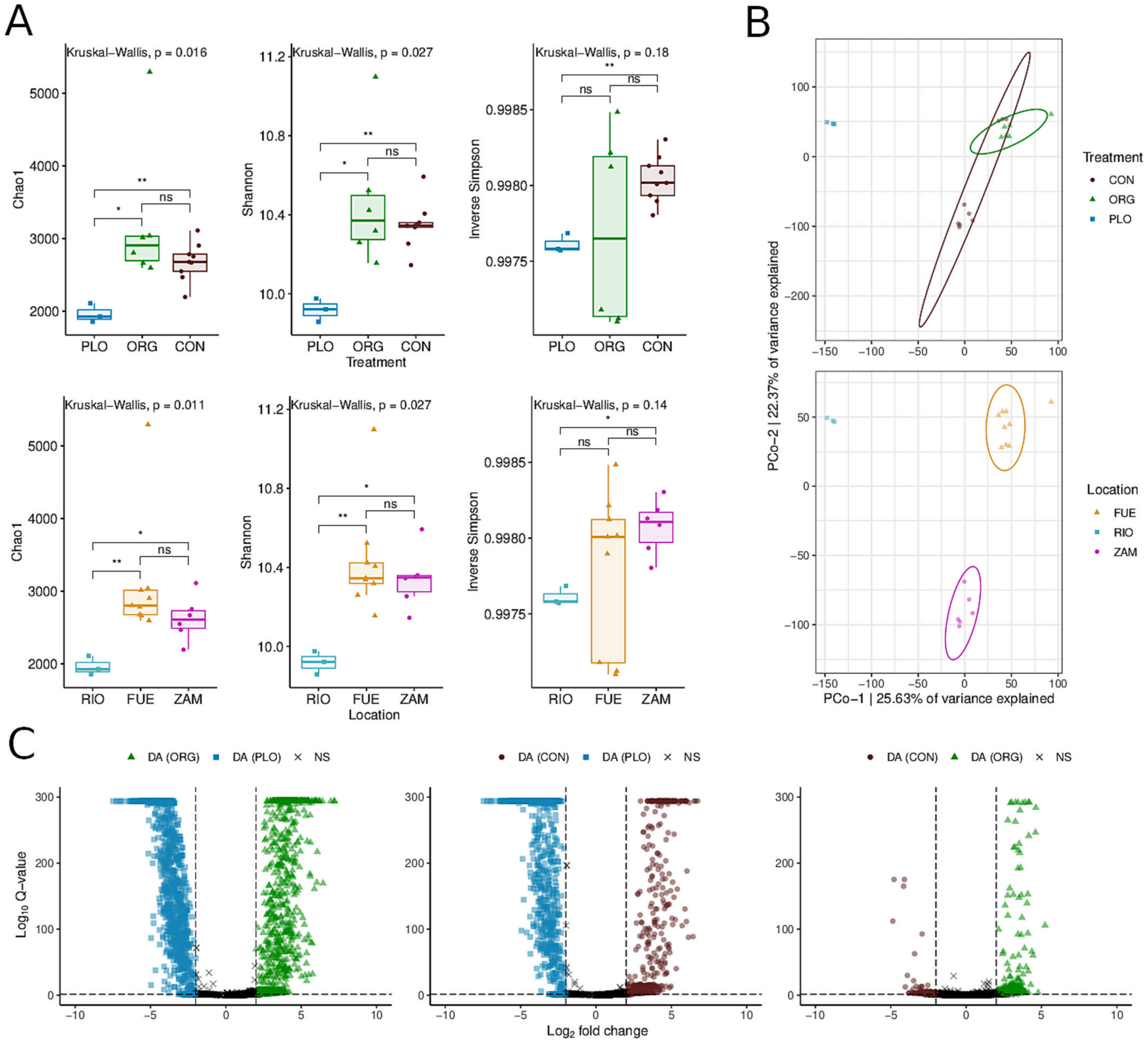

Fig. 3. Bacterial diversity is not associated with the farming system. **(A)** Alpha diversity indices are not dependent on the farming system. Box plots of Chao1 (left), Shannon (center) and inverse Simpson (right) indices aggregated by farming system (above) and location (below). **(B)** Beta diversity indices are not dependent on the farming system. Aitchison distances represented in a Principal Coordinates Analysis (PCoA) ordination aggregated by farming system (left) and location (right). **(C)** Volcano plots of differential abundance analysis comparing ploughed and organic (left), ploughed and conventional (center) and conventional and organic farming systems (right). DA, differentially abundant; NS, not significant. Farming systems: CON, conventional; ORG, organic; PLO, ploughed. Locations: RIO, Riofrío; FUE, Fuentelapeña; ZAM, Zamadueñas.

samples. The potential BCA activity was expected to be reflected as a negative correlation: as the bacterial abundance increased, fungal abundance would decrease, and vice versa. The average correlation by genus is shown in Fig. 5. The more extreme correlation average values were found in the organic soil samples (Fig. 5B), between the fungal genera *Aspergillus* and *Alternaria* and the bacterial genera *Pseudarthrobacter* (highest correlation values) and *Streptomyces* (lowest correlation values). Statistical analysis was conducted using the whole distributions of correlation values to detect possible differences in the cross-domain correlations between the conventional and organic samples. The results are presented in Table 5. Although most fungi–bacteria pairs do not show different correlation values between farming systems, the pairs *Aspergillus–Bacillus*, *Aspergillus–Streptomyces*, *Alternaria–Bacillus*, and *Alternaria–Streptomyces* may exhibit significantly higher BCA activity in organic samples. By contrast, *Aspergillus–-Pseudarthrobacter* and *Alternaria–Pseudarthrobacter* may show the same in conventional samples. See Fig. S4 for a full visualisation of correlation distributions by farming system.

4. Discussion

Cultivable soil plays a crucial role in both animal and human health and requires comprehensive study, particularly regarding its microbiota, which may include pathogens and mycotoxin producers. However, traditional culture techniques cannot fully capture the microbial diversity in soil owing to the presence of unculturable or difficult-to-isolate species (Ravin et al., 2015). These limitations can be overcome by high-throughput sequencing, enabling a more comprehensive

Table 2
Species-level classified ASVs belonging to known toxigenic fungal species differentially abundant (Q-value $\leq$0.05) in organic versus conventional farming systems. ASV ID: first 7 characters of the unique identifier (MD5 hash) of the ASV. Farming system: condition where the ASV is differentially abundant. $\log_2$FC: effect size (fold-change in log scale). Taxonomy: taxonomy assigned by the classifier. Confidence: confidence of the taxonomic classification, in a scale of 0–1 (threshold was set at 0.7).

ASV ID	Farming system	$\log_2$FC	Taxonomy	Confidence
7,124,022	Conventional	3.73 ± 0.85	*Penicillium chrysogenum*	0.7012
86704d9	Conventional	2.94 ± 0.74	*Fusarium venenatum*	0.9199
39d8dd8	Conventional	2.42 ± 0.77	*Fusarium algeriense*	0.8491
e554621	Conventional	2.29 ± 0.72	*Penicillium simplicissimum*	0.7754
c72588d	Conventional	2.27 ± 0.70	*Penicillium bilaiae*	0.9532
e9d861a	Organic	5.50 ± 0.95	*Fusarium tricinctum*	0.7779
5bcf745	Organic	2.53 ± 0.62	*Aspergillus insuetus*	0.9085
35a638e	Organic	2.32 ± 0.73	*Aspergillus flavus*	0.7319
f0eab31	Organic	2.16 ± 0.61	*Penicillium bilaiae*	0.9570

Table 3
ASVs belonging to potential BCA bacterial genera differentially abundant (Q-value $\leq$0.05) in organic versus conventional farming systems. ASV ID: first 7 characters of the unique identifier (MD5 hash) of the ASV. Farming system: condition where the ASV is differentially abundant. $\log_2$FC: effect size (fold-change in log scale). Taxonomy: taxonomy assigned by the classifier. Confidence: confidence of the taxonomic classification, in a scale of 0–1 (threshold was set at 0.7).

ASV ID	Farming system	$\log_2$FC	Taxonomy	Confidence
a56a811	Conventional	4.78 ± 0.17	*Streptomyces* sp.	0.9923
13cbdb2	Conventional	3.06 ± 0.74	*Bacillus* sp.	0.9973
c59dc65	Conventional	2.97 ± 0.57	*Bacillus* sp.	0.9996
e9f77f8	Conventional	2.94 ± 0.61	*Streptomyces* sp.	0.9951
9e155bf	Conventional	2.79 ± 0.67	*Streptomyces* sp.	0.9934
8f960ad	Conventional	2.43 ± 0.59	*Bacillus* sp.	0.9999
286d7f3	Conventional	2.26 ± 0.75	*Bacillus* sp.	0.9985
a475021	Conventional	2.62 ± 0.48	*Streptomyces* sp.	0.8168
ef95b65	Conventional	2.03 ± 0.67	*Streptomyces* sp.	0.9998
2adc093	Organic	3.73 ± 0.53	*Bacillus nealsonii*	0.7008
df84fc8	Organic	3.59 ± 0.97	*Bacillus* sp.	0.9989
2c5dd95	Organic	2.21 ± 0.63	*Streptomyces* sp.	0.9995
7,368,528	Organic	2.11 ± 0.73	*Streptomyces* sp.	0.9967
0f9dc74	Organic	2.03 ± 0.72	*Arthrobacter* sp.	0.7532

analysis of the occurrence and diversity of both fungal and bacterial communities in soil. We have successfully applied this approach in previous studies, discovering new species and revealing shifts in mycotoxigenic species distribution (Gil-Serna et al., 2022). In this study, we used metataxonomics to compare the fungal and bacterial diversity between organic and conventional cereal fields. This comparison was performed to assess the potential effects of farming practices on soil microbiota—in general, as well as on potential BCAs and toxigenic fungi, particularly *Fusarium* spp.

Our study is one of the few to employ a high-throughput sequencing approach to analyze samples from soil that has undergone two decades of organic farming practices. Consequently, finding cereal fields with a sufficient history of organic farming was a difficult task, which limited the number of samples analysed. To maintain a balanced design, a similar number of conventional samples was included in the study. However, detecting subtle differences between smaller samples is difficult, resulting in an increased risk of Type II errors. Nevertheless, the observed differences between farming systems cannot be considered reliable if the tested fields tested have not been subjected to a sufficiently long duration of organic farming. Expanding the sample size in future studies—when more organic fields have accumulated substantial time under this farming system—could help address this limitation by enhancing statistical power and reducing within-group variability.

Mycotoxins commonly contaminate cereal crops and their derivatives, posing serious health risks that climate change appears to exacerbate. In this scenario, organic farming, driven by growing consumer demand for perceived environmental and health benefits, emerges as a possible solution to mycotoxin contamination. In general, studies focused on the differences between farming systems claim that organic soils are more diverse than conventional soils in terms of microbiota (Lupatini et al., 2017). However, studies on the difference in mycotoxin contamination between organic and conventional cereals have shown mixed results (Wang et al., 2024). Therefore, analysing fungal diversity across farming systems using metagenomic approaches, such as amplicon sequencing, has emerged as a useful tool to clarify this impact. Furthermore, bacterial diversity is also relevant because of the activity of some naturally occurring bacterial BCAs against toxigenic fungi.

As mentioned in Section 3, both fungal and bacterial communities exhibited high Chao1, Shannon, and inverse Simpson indices, indicating the presence of rich, uniform, and diverse microbiomes. Comparing alpha diversity values between farming systems showed that the conventional and organic samples did not differ significantly in their indices. In the case of fungi, when we compared alpha indices between locations, we found significant differences in all cases, suggesting that the geographical origin of the samples, and not the farming system, is the factor responsible for the alpha diversity differences. For bacterial alpha diversity indices, a similar conclusion was reached, except for two points: i) samples from FUE and ZAM were not significantly different for any of the indices, and ii) values of the inverse Simpson index were not significantly different between the RIO and FUE samples. A possible explanation for these observations is the high variance observed in one replicate of the FUE organic sample and, more generally, the inverse Simpson values for all FUE samples compared to those of the other conditions. This could have affected the Kruskal–Wallis test, reducing its ability to detect differences and potentially leading to a non-significant result.

Nonetheless, clarifying that alpha indices represent only within-sample diversity is important. Therefore, they provide a snapshot of how diverse an individual sample is compared with itself alone. To understand the differences in diversity across multiple samples or conditions (e.g. farming systems), we must account for beta diversity. For both fungi and bacteria, we found significantly low PERMANOVA permutative *p*-values in all cases ($P = 0.001$, the lowest possible p-value with 999 permutations). Permutative p-values are distinct from classical p-values, as they reflect how extreme the observed data are compared to their random rearrangements. While classical p-values must be interpreted in a binary manner (a p-value is either significant or not significant, with no additional meaning assigned to smaller p-values beyond the threshold of significance), permutative p-values can be numerically compared to provide a more nuanced measure of effect size. In our study, given that all PERMANOVA test results were significantly low, we concluded that differences exist between groups. However, these differences could have been due to diversity itself or due to inter-group variance. To exclude this variance, PERMDISP tests were conducted, resulting again in significantly low p-values. Given that an analytical conclusion cannot be drawn with our study design when both PERMANOVA and PERMDISP are significant, we assessed the 95 % confidence ellipses plotted for every condition in the PCoA ordination. An overlap of two ellipses indicates that the centroids are not statistically different, implying no differences in beta diversity between the two groups. For both fungi and bacteria, ellipses corresponding to conventional and organic farming systems overlapped, indicating that the beta diversity was not significantly different between these conditions. For the comparison by location, no overlap was observed. This confirms our earlier notion that the geographical origin of the samples, and not the farming system, drives the beta diversity differences.

When referring to geographic location, we are in fact capturing a

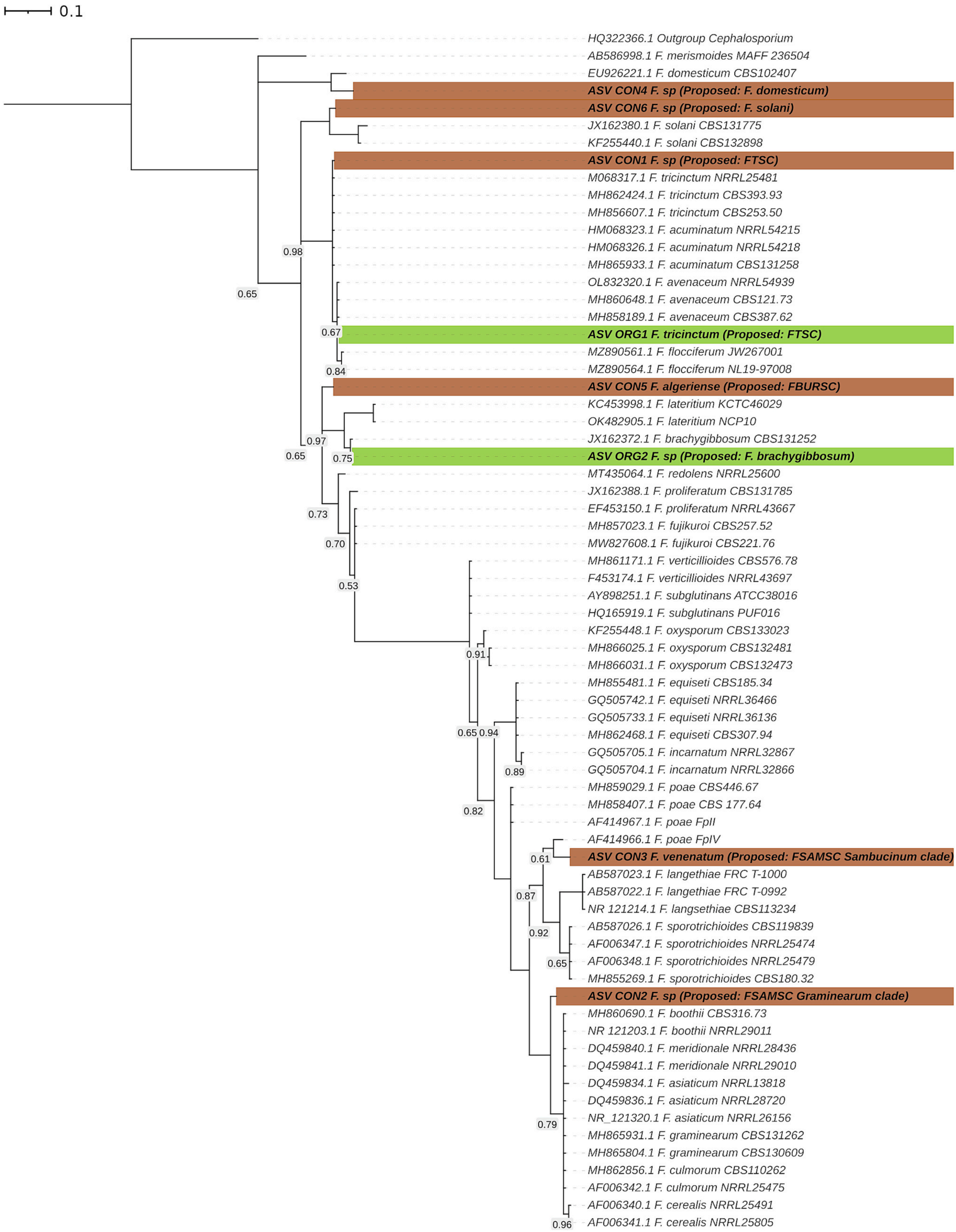

Fig. 4. Bayesian inference tree of the ITS2 sequences from differentially abundant *Fusarium* ASVs obtained in this work together with *Fusarium* sequences retrieved from NCBI (accession numbers are annotated in each sequence name). Taxa obtained in this work are highlighted. Taxa shown in ASVs represent the taxonomic assignment performed by the classifier and proposed based on the tree. Posterior probability values (0–1) are shown in the nodes. Nodes not annotated have a posterior probability of 1. FTSC: *Fusarium tricinctum* species complex. FSAMSC: *Fusarium sambucinum* species complex. FBURSC: *Fusarium burgessii* species complex.

Table 4
Taxonomic classification (assigned by the classifier and proposed according to phylogenetic inference) of *Fusarium* ASVs differentially abundant in organic versus conventional farming systems. ASV ID: first 7 characters of the unique identifier (MD5 hash) of the ASV. Tag: name used in phylogenetic inference, including the farming system where the ASV is differentially abundant (CON, conventional; ORG, organic). Confidence of the assigned taxonomy is shown in a scale of 0–1 (threshold was set at 0.7). FTSC: *Fusarium tricinctum* species complex. FSAMSC: *Fusarium sambucinum* species complex. FBURSC: *Fusarium burgessii* species complex.

ASV ID	Tag	Assigned taxonomy	Confidence of assigned taxonomy	Proposed taxonomy
1e4911d	ASV CON1	*Fusarium* sp	0.9789	FTSC
243070c	ASV CON2	*Fusarium* sp	0.9976	FSAMSC *Graminearum* Clade
86704d9	ASV CON3	*F. venenatum*	0.9199	FSAMSC *Sambucinum* Clade
ad8600b	ASV CON4	*Fusarium* sp	0.9963	*F. domesticum*
39d8dd8	ASV CON5	*F. algeriense*	0.8491	FBURSC
60dcdd7	ASV CON6	*Fusarium* sp	0.8703	*F. solani*
e9d861a	ASV ORG1	*F. tricinctum*	0.7779	FTSC
29f950c	ASV ORG2	*Fusarium* sp	0.9929	*F. brachygibbosum*

complex set of factors that together define the identity of each site. These include physical and chemical soil properties, such as pH, texture, organic matter content, and nutrient availability, as well as local climatic conditions like temperature and precipitation (Islam et al., 2020). Such environmental variables likely exert a stronger influence on soil microbial communities than farming system alone, and may explain the diversity patterns observed in our study.

Bonanomi et al. (2016) also examined microbial diversity over two decades of organic farming, concluding that conventional soils exhibit greater bacterial diversity, whereas eukaryotic diversity, particularly fungi, is higher in organic soils. However, notably, all samples in their analysis come from cultivations under plastic tunnels. Furthermore, the methodology employed in their findings had several limitations: i) the interpretation of results was predominantly based on observed differences in alpha diversity indices—which assess the diversity within individual samples—rather than on beta diversity; ii) beta diversity analysis was restricted to Jaccard and Bray–Curtis distances, which may not have effectively captured the true differences compared to those of other compositional methods such as Aitchison distance (Gloor et al., 2017); iii) dispersion of beta diversity values was not evaluated to determine whether the observed differences stem from genuine diversity or high inter-group variance. The non-parametric MANOVA used in their study is less sensitive to dispersion than PERMANOVA. However, it relies on rank-transformed Euclidean distances. Neither Jaccard nor Bray–Curtis distances are Euclidean, whereas Aitchison distance represents the Euclidean distance between samples after a centred log-ratio transformation (Aitchison et al., 2000). Considering these limitations, our findings are particularly relevant as, to our knowledge, this is one of the few studies on fungal and bacterial diversity in soils with sufficient years under organic farming and employing methodologies that account for the compositional characteristics of microbiome data.

Although our research focuses on the potential influence of farming system and geographic location on the composition of soil microbial communities, we acknowledge that these are not the only factors that can affect microbial diversity. An additional factor impacting diversity is the cropping history of the field. However, comprehensive crop history data were not available for all sampling points in our study, which limited our capacity to evaluate this aspect. Future research that includes detailed cropping records would help clarify these effects.

Regarding the distribution of fungal and bacterial genera, we found consistent classifications within and across samples. For instance, the bacterial genus with the highest relative abundance within all samples was the WD2101 soil group, comprising *Phycisphaera*-like planctomycetes commonly detected in various terrestrial habitats (Dedysh et al., 2021). Notably, these relative abundances were compositional data, and

Table 5
Toxigenic fungal and potential BCA bacterial genera whose abundance is negatively correlated (possible BCA activity) in one farming system significantly more than in another. P-values were obtained from permutation tests (10,000 iterations). Significance levels: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***).

Fungi	Bacteria	Farming system with potential BCA activity
Aspergillus	*Bacillus*	Organic (***)
Aspergillus	*Pseudarthrobacter*	Conventional (*)
Aspergillus	*Streptomyces*	Organic (***)
Alternaria	*Bacillus*	Organic (***)
Alternaria	*Pseudarthrobacter*	Conventional (*)
Alternaria	*Streptomyces*	Organic (***)

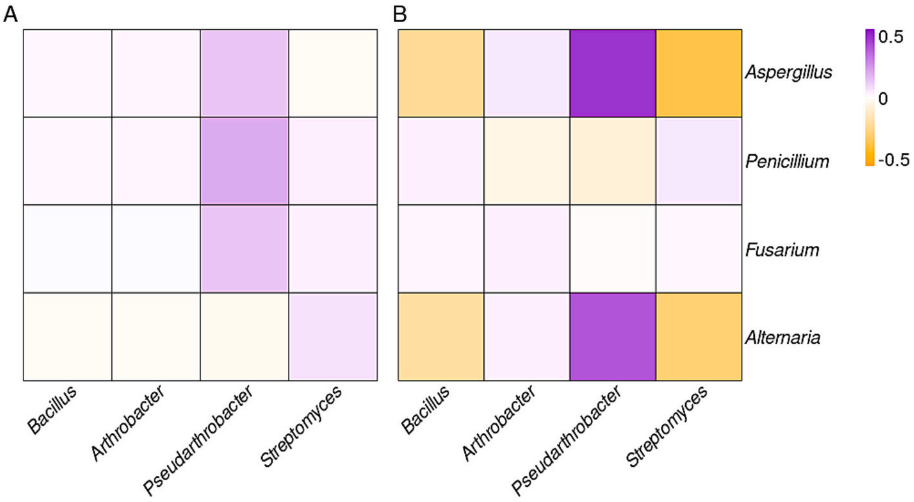

Fig. 5. Correlation of toxigenic fungal ASVs (rows) and potential BCA bacterial ASVs (columns) aggregated by genus. **(A)** Cross-domain correlations in conventional soil samples. **(B)** Cross-domain correlations in organic soil samples. Although correlation values range from −1 to 1, color scale is fixed from −0.5 to 0.5 to ensure visibility.

therefore comparing them directly and drawing statements of the type “Taxon X is more abundant in sample A compared to sample B" is not advisable. To draw meaningful conclusions, a compositional differential abundance approach is necessary (see Section 2.2.5) (Gloor et al., 2017).

Some well-known mycotoxin-producing fungal species were detected in both the conventional and organic samples. These included the aflatoxin producers *Aspergillus flavus and A. parasiticus* and the fumonisin producer *F. oxysporum* (Gil-Serna et al., 2019). Controlling the prevalence of these species in cereal fields is essential for reducing health risks associated with mycotoxins.

Although our results indicated that the differences in diversity between conventional and organic samples were not substantial enough to be deemed significant, we aimed to identify the taxa responsible for these minor differences. For this, we employed differential abundance methods that account for the compositional nature of microbiome data (Gloor et al., 2017). One of these methods is the ANCOM-BC method, which was utilised for both fungi and bacteria at the ASV level. As expected from the beta diversity results, the comparison between conventional and organic farming yielded low differentially abundant ASV numbers. Among these, we focused on the species-level annotated ASVs belonging to the mycotoxin-producer genera. Two ASVs, classified as *Fusarium tricinctum* (potential producer of trichothecenes A and B) and *A. flavus* (potential aflatoxin producer) (Gil-Serna et al., 2019), were differentially abundant in the organic sample and had low confidence values. Given that the abundance of *A. flavus* has gradually increased because of climate change (Melguizo et al., 2023; Molnár et al., 2023; Moretti et al., 2019), the contribution of emerging organic farming practices to its proliferation must be considered. However, further research is needed to accurately assess the potential risks associated with this trend. Notably, two different ASVs classified as *Penicillium bilaiae* (potential ochratoxin A and patulin producer) were differentially abundant in both conventional and organic samples, with high classification confidence. This may indicate that the differentially abundant ASVs correspond to different *P. bilaiae* strains, but the database used did not include strain-level annotations. Additionally, we focused on differentially abundant ASVs belonging to potential BCA bacterial genera. Notably, most differentially abundant ASVs could not be classified at the species level. In most cases, the 16S rRNA gene alone did not provide sufficient resolution to differentiate between species, and sometimes even between genera (Edgar, 2018). For example, the 16S sequence of the bacterial genera *Escherichia* and *Shigella* share over 99 % identity, making it impossible to distinguish between them using this gene alone (Jenkins et al., 2012). Therefore, further investigation is needed to assign species labels to the identified differentially abundant ASVs and determine whether they possess BCA activity.

In addition to conventional and organic samples, we used as control a ploughed field that was previously pastureland. At the time of sampling, the field was converted to tilled soil for agricultural use. In the case of fungal alpha diversity indices, the values were higher in the ploughed sample compared to those in the cultivated samples (conventional and organic). Conversely, bacterial alpha diversity values were lower in the ploughed sample compared to those in the other samples, except for inverse Simpson values, which were comparable between the ploughed and organic samples. This can be attributed to the large within-group variance in the inverse Simpson indices. One possible interpretation for these observed differences between ploughed and cultivated soils is that, before cultivation, fungi are typically the dominant group in soil. However, following the initiation of farming activity, the bacterial community reduces the fungal community to a minoritarian role. In our study, regarding beta diversity, the PCoA ordinations did not show any overlap between the ploughed and cultivated samples, indicating significant differences in both fungal and bacterial communities. Notably, the ploughed sample showed the smallest proportion of fungal and bacterial ASVs classified at least at genus level (approximately 35 % and 20 %, respectively).

Taxonomic classifiers rely on databases that are ultimately curated by researchers, whose scientific interests lean more towards the microbiome of food-producing fields than that of uncultivated soils. Therefore, species that are not related to agriculture are typically underrepresented. An example of misclassification due to underrepresentation is the bacterial clade B12-WMSP1, which is only found in ploughed samples and is probably a classifier error, considering the extreme environmental conditions where it has been detected (Costello and Schmidt, 2006; Schmidt et al., 2018). This underlines the importance of comprehensive and curated databases to exploit the full potential of metataxonomic and metagenomic tools. Finally, we also assed the relative abundance of differentially abundant ASVs between the ploughed and other farming systems, which have shown differences in beta diversity. As expected, the comparisons involving the ploughed sample yielded more differentially abundant ASVs than those between conventional and organic farming.

Databases of marker genes used in amplicon metagenomics usually lack comprehensive genus- and species-level annotations. Even when the taxonomy yielded by the classifier exhibits a high confidence value, they are still prone to inaccuracies at these levels. While this is particularly relevant for 16S, ITS also presents these problems. In our study, we aimed to ensure that differentially abundant ASVs assigned to *Fusarium*, one of the main mycotoxin producer genera, were correctly identified at the species level. Moreover, we assigned a species to the *Fusarium* differentially abundant ASVs classified as *Fusarium* sp. Phylogenetic inference, along with curated sequences from NCBI Nucleotide, allowed us to re-classify several of these ASVs. The ASV initially assigned to *Fusarium tricinctum* by the taxonomic classifier is indeed a member of the *Fusarium tricinctum* species complex; However, we could not confidently assign a specific species to it. One ASV, initially classified as *Fusarium* sp., was re-classified as *F. brachygibbosum*. This species is particularly relevant since some of its strains are expected to produce T-2 and HT-2 toxins along with beauvericin, (Rabaaoui et al., 2021). Although this ASV was differentially abundant in organic samples, it was also present in the conventional samples. We also detected this species in oat fields in Spain during a previous study (Gil-Serna et al., 2022). The European Food Safety Authority has identified *F. brachygibbosum* as a major plant pathogen and recommended treating it as a potential quarantine pest, highlighting the necessity to clarify its distribution and assess its potential impact on food safety (EFSA, 2021). Therefore, investigating the distribution and mycotoxin-production potential of *F. brachygibbosum* is essential.

Regarding the remaining ASVs re-classified using phylogenetic inference, one *Fusarium* sp. was re-classified as *F. solani*, which is not considered a significant contributor to mycotoxin contamination in crops (Munkvold et al., 2021). Another *Fusarium* sp. was reclassified as *F. domesticum*, a species not known to produce mycotoxins. The remaining *Fusarium* ASVs could only be categorised within a species complex, which limits the ability to determine their mycotoxin producing capabilities. This emphasizes the issue that the lack of species-level discrimination in the ITS2 region depletes the taxonomic classification of some ASVs. Using a different gene marker, such as the elongation factor 1-α (Boutigny et al., 2019), could help enhance the taxonomic classification of *Fusarium* ASVs.

To assess the role of bacteria as BCAs against toxigenic fungi, we performed a cross-domain correlation analysis for both organic and conventional samples. We selected *Bacillus*, *Arthrobacter*, *Pseudarthrobacter*, and *Streptomyces* as potential BCA genera owing to their frequent occurrence in soil and demonstrated activity against toxigenic fungi (de la Huerta-Bengoechea et al., 2022; Higazy et al., 2021; Strub et al., 2021). Clarifying that correlation does not imply causation is important; therefore, all interactions described in this study should be regarded as potential. If a bacterial BCA is effective against a specific fungal genus, we would expect a negative correlation between their abundances. In general, our analysis revealed correlation values approaching zero across nearly all comparisons, suggesting a lack of correlation. Furthermore, most correlations did not show significant differences between the organic and conventional samples, except for the fungal genera *Aspergillus* and *Alternaria*. In these cases, correlation values were significantly lower in organic samples compared to those in the bacterial genera *Bacillus* and *Streptomyces*, as well as in conventional samples compared to those in the bacterial genus *Pseudarthrobacter*. This may imply that bacterial species from these genera could potentially function as BCAs against the aforementioned fungal genera; however, further research is required to confirm this hypothesis. Additionally, when correlation values were not aggregated by genera, the various ASVs within each fungal and bacterial genus yielded mixed results, even when the overall comparison was significant. Thus, while correlation analysis serves as a valuable preliminary tool for identifying potential correlations, more targeted studies are required to validate these interactions.

An important point to consider is that the presence of a fungal taxon does not necessarily involve its ability to produce mycotoxins, as many strains, such as those of *Aspergillus flavus*, can be non-aflatoxigenic or may even act as biocontrol agents (Moore et al., 2022). Similarly, the bacterial taxa identified as potential BCAs in our co-occurrence analysis were not directly tested for antifungal activity in this study. These limitations underline the importance of complementing amplicon sequencing with functional approaches, such as shotgun metagenomics, to better assess the role and activity of these microorganisms. Future research should aim to integrate taxonomic and functional data to clarify these relationships.

Finally, we consider it important to highlight that our metataxonomic approach generated a vast amount of data encompassing a wide range of taxa, including mycotoxin producers and potential BCAs. These data provide a valuable resource that can be explored from multiple perspectives, depending on the research focus and interest. For example, researchers can use them to investigate broader ecological aspects such as soil health and nutrient cycling (Mushinski et al., 2018) or to study fungi that act as plant pathogens without necessarily producing mycotoxins. *F. oxysporum*, which was detected in both conventional and organic samples, serves as an illustrative case. While *F. oxysporum* is a mycotoxin producer, it is primarily known for causing vascular wilt diseases in a wide range of economically important crops worldwide (Gordon, 2017). This underscores the importance of high-throughput sequencing studies for obtaining a more comprehensive understanding of soil microbiota and its role in agroecosystems.

5. Conclusions

Using a metataxonomics approach, we found that the farming system of cereal fields (conventional or organic) does not drive diversity differences large enough to be considered significant. Instead, the geographical origin of samples provides a better understanding of the community differences in both fungi and bacteria. Cereals form the basis of animal and human diet and carry a high risk of mycotoxin contamination. Thus, identifying and controlling the abundance of potential mycotoxin producers in soil is essential to mitigate contamination. Although our results suggest a limited effect of farming systems, further analyses using different study designs, marker genes, and databases will provide greater insights into a field with huge implications for both human and animal health.

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CRediT authorship contribution statement

Sergio Alías-Segura: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Data curation. **Jéssica Gil-Serna:** Writing – review & editing, Resources, Methodology, Conceptualization. **Belén Patiño:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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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Data availability

Raw sequencing data have been deposited in the European Nucleotide Archive at EMBL–EBI under accession number PRJEB88264. Feature tables and consensus tree in Newick format have been archived on Zenodo (https://doi.org/10.5281/zenodo.15174484). Metataxonomic analysis was performed with code from https://github.com/SergioAlias/sporeflow. Visualisation, phylogenetic inference, and cross-domain correlations were determined using code from https://github.com/SergioAlias/diversity-cereal. Precomputed QIIME 2 naive Bayes classifiers can be found at https://library.qiime2.org/data-resources for the SILVA database and at https://github.com/colinbrislawn/unite-train/releases/ for the UNITE database.

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