



RESEARCH

Impact of Growing Conditions and Potato Cultivars on the Diversity of Endophytic Microbial Communities

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Abstract

Endophytes inhabit plant tissues, offering various benefits to their hosts. Understanding their roles in sustainable agriculture is a key focus of research. Using 16S rRNA gene and fungal-specific ITS2 region amplicon sequencing, we investigated how 11 different potato cultivars and 3 different growing conditions influence the diversity of microbial endophytes in potato roots. We compared plants grown on two different soils in greenhouse conditions and plantlets grown in agar media (i.e., in vitro), representing the planting material without the effect of the soil microbiome. Our results revealed that growing conditions significantly influenced the alpha and beta diversity of endophytic bacteria. In plants grown in soils, the bacterial endophytic community was mainly represented by the *Pseudomonadaceae* family, whereas for in vitro plants, the *Paenibacillaceae*, a spore-forming bacteria family, was the main

representative. The fungal community comprised many possible fungal pathogens, such as *Colletotrichum*, *Fusarium*, and *Verticillium*. For the endophytic fungi, both soil types and cultivars affected fungal diversity, and a stronger effect for cultivars was seen for fungi as compared with bacteria. Overall, our findings indicate that endophytic bacteria exhibit strong recruitment potential from soil communities, and the identity of cultivars has also influenced fungal dynamics in communities. These findings shed light on the intricate interactions among potato cultivars and soil microbiomes, which can affect the design of sustainable agricultural strategies.

Keywords: amplicon sequencing, holobiont, plant-microbiome interactions, sustainable agriculture

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Data availability: Sequences are deposited in the NCBI Sequence Read Archive under the accession number PRJNA1140627. The code used in the bioinformatics

analysis of this study is available on GitHub (https://github.com/jyotsnepal/potatometabiome_endophytes).

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Potato (*Solanum tuberosum*) is among the top five food crops, with an annual production of 368 million tons globally, as per Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) data for the year 2018 (Shi et al. 2021). Managing potato pests and diseases mainly depends on breeding strategies and the intensive use of chemical fertilizers and pesticides (Cooke et al. 2011). To achieve the UN's Sustainable Development Goals, particularly Goal 2 (Zero Hunger) by 2030, which promotes sustainable agricultural practices, it is imperative to explore production approaches that reduce the environmental footprint of potato production (United Nations 2015). One possible approach is to rely on functions provided by microbial communities, whether free-living or associated with plants. The latter, collectively known as the plant microbiome, contributes to nutrient cycling, plant growth, and disease suppression, thus playing a significant role in supporting plant health.

Among the plant-associated microbiomes, endophytic microbiota are of great interest due to their close interactions with the host plant. Microbial endophytes have been found in every host plant tissue studied (Lodewyckx et al. 2002; Ryan et al. 2008), providing several functions to the host plants, such as protection from pests (Grabka et al. 2022; Panaccione et al. 2014; Shikano et al. 2017; Vu et al. 2006) and pathogens (Arnold et al. 2003; Bae et al. 2011; Collinge et al. 2022; Mousa et al. 2016; Pavlo et al. 2011), phytohormone modulation (Egamberdieva et al. 2017; González Ortega-Villaizán et al. 2024; Sun et al. 2009), stress tolerance (Baltruschat et al. 2008; Hardoim et al. 2008; Ravel et al. 1997; Waller et al. 2005), and nutrient acquisition (Carvalho et al. 2014; Pankievicz et al. 2015). The bacterial endophytes associated with potato plants have several beneficial traits (Reiter et al. 2002; Sessitsch et al. 2004), and tapping into these resources could represent a sustainable strategy to reduce the footprint of potato production through the application of beneficial microbial species (Coleman-Derr and Tringe 2014). These endophytes exploit the endosphere, or internal environment of the plant, as a specialized niche to protect themselves from severe changes in external surroundings (Wu et al. 2021). Thus, they may represent potential sources of biofertilizers and biopesticides that could enhance plant resilience to environmental stresses (Bamisile et al. 2021; Collinge et al. 2019; Koskimäki et al. 2015). In addition, the vertical transmission of the endophytes through seeds makes the microbiota interesting for commercial applications, as microbial traits can be transferred between generations without a costly inoculation procedure each season (Berg and Raaijmakers 2018; Bergna et al. 2018; Mitter et al. 2017).

Despite their potential, endophytes beneficial to one plant species can be pathogenic to other hosts (Collinge et al. 2022). For example, *Verticillium dahlia*, which causes disease in potatoes, exists without symptoms in mustard and barley (Wheeler et al. 2019). This dual nature of endophytes implies the need to identify host-specific endophytic species, their function, and the environment in which these interactions occur (Brader et al. 2017). Thus, it is important to understand the host-specific diversity of the endophytes before selecting them for sustainable management practices.

Most of the relevant studies have reported soil physicochemical parameters as being the major factors determining the rhizosphere microbiome, far more important than plant cultivars or agricultural practices (Berg and Smalla 2009; Fierer and Jackson 2006; Lundberg et al. 2012; Simonin et al. 2020). For endophytes, the roles of the plant cultivar and the environment as triggers for community structure are far less clear. For instance, specific mechanisms are required for soil-derived endophytes to access the entry into the endosphere via the roots (Hardoim et al. 2015). Endophytes enter plants through root openings, stomata, hydathodes,

and wounds. They secrete various cell-degrading enzymes, including cellulases, xylanases, and endoglucanases, to facilitate their entry and dissemination within the plant (Compant et al. 2005; Hardoim et al. 2015). Moreover, endophytes must navigate different host-associated signals and defense mechanisms for successful colonization (Lahrman et al. 2013). This suggests a greater level of specificity in this process, where the plant cultivars may exert a more significant influence. Studies have shown that root parameters such as root length, as well as the quality and quantity of root exudates, influence the recruitment of plant microbiomes inside and around the roots (rhizosphere) (Pérez-Jaramillo et al. 2016). These differences in root exudation patterns can lead to variations in endophytic selection by different cultivars, as observed for rhizosphere communities. In the case of potatoes, it has been shown that cultivars influence the bacterial communities associated with the rhizosphere (İnceoğlu et al. 2011), with cultivar effects being observed only at an early stage (30 days) of plant development. Moreover, the plant cultivars significantly affected bacterial endophytic communities in potato tubers (Buchholz et al. 2019). The historical context in which plants are grown, soil type, and agricultural management also have important effects on the plant-associated microbiome, both inside plant tissues and in the rhizosphere (Lau and Lennon 2011, 2012). A recent study on potato seeds also confirmed that the effect of soil composition was greater than that of cultivars (up to 64% of the variation explained for soils versus 18% for cultivars) for both bacterial and fungal communities (Song et al. 2024).

Here, we applied a culture-independent approach to assess the effect of potato cultivars and growth conditions on both bacterial and fungal root endophytic microbial communities. For this, we processed potato root samples from 11 cultivars grown in 2 different soils under greenhouse conditions. In addition, we processed root samples of the same 11 cultivars, grown in vitro (i.e., grown in agar media in tubes), to identify the endophytic communities present in the planting material. These in-vitro-grown plantlets represented the planting materials that exclude the soil effect, providing an overview of the communities in potato plantlets grown in sterilized growth conditions before transplanting them into the soil. The two soils plus the in vitro media are hereafter called the “growing conditions.” We sampled plantlets at an early stage of plant development, 5 to 6 weeks after the beginning of the experiments. Our aim was to identify to what extent growing conditions and/or cultivars determine the composition of the endophytic bacterial and fungal communities in potato roots.

Materials and Methods

Soil properties and sampling

Potato plantlets were obtained from tissue culture from the Institute of Plant Breeding and Acclimation in Bonin (Poland). The information on the cultivars' origin, purpose, year, pulp color, skin color, and root exudate metabolite level are mentioned in the studies by Martins et al. (2024) and Zhao et al. (2025) (Supplementary Table S1), while the details on disease resistance can be found in The European Cultivated Potato Database (<https://www.europotato.org/>). Plantlets of the 11 cultivars were prepared as follows: after 8 weeks of growth in the in vitro conditions supplemented by Murashige and Skoog nutrient media, plantlets were shipped to greenhouses in Germany and the Netherlands. For in vitro samples, these agar-grown plantlets of 11 cultivars were used for analysis.

Once the remaining samples for greenhouse experiments were shipped to the Netherlands and Germany, gentle removal of the attached agar to the roots was performed, and plantlets were transferred to homogenized soil in small pots (7 × 7 × 8 cm). The greenhouse experiments were conducted using two different soils grown

in two locations: Germany (named GER soil) and the Netherlands (named NL soil). For GER soil, in spring 2020, the top of a luvisol (0 to 20 cm) characterized as a loamy sand was obtained from the Gut-Roggenstein experimental station (latitude 48.1879670, longitude 11.3342012; 508 m above sea level), Technical University of Munich, in Southern Germany. The soil contained 1.27% total carbon and 0.1% total nitrogen, resulting in a ratio of 12.7. The NL soil was taken from a field in the Valthe village located in the Drenthe province (latitude 52.849866, longitude 6.866878; 20 m above sea level). The NL soil was sandy and had a pH of 5.83, organic matter 3.35%, NO₃ 3.80 mg/kg, and NH₄ 3.34 mg/kg before planting. The field in Germany (GER soil) was subjected to crop rotations, which consisted of summer barley in 2020, maize in 2019, wheat in 2018, rapeseed in 2017, wheat in 2016, and beans in 2015. The soil used in the NL soil was sourced from the field with sugar beet. Stones and crop debris were removed from the soil, homogenized using a 2-mm-diameter mesh sieve, and stored at 4°C until further use.

We selected 10 cultivars representing a range of microbiome interactive traits characterized by their ability to resist several pathogens and their root exudate metabolite capabilities (quality and quantity). The commercial and widespread cultivar Desiree was selected a priori as a control in the potatoMETAbiome project (<https://www.suscrop.eu/projects-first-call/potatometabiome>). After 2 weeks of acclimatization, these plants were then transferred to pots with a size of 11 × 11 × 12 cm, with one plant per pot. The temperature in the greenhouse was maintained at 22°C during the day and 18°C at night, with a photoperiod of 14 h of light and 10 h of darkness in the Netherlands and 16 h of light and 8 h of darkness in Germany. The soil moisture was kept at 60% in both greenhouses. Sampling was made at 6 and 5 weeks of plantation in Germany and the Netherlands, respectively, with three replicates per cultivar.

Surface sterilization of root samples

After carefully removing the rhizosphere soil from plants grown in soil or cultivation media from *in vitro* plants, roots were washed with tap water to remove soil adhering to the roots. The roots were surface sterilized by washing with 0.1% Tween for 5 min, followed by immersion in 70% ethanol for 3 min and 5% sodium hypochlorite solution for 3 min, and, finally, rinsing with sterile water five times each for 5 min. The sterilization efficacy was checked by immersing the roots in R2A agar plates for 15 s, and the R2A agar plates were then incubated at 28°C for 4 days. Roots were stored at –20°C until DNA extraction.

DNA extraction, PCR, and sequencing

For DNA extraction, the sterilized roots were homogenized in a mortar and pestle in the presence of liquid nitrogen. DNA was extracted using Qiagen's DNeasy Plant Pro Kit using DNA extraction kit protocols, starting with 100 mg of frozen roots (Qiagen, Courtaboeuf, France). The obtained DNA was stored at –20°C until further processing.

For characterization of the bacterial community, the 16S rRNA gene was amplified using the primers 515F (5'-GTGYCAGCMG CCGCGGTA-3') and 806R (5'-GGACTACNVGGGTWCTA AT-3') (Earth Microbiome Project) (Apprill et al. 2015; Caporaso et al. 2011, 2012; Parada et al. 2016). For characterization of the fungal community, the internal transcribed spacer 2 (ITS2) region was amplified using the primers 5.8SR (5'-TCGATGAAGAA CGCAGCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). PCR was conducted with 25 ng of template in a final volume of 25 µl using 12.5 µl of Platinum Hot Start PCR 2× Master Mix (Fisher Scientific S.A.S., Illkirch-Graffenstaden, France) with 0.625 µl of forward and reverse primers at 10 µM each

under the following PCR conditions: 94°C for 3 min, 35 cycles of 45 s at 94°C, 54°C for 60 s and 72°C for 1 min 30 s, and 10 min at 72°C. Similarly, the ITS PCR was conducted with 25 ng of template in a final volume of 25 µl using Platinum Hot Start PCR 2× Master Mix, (Fisher Scientific S.A.S.) with 0.625 µl of forward and reverse primers at 10 µM each under the following PCR conditions: 94°C for 2 min, 35 cycles of 30 s at 94°C, 59°C for 30 s and 72°C for 1 min, followed by 10 min at 72°C. The amplified products were sent for high-throughput sequencing with the MiSeq technology performed at the Plateforme Génome Transcriptome de Bordeaux (PGTB) (<https://doi.org/10.15454/1.5572396583599417E12>; University of Bordeaux, INRAE, BIOGECO, F-33610 Cestas, France).

Bioinformatics and statistical data analysis

The acquired 16S rRNA gene sequences were processed using QIIME2 (2021.4 version). The qiime2-cutadapt plugin was used to remove the forward and reverse primer sequences. This was followed by filtering, dereplication, chimera removal, and merging of the paired sequences using the Divisive Amplicon Denoising Algorithm (DADA2) workflow package (Callahan et al. 2017). Taxonomic assignment of the 16S rRNA gene amplicon sequence variant (ASV) sequences was performed using the q2-feature-classifier plugin (Bokulich et al. 2018) against the SILVA DATABASE (version 138) (Yilmaz et al. 2014). For the processing of the ITS region sequences, PIPITS software version 3.0 was used following the steps suggested by Gweon et al. (2015). The process involved the importing of raw sequencing data in FASTQ format followed by cleaning and then trimming of sequences for the removal of low-quality sequence reads. Then, the ITS2 regions were identified and extracted from the data, and the sequences were grouped into operational taxonomic units (OTUs). The taxonomic assignment of these fungal sequences was done using the RDP Classifier against UNITE database version 27.10.2022. All the analyses in this study were performed using unique ASVs for the bacterial endophytes and OTU level with 97% sequence identity for the fungal endophytes.

For the 16S rRNA gene, an initial input of 3,906,133 sequences was used for data processing. Quality filtering, denoising, and merging were performed using the DADA2 plugin in QIIME2, with forward reads truncated to 160 bp and reverse reads truncated to 200 bp. The minimum sequence length retained was 231 bp. The quality filtering, denoising, and merging resulted in 3,043,845 sequences. Following chimera removal, 2,739,732 non-chimeric sequences were present. The removal of mitochondria and chloroplast sequences left 720,696 sequences remaining. For the ITS2 region concerning fungal data, an initial input of 17,182,855 reads (comprising 359 samples in the original dataset for 51 cultivars) was used for data processing. The quality filtering (Q = default setting; minimum read length = 100 bp) resulted in 9,951,511 sequences. After dereplication and removing chimeras and unique sequences, 9,632,350 sequences were obtained. In this step, 273 out of 1,411 unique sequences were identified as chimeras (19.3% of the unique sequences). When abundance was considered, 5.9% of total sequences were removed as chimeras, and the remainder were non-chimeric. The removal of nontargeted sequences led to 2,258,687 remaining sequences.

Blank samples were included during DNA extraction to identify potential contaminants, which were removed using the DECONTAM package (Davis et al. 2018) in R using the prevalence method (prevalence = 0.1; 34 ASVs and 23 OTUs were discarded). From this OTU table for fungal data, data for 11 cultivars with their 3 replicates were extracted for further downstream analysis of fungal endophytic microbiome analysis. The singletons (ASVs or OTUs represented with one read in total across all samples) were removed. For the bacteria, 702,305 total reads with 1,138 ASVs

were present in the final dataset, whereas for the fungal community, 720,256 reads with 452 OTUs were present in the final dataset. The analysis was conducted on the rarefied data, except for core and shared taxa analyses. Samples were rarefied to 1,500 reads and 1,000 reads for analysis of bacterial and fungal endophytes, respectively, using the function `rarefy_even_depth` from the phyloseq package (McMurdie and Holmes 2013), leading to the removal of 20 and 29 samples from the bacterial and fungal datasets, respectively, and resulting in a total of 731 bacterial ASVs and 364 fungal OTUs. Rarefaction curves are shown in Supplementary Figure S1.

Statistical analyses were performed in R version 4.1.2 (R Core Team 2021). Phyloseq objects were made for both bacterial and fungal communities using the package phyloseq (v.1.38.0) (McMurdie and Holmes 2013) and used in subsequent analyses. Endophytic bacterial and fungal alpha diversities were measured by calculating the observed richness and Shannon Diversity Indexes and visualized with ggplot2 (v.3.4.2) (Wickham 2016). Principal coordinate analysis using Bray-Curtis distance was performed to visualize the structure of bacterial and fungal endophytes in different conditions. Permutational analysis of variance (PERMANOVA) (Anderson 2017) was performed to check the effect of the soil type and cultivars on the bacterial and fungal endophytes with the use of the `Adonis2` function in the package `vegan` (v.2.6-4) (Dixon 2003). For the analysis of the shared and core communities, non-rarefied data after the removal of singletons were taken. Core members were identified using the function `core_members` with detection and prevalence thresholds of 1 and 50%, respectively, in the package `microbiome` (v.1.16.0) (Lahti and Shetty 2017). For the analysis of the shared community, after the removal of singletons, we considered all the non-rarefied sequences that were observed in both soils and compared them with the non-rarefied sequences observed in the in vitro conditions. Their abundance percentage in both soils and in vitro conditions was calculated and presented in the form of a bubble plot using the package `ggplot2` (v.3.4.2) (Wickham 2016).

Results

Alpha diversity of endophytic microbial communities in 11 potato cultivars

We compared microbial endophytes' diversity (Shannon index and observed species richness) obtained from 11 different cultivars grown in 3 growing conditions. GER soil and NL soil refer to the different soil conditions in Germany and the Netherlands, respectively, whereas growing condition in vitro refers to the microbiomes present in the in vitro plants representing the endophytic microbiome without the soil effect, as they were grown in agar media (sterile conditions).

The Shannon diversity and species observed richness of the endophytic bacterial communities were influenced by growing conditions but not by cultivars (Fig. 1A and B; Supplementary Table S2; Supplementary Fig. S2; $P < 0.05$). For the in vitro plants, the commercial cultivar Desiree exhibited greater diversity (observed richness and Shannon indexes) than the other 10 cultivars. For fungal endophytic diversity, growing conditions had a significant effect on the observed richness and Shannon diversity. The impact of cultivars influenced the Shannon index but did not affect observed richness (Fig. 1C and D; Supplementary Fig. S2; $P < 0.05$). The differences in alpha diversity between plants grown in soil and those in vitro were less distinct than for the bacterial communities, with values from in vitro samples being intermediates between those of the GER and NL soil growing conditions (Fig. 1C and D). Overall, the results indicate that growing conditions are the dominant factor determining the diversity of the endophytic microbial communities.

Growing conditions have a significant effect in determining the structure of potato endophytes

When considering the community composition, the bacterial and fungal endophytes responded differently to growing conditions and cultivars. A principal coordinate analysis of all bacterial data clearly indicated a separation of endophytic bacterial communities from samples grown in GER soil and NL soil against samples grown in vitro (Fig. 2A, separation along the first axis). PERMANOVA proved that 66% of the variation in bacterial endophytes was due to growing conditions ($P = 0.001$; Table 1). Removing the in vitro samples from the analyses reduced the effect of growing conditions, but still, 42% variation was observed between GER soil and NL soil (Supplementary Table S3). A significant effect of cultivars and their interaction with growing conditions was observed for bacterial endophytes when samples from three growing conditions were taken (5 and 10% of the variance, respectively, in community composition; Table 1). This significant effect of cultivars and their interaction with growing conditions was not seen when the samples from in vitro growing conditions were removed (Supplementary Table S3; Supplementary Fig. S3). When compared separately for each growing condition, no significant effect of cultivars was observed on the structure of bacterial endophytes (Fig. 3A to C; Table 1). Overall, our data on bacterial community suggest that growing conditions (i.e., soil and location effect) are the main driver in determining the endophytic bacterial composition inside potato roots.

The patterns associated with the fungal community structure showed less contrast than those observed in bacterial endophytes. PERMANOVA indicated that growing conditions and their interactions with cultivars significantly affect fungal community composition by 36% ($P = 0.001$) and 22% ($P = 0.002$), respectively (Table 1). However, no significant effect was seen for cultivars, even though it was close to the threshold ($P = 0.056$). When comparing the fungal communities grown in GER and NL soils without the in vitro material, we observed a similar influence of growing conditions (38%; $P = 0.001$) but an increase in the importance of cultivar (18%; $P = 0.02$) (Supplementary Table S3) and an absence of the significant effect of their interactions. The comparisons of the cultivar effect on each individual growing condition revealed the significant effect of cultivars on the fungal endophytic communities grown in NL soil (57%; $P = 0.012$) and in vitro (46%; $P = 0.004$) (Table 1). However, no significant effect of cultivars was observed on the structure of fungal endophytes grown in GER soil (Table 1). In summary, our data showed that the structures of fungal endophytic communities were mainly driven by growing conditions and the interaction between growing conditions and cultivars. Cultivars alone have a smaller but significant effect on the fungal community structure of root endophytes.

Composition of bacterial and fungal endophytic communities in 11 cultivars of potato

The result from 16S rRNA gene amplicon sequencing data showed a high abundance of ASVs linked to the family *Pseudomonadaceae* in the samples grown in soil-growing conditions (growing conditions GER and NL soil; Fig. 4). On the contrary, plants grown in vitro were highly dominated by the bacterial family *Paenibacillaceae*. The bacterial endophytes associated with the cultivar Desiree from in vitro conditions were more diverse and showed lower dominance of the family *Paenibacillaceae* (39.9%), followed by *Pseudomonadaceae* (29.9%) and *Bacillaceae* (13.2%) (Fig. 4). In general, the cultivar Desiree showed a higher diversity of bacterial families also in the other growing conditions compared with other potato cultivars.

Fungal samples were highly dominated by unknown fungal families, followed by the families *Chaetomiaceae*, *Glomerellaceae*,

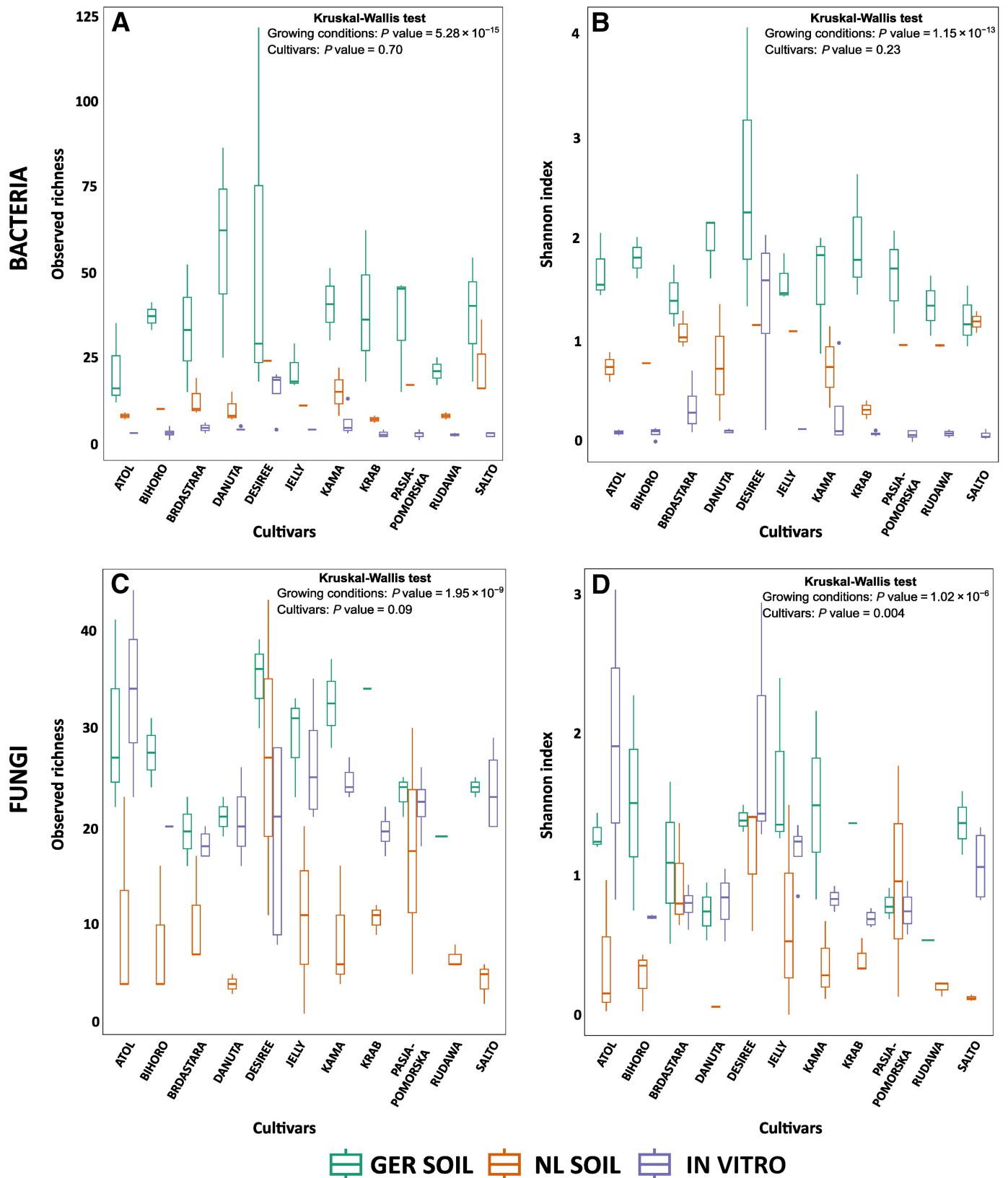


Fig. 1. Comparison of the alpha diversity of root endophytic bacteria and fungi between 11 potato cultivars grown in three different growing conditions: GER soil representing Germany ($n = 3$ replicates per cultivar), NL soil representing the Netherlands ($n = 3$ replicates per cultivar), and in vitro conditions ($n = 5$ replicates per cultivar). For each growing condition, we determined the **A and C**, observed richness and **B and D**, Shannon index of bacterial and fungal endophytes present in 11 potato cultivars, represented with boxplots. Boxplots display the medians, tops and bottoms of the boxes represent the 75th and 25th quartiles, and whiskers outside this range show variability outside the upper and lower quartiles. Small dots show the outliers. A Kruskal-Wallis test ($P < 0.05$) followed by Dunn's post-hoc tests with Benjamini-Hochberg (BH) correction was applied to calculate significant differences between different growing locations.

Nectriaceae, and *Lasiosphaeriaceae* in the samples grown in GER soil and plants grown in vitro, with a low abundance of other families (Fig. 5). However, in the samples grown in NL soil, the family *Glomerellaceae*, followed by an unknown fungal family, was dominant (Fig. 5). Desiree seems to have highly diverse fungal communities, such as bacterial endophytes, but with low relative abundance.

Shared and core communities between different locations and cultivars

To identify the shared bacterial ASVs or fungal OTUs across soils (growing conditions GER soil and NL soil) and in vitro plants, we used the non-rarefied dataset. In the case of bacteria, out of 1,138 ASVs that were observed, 141 ASVs were present in the in vitro plants and 1,017 in samples grown in the two soils. Among these

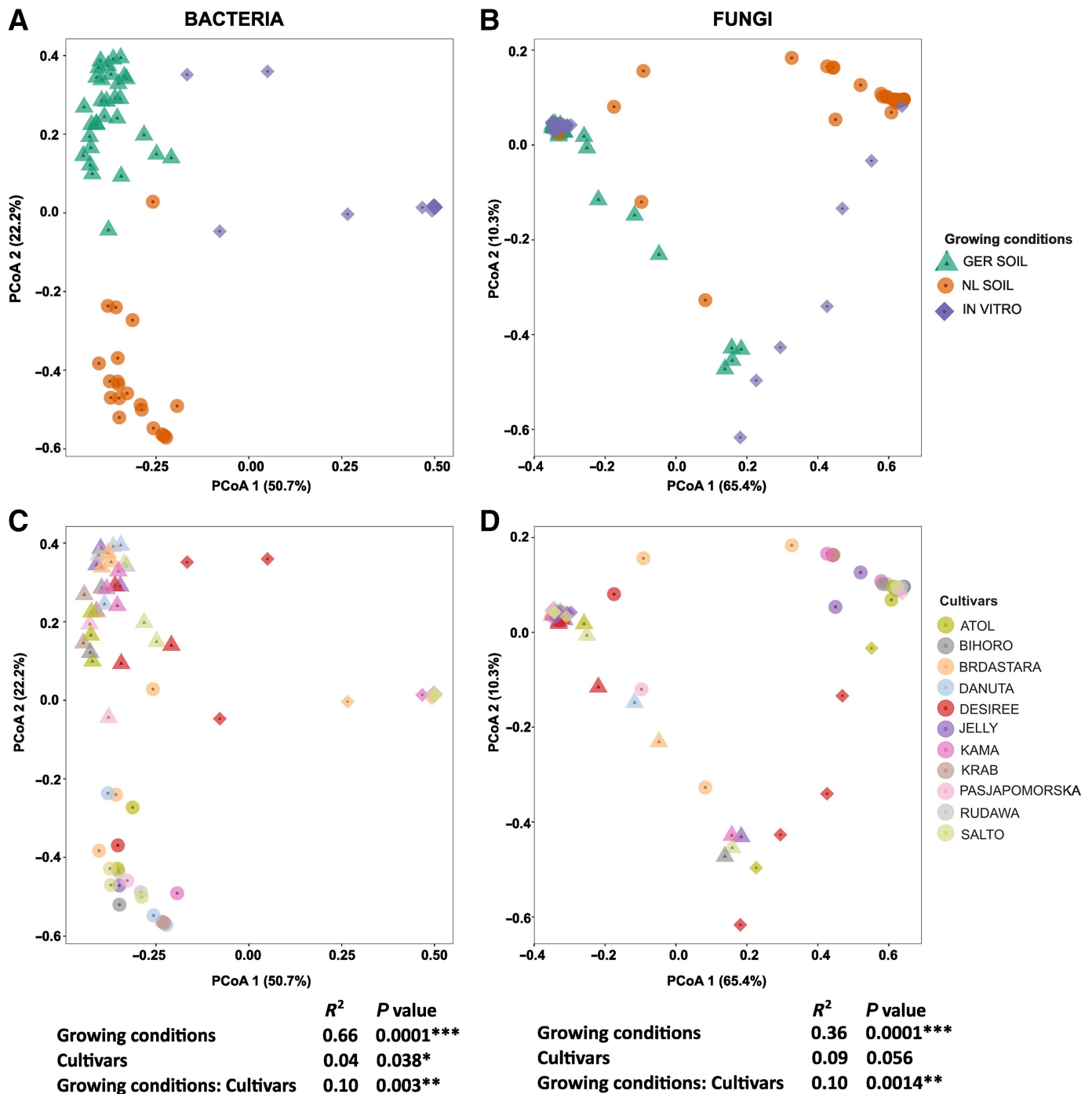


Fig. 2. Principal coordinates analysis (PCoA) plots based on Bray-Curtis distance matrices showing the structure of the **A and C**, root-associated bacterial and **B and D**, fungal endophytes between 11 potato cultivars across three growing conditions: GER soil representing Germany ($n = 3$ replicates per cultivar), NL soil representing the Netherlands ($n = 3$ replicates per cultivar), and in vitro conditions ($n = 5$ replicates per cultivar). In A and B, the samples are colored according to growing conditions, and in C and D, samples are colored according to the cultivars with shapes representing the growing conditions. Permutational multivariate analysis of variance (PERMANOVA) values are shown in the lower panel. R^2 shows the variance explained by each factor, and P value indicates which components significantly affect the structure of bacterial and fungal endophytes. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. Analysis was computed with 999 permutations. Table 1 shows PERMANOVA values indicating which components significantly affected the distribution.

ASVs, only 20 were shared between the soil samples and the in vitro plants, representing only 1.76% of the total bacterial ASVs observed. For fungi, out of 452 OTUs, 279 were present in the in vitro plants and 349 in samples grown in soils. Among these OTUs, 176 were shared between the soil samples and in-vitro-grown plants, which represented 38.94% of the total fungal OTUs observed.

Non-shared ASVs represented 14.8% in soils and 3.8% of the total ASVs in the in vitro samples. Among the shared taxa, samples originating from plants grown in soil conditions were dominated by the genus *Pseudomonas* (7 ASVs; 84.2% of the total abundance). In contrast, those coming from in vitro plants were highly dominated by one ASV associated with *Paenibacillus* (91.8%) (Fig. 6). Likewise, for the fungal community, samples originating from plants grown in soils were dominated by OTUs belonging to the genus *Colletotrichum* (2 OTUs; 64.2%), followed by unidentified fungi (23.9%), whereas those coming from in vitro plants were highly dominated by OTUs associated with unidentified fungi (62.7%), followed by *Colletotrichum* (10.8%) (Fig. 7).

Among the observed bacterial and fungal endophytes, only 3 bacterial ASVs and 12 fungal ASVs were found as core communities present in more than 50% of the total number of samples. Table 2 presents a list of these core communities, along with their prevalence and abundance.

Discussion

Microbial endophytes represent an exciting source of potentially beneficial microbes to reduce agriculture's environmental footprint

by supporting plant health and resistance to biotic and abiotic stress. To ensure the proper functioning of these beneficial microbes, it is essential to ensure their ability to colonize the tissues of the plants of interest, regardless of their genetic background. Here, we examined the endophytic communities in the roots of 11 different potato cultivars grown in two different soil conditions and sampled within 6 weeks of planting. Additionally, we determined the endophytes in the plantlets of potatoes that were grown in sterile in vitro conditions. Our data allowed us to identify core bacterial and fungal species associated with these cultivars, which can be used in future quests to optimize the interaction between beneficial microbes and potato production.

Soil parameters influence the bacterial and fungal endophytic communities in potato plants

It is well known that the soil microbiome drives plant-associated communities (Bulgarelli et al. 2012; Escobar Rodríguez et al. 2020, 2024; Zarrainaindia et al. 2015). In addition, soil parameters, including soil pH, nitrogen levels, agricultural practices, and cultivation history impact plant-associated microbiota (Girvan et al. 2003; Rousk et al. 2010). For instance, a history of soil cultivation leads to a plant-mediated legacy, contributing to variation in the endophytic community (Hannula et al. 2021). Our research underscores the significant influence of soils on the alpha and beta diversity of endophytes within potato roots. Using distinct growing conditions while maintaining similar greenhouse conditions and consistent fertilizer applications across both trials, we concluded

TABLE 1
Two-factor permutational analysis of variance (PERMANOVA) results showing the effect of growing conditions and cultivars in the structure of bacterial and fungal endophytic communities in potato roots^a

Community	Factor	PERMANOVA			
		Df	F value	R ²	P value
Bacteria (with in vitro samples)	Growing conditions	2	105.66	0.66	0.0001
	Cultivars	10	1.55	0.05	0.038
	Growing conditions × cultivars	20	1.66	0.10	0.003
	Residuals	59			
	Total	91			
Fungi (with in vitro samples)	Growing conditions	2	31.83	0.36	0.0001
	Cultivars	10	1.61	0.09	0.056
	Growing conditions × cultivars	19	2.03	0.22	0.0014
	Residuals	60			
	Total	91			
Bacteria (for each growing condition)	Cultivars (GER soil)	10	1.35	0.40	0.08
	Residuals	20			
	Cultivars (NL soil)	10	1.32	0.57	0.25
	Residuals	10			
	Cultivars (in vitro)	10	2.09	0.42	0.058
	Residuals	29			
Fungi (for each growing condition)	Cultivars (GER soil)	10	0.83	0.39	0.70
	Residuals	13			
	Cultivars (NL soil)	10	2.70	0.57	0.012
	Residuals	20			
	Cultivars (in vitro)	9	2.60	0.46	0.007
	Residuals	27			

^a Significant *P* values (*P* ≤ 0.05) are shown in bold. GER soil represents Germany; NL soil represents the Netherlands.

that the observed growing condition's effect on the bacterial and fungal endophytic community likely stems from variations in soil physicochemical and microbial properties mostly from the rhizosphere (Compant et al. 2012). As shown for *Arabidopsis thaliana* (Bulgarelli et al. 2012), our results further emphasize the broader implications of location on plant-associated microbiota and highlight the complex interplay between environmental factors and the plant microbiome.

The influence of cultivars is more prominent for fungal than for bacterial communities

In addition to the effect of growing conditions, we also observed an effect of the cultivar, which was more substantial for the fungal communities in the in vitro conditions. Our findings align with previous studies suggesting that cultivars have a more pronounced effect on the fungal than the bacterial microbiome, which is highly influenced by soil (Leff et al. 2017; Liu et al. 2022). This significant influence of cultivars on the community diversity and structure of fungal endophytes is confirmed by studies performed on the root endophytic mycobiome of other crops, such as tomatoes (Manzotti et al. 2020), wheat (Latz et al. 2021), and sunflowers (Brown and Mandel 2024). Conversely, the impact of cultivars on bacterial endophytes appears to be limited, mainly affecting their diversity. Similar limited effects of cultivars on the plant microbiome, including endophytes, have been observed in *Arabidopsis thaliana* (Lundberg et al. 2012) but also in potatoes, where the tuber's sur-

face bacterial microbiome showed to be weakly affected by potato cultivars (Weinert et al. 2010).

The varying effects of cultivars on the plant microbiome have been documented and can be attributed to factors such as plant compartment, developmental stage, and sampling year (Garcia et al. 2024; İnceoğlu et al. 2011; Quiza et al. 2023). Additionally, differences in root architecture among potato cultivars (Zarzyńska et al. 2017) and variations in the secretion of phytohormones and root exudates under different growth stages and stress conditions, such as drought, pathogen attacks, and herbivory, further contribute to the complexity of the relationships between cultivars and the endophytic microbiome (Manzotti et al. 2020). Given that this experiment spanned a period of 6 weeks, representing the early developmental stage, some effects might not yet have been strong enough to show significant differences between cultivars. The developmental stage at which experiments are conducted plays a crucial role in determining which aspects of the microbiome are observed and which might require long-term studies to fully understand. Future studies could aim to enhance our understanding of these dynamics by exploring the endophytic microbiome under various stress conditions, such as drought or fertilizer application, to assess how these factors might interact with cultivar-specific traits (Pérez-Jaramillo et al. 2016). Researchers could gain a deeper knowledge of cultivar effects by identifying crucial elements of plant-microbe interactions that drive the formation and evolution of the endophytic microbiome through long-term and more varied experimentation.

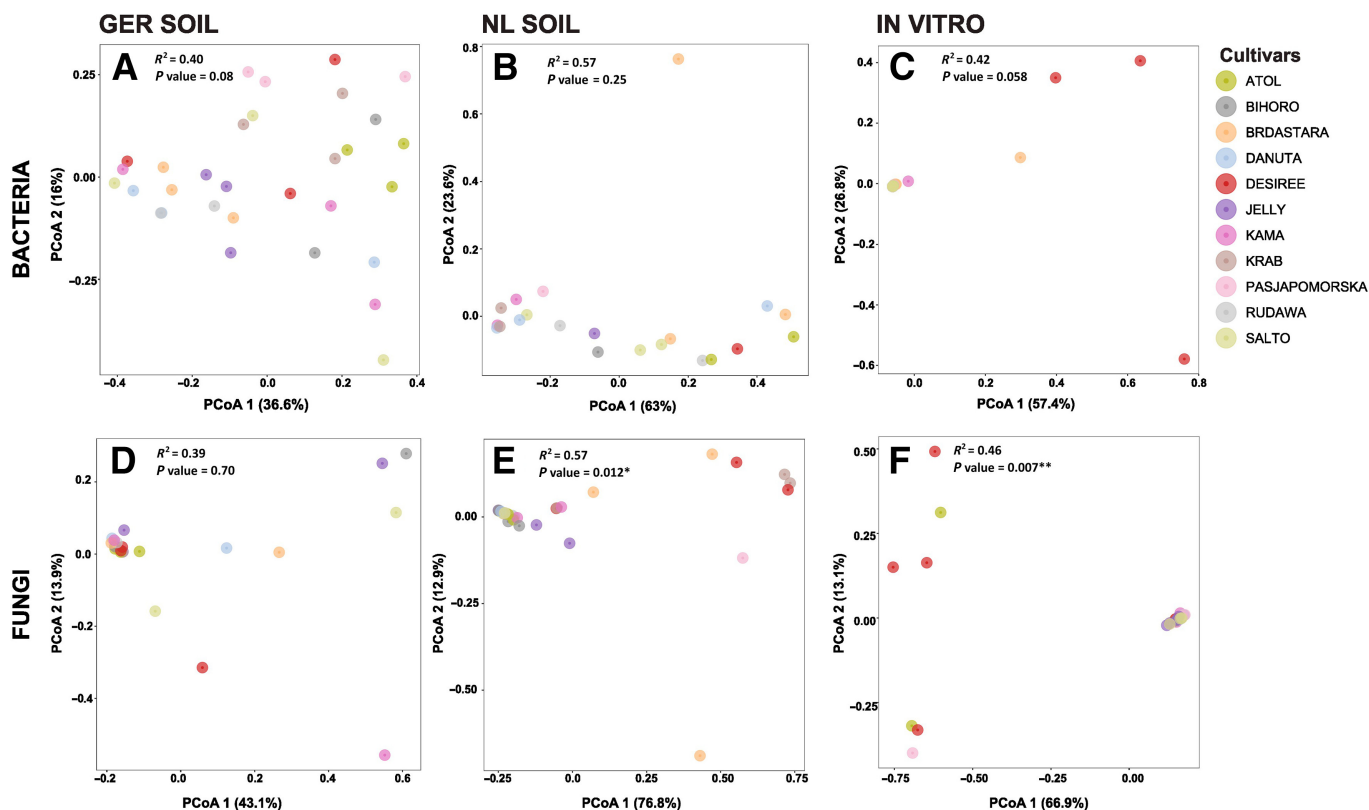


Fig. 3. Principal coordinates analysis (PCoA) plots based on Bray-Curtis distance matrices show the structure of the root-associated **A to C**, bacterial and **D to F**, fungal endophytes between 11 potato cultivars in the three growing conditions separately: GER soil representing Germany ($n = 3$ replicates per cultivar), NL soil representing the Netherlands ($n = 3$ replicates per cultivar), and in vitro conditions ($n = 5$ replicates per cultivar). Permutational multivariate analysis of variance (PERMANOVA) values are shown in the upper left corner of each panel. R^2 shows the variation in bacterial and fungal endophytic community structure explained by cultivars in each location. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. Analysis was computed with 999 permutations. Table 1 shows PERMANOVA values indicating which components significantly affect the distribution.

The community composition of the cultivar Desiree is different from that of the other 10 cultivars

The composition analysis of the endophytic microbiome in our study suggests that the cultivar Desiree exhibits a more diverse array of bacterial and fungal communities than the 10 cultivars selected from the culture collection. Desiree is a cultivar used commercially that gives quality yield but still relies on high application of nutrients. This high microbial diversity in Desiree roots hints at the idea that current agricultural practices have inadvertently considered the microbiome. This is consistent with a study demonstrated that breeding common beans against *Fusarium oxysporum* unintentionally led to selecting beneficial bacterial genera such as *Paenibacillus*, known for their antagonistic effects against *Fusarium* and other soil pathogens (Mendes et al. 2018). However, other research suggests that breeding has reduced selection pressure in modern wheat cultivars, enriching fungal pathogens compared with ancient cultivars (Kinnunen-Grubb et al. 2020). Similar results were observed in a study on sunflowers (Leff et al. 2017). Although our data, limited to the genus level, do not provide conclusive evidence for the concepts above, they do indicate distinct microbial community patterns in commercial cultivars compared with others. This divergence may be linked to their reliance on increased nutrient and chemical inputs. This highlights the importance of considering the interaction between the microbiome and potato plants in future breeding strategies.

Higher ASV number and differences in the composition of bacterial communities in the in vitro plants compared with those grown in the soil

The number of bacterial endophyte ASVs in the in-vitro-grown plant materials was lower than in samples cultivated in soil, and their composition differed significantly between the two conditions. These findings align with results from a bacterial endophytic study conducted on micro-propagated cultures of poplar plants, in which the diversity of the endophytic community of the micro-propagated plants was lower and composed of different communities as compared with field-grown plants (Ulrich et al. 2008a, b). The disparity could be attributed to two factors. First, the bulk and rhizosphere soil are significant sources of root-associated microbial communities (Attia et al. 2022), leading to the decreased diversity of endophytes in the in vitro plants. Second, the limited natural nutrient sources in the in vitro medium may favor the growth of specific microbial communities that can thrive under stressed conditions. In our investigation, the in vitro samples exhibited a higher abundance of *Paenibacillus* than root samples grown in soil. This could be attributed to the endospore-forming nature of *Paenibacillus*, enabling them to persist in controlled sterilized conditions. The elevated prevalence of *Paenibacillus* in the in-vitro-grown samples in our study aligns with similar observations in tissue cultures of woody (Ulrich et al. 2008a) and bulb plants (Ptak et al. 2022).

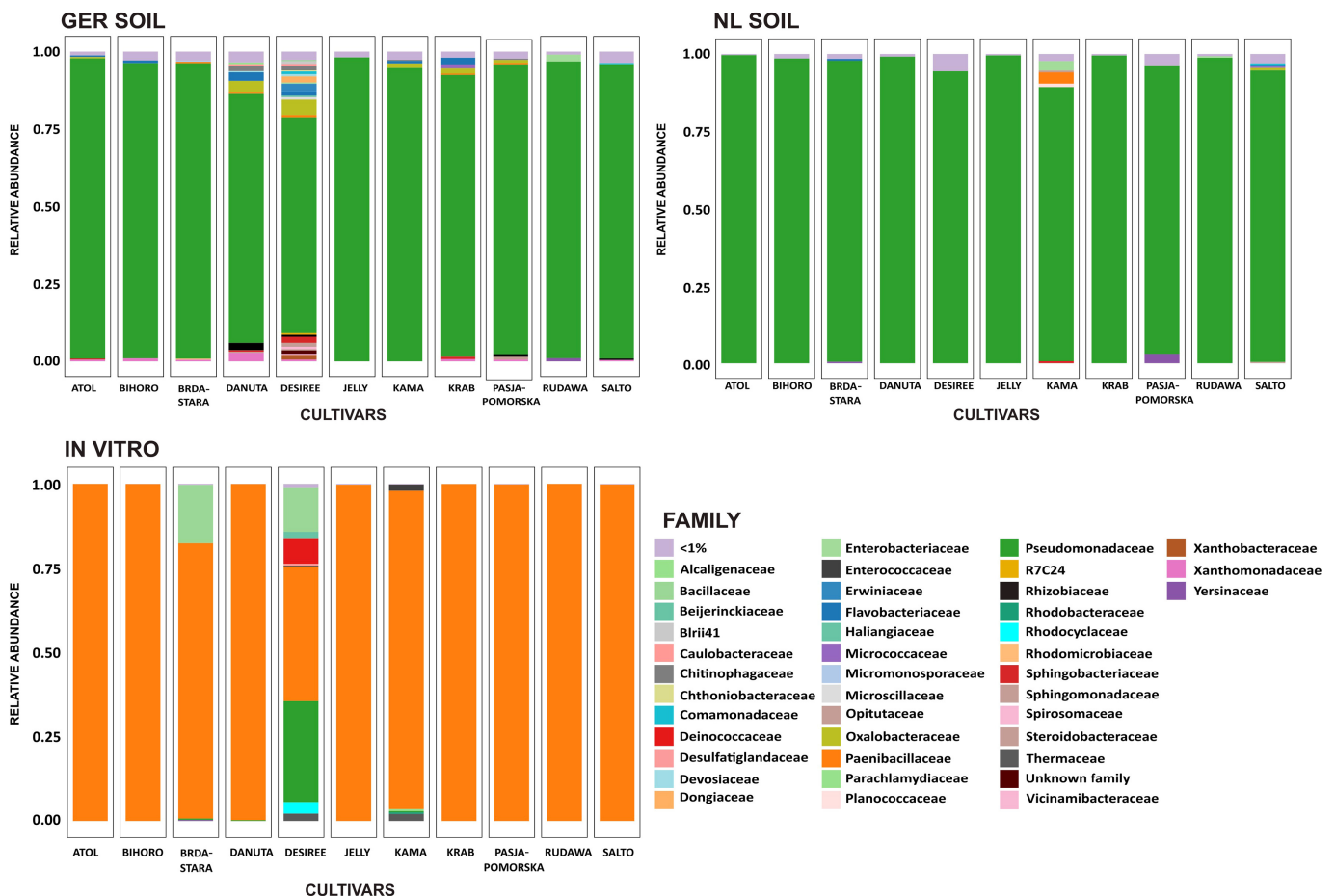


Fig. 4. Composition of the root-associated bacterial endophytic community of 11 potato cultivars in samples grown in three different growing conditions: GER soil representing Germany ($n = 3$ replicates per cultivar), NL soil representing the Netherlands ($n = 3$ replicates per cultivar), and in vitro conditions ($n = 5$ replicates per cultivar). The relative abundances (%) are calculated for major bacterial endophytic families present in the 11 potato cultivars ($n = 3$).

High dominance of a few taxa in all growing conditions

Being inside the plants, the endosphere is supposed to be less influenced by external environmental factors. However, this area also limits the source of surrounding soil nutrients. Likewise, to be able to enter plants, microbes have to overcome different responses/mechanisms of defense shown by the plants, which also explains the differences between rhizome and atmospheric microbiomes. In our study, we found a high abundance of a few genera in the roots of plants grown in soils and in vitro conditions. For in vitro plants, we saw a high abundance of the genus *Paenibacillus*, and in soil, this shifted to the genus *Pseudomonas*. Similarly, in fungi, we saw a high abundance of a few species, even though there was no trend of specific microbial communities in soil versus in vitro conditions. This is consistent with a study showing a high abundance of few microbial species in *Nicotiana tabacum* (Chen et al. 2020) and confirming that plants “filter” or at least “minimize” the entry of microorganisms inside their cells.

Identifying the shared and core bacterial communities has highlighted the importance of the genus *Pseudomonas*, which was predominantly observed in the samples grown in soil. The genus *Pseudomonas* has been documented as being an endophyte in various crops, such as tomatoes (Dong et al. 2019) and potatoes (Pageni et al. 2013). Several strains of the genus *Pseudomonas* have been shown to have the ability to combat various plant diseases, including the highly devastating potato pathogen *Phytophthora infestans* (Hunziker et al. 2015; Sessitsch et al. 2004). The greater presence of

the genus *Pseudomonas* in soils and its potential to control diseases might have conferred a selective advantage for plants that interact with those species, as it might promote plant response to overcome biotic stress. Whether the high abundance of *Pseudomonas* in plants grown in soil growing conditions is due to active plant selection or random chance remains to be tested.

For fungi, we observed far more OTUs shared between soils and in vitro conditions compared with bacteria (176 versus 20, respectively), and *Colletotrichum* is the most important genus in soils (64.5% of all the sequences). Overall, we observed a stronger relationship between bacterial endophytes and growing conditions ($R^2 = 0.66$, $P = 0.001$) than fungal endophytic communities ($R^2 = 0.36$, $P = 0.001$). One potential explanation for the lower variability of fungi relies on their ability to form mutualistic interactions with plants (such as mycorrhizae), in which both parties benefit from each other (Bonfante and Genre 2010). This close association, in addition to providing benefits to host plants, can also help stabilize fungal populations by supplying nutrients and a niche that protects them from environmental fluctuations. In contrast, bacterial communities may be more directly influenced by changes in environmental conditions due to their more generalized roles in soil ecosystems. Moreover, the lower variability of fungal communities could also be attributed to their structural and functional diversity, which allows them to sustain in a wide range of environmental and stressful conditions. For instance, fungal spores or resistant structures such as sclerotia can remain dormant until they have favorable

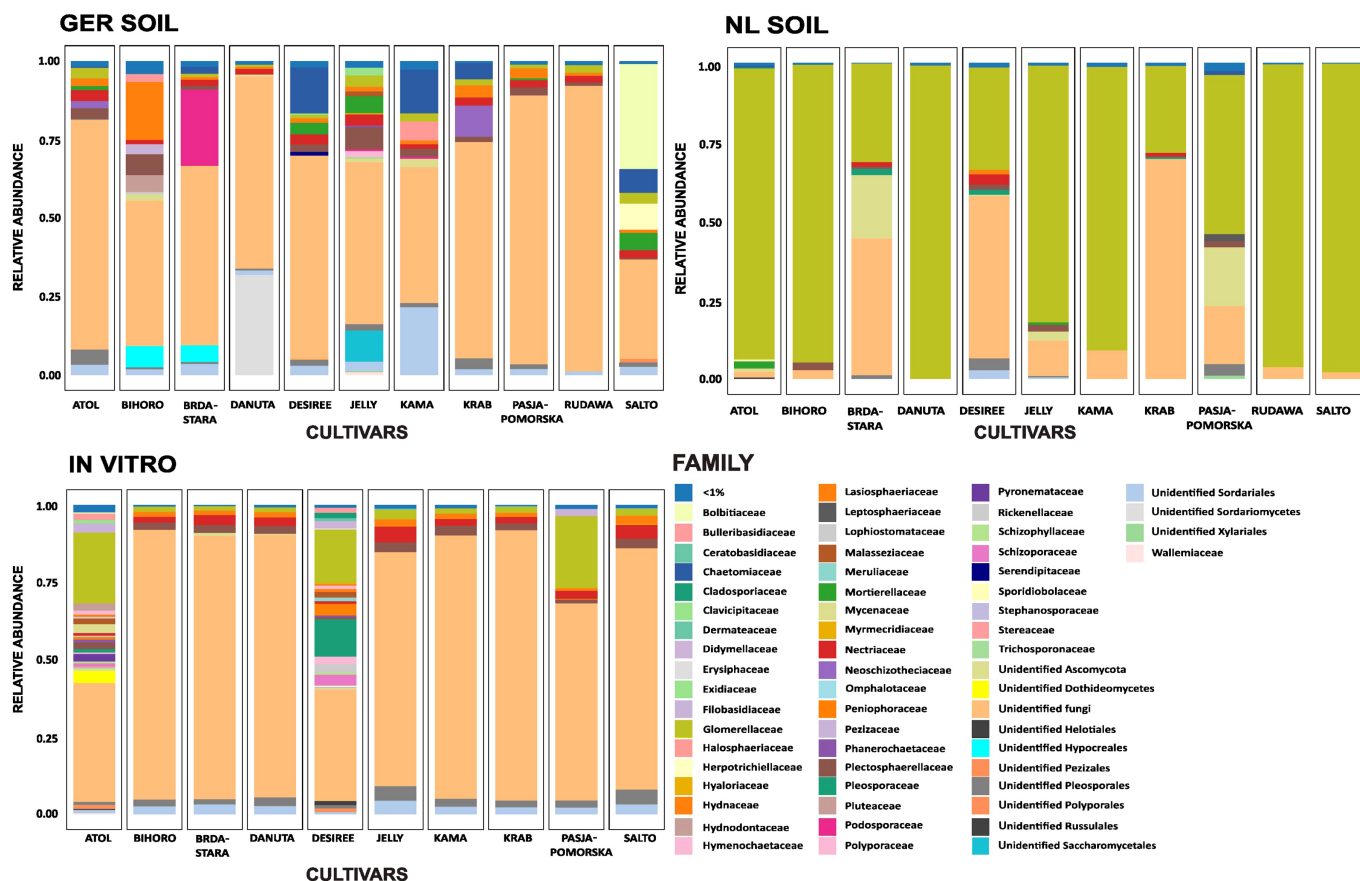


Fig. 5. Composition of the root-associated fungal endophytic community of potato plants in samples grown in three different growing conditions: GER soil representing Germany, NL soil representing the Netherlands, and in vitro conditions. The relative abundances (%) are calculated for major fungal endophytic families present in the 11 potato cultivars ($n = 3$). Taxa are shown at the nearest classified level when family-level classification was unavailable.

growth conditions, which might not be as pronounced for bacterial communities (Corona Ramirez et al. 2023; Smith et al. 2015).

A high abundance of fungal species, such as *Colletotrichum coccodes*, *Fusarium*, and *Verticillium dahlia*, has been observed as en-

dophytes in potatoes (Götz et al. 2006). *Colletotrichum coccodes*, belonging to the family *Glomerellaceae*, has been reported as a devastating fungal pathogen in potatoes that is responsible for causing black dot disease in potato tubers and causing significant yield loss.

Fig. 6. Diagram showing the number and the abundance percentage of shared bacterial taxa between samples grown in soil (combining soil from Germany and the Netherlands and cultivars belonging to these soils) and in vitro samples. The bubble's size reflects each community's relative abundance, providing a visual comparison of their prevalence. ASV, amplicon sequence variant.

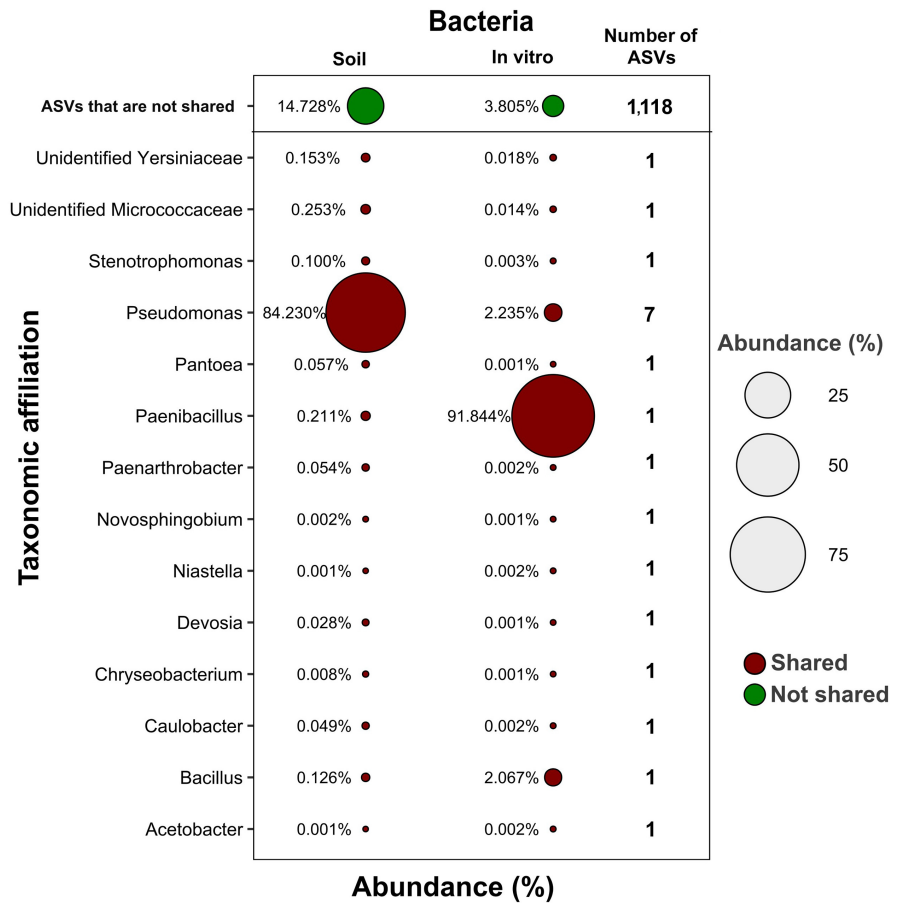


TABLE 2
List of core bacterial and fungal amplicon sequence variants and operational taxonomic units from root endophytic communities of potato observed in more than 50% of samples

Taxonomic affiliation	Abundance	Prevalence
Fungi		
<i>Sordariales</i> sp. SH0962352.09FU	0.94	61.98
<i>Lasiosphaeriaceae</i> sp. SH0932574.09FU	0.73	59.5
Unidentified <i>Sordariales</i>	0.09	52.07
Unidentified <i>Fusarium</i>	0.74	60.33
Unidentified <i>Nectriaceae</i>	0.52	59.5
Unidentified <i>Gibellulopsis</i>	0.65	71.07
<i>Chordomyces antarcticus</i> SH1451459.09FU	0.18	58.68
<i>Colletotrichum coccodes</i> SH0899393.09FU	49.66	100
<i>Pleosporales</i> sp. SH0997149.09FU	0.25	61.16
Unidentified <i>Pleosporales</i>	1.3	61.98
Unidentified <i>Fungi</i>	1.59	57.85
Unidentified <i>Fungi</i>	32.71	95.04
Bacteria		
<i>Pseudomonas</i> (f8aeec3c1ff0f4e7e1c829e4db0618d9)	10.56	50.89
<i>Pseudomonas</i> (9af3467db68cf6063627304cecd46a65)	14.43	54.46
<i>Pseudomonas</i> (6a42f6b31b1958a4fd7c015ad7affbf4)	28.43	71.43

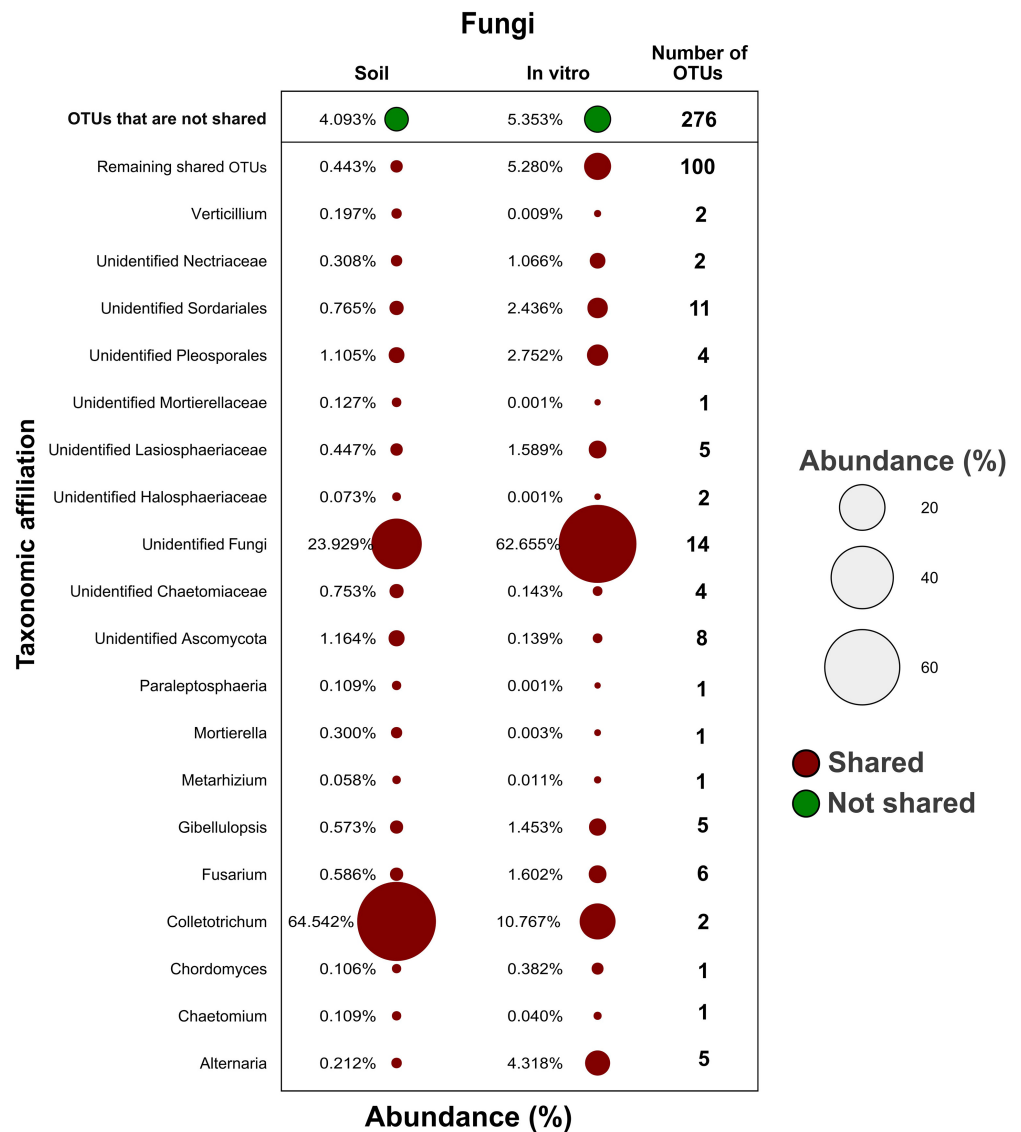
Likewise, many species of *Fusarium* and *Alternaria* are known to cause diseases such as dry rot and early blight, respectively, in potatoes (Niu et al. 2022). The presence of OTUs associated with these genera suggests that the prevalence of pathogenic fungi as endophytes is higher than that of the beneficial ones. Nevertheless, despite its low abundance, the presence of the beneficial fungal genus *Metarhizium* was observed and could be interesting to consider in future studies. In our study, we observed many unidentified fungal OTUs, which hints toward the complexity of fungal species identification and characterization. Thus, this opens the scope for improving the molecular biology and bioinformatics methods for taxonomic, phylogenetic, and functional studies of these unidentified fungal species. There are few studies focusing on fungal endophytes compared with bacteria, and even fewer when it comes to studies of bacterial and fungal endophytes together.

Conclusions

Here, we show that even though growing conditions have a strong effect on the endophyte microbiome of potato roots, the comparison across cultivars suggests a plant selection for root endophytes, specifically in the context of fungal microbiota. These different patterns between endophytic bacteria and fungi can be linked to

the ability of fungi to sporulate, which can allow them to maintain in the plant whatever the changes due to the plant developmental stages. Besides, if fungi are better transmitted across generations, it will be difficult for other fungi present in the soil to settle into the plant, again increasing the cultivar effect. For bacteria, on the other hand, our results suggest that soil recruitment might be more important. In terms of breeding or biocontrol strategies, our result implies that (i) a successful inoculation of beneficial fungi into plants could have an advantage that lasts across generations and (ii) bacterial biocontrol agents should be preferentially supplied at early plant stages when bacteria recruitment from the soils is important. As interactions between “existing” endophytic fungi and bacteria inoculated will occur, future studies should try to better understand this synergy or antagonism to improve the efficiency of breeding and/or biocontrol strategies. The use of culture-dependent methods could help in selecting potato-specific beneficial microbes. Moreover, future research should focus on analyzing these endophytic communities from earlier stages of plants to the harvesting phase, as well as changes in the bulk and rhizosphere communities over time, to see if any specific growth stage of plants and changes in soil communities are more critical for microbiome recruitment in plants. Also, studies should target the ideal trans-

Fig. 7. Diagram showing the number and the abundance percentage of shared fungal merged operational taxonomic units (OTUs) between samples grown in soil (combining soil from Germany and the Netherlands and cultivars belonging to these soils) and in vitro samples. The bubble's size reflects each community's relative abundance, providing a visual comparison of their prevalence.



mission route of these beneficial microbes to potato plants, either through tuber coating, as endophytes during potato seed production, or as soil inoculants. Together, these approaches will contribute to the reduction of the environmental footprint of potato production worldwide.

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