Impact of growing conditions and potato cultivars on the diversity of endophytic microbial communities

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Keywords: Holobiont; Plant-microbiome interactions; sustainable agriculture; amplicon-sequencing

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 2 different soils in greenhouse conditions and plantlets grown in agar medium, i.e., *in vitro*, representing the planting material without the effect of the soil microbiome. Our study reveals 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 *Pseudomonodacae* 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, a stronger effect for cultivars was seen for fungi as compared to bacteria.

Overall, our findings indicate that endophytic bacteria exhibit strong recruitment potential from soil communities, while the identity of cultivars has also influenced fungal dynamics communities. These findings shed light on the intricate interactions among potato cultivars and soil microbiomes, which can affect the design of sustainable agricultural strategies.

Introduction

Potato (*Solanum tuberosum* L.) 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, the endophytic microbiota is 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, like protection from pests (Grabka et al., 2022; Panaccione et al., 2014; Shikano et al., 2017; Vu et al., 2006) and pathogens (Ardanov et al., 2011; Arnold et al., 2003; Bae et al., 2011; Collinge et al., 2022; Mousa et al., 2016), 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) or 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 & 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 that microbiota interesting for commercial applications, as microbial traits can be transferred between generations without costly inoculation procedure each season (Berg & 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 where these interactions

happen (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 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 & Smalla, 2009; Fierer & Jackson, 2006; Lundberg et al., 2012; Simonin et al., 2020). For endophytes, the role 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 (Lahrmann 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 cultivar 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 an important effect on the plant-associated microbiome, both inside plant tissues and in the rhizosphere (Lau et al., 2011; Lau & Lennon, 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 vs. 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-medium 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 2 soils plus the *in vitro* medium are called thereafter “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 which 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 (Bonin, Poland). The information on the cultivars' origin,

purpose, year, pulp color, and skin color are mentioned in the study provided by Martins et al. (2024), while the details on disease resistance can be found in The European Cultivated Potato Database 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 medium, plantlets were shipped to the 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 done 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 (0–20 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 Valthe village located in Drenthe province (latitude 52.849866, longitude 6.866878, 20 m from 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. The 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 ten cultivars representing a range of microbiome interactive traits characterized by their ability to resist several pathogens and their root exudate metabolites capabilities (quality and quantity). Besides, the commercial and widespread cultivar Desiree was selected a priori as a control in the potatoMETAbiome project, were selected (https://www.potatometabiome.eu/project-structure). After two weeks of acclimatization, these plants were then transferred to pots with diameter 11*11*12cm 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 hours of light and 10 hours of darkness in the Netherlands and 16 hours of light and 8 hours 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 Netherlands, respectively, with 3 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 minutes, followed by immersion in 70% ethanol for 3 minutes, 5% sodium hypochlorite solution for 3 minutes, and finally, by rinsing with sterile water five times each for 5 minutes. The sterilization efficacy was checked by immersing the roots in the R2A agar plates for 15 seconds, and R2A agar plates were then incubated at 28°C for 4 days. Roots were stored at -20°C till 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 till further processing.

For characterization of the bacterial community, the 16S rRNA gene was amplified using the primers 515F (5'- GTGYCAGCMGCCGCGGTAA-3') and 806R (5'GGACTACNVGGGTWTCTAAT 3') (Earth microbiome project) (Apprill et al., 2015; Caporaso et al., 2011, 2012; Parada et al., 2016). For characterization of the fungal community, the ITS2 region was amplified using the primers 5.8SR (5'-TCGATGAAGAACGCAGCG-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 HotStart PCR 2X mastermix (Fisher Scientific S.A.S., Illkirch-Graffenstaden, France) with 0.625 µl of forward and reverse primers at 10µM 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, 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 HotStart PCR 2X mastermix, (Fisher Scientific S.A.S., Illkirch-Graffenstaden, France) with 0.625 µl of forward and reverse primers at 10µM 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 PGTB (doi:10.15454/1.5572396583599417E12), Univ. 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, chimaera 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 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 ASV (amplicon sequence variants) for the bacterial endophytes and OTUs (operational taxonomic units) 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, while the remainder were non-chimeric. The removal of non-targeted sequences led to 2258687 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, 702305 total reads with 1138 ASVs were present in the final data set, whereas, for fungal community, 720256 with 452 OTUs were present in the final data set. The analysis was conducted on the rarefied data, except for core and shared taxa analyses. Samples were rarefied to 1500 reads and 1000 reads for analysis of bacterial and fungal endophytes respectively using the function rarefy_even_depth from the phyloseq package (McMurdie & 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.

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 & 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 (PCoA) 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 Adonis2 function in 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 was taken. Core members were identified using function "*core_members*" with detection and prevalence thresholds 1% and 50%, respectively, in package microbiome (v. 1.16.0) (Lahti & 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 to 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 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 on 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-medium (sterile conditions).

The Shannon diversity and species observed richness of the endophytic bacterial communities were influenced by growing conditions but not by cultivars (Figure 1A and

1B; p-values <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 (Figure 1C and 1D; p-values <0.05). The differences in α-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 (Figure 1C and 1D). 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 (PCoA) 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* (Figure 2A, separation along the first axis). PERMANOVA analysis 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 S1). 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 S1). When compared separately for each growing condition, no significant effect of cultivars was observed on the structure of bacterial endophytes (Figure 3 A, B, C, and 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 S1) 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 structures of fungal endophytic communities were mainly driven by growing conditions and the interaction between growing conditions and cultivars. Cultivars alone play 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, Figure 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%) (Figure 4). In general, the cultivar Desiree showed a higher diversity of bacterial families also in the other growing conditions compared to other potato cultivars.

Fungal samples were highly dominated by unknown fungal families, followed by the families *Chaetomiaceae*, *Glomerallaceae*, *Nectriaceae*, *Lasiosphaeriaceae* in the samples grown in GER soil and plants grown *in vitro* with a low abundance of other families (Figure 5). However, in the samples grown in NL soil, the family *Glomerallaceae*, followed by an unknown fungal family, was dominant (Figure 5). Desiree seems to have highly diverse fungal communities like 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 1138 ASVs that were observed, 141 ASVs were present in the *in vitro* plants and 1017 in samples grown in the 2 soils. Among these 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 OTUs were shared between the soil samples and *in vitro* grown plants which represented 38.94% of 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%) (Figure 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%) (Figure 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 also 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., 2024; Rodríguez et al., 2020; Zarraonaindia 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. In distinct growing conditions while maintaining similar greenhouse conditions and consistent fertilizer applications across both trials, we conclude 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 the *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 plant microbiome.

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

In addition to the effect of growing conditions, we could also observe an effect of the cultivar, which was more substantial for the fungal communities in the *in vitro*. 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 & Mandel, 2024). Conversely, the impact of cultivars on bacterial endophytes appears to be limited, mainly affecting the 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 surface 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 spans a period of 6 weeks, representing the early developmental stage, some effects might not yet be 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.

The community composition of the cultivar Desiree is different to 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 these days 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 oxysporium* 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 to ancient cultivars (Kinnunen-Grubb et al., 2020). Similar results were observed in a study on sunflowers (Leff et al., 2017). While our data, limited to the genus level, don't provide conclusive evidence for the concepts above, it does indicate distinct microbial

community patterns in commercial cultivars compared to 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 to those grown in the soil

The number of bacterial endophytes 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 where the diversity of the endophytic community of the micro-propagated plants was lower and composed of the different communities as compared to field-grown plants (Ulrich, Stauber, et al., 2008; Ulrich, Ulrich, et al., 2008). 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), therefore leading to the decrease diversity of endophytes in the *in vitro* plants. Secondly, 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, Stauber, et al., 2008) and bulb plants (Ptak et al., 2022).

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. But 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 show a high abundance of a few genera in the roots of plants grown in soils and *in vitro* conditions. For *in vitro* plants, we see a high abundance of the genus *Paenibacillus*, and in soil, this shifts to the genus *Pseudomonas*. Similarly, in fungi, we see a high abundance of a few species, even though there was no trend of specific microbial communities in soil vs. *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 endophyte in various crops, such as tomatoes (Dong et al., 2019) as well as 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 their potential to control diseases might have conferred a selective advantage for plants that interact with those species, as they 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 to bacteria (176 vs 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 with 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), where both parties benefit from each other (Bonfante & 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 like sclerotia can remain dormant until they have favorable 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 endophytes 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. Likewise, many species of

Fusarium and *Alternaria* are known to cause diseases like dry rot and early blight, respectively, in potatoes (Niu et al., 2022). The presence of OTUs associated with these genera suggests the prevalence of pathogenic fungi as endophytes is higher than the beneficial ones. Nevertheless, despite its low abundance, the presence of the beneficial fungal genus *Metarhizium* was observed and can be interesting 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 these unidentified fungal species' taxonomic, phylogenetic, and functional studies. There are few studies focusing on the fungal endophytes compared to the 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, increasing again 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 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 transmission 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.

Availability of Data

Sequences are deposited in (NCBI) Sequence Read Archive (SRA) under the accession number PRJNA1140627. The code used in the bioinformatics analysis of this study is available on github with following link: https://github.com/jyotsnepal/potatometabiome_endophytes

Acknowledgements

This research was financially backed by the ERA-NET Cofund SusCrop project potatoMETAbiome, which received support from the European Union’s Horizon 2020 research and innovation program (grant agreement No. 771134; French National Agency, grant number ANR-18-SUSC-0001). It is also affiliated with the Joint

Programming Initiative on Agriculture, Food Security, and Climate Change (FACCE-JPI). J.N. was supported by a scholarship from the Local Council of Pyrénées-Atlantiques, E2S UPPA and the University of Groningen scholarship program. T.Z. received financial support through a scholarship from the China Scholarship Council (CSC) and a scholarship program at the University of Groningen.

We are grateful to PGTB for sequencing (Genome Transcriptome Platform of Bordeaux, doi:10.15454/1.5572396583599417E12) (Univ. Bordeaux, INRAE, BIOGECO, F-33610 Cestas, France) and especially to Gaelle Chancerel, Erwan Guichoux and Zoe Delporte.

Author contributions

EA, RG, MS, SV, and JFS were responsible for designing the study. KT, DM and AP supplied the plantlets grown *in vitro* for the research. TZ, SV, and XJ conducted greenhouse experiments in the Netherlands, while BRM, MS, and VR carried out similar experiments in Germany. JN and FG were in charge of processing the root samples in the lab. JN analyzed the datasets, created the figures, and drafted the manuscript. Additionally, all co-authors contributed to reviewing and editing the final manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

References

Anderson, M. J. (2017). Permutational multivariate analysis of variance (PERMANOVA). In Wiley StatsRef: Statistics Reference Online (pp. 1–15). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118445112.stat07841

Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquatic Microbial Ecology, 75(2), 129–137. https://doi.org/10.3354/ame01753

Ardanov, P., Ovcharenko, L., Zaets, I., Kozyrovska, N., & Pirttilä, A. M. (2011). Endophytic bacteria enhancing growth and disease resistance of potato (Solanum tuberosum L.). Biological Control, 56(1), 43–49. https://doi.org/10.1016/J.BIOCONTROL.2010.09.014

Arnold, A. E., Mejía, L. C., Kyllo, D., Rojas, E. I., Maynard, Z., Robbins, N., & Herre, E. A. (2003). Fungal endophytes limit pathogen damage in a tropical tree. Proceedings of the National Academy of Sciences of the United States of America, 100(26), 15649–15654. https://doi.org/10.1073/PNAS.2533483100

Attia, S., Russel, J., Mortensen, M. S., Madsen, J. S., & Sørensen, S. J. (2022). Unexpected diversity among small-scale sample replicates of defined plant root compartments. The ISME Journal, 16(4), 997–1003. https://doi.org/10.1038/S41396-021-01094-7

Bae, H., Roberts, D. P., Lim, H. S., Strem, M. D., Park, S. C., Ryu, C. M., Melnick, R. L., & Bailey, B. A. (2011). Endophytic Trichoderma Isolates from Tropical Environments Delay Disease Onset and Induce Resistance Against Phytophthora capsici in Hot Pepper Using Multiple Mechanisms. Molecular Plant-Microbe Interactions, 24(3), 336–351. https://doi.org/10.1094/MPMI-09-10-0221

Baltruschat, H., Fodor, J., Harrach, B. D., Niemczyk, E., Barna, B., Gullner, G., Janeczko, A., Kogel, K. H., Schäfer, P., Schwarczinger, I., Zuccaro, A., & Skoczowski, A. (2008). Salt tolerance of barley induced by the root endophyte Piriformospora indica is associated with a strong increase in antioxidants. New Phytologist, 180(2), 501–510. https://doi.org/10.1111/J.1469-8137.2008.02583.X

Bamisile, B. S., Akutse, K. S., Siddiqui, J. A., & Xu, Y. (2021). Model Application of Entomopathogenic Fungi as Alternatives to Chemical Pesticides: Prospects, Challenges, and Insights for Next-Generation Sustainable Agriculture. Frontiers in Plant Science, 12, 741804. https://doi.org/10.3389/FPLS.2021.741804

Berg, G., & Raaijmakers, J. M. (2018). Saving seed microbiomes. The ISME Journal, 12(5), 1167–1170. https://doi.org/10.1038/S41396-017-0028-2

Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology, 68(1), 1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x

Bergna, A., Cernava, T., Rändler, M., Grosch, R., Zachow, C., & Berg, G. (2018). Tomato seeds preferably transmit plant beneficial endophytes. Phytobiomes Journal, 2(4), 183–193. https://doi.org/10.1094/PBIOMES-06-18-0029-R

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., & Gregory Caporaso, J. (2018). Optimizing taxonomic classification of

marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome, 6(1), 1–17. https://doi.org/10.1186/S40168-018-0470-Z

Bonfante, P., & Genre, A. (2010). Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nature Communications, 1(1), 48. https://doi.org/10.1038/ncomms1046

Brader, G., Compant, S., Vescio, K., Mitter, B., Trognitz, F., Ma, L. J., & Sessitsch, A. (2017). Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. Annual Review of Phytopathology, 55(Volume 55, 2017), 61–83. https://doi.org/10.1146/ANNUREV-PHYTO-080516-035641

Brown, S. P., & Mandel, J. R. (2024). Rapid restructuring of rhizosphere and endospheric fungal communities with drought in multiple lines of domesticated sunflower. Phytobiomes Journal, 8(2), 179–191. https://doi.org/10.1094/PBIOMES-06-23-0049-R

Buchholz, F., Antonielli, L., Kostić, T., Sessitsch, A., & Mitter, B. (2019). The bacterial community in potato is recruited from soil and partly inherited across generations. PLOS ONE, 14(11), e0223691. https://doi.org/10.1371/JOURNAL.PONE.0223691

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F. O., Amann, R., Eickhorst, T., & Schulze-Lefert, P. (2012). Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature, 488(7409), 91–95. https://doi.org/10.1038/nature11336

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. The ISME Journal, 11(12), 2639–2643. https://doi.org/10.1038/ismej.2017.119

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal, 6(8), 1621–1624. https://doi.org/10.1038/ISMEJ.2012.8

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America, 108(SUPPL. 1), 4516–4522. https://doi.org/10.1073/PNAS.1000080107

Carvalho, T. L. G., Balsemão-Pires, E., Saraiva, R. M., Ferreira, P. C. G., & Hemerly, A. S. (2014). Nitrogen signalling in plant interactions with associative and endophytic diazotrophic bacteria. Journal of Experimental Botany, 65(19), 5631–5642. https://doi.org/10.1093/JXB/ERU319

Chen, X., Krug, L., Yang, H., Li, H., Yang, M., Berg, G., & Cernava, T. (2020). Nicotiana tabacum seed endophytic communities share a common core structure and genotype-specific signatures in diverging cultivars. Computational and Structural Biotechnology Journal, 18, 287–295. https://doi.org/10.1016/j.csbj.2020.01.004

Coleman-Derr, D., & Tringe, S. G. (2014). Building the crops of tomorrow: Advantages of symbiont-based approaches to improving abiotic stress tolerance. Frontiers in Microbiology, 5(JUN), 96317. https://doi.org/10.3389/FMICB.2014.00283

Collinge, D. B., Jensen, B., & Jørgensen, H. J. (2022). Fungal endophytes in plants and their relationship to plant disease. Current Opinion in Microbiology, 69, 102177. https://doi.org/10.1016/J.MIB.2022.102177

Collinge, D. B., Jørgensen, H. J. L., Latz, M. A. C., Manzotti, A., Ntana, F., Rojas, E. C., & Jensen, B. (2019). Searching for Novel Fungal Biological Control Agents for Plant Disease Control Among Endophytes. Endophytes for a Growing World, 25–51. https://doi.org/10.1017/9781108607667.003

Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clément, C., & Barka, E. A. (2005). Endophytic colonization of Vitis vinifera L. by plant growth-promoting bacterium Burkholderia sp. strain PsJN. Applied and Environmental Microbiology, 71(4), 1685–1693. https://doi.org/10.1128/AEM.71.4.1685-1693.2005

Compant, S., Sessitsch, A., & Mathieu, F. (2012). The 125th anniversary of the first postulation of the soil origin of endophytic bacteria - a tribute to M.L.V. Galippe. Plant and Soil, 356(1–2), 299–301. https://doi.org/10.1007/S11104-012-1204-9

Cooke, L. R., Schepers, H. T. A. M., Hermansen, A., Bain, R. A., Bradshaw, N. J., Ritchie, F., Shaw, D. S., Evenhuis, A., Kessel, G. J. T., Wander, J. G. N., Andersson, B., Hansen, J. G., Hannukkala, A., Nærstad, R., & Nielsen, B. J. (2011). Epidemiology and integrated control of potato late blight in Europe. Potato Research, 54(2), 183–222. https://doi.org/10.1007/s11540-011-9187-0

Corona Ramirez, A., Bregnard, D., Junier, T., Cailleau, G., Dorador, C., Bindschedler, S., & Junier, P. (2023). Assessment of fungal spores and spore-like diversity in environmental samples by targeted lysis. BMC Microbiology, 23(1), 68. https://doi.org/10.1186/s12866-023-02809-w

Davis, N. M., Proctor, Di. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome, 6(1), 1–14. https://doi.org/10.1186/S40168-018-0605-2

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. Journal of Vegetation Science, 14(6), 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

Dong, C.-J., Wang, L.-L., Li, Q., & Shang, Q.-M. (2019). Bacterial communities in the rhizosphere, phyllosphere and endosphere of tomato plants. PLOS ONE, 14(11), e0223847. https://doi.org/10.1371/journal.pone.0223847

Egamberdieva, D., Wirth, S. J., Shurigin, V. V, Hashem, A., & Abd_Allah, E. F. (2017). Endophytic bacteria improve plant growth, symbiotic performance of chickpea (Cicer arietinum L.) and induce suppression of root rot caused by Fusarium solani under salt stress. Frontiers in Microbiology, 8, 1887. https://doi.org/10.3389/fmicb.2017.01887

Escobar Rodríguez, C., Antonielli, L., Mitter, B., & Sessitsch, A. (2024). Assembly of endophytic communities of Setaria viridis plants when grown in different soils and derived from different seeds. Phytobiomes Journal, 8(1), 34–45. https://doi.org/10.1094/PBIOMES-08-22-0054-MF

Fierer, N., & Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences, 103(3), 626–631. https://doi.org/10.1073/pnas.0507535103

Garcia, C., Pauli, D., Plecki, C., Alnasser, H., Rozzi, B., Calleja, S., & Arnold, A. E. (2024). The Root Endophytic Microbiome Shifts Under Drought in High-Performing Sorghum. Phytobiomes Journal, 8(3), 282–296. https://doi.org/10.1094/PBIOMES-09-23-0095-R

Girvan, M. S., Bullimore, J., Pretty, J. N., Osborn, A. M., & Ball, A. S. (2003). Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Applied and Environmental Microbiology, 69(3), 1800–1809. https://doi.org/10.1128/AEM.69.3.1800-1809.2003

González Ortega-Villaizán, A., King, E., Patel, M. K., Pérez-Alonso, M. M., Scholz, S. S., Sakakibara, H., Kiba, T., Kojima, M., Takebayashi, Y., Ramos, P., Morales-Quintana, L., Breitenbach, S., Smolko, A., Salopek-Sondi, B., Bauer, N., Ludwig-Müller, J., Krapp, A., Oelmüller, R., Vicente-Carbajosa, J., & Pollmann, S. (2024). The endophytic fungus Serendipita indica affects auxin distribution in Arabidopsis thaliana roots through alteration of auxin transport and conjugation to promote plant growth. Plant, Cell & Environment, 47(10). https://doi.org/10.1111/PCE.14989

Götz, M., Nirenberg, H., Krause, S., Wolters, H., Draeger, S., Buchner, A., Lottmann, J., Berg, G., & Smalla, K. (2006). Fungal endophytes in potato roots studied by traditional isolation and cultivation-independent DNA-based methods. FEMS Microbiology Ecology, 58(3), 404–413. https://doi.org/10.1111/j.1574-6941.2006.00169.x

Grabka, R., D'entremont, T. W., Adams, S. J., Walker, A. K., Tanney, J. B., Abbasi, P. A., & Ali, S. (2022). Fungal Endophytes and Their Role in Agricultural Plant Protection against Pests and Pathogens. Plants 2022, Vol. 11, Page 384, 11(3), 384. https://doi.org/10.3390/PLANTS11030384

Gweon, H. S., Oliver, A., Taylor, J., Booth, T., Gibbs, M., Read, D. S., Griffiths, R. I., & Schonrogge, K. (2015). PIPITS: an automated pipeline for analyses of fungal internal transcribed spacer sequences from the Illumina sequencing platform. Methods in Ecology and Evolution, 6(8), 973–980. https://doi.org/10.1111/2041-210X.12399

Hannula, S. E., Heinen, R., Huberty, M., Steinauer, K., De Long, J. R., Jongen, R., & Bezemer, T. M. (2021). Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. Nature Communications, 12(1), 5686. https://doi.org/10.1038/s41467-021-25971-z

Hardoim, P. R., van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., & Sessitsch, A. (2015). The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. Microbiology and Molecular Biology Reviews, 79(3), 293–320. https://doi.org/10.1128/MMBR.00050-14

Hardoim, P. R., van Overbeek, L. S., & Elsas, J. D. van. (2008). Properties of bacterial endophytes and their proposed role in plant growth. Trends in Microbiology, 16(10), 463–471. https://doi.org/10.1016/j.tim.2008.07.008

Hunziker, L., Bönisch, D., Groenhagen, U., Bailly, A., Schulz, S., & Weisskopf, L. (2015). Pseudomonas strains naturally associated with potato plants produce volatiles with high potential for inhibition of Phytophthora infestans. Applied and Environmental Microbiology, 81(3), 821–830. https://doi.org/10.1128/AEM.02999-14

İnceoğlu, Ö., Al-Soud, W. A., Salles, J. F., Semenov, A. V, & Elsas, J. D. van. (2011). Comparative analysis of bacterial communities in a potato field as determined by pyrosequencing. PLOS ONE, 6(8), e23321. https://doi.org/10.1371/journal.pone.0023321

Kinnunen-Grubb, M., Sapkota, R., Vignola, M., Nunes, I. M., & Nicolaisen, M. (2020). Breeding selection imposed a differential selective pressure on the wheat root-associated microbiome. FEMS Microbiology Ecology, 96(11), fiaa196. https://doi.org/10.1093/femsec/fiaa196

Koskimäki, J. J., Pirttilä, A. M., Ihantola, E.-L., Halonen, O., & Frank, A. C. (2015). The intracellular scots pine shoot symbiont Methylobacterium extorquens DSM13060 aggregates around the host nucleus and encodes eukaryote-like proteins. MBio, 6(2), 1110–1128. https://doi.org/10.1128/mbio.00039-15

Lahrmann, U., Ding, Y., Banhara, A., Rath, M., Hajirezaei, M. R., Döhlemann, S., von Wirén, N., Parniske, M., & Zuccaro, A. (2013). Host-related metabolic cues affect colonization strategies of a root endophyte. Proceedings of the National Academy of Sciences, 110(34), 13965–13970. https://doi.org/10.1073/pnas.1301653110

Lahti, L., & Shetty, S. (2017). microbiome R package. Bioconductor, 3.19. https://doi.org/10.18129/B9.bioc.microbiome

Latz, M. A. C., Kerrn, M. H., Sørensen, H., Collinge, D. B., Jensen, B., Brown, J. K. M., Madsen, A. M., & Jørgensen, H. J. L. (2021). Succession of the fungal endophytic microbiome of wheat is dependent on tissue-specific interactions between host genotype and environment. Science of The Total Environment, 759, e143804. https://doi.org/10.1016/j.scitotenv.2020.143804

Lau, J. A., & Lennon, J. T. (2012). Rapid responses of soil microorganisms improve plant fitness in novel environments. Proceedings of the National Academy of Sciences of the United States of America, 109(35), 14058–14062. https://doi.org/10.1073/pnas.1202319109

Lau, J. A., Lennon, J. T., Kellogg, W. K., & Lau, J. (2011). Evolutionary ecology of plant–microbe interactions: soil microbial structure alters selection on plant traits. New Phytologist, 192(1), 215–224. https://doi.org/10.1111/J.1469-8137.2011.03790.X

Leff, J. W., Lynch, R. C., Kane, N. C., & Fierer, N. (2017). Plant domestication and the assembly of bacterial and fungal communities associated with strains of the common sunflower, Helianthus annuus. New Phytologist, 214(1), 412–423. https://doi.org/10.1111/nph.14323

Liu, J., Abdelfattah, A., Wasserman, B., Wisniewski, M., Droby, S., Fazio, G., Mazzola, M., & Wu, X. (2022). Contrasting effects of genotype and root size on the fungal and bacterial communities associated with apple rootstocks. Horticulture Research, 9, uhab013. https://doi.org/10.1093/hr/uhab013

Lodewyckx, C., Vangronsveld, J., Porteous, F., Moore, E. R. B., Taghavi, S., Mezgeay, M., & Van der Lelie, D. (2002). Endophytic bacteria and their potential applications. Critical Reviews in Plant Sciences, 21(6), 583–606. https://doi.org/10.1080/0735-260291044377

Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., Rio, T. G. Del, Edgar, R. C., Eickhorst, T., Ley, R. E., Hugenholtz, P., Tringe, S. G., & Dangl, J. L. (2012). Defining the core Arabidopsis thaliana root microbiome. Nature 2012 488:7409, 488(7409), 86–90. https://doi.org/10.1038/nature11237

Manzotti, A., Bergna, A., Burow, M., Jørgensen, H. J. L., Cernava, T., Berg, G., Collinge, D. B., & Jensen, B. (2020). Insights into the community structure and lifestyle of the fungal root endophytes of tomato by combining amplicon sequencing and isolation approaches with phytohormone profiling. FEMS Microbiology Ecology, 96(5), fiaa052. https://doi.org/10.1093/femsec/fiaa052

Martins, B. R., Radl, V., Treder, K., Michałowska, D., Pritsch, K., & Schloter, M. (2024). The rhizosphere microbiome of 51 potato cultivars with diverse plant growth characteristics. FEMS Microbiology Ecology, 100(8). https://doi.org/10.1093

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLOS ONE, 8(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Mendes, L. W., Raaijmakers, J. M., De Hollander, M., Mendes, R., & Tsai, S. M. (2018). Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. The ISME Journal, 12(1), 212–224. https://doi.org/10.1038/ISMEJ.2017.158

Mitter, B., Pfaffenbichler, N., Flavell, R., Compant, S., Antonielli, L., Petric, A., Berninger, T., Naveed, M., Sheibani-Tezerji, R., Maltzahn, G. von, & Sessitsch, A. (2017). A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. Frontiers in Microbiology, 8(11), e230405. https://doi.org/10.3389/FMICB.2017.00011

Mousa, W. K., Shearer, C., Limay-Rios, V., Ettinger, C. L., Eisen, J. A., & Raizada, M. N. (2016). Root-hair endophyte stacking in finger millet creates a physicochemical barrier to trap the fungal pathogen Fusarium graminearum. Nature Microbiology, 1(12), 1–12. https://doi.org/10.1038/nmicrobiol.2016.167

Niu, Z., Zheng, L., Yang, P., Wang, J., Tian, M., Pan, Y., Zhao, D., Yang, Z., & Zhu, J. (2022). Detection of Alternaria solani with high accuracy and sensitivity during the latent period of potato early blight. Frontiers in Microbiology, 13, e1016996. https://doi.org/10.3389/FMICB.2022.1016996

Pageni, B. B., Lupwayi, N. Z., Larney, F. J., Kawchuk, L. M., & Gan, Y. (2013). Populations, diversity and identities of bacterial endophytes in potato (Solanum tuberosum L.) cropping systems. Canadian Journal of Plant Science, 93(6), 1125–1142. https://doi.org/10.4141/cjps2013-166

Panaccione, D. G., Beaulieu, W. T., & Cook, D. (2014). Bioactive alkaloids in vertically transmitted fungal endophytes. Functional Ecology, 28(2), 299–314. https://doi.org/10.1111/1365-2435.12076

Pankievicz, V. C. S., Do Amaral, F. P., Santos, K. F. D. N., Agtuca, B., Xu, Y., Schueller, M. J., Arisi, A. C. M., Steffens, M. B. R., De Souza, E. M., Pedrosa, F. O., Stacey, G., & Ferrieri, R. A. (2015). Robust biological nitrogen fixation in a model grass–bacterial association. The Plant Journal, 81(6), 907–919. https://doi.org/10.1111/TPJ.12777

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology, 18(5), 1403–1414. https://doi.org/10.1111/1462-2920.13023

Pérez-Jaramillo, J. E., Mendes, R., & Raaijmakers, J. M. (2016). Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Molecular Biology, 90(6), 635–644. https://doi.org/10.1007/s11103-015-0337-7

Ptak, A., Morańska, E., Warchoł, M., Gurgul, A., Skrzypek, E., Dziurka, M., Laurain-Mattar, D., Spina, R., Jaglarz, A., & Simlat, M. (2022). Endophytic bacteria from in vitro culture of Leucojum aestivum L. a new source of galanthamine and elicitor of alkaloid biosynthesis. Scientific Reports, 12(1), e13700. https://doi.org/10.1038/s41598-022-17992-5

Quiza, L., Tremblay, J., Pagé, A. P., Greer, C. W., Pozniak, C. J., Li, R., Haug, B., Hemmingsen, S. M., St-Arnaud, M., & Yergeau, E. (2023). The effect of wheat genotype on the microbiome is more evident in roots and varies through time. ISME Communications, 3(1), 1–10. https://doi.org/10.1038/s43705-023-00238-4

Ravel, C., Courty, C., Coudret, A., & Charmet, G. (1997). Beneficial effects of Neotyphodium lolii on the growth and the water status in perennial ryegrass cultivated under nitrogen deficiency or drought stress. Agronomie, 17(3), 173–181. https://doi.org/10.1051/AGRO:19970304

R Core Team. (2021). R: a language and environment for statistical computing (R foundation for statistical computing, Vienna).

Reiter, B., Pfeifer, U., Schwab, H., & Sessitsch, A. (2002). Response of endophytic bacterial communities in potato plants to infection with Erwinia carotovora subsp. atroseptica. Applied and Environmental Microbiology, 68(5), 2261–2268. https://doi.org/10.1128/AEM.68.5.2261-2268.2002

Rodríguez, C. E., Antonielli, L., Mitter, B., Trognitz, F., & Sessitsch, A. (2020). Heritability and functional importance of the Setaria viridis bacterial seed microbiome. Phytobiomes Journal, 4(1), 40–52. https://doi.org/10.1094/PBIOMES-04-19-0023-R

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R., & Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal, 4(10), 1340–1351. https://doi.org/10.1038/ISMEJ.2010.58

Ryan, R. P., Germaine, K., Franks, A., Ryan, D. J., & Dowling, D. N. (2008). Bacterial endophytes: recent developments and applications. FEMS Microbiology Letters, 278(1), 1–9. https://doi.org/10.1111/j.1574-6968.2007.00918.x

Sessitsch, A., Reiter, B., & Berg, G. (2004). Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities. Canadian Journal of Microbiology, 50(4), 239–249. https://doi.org/10.1139/w03-118

Shikano, I., Rosa, C., Tan, C. W., & Felton, G. W. (2017). Tritrophic Interactions: Microbe-Mediated Plant Effects on Insect Herbivores. Annual Review of Phytopathology, 55(Volume 55, 2017), 313–331. https://doi.org/https://doi.org/10.1146/annurev-phyto-080516-035319

Shi, W., Su, G., Li, M., Wang, B., Lin, R., Yang, Y., Wei, T., Zhou, B., & Gao, Z. (2021). Distribution of bacterial endophytes in the non-lesion tissues of potato and

their response to potato common scab. Frontiers in Microbiology, 12. https://doi.org/10.3389/fmicb.2021.616013

Simonin, M., Dasilva, C., Terzi, V., Ngonkeu, E. L. M., Diouf, D., Kane, A., Béna, G., & Moulin, L. (2020). Influence of plant genotype and soil on the wheat rhizosphere microbiome: evidences for a core microbiome across eight African and European soils. FEMS Microbiology Ecology, 96(6), fiaa067. https://doi.org/10.1093/femsec/fiaa067

Smith, M. E., Henkel, T. W., & Rollins, J. A. (2015). How many fungi make sclerotia? Fungal Ecology, 13, 211–220. https://doi.org/10.1016/j.funeco.2014.08.010

Song, Y., Spooren, J., Jongekrijg, C. D., Manders, E. H. H., de Jonge, R., Pieterse, C. M. J., Bakker, P. A. H. M., & Berendsen, R. L. (2024). Seed tuber imprinting shapes the next-generation potato microbiome. Environmental Microbiome, 19(1), 1–16. https://doi.org/10.1186/s40793-024-00553-w

Sun, Y., Cheng, Z., & Glick, B. R. (2009). The presence of a 1-aminocyclopropane-1-carboxylate (ACC) deaminase deletion mutation alters the physiology of the endophytic plant growth-promoting bacterium Burkholderia phytofirmans PsJN. FEMS Microbiology Letters, 296(1), 131–136. https://doi.org/10.1111/J.1574-6968.2009.01625.X

Ulrich, K., Stauber, T., & Ewald, D. (2008). Paenibacillus—a predominant endophytic bacterium colonising tissue cultures of woody plants. Plant Cell, Tissue and Organ Culture, 93(3), 347–351. https://doi.org/10.1007/s11240-008-9367-z

Ulrich, K., Ulrich, A., & Ewald, D. (2008). Diversity of endophytic bacterial communities in poplar grown under field conditions. FEMS Microbiology Ecology, 63(2), 169–180. https://doi.org/10.1111/j.1574-6941.2007.00419.x

United Nations. (2015). Transforming our world: the 2030 Agenda for Sustainable Development. General Assembly Resolution A/RES/70/1.

Vu, T., Hauschild, R., & Sikora, R. A. (2006). Fusarium oxysporum endophytes induced systemic resistance against Radopholus similis on banana. Nematology, 8(6), 847–852. https://doi.org/10.1163/156854106779799259

Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Hückelhoven, R., Neumann, C., Von Wettstein, D., Franken, P., & Kogel, K. H. (2005). The endophytic fungus Piriformospora indica reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proceedings of the National Academy of Sciences of the United States of America, 102(38), 13386–13391. https://doi.org/10.1073/PNAS.0504423102

Weinert, N., Meincke, R., Gottwald, C., Heuer, H., Schloter, M., Berg, G., & Smalla, K. (2010). Bacterial diversity on the surface of potato tubers in soil and the influence of the plant genotype. FEMS Microbiology Ecology, 74(1), 114–123. https://doi.org/10.1111/j.1574-6941.2010.00936.x

Wheeler, D. L., Dung, J. K. S., & Johnson, D. A. (2019). From pathogen to endophyte: an endophytic population of Verticillium dahliae evolved from a sympatric pathogenic population. New Phytologist, 222(1), 497–510. https://doi.org/10.1111/nph.15567

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

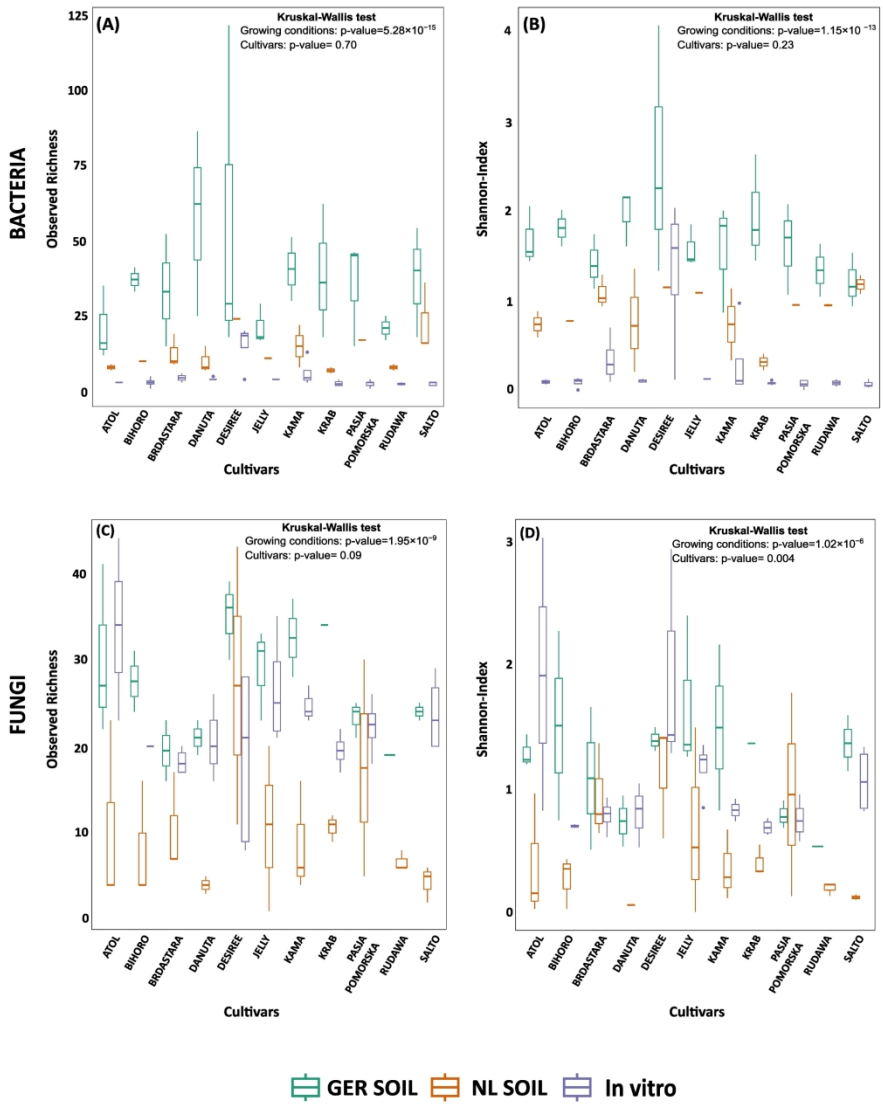
Wickham, H. (2016). ggplot2: elegant graphics for data analysis. In Springer Cham (2nd ed.). Springer Cham. https://doi.org/10.1007/978-3-319-24277-4

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., & Glöckner, F. O. (2014). The SILVA and “All-species Living Tree Project (LTP)” taxonomic frameworks. Nucleic Acids Research, 42(D1), D643-648. https://doi.org/10.1093/nar/gkt1209

Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N. A., Mills, D. A., Martin, G., Taghavi, S., van der Lelie, D., & Gilbert, J. A. (2015). The soil microbiome influences grapevine-associated microbiota. MBio, 6(2). https://doi.org/10.1128/MBIO.02527-14

Zarzyńska, K., Boguszewska-Mańkowska, D., & Nosalewicz, A. (2017). Differences in size and architecture of the potato cultivars root system and their tolerance to drought stress. Plant, Soil and Environment, 63(4), 159–164. https://doi.org/10.17221/4/2017-PSE

Zhao, T., Vink, S. N., Jia, X., Erban, A., Schaarschmidt, S., Kopka, J., Zuther, E., Treder, K., Michałowska, D., Guyoneaud, R., Elzenga, J. T. M., Attard, E., & Salles, J. F. (2024). Unveiling the potato cultivars with microbiome interactive traits for sustainable agricultural production. BioRxiv, 2024.08.21.609084. https://doi.org/10.1101/2024.08.21.609084



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) and 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 observed richness (A and C) and Shannon index (B and D) of bacterial and fungal endophytes present in 11 potato cultivars and was represented with boxplots. Boxplots display the medians, tops, and bottoms of the boxes represent 75th and 25th quartiles, and whiskers outside this range show variability outside the upper and lower quartiles. Small dots shows the outliers. Kruskal wallis test ($P < .05$) followed by Dunn's post-hoc tests with Benjamini–Hochberg (BH) correction was applied to calculate significant differences between different growing locations.

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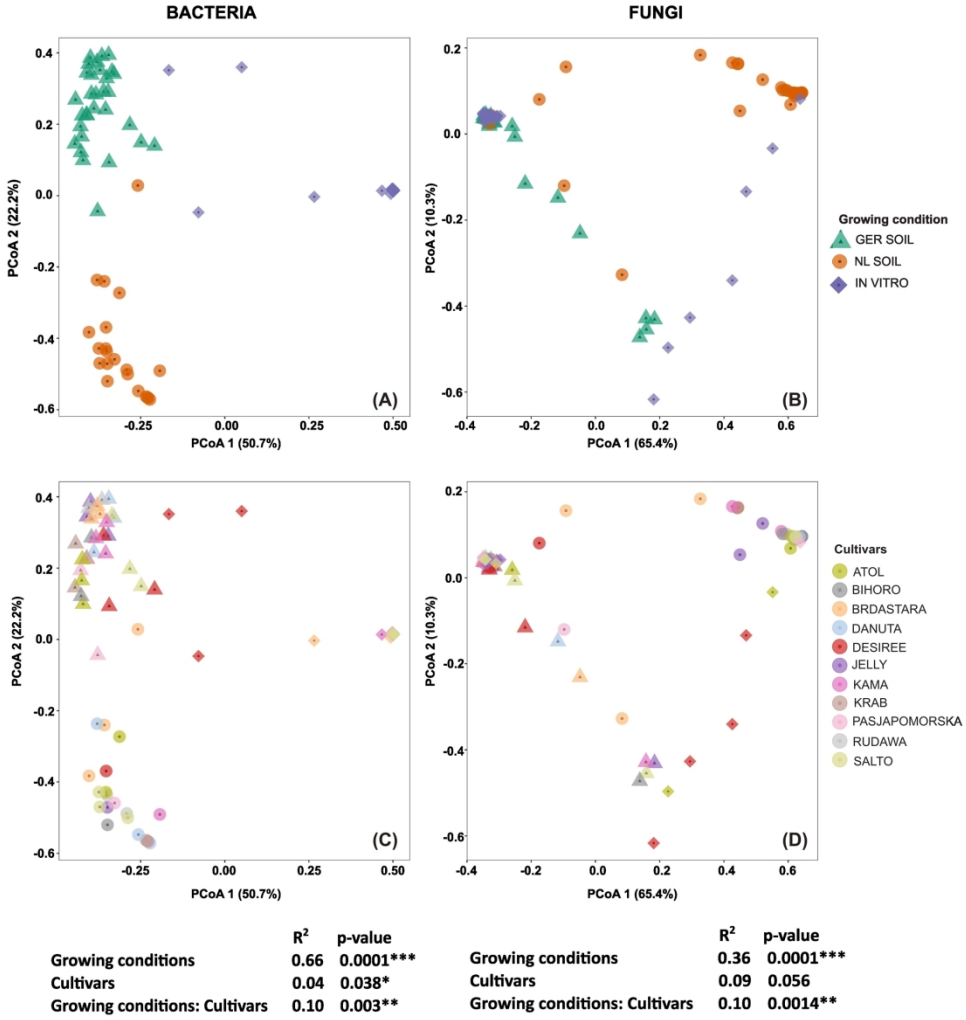

	R^2	p-value
Growing conditions	0.66	0.0001***
Cultivars	0.04	0.038*
Growing conditions: Cultivars	0.10	0.003**

	R^2	p-value
Growing conditions	0.36	0.0001***
Cultivars	0.09	0.056
Growing conditions: Cultivars	0.10	0.0014**

Principal coordinates analysis (PCoA) plots based on Bray-Curtis distance matrices showing the structure of the root-associated bacterial (A and C) and fungal (B and D) endophytes between 11 potato cultivars across 3 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. Permanova values are shown in the lower panel. R2 shows the variance explained by each factor and p-value indicates which components significantly affect the structure of bacterial and fungal enophytes. *P < .05, **P < .01, and ***P < .001. Analysis was computed with 999 permutations. Table 1 shows Permanova values indicating which components significantly affect the distribution.

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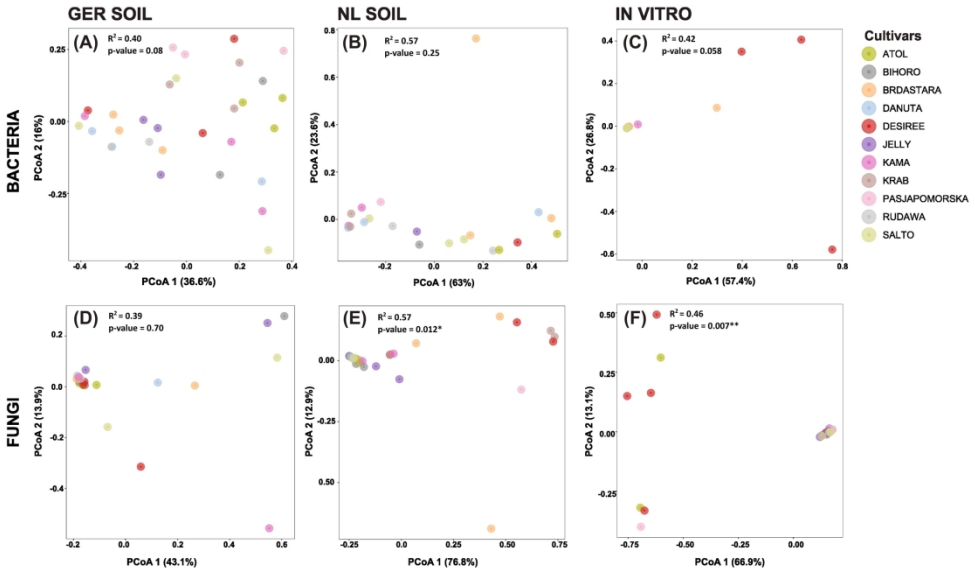
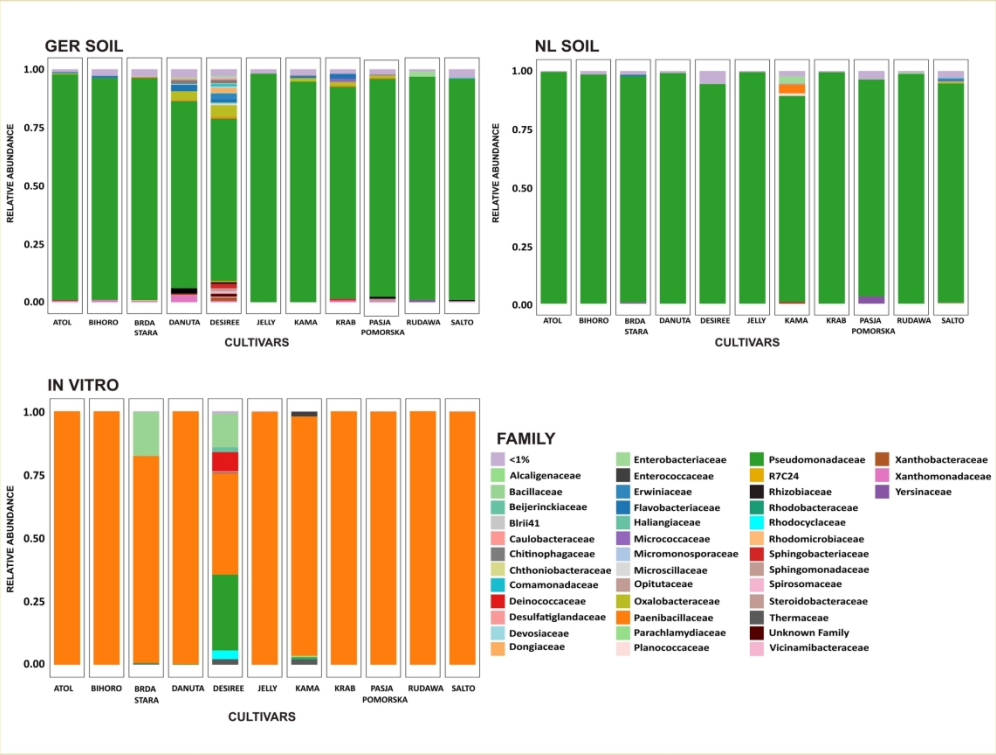


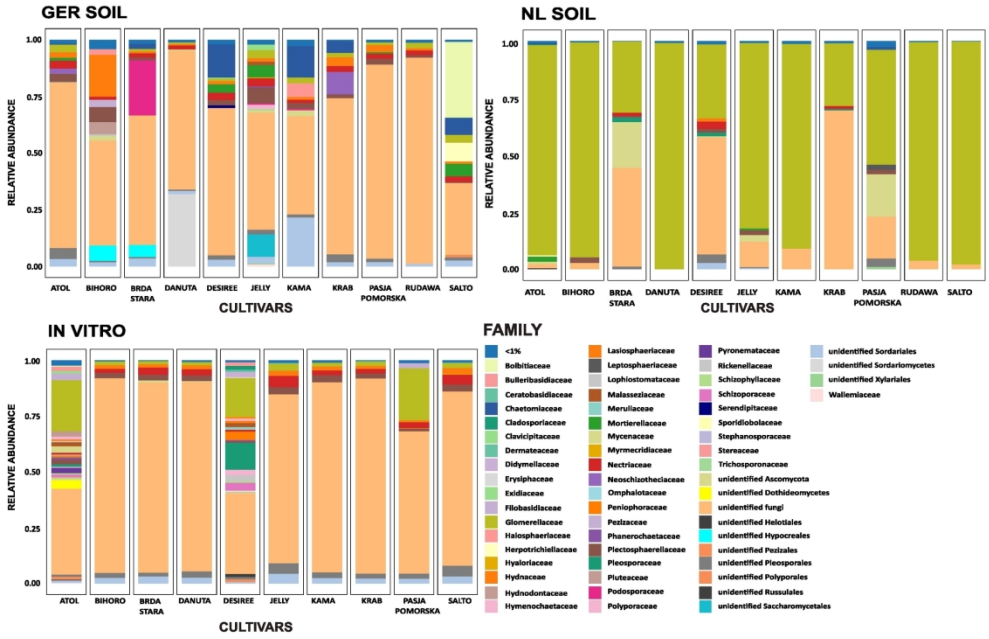
Principal coordinates analysis (PCoA) plots based on Bray-Curtis distance matrices show the structure of the root-associated bacterial and fungal endophytes between 11 potato cultivars in the three growing conditions separately (GER soil representing Germany (n=3 replicates per cultivar), NL soil representing the Netherlands (n=3 replicates per cultivar) and in vitro conditions (n=5 replicates per cultivar). Permanova values are shown in the lower panel. R2 shows the variation in bacterial and fungal endophytic community structure explained by cultivars in each location. *P < .05, **P < .01, and ***P < .001. Analysis was computed with 999 permutations.Table 1 shows Permanova values indicating which components significantly affect the distribution.

209x139mm (300 x 300 DPI)



Composition of the root-associated bacterial endophytic community of 11 potato cultivars in samples grown in 3 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).

209x158mm (300 x 300 DPI)



Composition of the root-associated fungal endophytic community of potato plants in samples grown in 3 different growing conditions. GER soil represents Germany, NL soil represents 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.

209x159mm (300 x 300 DPI)

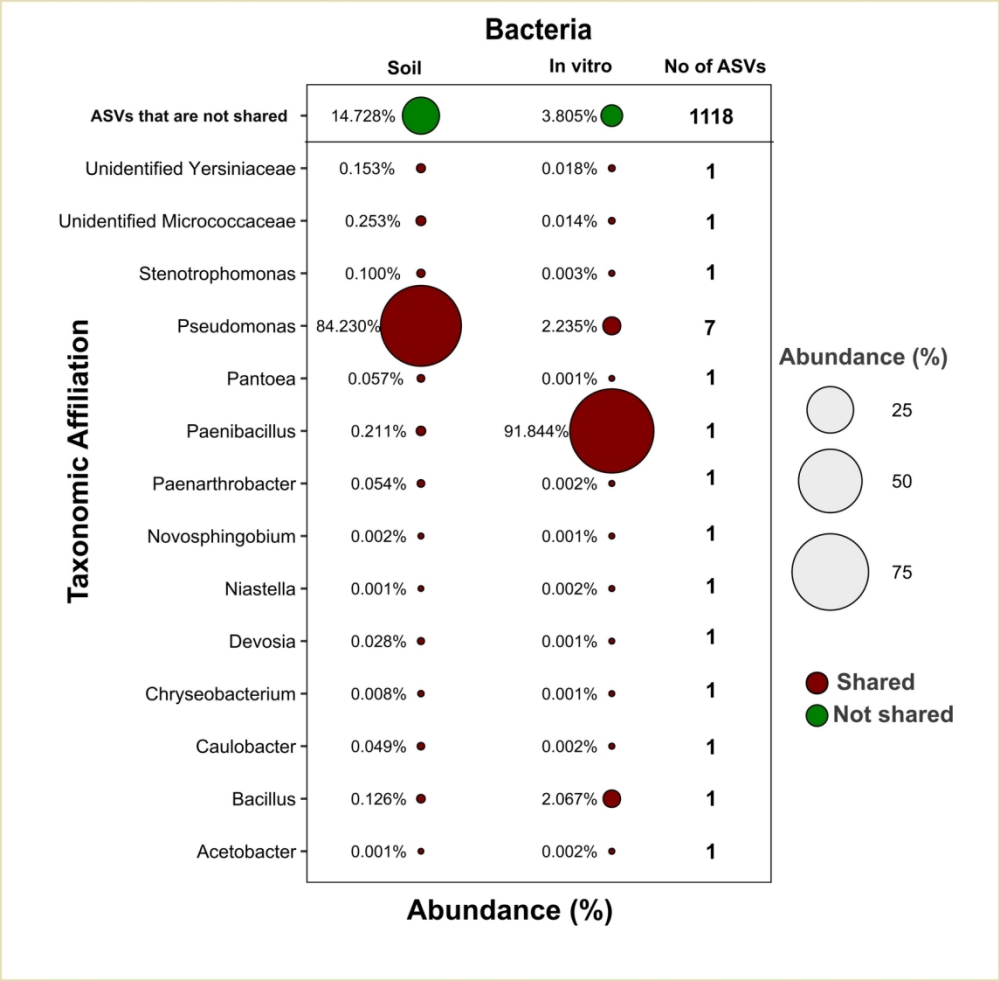


Diagram showing the number and the abundance percentage of shared bacterial taxa between samples grown in soil (combining GER and NL soils and cultivars belonging to these soils) and in vitro samples. The bubble's size reflects each community's relative abundance, providing a visual comparison of their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

149x146mm (300 x 300 DPI)

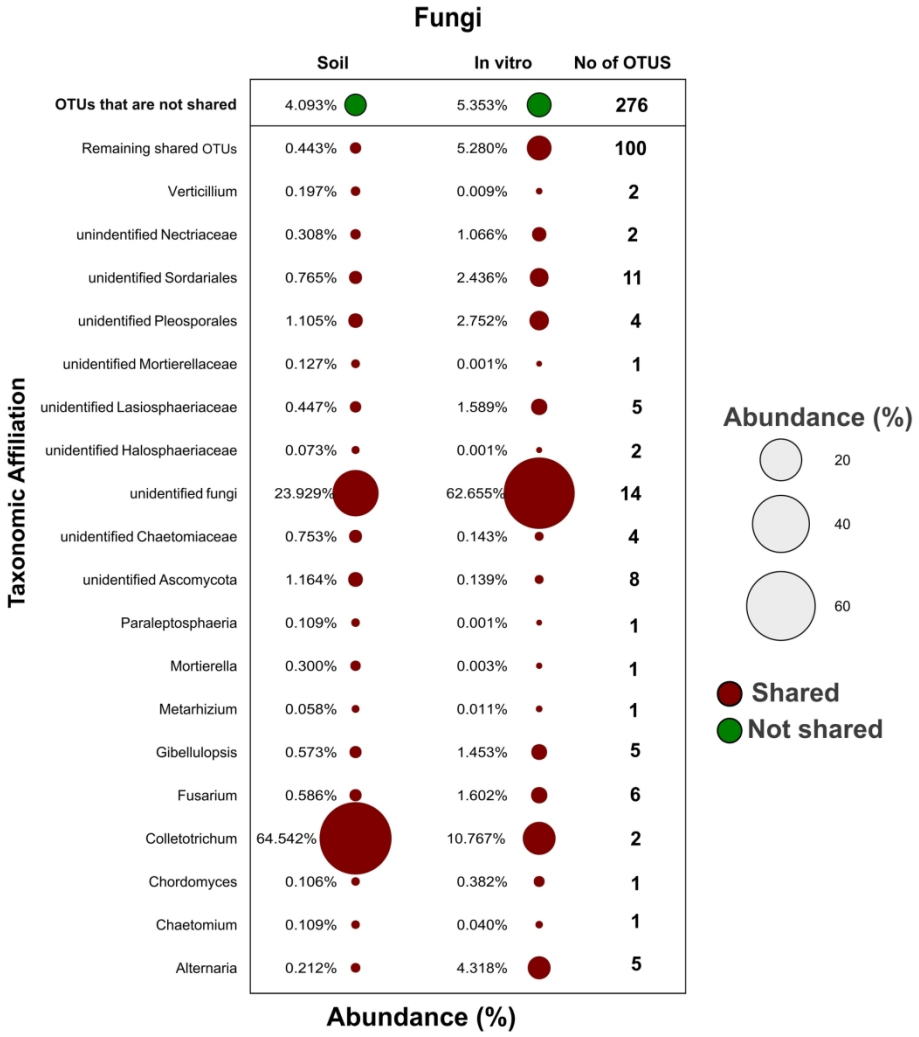


Diagram showing the number and the abundance percentage of shared fungal merged OTUs between samples grown in soil (combining GER and NL soils and cultivars belonging to these soils) and in vitro samples. The bubble's size reflects each community's relative abundance, providing a visual comparison of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

184x209mm (300 x 300 DPI)

	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 conditions)	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 conditions)	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			

Taxonomic Affiliation	Abundance	Prevalence
Fungi		
Sordariales_sp_SH0962352.09FU	0.94	61.98
Lasiosphaeriaceae_sp_SH0932574.09FU	0.73	59.5
Unidentified Sordariales	0.09	52.07
Unidentified Fusarium	0.74	60.33
Unindentified Nectriaceae	0.52	59.5
Unidentified Gibellulopsis	0.65	71.07
Chordomyces_antarcticus_SH1451459.09FU	0.18	58.68
Colletotrichum_coccodes_SH0899393.09FU	49.66	100
Pleosporales_sp_SH0997149.09FU	0.25	61.16
Unidentified Pleosporales	1.3	61.98
Unidentified fungi	1.59	57.85
Unidentified fungi	32.71	95.04
Bacteria		
Pseudomonas (f8aeec3c1ff0f4e7e1c829e4db0618d9)	10.56	50.89
Pseudomonas (9af3467db68cf6063627304cecd46a65)	14.43	54.46
Pseudomonas (6a42f6b31b1958a4fd7c015ad7affbf4)	28.43	71.43

Supplementary Tables

Supplementary Table S1 : 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.

	Factor	**PERMANOVA**			
		Df	**F value**	**R2**	**p-value**
Bacteria (Without in vitro grown samples)	Growing conditions	1	40.62	0.42	**0.0001**
	Cultivars	10	1.32	0.14	0.13
	Growing conditions: Cultivars	10	1.25	0.13	0.16
	Residuals	30			
	Total	51			
Fungi (Without in vitro grown samples)					
	Growing conditions	1	40.42	0.38	**0.0001**
	Cultivars	10	1.88	0.18	**0.023**
	Growing conditions: Cultivars	10	1.23	0.12	0.24
	Residuals	33			
	Total	54			

Supplementary Table S2: Characteristics of 11 cultivars used in the study modified from (Martins et al., (2024, Tianci et al.,(unpublished greenhouse data) (Zhao et al., 2024)

Cultivar	Origin	Year	Earliness	Purpose	The color of the pulp	Skin color	Shape of tuber	Root Exudate metabolite level
ATOL	Poland	1978	Medium late	Table	Pale yellow	White	Round oval	Middle
BIHORO	Japan	1969	Very late	General purpose	Yellow	Yellow	Round oval	Middle
BRDA STARA	Poland	1964	Late	Table	Yellow	White	Round	Low
DANUTA	Germany	2009	Medium late	Starch	Yellow	Yellow	Round oval	High
DESIREE	The Netherlands	1962	Medium late	Table	Pale yellow	Red	Long oval	Middle
JELLY	Germany	2005	Medium late	Table	Yellow	White	Round oval	Middle
KAMA	Poland	1978	Medium late	Table	White	White	Oval	High
KRAB	Poland	1967	Medium late	General purpose	Pale yellow	White	Round	High
PASJA POMORSKA	Poland	2000	Medium late	Starch	Pale yellow	White	Round oval	Low
RUDAWA	Poland	2002	Late	Starch	Creamy	Yellow	Round oval	Low
SALTO	Poland	1998	Medium late	Table	Pale yellow	Yellow	Round oval	High

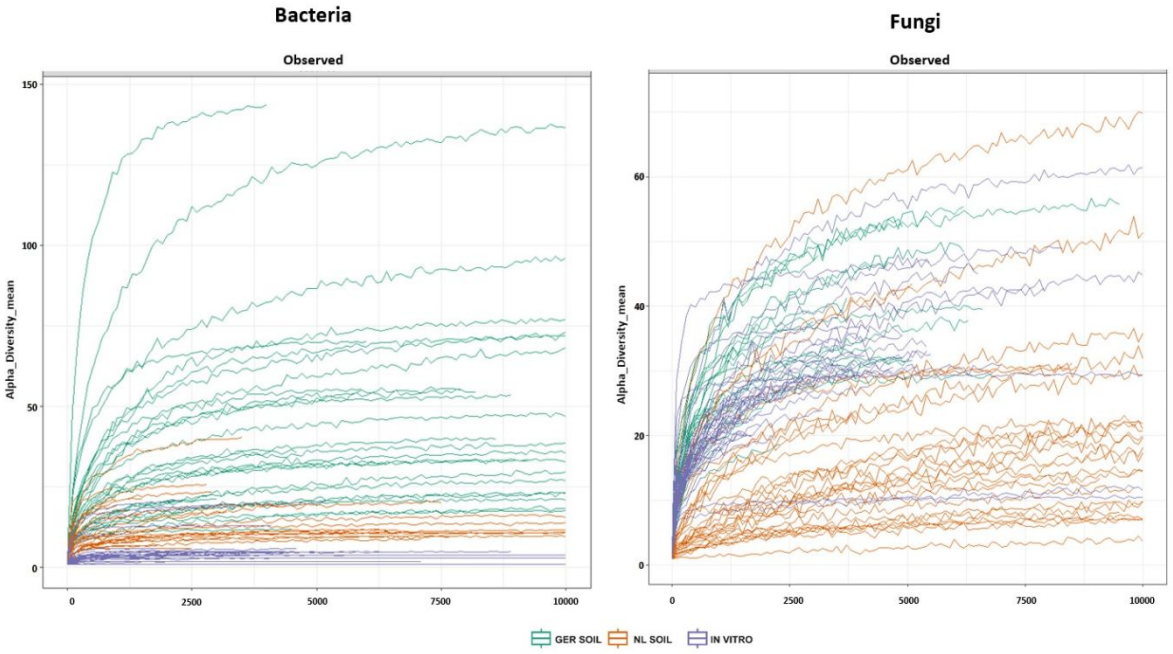
Supplementary Table S3: Kruskal-wallis test and post hoc (Dunn test) results showing the effect of growing conditions and cultivars on alpha diversity indices.

Kruskal-Wallis test					
	Factors	**Indices**	**Chi-squared (χ2)**	**df**	**p-value**
BACTERIA	Growing conditions	Observed richness	65.75	2	5.28E-15***
		Shannon	59.58	2	1.15E-13***
		Simpson	55.87	2	7.36E-13***
	Cultivars	Observed richness	7.26	10	0.7
		Shannon	12.9	10	0.23
		Simpson	14.52	10	0.15
FUNGI	Growing conditions	Observed richness	40.1	2	1.959E-09***
		Shannon	26.58	2	1.028E-06***
		Simpson	17.96	2	0.00013***
	Cultivars	Observed richness	15.99	10	0.09
		Shannon	25.6	10	0.004**
		Simpson	27.8	10	0.002**
Pairwise comparisons: Dunn test					
BACTERIA (Growing conditions)			Observed Richness		
	Comparisons	Chi-squared (χ2)	Z	P	P.adjusted (BH)
	Germany - Netherlands	65.75	3.07	0.0011	0.00105**
	Germany – In vitro	65.75	8.06	3.78E-16	1.13E-15***
	Netherlands -In vitro	65.75	3.93	4.19E-05	0.00006***
			Shannon-Diversity		
	Germany - Netherlands	59.58	3.09	0.0010	0.00099***
	Germany - In vitro	59.58	7.69	7.28E-15	2.182E-14***
	Netherlands -In vitro	59.58	3.59	0.00016	0.00025***
			Simpson		
	Germany - Netherlands	55.87	2.84	0.00227	0.0023**
	Germany - In vitro	55.87	7.43	5.37E-14	1.612E-13***
	Netherlands -In vitro	55.87	3.62	0.000145	0.00022***
FUNGI (Growing conditions)			Observed Richness		
	Germany - Netherlands	40.1	6.055	7.01E-10	2.10E-09***
	Germany - In vitro	40.1	2.037	2.08E-02	0.021*
	Netherlands -In vitro	40.1	-4.57	2.45E-06	3.6E-06***
			Shannon-Diversity		
	Germany - Netherlands	27.58	4.85	6.03E-07	0.000001***
	Germany - In vitro	27.58	1.21	1.12E-01	0.11242
	Netherlands -In vitro	27.58	-4.11	0.00002	0.000029***

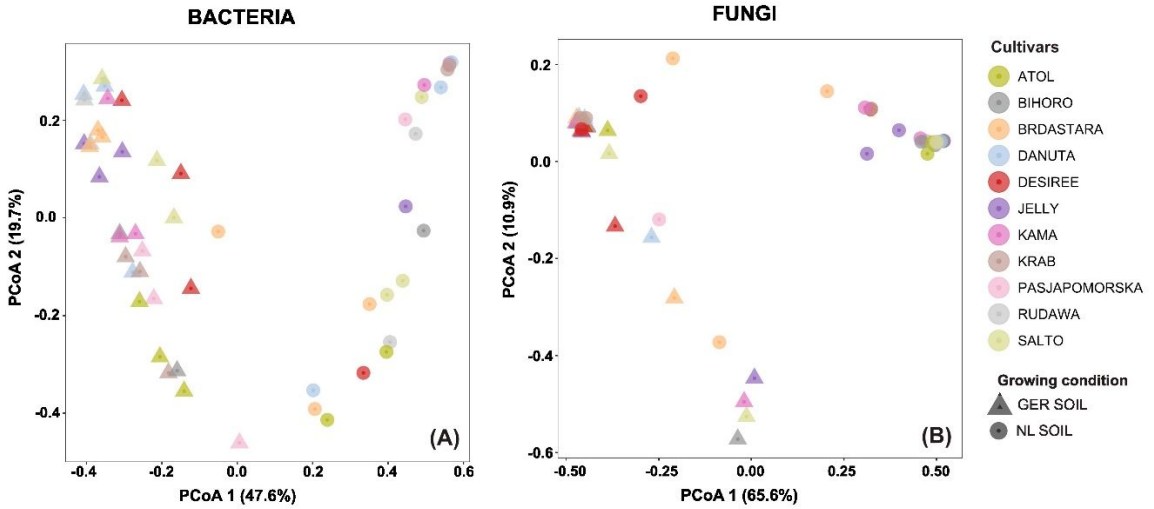
			Simpson		
	Germany - Netherlands	17.96	4.023	0.00003	0.00009***
	Germany - In vitro	17.96	1.27	0.102	0.1
	Netherlands -In vitro	17.96	-3.13	0.0009	0.0013**
FUNGI (Cultivars)			Shannon-Diversity		
	DESIREE - RUDAWA	25.6	3.745	9.02E-05	0.0049**
	DESIREE - BIHORO	25.6	-3.159	7.91E-04	0.011*
	DESIREE - KRAB	25.6	3.215	6.53E-04	0.012*
	DESIREE - DANUTA	25.6	-3.247	5.83E-04	0.02*
	DESIREE - PASJAPOMORSKA	25.6	2.553	5.34E-03	0.04*
	DESIREE - KAMA	25.6	2.570	5.09E-03	0.04*
	JELLY - RUDAWA	25.6	2.725	3.21E-03	0.03*
	ATOL - RUDAWA	25.6	2.543	5.49E-03	0.03*
			Simpson		
	DESIREE - RUDAWA	27.80	3.90	4.85E-05	0.003**
	DESIREE - BIHORO	27.80	-3.48	2.48E-04	0.007**
	DESIREE - KRAB	27.80	3.24	6.07E-04	0.008**
	DESIREE - DANUTA	27.80	-3.31	4.71E-04	0.009**
	DESIREE - PASJAPOMORSKA	27.80	2.90	1.85E-03	0.02*
	DESIREE - KAMA	27.80	2.74	3.05E-03	0.03*
	DESIREE - SALTO	27.80	2.52	5.83E-03	0.04*
	ATOL - RUDAWA	27.80	2.43	7.54E-03	0.04*
	JELLY - RUDAWA	27.80	2.74	3.06E-03	0.02*

Note: *P < .05, **P < .01, and ***P < .001. p-adjusted (BH) shows the adjustments were made using the Benjamini-Hochberg correction method to control for multiple comparisons.

Supplementary Figures



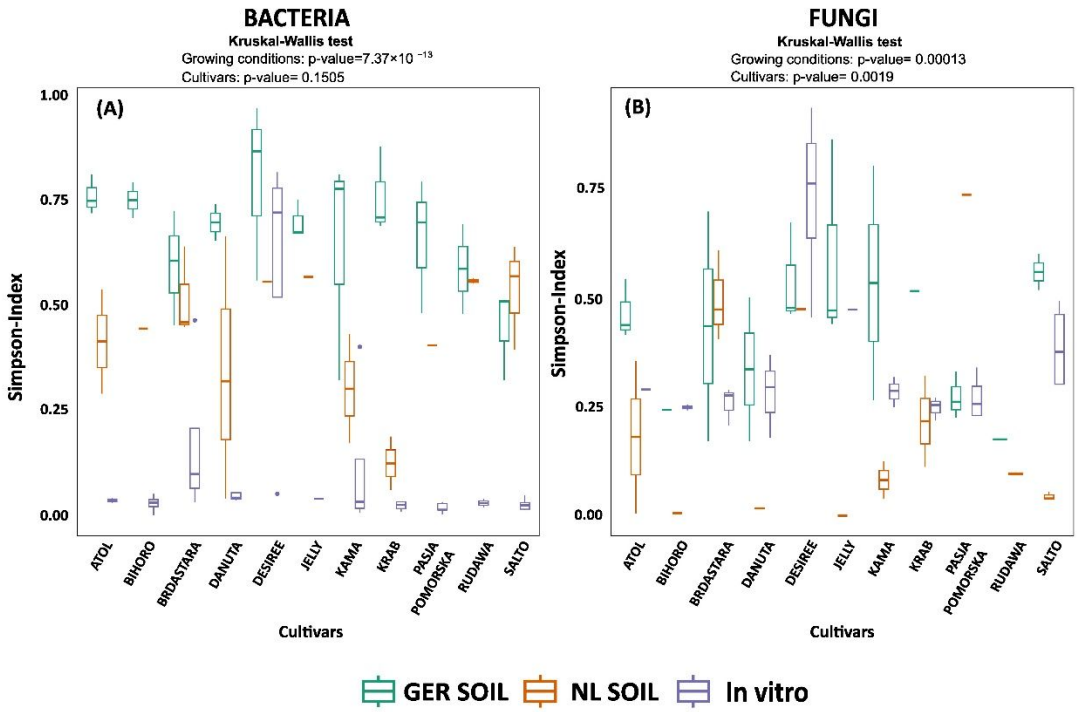
Supplementary Figure S1: Rarefaction curve showing the observed richness of bacteria and fungi in three growing conditions



	R^2	p-value
Growing conditions	0.42	0.0001***
Cultivars	0.14	0.13
Growing conditions: Cultivars	0.13	0.16

	R^2	p-value
Growing conditions	0.38	0.0001***
Cultivars	0.18	0.023*
Growing conditions: Cultivars	0.12	0.24

Supplementary Figure S2: Principal coordinates analysis (PCoA) plots based on Bray-Curtis distance matrices showing the structure of the root-associated bacterial and fungal endophytes between 11 potato cultivars grown in two different soils (GER soil representing Germany (n=3 replicates per cultivar), NL soil representing the Netherlands (n=3 replicates per cultivar). In A and B the samples are coloured according to cultivars. 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 enophytes. *P < .05, **P < .01, and ***P < .001. Analysis was computed with 999 permutations.



Supplementary Figure S3: Comparison of the Simpson evenness of root endophytic bacteria (A) and fungi (B) between 11 potato cultivars grown in three different growing conditions (GER soil representing Germany (n=3 replicates per cultivar) and NL soil representing the Netherlands (n=3 replicates per cultivar) and *in vitro* conditions (n=5 replicates per cultivar was represented with boxplots. Boxplots display the medians, tops, and bottoms of the boxes represent 75th and 25th quartiles, and whiskers outside this range show variability outside the upper and lower quartiles. Small dots shows the outliers. Kruskal wallis test ($P < .05$) followed by Dunn's post-hoc tests with Benjamini–Hochberg (BH) correction was applied to calculate significant differences between different growing locations.