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Changes in the potato rhizosphere microbiota richness and diversity occur in a growth stage-dependent manner

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Plant root and soil-associated microbiomes are influenced by niches, including bulk and rhizosphere soil. In this work, we collected bulk and rhizosphere soil samples at four potato developmental stages (leaf growth, flowering, tuber elongation and harvest) to identify whether rhizosphere microbiota are structured in a growth stage-dependent manner. The bacterial and fungal microbiota showed significant temporal differences in the rhizosphere and bulk soil. Rhizobacteria were most diverse at the tuber elongation stage, and dominant ASVs identified as *Sphingomonas*, *Rhodanobacter*, *Sphingobium*, *Hyphomicrobium*, and *Solirubrobacter* spp. In contrast, rhizosphere fungal diversity peaked at flowering stage, with *Lecanicillium* spp. being prominent. Furthermore, the abundance of saprophytic fungal genera, including *Colletotrichum* and *Fusarium*, and *Alternaria*, sharply increased at harvest stage, likely contributing to plant residue decomposition. Indicator taxa analysis highlighted the dominance of these genera at harvest. Network analysis revealed increased microbial complexity during the later growth stage, with 721 edges compared to 521 edges in the early growth stage. This increase included positive correlations between bacteria and negative correlations between bacteria and fungi. These changes suggest that microbial interactions become more interconnected and complex as potato plants mature. Our findings highlight the potential role of saprophytic fungi in shaping microbial dynamics during the later growth stage in rhizosphere soil.

Keywords Potato rhizosphere microbiota, Developmental stage, Bacteria, Fungi

The relationship between plant and soil microbes is highly complicated. Soil microbiome constituent members can interact with each other with positive or negative consequences for plant health and productivity. Previous work has revealed that in addition to niche, the soil and plant microbiome structure is influenced by plant genotype, soil type, field management, cropping system, as well as a range of biotic and abiotic factors[1–3]. It, therefore, follows that to optimise positive microbiome impacts on crop health, a thorough understanding of the impact that these factors have on soil and plant microbiome status is pivotal to achieve microbiome-facilitated crop production.

Previous research has identified temporal changes in microbiome structure during the plant growth cycle and it is also known that the root microbiome is influenced by plant root exudates[4–8]. The functions of growth stage-related microbiomes are still unclear, though recent studies suggest that microbiome networks may affect plant development and health in a growth stage-dependent manner[5–8].

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Potato (*Solanum tuberosum* L.) is one of the world's most cultivated crops and much potato microbiome research works have been conducted to identify microbiome characteristics and to understand their roles in potato productivity. Potato microbiome structure depends on soil amendment, microbe application, potato variety as well as environmental diversity[6,9–17].

Previous studies demonstrate that bacterial and fungal microbiota are influenced by potato growth stage, and it has also been shown that plants establish a beneficial microbial association within their rhizosphere as a defence mechanism against pathogens or to overcome stressful conditions[18]. Rhizosphere soil is more affected by root exudates than bulk soil[1]. However, the comprehensive study examining the temporal dynamics of both bacterial and fungal microbiota in the potato rhizosphere soil and bulk soil simultaneously has yet to be achieved. Therefore, investigating the distinctions in bacterial and fungal microbiota between bulk soil and rhizosphere soil under the same conditions is meaningful in terms of advancing our understanding of the interactions among host plants and the rhizosphere soil microbiome.

In this research, we aimed to fill this knowledge gap in understanding the temporal dynamics of bacterial and fungal microbiota in potato cultivation systems by conducting a parallel study of bulk and rhizosphere soil microbiomes. To investigate the specific functions and roles of the microbiome, it is essential to study the changes in bacterial and fungal composition under the same conditions. Specifically, we hypothesized that microbial communities (fungal and bacterial) are influenced by niche (bulk and rhizosphere soil) and that rhizosphere microbiomes are further structured in a growth stage dependent manner. Our study focuses on elucidating the bacterial and fungal composition corresponding to niches and growth stages and provides novel insights into the simultaneous temporal fluxes of bacterial and fungal communities in the potato rhizosphere. The work presented represents an advancement in the understanding how microbial communities interact with potato plants throughout their growth cycle, offering potential strategies for optimizing microbiome management to improve crop yield, resilience, and sustainable agricultural practices.

Results and discussion

Taxonomic distribution of potato rhizosphere microbiota

When analyzing microbiome data from bulk and rhizosphere soil samples, a total of 7,203 bacterial and 3,106 fungal ASVs were detected after denoising with QIIME2. To assess overall microbial changes across potato planting stages and niches, we compared the bacterial and fungal ASVs detected in bulk soil and rhizosphere soil at four specific growth stages (leaf growth, flowering, tuber elongation and harvest) with those detected in a pre-planting bulk soil. In this study, bulk soil samples were taken from between two potato plants at a depth of 5 to 25 cm. When comparing ASVs from bulk and rhizosphere soils during the cultivation period to those from the pre-planting soil, we observed that approximately 15% of ASVs were shared. Furthermore, we found that approximately 40% of the total ASVs detected were common between bulk and rhizosphere soil during the cropping period (Supplemental Figure S1).

Across the entire potato cultivation period, including the pre-planting phase, 516 bacterial ASVs were common across all bulk soil samples, whereas 438 ASVs were common across all rhizosphere samples. However, for fungal communities, 187 ASVs were common across bulk soil samples, and 185 ASVs were common in rhizosphere samples regardless of sampling time (Supplemental Figure S2). Venn diagram analysis of detected ASV numbers in the rhizosphere at different potato growth stages revealed that the lowest number of bacterial ASVs was detected at the leaf growth stage, whereas the highest number of fungal ASVs was found at flowering (Supplemental Figure S2). In bulk soil samples, the leaf growth stage supported the highest number of bacterial ASVs, while fungal ASVs showed less fluctuation across the sampling times.

The relative abundances of bacterial and fungal microbiota at the order level are shown in Supplemental Figure S3. For the bacterial community, Sphingomonadales was the most abundant in the leaf growth stage, while Micrococcales was abundant from flowering to tuber elongation stage. Rhizobiales showed a higher abundance from leaf growth to tuber elongation stages (Supplemental Figure S3A). In rhizosphere soil, the trends were different. These three orders were more abundant from leaf growth to tuber elongation, but they decreased in harvest (Supplemental Figure S3B). Specifically, compared to the pre-planting period, Sphingomonadales had three times higher relative abundance at 7.6% during the leaf growth stage, peaking at the tuber elongation stage with 10% relative abundance. Similarly, Micrococcales increased by 6.5 times at the leaf growth stage, reaching its highest abundance of 5.3% at the tuber elongation stage. Rhizobiales doubled in relative abundance at the leaf growth stage compared to the pre-planting and maintained this level until tuber elongation stage. The increased abundance of Sphingomonadae family, belonging to the order Sphingomonadales, was detected at the later growth stage in previous research[6]. In addition, *Sphingobium*, a genus within the Sphingomonadales, along with *Bradyrhizobium*, a genus of rhizobial order, were identified as stable core genera in rhizosphere soil throughout the potato cultivation period[6]. These findings suggest a close relationship between these bacterial groups and the vigorous growth of potatoes.

For fungal communities (Supplemental Figure S3C and S3D), the abundance of Sordariales decreased after cropping, maintaining a similar proportion throughout the potato cropping period in rhizosphere soil. In the case of bulk soil, on the other hand, the proportion of Sordariales was around 12% until the tuber elongation stage and peaked at 20% relative abundance at the harvest. Mortierellales increased at later growth stage in bulk soil, while it decreased at this growth stage in rhizosphere soil. Helotiales, Pleosporales, Agaricales, and Chaetothyriales showed the highest abundance at the leaf growth stage in bulk soil. Glomerellales, Cantharellales, and Agaricales showed the highest abundance at the harvest stage in rhizosphere soil. Hypocreales exhibited a higher relative abundance (13–20%) throughout the potato cropping period in rhizosphere soil, while its relative abundance was slightly higher at the flowering stage in bulk soil. In a previous study that conducted a comparative analysis of fungal microbiota across different potato growth stages, the Nectriaceae family, belonging to the Hyprocrealees order, was identified as one of the dominant families in the root during the potato growth period[12]. Additionally,

a significant increase in soil pathogen or saprotroph richness was reported at harvest[12,16]. Therefore, in this current study, these findings suggest that fungal orders showing increased abundance at harvest could be pathogens or saprotrophs.

Niche and developmental stage-related abundance patterns

The comparisons of bacterial phyla and fungal classes according to the growth stages are described in Fig. 1A and B. Nine bacterial phyla had a relative abundance of greater than 1% at all sampling times, and all showed significant differences in their relative abundance based on combinations of niche and growth stage. Most phyla showed different abundances patterns between bulk and rhizosphere soils.

Acidobacteriota and Verrucomicrobiota had the highest relative abundances at pre-planting. Myxococcota and Gemmatimonadota showed the highest relative abundance at harvest regardless of niche throughout potato cultivation period. Planctomycetota and Chloroflexi were more abundant at the tuber elongation stage within both bulk soil and rhizosphere soil. Proteobacteria had significantly higher abundance in rhizosphere soil compared to bulk soil throughout the potato growth period. The nine phyla with > 1% relative abundance demonstrated diverse trends in relation to niche and growth stage. Although the bacterial and fungal microbiota are host-dependent, Proteobacteria, Actinobacteriota, Acidobacteriota, and Chloroflexi have previously been reported as dominant in agricultural fields[19,20]. A previous study on bacterial microbiota in potato rhizosphere soil also identified Proteobacteria, Firmicutes, and Actinobacteria as dominant phyla during potato cultivation[6].

Seven fungal classes had a relative abundance greater than 1% throughout the potato growth period (Fig. 1B). Among these, four classes of Dothideomycetes, Leotiomycetes, Tremellomycetes, and Eurotiomycetes showed significant differences in their relative abundances between sampling times and niches. The trends of Sordariomycetes, Tremellomycetes, Eurotiomycetes, Dothideomycetes, and Leotiomycetes were similar between bulk and rhizosphere soil according to the growth stages, while Mortierellomycetes and Agariomycetes showed opposite trends between bulk and rhizosphere soil though without significant differences. Sordariomycetes and Agaricomycetes had the highest abundances at harvest in rhizosphere soil, while Tremellomycetes, Dothideomycetes, and Leotiomycetes were more abundant during the flowering or tuber elongation stage in rhizosphere soil. Previous studies have reported that Ascomycota, including Sordariomycetes, Eurotiomycetes, Dothideomycetes, and Leotiomycetes, consistently present at a higher relative abundance than other phyla, regardless of plant species including potato[16,21].

Microbiota diversity comparisons according to the potato growth stage

It is known that microbiota are affected by both plant niche and growth stage, with root exudate production playing a key role in their composition and abundance[21,22]. In this study, bulk soil was collected between two potato plants at a depth of 5–25 cm. Although bulk soil is less influenced by the rhizosphere effect, the bulk soil samples collected in this study may still have a mild rhizosphere effect due to their proximity to potato roots. The PERMANOVA analysis revealed significant differences in bacterial microbiota composition according to niche and growth stage, while fungal microbiota diversity was significantly influenced by growth stage, but not by niche (Table 1). Significant differences were observed in bacterial and fungal microbiota according to different growth stages in both bulk soil and rhizosphere soil. Although no study has compared bulk and rhizosphere soil fungal microbiota in potatoes, previous studies reported a weaker response of fungal rhizosphere microbiota compared to bacterial rhizosphere microbiota in *Chrysanthemum* and *Arabidopsis*[23,24]. There are a few studies comparing fungal microbiota between bulk soil and rhizosphere soils have shown different trends in snakewood[25] and sorghum[26], Chinese medicinal herb[27] and lisianthus[28]. Although the exact factors responsible for the differences in fungal and bacterial microbiota between the bulk soil and rhizosphere soil remain unclear, both communities are influenced by many factors, such as host plant, soil characteristics, agricultural management and environmental conditions[1–3]. A previous study regarding soil microbiota in plants that are toxic and non-toxic to grazing animals explained that differences in bacterial microbiota were more pronounced than fungal microbiota differences, even when considering niche, habitats and species[29].

Even if no significant differences were found in the α-diversity index between bulk soil and rhizosphere soil (Supplemental Figure S4), bacterial richness was higher in bulk soil, whereas for fungi species richness was greater in rhizosphere soil. Previous studies of comparisons of bacterial and fungal α-diversity between bulk and rhizosphere soil have shown contradictory results, with some reporting higher richness in the rhizosphere, others in bulk soil, and some finding no differences in α-diversity between rhizosphere and bulk soil[23,26].

For bacterial α-diversity, according to the growth stages (Fig. 2A and B), different patterns were observed between bulk and rhizosphere soil, although there were no significant differences within the rhizosphere samples. However, bacterial species richness was significantly higher in the leaf growth stage in bulk soil. The patterns of fungal diversity were similar between bulk soil and rhizosphere soil but differed from bacterial communities (Fig. 2C and D). Fungal diversity and species richness peaked in the middle growing periods for both niches, and significantly decreased at the harvest stage in rhizosphere soil. These results suggest that the bacterial community in rhizosphere soil is predominantly influenced by root-associated factors, while the fungal community is more affected by soil or other environmental factors[30].

Although this is the first study to investigate microbiota change in both bulk and rhizosphere soil throughout the potato cropping period, two previous studies have examined microbiota change in the rhizosphere soil during potato cultivation[6,17]. Pfeiffer et al. (2017) focused on bacterial microbiota, collecting rhizosphere soil samples from three distinct mountainous regions. It revealed different patterns of α-diversity among these regions, though no significant differences were observed during the potato cultivation period[6]. Another study explored both bacterial and fungal microbiota change throughout the potato cultivation period and reported results consistent with our findings, indicating higher α-diversity values during the flowering to tuber elongation stage, followed by a decrease at harvest stage[17]. Our results suggested that the fungal community in rhizosphere soil

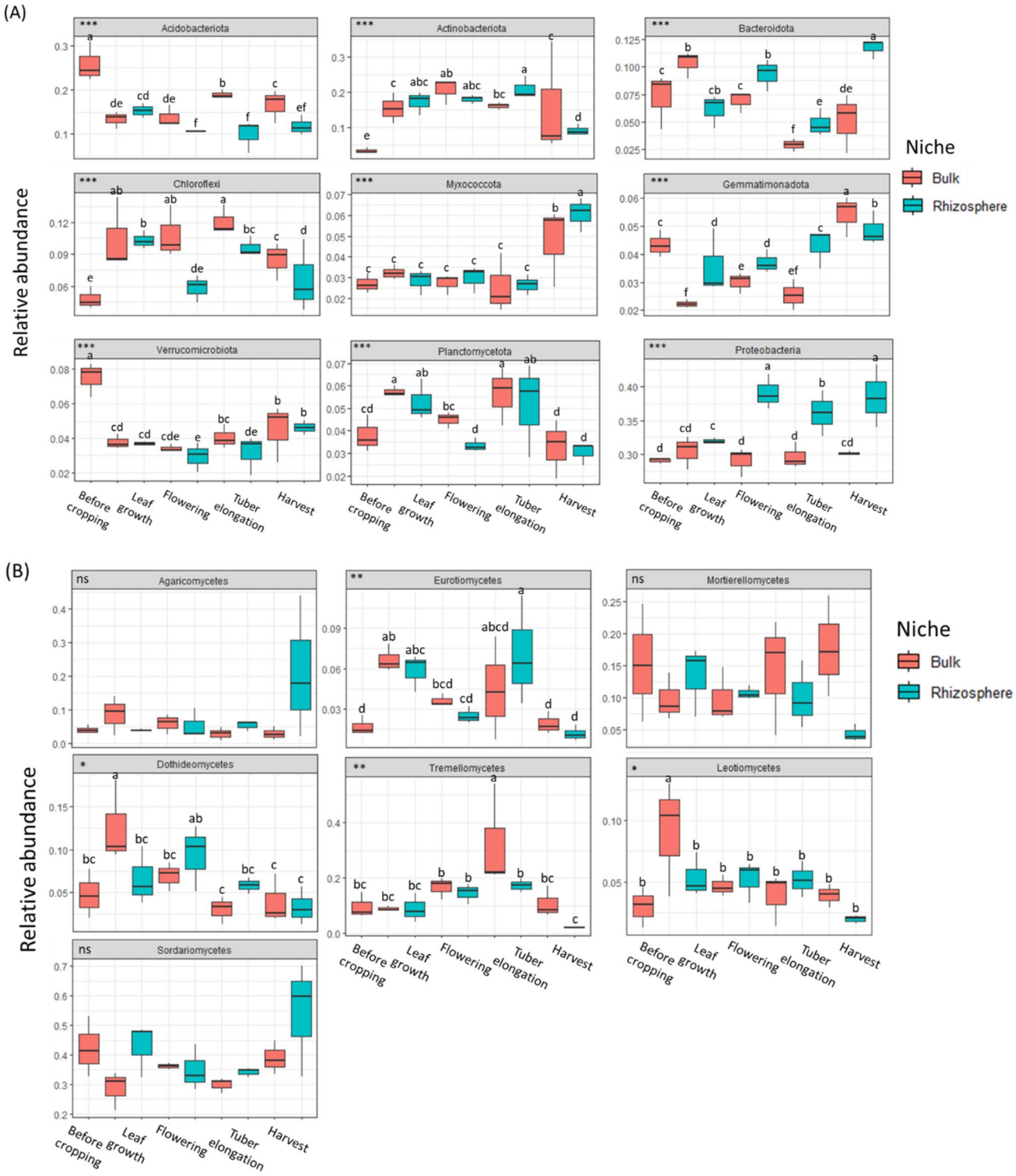

Fig. 1. The comparisons of abundances of bacterial phyla (**A**) and fungal class (**B**) according to the niches and potato growth stages. ANOVA, ns: no significance, **p-value* < 0.05, ***p-value* < 0.01, ****p-value* < 0.001, Post hoc Fisher's Least Significant Difference (LSD) test was conducted after ANOVA analysis.

is simplified at the harvest stage, as seen in the relative abundance at the class level (Supplemental Figure S3D). Given that microbial diversity can be influenced by various factors other than growth stage[31], it is imperative to conduct further studies that consider many factors in addition to potato genotype, biotic factor of indigenous microbial population or pathogen population, including edaphic factors such as soil organic matter, texture and porosity.

Treatments	*d.f*	*SS*	*MS*	*Pseudo*F	R^2	Pr (> F)
Bacteria						
Niches	1	0.389	0.389	2.407	0.09	**
Developmental stages	4	1.449	0.362	2.679	0.33	***
Developmental stages						
Bulk soil	4	1.115	0.2787	2.219	0.47	***
Rhizosphere soil	4	1.427	0.357	3.105	0.55	***
Fungi						
Niches	1	0.262	0.262	1.220	0.047	ns
Developmental stages	4	1.384	0.346	1.794	0.246	***
Developmental stages						
Bulk soil	4	1.110	0.278	1.352	0.351	**
Rhizosphere soil	4	1.407	0.352	1.967	0.440	***

Table 1. The results of PERMANOVA multivariate analysis for the abundance patterns of the detected bacterial and fungal ASVs. MS: mean sum of squares, SS: sum of squares, significance value based on 999 permutations. ns: no significance, **p-value* < 0.05, ***p-value* < 0.01, *** *p-value* < 0.001.

Ordination analysis revealed distinct bacterial and fungal microbiota according to potato growth stage (Fig. 3). The bacterial community exhibited higher CAP values, with approximately 25% of the variance explained by CAP1 in bulk soil and 28% in rhizosphere soil. In the fungal community, around 13% and 17% of CAP1 variances were observed in bulk soil and rhizosphere soil, respectively. Both bacterial and fungal microbiota showed little change in microbiome structure during the leaf growth stage to tuber elongation stage in rhizosphere soil (Fig. 3B and D).

Microbiota correlated to potato growth stage

To identify dominant temporal shifts in the microbiota linked to particular potato growth stages, we conducted a time-series analysis using the STEM (Short Time-series Expression Miner) program. This program is generally applied to gene expression data. However, here we applied ASV abundance data as input, and significant profiles were detected (Supplemental Figure S5). The time-series analysis of bacterial communities showed 11 and 13 profiles with significant pattern of bulk and rhizosphere soil, respectively (Supplemental Figure S5A, S5B). Among these, 8 profiles were statistically matched in both bulk soil and rhizosphere soil. In the bulk soil, 470 ASVs corresponded to profile 45, which described the highest abundance at the leaf growth stage, and subsequently decreased (Supplemental Table S2). Regarding the fungal communities, 5 profiles from each niche matched (Supplemental Figure S5C, S5D), with similar trends in both bulk soil and rhizosphere soil.

The time-series analysis showed that many bacterial ASVs had higher abundance at the leaf growth stage in bulk soil, while in rhizosphere soil, the abundance peaked from the leaf growth to the tuber elongation stages. To identify families associated with dominant profiles, we selected profiles 40, 45, 47 and 49 for bulk soil, which showed higher abundances during potato cultivation period compared to pre-cropping, and profiles 34, 40 and 49 for rhizosphere soil, which showed higher abundances during the mid-growth stage. Even though a large proportion of ASVs were unidentified ASVs at the family level, many of the bacterial ASVs exhibiting the highest relative abundance at the leaf growth stage in bulk soil were assigned to Sphingomonadaceae, Caulobacteraceae, Rhodanobacteraceae, Devosiaceae, Nocardioidaceae, and Xanthobacteraceae (Fig. 4A). On the other hand, Xanthobacteraceae, Comamonadaceae, Sphingomonadaceae, Micrococcaceae, Rhodanobacteraceae, Chthoniobacteraceae, and Devosiaceae were dominant from the leaf growth stage to the tuber elongation stage in rhizosphere soil (Fig. 4B). Xanthobacteraceae, Micrococcaceae and Sphingomonadaceae which exceed 1% of relative abundance during the mid-growth stage, were matched to significant profile in rhizosphere microbiota. A previous study reported that Xanthobacteraceae and Sphingomonadaceae were common in tomato rhizosphere soil, suggesting their role in promoting plant growth and protection against fungal pathogens[32,34].

In bulk soil, fungal ASVs correlated to three profiles 39, 41, 42 and 43, which increased after potato planting, were selected (Supplemental Figure S5C). In rhizosphere soil, profiles 34, 39, 40 and profile 49, which described higher abundance during potato cultivation period and a decrease at the harvest, were selected for further analysis (Supplemental Figure S5D). Fungal ASVs correlated with the time-series analysis profiles, accounted for 8-30% of the total relative abundances, while bacterial ASVs accounted for 5-40%. Although many fungal ASVs remained unidentified at the family level, Nectriaceae, Mortierellaceae, Holtermanniales, Vibrisseaceae, Chaetomiaceae, Herpottichiellaceae and Lasiophaeriaceae were more abundant from flowering to harvest in bulk soil (Fig. 4C). In rhizosphere soil, Holtermanniaceae, Mortierellaceae, Nectriaceae, and Vibrisseaceae were more prominent (Fig. 4D) with Holtermanniaceae showing a larger proportion in rhizosphere soil, particularly at the flowering stage. However, the ecological function of these families remain unclear.

The differences in the timing of bacterial ASVs abundance peaks between bulk soil and rhizosphere soil could be related to several environmental factors. In the bulk soil, microbiota which exhibited a higher ASV count at the early growth stage may be more sensitive to environmental conditions rather than being influenced by plant effects. Temperature is a crucial factor influencing microbial growth. Indeed, previous studies have reported a substantial increase in soil microbial growth and activities as the temperature rises from 5 °C to 35 °C

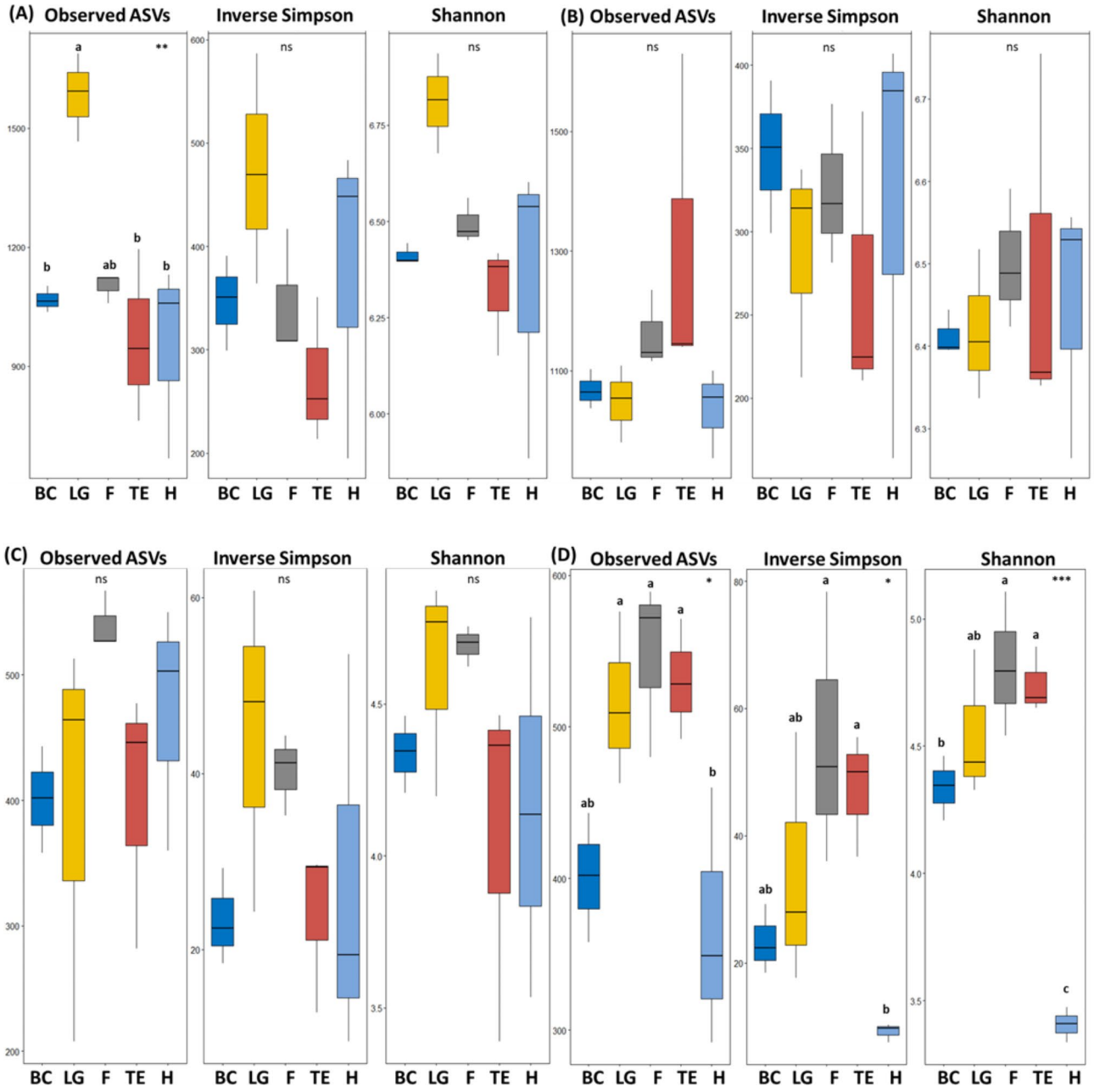

Fig. 2. α-diversity of bacterial (**A**: bulk soil, **B**: rhizosphere soil) and fungal (**C**: bulk soil, **D**: rhizosphere soil) microbiota depending on the potato growth stages, BC: before cropping, LG: leaf growth, F: flowering, TE: tuber elongation, H: harvest, ANOVA, NS: no significance, **p-value* < 0.05, ***p-value* < 0.01, ****p-value* < 0.001, ns: no significance.

from most regions across various sites including Arctic, boreal, temperate, and tropical ecosystems[32]. In this study, the highest number of bacterial ASVs in bulk soil was observed at leaf growth stage, which occurred in early June. This increase can be attributed to a 8 °C temperature rise during the leaf growth stage, compared to the pre-cropping temperature of around 15 °C in late April (Supplemental Figure S6).

Conversely, in rhizosphere soil, the higher number of bacterial ASVs detected during the later growth stage suggests stronger plant-related influences (Supplemental Table S2). Previous studies have highlighted the influence of root exudates on rhizosphere microbiota, with significant variations in several plants including *Arabidopsis*, rice, *Medicago truncatula* and wheat[20,33,34]. Studies comparing bulk and rhizosphere soil microbial communities in agricultural fields have reported that plant roots influence microbial community composition through selective recruitment[1].

We performed an indicator taxa analysis to identify microbial ASVs significantly correlated with different potato growth stages in both bulk and rhizosphere soil (Supplemental Table S3). In bulk soil, 22, 16, 30, and 36 bacterial ASVs were identified as indicators for leaf growth, flowering, tuber elongation, and harvest stages,

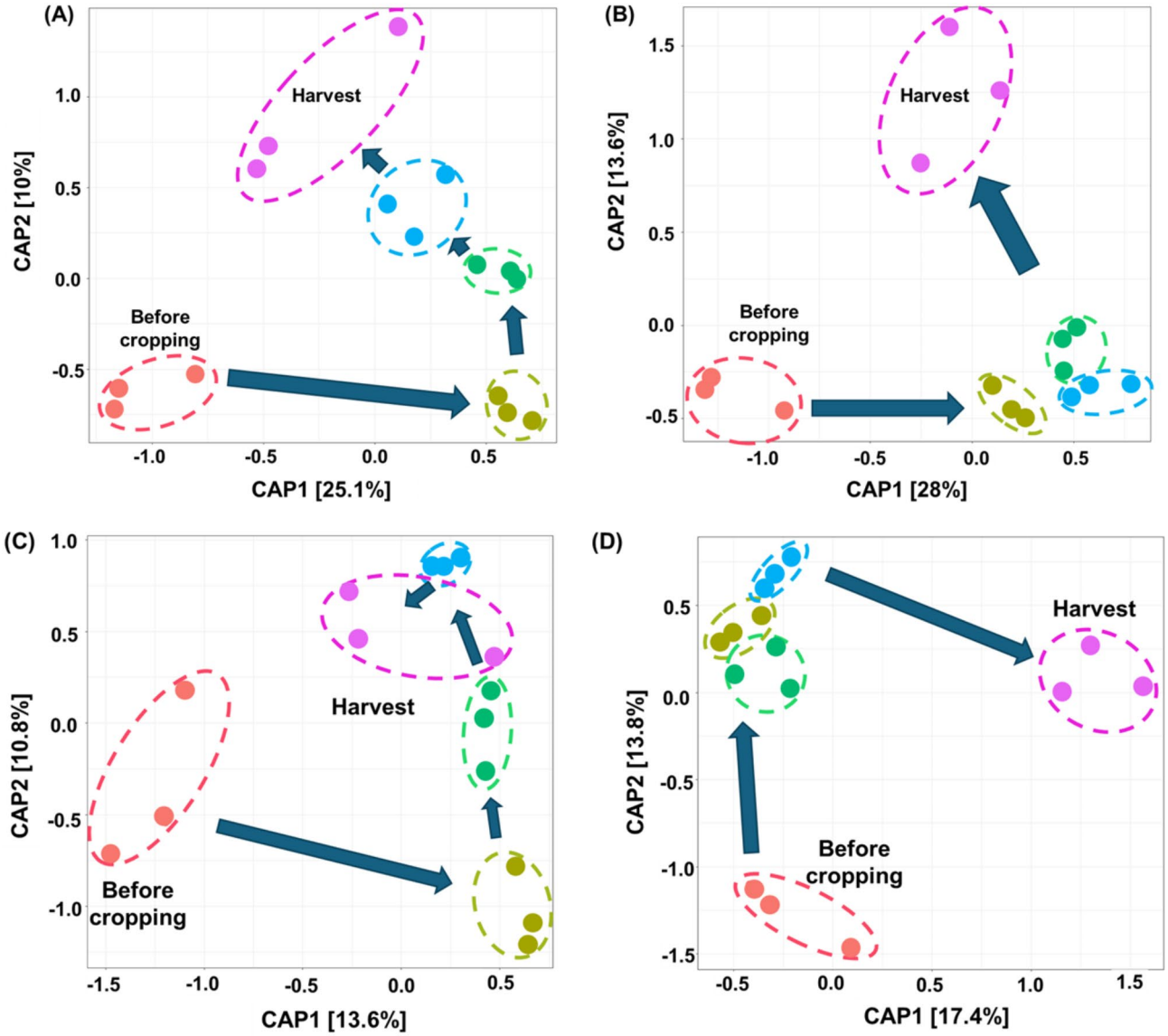

Fig. 3. Constrained analysis of principal coordinates analysis (CAP) on bacterial (**A**: bulk soil, **B**: rhizosphere soil) and fungal (**C**: bulk soil, **D**: rhizosphere soil) microbiota across different potato growth stages. Using Bray-Curtis distance, dot colors represent growth stages.

respectively. In rhizosphere soil, 36, 23, 32, and 45 bacterial ASVs were detected as indicator of each stage, respectively. In the bulk soil, *Rhodanobacter* was consistently identified as an indicator ASV, with relative abundance of over 0.3% throughout the potato cultivation period, except at the flowering stage. The highest proportion of indicator ASVs was observed at the tuber elongation stage, with a relative abundance of 7.8%, driven by increased proportions of *Udaeobacter*, *Dokdonella*, *Rhizobium*, *Sphingomonas*, *Nocardioides*, and *Rhodanobacter*. In the rhizosphere soil, *Sphingomonas* was identified as an indicator ASV with over 0.5% relative abundance during the cultivation period, except at the harvest. The highest proportion of indicator ASVs was observed at the harvest stage, with a relative abundance of 8.2%, primarily contributed by *Mesorhizobium*, *Gemmatimonas*, and *Ferruginibacter* (Supplemental Table S3).

In contrast, fewer fungal ASVs were detected as indicators. In bulk soil only 6, 9, 23, and 8 ASVs were detected at leaf growth, flowering, tuber elongation, and harvest stages respectively, while in rhizosphere soil, 7, 19, 27, and 11 ASVs were identified as indicators of each growth stage. No fungal genera were commonly detected throughout the potato cultivation period in either bulk soil and rhizosphere soil. However, significant increases in the proportions of *Tausonia* and *Solicoccozyma* were observed at the tuber elongation stage in bulk soil, with relative abundances of 8.2% and 2.7%, respectively. In the rhizosphere soil, *Phialocephala* was detected as an indicator ASV at the tuber elongation stage with a relative abundance of 2.5%. Additionally, *Colletotrichum* and *Fusarium* were detected at the harvest stage, showing substantial proportions of 18.63% and 11.52% relative abundance, respectively (Supplemental Table S3). Hence, this finding suggested that the microbial communities in bulk and rhizosphere soils are distinct, with differences observed across all growth stages.

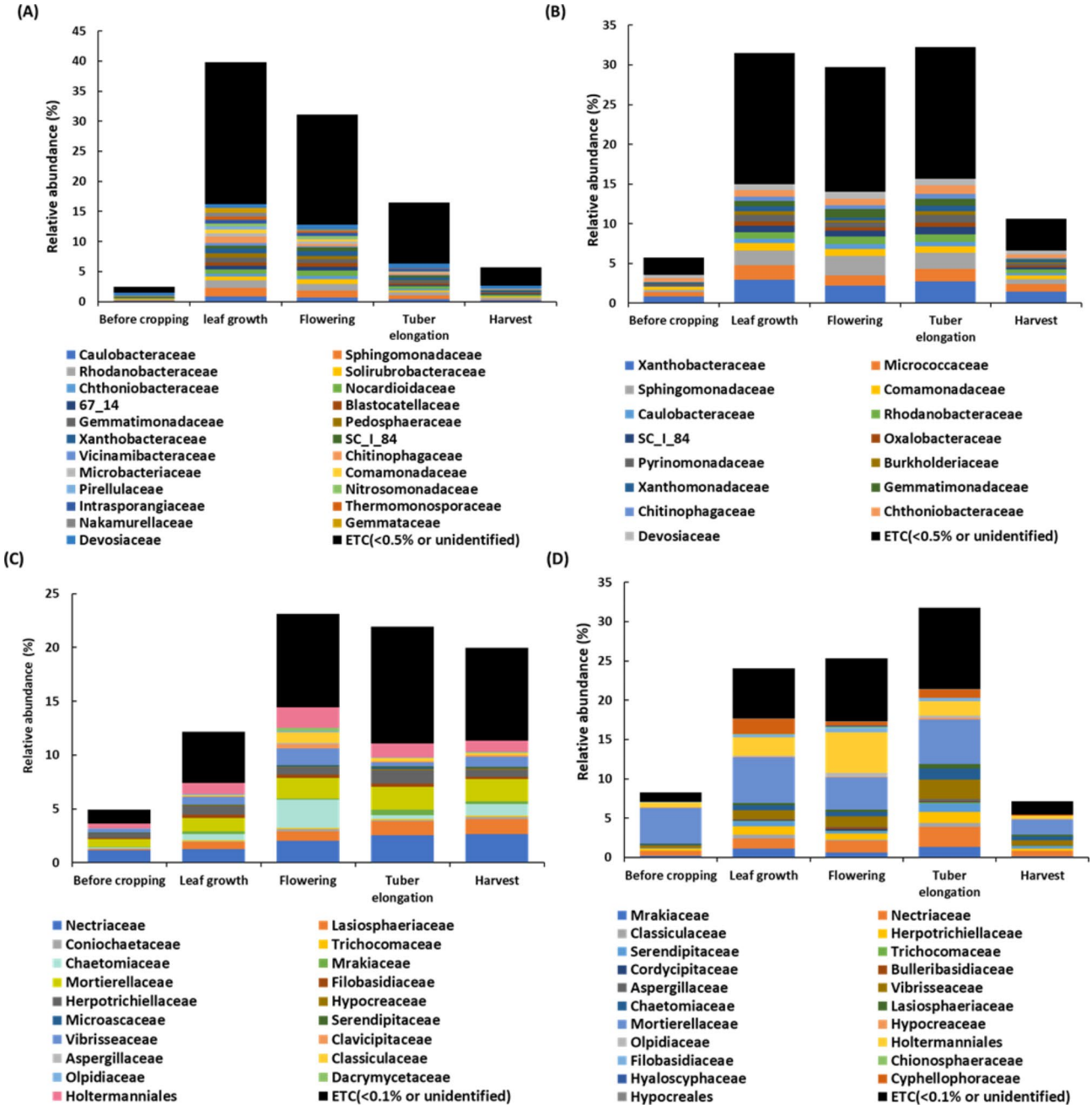

Fig. 4. The relative abundance of bacterial (**A**: bulk soil, **B**: rhizosphere soil) and fungal (**C**: bulk soil, **D**: rhizosphere soil) ASVs which correlated to the results of the time-series analysis at the family level.

Previous studies have reported that the highest number of OTUs or ASVs were detected at the middle growth stage[6], which is also considered critical for potato productivity due to its strong association with tuber bulking[35]. Some studies on other crops, such as soybean, rice, and maize[5,36,37], described more complex microbiota in rhizosphere soil during later growth stage. However, conflicting results have been reported in soybean[5,38], where one study reported increased diversity at the later growth stage[5], while another observed the opposite trend[38]. The selection of rhizosphere microbiome depends on environmental conditions and host genotype[1,3,4]. The host plant stimulates specific functional microorganisms to adapt to challenging environments, such as nutrient-poor soils[39,40] or pathogen invasion[41,42]. This adaptation often leads to a reduction in microbial diversity as certain microbial groups dominate. Although many studies on beneficial roles of rhizosphere microbiome[2], the assembly patterns of these microbial communities are still not fully understood.

In our study, bacterial indicator ASVs accounted for between 4% and 8% of the relative abundance at each growth stage in both bulk and rhizosphere soil, while fungal indicator ASVs accounted for up to 35% of relative abundances, with certain groups increasing significantly (Supplemental Figure S7). In the fungal community, particularly at the leaf growth stage (an early growth phase), only a small number of indicator ASVs were detected,

accounting for approximately 2% of the relative abundance in both rhizosphere soil and bulk soil. However, at the flowering stage, the number of indicator ASVs increased to 19, and this trend continued into the tuber elongation stage, where the number of indicator ASVs reached 27, comprising 16% of the relative abundance in the rhizosphere soil (Supplemental Table S3). Notably, most of these indicator ASVs constitute small proportions, accounting for less than 1% of the total microbiota at any given specific growth stage (Supplemental Figure S7). *Tausonia* and *Solicoccozyma* at the tuber elongation stage in the bulk soil, and *Phialocephala* in the same stage in rhizosphere soil, comprised larger proportions.

For comprehensive analysis, we considered all growth stages in our study. In the bacterial microbiota, 35 genera were identified in bulk soil, and 45 genera were identified in rhizosphere soil. In contrast, in the fungal community, 11 and 22 genera were identified in bulk soil and rhizosphere soil, respectively. Dominant genera were selected based on higher relative abundances at each growth stage (Supplemental Table S3). The abundance of ten selected bacterial genera in each niche was described in Fig. 5. Among these, *Sphingomonas*, *Rhodanobacter*, *Nocardioides*, *and* JGI_0001001_H03 (a member of Blastocatellaceae family) were detected in both bulk and rhizosphere soil. *Massilia* and *Rhizobium* showed significantly higher abundances at the leaf growth stage in bulk soil (Fig. 5A), while *Nocardioides*, *Phycicoccus*, and *Rhodanobacter* showed significantly higher abundances at the flowering stage in rhizosphere soil. (Fig. 5B). These genera are likely more closely related to potato cropping than others detected as indicator ASVs in bulk soil. Since the previous research on potato microbiota discussed the significant OTUs in the phylum or class level, direct comparisons with those studies are challenging. However, given that *Sphingomonas*, *Massilia*, *Rhodanobacter* and *Nocardioides* have been

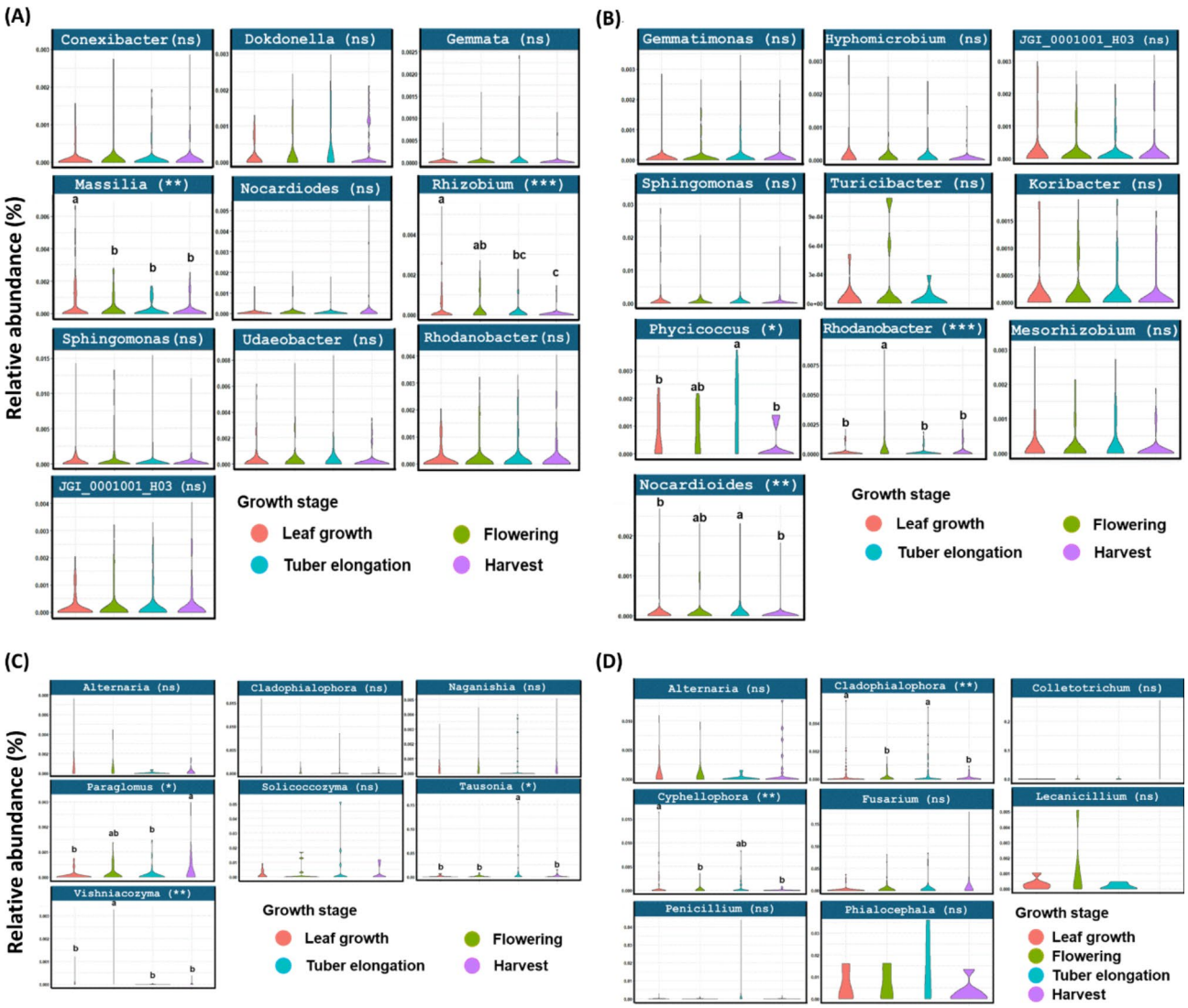

Fig. 5. The abundances of dominant indicator genera according to the growth stage. **A**: bacterial bulk soil, **B**: bacterial rhizosphere soil, **C**: fungal bulk soil, **D**: fungal rhizosphere soil, LG: leaf growth, F: flowering, TE: tuber elongation, H: harvest, ANOVA, NS: no significance, **p-value* < 0.05, ***p-value* < 0.01, ****p-value* < 0.001, ns: no significance. The top ten bacterial genera with relative abundances above 0.3% were selected for both bulk and rhizosphere soils. For fungi, genera with relative abundances below 0.1% were excluded.

reported as plant growth-promoting bacteria[43–46], it is likely that these genera closely related to enhancing potato productivity. The temporal increase in the abundance of these genera coincided with the tuber elongation period, suggesting that they may be influenced by potato root exudates released by potato plants during this stage.

Since fungal indicator taxa associated with a limited number of genera, abundance patterns of seven and eight genera were described in bulk soil (Fig. 5C) and rhizosphere soil (Fig. 5D), respectively. For bulk soil, *Tausonia* showed the highest abundances at the tuber elongation stage, with 8.2% of relative abundance. Although there was no significant difference, two genera of *Colletotrichum* and *Fusarium* showed high proportions at the harvest stage accounting for 18.6% and 11.5% of the relative abundance, respectively, in rhizosphere soil (Fig. 5D, Supplemental Table S3).

A previous study[16] identified the ten most dominant indicator fungal OTUs during potato cropping period in the rhizosphere soil. Notably, *Alternaria solani*, *Colletotrichum coccodes*, *and Fusarium equiseti* were among the detected OTUs. Furthermore, *A. solani* and *C. coccodes* exhibited increased abundance at later growth stage, while *F. equiseti* displayed a decline in abundance during the same stage. In our current study, we did not identify these genera at the species level; however, we found that the abundance of three genera -*Althernaria*, *Colletotrichum*, and *Fusarium* peaked at the harvest stage. Many species belonging to these genera have been reported as saprophytic fungi[47,48]. Furthermore, these genera include cellulolytic saprophytic species[49,50], which are capable of degrading dead plant tissue produced as the host plant ages. In addition, a previous study on *Eustoma* cultivation demonstrated that the fungal community, including pathotrophs, saprotrophs and symbiotrophs, exhibited higher abundance at the harvest stage compared to the flowering stage[28]. These findings are consistent with our results, suggesting that the observed increase in abundance of *Alternaria*, *Colletotrichum*, and *Fusarium* during the harvest stage may be related to increased saprophyte abundance.

Microbial associations of rhizosphere microbiota

Network analysis was carried out to understand the relationships between bacterial and fungal microbiota in potato rhizosphere soil. Nodes and edges were selected based on strong correlation coefficients, with an absolute value greater than 0.7. The network analysis revealed differences in network complexity and microbial interactions between the early and later growth stages (Fig. 6). Although the network of the later growth stage had a lower number of nodes (156 nodes) compared to the early growth stage (212 nodes), it had a greater number of significant associations (Early growth stage, 529 edges; later growth stage, 721 edges) (Supplemental Table S4). This increase in edges was primarily driven by more positive correlations within bacterial communities and higher number of negative correlations between fungi and bacteria (Supplemental Table S4). Closeness centrality, in particular, showed significantly increases in both bacterial and fungal communities (Supplemental Figure S8) during the later growth stage compared to the early growth stage, indicating a shift towards a more interconnected microbial network.

Hub nodes play important roles in the microbial structure[51]. It was found that growth stage influenced the number of hub nodes. During the early growth stage, only one hub node was identified, whereas nine hubs were detected in the later growth stages. Furthermore, most of the hub nodes were not classified as indicator ASVs (Supplemental Table S5), though four hub ASVs during the later growth stage were identified as indicator ASV at the tuber elongation stage. This discrepancy suggests that microorganisms important for the network's structure may not always align with those responding to plant developmental cues in potato rhizosphere soil. The previous study described consistent results that reported no direct correlation between ASV abundance and role in microbial networks[52].

Bradyrhizobium ASVs, were identified as two of the nine hub ASVs in this study. These genera are commonly isolated from various plant rhizosphere soil and has been previously reported as a core taxon in potato rhizosphere soil[6]. This highlights its potential importance not only in network structure but also in its association with potato growth. While earlier analyses concerning diversity, time-series analysis and indicator ASV analysis indicated that the proportion and total number of fungal ASVs were less than half of the bacterial ASVs, the network analysis revealed that fungal community plays a crucial role in the network's complexity. Specifically, the number of edges increased from 529 during the early growth stage to 721 in the later growth stage, despite the decrease in the number of nodes from 212 to 156 over the same period (Supplemental Table S4). This shift pointed to a more interconnected network, particularly within bacterial communities, where the number of positive edges increased by 148. Additionally, the emergence of 79 negative edges involving fungi during the later growth stage, compared to none in the early growth stage, suggests that fungi may mediate negative interactions within the microbial community as the potato plant matures. Furthermore, the increased abundances of saprophytic fungal groups at the harvest (Fig. 5D) suggest their substantial contribution to the network dynamics during the later growth stage. Previous studies on microbial networks reported that increased complexity among fungal communities and between bacterial and fungal relations upon fungal pathogen invasion on lisianthus and tobacco[53,54]. While we not directly observe pathogenic activity, the increase in negative interactions involving fungal ASVs during the later growth stage in our study might indicate similar dynamics in the microbial community, with competitive or saprophytic fungi potentially outcompeting other microbes as the plant ages. The observed increase in the relative abundances of genera such as *Alternaria*, *Fusarium* and *Colletotrichum* at the harvest stage (Fig. 5D), which include both pathogenic and saprophytic species, supports this interpretation.

In our research, we primarily focused on analysing microbial changes in the rhizosphere soil throughout different growth stages of potato growth while also examining the bulk soil. Both microbial diversity and abundance trends were influenced by both environmental conditions (e.g., niche and growth stage), and the distinct characteristics of the microbial groups. The results of α-diversity, time-series and indicator taxa analysis revealed that bacterial communities showed higher dynamics and abundance at the tuber elongation stage, whereas fungal dynamic and abundances were greatest at the flowering stage, in rhizosphere soil. The higher number of bacterial ASVs compared to fungal ASVs was detected and network analysis revealed that bacterial

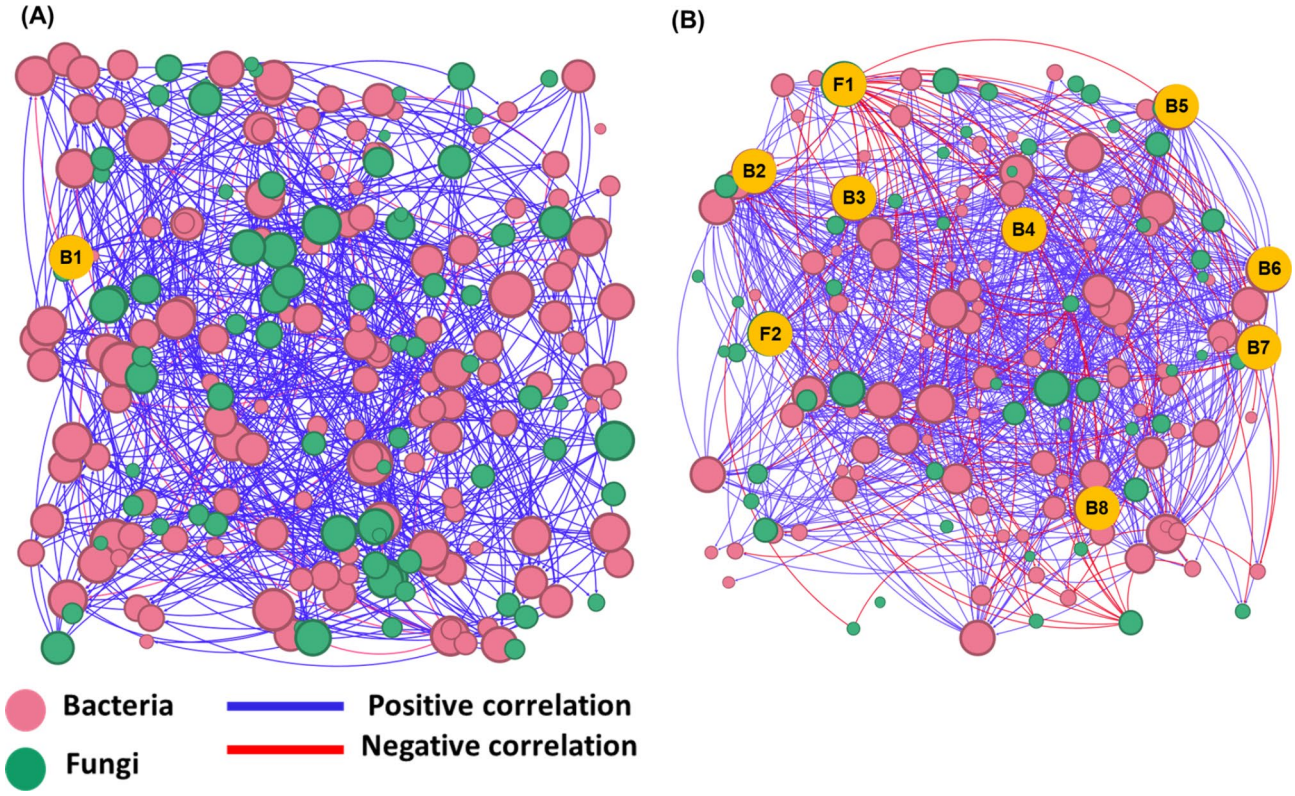

Fig. 6. Results of co-occurrence network analyses for each growth stage in rhizosphere soil. To construct networks, ASVs were filtered based on the relative abundance and prevalence (0.001 of detection and 0.8 of prevalence for bacterial microbiota and 0.001 of detection and 0.6 of prevalence for fungal microbiota) (**A**: Early growth stage from the leaf growth to the flowering stages, node: 212, edge: 529 **B**: Later growth stage from the tuber elongation to the harvest stages, node: 156, edge: 721). The nodes represent ASVs and the edges represent correlations between the nodes with Spearman's correlation coefficients greater than 0.7. Hub ASVs were selected using 92% of degree, betweenness centrality and closeness centrality for the early growth stage while 90% of degree, betweenness centrality and closeness centrality for the later growth stage. The 10 Hub ASVs are described as yellow color. B1: Gemmatimonadaceae, B2: Sphingomonadaceae, B3: *Bradyrhizobium*, B4: *Phycicoccus*, B5: *Nocardioides*, B6: *Bradyrhizobium*, B7: *Cellulomonas*, B8: *Gemmatimonas*, F1: Sordariomycetes, F2: *Tausonia*.

nodes were twice as abundant than fungal nodes. However, the increase in negative edges during later growth stage, closely related to fungal nodes. This finding suggests that fungal communities may have an expanded role in shaping microbial interactions as potato matures.

Materials and methods

1. Crop management and potato field conditions

Potato cv. Sumi was cultivated in Pyeongchang (37.6825°N, 128.7282°E), a mountainous area at an altitude of 700 m, from the middle of May to the end of August in 2016. The seed potatoes were produced by the Highland Agriculture Research Institute in the previous year. The experimental field consisted of rectangular plots, each measuring 4 m by 6 m, with three replicate plots arranged randomly. Due to the limited cultivation period in this mountainous area, only one crop was grown per year. Potatoes were planted during the previous growing season (2015), from middle of May to the end of August. No further cultivation and management occurred after the harvest prior to soil sampling in April 2016. Additionally, the field had not been cultivated for over three years before 2015. Rotary tillage was applied at the end of April, and chemical fertilizer (NH_2-P_20_5-K_2O at 10, 10, and 12 kg/1000 m^2, respectively) and compost (2000 kg/1000 m^2) were added to the potato field. Seed potatoes were planted at 80 × 25 cm distances with black plastic mulching. Potato plants were rainfed, and agrochemicals were applied weekly from three weeks after planting until one month before harvest to control pathogens and insects. Soil chemical conditions are described in Supplemental Table S1. In accordance with the guidelines established by the Rural Development Administration (RDA), it suggests the specific soil parameters for optimal potato cultivation. These parameters include within 5.5–6.2 of soil pH, 20–30 (g/kg) of organic matter, 250–350 (mg/kg) of available phosphate, 0.5–0.6 (cmol/kg) of potassium, 4.5–5.5 (cmol/kg) of calcium, and 1.5-2.0 (cmol/kg) of magnesium[55]. The experimental field site is mildly acidic with a pH of 6.4.

2. Soil sampling

Soil samples were collected for chemical property analysis with an auger at a depth of up to 15 cm. Samples were collected in five randomly selected points in the field at each sampling time before fertilization and after harvest. Collected soils were well mixed in plastic bags and dried over two days before being passed through a 2 mm sieve.

Rhizosphere and bulk soil was collected at four different growth periods of leaf growth (planting after 3 weeks), flowering (planting after 6 weeks), tuber elongation (planting after 9 weeks), and harvest (planting after 12 weeks). The soil before planting was collected as a control. All tools used in the sampling, including spade, tweezers and scissors were sterilized with 70% ethanol before sampling and between samples. To conduct a comparative assessment between bulk soil and rhizosphere soil microbiota, soil samples were taken at an equivalent depth ranging from 5 to 15 cm. Bulk soils samples were collected between the potato plants, spaced 25 cm apart, at a depth of 5 cm to 25 cm. For the collection of rhizosphere soil, the entire plant was carefully extracted from the soil, and the soil adhering firmly to the roots was obtained through vigorous shaking. The root-adhering rhizosphere soil was submerged in 15 ml of water in a 50 ml Falcon tube. Tubes were shaken for 30 s by hand. The soil suspension was centrifuged at 5000 x g for 5 min, and the supernatant was discarded. Collected bulk soil and rhizosphere soil were stored in a -70 ^{0}C freezer prior to DNA extraction.

3. Soil microbiota analysis

The DNA from bulk or rhizosphere soil was extracted using an ISOILIIDNA extraction kit (Nippongene, Japan) following the manufacturer's instructions. Extracted DNA was stored at -20 ^{0}C prior to PCR amplification. DNA was amplified with universal primer pairs targeting the V4 region for bacterial 16 S rRNA gene and fungal ITS1 spacer regions, respectively, from the Illumina metagenomic sequencing library preparation guide (Illumina, 2013). The 16 S rRNA gene primer pair (515 F and 806R) and ITS region primers (ITS1F and ITS2R) were used for PCR amplification. The sequences of each primer are as follows:

515F[56] (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-GTGCCAGCMGCCGCGGTAA-3′) and 806R[57] (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GGACTACHVGGGTWTCTAAT-3′) for V4 region.

ITS1F (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GCTGCGTTCTTCATCGATGC-3′) for ITS1 amplification[58].

PCRs were performed using AmpliTaq GOLD (Applied Biosystems; Carlsbad, CA, USA), and PCR conditions were as follows: (1) 95 ^{0}C for 10 min, (2) 95 ^{0}C for 30 s, (3) 55 ^{0}C for 30 s, (4) 72 ^{0}C for 1 min and (5) 72 ^{0}C for 7 min, repeated 2) to 4) 30 cycles. The PCR amplicons were applied to carry out library production with Nextera barcode (Illumina, 2013, Illumina Co., California, USA), and the libraries were sent to Macrogen Co. (Seoul, South Korea) to conduct sequencing analysis (MiSeq, Illumina Co., California, USA). The sequence data were provided as demultiplexed fastq paired-end files.

4. Data analysis

The microbial sequence data were handled with QIIME2 platform for merging, denoising, and taxonomic assignment using ASV with SILVA 138 (for bacteria) and UNITE v8 (for fungi) databases. Analyses of microbiota α-diversity (Shannon, Simpson, observed ASVs), β-diversity (constrained analysis of principal coordinates by developmental stages, Bray-Curtis distance metric was used), permutational multivariate analysis of variance (PERMANOVA, Bray-Curtis dissimilarity was used), Venn diagram, and indicator taxa analysis were conducted with phyloseq, microbiome, vegan, and labdsv packages in R (4.1.1). Venn diagrams were generated with Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) and R. Relative abundance analysis was calculated and described using Excel. The statistical analysis of the abundance comparison of each phylum according to the growth stages was conducted using Fisher's LSD (Least Significant Difference) test after ANOVA using R. Time series analysis of microbiota abundance profile correlated with the potato growth period was conducted with Short Time-series Expression Miner (STEM. https://www.cs.cmu.edu/~jernst/stem/) with normalized ASV table using metagenomeSeq package of R.

In order to perform network analysis four replicate samples are required. It was necessary to recategorize rhizosphere samples into early growth stage (leaf growth and flowering) and later growth stage (tuber elongation and harvest) to achieve this. Dominant ASVs with a relative abundance of 0.001 and a prevalence of 0.8 (for bacteria) or 0.6 (for fungi) were used to construct the networks for each stage. The value of Spearman's rank correlation coefficient was calculated using R and selected with over 0.7 absolute value of correlation coefficient and below 0.01 of the *p-value*. Hub ASVs were selected by degree, betweenness centrality, and closeness centrality, which were calculated with Hmisc package in R. For the early growth stage, hub ASVs were selected from the top 92% of these metrics, while for the later growth stage, the threshold was set at the top 90%. The selected data was applied to Gephi to visualize the network. Dunn's Kruskal-Wallis multiple comparisons was conducted to compare degree, betweenness centrality, and closeness centrality of bacterial and fungal groups with FSA package in R.

Data availability

The datasets generated and/or analysed during the current study are available in the KNB (The Knowledge Network for Biocomplexity) repository, [urn: uuid: c42441b4-3672-4836-8b23-54a125cd2673].https://knb.ecoinformatics.org/view/urn%3Auuid%3Ac42441b4-3672-4836-8b23-54a125cd2673.

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Author contributions

YHL and GRB designed the experimental setup and supervised the research process. GRB conducted the experiments, collected samples and gathered data. SJ and JTL assisted with preparation for of the field experiment. GRB, HK, IMC, THM, KKL and VNK collaborated on data analysis and interpretation. GRB and HK wrote the initial draft of manuscript and GRB integrated inputs from all authors. YHL, IMC and THM contributed substantially to manuscript revision and editing.

Declarations

Ethical approval

The research conducted adhered to the guidelines outlined in the IUCN Policy Statement on Research Involving Species at Risk of Extinction and Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Competing interests

The authors declare no competing interests.

Additional information

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