Unveiling the potato cultivars with microbiome interactive traits for sustainable agricultural production

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Abstract

Root traits significantly influence the composition and functionality of the rhizosphere microbiome, shaping nutrient cycling and plant health. Despite the critical role of root morphology and physiology in shaping plant-microbiome interactions, they are often overlooked in plant breeding. Here, we propose that selecting modern cultivars based on microbiome interactive traits (MITs), such as root biomass and exudation patterns, can enhance agricultural sustainability by interacting effectively with soil microbiomes, which in turn, promotes plant growth and resistance to stress, thereby reducing reliance on synthetic crop protectants. Through a stepwise selection process, we chose 51 cultivars based on their MITs and verified their capacity to interact with the soil microbiome in a greenhouse experiment. These were further clustered into functional groups based on their MITs and leaf metabolites and further characterised by their rhizosphere microbiome (bacterial and fungal communities). Our results indicate a positive correlation between microbial community diversity and root biomass. A potential influence of leaf metabolites on the composition of rhizosphere bacteria was observed, supporting the plant holobiont framework. We highlight the significance of incorporating positive effects on microbiomes into the breeding targets to advance the objectives of sustainable agriculture. A set of 10 potato cultivars was selected, based on their MITs, that should be evaluated genetically to identify future potential markers for breeding strategies, to verify whether those cultivars with microbiome interactive traits are conducive to sustainable agricultural production.

1. Introduction

As a staple crop, potatoes (*Solanum tuberosum* L.) rely heavily on conventional agricultural practices. Conventional management, which includes the widespread use of synthetic fertilisers and pesticides, is frequently used to enhance yields and protect crops from pests and diseases (Timsina, 2018). However, this dependency on synthetic compounds comes with substantial environmental issues. Overusing chemical inputs in conventional agriculture leads to water pollution, soil degradation, and soil microbial biomass and activity reduction, which are crucial

in maintaining soil fertility and nutrient cycling (AL-Ani et al., 2019; Tripathi et al., 2020). Balancing the imperative for high yields with care for our environment is a critical challenge in the breeding of crops.

In conventional breeding, plants are bred for traits such as high yields, disease resistance, tolerance to environmental stresses, and improved agronomic traits (Breseghello and Coelho, 2013). While pivotal in crop development, conventional breeding has inadvertently led to the dissociation between plant and soil microbiomes, negatively affecting beneficial plant-microbiome interactions (Spor et al., 2020). Comparisons between domesticated cultivars and their ancestral plants revealed that the rhizosphere microbiome of the latter exhibited higher complexity and connectivity (Rossmann et al., 2020; da Silva et al., 2023). However, relying solely on ancestral or wild plants may not effectively address the challenge of optimising plant-soil microbiome interactions in modern agricultural systems. After generations of crop selection, hosts have decreased their ability to regulate microbe-to-host interactions. Identifying the specific genes associated with beneficial microbiomes, with the aim of subsequently transferring to modern crops, is complex (Raaijmakers and Kiers, 2022). Considering environmental effects on gene expression, it is important to identify beneficial microbiome gene markers among modern crop cultivars and harness them in real-world scenarios, particularly in the context of climate change.

The connection between root traits and plant productivity in crops has long been acknowledged (Lynch, 1995), and it is increasingly evident that our understanding of this connection should extend to how root traits shape the composition and functionality of the rhizosphere microbiome. While this aspect has received somewhat limited attention within plant breeding (Herms et al., 2022), many studies highlight the role of root traits in the rhizosphere microbiome. For instance, a study on maise revealed that wild maise had a significant impact on the structure of the microbial community, which was attributed to a high root-to-shoot biomass ratio (Szoboszlay et al., 2015). Plant root traits influence the soil microbial community by modulating the fungi-to-bacteria ratio, contributing to nutrient cycling (Wan et al., 2021). Moreover, a study involving beans (Pérez-Jaramillo et al., 2017) indicated that root architecture is linked to increased specific bacterial taxa, which could be connected to plant health. In addition to the root system, root exudates substantially impact the rhizosphere microbiome. As a

survival strategy, plants secrete an abundance of compounds in their root exudates that can influence the diversity of the rhizosphere microbial community, promote the development of a more complex microbial network and enhance microbial carbon cycling, thereby facilitating the growth and survival of the host (Wang et al., 2022). A study on wheat has demonstrated that cultivars with a strong capacity for root exudates contribute to increased soil microbial diversity (Iannucci et al., 2021). These specific cultivars can be further explored for their resource utilisation potential in agricultural systems.

Here, we approach the challenge of dissociation between plants and soil microbes by exploring the microbiome interactive traits (MITs) of existing potato cultivars in the European potato germplasm bank. The root system architecture and exudation patterns, identified as pivotal MITs, guide our stepwise selection process for cultivars exhibiting high to low MITs. We hypothesise that cultivars with high MITs will select a more diverse rhizosphere microbiome, exhibiting superior growth to those with low MITs. A greenhouse experiment was conducted to investigate the relationship between the plant traits of 51 potato cultivars and the rhizosphere microbial community. We propose that MITs capable of recruiting beneficial microbes to the rhizosphere should be considered in the breeding and developing new crop cultivars. By prioritising these interactive traits, we aim to contribute not only to the enhancement of crop performance but also to the interaction between plants and their microbiomes, unravelling valuable insights for innovative and sustainable agricultural practices.

2. Materials and methods

In this project, we initially narrowed down a selection of 151 late potato cultivars, including a commercial cultivar Desiree as a reference, selected from a pool of a thousand potato cultivars based on their resistance to pathogens, especially viruses. These 151 cultivars were then further selected based on the quality of the root exudation, determined under sterile conditions using 3-week-old in *vitro* plants (Supplementary information I). From those, 1/3 of the cultivars (50 plus Desiree) were tested for their ability to interact with soil microbiome in a greenhouse experiment.

2.1. Root exudate and leaf metabolites profiling and processing

The metabolites in the root exudate samples from the in *vitro* experiment (Supplementary I) and leaf samples from the greenhouse experiment were measured using gas chromatography and electron impact ionisation-time of flight mass spectrometry (GC/EI-TOF-MS). Before GC/EI-TOF-MS analysis, metabolites in both types of samples were subjected to manual methoxyamination and trimethylsilylation (Lisec et al., 2006; Luedemann et al., 2008). The freeze-dried sample was processed per individual sample for the root exudate metabolite profiling. For leaf metabolite profiling, 120 mg of snap-frozen leaf material per sample was extracted following the procedures outlined by Erban et al. (2020). A 30 µl internal standard addition of 0.02 mg/ml $^{13}C_6$-sorbitol was incorporated into all samples. The chromatograms were obtained and adjusted for baseline using the ChromaTOF software (LECO Instrumente GmbH, Mönchengladbach, Germany). Metabolite annotation was performed using TagFinder (Luedemann et al., 2008), the NIST08 platform (U.S. Department of Commerce, Gaithersburg, USA), and the Golm Metabolome Database (Kopka et al., 2005; Hummel et al., 2010). The comprehensive methodological description can be found in Erban et al. (2020). Metabolites identified as known contaminants or those absent in over 75% of the samples were excluded, with missing values imputed as zero. A total of 84 leaf metabolites and 49 root exudate metabolites were characterised.

The concentration level of root exudate metabolites was determined using the median values of three replicates. To obtain an approximately normal distribution, concentrations were standardised through dividing them by the median concentration across all cultivars (Schaarschmidt et al., 2020). All presented metabolite data reflect relative metabolite abundance. We ranked the relative abundance of metabolites among cultivars and calculated the sum of all metabolites per cultivar. The order of these sums represents the quality and quantity of metabolites (Supplementary II, Table S1).

2.2. Greenhouse experiment

Fifty-one potato cultivars (including Desiree) were selected for a greenhouse experiment based on root exudate metabolite quality and quantity levels from the in *vitro* experiment (Supplementary II, Table S1). The soil in this study originated from a sugar beet field and was sieved with a 2 mm sieve. Before starting the experiment, six bulk soil samples were kept for physicochemical analyses and DNA extraction.

Potato plantlets obtained from tissue culture from the Institute of Plant Breeding and Acclimation in Bonin (Bonin, Poland) were transplanted from agar tubes to small pots filled with homogenised soil. Three replicates were established for each cultivar. Additionally, six control pots without plants were prepared. The greenhouse temperature was 22 °C during the day and 18 °C at night, with a photoperiod of 14 hours of light and 10 hours of darkness.

After two weeks, the potato plants were transplanted into larger pots (1.45 L). Plant leaves, specifically the second or third leaf from the shoot top, were harvested for metabolomics analysis in the sixth week. The harvested leaves were snap-frozen in liquid nitrogen and then transferred to a freezer at -80 °C. The rhizosphere soil was obtained by gently brushing off adhering soil from the roots with a disposable toothbrush. Additionally, six bulk soil samples were collected from pots without plants. These soil samples were frozen at -20 °C until DNA extraction the following day. Shoot height and root length were measured on the day of harvesting, and shoot and root dry weights were measured after drying at 65 °C for 72 hours.

2.3. Soil sample sequencing and processing

DNA was extracted from 12 bulk soil samples and 153 rhizosphere samples with DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) on 0.25 g of soil. DNA extraction followed the kit's instructions except for the initial stage of bead beating, which was conducted with a FastPrep-24TM 5G Instrument at 6000 rpm/s for 40 s (MP Biomedicals, Santa Ana, USA). For sequencing of the bacterial community, the V4 region of the 16S rRNA gene was targeted using the primer sets 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011, 2012). For the fungal community, the ITS2 region was sequenced with the primer sets 5.8SR (5'-TCGATGAAGAACGCAGCG-3')

and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). The Illumina MiSeq platform was used for paired-end sequencing (2 × 250bp for 16S, 2 × 300bp for ITS) at the PGTB (Genome Transcriptome Platform of Bordeaux, Cestas, France).

The 16S rRNA gene sequencing data were processed using a QIIME2 (version 2020.8) pipeline (Bolyen et al., 2019). The removal of adaptors and primer sequences was performed with the Cutadapt plugin (Martin, 2011). Subsequently, sequences were filtered, denoised, and dereplicated using the default setting of the Divisive Amplicon Denoising Algorithm (DADA2) plugin (Callahan et al., 2016). 16S rRNA taxonomic classification was performed using the q2-feature-classifier plugin (Bokulich et al., 2018) against the SILVA database (version 138) (Yilmaz et al., 2014). ITS sequencing data was processed using the PIPITS / 2.4 pipeline (Gweon et al., 2015). In brief, the PEAR plugin was used to join read pairs (Zhang et al., 2014). The FASTX-Toolkit was utilised for quality filtering (Gordon and Hannon, 2010). The fungal-specific ITS2 region was extracted via ITSx (version 1.1b) (Bengtsson-Palme et al., 2013). The VSEARCH 2.13.3 plugin (Rognes et al., 2016) was used for the dereplication of unique sequences, clustering to 97% sequence identity, and the UNITE Uchime reference dataset was used for chimera detection (Nilsson et al., 2015). Ultimately, the taxonomy was assigned with the RDP Classifier against the UNITE database (version 8.0) (Kõljalg et al., 2013).

2.4. Statistical Analysis

A one-way analysis of variance (ANOVA) was used to test variation in plant performance (shoot and root growth), plant metabolite diversity, and rhizosphere microbial alpha diversity. Post hoc comparisons were performed through Tukey's honest significant differences or Duncan’s multiple range tests.

Analysis of soil microbial community structure (bacteria and fungi) was performed in R (version 4.2.0). Feature tables were rarefied at 14400 reads for bacterial 16S rRNA gene and 6361 for fungal ITS sequences, resulting in 8970 amplicon sequence variants (ASVs) for the bacterial community and 2006 operational taxonomic units (OTUs) for the fungal community after excluding non-microbial OTUs. The rarefactions of feature tables were generated via

'rarefy' function in the package 'vegan' (Dixon, 2003). The soil microbial community, root exudate and leaf metabolite compositions were visualised through Principal Coordinate Analysis (PCoA) based on Bray-Curtis distance. This analysis used the 'ape' package in R (Paradis et al., 2004). PCoA scores per axis were averaged for each sample to illustrate the distribution of distinct cultivars in PCoA plots more clearly. To assess the impact of cultivars on soil microbial communities and metabolite profiles, permutational multivariate analysis of variance (PERMANOVA) was conducted using the 'adonis' function within the 'vegan' package (Dixon, 2003).

Principal Component Analysis (PCA) based on the covariance matrix was performed with the R package 'FactoMineR' to visualise the distribution of plant cultivars (Lê et al., 2008). The variables included in the analysis were shoot and root length, shoot and root dry weight, total length and dry weight, leaf metabolite concentration, and root exudation concentration. Due to significant variation in root exudate concentration observed in the in *vitro* experiment, median values were used for PCA plotting. Normalisation of plant data and metabolite richness was performed before analysis by calculating Z-scores. Subsequently, a PERMANOVA was conducted to assess the influence of variables on the distribution of 51 cultivars.

To investigate the relationship between plant cultivars and rhizosphere microbiome composition, we categorised the 51 potato cultivars into four distinct functional groups. The category was determined by the distribution of cultivars and variables along the first two axes in the PCA plot. The Spearman's rank correlation coefficient was used to assess the relationship between plant growth and microbial kingdoms under different functional cultivar groups.

The correlation between plant growth and related omics datasets was calculated using the Spearman correlation coefficient via the 'corr.test()' function from the 'psych' package in R (Revelle, 2024). The resulting p-values were adjusted using the False Discovery Rate (FDR) method to control for multiple comparisons and minimise false positives. We used the Mantel test with Spearman's rank correlation to assess the correlation between microbial community compositions and metabolite profiles, utilising Bray-Curtis dissimilarity matrices. This analysis aimed to investigate the relationship between plant leaf metabolites and rhizosphere soil community.

Using the 'ggsankey' R package (Sjoberg, 2023), a Sankey plot was generated based on different root exudation levels and functional groups to summarise the selection of the 11 cultivars with the most potential for future research. The distribution of rhizosphere bacterial and fungal community compositions was illustrated for the 11 selected cultivars using Spearman correlation analysis. The first axis of the PCoA plot of bacterial and fungal communities served as the indicator of community composition.

3. Results

3.1 Plant growth

Plant growth varied across the 51 potato cultivars. Analysis of variance (ANOVA) revealed that most of these cultivars' growth patterns differed significantly according to their identity. Among them, the cultivars showed a more pronounced effect on root biomass than on root length (root biomass: $p = 0.001$, $F = 2.26$; root length: $p = 0.003$, $F = 1.91$; Figure 1). Specifically, the cultivars Inwestor, Salto and Szyper demonstrated higher root biomass than others. For shoot growth, the cultivars had a more pronounced effect on shoot length than on shoot biomass (shoot length: $p = 0.001$, $F = 6.80$; shoot biomass: $p = 0.009$, $F = 1.75$; Figure S1). The cultivars Astrid, Inwestor, and Tewadi were the top three in terms of shoot biomass. Regarding the root-to-shoot ratio, the length ratio exhibited a significant response to distinct cultivars ($p = 0.001$; Figure S2). Conversely, the ratio of root-to-shoot dry weight did not exhibit a substantial response to different cultivars ($p = 0.36$; Figure S2).

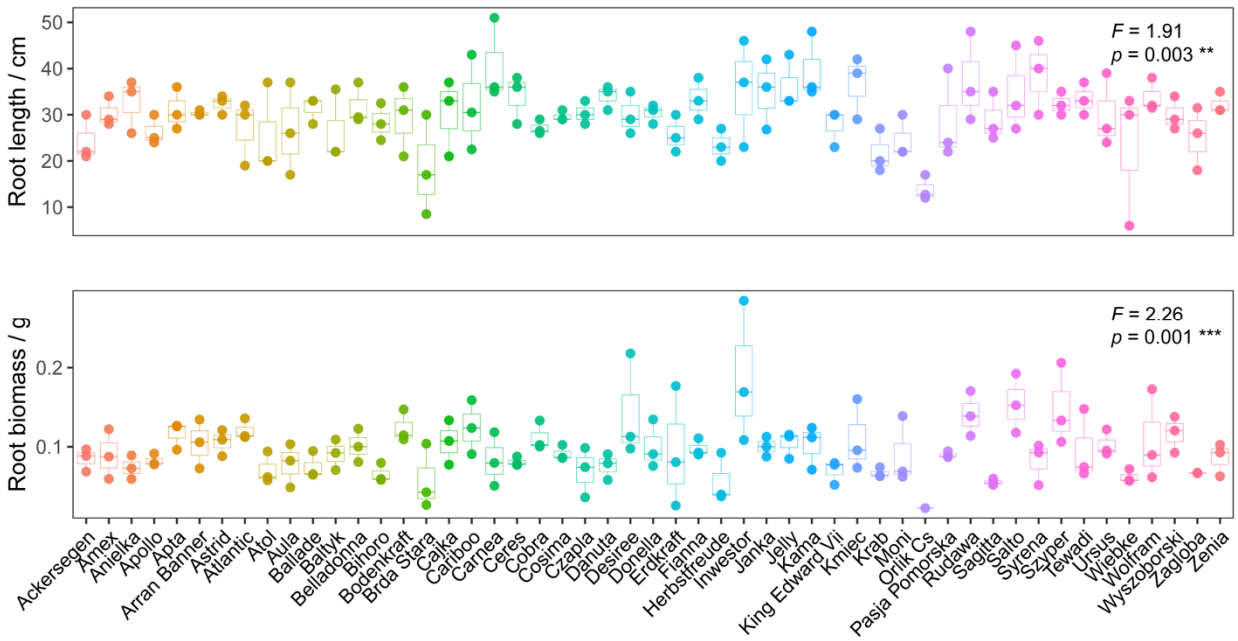

Figure 1. Root growth analysis of 51 potato cultivars. The upper panel displays root length, while the lower panel illustrates root biomass. Each colour represents a distinct potato cultivar. The upper right corner of each plot displays one-way ANOVA results, where the F-value explains the variation among different cultivars, and the p-value indicates the statistical relationship among cultivars. Significance levels are denoted as ** (p = 0.01) and *** (p = 0.001).

3.2 Plant leaf and root exudate metabolites

To understand the variation in metabolites produced and released by the 51 potato cultivars, we conducted a Principal Coordinates Analysis (PCoA) and visualised the distribution of metabolites among different cultivars (Figure 2). The cultivars differed significantly in metabolite composition from both root exudates and leaves ($p_{\text{root exudate}}$ = 0.001 and p_{leaf} = 0.001; Figure 2).

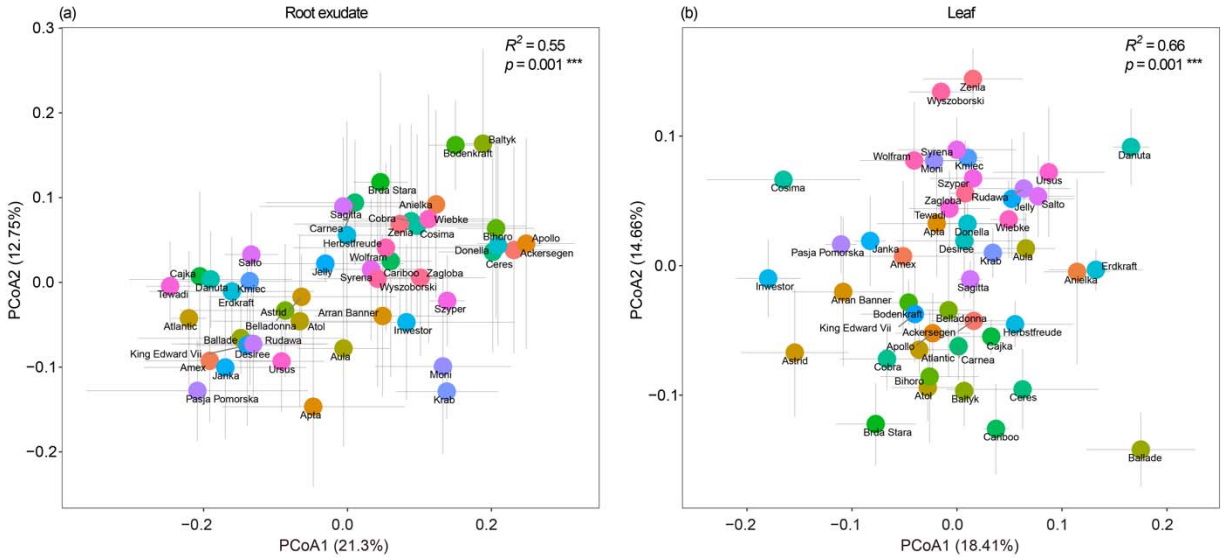

Figure 2. Distribution of metabolites in root exudates and leaves across different cultivars. Principal Coordinates Analysis (PCoA) based on mean Bray-Curtis dissimilarity of metabolite composition of root exudates (a) and leaves (b). Distinct potato cultivars are represented by different colours, with the error bars for each cultivar displayed in grey. PERMANOVA results are shown in the upper right corner of each panel, indicating variations in metabolite composition among different cultivars. R^2 quantifies the explained variation, and p-values are derived from 9999 permutations; *** denotes statistically significant p-values (p = 0.001). Three outlier cultivars (Kama, Czapla, Fianna, Orlik Cs) were excluded to illustrate the distribution of distinct cultivars better. The distribution of the full set of 51 potato cultivars is presented in Figure S3.

Notably, leaf metabolite structure responded more strongly to plant cultivar identity compared to root exudate metabolites ($R^2_{\text{leaf metabolite}}$ = 0.66, and $R^2_{\text{root exudates}}$ = 0.55; Figure 2). The first two axes of the PCoA plot explained more than 30% of the variation for both metabolites. Although most cultivars tended to cluster together, several cultivars stood out, suggesting a unique and pronounced influence on the metabolite profiles. Furthermore, we found that the alpha diversity of plant metabolites was significantly affected by cultivars (Table S2). These results indicate that different potato cultivars exhibit distinct metabolites, contributing to variations in metabolite composition among cultivars.

3.3 Plant cultivar functional groups

The analysis of plant cultivar growth characteristics, including shoot and root growth, leaf metabolites and root exudates, revealed that the distribution of these traits differed significantly among different potato cultivars, as evidenced by the results of the PERMANOVA analysis (p = 0.001; Figure 3). Specifically, the first axis of the PCA biplot (Figure 3) prominently reflected the impact of root exudate metabolite diversity and composition. In contrast, plant performance metrics and leaf metabolite composition mainly drove the second axis.

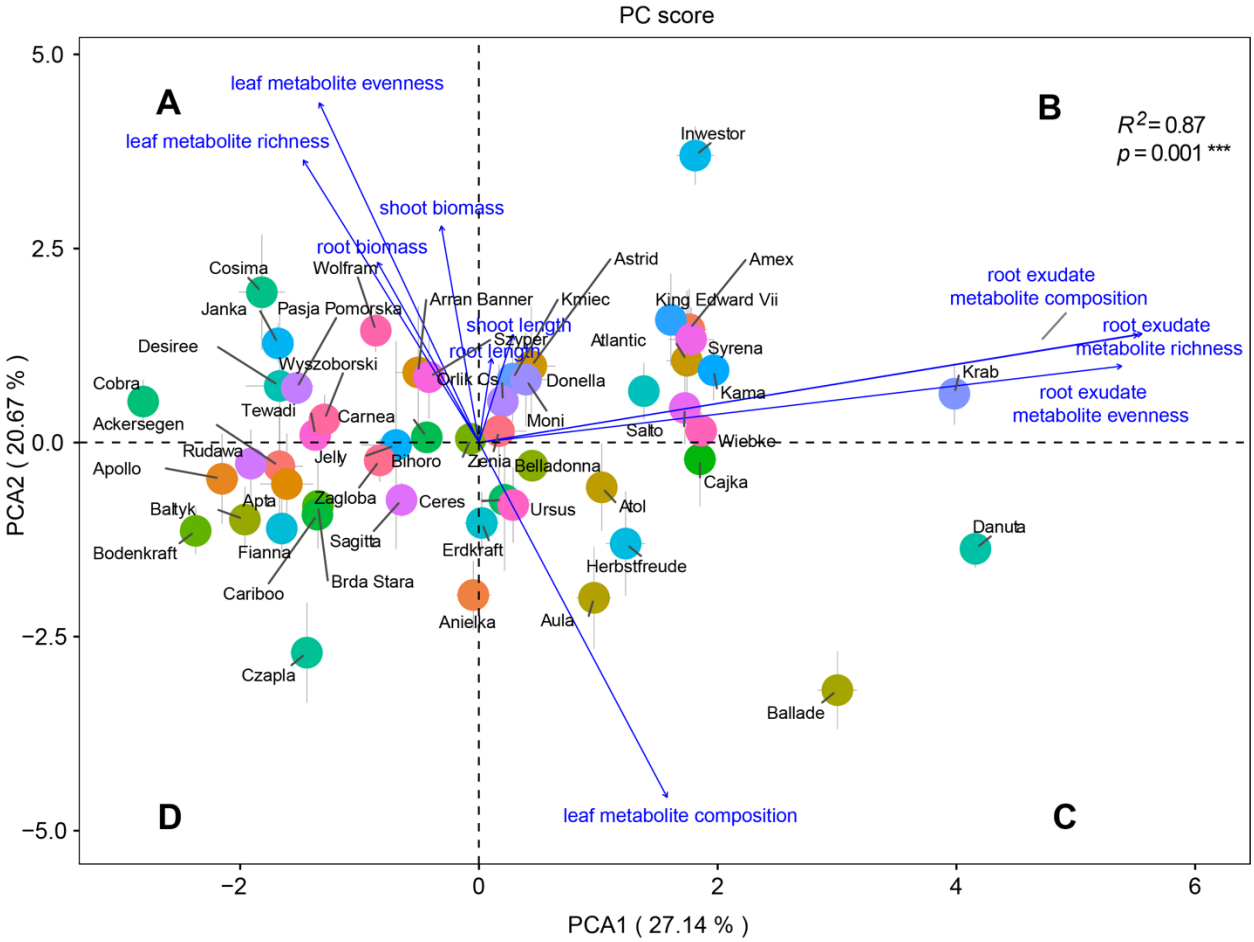

Figure 3. Distribution of 51 potato cultivars based on plant performance, root exudates and leaf metabolite profiles. Principal Component Analysis (PCA) based on the covariance matrix,

highlighting variables impacting the distribution of potato cultivars. Distinct potato cultivars are represented by different colours, with the error bars for each cultivar displayed in grey. The result of PERMANOVA is shown in the upper right, elucidating the cultivar's influence on the distribution. R^2 quantifies the explained variation, and p-values, derived from 9999 permutations, are denoted with *** for statistically significant results (p = 0.001).

Based on the distribution of cultivars and variables across the first two axes, the 51 potato cultivars were systematically categorised into four distinct functional groups (Figure 3). Group A featured cultivars exhibiting high leaf metabolite diversity and plant biomass, suggesting strong performance in leaf metabolic diversity and plant development. Group B included cultivars with high root exudates and plant length, indicating a focus on belowground metabolic processes and vertical growth of plants. Group C consisted of cultivars characterised by complex leaf metabolite composition. These cultivars may have a distinct composition of leaf metabolites compared to other groups, indicating specific leaf metabolic profiles that differentiate them from other cultivars. Group D comprised cultivars with low root exudate metabolite diversity and different leaf metabolite compositions.

3.4 Soil microbial communities

The analysis of microbial communities in bulk and rhizosphere soil revealed a significant impact of plant presence on microbial communities. At the diversity level, there was a significant difference in bacterial evenness between different soil compartments (p = 0.001; Figure S4), whereas no significant effect on fungal diversity was observed. Regarding the composition level, bacterial and fungal communities responded to the presence of plants (Figure S5). Specifically, all bulk soil samples were found to cluster together in bacterial communities, separate from the rhizosphere samples (Figure S5a). Some potato cultivars' rhizosphere fungal communities were grouped distinctly apart from the bulk soil (Figure S5b).

Comparison across the 51 potato cultivars revealed large variation and no significant effect of cultivar on bacterial and fungal alpha diversities, except for the cultivar effect on fungal evenness (p = 0.03; Figure S6). At the composition level, plant cultivars showed no significant impact but a trend towards influencing bacterial community distribution (p = 0.093 and R^2 = 0.34; Figure 4a). However, cultivars exhibited significant variation in fungal community composition (p = 0.001 and R^2 = 0.37; Figure 4b), indicating that cultivars can significantly influence the fungal community composition within their rhizosphere.

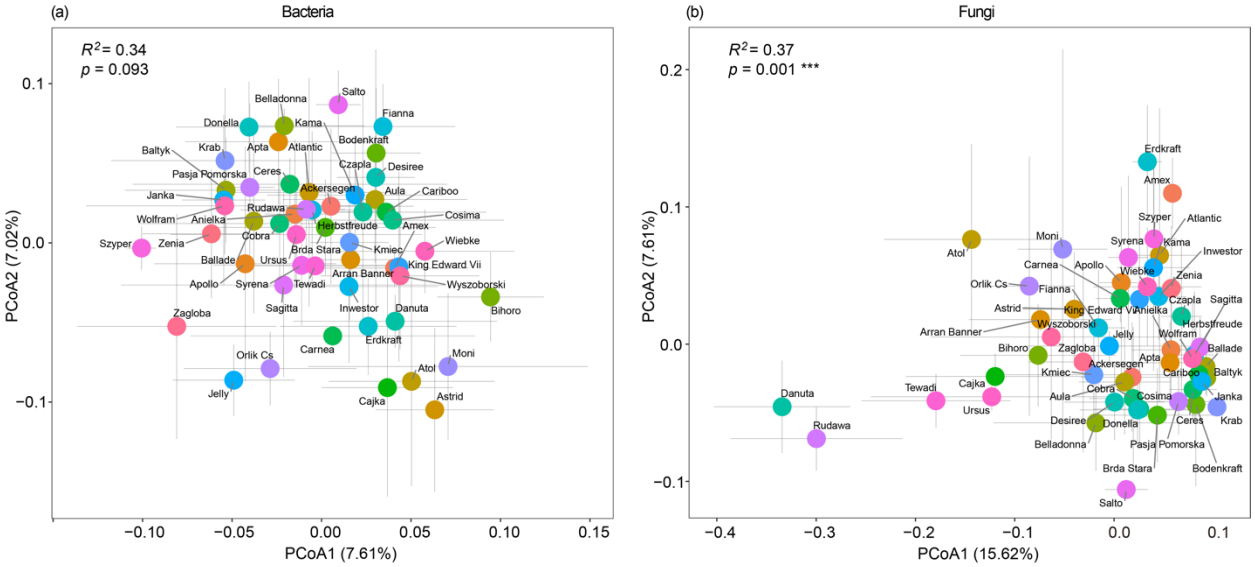

Figure 4. Rhizosphere microbial community composition. Principal Coordinates Analysis (PCoA) based on mean Bray-Curtis dissimilarity of community composition of bacteria (a) and fungi (b). Distinct potato cultivars are represented by different colours, with the error bars for each cultivar displayed in grey. PERMANOVA results in the upper left corner of each panel elucidate the influence of cultivars on community composition. R^2 quantifies the explained variation, and p-values are derived from 9999 permutations. The symbol *** denotes statistically significant p-values (p = 0.001).

Grouping the cultivars in the functional groups determined by MIT revealed no significant differences in the alpha diversity of bacterial and fungal communities between the different functional groups (p > 0.05; Figure S7). Similarly, the composition of rhizosphere microbial

communities did not vary among the different functional groups ($p_{bacteria}$ = 0.49 and p_{fungi} = 0.14; Figure S8).

3.5 Correlation between plant traits, metabolites and rhizosphere microbiome

To evaluate how different plant traits relate to root-associated microbes and metabolite release, we correlated plant shoot growth, MITs (i.e., root biomass and root exudate metabolites) with leaf metabolites and rhizosphere microbial communities of the 51 potato cultivars. Positive correlations were observed between plant biomass and leaf metabolite richness. In contrast, negative correlations were identified between plant shoot growth and leaf metabolite composition (Figure 5). Regarding the microbiome, we observed positive correlations between rhizosphere bacterial species richness and both root biomass and the root-to-shoot biomass ratio (Figure 5). Similarly, rhizosphere fungal species richness exhibited a positive correlation with the root-to-shoot biomass ratio. However, the composition of the rhizosphere microbial community showed no significant correlations with root growth. Notably, the bacteria-to-fungi species richness ratio (B/F) was positively associated with root and root-to-shoot biomass ratios. Additionally, we did not observe a pronounced correlation between root exudate metabolites and the rhizosphere microbial community (Figure 5).

We performed Mantel tests based on Bray-Curtis dissimilarity matrices to further investigate the relationship between plant leaf metabolites and soil microbial communities. Our analysis revealed a subtle yet statistically significant correlation between bacterial community compositions and leaf metabolite profiles (r = 0.10, p = 0.02). In contrast, the Mantel test for fungi showed no significant correlation between fungal community compositions and leaf metabolite profiles (r = 0.04, p = 0.22).

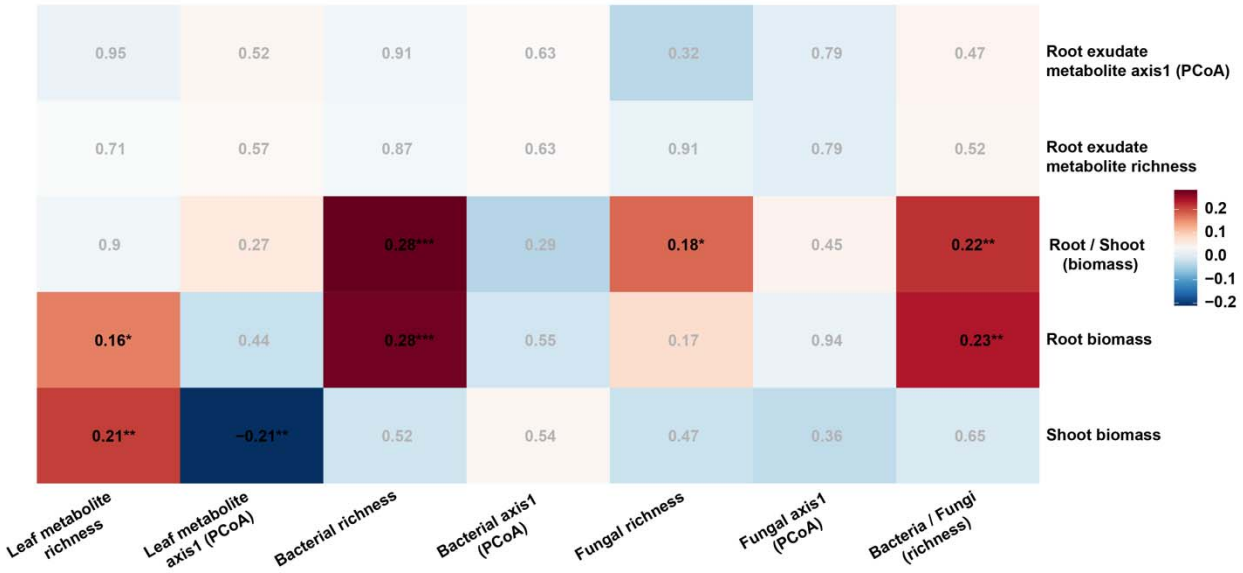

Figure 5. Correlation analysis between shoot biomass, MITs, leaf metabolite, and rhizosphere microbial community of the 51 potato cultivars. Correlations are based on the Spearman's rank correlation coefficient. Dark-coloured boxes indicate a significant correlation ($p < 0.05$), with the colour intensity reflecting the strength of the correlation coefficient. Red represents a positive correlation, while blue represents a negative correlation. The numbers displayed within the boxes represent the correlation coefficient. Significance levels are denoted as follows: * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

When examining the correlations between plant root biomass and rhizosphere bacterial community diversity across different functional groups, functional groups A and D showed significant positive correlations ($p < 0.05$; Figure S9). Functional groups B and C show no significant correlation between these variables. A positive correlation between leaf metabolite and bacterial Shannon index was found in functional group B ($p < 0.05$; Figure S10). A negative correlation was observed between leaf metabolite and fungal composition (PCoA1) ($p < 0.05$; Figure S10).

In exploring the relationship between bacterial and fungal community composition (PCoA1), a negative correlation was identified for functional group A ($p < 0.05$; Figure S11). Regarding the

relationship between bacterial and fungal richness, positive correlations were found for functional groups B and C ($p < 0.05$; Figure S11).

3.6 MIT-selected cultivars and their characterisation

The selection workflow and the chosen cultivars are visualised in Figure 6a. Classifying the 51 potato cultivars into functional groups allowed us to identify 10 representative cultivars across the four functional groups according to their microbiome diversity and composition variation, keeping the commercial cultivar Desiree as a reference. We illustrated the distribution of the rhizosphere community composition of the 51 cultivars in Figure 6b, which shows a substantial separation in bacterial and fungal compositions among the MIT-selected cultivars. These selected cultivars can undergo further exploration in real-world conditions to evaluate their potential for interacting with beneficial soil microbiomes. Furthermore, the developmental traits of the MIT-selected cultivars were comprehensively characterised in Figure S12.

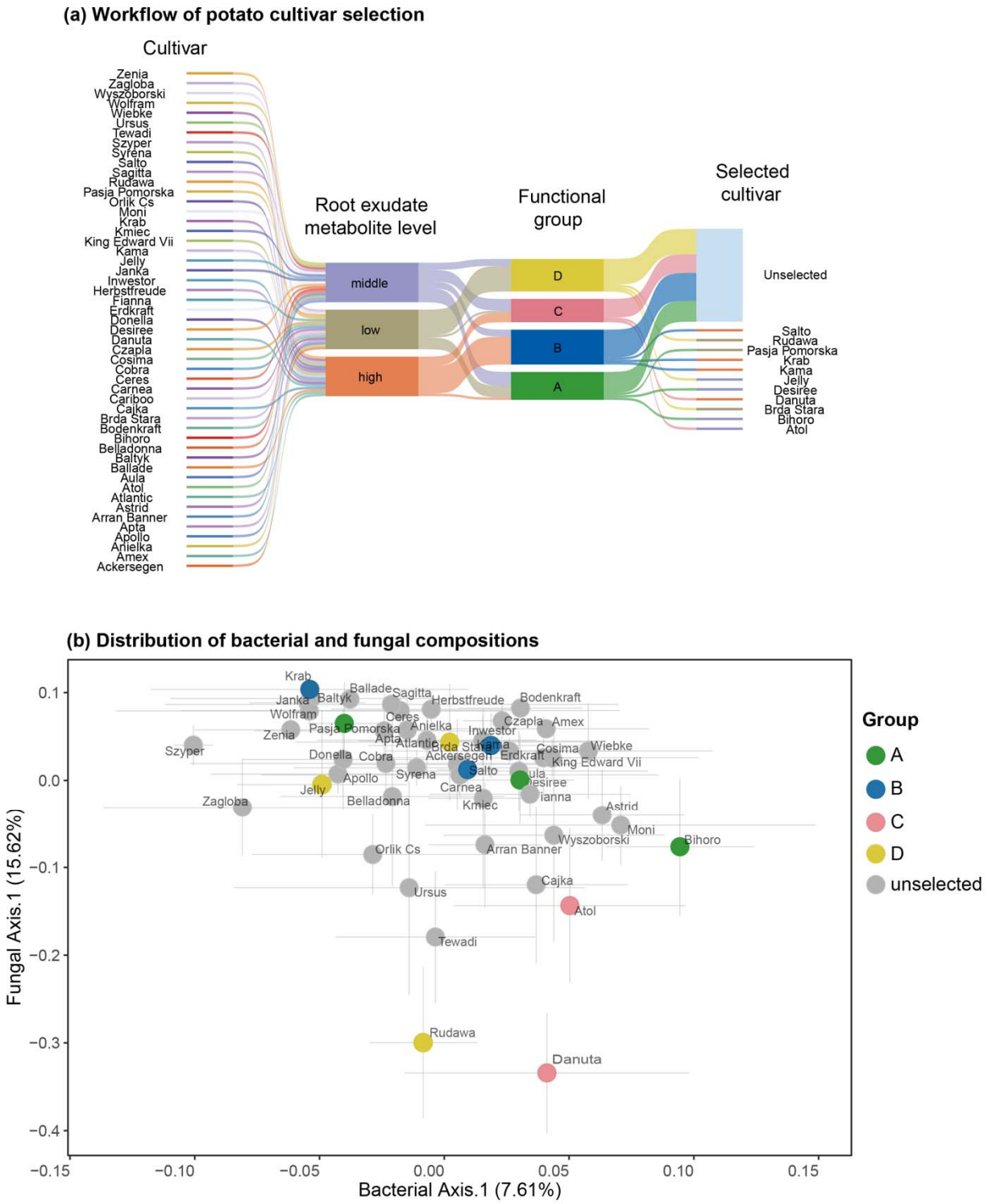

Figure 6. (a) Workflow of the potato cultivar selection by Sankey plot. The colour of selected cultivars corresponds to the level of root exudate metabolites. (b) Distribution of rhizosphere bacteria and fungi community composition through Spearman correlation analysis. The first axis

of principal coordinate analysis (PCoA1) of microbial community beta-diversity (bacteria and fungi) serves as the indicator of community composition. The cultivars that were not selected are depicted in grey. Selected potato cultivars from different functional groups are represented by different colours, with the error bars for each cultivar displayed in grey.

4. Discussion

Conventional breeding primarily harnesses genetic variation to achieve the desired traits of plants. However, the impact of a cultivar's genetic information on associated microbial organisms has yet to receive much consideration, particularly those associated with the rhizosphere. Here, we emphasise that modern breeding should consider the soil microbiome as a strategy to reduce its environmental footprint. Understanding the importance of microbiome interactive traits (MITs), such as root traits and exudates, is vital to comprehending how plants and microbiomes interact. This understanding will, in the future, contribute to informing the breeding process, providing valuable insights for developing new microbiome-based cultivars.

4.1 Correlation between plant traits, metabolites and rhizosphere microbiome

Our findings underscore the significant role of plant genetics in shaping both root exudate and leaf metabolite profiles. We observed substantial cultivar-dependent variation in root exudate metabolite composition consistent with previous studies. This genetic influence extends to leaf metabolites as well, aligning with earlier research on rice (Schaarschmidt et al., 2020). These results collectively demonstrate that the genetic makeup of different plant cultivars significantly influences the metabolite composition of different plant tissues.

We observed a positive correlation between leaf metabolite richness and plant biomass, a relationship that can be explained through considerations of resource availability and plant growth strategies. Higher plant biomass often indicates improved resource availability, including nutrients, water, and light. In optimal environmental conditions, biomass allocation primarily aboveground to compete for light can enhance the diversity of leaf metabolites instead of root (Chapin et al., 2005). The crucial role of plant secondary metabolites in host defence may also explain this correlation. These compounds protect hosts against herbivores, pathogens, and (biotic) stresses (Zaynab et al., 2018; Yadav et al., 2021; Divekar et al., 2022; Anjali et al., 2023). Plants with higher biomass may invest more in defence mechanisms, producing a wider range of leaf metabolites for protection. This establishes positive feedback loops, wherein increased

biomass boosts photosynthetic activity and energy production, consequently supporting the synthesis of more diverse metabolites.

Previous studies have demonstrated that plant root traits are crucial in regulating rhizosphere microbial communities (Szoboszlay et al., 2015; Wan et al., 2021). This relationship is further supported by Eisenhauer et al. (2017), who showed that microbial diversity increases with increasing root biomass and exudate amount. Our results align with these findings, revealing positive correlations between plant root biomass and root-to-shoot biomass ratio, rhizosphere bacterial community diversity, and bacteria-to-fungi species richness ratio (B/F). A more diverse microbial community, in turn, can enhance soil nutrient cycling and availability (Jiao et al., 2021), potentially promoting plant growth.

Previous studies have demonstrated that root exudates significantly influence bacterial and fungal communities in the rhizosphere (Hartmann et al., 2009). In contrast, our study did not find a significant correlation between root exudate metabolites and the rhizosphere microbial community. This unexpected result may be because we collected root exudate metabolites from 151 potato cultivars under in *vitro* conditions, while the microbial community data were obtained from a greenhouse experiment. The differences in substrates between these environments may have influenced the root exudate profiles, leading to variances that could explain the lack of correlation.

Given the complexity of collecting root exudates from soil plants, we collected leaf metabolites in our current experiment to explore the relationship between plant metabolites and the soil microbial community. The marginal but positive correlation observed between leaf metabolites and the rhizosphere bacterial community suggests a potential influence of leaf metabolites on the composition of rhizosphere bacteria. This finding indicates a possible link between aboveground plant tissues and belowground microbial communities, aligning with the holobiont concept, in which plants and their associated microorganisms are viewed as a holistic ecological unit (Vandenkoornhuyse et al., 2015).

Recent studies further support this interconnection. Korenblum et al. (2020) demonstrated that the composition of the rhizosphere microbial community affects the metabolomes and

transcriptomes of tomatoes' leaves and roots. Earlier research showed that rhizobacterium Bacillus strains can influence photosynthesis, leaf growth, and overall plant phenotypes by producing phytohormones or volatile organic compounds (Pang et al., 2021), potentially impacting leaf metabolomes. These findings collectively emphasise the concept of metabolites as primary mediators regulating plant-microbiome interactions within the holobiont framework (Carper et al., 2022). As described, the transport of leaf-produced metabolites to the roots via the phloem (Broussard et al., 2023) suggests a potential mechanism for how aboveground metabolites might influence root exudation patterns and, consequently, the rhizosphere microbiome. However, it's important to note that our study only demonstrates correlation, not causation. The complex interactions between leaf metabolites, root exudates, and microbial communities require further investigation to elucidate the holobiont concept fully.

4.2. Enhancing plant-microbiome interactions for sustainable agriculture

The soil microbiome promotes plant growth by promoting carbon, nutrient, and phosphorus cycling (Hartmann and Six, 2022). It also contributes to plant resistance by producing hormones that protect against abiotic and biotic stress (Eichmann et al., 2021). Despite its critical functions, the soil microbiome has received limited attention in conventional breeding (Mitter et al., 2019). Compounding this issue is the fact that conventional agricultural management not only negatively affects the environment but also substantially impacts the soil microbiome (Longepierre et al., 2021). This dual impact increases the decoupling between plants and soil microbiomes (Huang et al., 2019; Spor et al., 2020).

Studies addressing the role of plants in regulating their associated microbiome remain relatively limited (Wei and Jousset, 2017). More precisely, plant roots, serving as the primary interface for interaction with soil microbes, are underexplored in plant breeding (Reinhold-Hurek et al., 2015; Herms et al., 2022). Here, we consider morphological root characteristics and root exudate metabolites as microbiome interactive traits (MITs) to explore their interaction with the rhizosphere microbiome. We aim to supply a strategy for breeding that considers plant-associated microbiota.

Indeed, the positive correlation between root growth and rhizosphere microbial diversity indicates that MITs can aid in identifying plant cultivars with the potential to interact effectively with root-associated microbiomes. Cultivars exhibiting high MITs are likely to select for a more diverse rhizosphere microbiome, which in turn can lead to enhanced plant growth through the support of beneficial microorganisms. Identifying the genes associated with beneficial microbiomes in modern cultivars and using them in selective breeding efforts to achieve microbial-assisted cultivars, can serve as a new plant breeding strategy. This approach represents a promising avenue for sustainable agriculture, as it harnesses the power of beneficial microorganisms to improve crop performance while reducing the need for chemical inputs.

4.3. Integrating additional root traits and phyllosphere microbiome in future studies

We suggest expanding future research beyond the MITs examined in this study to include a broader range of root phenotypic traits. While the current study focuses on root biomass and length, future investigations should also include root diameter, surface area, and root type. Although less studied, evidence suggests that fine roots with smaller diameters have larger surface areas, potentially recruiting a greater diversity and abundance of microbes through enhanced nutrient and metabolite exchange (Saleem et al., 2018; Wan et al., 2021). Pérez-Jaramillo et al. (2017) linked root types (thin or thick) to specific bacterial phyla, highlighting the importance of root morphology in shaping microbial communities. Additionally, the spatial distribution of microbial communities along the root should be considered. Kawasaki et al. (2016) observed that the functional genes detected in microorganisms near the root tip were distinct from those isolated near the root base. Collectively, these various root phenotypic traits should be considered to improve plant-microbiome interactions.

In addition to expanding our focus on root phenotypic traits, we propose incorporating the phyllosphere microbiome into future studies. The phyllosphere microbiome, which includes microorganisms inhabiting the aboveground parts of plants, plays a crucial role in plant health and function (Vorholt, 2012; Thapa and Prasanna, 2018). Previous studies have demonstrated that host genotypes significantly influence the composition of phyllosphere microbial

communities (Bodenhausen et al., 2014; Thapa et al., 2017). The phyllosphere microbiome is involved in nitrogen fixation (Abadi et al., 2021), enhancing stress tolerance (Stone et al., 2018), and suppressing plant diseases (Fan et al., 2019; Das et al., 2023). Additionally, they can regulate plant growth through the production of plant hormones (Stone et al., 2018). These diverse functions highlight the importance of the phyllosphere microbiome in plant health and productivity.

By considering the phyllosphere microbiome together with the rhizosphere microbiome and plant metabolites, we can establish a more comprehensive understanding of the plant holobiont. This approach will allow us to bridge the gap between the plant's aboveground and belowground components. By harnessing the functions of phyllosphere and rhizosphere microbiomes, we may be able to enhance crop yields, improve plant resilience, and reduce reliance on chemical inputs, ultimately contributing to more sustainable agricultural systems.

5. Conclusion

This study underscores the significant impact of plant cultivars on leaf metabolites and root exudate metabolites. We also observe a positive correlation between leaf metabolites and rhizosphere bacterial community; further studies are needed to verify the causation and involve root exudates to expand our knowledge of the holobiont framework. We systematically selected potato cultivars to identify those with diverse microbiome interactive traits (MITs). We lay the foundation for further studies to evaluate the performance of MIT-selected cultivars in field conditions. This is needed to provide a promising strategy for future breeding programs, including identifying gene markers associated with a beneficial microbiome and utilising these genes to increase plant-microbiome interactions. This breeding strategy could promote host growth while reducing the reliance on synthetic chemicals in conventional agriculture. Finally, we suggest integrating additional root phenotypic traits and the phyllosphere microbiome in future studies to establish a more comprehensive understanding of the plant holobiont, which can benefit plant-microbiome interactions.

Author contributions

J.F.S., S.N.V., J.T.M.E., A.E., and E.A. designed the experiment; T.Z. and S.N.V. performed the experiment, laboratory work and data analysis; X.J. participated in data analysis; E.A., and R.G. contributed to plant sampling; D.M. and K.T. provided the potato materials; A.E., S.S., J.K., and E.Z. collected and explored plant metabolites. T.Z. drafted the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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