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Research Article

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Cultivar-dependent differences in plant bud microbiome and functional gene pathways in woody plants commonly used in urban green space

Marja I. Roslund [1], Polina Galitskaya[2], Mika Saarenpää[1,3], Aki Sinkkonen[1,*]

[1]Natural Resources Institute Finland Luke, Latokartanonkaari 9, FI-00790 Helsinki, Finland
[2]Research Institute for Environmental Studies, 2775-083 Parede, Portugal
[3]Ecosystems and Environment Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Niemenkatu 73, FI-15140 Lahti, Finland

*Corresponding author. Horticulture technologies, Natural Resources Institute Finland, Itäinen Pitkäkatu 4A, FI-20520 Turku, Finland.
E-mail: aki.sinkkonen@luke.fi

Abstract

Plant richness and microbiota have been associated with plant health; hardly any studies have investigated how plant taxa differs in microbiota in the context of human health. We investigated the microbial differences in buds of 83 woody plant taxa used in urban green spaces in hemiboreal climate, using 16S rRNA and whole metagenome shotgun sequencing. Bud microbial community was the richest in *Cotoneaster Nanshan* and *C. integerrimus,* and *Malus domestica* cultivars "Sandra" and "Lobo" and poorest in *Ribes glandulosum*. Metagenomic shotgun sequencing of two *M. domestica* and four *Ribes* varieties confirmed differences in taxa in bud microbiota and indicated higher siderophore synthesis in *Malus*. Microbial richness, including bacteria, archaea, and viruses, and functional richness of gene pathways was higher in *Malus* compared to *Ribes*. The 10 most abundant amplicon sequence units, often referred as species, belonged to the phylum *Proteobacteria*. The differences between plant taxa were evident in classes *Alpha-* and *Gammaproteobacteria*, known for potential human health benefits. Since environmental microbiota contributes to human microbiota and immunoregulation, horticultural cultivars hosting rich microbiota may have human health benefits. Further studies are needed to confirm the effectiveness of microbially-oriented plant selection in optimizing human microbiota and planetary health.

Impact Statement

We demonstrated differences in richness of bud bacteria in 83 woody ornamental and fruit cultivars suitable for urban rewilding. Bud microbial community was the richest in many Rosaceae and poorest in Ericaceae and Grossulariaceae cultivars. The differences between plant taxa were striking in classes *Alpha-* and *Gammaproteobacteria*, known for potential human health benefits. The 10 most abundant amplicon sequence units belonged to *Proteobacteria*. Metagenomic shotgun sequencing of *Malus and Ribes* confirmed these differences and indicated a siderophore synthesis in *Malus*. This is the first report to suggest human health associated differences in microbiota of green space plants and warrant further research.

Keywords: plant bud microbiome; shotgun metagenomes; planetary health; urban green space

Introduction

Plants and humans are both holobionts, consisting of a host and trillions of symbiotic microorganisms living on and inside us, or hologenomes, comprising the host and microbial genomes (Simon et al. 2019). Urban environment characterized by sealed surface shapes the microbiota of these holobionts (Mills et al. 2020, Robinson et al. 2021). House plants, indoor gardening and outdoor vegetation can modify indoor microbial communities, which is associated with reduced pro-inflammatory markers in serum (Dockx et al. 2021, 2022, Soininen et al. 2022, Saarenpää et al. 2024, Zhao et al. 2024a). Diverse home yard and daycare yard vegetation is associated with microbiota on human skin and in gastrointestinal tract, which in turn is linked to enhanced immune regulation (Parajuli et al. 2020, Roslund et al. 2020, 2021). Plant microbiota in urban green spaces, like private gardens, are interlinked with human microbiota and health (Berg et al. 2014, Grönroos et al. 2019, Krishna et al. 2019, Wu et al. 2022, Y.-D. Zhang et al. 2023a, Zhao et al. 2024b). Although plants are known to contribute to human microbiota, there is little to no information regarding the microbial communities of different horticultural plant species and cultivars in the context of plant and human health.

Biodiversity loss, including the visible plant species and invisible microbiological diversity, is linked to the rising burden of immune mediated diseases among urban dwellers (Ege et al. 2011, Haahtela et al. 2021, Hanski et al. 2012, Kirjavainen et al. 2019, Ruokolainen et al. 2015). The main reason for the increased prevalence of immune mediated diseases is suggested to be the reduced contact with environmental microbial diversity, which prevents the priming of the immune system in childhood (Rook and Brunet 2002, Rook 2009, Kondrashova et al. 2013, Haahtela 2019). Therefore, there is an unmet need for microbial rewilding of urban areas to enhance planetary health.

Particularly, the diversity of *Alpha-* and *Gammaproteobacteria* could be beneficial both to plant and human health for several reasons. First, microbial diversity has been identified as a key factor in plant disease resistance (Berg et al. 2017, Yan et al. 2017, Wang et al. 2021). *Alpha-* and *Gammaproteobac-*

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teria include plant growth promoting bacteria, such as species in *Pseudomonas*, which are also indicators for horticulture in fruits (Glick 2012, Köberl et al. 2015, Wicaksono et al. 2023). *Alpha-* and *Gammaproteobacteria* seem to play an important role in immunological resilience of humans (Fyhrquist et al. 2014, Hanski et al. 2012, Roslund et al. 2020, 2024, Ruokolainen et al. 2015). Many species of *Alpha-* and *Gammaproteobacteria* are opportunistic pathogens, which indicates their potential role in the priming of the immune system in childhood (Ruokolainen et al. 2015). The diversity of these bacterial taxa is observed to be higher in natural environments with high plant species richness compared to urban environments with low plant species richness (Parajuli et al. 2018, Hui et al. 2019, Roslund et al. 2021). However, these previous studies did not estimate the microbial diversity of plant species. Understanding and harnessing the microbial communities and functions of microbiota between different plant species and cultivars thus holds great potential for sustainable agriculture and ecosystem management as well as human health. As microbiological diversity supports the health of the plants and humans, there is an unmet need to estimate microbial communities in different plant species and cultivars. Plant cultivars with high microbial diversity could be used in urban rewilding approaches to support human immune system.

To address this need, we analyzed metagenomes of horticultural bud samples of woody plant taxa, covering 59 species with altogether 83 cultivars. Bud microbiota was analyzed because they represent an early developmental stage that harbors potential leaf colonizers, and buds are less exposed to external environmental factors, like UV radiation and pollutants. Since previous studies indicate that the diversity of *Alpha-* and *Gammaproteobacteria* on the skin is associated with enhanced immune regulation (Hanski et al. 2012, Roslund et al. 2020, 2022, Soininen et al. 2022), and since these classes include many plant health promoting bacteria (Glick 2012), we specifically focused on the richness and diversity of these bacterial taxa between plant cultivars. In addition, since an earlier study indicated differences in functional gene pathways in apples (Wicaksono et al. 2023), and since optimal growing conditions of fruit producing species vary, we analyzed bud functional gene pathways of edible apple and currant (*Ribes*) cultivars. The hypotheses were as follows:

(1) Bacterial diversity (Alpha and Beta) and richness, estimated from 16S rRNA, particularly alpha- and gammaproteobacterial, differ between plant taxa, including conspecific cultivars.
(2) Shotgun metagenomic analysis reveals differences in microbial taxa and functional gene pathways of edible apple (*Malus*) and currant (*Ribes*) cultivars.
(3) Differences between microbiota of different plants are related to the frequency of human health-associated functional gene pathways in plants.

Materials and methods

Bud sample collection

83 different cultivars belonging to 72 unique plant species and 38 genera were included in the study (Fig. S1). The brunches with buds were sampled in February 2022 in two research stations of Natural Resources Institute Finland—Tuorla (60.4145, 22.4367) and Yltöinen (60.3875, 22.5534). The cultivars represented the following plant families: Adoxaceae, Betulaceae, Caprifoliaceae, Elaeagnaceae, Ericaceae, Grossulariaceae, Hamamelidaceae, Hydrangeaceae, Magnoliaceae, Oleaceae, Rosaceae, Salicaceae, Ulmaceae (Fig. S1). Plants at Yltöinen research station were planted earlier (plant mean age 46 ± standard deviation 25 years) compared to plants in Tuorla research station (12 ± 5 years) (Table S1). They belonged to commercially available cultivars and were thus grown from cuttings. Three plants per cultivar were haphazardly selected for the study. From each plant, a 5–10 cm long branch that contained at least three buds was cut (Fig. S3). The branches were cut from the plants using sterilized equipment, placed in sterilized plastic bags and transported to −80°C for further laboratory analysis. In general, soils in Yltöinen and Tuorla are fine-grained silt and clay characterized by rich organic content, but all plants were grown in species-specific substrates, e.g. *Malus* and *Ribes* were grown in slightly acidic (pH 5.5–6.5) while *Vaccinium* and *Rhododendron* in acidic (pH < 5.5) organic substrates. More detailed information on the cultivars is presented in Supplement Table S1.

DNA extraction and 16S rRNA sequencing

DNA extraction and sequencing were conducted in BGI lab in Hong Kong. Before DNA extraction, outermost brown bud scales were peeled, and DNA was extracted from the axillary vegetative naked buds, three buds per sample and three samples from each cultivar (Fig. S2).

DNA was extracted with DNeasy® PowerSoil® Pro Kit (Qiagen). Thirty nanogram qualified DNA template and the 16S rRNA fusion primers were added for Polymerase chain reaction (PCR). All PCR products were purified by Agencourt AMPureXP beads, dissolved in Elution Buffer and eventually labeled to finish library construction. Library size and concentration were detected by Agilent 2100 Bioanalyzer. Qualified libraries are sequenced on HiSeq platform according to their insert size.

Raw paired-end sequence files were processed into amplicon sequence variants (ASVs) with the non-redundant Silva database version 138 (Quast et al. 2012) using DADA2 (Callahan et al. 2016). To conceptualize the different sequence read depths, the data were normalized with centered clr transformation.

Shotgun metagenomic analysis

Whole metagenome shotgun sequencing was done for two apple species (*Malus domestica* "Lobo" and "Sandra") and for four Ribes cultivars (*Ribes Rubrum* cultivars: "Piikkiön Helmi" and "Punahilkka," *Ribes Nigrum* "Venny" and *Ribes uva Crispa* cultivar "Lepaan punainen") to test species-level resolution of the microbial content of each sample while also enabling comparisons at the functional gene level.

Libraries generated from total genomic DNA extracted from bud samples was sequenced on the Noveseq S1/300c (Illumina) platform using the 2 × 150 bp paired-end read protocol. The quality of the sequencing reads was assessed with FastQC and low-quality, ambiguous sequences, adapters and contaminants were removed. Taxonomic profiling of the final read set was performed using Kraken2 (Wood et al. 2019), while functional profiling was carried out using HUMAnN3 (Beghini et al. 2021).

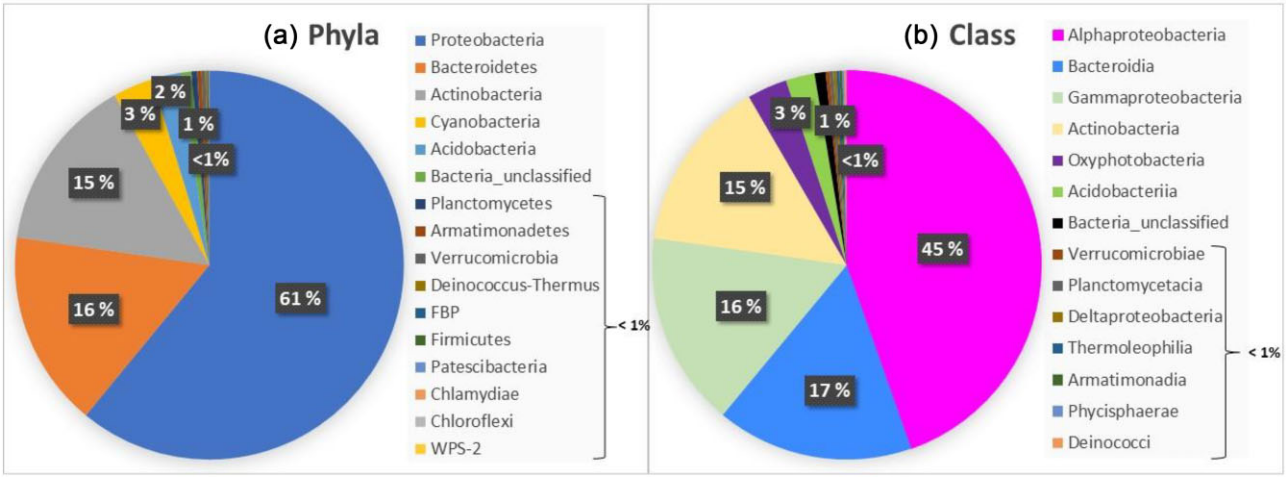

Figure 1. Dominating (a) phyla and (b) classes in the plant bud microbiomes.

Statistical analysis

All the statistical tests were done with R v4.3.1 (R core team, 2018).

Analysis of variance (ANOVA) (function *aov*) was used to analyze differences in microbial richness, diversity, and relative abundance (taxa and functional gene pathways) or in case of non-normally distributed data with the Kruskal–Wallis test (function *Kruskal.test*). Pairwise analyses were done with *pairwisePermutationTest* in *rcompanion* package with Benjamini–Hochberg correction to conceptualize the false discovery rate (FDR). ANOVA and pairwise tests were used for 16S rRNA and whole metagenome shotgun sequencing data.

Bud 16S rRNA bacterial beta diversity was analyzed with Permutational Multivariate Analysis of Covariance (PERMANOVA, function *adonis2* in *vegan* package) (Anderson 2017). Principal coordinates analysis (PCoA) with Euclidian distance was used to visualize the difference in bacterial community composition (*cmdscale* function). All the statistical tests were considered significant when permuted *P*-value was < 0.05 level.

Results and discussion

Differences in bud microbial communities in 16S rRNA sequencing

We selected 83 horticultural cultivars common in urban greening for 16S rRNA microbiome screening (Table S1). Sequencing of the amplicon libraries of plant bud microbiome resulted in a total of 2 803 416 bacterial high-quality reads from all the 250 samples (mean 11 213 ± standard deviation 19 890 per sample), and total of 6 633 974 (26 536 ± 14 325) mitochondrial and 26 030 042 (104 120 ± 23 790) chloroplast reads. Among all samples, 4 225 ASVs were detected. The most abundant phyla were *Proteobacteria* (61%), *Bacteroidetes* (16%) and *Actinobacteria* (15%) at the phylum level and *Alphaproteobacteria* (45%), *Bacteroidia* (17%), *Gammaproteobacteria* (16%) and *Actinobacteria* (15%) at the class level (Fig. 1). The dominating ASVs were found in every bud sample and these ASVs belonged to the classes *Alpha-* and *Gammaproteobacteria* (Table 1). These findings indicate that plants harbor and potentially are a source of previously health-associated microbiota for humans.

The bacterial richness depended on genus, species and genera (Fig. 2; Table S1; ANOVA and pairwise permutation results are in Table S2A). Bacterial richness (number of ASV with 3 and more sequences in the buds) differed between cultivars ranging between 19 (*Ribes glandulosum*) and 1036 (*Cotoneaster nanshan*) (Fig. 2). These findings indicate that some plant taxa host superior bacterial richness, as compared to other plant taxa. Particularly several cultivars within Rosaceae seem to carry rich bacterial community in buds. Interestingly, *Rosa* sp. contained poorer richness.

The highest Shannon index was observed in *C. nanshan* buds (mean 5.45 ± SD 0.07) and the lowest in *Vaccinum uliginosum* (0.71 ± 0.91). The pairwise permutation test revealed that Shannon index' values in *C. nanshan* and *C. integerrimus* buds differed significantly from 66 cultivars while that in *R. glandulosum* differed significantly from 52 cultivars ($P \leq 0.05$; Table S2B). For 18 cultivars the Shannon diversity index exceeded 4.01. The majority (14) of these belong to Rosaceae, 2 Salicaceae and a single cultivar to Grossulariaceae and Caprifoliaceae. This may partly reflect the uneven distribution of different Families and be also related to family level differences in diversity of plant bacterial communities. Interestingly, most data published on Shannon index values for bacterial communities associated with plants are lower than ours, most likely because they were calculated on the basis of abundances of OTU but not ASV as done in our study (Elmagzob et al. 2019, Patturaj et al. 2021).

The lowest bacterial diversity and richness were observed in *Ribes* species known for phytochemicals with strong odor (black currant *R. nigrum* and skunk currant *R. glandulosum*, and Ericaceous genera known for phytochemicals and wax, e.g., *Rhododendron* and *Vaccinum* (Fig. 2; Table S1). Unfortunately, earlier bacterial ASV data on bud bacteria are not available, but earlier studies on leaf and bark bacterial ASVs of tea, tobacco and beech resulted in as low richness and Shannon diversity index levels as we found in Ericaceous species and *Ribes* with strong odor (Chen et al. 2021, Dreyling et al. 2022, Zhang et al. 2023b). Since the earlier studied plants are known for phytochemicals, the results hint that phytochemicals contribute to microbial diversity.

We used PERMANOVA to compare bacterial community (16S rRNA data) of cultivars and visualized the centroids and dispersion, i.e. beta diversity, of the microbial

Table 1. Dominating ASVs that were found in every bud sample.

Class	Order	Family	Genus	Average relative abundance%
Alpharoteobacteria	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	10.0
Alpharoteobacteria	*Rhizobiales*	*Beijerinckiaceae*	*1174_901_12*	9.3
Gammaproteobacteria	*Betaproteobacteriales*	*Burkholderiaceae*	*Massilia*	9.3
Alpharoteobacteria	*Rhizobiales*	*Beijerinckiaceae*	*Methylobacterium*	8.2
Gammaproteobacteria	*Betaproteobacteriales*	*Burkholderiaceae*	-	7.7
Alpharoteobacteria	*Acetobacterales*	*Acetobacteraceae*	-	7.7
Alpharoteobacteria	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	7.6
Alpharoteobacteria	*Acetobacterales*	*Acetobacteraceae*	*Acidiphilium*	7.6
Gammaproteobacteria	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*	7.5
Gammaproteobacteria	*Betaproteobacteriales*	*Burkholderiaceae*	*Massilia*	7.0

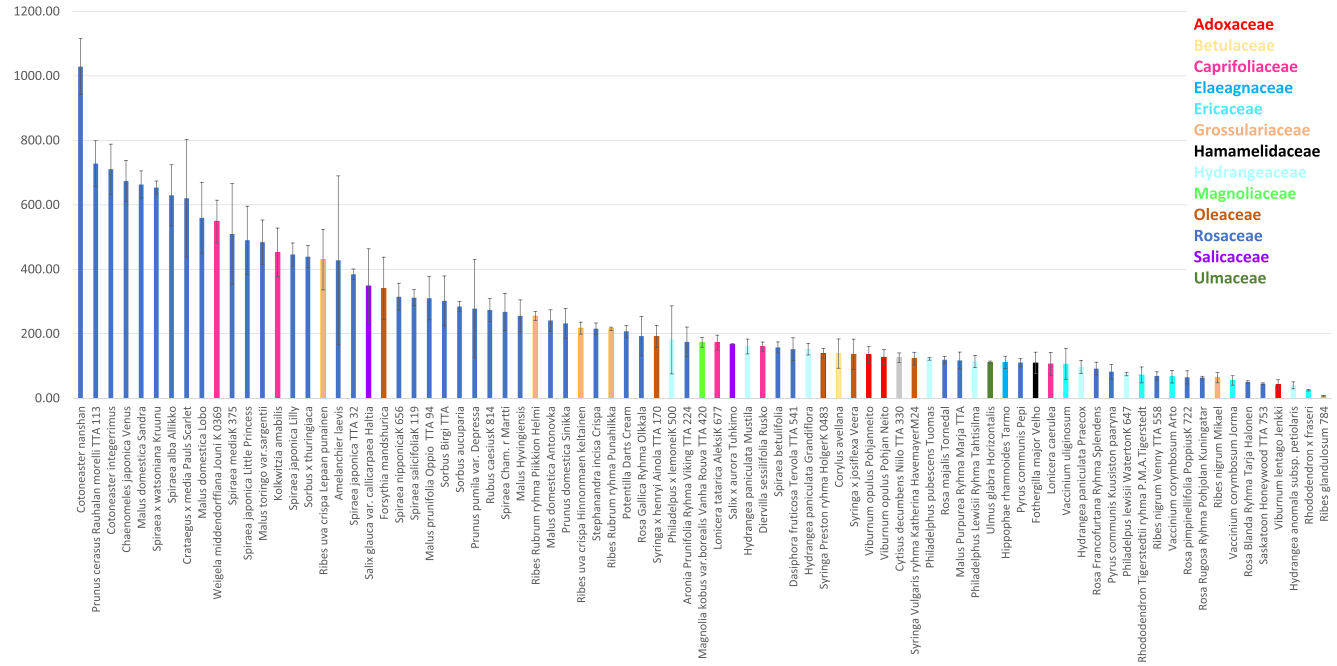

Figure 2. Bacterial richness (number of ASVs) found in buds of different plant cultivars. Data are presented as mean ± standard error ($n = 3$ per cultivar) for 65 cultivars (richness of all the 85 cultivars can be found in Table S1). Colors represent the plant families.

communities with principal component analysis (Fig. 3). Beta diversity of bacteria depended on horticultural cultivar, plant species, genus and family (Fig. 3; PERMANOVA: $P = 0.001$). The bud bacterial communities of most genera overlap with several other genera (Fig. 3). Magnoliaceae (represented by 1 species) has a distinctive, non-overlapping community. Two families represented by a single native species (Betulaceae and Ulmaceae) and Fabaceae (1 species) have almost separate ellipses in the opposite corner to Magnoliaceae (Fig. 3). These differences may be related to plant characteristics, such as evolutionary history, leaf morphology and phytochemistry. The general trend is that ellipse size is related to the number of cultivars in a family in our study (Fig. 3).

The ten most dominant ASVs belonged to the phylum Proteobacteria (Table 1). Among the other abundant ASV were bacteria in Actinobacteria and Bacteroidetes. Those phyla were found to predominate in the aboveground plant vegetative organs earlier as well (Elmagzob et al. 2019, Chen et al. 2021, Patturaj et al. 2021); Siegenthaler et al. (2024) compared the bacterial communities of two temperate forest trees (beech and spruce) and showed that dominating taxa were relatively uniform while minor ASVs were host-specific. Our study is the first to perform a large-scale survey of 83 woody plants: Remarkable differences exist in the bud microbiota in many abundant bacterial classes between woody plant taxa and between genotypes of the same taxa.

ASV from genera *Sphingomonas*, *Massilia*, *Beijerinckiaceae 1174_901_12* and *Methylobacterium* were the most abundant in the bud samples investigated (Table 1). *Sphingomonas* species are commonly found in the plant rhizosphere, but they have also been detected in the plant endosphere. They are known to possess plant growth-promoting properties. Specifically, they can produce auxin and nitric oxide, create siderophores, and modulate plant hormones such as abscisic acid, jasmonic acid, and salicylic acid. These functions are crucial for plant development and defense mechanisms (Khan et al. 2017, Mazoyon et al. 2023).

Massilia species have been documented in various environmental contexts, including rhizosphere and phyllosphere

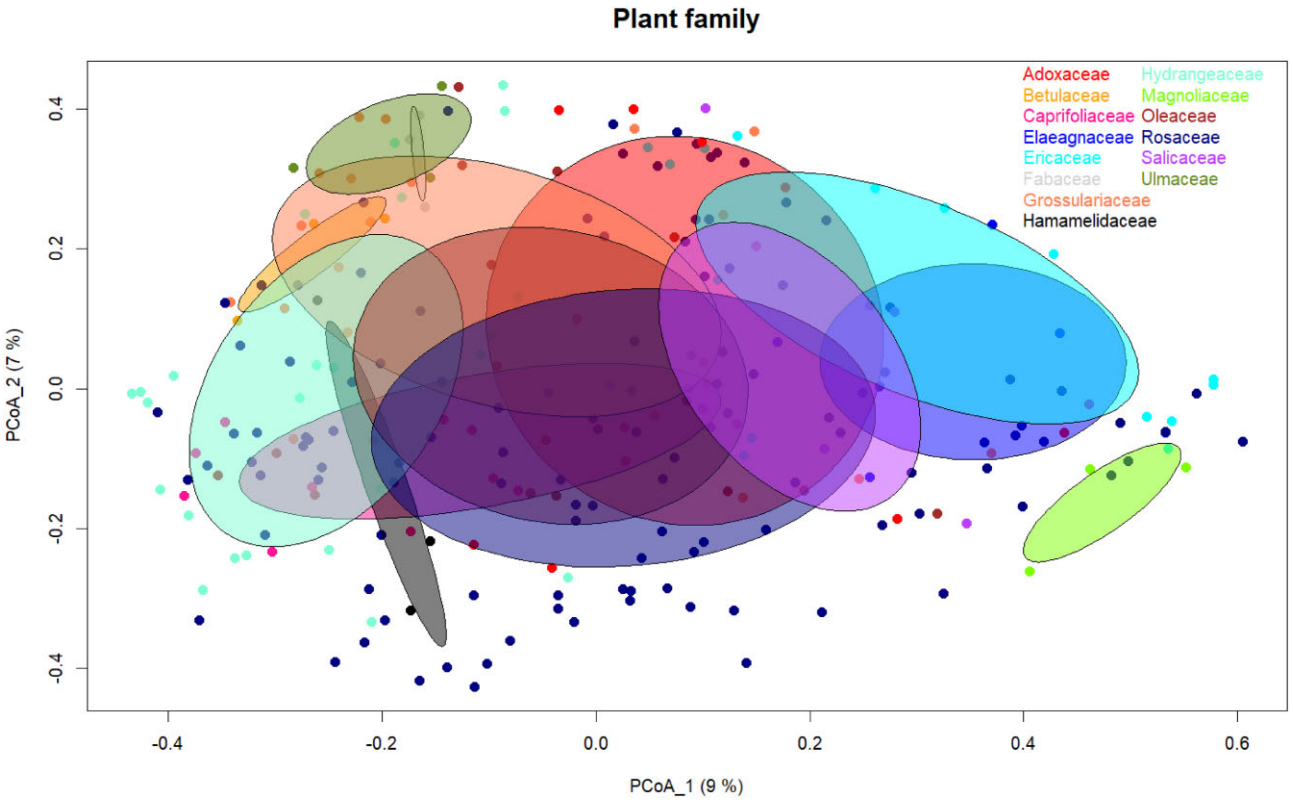

Figure 3. Beta diversity of bacteria in plant buds at family level.

of plants and soil. *Massilia* are enriched in the rhizosphere of lateral roots and cortex tissue, highlighting their potential involvement in root–microbe interactions and influence on plant development, and they play a dominating role in *Cannabis* endophytic microbiome (Jeon et al. 2023, Xu et al. 2023). Since we found *Massilia* abundantly in spring buds, we assume that the prevalence of the genus in soil is partly reflected in bud microbiota, and on the other hand, the prevalence of *Massilia* in bud microbiota may aid to become abundant in soil and roots; deciduous leaves and inflorecenses fall down and eventually form a major part of organic top layer in temperate and hemiboreal soils.

The high abundance of *Methylobacterium* may be beneficial for plant health. *Methylobacterium* representatives exhibit plant growth-promoting characteristics such as phytohormone production, nutrient provision, and inhibition of the phytopathogens (Dourado et al. 2023, Tani et al. 2023, Walitang et al. 2023). A *1174–901-12* representative of Beijerinckiaceae was beneficial for plant growth and inhibitive for plant pathogens (Ares et al. 2021, Jo et al. 2022, Chen et al. 2023, Mori et al. 2023, Wei et al. 2023, Siegenthaler et al. 2024).

Since our analysis separated bud surface from the interior, we analyzed only endophytic bacteria inside buds. Interestingly, Lee et al. (2023) revealed 232–546 ASVs in leaf bud endosphere, which is comparable with our data for *Malus* cultivars (117–667 ASVs). To summarize the findings of our survey of bacterial taxa of 83 woody plant cultivars, bud microbiota of some taxa is surprisingly poor while most taxa have rich microbial community in spring buds. Poor bacterial community was found in species known for surface wax or strong defense chemicals. Several of the most abundant bacteria were earlier observed to be beneficial for plants per se, which is promising in the context of stress tolerance in urban green space. The plant growth promoting bacteria were found abundantly in taxa that host rich bud microbiota.

Shotgun metagenomics revealed differences in microbial taxa and functional gene pathways within and between plant species

Six representative cultivars were selected for whole metagenome shotgun sequencing, we selected cultivars in *Malus* and *Ribes* because they are the most commonly grown fruit and berries in Finland. Within these genera, we selected cultivars with high and low bacterial richness and diversity. Therefore, the cultivars included were *Malus domestica* "Lobo" and "Sandra," and *R. rubrum* "Piikkiön Helmi" and "Punahilkka," *R. uva-crispa* "Lepaan punainen," *R. nigrum* "Venny." Shotgun metagenome sequencing provides higher resolution compared to 16S rRNA sequencing, therefore, it can identify microbes to strain level and the functional gene pathways. In addition, this method captures DNA from all organisms in the sample, such as viruses, fungi and archaea, providing a more complete picture of the microbial community.

Shotgun sequences resulted in a total of 712 438 771 high-quality clean reads (quality scores% = 38–60, Q20% = 92–97) from 18 samples (mean 39 579 931 ± standard deviation 1 917 801 per sample).

Microbial species richness, including bacteria, archaea and viruses, in apple varieties *Malus domestica* "Lobo" (mean ± SD = 7289 ± 284) and *M. domestica* "Sandra" (7372 ± 232) was higher compared to *Ribes* varieties (4003–6255 ± 85–223) (ANOVA $P < 0.0001$, pairwise permuted

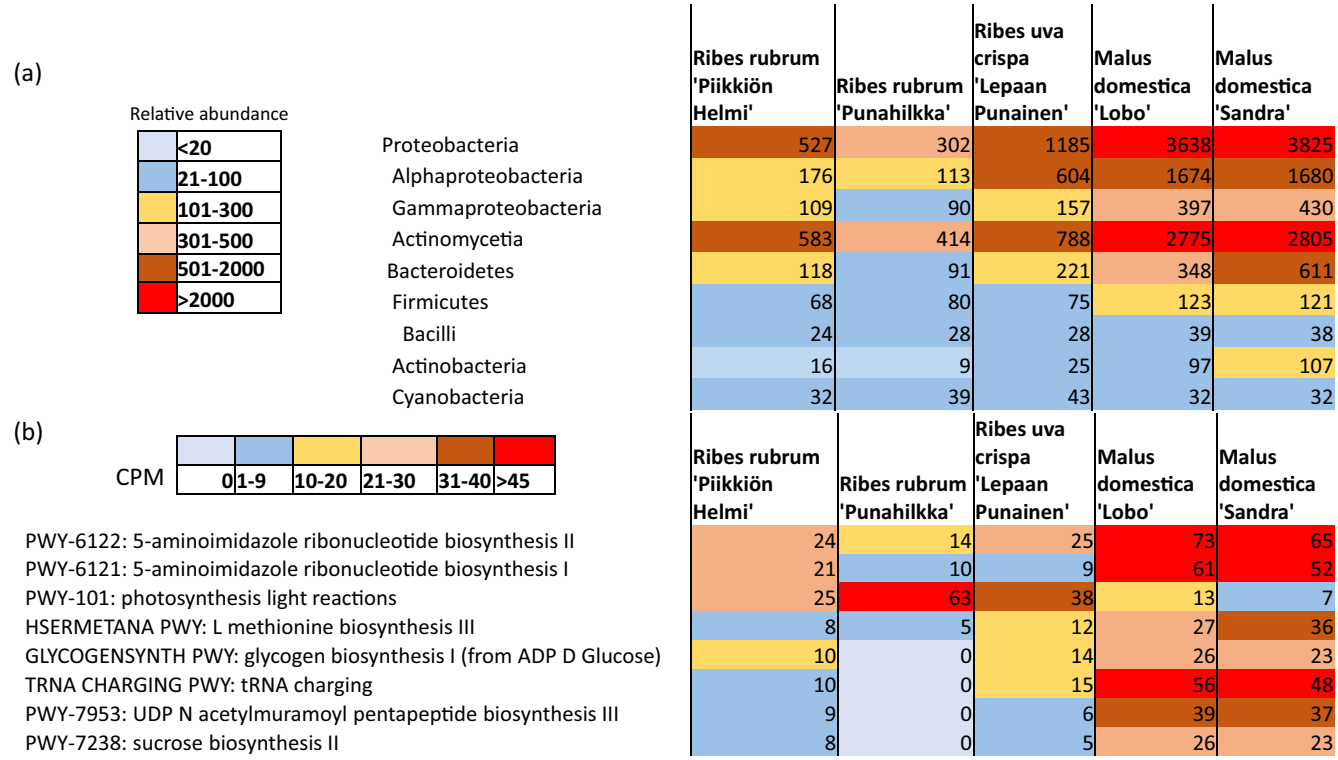

Figure 4. (a) Heatmap of major phyla and class of *Ribes* and *Malus* cultivars. Heatmap at genus level is provided in the supplementary Fig. S3. (b) Heatmap of functional pathways that differed between Ribes and Malus cultivars. HUMAnN's default values are normalized to relative abundance "copies per million" (CPM).

$P < 0.03$), while *Ribes Nigrum* "Venny" (4003 ± 223) had lowest richness (pairwise permuted $P < 0.05$) (Table S3). In addition, functional richness and Shannon diversity index was highest in *M. domestica* varieties, and lowest in *R. nigrum* "Venny" (Table S3).

Whole shotgun metagenome analysis revealed that apple cultivars "Lobo" and "Sandra" had more strains in *Proteobacteria*, including classes *Alpha-* and *Gammaproteobacteria*, when compared to *Ribes* species (*R. rubrum* "Piikkiön Helmi" and "Punahilkka," *R. uva-crispa* "Lepaan punainen," *R. nigrum* "Venny") Kruskal–Wallis test: $P = 0.01$, pairwise permuted $P < 0.04$) (Tables S4 and S5; Fig. 4a). Interestingly, previously health related acinetobacterial and mycobacterial abundances were higher in these two apple varieties compared to *Ribes* varieties (ANOVA $P < 0.001$, pairwise permuted $P < 0.05$; Table S4; Fig. S3). At the bacterial species level, the abundance of particularly *Acinetobacter lwoffii*, previously related to a low risk of atopy and allergies (Fyhrquist et al. 2014, Hanski et al. 2012), was higher in apples (ANOVA $P < 0.001$; Table S4; Fig. S3). However, only *M. domestica* "Sandra" had significantly higher *A. Lwoffii* abundance when compared to *Ribes* varieties (pairwise permuted $P = 0.03$; Fig. S3).

Differences in functional gene pathways between apple and *Ribes* species included 5-aminoimidazole ribonucleotide biosynthesis I (ANOVA P = 0.02) and II, photosynthesis light reactions, glycogen biosynthesis I (from ADP-D-Glucose) (ANOVA P = 0.007), L-methionine biosynthesis III, tRNA charging (ANOVA $P = 0.004$), UDP-N-acetylmuramoyl-pentapeptide biosynthesis III (meso-diaminopimelate containing) (ANOVA $P = 0.008$), and sucrose biosynthesis II (ANOVA $P = 0.002$) (Table S5; Fig. 4b). In detail, *Malus* cultivars "Lobo" and "Sandra" had higher abundance of these gene pathways compared to *R. rubrum* "Punahilkka" (pairwise permuted $P < 0.05$). *Malus* and *R. uva-crispa* "Lepaan Punainen" varieties had gene pathways related to glycogen degradation II, while *Ribes* cultivars "Piikkiön helmi" and "Punahilkka" had not (ANOVA $P = 0.0003$; Fig. 4b). *R. nigrum* "Venny" had only five abundant functions, therefore, it was excluded from the functional analyses.

The biosynthesis of 5-aminoimidazole ribonucleotide (AIR) is highly important in the context of plant and human health, because AIR contributes to the overall metabolic capabilities of siderophore-producing microbes by providing the necessary purine nucleotides for various cellular processes (Stevens et al. 2000). In the context of plant health, by supporting siderophore production, microbes can significantly enhance iron availability, suppress plant pathogens, stimulate plant hormone production, and improve soil health (Timofeeva et al. 2022). These combined effects can lead to improved plant growth and productivity, highlighting the importance of microbial functions in horticultural cultivars. Because microbiota of plants in urban green spaces are interlinked with human microbiota, it would be interesting to investigate if cultivars with high abundance of important gene pathways, such as AIR, could contribute to human health. Indeed, in the context of human health, the biosynthesis of AIR, a crucial intermediate in the biosynthesis of purine nucleotides, can contribute to cellular repair and genomic stability (Corton et al. 1995); purine nucleotides are involved in the synthesis of neurotransmitters that participate in regulating neuronal activity and eventually mood (Mutafova-Yambolieva and Durnin 2014). Thus, our findings support the hypothesis that differences in functional metagenomes are connected to potential synthesis of human health-associated compounds in

Table 2. Richness and Shannon diversity index of 16 of most promising cultivars in the context of human microbial exposure.

	Richness		Shannon index	
	Mean	SD	Mean	SD
Crataegus x media 'Pauls Scarlet'	620.33	258.7	4.33	0.8
Kolkwitzia amabilis	452.33	106.71	4.31	0.15
Spiraea media	509.33	221.07	4.19	0.09
Spiraea japonica 'Lilly'	445.67	50.89	4.14	0.09
Ribes uva crispa 'Lepaan punainen'	429.67	132.71	4.12	0.19
Malus domestica 'Sandra'	663	59.67	4.11	0.16
Chaenomeles japonica 'Venus'	673.67	90.26	4.07	0.29
Spiraea japonica ' Little Princess'	490	149.53	4	0.19
Weigela middendorffiana 'Jouni'	549	92.4	3.92	0.36
Malus domestica toringo var. sargentii	484	97.2	3.9	0.19
Spiraea x watsoniana 'Kruunu'	653.33	28.55	3.88	0.04
Prunus cerasus 'Rauhalan morelli'	727.67	100.45	3.81	0.4
Sorbus x thuringiaca	439	49	3.81	0.21
Spiraea alba 'Allikko'	629.33	134.11	3.73	0.19
Amelanchier laevis	427.67	370.61	3.56	0.86
Malus domestica 'Lobo'	559.33	155.16	3.55	0.53

plants. Noteworthily, our results do not show that edible plant parts have human health-related differences in functional microbiome; our study covered only bud microbiota to identify plant species that have the potential to provide microbial diversity.

Taxonomic differences in the context of human health and wellbeing

The dominating classes in *Malus and Ribes* buds were *Gammaproteobacteria* and *Alpharoteobacteria* in our metagenomic dataset (Table S4), while in the larger 16S dataset *Proteobacteria* were the most abundant and richest phylum and its classes *Alpha-* and *Gammaproteobacteria* the richest and third richest classes (Fig. 1). Further, all the dominating ASVs in our study belonged to *Alpha-* and *Gammaproteobacteria* (Table 1). In our earlier publications, the richness of environmental bacteria in general, and *Gammaproteobacteria* and *Alpharoteobacteria* in particular, were associated with skin bacterial community and proper immune modulation (Roslund et al. 2020, 2021, 2022, Saarenpää et al. 2024). However, many species belonging to these taxa are known as opportunistic pathogens (e.g. *Escherichia*, *Klebsiella*, *Yersinia*, etc.). Human pathogens have often been studied in clinical context and a high abundance of *Proteobacteria* in human stool is usually regarded as a typical sign for gut microbiome dysbiosis (Shin et al. 2015). In parallel, we earlier found that the high richness of woody plants at home yards was associated with a balanced stool bacterial community, particularly during winter when soil was covered by snow and only woody plant parts, including buds, were reachable (Parajuli et al. 2020). Moreover, several studies have found evidence that high prevalence of land cover dominated by woody species or herbaceous vegetation is important in the prevention of certain immune-mediated diseases, like atopy and type1 diabetes (Nurminen et al. 2018, Vari et al. 2021). These contrasting findings are explained if plant associated *Proteobacteria* enrich skin microbiota, and potentially upper intestine, instead of surviving through human gut.

There is no doubt that multiple factors have to be taken into account in selecting the best candidates for urban planning in the context of human health. One of the factors is the microbiota of above-ground plant parts, which is surveyed as differences in bud microbiota of woody horticultural plants in the current study. Environmental microbiota covers also soil microbiota and microbiota in ambient air (Roslund et al. 2022, Kummola et al. 2023, Saarenpää et al. 2024). In addition to microbiota, some plant taxa are known to release phytochemical in ambient air, which was recently stated to be related to positive health effects (Zhao et al. 2024b). Despite these confounding factors we suggest that since woody plants have significant differences in above-ground microbiota, the screening of cultivars to find those hosting rich microbiota is proposed as a potential method to find candidate for immunomodulatory green space design. However, the potential immunomodulatory benefits require further investigation to determine whether urban dwellers can gain immunological advantages from exposure to plant species with high bud microbial diversity. Additionally, future research should assess whether the phyllosphere microbiome that originates from the bud microbiome retains the diversity patterns after bud opening, when it becomes exposed to environmental factors such as temperature fluctuations, UV radiation, oxygen, and plant secondary metabolites.

Since our group largely focuses on investigating the wellbeing of children, we dropped out plants producing toxic fruit before building a list of cultivars suitable for urban green space (Table 2). Also the selection of cultivars dominated by *Alpha-* and *Gammaproteobacteria* could open new avenues for horticulture and applied environment microbiology; in our study all cultivars hosting rich microbiota had rich alpha- and gammaproteobacterial community in buds as well.

Conclusion

This study is the first of its kind to compare the microbiota of 83 cultivars of woody plants commonly used in urban green space in the context of their suitability for microbially oriented green space design where the target is to enrich microbial diversity and consequently to improve plant health and human immunomodulation and wellbeing.

Our study demonstrated that microbial community composition and the abundance of functional gene pathways are cultivar dependent. Our study showed that *Malus domestica* "Lobo" and "Sandra" had high microbial richness and di-

versity, and higher biosynthesis of 5-aminoimidazole ribonucleotide that can contribute to plant and human health.

Understanding and leveraging microbial community composition and functional gene pathways in different horticultural cultivars can be a valuable strategy in sustainable urban planning aimed at promoting planetary health. Since our study did not explore the health effects of differences in plant-associated microbial communities, future research should examine the potential associations between these cultivars, urban rewilding efforts, and their impact on human commensal microbiota and immune regulation.

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Author contributions

Marja I. Roslund (Conceptualization [supporting], Data curation [equal], Formal analysis [lead], Funding acquisition [supporting], Investigation [equal], Methodology [lead], Software [equal], Validation [equal], Visualization [lead], Writing – original draft [lead], Writing – review & editing [equal]), Polina Galitskaya (Data curation [supporting], Formal analysis [supporting], Investigation [supporting], Methodology [supporting], Writing – original draft [equal]), Mika Saarenpää (Data curation [equal], Formal analysis [supporting], Investigation [supporting], Methodology [supporting], Software [supporting], Writing – review & editing [supporting]), and Aki Sinkkonen (Conceptualization [equal], Data curation [equal], Formal analysis [supporting], Funding acquisition [equal], Investigation [equal], Project administration [lead], Supervision [lead], Validation [supporting], Visualization [supporting], Writing – original draft [equal], Writing – review & editing [equal])

Supplementary data

Supplementary data is available at *LAMBIO Journal* online.

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Data availability

All bacterial 16S rRNA sequence data were accessioned into the at IDA—Research Data Storage: https://doi.org/10.23729/fd816e25-556f-4ffa-8f96-6c154139cb30 and shotgun sequences https://doi.org/10.23729/5c99c0be-6902-43fb-acf9-80bb4b2f29b8

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