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Impact of Cultivation and Origin on the Fruit Microbiome of Apples and Blueberries and Implications for the Exposome

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Abstract

Vegetables and fruits are a crucial part of the planetary health diet, directly affecting human health and the gut microbiome. The objective of our study was to understand the variability of the fruit (apple and blueberry) microbiome in the frame of the exposome concept. The study covered two fruit-bearing woody species, apple and blueberry, two countries of origin (Austria and Finland), and two fruit production methods (naturally grown and horticultural). Microbial abundance, diversity, and community structures were significantly different for apples and blueberries and strongly influenced by the growing system (naturally grown or horticultural) and country of origin (Austria or Finland). Our results indicated that bacterial communities are more responsive towards these factors than fungal communities. We found that fruits grown in the wild and within home gardens generally carry a higher microbial diversity, while commercial horticulture homogenized the microbiome independent of the country of origin. This can be explained by horticultural management, including pesticide use and post-harvest treatments. Specific taxonomic indicators were identified for each group, i.e., for horticultural apples: *Pseudomonas*, *Ralstonia*, and *Stenotrophomonas*. Interestingly, *Ralstonia* was also found to be enriched in horticultural blueberries in comparison to such that were home and wildly grown. Our study showed that the origin of fruits can strongly influence the diversity and composition of their microbiome, which means that we are exposed to different microorganisms by eating fruits from different origins. Thus, the fruit microbiome needs to be considered an important but relatively unexplored external exposomic factor.

Keywords Fruit microbiome · Apple · Blueberry · Amplicon sequencing · Growing system · Naturally grown · Commercial horticulture

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Abbreviations

ANOSIM	Analysis of similarities
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
ASV	Amplicon sequences variant
NMDS	Non-metric multidimensional scaling (NMDS)

Introduction

Vegetables and fruits are a crucial part of the planetary health diet EAT-Lancet Commission [1]. This diet aims to simultaneously provide health to the population and the planet as required by the Food and Agriculture Organization (FAO) and World Health Organization [2]. In addition to vegetables and fruits, this diet is based on the predominant consumption of greens and whole grains, and reduced consumption of meat, fish, eggs, refined cereals, and tubers [3]. Vegetables and fruits contain various ingredients and bioactive plant-derived secondary metabolites which are considered to have beneficial health effects. Moreover, they harbor millions of microorganisms [4], which potentially serve as one of the main direct sources of environmental microbiota. The human gut microbiome is regarded as an internal environmental factor, while the impact of the external environment, including the food microbiota, is less well understood in the exposome concept. The exposome concept was first suggested by Wild [5] to encompass the totality of human environmental exposures from conception onwards, complementing the genome. The concept differentiates three categories of non-genetic exposures: internal, specific external, and general external [6]. Recently, intervention trials to demonstrate the importance of external, nature-based microbe exposures on the human microbiota and immune functions were reported [7, 8].

as well as factors that shape them are still scarce. During the last years, an accumulating amount of evidence has shown that fruits are colonized by distinct microbial communities. Studies have examined the impact of host genetics [9] and environmental influences, e.g., soil and climate [10, 11] on the composition of the fruit microbiota. Moreover, post-harvest treatments, such as washing, waxing, storage, and thermal treatment, were shown to strongly influence the composition of fruit microbiota [12–14]. Recently, the beneficial effects of fruit consumption on the gut microbiota and human health have been increasingly recognized [15, 16]. However, our knowledge of the microbial composition of fruits produced in different growing systems and geographic regions is still very limited.

Despite the importance of the fruit and vegetable associated microbiota, studies on fresh fruits and vegetables. Our objective was to understand the fruit microbiome in the context of the exposome concept. We expected that the fruit microbiome is an important external factor that influences the gut microbiome especially during the early life. First, however, it is important to understand the variability of the fruit microbiome between different growing systems. Therefore, we have selected apples and blueberries, which are among the most commonly consumed raw fruits in the world and, more importantly, are commonly eaten in early childhood. Apples can be grown in home gardens as well as in commercial orchards. Blueberries are grown in commercial farms and can be also found in the wild. These different growing systems make both fruits ideal models to further study the variability of the fruit microbiota that are commonly consumed. The growing systems are characterized by different management practices. While no chemicals, nor fertilization were used in fruits that were grown in the wild and within home gardens, typical horticultural systems are intensively treated. In this study, we attempted to address the following questions: (i) do fruits of natural origin have a different microbial diversity compared to horticulturally grown fruits; (ii) are there differences in the microbial composition between these two groups; (iii) are there differences between fruits from distinct geographical locations; and (iv) which taxa explain the differences between the two groups? Overall, this study provides important insights into the impacts of growing systems on the apple and blueberry microbiota.

Materials and Methods

Sampling Procedures and DNA Extraction

Apple (*Malus domestica*) and blueberry samples were collected between July and August 2020 at 29 locations (Austria — 20 locations; Finland — 9 locations, Supplementary Table S1) in Austria and Finland. We chose these countries to test if geographic distance had an effect of fruit microbiota compositions. Here, we have defined naturally grown fruits as those grown in the wild or in private gardens, away from commercial orchards, and have not undergone any post-harvest treatment. A total of 15 and 6 naturally grown apple and blueberry samples, respectively, were collected in Austria while a total of 5 and 4 naturally grown apple and blueberry samples, respectively, were collected in Finland. The naturally grown apple and blueberry samples were collected from ecologically isolated individuals. Ripe fruits were collected using sterile gloves and instruments. We

randomly selected at least two apple fruits per sampling tree. For blueberry samples, we collected four composite samples (containing at least 10 berries) from four adjacent bushes/shrubs in one location. All fruits that represented horticultural production, were obtained from local supermarkets in Austria and Finland. We decided to obtain fruits from the local supermarket because it is the point that fruits are purchased and consumed. It should be noted that, in this study, most of the naturally grown blueberries belong to *Vaccinium myrtillus* whereas horticultural blueberries mostly belong to *Vaccinium corymbosum* (Supplementary Table S1). Sampling was carried out by using hand gloves and changing the hand gloves between handling various samples. All samples were put in sterile bags, kept in a cooling box during transportation, and stored at 4 °C before processing. Upon arrival in the laboratory, all samples were processed under sterile conditions. A total of 108 apple samples and 100 blueberry samples were analyzed. Details related to the samples and the associated metadata are presented in Supplementary Table S1.

To extract microorganisms from the fruits, approx. 10 g of each fruit sample was homogenized in a BagMixer laboratory blender (Interscience, Saint-Nom-la-Bretèche, France) with 10 ml sterile NaCl (0.85%) solution for 3 min. A total of 2 ml of homogenized suspensions was then centrifuged for 20 min at 16,000 g and pellets were used for DNA extraction. DNA extraction was carried out using the FastDNA SPIN Kit for soil and the FastPrep Instrument (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's protocol. DNA quality and yield were determined using the Nanodrop 2000 UV–Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and then stored at − 20 °C for further PCR reactions.

Bacterial and Fungal Quantification Using Quantitative Real-Time PCR (qPCR)

By implementing a qPCR-based analysis, we first calculated microbial abundance in the fruit samples (copies maker genes/gram). The qPCR analysis was based on SYBR Green fluorescence using KAPA SYBR FAST qPCR Kit (Kapa Biosystems, Woburn, USA) using the primer pair 515f–806r [17] and ITS1f-ITS2r [18] for bacterial and fungal quantification, respectively. The qPCR reactions and standard preparations were conducted as described previously [19]. Fluorescence quantification was performed using the Rotor-Gene 6000 real-time rotary analyzer (Corbett Research, Sydney, Australia) with initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 30 s and a final melting curve. The calculated PCR efficiencies were in a range of 94–98% ($R^2 = 0.955–0.965$) for 515f–806r primers and 80–88% ($R^2 = 0.993–0.995$) for ITS1f-ITS2r primers.

16S rRNA Gene Fragment and Internal Transcript Spacer (ITS) PCR Amplification and Illumina Sequencing

A one-step PCR approach using primers 515F/806R [17] and ITS1f-ITS2r [18] was employed for targeted amplification of the bacterial 16S rRNA V4 region and fungal ITS1 region. The primers contained Illumina indexes (barcode sequences) for multiplexing. Peptide nucleic acid (PNA) clamps were included in the PCR mix to block amplification of the plant's plastid and mitochondrial DNA. To verify successful amplification, PCR products were visualized on 1% agarose gels and subsequently combined and purified using the Wizard® SV Gel and PCR Clean-Up kit (Promega). All barcoded amplicons were pooled in equimolar concentrations. The pooled samples were sequenced by the commercial sequencing provider Eurofins (Ebersberg, Germany) using the Illumina MiSeq platform (2 × 300 bp paired end reads). Amplicon sequences were deposited at the European Nucleotide Archive (ENA) under the project number PRJEB51939.

Bioinformatic and Statistical Analysis

Cutadapt was used to remove low quality reads, primer sequences and demultiplex the reads according to the assigned barcode [20]. The DADA2 algorithm [21] was executed in QIIME2 [22] to quality filter, denoise, and remove chimeric sequences. The resulting representative sequences, known as amplicon sequences variants (ASVs), were further classified using the vsearch algorithm against the SILVA v132 and UNITE v7.1 database [23–25].

Statistical analysis and graph rendering were conducted in R studio version 2021.09.0 [26] unless stated otherwise. Prior to statistical analysis, a normality test was performed using the Shapiro test. The data were not normally distributed; therefore, the non-parametric Kruskal–Wallis test was carried out to determine significant differences ($P < 0.05$) of bacterial gene copy numbers per gram of fruits countries and growing systems (commercial versus wild/home-grown). Groups were compared using Dunn's test of multiple comparisons and the P values were adjusted using Benjamini–Hochberg procedure. ASV tables and taxonomic classifications that were generated with the DADA2 algorithm were used as an input for bacterial community analysis. The bacterial community analysis was performed using the software packages Phyloseq and MicrobiomeAnalyst [27, 28]. After removing non-bacterial reads, i.e., mitochondrial and chloroplast sequences, the overall bacterial community, assessed by 16S rRNA gene fragment amplicon sequencing, contained 1,045,695 sequences from the apple dataset and 1,640,743 from the blueberry dataset. These sequences were assigned to 1659

bacterial ASVs. The overall fungal community, assessed by ITS gene fragment amplicon sequencing, contained 911,875 sequences from the apple dataset and 1,896,835 from the blueberry dataset. These sequences were assigned to 1778 fungal ASVs.

The amplicon sequencing datasets were normalized by randomly selecting subsets of sequences (1000 reads for bacterial datasets and 400 reads for fungal datasets). A total of one (16S rRNA dataset) and five (ITS dataset) samples had to be removed due to low read numbers. Respective rarefraction curves indicate that the sampling size was sufficient to capture overall bacterial and fungal diversity (Supplementary Fig. S1). Taxonomical compositions at family level were summarized using bar plots. Based on normalized datasets, differences in microbial alpha diversity (Shannon diversity index – H') were assessed using the Kruskal–Wallis test. For beta diversity analysis, Bray–Curtis dissimilarity matrices were generated from normalized datasets. We used the *betadisper* function in the vegan package to calculate the distance of each sample to the respective group centroid from the Bray–Curtis distance matrix. The matrix distances were further subjected to analysis of similarities (ANOSIM) to test for significant effects of experimental factors on microbial community structures. Finally, microbial biomarkers at bacterial genus level for horticultural and naturally grown fruits were identified using a linear discriminant analysis effect size (LefSe) analysis [29].

Results

Microbial Abundance, Diversity, and Community Structures Were Influenced by Growing Systems and the Country of Origin

We observed a significantly higher bacterial and fungal abundance in blueberry samples (copy number of genes/grams: bacterial 2.4×10^6 and fungal 3.7×10^5) in comparison to apple samples (bacteria: 1.6×10^5 and fungi: 2.7×10^4, Kruskal–Wallis test, $P < 0.05$; Fig. 1A and B). A higher bacterial abundance was generally observed in horticultural apples in comparison to naturally/home grown apples whereas the opposite pattern was observed in blueberry samples (Kruskal–Wallis test, $P_{adj} < 0.05$; Fig. 1A). Interestingly, a higher fungal abundance was consistently

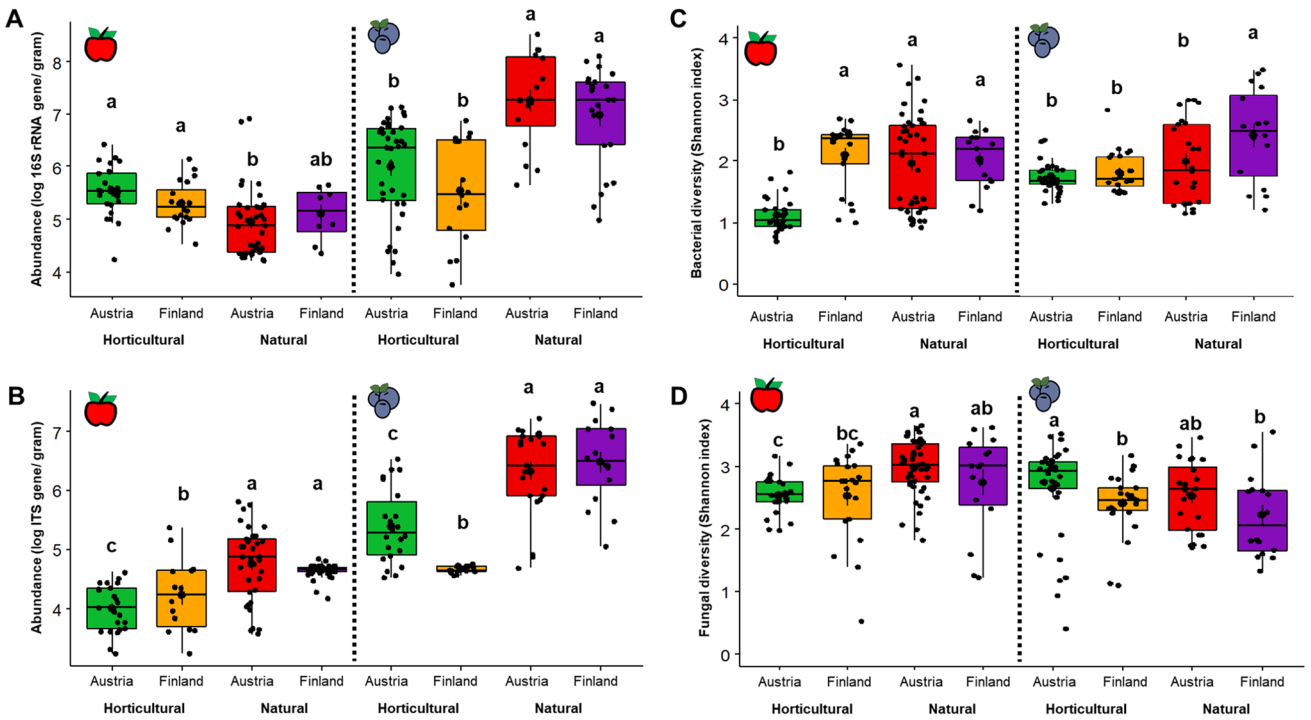

Fig. 1 Comparison of microbial abundance and diversity between naturally grown and horticultural fruits. The box plots visualize the variability of microbial abundance (**A** and **B**) and diversity (**C** and **D**). Bacterial (**A**) and fungal (**B**) abundances were quantified using a qPCR-based approach and transformed into log values. In order to compare bacterial (**C**) and fungal (**D**) alpha diversity, the Shannon index was calculated for each sample type. The different letters above each bar indicate statistical significance at $P_{adj} < 0.05$ based on Dunn's test of multiple comparisons

detected in naturally/home grown apples and blueberries in comparison to the horticultural fruits (Kruskal–Wallis test, $P < 0.05$; Fig. 1B).

By using 16S rRNA and ITS genes fragment amplicon sequencing, we further compared bacterial and fungal diversity between wild/home grown and horticultural fruits. Wild/home grown apples that were obtained in Austria had a higher bacterial and fungal diversity (bacteria: $H' = 2.1$; fungi: $H' = 3.0$, Kruskal–Wallis test, $P_{adj} < 0.05$) in comparison to horticultural apples that were obtained from the same country (bacteria: $H' = 1.3$; fungi: $H' = 2.5$, Fig. 1C). These differences were not observed from apple samples that were obtained in Finland. When comparing blueberry samples (Fig. 1C and D), homegrown/wild blueberries that were obtained in Finland had a higher (Kruskal–Wallis test, $P_{adj} < 0.05$) bacterial diversity ($H' = 1.9$) in comparison to horticultural ones obtained in Austria ($H' = 1.2$) or Finland ($H' = 1.3$).

Based on ANOSIM, the two tested factors, i.e., growing system and country of origin, had substantial effects on the microbial community structures associated with the fruits ($P < 0.05$; Table 1). Differences between horticultural and naturally grown fruits were observed when the comparison was performed from samples that were obtained from the same country. For examples, horticultural apples and blueberries that were obtained from Austria had different bacterial and fungal community structures in comparison to naturally grown apples and blueberries from Austria ($P_{adj} < 0.05$; Supplementary Table S2). Different fungal community structures were also observed between horticultural and naturally grown apples ($P_{adj} = 0.010$) and blueberries ($P_{adj} = 0.002$) that were obtained in Finland (Supplementary Table S2). Moreover, the country of origin also had an effect on bacterial community composition. For instance, horticultural apples and blueberries obtained in Austria had different bacterial and fungal community composition in comparison to samples that were obtained in Finland ($P_{adj} < 0.05$).

Table 1 ANOSIM results on the effects of the growing system and country of origin on bacterial community composition

		Factor	R value	*P* value
Bacteria	Apple	Growing system	0.107	0.001
		Country	0.092	0.006
	Blueberry	Growing system	0.178	0.001
		Country	0.147	0.002
Fungi	Apple	Growing system	0.048	0.024
		Country	0.140	0.003
	Blueberry	Growing system	0.323	0.001
		Country	0.141	0.001

Horticultural Fruits Harbor a More Homogenous Bacterial and Fungal Community Structure Compared to Naturally Grown Fruits

NMDS plots supported the obtained ANOSIM results as indicated by a clear clustering of naturally grown and horticultural fruits (Fig. 2). Horticultural apple samples showed a tendency to cluster together whereas naturally grown apple samples were more scattered for both bacterial and fungal datasets (Fig. 2A and C, respectively). This pattern was also more apparent when the apple samples originating from Austria and Finland were analyzed separately (Supplementary Fig. S2A, B and Supplementary Fig. S3A, B). However, the different clustering between horticultural and naturally grown apple were less apparent from the samples that were obtained from Finland. Bacterial and fungal communities from blueberry datasets showed a similar clustering where all the horticultural blueberry samples that were obtained from Austria and Finland clustered together (Fig. 2B and D, Supplementary Fig. S2C and D, and Supplementary Fig. S3C and D).

A complementary analysis using *betadisper* indicated that variability in bacterial and fungal community structures, or the distance of each sample point to the respective group centroid, was higher in naturally grown fruits in comparison to horticultural fruits (Fig. 2E–H). For instance, variability in bacterial communities of horticultural apples (Fig. 2E) that were obtained in Austria was lower (0.276) in comparison to naturally grown apples (0.545). A similar pattern was also observed in the blueberry datasets (Fig. 2F), where a smaller average distance to the centroid was observed in horticultural blueberries (Austria: 0.192 and Finland: 0.285, respectively) compared to naturally grown blueberries (Austria: 0.359 and Finland: 0.419). Similar results were also obtained for the fungal community of blueberries where the variability in fungal communities of naturally grown blueberries was higher in comparison to horticultural blueberries. However, the variability in fungal communities between horticultural and naturally grown apples that were obtained in Austria and Finland was relatively similar (Fig. 2G and H).

Microbial Community Analysis Revealed Bacterial Indicators Associated with Horticultural and Naturally Grown Fruits

To identify bacterial taxa that are specifically enriched in either horticultural or naturally grown fruits, we first visualized the overall bacterial and fungal composition. Apple and blueberry bacterial communities were dominated by *Gammaproteobacteria*, i.e., *Pseudomonadaceae*, *Burkholderiaceae*, and *Enterobacteriaceae* as well as *Alphaproteobacteria*, i.e., *Sphingomonadaceae* (Fig. 3).

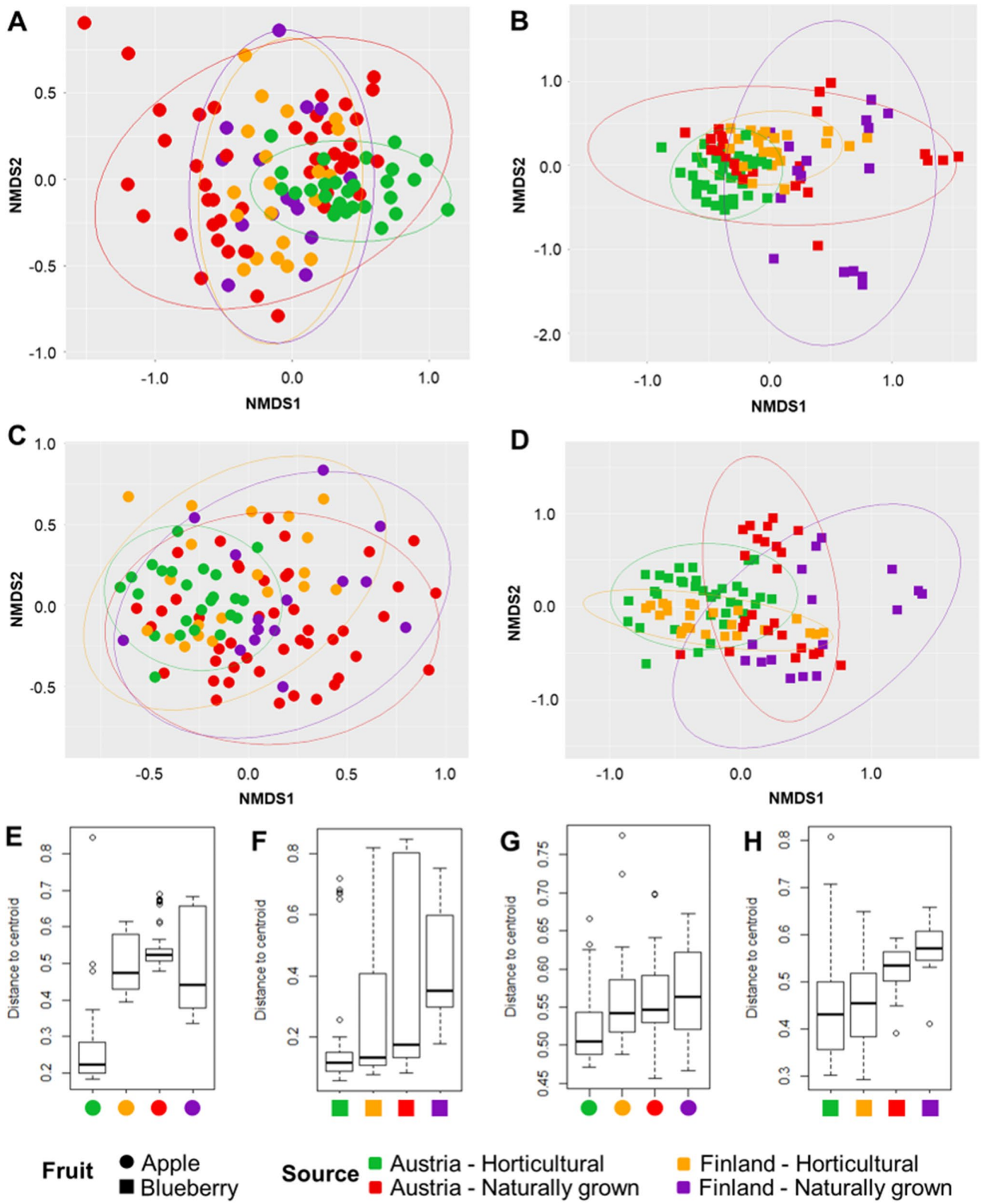

Fig. 2 Community clustering and variability in microbial community compositions of naturally grown and horticultural fruits. The non-metric multidimensional scaling (NMDS) plots show clustering of bacterial (**A** and **B**) and fungal (**C** and **D**) communities from apples and blueberries. The box plots visualize the variability of bacterial (**E** and **F**) and fungal (**G** and **H**) community compositions. Each box-plot shows the distance of the respective sample type to the respective group centroid

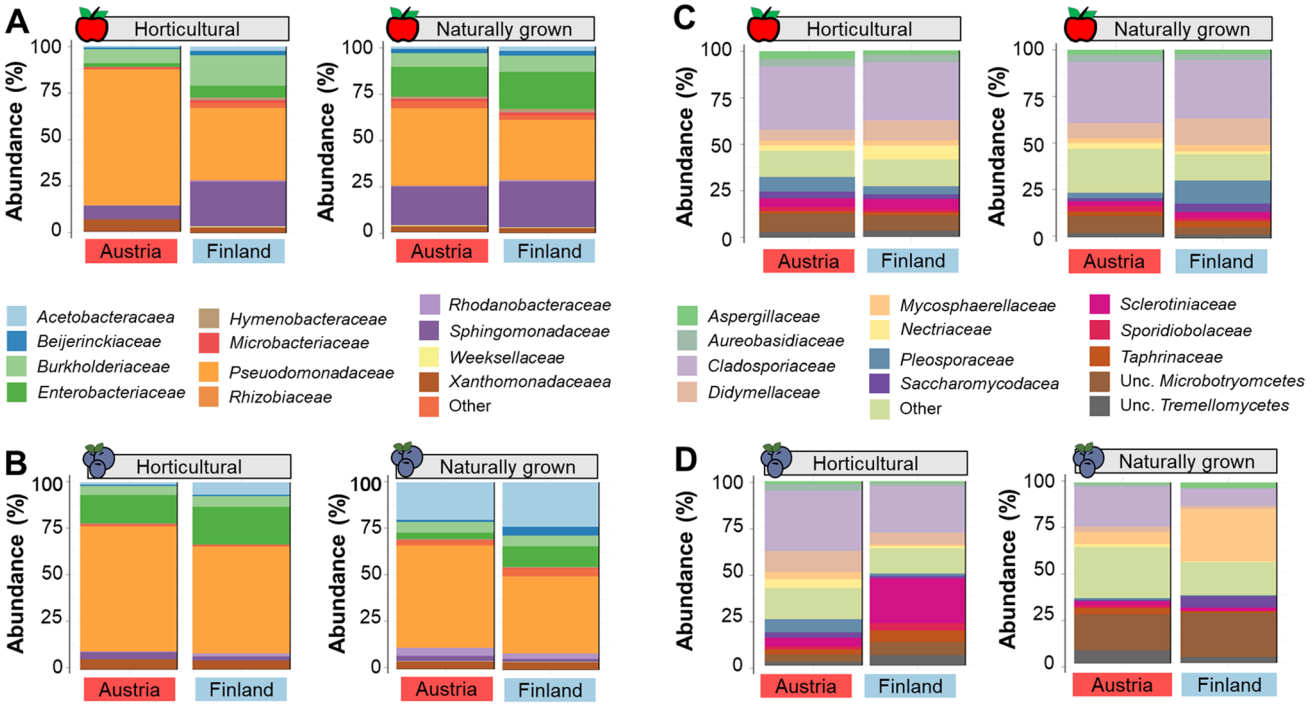

Fig. 3 Microbial community composition for naturally grown and horticultural fruits. Bar plots show bacterial (**A** and **B**) and fungal (**C** and **D**) composition at family level for different apple and blueberry samples

These taxa contributed 81.5% of the total sequencing reads. A distinct bacterial composition between naturally grown and horticultural apples was indicated by differences in relative abundance of *Pseudomonadaceae*, *Burkholderiaceae*, and *Enterobacteriaceae* (Fig. 3A). Horticultural apples that were obtained from local supermarkets in Austria and Finland harbored a high proportion of *Pseudomonadaceae* and *Burkholderiaceae* that contributed to a total of 80.2 and 55.7% of total reads, respectively with a low abundance of *Enterobacteriaceae* (0.7 and 5.8%, respectively, Fig. 3A). In contrast, the relative abundance of *Enterobacteriaceae* was higher in naturally grown apples that were obtained from both countries (Austrian apples: 15.6% and Finnish apples: 19.6% of total reads). Differences in bacterial community composition between naturally grown and horticultural fruits was also observed for blueberry samples (Fig. 3B). A higher abundance of *Acetobacteraceae* was observed in naturally grown blueberries that were obtained from both countries (Austria: 19.9% and Finland: 23.1%) in comparison to horticultural blueberries (Austria: 1.3% and Finland: 7.3%). In contrast, a higher abundance of *Enterobacteriaceae* was observed in horticultural blueberries (Austria: 15.1% and Finland: 19.8%) in comparison to those naturally grown (Austria: 3.5% and Finland: 11.5%).

Three fungal families, namely *Cladosporiaceae*, *Didymellaceae*, and *Pleosporaceae* were highly abundant in apple samples (49.5%), whereas *Cladosporiaceae*, *Sclerotiniaceae*, and *Mycosphaerellaceae* were highly abundant in blueberry samples (40.9%). The differences in fungal community compositions between horticultural and naturally grown apples were not apparent. A distinct composition was observed for the two countries. For instance, apple samples that were obtained in Finland had a higher abundance of *Didymellaceae* (naturally grown: 14.6% and horticultural: 10.9%) in comparison to apple samples that were obtained in Austria (naturally grown: 8.5% and horticultural: 5.4%, Fig. 3C). In comparison to the apple samples, fungal compositions in blueberries varied more between different groups (Fig. 3D). In horticultural blueberries obtained in Austria and Finland, the relative abundance of *Didymellaceae* (10.6% and 6.3%, respectively) was higher in comparison to naturally grown blueberries that were obtained in the respective countries (*Didymellaceae* 3.0% and 1.6%). In contrast, unclassified members of the fungal class *Microbotryomycetes* were more abundant in naturally grown blueberries (Austria: 18.9% and Finland: 23.4%) in comparison to horticultural blueberries (Austria: 4.4% and Finland: 6.9%).

Because differences at higher taxonomic levels may not provide sufficient information to infer ecological relevance, taxa that are indicators for naturally grown or horticultural fruits were identified on ASV level using

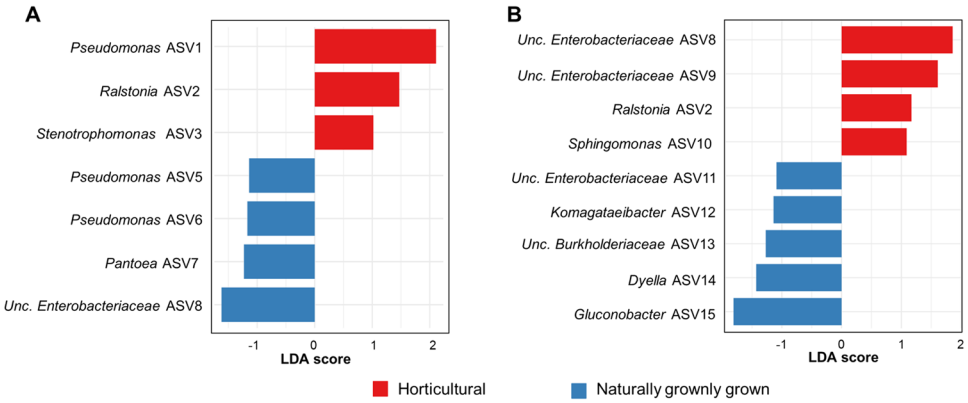

Fig. 4 Identification of significantly enriched bacterial taxa in apples and blueberries. Linear discriminant analysis effect size (LEfSe) analysis was implemented to identify microbial biomarkers for naturally grown and horticultural fruits at ASV level. **A** Apple; **B** blueberry

LEfSe analysis. Three bacterial ASVs, i.e., *Pseudomonas* ASV1, *Ralstonia* ASV2, and *Stenotrophomonas* ASV3 (LDA score > 1, $P < 0.05$) that are closely related to *Pseudomonas mandelii*, *Ralstonia insidiosa*, and *Stenotrophomonas maltophilia*, respectively, were found to be enriched in horticultural apples (Fig. 4A, Supplementary Table S3). Interestingly, *Ralstonia* ASV2 was also found to be enriched in horticultural blueberries in comparison to naturally grown blueberries (Fig. 4A). Three other bacterial ASVs that were further identified as *Tatumella ptyseos* (ASV ID: *Unc. Enterobacteriaceae* ASV8), *Serratia* sp. (ASV ID: *Unc. Enterobacteriaceae* ASV8), and *Sphingomonas aerolata* (ASV ID: *Sphingomonas* ASV10) were also enriched in horticultural blueberries. In contrast, three ASVs that are closely related to *Pseudomonas viridiflava*, *Pseudomonas gingeri*, and *Pantoea agglomerans*, respectively, were enriched in naturally grown apples (Supplementary Table S3). With respect to the blueberry dataset, bacterial ASVs that were enriched in naturally grown berries were *Gluconobacter cerinus* (*Gluconobacter* ASV15), *Dyella japonica* (*Dyella* ASV14), *Robbsia* sp. (Unc. *Burkholderiaceae* ASV13), *Komagataeibacter intermedius* (*Komagataeibacter* ASV12), and *Enterobacter hormaechei* (Unc. *Enterobacteriaceae* ASV11).

Only a low number of fungal ASVs (1 ASV from the apple dataset and 4 ASVs from the blueberry dataset; Supplementary Table S3) were identified as indicator taxa for naturally grown and horticultural fruits. These taxa were only enriched in horticultural fruits in comparison to those grown naturally. One fungal ASV was enriched in both horticultural fruits in comparison to naturally grown fruits and further identified as *Cladosporium cladosporioides* (Supplementary Table S4). Additionally, three fungal ASVs, i.e., Unc. *Sclerotiniaceae* ASV2, *Cladosporium* ASV3, and Unc. *Didymellaceae* ASV4 that were further identified as *Botrytis cinerea*, *Cladosporium allicinum*, and *Epicoccum layuense* were also enriched in horticultural blueberries.

Discussion

In the present study, we characterized microbial communities of naturally grown and horticultural apples and blueberries in order to identify the impact of the growing system on fruit microbiomes as well as specific signatures for each of the two origins. After subjecting a representative set of samples to various comparative analyses, clear differences in their microbial community compositions were identified suggesting a strong influence of the growing system. We were able to provide insights into the impact of growing systems on the native microbiota consumed with fruits and their potential implications for the exposome.

In detail, we showed that the growing system as well as the country of origin have an impact on microbial abundance and diversity. Previously, it was reported that the type of commercial farming (organic or conventional) affects microbial diversity [4, 30]. However, detailed insights into specific impacts of growing systems remained unresolved so far. Here, we observed that fruits from horticultural production generally carried a lower microbial diversity in comparison to naturally grown fruits. It is known that intensively managed agricultural soils generally have a lower soil biodiversity, while natural ecosystems harbor more complex microbial communities [31, 32]. As soil is widely recognized as a source for microbial communities in the phyllosphere, including fruits [33, 34], it can be assumed that intensive farming practices would not only have an impact on soil microbial diversity but also fruit microbial diversity grown under these growing systems. Detailed insights into impacts of the country of origin and even local environments on microbial communities in apples and other fruits, e.g., grapevine, were provided in foregoing studies [35–37]. The present study confirmed that previously observed patterns also apply for bacterial communities in blueberries which have not been investigated so far. However, variation in the fungal community was explained to a greater extent by these factors. This observation indicates that fungal communities are more responsive towards the growing system and

country of origin in comparison to bacterial communities in case of blueberry.

In general, microbial communities from horticultural fruits were observed to be more homogenous in the present study. Commercial farming practice and plant breeding have led to highly productive and consistent quality of fruits [38–40] which is required to meet the consumer demand. Interestingly, these factors also result in profound changes of the microbiome in agricultural crops (reviewed in [41]. Recently, a study by Lupatini and colleague [42] indicated that organic farming systems harbor a more heterogenous soil microbiome than conventional farming systems. As soil is known to be a major source of microbes for aboveground habitats, we speculate that more homogenous microbial communities from fruits produced under commercial management practices could be a result of the more homogenous soil microbiome under this management in comparison to natural soil microbiomes. We hypothesize that this finding is also relevant for human health. Exposure to highly diverse environmental microbiota can shape commensal microbiota and consequently promote immune modulation [8, 43]. In terms of the gut microbiota, microorganisms associated with fresh produce, including fruits, constitute an important exposome. The consumption of fresh fruits, which harbor trillions of microorganisms, is likely influencing the gut microbiota composition. Hence, consuming naturally grown fruits likely exposes our gut to a more diverse environmental microbiota. Recently, Wankhade et al. [44] demonstrated a changed gut microbiome composition following blueberry consumption in mice. They speculated that it is caused by active compounds, e.g., antioxidants in the fruit; however, we suggest that it could also be partially caused by the fruit microbiota. As a growing body of literature suggests that gut microbial diversity is linked to human health [45], it is critical to understand the impact of farming systems on the microbial diversity and composition of the fruits we consume.

In a detailed data assessment, we identified taxonomic indicators for naturally grown and horticultural fruits which include known beneficial plant associated bacteria but also potential human pathogens. For example, *Stenotrophomonas maltophilia*, which is recognized as an emerging global opportunistic pathogen [46], was enriched in horticultural apples. This bacterium was also recently isolated from diseased fruits [47, 48]. Interestingly, *Pantoea agglomerans*, a biocontrol agent against fire blight caused by *Erwinia amylovora* [49], but also a potential opportunistic human pathogen [50], was enriched in naturally grown apples. Presence of potential opportunistic pathogens in fresh produce was reported previously [51–53]. Our results reinforce the notion that fresh produce is a reservoir of opportunistic human pathogens. We also found that an ASV closely related to *Pseudomonas viridiflava*, the causal agent of bacterial shoot blight in apples [54], was enriched in naturally grown apples. This taxon can occur as an endophyte, epiphyte, and saprophyte in both agricultural and natural environments [55]. A similar pattern was also observed in commercial blueberries where putative pathogens and beneficial taxa were present. For instance, *Botrytis cinerea*, a causal agent of gray mold [56, 57], and *Epicoccum layuense*, a potential biocontrol agent against fungal diseases [58], were enriched in commercial blueberries. Due to the fact that we only analyzed healthy fruits, we assume that a balanced proportion of beneficial and pathogenic microorganisms provides resilience towards disease outbreaks as previously shown in seed microbiomes of native plants [59].

It should also be highlighted that although a clear distinction of microbial community structures between commercial and naturally grown fruits was observed, there was a fraction of microbial variation that was explained by the tested factors. Our study does not include data on post-harvest handling, i.e., precooling, packaging, and the storing period that can induce microbial community changes [60, 61]. Interestingly, high abundances of ASVs closely related to *Ralstonia insidiosa* and *Sphingomonas aerolata* that were observed in horticultural fruits might be due to post-harvest handling. *Ralstonia Insidiosa* is a strong biofilm producer and was frequently isolated in various fresh produce [62, 63] whereas *S. aerolata* is able to grow at low temperatures [64]. Both traits provide an effective survival strategy during cold storage. Moreover, *P. mandelii* was previously reported to have the ability to grow at low temperature (10–12 °C) due to the cold-adapted physiology of this species [65, 66]. We, therefore, speculate that post-harvest handling could shape and proliferate taxa that are more adaptable towards low temperature and other storage conditions. Moreover, we assume that the observed differences in microbial communities are also additionally affected by other factors including the plant genotype (cultivar and species) and pedoclimatic conditions at the production site [67–69]. To answer this question, targeted studies would be required.

Conclusion

In conclusion, growing systems were shown to substantially affect the variability of the fruit microbiome. Horticultural production results in a more homogenous fruit microbiome in comparison to naturally grown fruits (wild or home gardens). Moreover, specific changes in the composition of the microbiomes were observed that could have implications for human health. The microbiota associated with fruits and other fresh produce is considered a potential source and a key exposome for the gut microbiota. Hence, consuming naturally grown fruits could potentially expose our gut to

diverse microbiota. Moreover, for future research, it is also important to consider the impact of management practices on the indigenous fruit microbiota, an element that is mostly overlooked.

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Author Contribution Gabriele Berg: conceptualization, supervision, project administration, funding acquisition, and writing — review and editing. Wisnu Adi Wicaksono: conceptualization, formal analysis, data curation, writing — original draft, writing — review and editing, and visualization. Aisa Buko: formal analysis and writing — original draft. Peter Kusstatscher: data curation and writing — review and editing. Tomislav Cernava: writing — review and editing. Aki Sinkkonen: funding acquisition and writing — review and editing. Olli H Laitinen: writing — review and editing. Suvi M. Virtanen: writing — review and editing. Heikki Hyöty: funding acquisition and writing — review and editing.

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Data availability Raw sequencing data for each sample used in this study was deposited at the European Nucleotide Archive (ENA) in the FASTQ format and is available under the Bioproject accession number PRJEB51939.

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interests The authors declare no competing interests.

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