# Genotype-Dependent Recruitment of the Strawberry Holobiome

**Antonio Cellini**
University of Bologna: Universita di Bologna
**Daniela Sangiorgio**
University of Bologna
**Irene Donati**
University of Bologna
**Erika Ferrari**
University of Modena and Reggio Emilia
**Benjawan Tanunchai**
Helmholtz-Zentrum für Umweltforschung UFZ: Helmholtz-Zentrum fur Umweltforschung UFZ
**Sara Fareed Mohamed Wahdan**
UFZ Centre for Environmental Research Leipzig-Halle: Helmholtz-Zentrum fur Umweltforschung UFZ
**Dolaya Sadubsarn**
UFZ Centre for Environmental Research Leipzig-Halle: Helmholtz-Zentrum fur Umweltforschung UFZ
**Brian Farneti**
Fondazione Edmund Mach Istituto Agrario di San Michele all'Adige
**Francois Buscot**
UFZ Centre for Environmental Research Leipzig-Halle: Helmholtz-Zentrum fur Umweltforschung UFZ
**Francesco Spinelli** ( francesco.spinelli3@unibo.it )
University of Bologna: Universita di Bologna https://orcid.org/0000-0003-3870-1227
**Witoon Purahong**
UFZ Centre for Environmental Research Leipzig-Halle: Helmholtz-Zentrum fur Umweltforschung UFZ

**Research**

**Keywords:** Fragaria x ananassa, Next Generation Sequencing, biological control, metagenome, plant growth promoting bacteria, arbuscular mycorrhiza, rhizosphere, phyllosphere, Pseudomonas fluorescens, Rhizophagus irregularis

**DOI:** https://doi.org/10.21203/rs.3.rs-104580/v1

# Abstract

## Background

Cultivated strawberry (*Fragaria* × *ananassa* Duch., fam. *Rosaceae*) is an important fruit crop, greatly appreciated for its aroma and nutraceutical properties. Niche-specific characterisation of plant microbiome, from rhizosphere to aboveground plant organs, is crucial to understand the influence of structure and function of the microbial communities on plant phenotype, performances and disease resistance. Strawberry cultivation is challenged by a large variety of pathogens, which cause substantial economic losses and require the frequent application of pesticides. Biological control is a promising and safer alternative to the use of xenobiotic pesticides. Biological control agents isolated from the microbiome of the host plant may have a superior efficacy in comparison to non-indigenous microbial inoculants. Therefore, the characterization of the native microbiome along different plant compartments is a key step for the successful microbial manipulation in farmlands.

## Results

Here, we provide the first comprehensive description of the soil, rhizosphere, root and aerial parts microbiome of three commercially important strawberry cultivars ('Darselect', 'Elsanta' and 'Monterey') under cultural conditions. The fungal and bacterial microbiomes were functionally characterised to investigate their influence on plant disease tolerance, plant mineral nutrient content and fruit quality. The core microbiome included 24 bacteria and 15 fungal operative taxon units which were present in all compartments and plant genotypes. However, both plant organ and genotype had a significant role in assembling the microbial communities. The microbial community assemblage across different soil and plant compartments significantly correlated with disease resistance, mineral nutrient content in the plant and with fruit quality parameters. Interestingly, only the disease tolerant genotype 'Monterey' was able to recruit *Pseudomonas fluorescens* in all plant organs and to establish symbiosis with the arbuscular mycorrhiza *Rhizophagus irregularis*. These two species include several strains acting as pathogen biocontrol agents, plant growth promoters and plant defence inducers.

## Conclusions

Altogether, our study provides the first comprehensive view of strawberry microbiome in relation to plant genotype, health and nutritional status and fruit quality parameters, shedding light on potential practical applications to increase the sustainability of crop production.

# Background

Crop plants are associated with a wide diversity of microorganisms in all their parts[1,2]. Such microbial biocoenosis influence plant phenotype fitness, growth, fruit production and quality, by contributing to plant nutrition, tolerance to abiotic stresses, and control of pathogenic or opportunistic species[1,3]. In this view, individual plants can be considered as an holobiont, i.e. the superorganism encompassing

individual host and its associated microbial community[4,5]. The association between terrestrial plants and microbes developed at least 460 million years ago, as suggested by the fossil evidence of arbuscular mycorrhizae on some of the earliest land plants[4]. To date, many important questions regarding these associations remain unanswered, especially concerning the factors determining the community assemblage and diversity of the plant microbiome[3]. Increasing evidence suggests that plants can actively recruit a beneficial microflora to facilitate their adaptation to environmental conditions and changes[3,6,7]. However, further studies are needed to generalize this hypothesis, and enable practical applications, especially for horticultural perennial crops grown in cultural conditions[8]. To date, most experiments on plant microbiome have focused either on specific model plants (i.e. *Arabidopsis thaliana*) or economically important, annual herbaceous monocotyledons[9]. Perennial plants, on the other hand, are exposed to radically changing environmental conditions (including freezing winter temperatures, dry seasons, periodic flooding)[10]. Therefore, in perennial plants, the microbial community has evolved to last for more than a growing season, thus suggesting an assembly with a more intimate connection with host allowing its endurance to changing environmental conditions. Furthermore, perennial crops may promote plant–microbial linkages, increasing richness of bacterial and fungal beneficial communities, due to their extensive root networks and allocation of belowground carbon[11–13]. In addition, microbiome research has so far primarily taken into consideration the rhizosphere, while other plant compartments have been relatively neglected[14]. Finally, bacterial community analysis dominates the microbiome studies[15]. The study of bacterial and fungal microbiomes colonizing different plant compartments under agronomic conditions provides key information to unfold agricultural constraints and achieve a successful microbial manipulation in farmlands[16].

Cultivated strawberry (*Fragaria* × *ananassa* Duch., fam. *Rosaceae*) is an important fruit crop, originated approximately 300 years ago from the hybridisation between ecotypes of wild octoploid species: *Fragaria chiloensis* subsp. *chiloensis* from South America and *Fragaria virginiana* subsp. *virginiana* from North America[17]. In the last decade, the global strawberry cultivation area has increased by 14% (2008–2018)[18]. In 2016, the global strawberry gross production valued 17 billions US$ with China having the biggest market-share (6,48 bn US$), followed by Europe (3,49 bn US$) and United States (3,47 bn US$)[18]. The high adaptability of strawberry to different conditions, allows the cultivation under a wide range of environments and agronomical managements (from Mediterranean to the Nordic climates) making the fruit available on the market, almost independently of the season[19]. For this reason, strawberry fruit represents an important and valuable portion of the daily fresh food consumption[20]. Strawberry is greatly appreciated for its aroma and nutraceutical properties. Among others, strawberry fruit contains phytochemicals, such as anthocyanins and ellagitannins which may prevent human health diseases induced by reactive oxygen species[21]. While strawberry productivity and quality can be positively improved by beneficial microorganisms[22], the cultivation is challenged by a large variety of pathogens, which cause substantial economic losses and require the frequent application of pesticides. Among these diseases, red stele (*Phytophthora fragariae*), powdery mildew (*Podosphaera aphanis*) and leaf spot are the ones most severely affecting strawberry production worldwide[23]. Powdery mildew mainly affects

photosynthetic ability of strawberries cultivated in humid environments[24], which leads to strong reduction of growth and productivity with major yield losses[25]. Leaf spot diseases, which in severe conditions may led to plant death, are caused by different pathogens, including bacteria (*Xanthomonas fragariae*) and fungi (*Colletotrichum gloeosporiodes*, *Mycosphaerella fragariae*, *Cercospora fragariae, Mycosphaerella louisianae*, *Septoria fragariae, S. aciculosa, S. fragariaecola*, etc.). Multiple resistance to a broad spectrum of diseases such as powdery mildew and leaf spot is still not available among commercial strawberry cultivars (i.e. human-selected clonal genotypes)[26]. Disease control is particularly challenging in strawberry production, since several cultivars present at the same time, flowers, fruit and leaves, and are therefore subjected to a high risk of pesticide residue accumulation on berries[19]. Biological control is a promising and safer alternative to the use of xenobiotic pesticides. Some commercially available, beneficial microorganisms (i.e. *Ampelomyces quisqualis*, *Bacillus subtilis*, *Trichoderma harzianum*, *Glomus* spp.) have been tested for disease control in strawberry, yet none of them has demonstrated characteristics of reliability, persistence and/or cost-effectiveness justifying their use as an alternative to chemical pesticides[23]. The unsatisfactory degree of disease control and the high variability of results obtained in different locations and seasons with commercial beneficial microorganisms can be explained by the fact that those microbes are in most cases non-native to the strawberry plant microbiome. Several studies suggest that biological control agents isolated from the microbiome of the host plant have a superior efficacy in comparison to non-indigenous microbial inoculants[27–29]. Thus, the characterization of the native microbiome is a key step for the successful selection of beneficial microorganisms against plant diseases[1]. Unfortunately, the complete microbiome of cultivated strawberry has not yet been described, hindering the identification and selection of the most effective indigenous microorganisms to improve plant fitness and fruit quality and/or provide resistance to biotic and abiotic stresses.

The aim of this study was to provide a complete picture of the strawberry holobiome, including both fungal and bacterial populations, and to identify a core microbiome, from soil, plant-soil interface (rhizosphere) and plant compartments (roots and above-ground organs) using Next Generation Sequencing (NGS). For this purpose, three commercially important strawberry genotypes (‘Elsanta’, ‘Darselect’ and ‘Monterey’) were used. Furthermore, the effects of strawberry genotypes, soil and plant compartments on the richness and community composition of the overall microbiome were studied, with a focus on pathogenic and beneficial microbes. Finally, the links between strawberry microbiomes, plant mineral nutrient content and fruit quality traits were investigated. To our knowledge, this study provides the first in depth and comprehensive view of horticultural crop microbiome in relation to plant genotype, health and nutritional status and fruit quality parameters, shedding light on potential practical applications to increase the sustainability of crop production.

## Results And Discussion

# Composition of strawberry microbiomes

Quadruplicate bulk soil, rhizosphere, root and above-ground organs samples were prepared for bacterial 16S rRNA and ITS gene community profiling for three strawberry genotypes (Fig. 1). In roots and above-ground organs, we targeted epiphytic and endophytic microorganisms jointly. In total, we generated 1,531,637 (average of 31,909 reads per sample) and 739,458 (average of 15,405 reads per sample) high quality reads excluding chimeric sequences for bacteria and fungi, respectively. We removed singletons which may come from sequencing errors and normalized all bacterial and fungal datasets to 10,930 sequences for bacteria and 8,077 for fungi. Rarefaction curves show the sufficient sequencing effort for most of the samples (Fig. S1b,c). Nevertheless, OTU richness estimates, predicted with Chao1 were also analysed and showed (Fig. S2). We used observed richness directly as diversity measure for both bacteria and fungi (Fig. 1c,e). In total, we detected 26,434 bacterial and 1,716 fungal OTUs. The total bacterial and fungal community assemblages were compared using two-way PERMANOVA to identify the main drivers of the microbiome composition (Table 1; Table S1). Notably, we found that microbial compositions are strongly dependent both on the analysed genotype (bacteria $F = 1.87$, $P = 0.002$; fungi $F = 2.93$, $P = 0.001$) and compartment (bacteria $F = 4.27$, $P = 0.001$; fungi $F = 3.56$, $P = 0.001$) (PERMANOVA values genotype × compartment bacteria $F = 1.44$, $P = 0.001$; fungi $F = 1.51$, $P = 0.001$; Fig. 1b,d; Table 1; Table S1). Similar results were obtained when we compared the effect either of genotype or compartment by means of two-way ANOSIM analysis (Table 1; Table S1).

Table 1
Effect of genotype, soil and plant compartment on richness and community composition of strawberry microbiome. Nd = not determined; Significant *P* values are highlighted in bold.

| Microorganisms/Factors | Richness (Two way ANOVA) | | Community composition (Two-way ANOSIM) | | Community composition (Two-way PERMANOVA) | |
|---|---|---|---|---|---|---|
| | F | P | R | P | PseudoF | P |
| **Total bacteria** | | | | | | |
| Genotype | 12.15 | **0.000** | 0.65 | **0.001** | 1.87 | **0.002** |
| Compartment | 32.47 | **0.000** | 0.83 | **0.001** | 4.27 | **0.001** |
| Genotype x compartment | 2.55 | **0.037** | nd | nd | 1.44 | **0.001** |
| **Potential beneficial bacteria** | | | | | | |
| Genotype | 4.92 | **0.013** | 0.34 | **0.001** | 1.61 | **0.001** |
| Compartment | 20.86 | **0.000** | 0.48 | **0.001** | 2.87 | **0.001** |
| Genotype x compartment | 1.81 | 0.125 | | | 1.35 | **0.001** |
| **Fungi** | | | | | | |
| Genotype | 1.74 | 0.191 | 0.78 | **0.001** | 2.93 | **0.001** |
| Compartment | 19.00 | **0.000** | 0.76 | **0.001** | 3.56 | **0.001** |
| Genotype x compartment | 2.00 | *0.092* | nd | nd | 1.51 | **0.001** |
| **Potential beneficial fungi** | | | | | | |
| Genotype | 9.23 | **0.001** | 0.22 | **0.001** | 2.05 | **0.004** |
| Compartment | 13.13 | **0.000** | 0.47 | **0.001** | 4.05 | **0.001** |
| Genotype x compartment | 3.46 | **0.008** | nd | nd | 1.33 | **0.033** |
| **Plant pathogenic fungi** | | | | | | |
| Genotype | 3.65 | **0.036** | 0.43 | **0.001** | 3.34 | **0.001** |
| Compartment | 4.30 | **0.011** | 0.51 | **0.001** | 3.90 | **0.001** |
| Genotype x compartment | 2.92 | **0.020** | nd | nd | 1.58 | **0.002** |

In agreement with previous studies[4,30], above-ground organs displayed the lowest bacterial and fungal OTU richness. 'Darselect' showed to be the genotype with the lowest bacterial richness in all compartments (Fig. 1c). OTU richness estimates, predicted with Chao1, also showed similar results as the observed data (Fig. S2). Diversity between above and below-ground microbial community composition (Fig. 1a; fig. S1a) and richness could be explained by the differences in the physical and chemical properties of the two environments. In fact, above-ground organs are subjected to oligotrophic and unstable conditions (with daily and seasonal fluctuations in temperature, humidity, UV light[31]), whereas the soil compartment is relatively more protected, stable and nutrient-rich[32].

Our study showed that Actinobacteria, Alphaproteobacteria, Gammaproteobacteria, Deltaproteobacteria and Bacteroidia are the bacterial groups representing the backbone of strawberry bacterial microbiome in all plant and soil compartments, all together accounting on average for 80% of total detected OTUs based on presence-absence data (Fig. 1a), which correspond to 93% of total bacterial abundance (Fig. S1a). Above-ground organs of 'Darselect' and 'Elsanta' were dominated by Actinobacteria (54 and 53% respectively), whereas 'Monterey' cv was mosty colonized by Gammaproteobacteria (Fig. 1a). Below-ground compartments of the three cvs were dominated by Alphaproteobacteria (Fig. 1a). Analyzing bacterial abundance, we found that Actinobacteria was the predominant group in all genotypes and compartments analysed. The lowest percentages were found in the rhizosphere and bulk soil of 'Elsanta' (31 and 33%, respectively), whereas 'Elsanta' and 'Darselect' above-ground compartments showed a high group homogeneity, being dominated by Actinobacteria for 98 and 99%, respectively (Fig. S1a). Alphaproteobacteria were homogenously represented in plant and soil compartments of the three genotypes. Gammaproteobacteria were almost absent in above ground compartments of 'Elsanta' and 'Darselect', while they were the second most represented group in 'Monterey' (26%) (Fig. S1a).

Regarding the strawberry mycobiome, Sordariomycetes, Dothideomycetes, Leotiomycetes and Agaricomycetes were the most represented fungal classes in all plant and soil compartments accounting for 64% of total OTUs based on presence-absence data (Fig. 1a), which correspond to 86% of total fungal OTU abundance (Fig. S1a), but their percentages varied depending on cv and compartment. Dothideomycetes were predominant in leaves of the three *F.* x *ananassa* genotypes (34% 'Elsanta', 28% 'Darselect', 27% 'Monterey'), whereas below-ground compartments of all genotypes were mostly dominated by Sordariomycetes (Fig. 1a).

# Identification Of The Core Microbiome

In this study, we report the existence of a core microbiome, common to the three genotypes (Fig. 2). The dominant bacterial and fungal groups in the overall and core microbiome are similar, at the fine taxonomic resolution. We identified ubiquitous microbes in all the studied environments from the soil to the above ground plant organs of all three strawberry genotypes. This observation suggests that they are either able to colonize all soil and plant compartments or they can move across soil and the plant organs with a passive or active translocation from roots to the above-ground organs (e.g. leaves, runners).

Among these core microbes, 24 OTUs were bacterial (mainly Micrococcales) and 15 fungal (mainly Ascomycota). Interestingly, several strawberry pathogens were found among the core fungal OTUs, namely *Plectosphaerella cucumerina* (fruit, root and collar rot), *Botrytis caroliniana* (gray mold) and *Alternaria alternata* (black leaf spot) (Table S2). The colonisation of plant organs by a pathogen is not sufficient, per se, to result in a successful infection causing disease symptoms[33]. This observation confirms that abiotic (e.g. temperature, humidity, nutrient availability)[34] and biotic (e.g. plant-associated microbial consortia, plant resistance)[23,35,36] factors play a crucial role in determining the fate of plant-pathogen interactions.

# Functions Potentially Expressed By The Microbiota

Based on their taxonomy, 3,845 bacterial (15% of all detected bacteria) and 706 fungal (41% of all detected fungi) OTUs were assigned to a putative functional group. Results showed that 20 bacterial and 16 fungal functional groups colonized different soil and plant compartments of strawberry plants (Table S3). Chemoheterotrophy, methanol oxidation, intracellular parasitism, predation/exoparasitism were the dominant bacterial functions while saprophytism, plant pathogenic and endophytic colonization were dominant among the fungal functions (Table S3). Within the bacterial OTUs we further explored specific functions relevant to plant health, fitness and growth. We identified 285 OTUs as potential N-fixing genera and 129 OTUs as species known for their activity as biological control agents (BCA) and/or plant growth promoter (PGPB) (Fig. 2; Fig. 3b; Table S4). Interestingly, both compartment and genotype had a significant role in defining plant-associated beneficial bacterial community, according to ANOSIM and PERMANOVA ($P < 0.001$) (Fig. S3a; Table 1; Table S1).

Likewise, different groups of potentially beneficial fungi (mycorrhizae, endophytes, dark septate endophytes, and fungal parasites) were found (Figs. 2 and 3d). In addition to the beneficial fungi recognized with FUNGuild, we highlighted some species previously documented as beneficial to plants (Table S5). As for bacteria, beneficial fungal community showed to be strongly correlated to both genotype ($F = 2.05$, $P = 0.004$) and compartment ($F = 4.05$, $P = 0.001$) (PERMANOVA values genotype × compartment $F = 1.33$, $P = 0.033$; Fig. S3b; Table 1; Table S1).

It has been suggested that domestication of crop plants has determined a reduction in the biodiversity of the associated microflora, in particular for functions regarding nutrition and stress tolerance[37]. On the other hand, it is also possible that cultivated plants recruit microbes specifically exerting beneficial functions under cultural conditions. In this view, the ability to interact with such microbes may be regarded as a trait selected by domestication[38]. In this work, we found that, even after centuries of domestication and complex hybridisation[17,39], cultivated strawberry plants are associated with 16 nitrogen fixing bacterial genera (Fig. 3a), which is more than what reported in wild strawberry plants relatives (*F. chiloensis*, *F. virginiana* ssp. *platypetala*, *F.* × *ananassa* ssp. *cuneifolia*) (7 genera)[40] and comparable to the number of nitrogen-fixing genera (18) reported in legumes, which are nodulating plants specialized for symbiosis with nitrogen-fixing bacteria (Table S6). The presence of nitrogen fixing

bacteria is confirmed by PCR on *nifH* gene in bulk soil, rhizosphere and root samples of the three strawberry genotypes (Fig. S4).

Although, bacterial taxa known to have N-fixing potential were surprisingly found in the above-ground habitat (Fig. 3a), we did not detect any *nifH* gene in this compartment (Fig. S4). Indeed, nitrogenase is inactivated by oxygen. This may indicate that the ability of these bacteria to interact with plant hosts is at least partially disconnected from the ability to fix nitrogen.

Our work also revealed the vast diversity of fungal partners of strawberry, which have not been thoroughly investigated so far, and include ectomycorrhizae, arbuscular mycorrhizae, ericoid mycorrhizae, endophytes, dark septate endophytes and mycoparasites (Figs. 2 and 3c,d ).

Remarkably, we found that both genotype ($F = 3.34$, $P = 0.001$) and plant compartment ($F = 3.90$, $P = 0.001$), as well as their interaction (PERMANOVA values genotype × compartment $F = 1.58$; $P = 0.002$; Fig. 4b; Fig. 3b; Table 1; Table S1) play a key role in the abundance of pathogens in the fungal community associated to strawberry.

The environmental factors, soil conditions and pool of natural microbial inoculum are assumed to be comparable for all three strawberry genotypes, as plants were grown in the same cultural and environmental conditions. Therefore, the observed differences in associated bacterial and fungal communities (Figs. 2, 3) can be explained with the ability of the plant to adjust the composition of the associated microflora[41]. In this view, the lower susceptibility to powdery mildew and leaf spot observed in ‘Monterey’ over the season (Table S8), may be at least partly due to its ability to establish exclusive beneficial microbial relationships (Fig. 4a,c).

Indeed, while most of the potentially beneficial fungal groups are similarly represented in the three strawberry genotypes, the arbuscular mycorrhizae *Rhizophagus irregularis* showed a high frequency only in ‘Monterey’, while being completely absent in ‘Elsanta’ and ‘Darselect’ (Fig. 3d). In several crop plants, the colonisation of the root systems by *R. irregularis* has been demonstrated to confer plant resistance to broad-spectrum of pathogens by induced systemic resistance (ISR) and mycorrhizal-induced resistance (MIR)[42,43]. Regarding the bacterial beneficial microbiome, in cultivar ‘Monterey’, 19% of beneficial OTUs were able to simultaneously colonise below and above-ground organs, whereas in “Elsanta” and “Darselect” only one OTU (identified as *B. megaterium*) was found to colonize both underground and above-ground organs. *B. megaterium* has attracted considerable attention as a functional microbe in several crop species, including strawberry, since it is able to solubilize phosphate and produce phytohormones[44]. Furthermore, it has been proven to be effective for the control of *B. cinerea*[45].

Surprisingly, *Pseudomonas fluorescens* has been detected only in the above-ground compartments of “Monterey”, the genotype showing the highest disease tolerance (Fig. 3b). We further investigated its colonization ability by PCR amplification. Indeed, we proved the ability of *Ps. fluorescens* to establish detectable populations in the soil and, remarkably, both in internal and external tissues of “Monterey” plants (Fig. S5). Many *Ps. fluorescens* strains have been proven to promote plant growth or protection, by

mechanisms such as phosphorus solubilization, phytohormone production, competition against phytopathogens, elicitation of ISR, or production of antimicrobial compounds, such as cyanide or phenolics[46,47]. Non-indigenous *Ps. fluorescens* strains have been already applied to strawberry plants, allowing to anticipate flowering and fruiting, increase fruit yield and vitamin content[48], and to control crown rot (*Phytophtora cactorum*)[49]. Notably, the inoculation of rice seed with a *Ps. fluorescent* strain for riceblast control resulted in the colonization of roots, stems and leaves[50], supporting that this species does not have strict organ preferences.

Interestingly, the combined action of *Pseudomonas* spp. and *Rhizophagus* spp. has been explored in several crop species[43,51,52]. In particular, a mixture of AMF, which included *Rhizophagus* sp., and *Pseudomonas fluorescens* was successfully applied to strawberry, resulting in increased fruit production and quality[48]. The combination of *Rhizophagus* sp. and *Ps. fluorescens* has been proven to elicit plant systemic defence system in tomato via the activation of ethylene response to pathogen attack[43].

Finding unique beneficial microbial patterns for a genotype that showed to be more tolerant than others to biotic stresses suggests an important contribution of the microbiota in the defence strategy of strawberry plants. Influence of rhizosphere microbiome on plant tolerance to root diseases is well known[53]. However, microbiome investigations focusing on specific soil or plant compartments may be less informative than studying the overall plant holobiont. In our work, we show a clear relationship between plant tolerance to above-ground diseases and overall plant colonization by specific microbes. Howbeit, further studies are required to deeply investigate, and finally agronomically exploit, the naturally occurring, genotype-specific plant beneficial microbiome.

### Interactions between strawberry microbiome, plant mineral composition and effect of microbiome on fruit quality

Besides finding significant effects of genotypes, soil and plant compartments on the taxonomic (Fig. 1b,d) and functional composition of both bacterial and fungal communities (Figs. 4b; S3, Table 1), we found significant correlations between the mineral composition of the plant organs and the microbial community assemblage of bacteria and fungi across the different soil and plant compartments (Fig. 5a). Indeed, plant associated microbiomes have been already proven to play a key role in improving plant nutrition both by promoting nutrient acquisition and nutrient use efficiency[54]. On the other side, the host plant and its nutrient preferences impact its microbiome recruitment[55].

In addition, microbes, and particularly those associated with soil and roots, contribute substantially, although indirectly to sensorial fruit quality (Table S11). In details, titratable acidity is mainly related to the below-ground microbiome (Fig. 5b), whereas total soluble solids content of fruits is linked to rhizospheric and above-ground bacterial microbiome and to below-ground fungal microbiome. Notably, inoculation of *Bacillus* sp. on flowers and leaves of sour cherry affected sugar content and titrable acidity of fruits[56]. Similar results were obtained applying bacteria and AMF, both alone or in combination, on strawberry plantlets[22]. Fruiting process and ripening are finely regulated by phytohormones, in particular

by ethylene, auxin and gibberellins that are known to be produced by both fungi and bacteria. Ethylene is a key regulator of fruit ripening thus influencing all the main quality traits. Despite strawberry has been considered as non-climacteric fruit, new genetic evidences suggest that ethylene is required for strawberry ripening[57,58]. Ethylene is produced by a wide range of microbes starting from two alternative precursors, 2-keto-4-methyl-thiobutyric acid (KMBA) or 2-oxoglutarate[59,60]. Furthermore, several bacterial species present the 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity. ACC deaminase degrades the ethylene precursor, thus, impairing its production in the plant tissues[8].

AMF have been proven to affect plant hormonal balance and metabolism, indeed their beneficial effect has been observed both in below and above-ground organs[48]. Besides AMF, PGPB are also able to affect fruit quality, mainly by modulating the interplay between ethylene and auxin metabolisms and proving essential nutrients[8,61]. Altogether, these correlations suggest that bacteria and fungi contribute to the host's adaptation to growing conditions and, consequently, to fruit development.

## Conclusions

Cultivated strawberry genotypes interact with a variety of microbial species. Such interactions have been demonstrated to be specific to genotypes and compartments. These microbiomes play a key role in the plant ability to cope with biotic stress and in modulating fruit quality. Our findings suggest that a comprehensive picture of plant holobiome is needed in order to shed light on the influence of microbial communities and key microbes on plant phenotype and performances. Further studies on microbiomes of crop plants can contribute to the advancement of plant production science, by providing a deeper insight in the interactions between crops and the microflora and evidencing applicative tools and strategies for an efficient and environmentally sustainable horticultural practice. However, the complexity and specificity of the patterns described in this work suggests that the idea to replace agro-chemicals by a few universal beneficial microorganisms is not realistic. Therefore, breeding programs should aim at the selection of high quality, climate-change resilient horticultural varieties with remarkable capacity to establish symbiotic relationships with useful microorganisms[8]. The inclusion of microbial markers in marker-assisted selection will represent a paradigm shift in plant breeding.

## Methods

# Strawberry cultivation, disease severity ranking and physicochemical analyses

Three *Fragaria* × *ananassa* cultivars (genotypes) were used: the everbearing varieties 'Elsanta' (E) and 'Darselect' (D) (widely cultivated in the Northern Italy), and the day-neutral variety 'Monterey' (M). Bareroot strawberries were bought from CREA Forlì, COVIRO Ravenna, SANTORSOLA Trento, for D, M and E genotype, respectively. Plants were transplanted at the beginning of June-July 2017 into 48.5 × 22 × 11 cm white plastic pots, filled with a commercial blond sphagnum peat moss soil (pH 5.2–5.8)

(company Vigorplant s.r.l, Lodi), each pot containing 6 plants with a distance of 16.7 cm between each plant. These pots were maintained at 1.2 m above ground under rainproof tunnel (18 m × 3.50 m × 5.60 ) located in field at the experimental station of Pergine Valsugana (frazione Vigalzano, TN, Italy; 46°07'N, 11°22'E, 450 a.s.l.). Plants were fertigated using a drip system (Table S9). Throughout the season, addition 100 plants of each genotype grown in same conditions were weekly monitored for powdery mildew and leaf spot symptoms. Symptom severity on leaves was visually ranked using a 0–5 scale (0 = no symptoms; 5 = plant death) (Table S7).

# Sampling

At the end of the production cycle (June, 2018), for each genotype, four asymptomatic plant replicates were collected, from different pots distributed in the field area and immediately brought to the laboratory. Definition of the plant-soil compartments were slightly modified from previous studies: 'bulk soil' is the soil domain explored by the roots, but not attached to them (i.e. approx. 1 cm radius form a feeder root); 'rhizosphere' includes only soil particles firmly adhering to root and extracted by washing; 'roots' are washed roots (without visible soil particles); 'above-ground organs of strawberry plant' are constituted by crown (short stem), petiole, leaves and runners. More in detail, bulk soil was collected from the growing pots, approx. 10 cm apart from any plant and at 5 cm depth, and suspended in sterile 10 mM $MgSO_4$ solution. Plants were divided in above-ground tissues (leaves, stems, crown) and roots. Roots were shaken to release loosely-associated soil, then washed in sterile 10 mM $MgSO_4$ solution under vigorous shaking to collect the rhizospheric soil. Above-ground tissues and root samples (further cleaned with a brush) were ground with mortar and pestle, and suspended in sterile 10 mM $MgSO_4$ solution. No bleaching agent was used neither for roots and aerial parts samples as it may enter inside the plant tissues and degrade the microbial DNA targets. All the samples were stored at -20 °C until DNA extraction.

# Dna Extraction And Illumina Sequencing

DNA was extracted from 250 mg of each homogenized bulk soil, rhizosphere, root, and aboveground organs the MoBio PowerSoil kit (MO BIO Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions. DNA quality and quantity were measured by spectrophotometric quantification with a NanoDrop ND-8000 V1.1.1 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany). DNA extracts were then stored at − 20 °C before further analysis. The extracted DNA samples were sent to RTL Genomics, Lubbock, TX, USA for Paired-end Illumina MiSeq sequencing. The V5, V6 and V7 regions of the 16S rRNA gene and ITS2 regions of the nuclear ribosomal internal transcribed spacer (ITS) rRNA gene were targeted for bacteria and fungi respectively. DNA extracts were amplified for sequencing in a two-step process. The forward primer was constructed with (5'-3') the Illumina i5 sequencing primer (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG) and the 799F (5'-AACMGGATTAGATACCCKG-3') (bacteria)[62] or the fITS7 primer (5'- GTGARTCATCGAATCTTTG-3', [1]) (fungi). The reverse primer was constructed with (5'-3') the Illumina i7 sequencing primer

(GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG) and the 1193r (5’-ACGTCATCCCCACCTTCC-3’) (bacteria)[63] or the ITS4 primer (5'-TCCTCCGCTTATTGATATGC-3', [2]) (fungi). The selected primer set for bacteria (799F and 1193r) can strongly reduce contamination from plastid DNA. Amplifications were performed in 25 µl reactions with Qiagen HotStar Taq master mix (Qiagen Inc, Valencia, California), 1 µl of each 5 µM primer, and 1 µl of template. Reactions were performed on ABI Veriti thermocyclers (Applied Biosytems, Carlsbad, CA, USA) under the following thermal profiles: 95●C for 15 min, then 35 cycles of 94●C for 30 sec, 55●C for 30 sec, 72●C for 1 min, followed by one cycle of 72●C for 10 min and 4●C hold (bacteria) and 95●C for 15 min, then 35 cycles of 94●C for 30 sec, 54●C for 40 sec, 72●C for 1 min, followed by one cycle of 72●C for 10 min and 4●C hold (fungi). Products from the first stage amplification were added to a second PCR based on qualitatively determine concentrations. Primers for the second PCR were designed based on the Illumina Nextera PCR primers as follows: Forward - AATGATACGGCGACCACCGAGATCTACAC[i5index]TCGTCGGCAGCGTC and Reverse - CAAGCAGAAGACGGCATACGAGAT[i7index]GTCTCGTGGGCTCGG. The second stage amplification was run the same as the first stage except for 10 cycles. Amplified products were visualized with eGels (Life Technologies, Grand Island, New York). The products were then pooled equimolar and each pool was size selected in two rounds using Agencourt AMPure XP (BeckmanCoulter, Indianapolis, Indiana) in a 0.75 ratio for both rounds. The size selected pools were then quantified using the Quibit 2.0 fluorometer (Life Technologies) and loaded on an Illumina MiSeq (Illumina, Inc. San Diego, California) 2 × 300 flow cell at 10pM.

nifH **gene and** Pseudomonas fluorescens **detection**

The presence of *nifH* gene in samples was verified by PCR using *nifH* gene-specific primers (PolF (5'- TGC GAY CCS AAR GCB GAC TC -3') /PolR (5’- ATS GCC ATC ATY TCR CCG GA -3')), as previously described[64]. *Pseduomonas fluorescens* detection in ‘Monterey’ genotype was performed as follows: bulksoil and rhizosphere DNA were extracted as above; roots and above ground parts of strawberry plants were surface sterilized two times with deionized water and 70% ethanol and washed 3 times sterile water, organs were let 3 h in sterile water. DNA was extracted as above and amplified using *Pseudomonas fluorescens* specific primers (16SPSEfluF (5'-TGC ATT CAA AAC TGA CTG-3') /16SPSER (5'-AAT CAC ACC GTG GTA ACC G-3')) as described elsewhere[65]. Both for *nifH* and *P. fluorescens*, amplification products were visualized through agarose gel 1.5% electrophoresis.

# Analysis Of Plant Mineral Composition And Fruit Quality Traits

Ultrapure 65% $HNO_3$ was obtained from analytical grade $HNO_3$ (Carlo Erba, Milan, Italy) by means of a SAVILLEX DST 1000 sub-boiling system (Savillex Corp., Eden Prairie, MN, USA). Standard solution and sample preparation were carried out by weight with a Mettler AE200 analytical balance (Mettler Toledo S.p.A, Milan, Italy) with ± 0.0001 g sensitivity. Elemental analysis on root and above-ground organs of

strawberry plants (percentage of C, H and N) was performed on Thermo ScientificTM FLASH 2000 organic elemental analyser, each sample was analysed in duplicate. Strawberry samples' digestion was performed by a microwave assisted procedure performed with a FKV autoclave, Ultrawave model, on a maximum sample aliquot of 0.4 g, accurately weighted in the microwave quartz vessels, before adding 1.5 mL $HNO_3$ and 3.5 mL $H_2O$. At the end of digestion process, an almost colourless, pale yellow sample was obtained. The resulting solutions were diluted up to a total mass of 15 g with Milli-Q water in polypropylene tubes, microfiltered (Ø 0.22 mm) and analysed. Measurements of the Mg, P, K, Ca, Fe, Mn, Co, Ni, Zn. Sr, Ba and Pb content in vegetal samples were performed by using an inductively coupled plasma interfaced to a quadrupolar mass analyzer, ICP/qMS, (XSeries II model, ThermoFisher Scientific, Bremen, Germany) equipped with Peltier cooled (3 °C) spray chamber. The collected samples were randomly acquired after being introduced by the autosampler CETAC ASX 520 into the nebulizer, and the positively charged ions were then produced by a high-temperature, inductively coupled plasma. The ions passed through a sampling cone interface into a high-performance quadruple mass spectrometer, which is computer controlled to carry out multi-element analysis. Data were analysed by PlasmaLab software. The instrument was tuned daily with an ICP-MS tuning solution. In in $HNO_3$ 4% (100 ppb) was used as internal standard. ICP-multi-element solution, IV-ICP-MS-71A (Inorganic Ventures, Christiansburg, VA, USA) was used for the determination of Mg, P, K, Ca, Fe, Mn, Co, Ni, Zn, Sr, Ba and Pb concentrations. Each sample was analysed at least in 3 independent measurements and each experiment comprised three repetitions. Results are given as mean value ± standard deviation (Table S10).

Strawberry fruit firmness was measured by a texture analyser (Zwick Roell, Italy) using the penetration test methodology that was previously developed for raspberry[66]. This penetration test outlined a mechanical force displacement using a 5 kg loading cell and a cylindrical flat head probe with a diameter of 4 mm entering into the berry flesh that was placed on the plate with the receptacle upright to the compression probe. Mechanical profiles were acquired with a resolution of 100 points per second with the following instrumental settings: test speed of 300 mm $min^{-1}$, post-test speed of 1000 mm $min^{-1}$, auto force trigger of 2 g and stop plot at target position. Each berry was penetrated until a 99% penetration strain. In this study only the maximum force value (N) was considered, since this parameter is usually highly related with berry firmness[66].

Soluble sugar content was measured on strawberry fruit juice with a hand-held Atago digital refractometer (Optolab, Modena, Italy). Titratable acidity was determined on strawberry juice diluted (1:2) in distilled water by titration with NaOH to pH 8.1, and expressed as citric acid equivalents.

# Bioinformatics

High quality reads from the paired-end sequences generated by Illumina MiSeq sequencing platform were extracted using MOTHUR[67] and OBI Tools[68] software suits. PANDAseq was used to merger forward and reverse raw reads from the same sample by using the simple-bayesian algorithm with a minimum overlap of 80 and 20 nucleotides for bacteria and fungi, respectively. All the merged reads were then trimmed with

the following parameters: (i) minimum length of 350 (bacteria) and 120 (fungi), (ii) minimum average Phred score of 25 on the trimmed length, (iii) no ambiguities in the sequence length, and (iv) maximum length of 20 homoploymers in the sequence. The reads were then pre-clustered using CD-HIT-EST, allowing a maximum of 1% of dissimilarity and with only one base allowed per indel[69], in order to merge those reads arising likely from sequencing errors[70]. Chimeric sequences were detected using the UCHIME algorithm[71] as implemented in MOTHUR and removed. Reads from each sample were pooled together and were dereplicated into unique sequences and sorted by decreasing abundance. The resulting reads were then clustered into operational taxonomic units (OTUs) using the CD-HIT-EST algorithm[72] at a threshold of 97% sequence similarity. The OTU representative sequences (defined as the most abundant sequence in each OTU) were taxonomically assigned against the reference sequences from the SILVA database v132 for prokaryote 16S[73] and from the Unite database (version unite.v7)[74] for fungal ITS using the naive Bayesian classifier[75] as implemented in MOTHUR using the default parameters. All the sequences identified as non-target organisms were removed from bacterial and fungal datasets. Rare OTUs (singletons), which potentially might represent artificial sequences were removed. The read counts were rarefied to the smallest read number per sample (10,930 and 8,077 reads for bacteria and fungi, respectively). Ecological functions were determined for each OTU using FAPROTAX for bacteria[76], and FUNGuild[77] for fungi. Ecological functions of bacteria obtained by FAPROTAX were also manually checked against other references for their present in terrestrial system. We grouped arbuscular mycorrhizae, ectomycorrhizae, ericoid mycorrhizae, endophytes, dark septate endophytes and mycoparasites as potential beneficial fungi. All fungal plant pathogens were checked again for their taxonomic identifications and their DNA-based Species Hypotheses (SH) are presented in Supplementary Table S8. Potential beneficial bacteria (N fixing, plant growth promoting and biological control agents) were manually assigned using all available references (Table S4). The Illumina sequencing of all bacterial and fungal datasets are deposited in The National Center for Biotechnology Information (NCBI) database under BioProject: PRJNA556362 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA556362?reviewer=4d8dskbpvkqqcci8o1inim36b).

# Statistical analysis:

To assess the coverage of the sequencing depths, individual rarefaction analysis was performed for each sample using the function ‘diversity’ in PAST. At the analyzed sequencing depths, all individual rarefactions shown to be sufficient to infer bacterial and fungal community composition and richness in our samples (Fig. S1). We defined core microbiome as the bacterial and fungal communities that are comprised of OTUs that were detected in all strawberry genotype and present in more than 75% of the samples[78]. The effects of strawberry genotype, soil plant compartment (bulk soil, rhizosphere, root and aboveground organs) on bacterial and fungal OTUs richness were analyzed using two-way analysis of variance (ANOVA), incorporating the Jarque-Bera JB test for normality. The effects of strawberry genotype, soil and plant compartment on bacterial and fungal community compositions were visualized using Non-metric multidimensional scaling (NMDS) based on the presence–absence data and Jaccard distance measure. Coloured ellipses in NMDS ordinations are 95% confidence intervals of species

centroids for each treatment level. The significant effect of the strawberry genotype, soil and plant compartment on bacterial and fungal community compositions were determined using two-way Analysis of Similarity (ANOSIM) and two-way Permutational multivariate analysis of variance (PERMANOVA) based on the presence–absence data and Jaccard distance measure over 999 permutations. Since relative abundance data from Next Generation Sequencing may not be fully used quantitatively[79], we analyzed the microbial community composition using both presence/absence and relative abundance data sets. The results from presence/absence data are presented in the main text and the corresponding results using relative abundance data (with Bray–Curtis distance measure) are presented in Supplementary Information (Table S1). NMDS ordination based on presence/absence data and the Jaccard dissimilarity measure coupled with the envfit function of the vegan package in R were used to investigate the links between each of bacterial and fungal community composition (bulk soil, rhizosphere, root and aboveground organs) and soil nutrient parameters, strawberry genotypes, fruit quality parameters (soluble sugar content and titratable acidity). NMDS stress values were between 0.06–0.13. All statistical analyses were performed using PAST[80] version 2.17. and R version 3.2.2[81].

# Abbreviations

AMF
Arbuscular Mychorrhiza Fungi
BCA
Biological Control Agent
cv
cultivar (in strawberry, a cultivar is a human-bred genotype)
NGS
Next Generation Sequencing
PGPB
Plant Growth Promoting Bacteria

# Declarations

- **Ethics approval and consent to participate**: Not Applicable
- **Consent for publication**: Not Applicable
- **Availability of data and materials**: The datasets Illumina sequencing of all bacterial and fungal datasets generated during the current study are available in The National Center for Biotechnology Information (NCBI) database under BioProject: PRJNA556362 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA556362?reviewer=4d8dskbpvkqqcci8o1inim36b).
- **Competing interests**: The authors declare that they have no competing interests
- **Funding**: This work did not receive any external funding.

- **Authors contribution**: FS and WP conceived project idea and designed the experiments. BF and ID carried out the field experiment. EF analyzed the mineral composition. WP, BT, DoS, ID and DS performed molecular analysis. BF, ID and AC performed the biochemical analysis. ID performed the classical microbiological and fruit quality analysis. SFW contributed for bioinformatics. WP, DS and BF contributed for statistical and data analysis. AC, DS, ID, EF, BT, SFW, DoS, BF, FB, FS and WP contributed to critical discussion and revision of final manuscript. All authors read and approved the final manuscript.
- **Acknowledgements:** The authors are grateful to L. Giongo for providing accession to the fields and to P. Martinatti for plants management.

# References

1. Purahong W, Orrù L, Donati I, Perpetuini G, Cellini A, Lamontanara A, et al. Plant microbiome and its link to plant health: Host species, organs and *Pseudomonas syringae* pv. *actinidiae* infection shaping bacterial phyllosphere communities of kiwifruit plants. Front. Plant Sci. 2018; 9:1563.
2. Compant S, Samad A, Faist H, Sessitsch A. A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. J. Adv. Res. 2019;19:29-37.
3. Busby PE, Newcombe G, Dirzo R, Whitham TG. Differentiating genetic and environmental drivers of plant–pathogen community interactions. J. Ecol. 2014;102:1300–1309.
4. Cregger MA, Veach AM, Yang ZK, Crouch MJ, Vilgalys R, Tuskan GA, Schadt CW.The Populus holobiont: dissecting the effects of plant niches and genotype on the microbiome. Microbiome. 2018;6.
5. Lemanceau P, Blouin M, Muller D, Moënne-Loccoz Y. Let the core microbiota be functional. Trends Plant Sci. 2017;22:583-595.
6. Xu J, Zhang Y, Zhang P, Trivedi P, Riera N, Wang, Y, et al. The structure and function of the global citrus rhizosphere microbiome. Nat. Commun. 2018;9:1-10.
7. Kong HG, Song CG, Ryu C-M. Inheritance of seed and rhizosphere microbial communities through plant–soil feedback and soil memory. Env. Microb. Reports. 2019;11:479-486.
8. Sangiorgio D, Cellini A, Donati I, Pastore C, Onofrietti C, Spinelli F. Facing Climate Change: Application of Microbial Biostimulants to Mitigate Stress in Horticultural Crops. Agronomy. 2020;10:794.
9. Busby PE, Soman C, Wagner MR, Friesen ML, Kremer J, Bennett A, et al. Research priorities for harnessing plant microbiomes in sustainable agriculture. PLoS Biol. 2017;15:e200179.
10. Gutschick VP, BassiriRad H. Extreme events as shaping physiology, ecology, and evolution of plants: toward a unified definition and evaluation of their consequences. New Phytologist. 2008;160: 21-42.
11. Hargreaves SK, Hofmockel KS. Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. Biogeochemistry. 2014; 117: 67-79.
12. Thompson KA, Bent E, Abalos D, Wagner-Riddle C, Dunfield KE. Soil microbial communities as potential regulators of in situ N2O fluxes in annual and perennial cropping systems. Soil Biol.

Biochem. 2016;103:262-273.
13. McGowan AR, Nicoloso RS, Diop HE, Roozeboom KL, Rice CW. Soil organic carbon, aggregation, and microbial community structure in annual and perennial biofuel crops. Agronomy Journal. 2019;111:128-142.
14. Grady KL, Sorensen JW, Stopnisek N, Guittar J, Shade A. Assembly and seasonality of core phyllosphere microbiota on perennial biofuel crops. Nat Commun. 2019;10:4135.
15. Bergelson J, Mittelstrass J, Horton MW. Characterizing both bacteria and fungi improves understanding of the *Arabidopsis* root microbiome. Sci. Rep. 2019;9:24.
16. Qiu Z, Egidi E, Liu H, Kaur S, Singh BK. New frontiers in agriculture productivity: Optimised microbial inoculants and in situ microbiome engineering. Biotech. Adv. 2019;37:107371.
17. Bertioli DJ. The origin and evolution of a favorite fruit. Nat. Genet. 2019;51:372-373.
18. Food and Agriculture Organization of the United Nations. (1997). FAOSTAT statistical database. [Rome] :FAO
19. Mezzetti B, Giampieri F, Zhang YT, Zhong CF. Status of strawberry breeding programs and cultivation systems in Europe and the rest of the world. J. Berry Res. 2018;8:205-221.
20. Battino M, Forbes-Hernandez TY, Gasparrini M, Afrin S, Mezzetti B, Giampieri F. The effects of strawberry bioactive compounds on human health. In VIII International Strawberry Symposium. 2018;1156:355-362.
21. Giampieri F, Forbes-Hernandez TY, Gasparrini M, Alvarez-Suarez JM, Afrin S, Bompadre S, et al. Strawberry as a health promoter: an evidence based review. Food Func. 2015;6:1386-1398.
22. Todeschini V, AitLahmidi N, Mazzucco E, Marsano F, Gosetti F, Robotti E, et al. Impact of beneficial microorganisms on strawberry growth, fruit production, nutritional quality, and volatilome. Front. Plant Sci. 2018;9.
23. Husaini AM, Neri D. Strawberry: growth, development and diseases. Eds; 2016.
24. Amsalem L, Freeman S, Rav-David D, Nitzani Y, Sztejnberg A, Pertot I, Elad Y. Effect of climatic factors on powdery mildew caused by *Sphaerotheca macularis* f. sp. *fragariae* on strawberry. Eur. J. Plant Pathol. 2006;114:283-292.
25. Sargent DJ, Buti M, Šurbanovski N, Brurberg MB, Alsheikh M, Kent MP, Davik J. Identification of QTLs for powdery mildew (*Podosphaera aphanis*; syn. *Sphaerotheca macularis* f. sp. *fragariae*) susceptibility in cultivated strawberry (*Fragaria*× *ananassa*). PloS one. 2019;14.
26. Whitaker VM, Knapp SJ, Hardigan MA, Edger PP, Slovin JP, Bassil NV, et al. A roadmap for research in octoploid strawberry. Hort. Res. 2020;7:1-17.
27. Mazzola M, Freilich S. Prospects for biological soilborne disease control: application of indigenous versus synthetic microbiomes. Phytopath. 2017;107:256–263.
28. Haney CH, Samuel BS, Bush J, Ausubel FM. Associations with rhizosphere bacteria can confer an adaptive advantage to plants. Nat. Plants. 2015;1.

29. Santhanam R, Luu VT, Weinhold A, Goldberg J, Oh Y, Baldwin IT. Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping. Proc. Natl. Acad. Sci. U. S. A. 2015;112:E5013–20.
30. De Souza RSC, Okura VK, Armanhi JSL, Jorrín B, Lozano N, Da Silva MJ, et al. Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. Sci. Rep. 2016;6:28774.
31. Redford AJ, Fierer N. Bacterial succession on the leaf surface: a novel system for studying successional dynamics. Microb. Ecol. 2009;58:189-198.
32. Lindow SE, Brandl MT. Microbiology of the phyllosphere. Appl. Environ. Microbiol. 2003; 69:1875-1883.
33. Donati I, Cellini A, Sangiorgio D, Vanneste JL, Scortichini M, Balestra GM, Spinelli F. *Pseudomonas syringae* pv. *actinidiae*: Ecology, Infection Dynamics and Disease Epidemiology. Microb. Ecol. 2020;1-22.
34. Pandey P, Senthil-Kumar M. Plant-pathogen interaction in the presence of abiotic stress: What do we know about plant responses?. Plant Physiology Reports. 2019;1-9.
35. Ab Rahman SFS, Singh E, Pieterse CM, Schenk PM. Emerging microbial biocontrol strategies for plant pathogens. Plant Sci. 2018; 267: 102-111.
36. Zhang Y, Lubberstedt T, Xu M. The genetic and molecular basis of plant resistance to pathogens. J. Genet. Genom. 2013; 40:23-35.
37. Pérez-Jaramillo JE, Mendes R, Raaijmakers JM. Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol. Biol. 2016;90:635-644.
38. Pérez-Jaramillo JE, Carrión VJ, de Hollander M, Raaijmakers JM.The wild side of plant microbiomes. Microbiome. 2018;6:143.
39. Edger PP, Poorten TJ, VanBuren R, Hardigan MA, Colle M, McKain M, et al. Origin and evolution of the octoploid strawberry genome. Nat. Genet. 2019;51:541–547.
40. Wei N, Ashman T-L. The effects of host species and sexual dimorphism differ among root, leaf and flower microbiomes of wild strawberries in situ. Sci. Rep. 2018;8:5195.
41. Morella NM. Multiple forces shape the phyllosphere microbiome: The importance of vertical transmission, environmental selection, and bacteriophages (Doctoral dissertation, UC Berkeley) (2019).
42. Cameron DD, Neal AL, van Wees SC, Ton J. Mycorrhiza-induced resistance: more than the sum of its parts?. Trends Plant Sci. 2013;18:539-545.
43. Velivelli SL., Lojan P, Cranenbrouck S, de Boulois HD, Suarez JP, Declerck S, et al. The induction of Ethylene response factor 3 (ERF3) in potato as a result of co-inoculation with *Pseudomonas* sp. R41805 and *Rhizophagus irregularis* MUCL 41833 - a possible role in plant defense. Plant Signal Behav. 2015;10:e988076.
44. Dias AC, Costa FE, Andreote FD, Lacava PT, Teixeira MA, Assumpçao LC,et al. Isolation of micropropagated strawberry endophytic bacteria and assessment of their potential for plant growth

promotion. World J. Microb. Biotechnol. 2009;25:189-195.

45. Donmez MF, Esitken A, Yildiz H, Ercisli S. Biocontrol of *Botrytis cinerea* on strawberry fruit by plant growth promoting bacteria. J. Anim. Plant Sci. 2011;21:758-763.
46. Bakker P, Pieterse CMJ, Van Loon LC. Induced systemic resistance by fluorescent *Pseudomonas* spp. Phytopathology. 2007;97:239-243.
47. David Baliah V, Chandrasehar G, Selvam PN. *Pseudomonas fluorescens*: a plant-growth-promoting rhizobacterium (PGPR) with potential role in biocontrol of pests of crops. Crop improvement through microbial biotechnology. 2018;221-243.
48. Bona E, Lingua G, Manassero P, Cantamessa S, Marsano F, Todeschini V, et al. AM fungi and PGP pseudomonads increase flowering, fruit production, and vitamin content in strawberry grown at low nitrogen and phosphorus levels. Mycorrhiza. 2015;25:181-193.
49. Agusti L, Bonaterra A, Moragrega C, Camps J, Montesinos E. Biocontrol of root rot of strawberry caused by *Phytophthora cactorum* with a combination of two *Pseudomonas fluorescens* strains. J. Plant Pathol. 2011;363-372.
50. Vidhyasekaran P, Rabindran R, Muthamilan M, Nayar K, Rajappan K, Subramanian N, Vasumathi K. Development of a powder formulation of *Pseudomonas fluorescens* for control of rice blast. Plant Pathol. 1997;46:291-297.
51. Ma Y, Látr A, Rocha I, Freitas H, Vosátka M, Oliveira RS. Delivery of inoculum of *Rhizophagus irregularis* via seed coating in combination with *Pseudomonas libanensis* for cowpea production. Agronomy. 2019;9:33.
52. Bahmani M, Naghdi R, Kartoolinejad D. Milkweed seedlings tolerance against water stress: Comparison of inoculations with *Rhizophagus irregularis* and *Pseudomonas putida*. Env. Tech. Innov. 2018;10:111-121.
53. Mendes R, Kruijt M, De Bruijn I, Dekkers E, van der Voort M, Schneider JH, et al. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science. 2011;332:1097-1100.
54. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. Plant–microbiome interactions: from community assembly to plant health. Nat. Rev. Microbiol. 2020;1-15.
55. Mendes R, Kruijt M, De Bruijn I, Dekkers E, van der Voort M, Schneider JH, et al. The nutrient preference of plants influences their rhizosphere microbiome. Appl. Soil Ecol. 2017;110:146-150.
56. Arikan Ş, Pirlak L. Effects of plant growth promoting rhizobacteria (PGPR) on growth, yield and fruit quality of sour cherry (*Prunus cerasus* L.). Erwerbs-obstbau 2016;58: 221-226.
57. Sun JH, Luo JJ, Tian L, Li CL, Xing Y, Shen Y. New Evidence for the Role of Ethylene in Strawberry Fruit Ripening. J. Plant Growth Regul. 2013;32:461–470.
58. Merchante C, Vallarino JG, Osorio S, Aragüez I, Villarreal N, Ariza MT, et al. Ethylene is involved in strawberry fruit ripening in an organ-specific manner. J. Exp. Bot. 2013;64:4421-4439.
59. Nagahama K, Ogawa T, Fujii T, Fukuda H. Classification of ethylene-producing bacteria in terms of biosynthetic pathways to ethylene J. Ferment. Bioeng. 1992;73:1–5.

60. Hausinger R.P. Fe(II)/α-ketoglutarate-dependent hydroxylases and related enzymes. Crit. Rev. Biochem. Mol. Biol. 2004;39:21–68.
61. Perpetuini G, Donati I, Cellini A, Orrú L, Giongo L, Farneti B, Spinelli F. Genetic and functional characterization of the bacterial community on fruit of three raspberry (*Rubus idaeus*) cultivars. J.Berry Research. 2019;9:227-24.
62. Chelius MK, Triplett EW. The diversity of archaea and bacteria in association with the roots of *Zea mays* L. Microb. Ecol. 2011;41:252–263.
63. Bodenhausen N, Horton MW, Bergelson J. Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. PLoS One. 2013;8:e56329.
64. Hoppe B, Kahl T, Karasch P, Wubet T, Bauhus J, Buscot F, Krüger D.Network analysis reveals ecological links between N-fixing bacteria and wood-decaying fungi. PLoS One. 2014;9:e88141.
65. Scarpellini M, Franzetti L, Galli A. Development of PCR assay to identify *Pseudomonas fluorescens* and its biotype. FEMS Microbiol. Lett. 2004;236:257-260.
66. Giongo L, Ajelli M, Poncetta P, Ramos-García M, Sambo P, Farneti B. Raspberry texture mechanical profiling during fruit ripening and storage. Postharvest Biol. Tec. 2019;149:177-186.
67. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 2009;75:7537–7541.
68. Boyer F, Mercier C, Bonin A, Le Bras Y, Taberlet P, Coissac E. Obitools: a unix-inspired software package for DNA metabarcoding. Mol. Ecol. Resour. 2016;16:176–182.
69. Niu B, Fu L, Sun S, Li W. Artificial and natural duplicates in pyrosequencing reads of metagenomic data. BMC Bioinformatics. 2010;11:187.
70. Huse SM, Welch DM, Morrison HG, Sogin ML. Ironing out the wrinkles in the rare biosphere through improved OTU clustering. Environ. Microbiol. 2010;12:1889–1898.
71. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinforma. Oxf. Engl. 2011;27:2194–2200.
72. Fu L, Niu B, Zhu Z, Wu S, Li W. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinforma. Oxf. Engl. 2012;28:3150–3152.
73. Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, et al. The SILVA and “All-species Living Tree Project (LTP)” taxonomic frameworks. Nucleic Acids Res. 2014;42:643–64.
74. Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AF, Bahram M. Towards a unified paradigm for sequence-based identification of fungi. Mol. Eco. 2013;22:5271-5277.
75. Bai HF, Li MW, Wang DS. Bayesian classifier based service-aware mechanism in 10G-EPON for smart power grid. Acta Photonica Sinica. 2013;42:668-673.
76. Louca S, Parfrey LW, Doebeli M. Decoupling function and taxonomy in the global ocean microbiome. Science. 2016;353:1272–1277.

77. Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, et al. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 2016;20:241–248.
78. Xu J, Zhang Y, Zhang P, Trivedi P, Riera N, Wang Y, et al. The structure and function of the global citrus rhizosphere microbiome. Nat. Commun. 2018;9:1-10.
79. Amend AS, Seifert KA, Bruns TD. Quantifying microbial communities with 454 pyrosequencing: does read abundance count?. Mol. Ecol. 2010;19:5555-5565.
80. Hammer Ø, Harper DAT, Ryan PD. PAST: Paleontological statistics software package for education and data analysis. Palaeontol. Electron. 2001;4:9.
81. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara RB, et al. Package 'vegan'. Community ecology package, version, 2 2013;1-295.
82. Khan MS, Zaidi A, Musarrat J. Microbes for legume improvement. Vienna: Springer; 2010.
83. Choudhary DK, Johri BN. Interactions of *Bacillus* spp. and plants–with special reference to induced systemic resistance (ISR). Microbiol. Research. 2009;164:493-513.
84. Nabti E, Bensidhoum L, Tabli N, Dahel D, Weiss A, Rothballer M, et al. Growth stimulation of barley and biocontrol effect on plant pathogenic fungi by a *Cellulosimicrobium* sp. strain isolated from salt-affected rhizosphere soil in northwestern Algeria. Eur. J. Soil Biol. 2014;61: 20-26.
85. Prapagdee B, Kuekulvong C, Mongkolsuk S. Antifungal potential of extracellular metabolites produced by *Streptomyces hygroscopicus* against phytopathogenic fungi. Int. J. Biol.Sci. 2008;4:330.
86. Raps A, Vidal S. Indirect effects of an unspecialized endophytic fungus on specialized plant-herbivorous insect interactions. Oecologia. 1998;114:541–547.
87. Mari M, Martini C, Spadoni A, Rouissi W, Bertolini P. Biocontrol of apple postharvest decay by *Aureobasidium pullulans*. Postharvest Biol. Tec. 2012;73:56-62.
88. Marín A, Cháfer M, Atarés L, Chiralt A, Torres R, Usall J, Teixidó N. Effect of different coating-forming agents on the efficacy of the biocontrol agent *Candida sake* CPA-1 for control of *Botrytis cinerea* on grapes. Biol. Control. 2016;96:108-119.
89. Shanthiyaa V, Saravanakumar D, Rajendran L, Karthikeyan G, Prabakar K, Raguchander T. Use of *Chaetomium globosum* for biocontrol of potato late blight disease. Crop Protection. 2013;52:33-38.
90. Soytong K, Kanokmedhakul S, Kukongviriyapa V, Isobe M. Application of *Chaetomium species* (Ketomium) as a new broad spectrum biological fungicide for plant disease control. Fungal Divers. 2001;7:1-15.
91. Ravnskov S, Jensen B, Knudsen IM, Bødker L, Jensen DF, Karliński L, Larsen J. Soil inoculation with the biocontrol agent *Clonostachys rosea* and the mycorrhizal fungus Glomus intraradices results in mutual inhibition, plant growth promotion and alteration of soil microbial communities. Soil Biol. Biochem. 2006;38:3453-3462.
92. Gholami M, Amini J, Abdollahzadeh J, Ashengroph M. Basidiomycetes fungi as biocontrol agents against take-all disease of wheat. Biol. Control. 2019;130:34-43.

93. Gil-Serna J, Patiño B, Cortés L, González-Jaén MT, Vázquez C. Mechanisms involved in reduction of ochratoxin A produced by *Aspergillus westerdijkiae* using *Debaryomyces hansenii* CYC 1244. Int. J. Food Microbiol. 2011;151:113-118.
94. Schisler DA, Yoshioka M, Vaughan MM, Dunla CA, Rooney AP. Nonviable biomass of biocontrol agent *Papiliotrema flavescens* OH 182.9 3C enhances growth of *Fusarium graminearum* and counteracts viable biomass reduction of Fusarium head blight. Biol. Control. 2019;128:48-55.
95. Sandberg C. Evaluation of the antagonistic activity of *Rhodotorula babjevae* and its extracellular compounds towards postharvest pathogens. (2019).
96. Yin J, Qian F, Huang J. New functions of *Ceriporia lacerate* in phytophthora blight control and growth promotion of eggplants. Scientia Agricultura Sinica. 2010;51:2300-2310.
97. Khan AL, Shinwari ZK, Kim YH, Waqas M, Hamayun M, Kamran M, Lee I. Role of endophyte *Chaetomium globosum* LK4 in growth of *Capsicum annuum* by producion of gibberellins and indole acetic acid. Pak. J. Bot. 2012;44:1601-1607.
98. Szwajkowska-Michałek L, Kwaśna H, Łakomy P, Perkowski J. Inhibition of *Armillaria* and *H eterobasidion* growth by *Penicillium adametzii* isolated *from Pinus sylvestris* forest soil. Forest Pathol. 2012;42:454-466.
99. Lopes MR, Klein MN, Ferraz LP, da Silva AC, Kupper KC. *Saccharomyces cerevisiae*: a novel and efficient biological control agent for *Colletotrichum acutatum* during pre-harvest. Microbiol Res. 2015;175:93-99.
100. Adnan M, Islam W, Shabbir A, Khan KA, Ghramh HA, Huang Z, et al. Plant defense against fungal pathogens by antagonistic fungi with *Trichoderma* in focus. Microb. Pathog. 2019;129:7-18.
101. Zimmermann G. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Sci. Tec. 2007;17:553-596.
102. Ownley BH, Kimberly DG, Fernando EV. Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. BioControl. 2010;55:113-128.
103. Khan AL, Hamayun M, Kim YH, Kang SM, Lee JH, Lee IJ. Gibberellins producing endophytic *Aspergillus fumigatus* sp. LH02 influenced endogenous phytohormonal levels, isoflavonoids production and plant growth in salinity stress. Process Biochem. 2011;46:440-447.
104. Hamayun M, Khan SA, Khan AL, Rehman G, Kim YH, Iqbal I, et al. Gibberellin production and plant growth promotion from pure cultures of *Cladosporium* sp. MH-6 isolated from cucumber (*Cucumis sativus* L.). Mycologia. 2010;102:989-995.
105. Li F, Chen L, Redmile-Gordon M, Zhang J, Zhang C, Ning Q, Li W. *Mortierella elongata*'s roles in organic agriculture and crop growth promotion in a mineral soil. Land Degrad. Devel. 2018;29:1642-1651.
106. Bilal L, Asa S., Hamayun M, Gul H, Iqbal A, Ullah I, et al. Plant growth promoting endophytic fungi *Aspergillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 produce gibberellins and regulates plant endogenous hormones. Symbiosis. 2018;76:117-127.

107. Yakti W, Kovács GM, Vági P, Franken P. Impact of dark septate endophytes on tomato growth and nutrient uptake. Plant Ecol. Div. 2018;11:637-648.
108. Zhang S, Yantai G, Bingliang X. Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. Front. Plant Sci. 2016;1405.