

A peer-reviewed version of this preprint was published in PeerJ on 26 February 2015.

[View the peer-reviewed version](https://doi.org/10.7717/peerj.804) (peerj.com/articles/804), which is the preferred citable publication unless you specifically need to cite this preprint.

Aleklett K, Leff JW, Fierer N, Hart M. 2015. Wild plant species growing closely connected in a subalpine meadow host distinct root-associated bacterial communities. PeerJ 3:e804 <https://doi.org/10.7717/peerj.804>

Distinct root-associated bacterial communities on three wild plant species growing in a common field

Plant roots are known to harbor large and diverse communities of bacteria. It has been suggested that plant identity can structure these root-associated communities, but few studies have specifically assessed how the composition of root microbiota varies within and between plant species growing under natural conditions. We sampled endophytic and epiphytic bacteria in root tissues from a population of a wild, clonal plant (Orange hawkweed – *Pilosella aurantiaca*) as well as two neighboring plant species (Oxeye daisy – *Leucanthemum vulgare* and Alsike clover – *Trifolium hybridum*) to determine if plant species hosted unique root microbiota. Our results show that plants of different species host distinct bacterial communities in their roots. In terms of community composition, Betaproteobacteria (especially the family Oxalobacteraceae) were found to dominate in the root microbiota of *L. vulgare* and *T. hybridum* samples, whereas the root microbiota of *P. aurantiaca* had a more heterogeneous distribution of bacterial abundances where gamma Proteobacteria and Acidobacteria occupied a larger portion of the community. Whether all plant species host their own distinct root microbiota and plants more closely related to each other share more similar bacterial communities still remains to be explored.

1 **Distinct root-associated bacterial communities on three wild plant species growing in a**
2 **common field**

3 Kristin Aleklett¹, Jonathan W. Leff², Noah Fierer², Miranda Hart^{1*}

4 ¹*Department of Biology, Irving K. Barber School of Arts and Sciences, University of British*
5 *Columbia – Okanagan, 3333 University Way, Kelowna, BC, V1V 1V7 CANADA, ²Cooperative*
6 *Institute for Research in Environmental Sciences and the Department of Ecology and*
7 *Evolutionary Biology, University of Colorado, Boulder, Colorado, United States of America*

8 *Corresponding author: Miranda Hart, miranda.hart@ubc.ca, +1-250-807-9398

9 **Abstract**

10 Plant roots are known to harbor large and diverse communities of bacteria. It has been suggested
11 that plant identity can structure these root-associated communities, but few studies have
12 specifically assessed how the composition of root microbiota varies within and between plant
13 species growing under natural conditions. We sampled endophytic and epiphytic bacteria in root
14 tissues from a population of a wild, clonal plant (Orange hawkweed – *Pilosella aurantiaca*) as
15 well as two neighboring plant species (Oxeye daisy – *Leucanthemum vulgare* and Alsike clover –
16 *Trifolium hybridum*) to determine if plant species hosted unique root microbiota. Our results
17 show that plants of different species host distinct bacterial communities in their roots. In terms of
18 community composition, Betaproteobacteria (especially the family Oxalobacteraceae) were found
19 to dominate in the root microbiota of *L. vulgare* and *T. hybridum* samples, whereas the root
20 microbiota of *P. aurantiaca* had a more heterogeneous distribution of bacterial abundances where
21 gamma Proteobacteria and Acidobacteria occupied a larger portion of the community.
22 Whether all plant species host their own distinct root microbiota and plants more closely related
23 to each other share more similar bacterial communities still remains to be explored.

24 **Keywords:** root microbiota, plant identity, bacteria

25 **Introduction**

26 Plant roots function as distinct habitats within the soil and bacterial communities in root systems
 27 have repeatedly been shown to differ from those of the surrounding bulk soil (Smalla et al. 2001;
 28 Haichar et al. 2008; Gottel et al. 2011; Lundberg et al. 2012). Even though root associated
 29 bacterial communities (both *rhizospheric* – in the soil surrounding the roots, *epiphytic* – living at
 30 the surface of roots and *endophytic* – living inside root tissues) have been under investigation for
 31 many years, there is still little consensus in how these communities are formed and what
 32 determines their composition (Berg & Smalla 2009; Aleklett & Hart 2013; Bulgarelli et al. 2013).

33 Traditionally, the composition of bacterial communities living in association with plants has
 34 been attributed to environmental factors. For example, soil type has been suggested as the
 35 strongest determinant of community structure in root associated microbial communities (De
 36 Ridder-Duine et al. 2005; Singh et al. 2007; Lundberg et al. 2012; Bulgarelli et al. 2013). At the
 37 same time, it has also been argued that the host plant may play an equally large role in
 38 determining the composition of its root microbiota (Marschner et al. 2005; Costa et al. 2006;
 39 Hartmann et al. 2008; Doornbos et al. 2011), especially endophytic bacterial communities
 40 (Haichar et al. 2008).

41 Recent work has demonstrated that hosts can alter their root microbiota by regulating soil
 42 conditions in the vicinity of the root system through root exudation of sugars, phenolics and
 43 amino acids that could also function as signaling molecules with the microbes in the surrounding
 44 soil (Chaparro et al. 2013). Since root exudation patterns and composition can be associated with
 45 plant gene expression, variation in host genetics has the potential to create large differences in the
 46 chemical profile of plants and consequently the composition of microbes able to inhabit the root
 47 system. Several studies have found that different plant species or genotypes of the same species
 48 host distinct microbial communities (Bailey et al. 2005; Marschner et al. 2005; van Overbeek &
 49 van Elsas 2008; Schweitzer et al. 2008; Micallef et al. 2009a; Manter et al. 2010; Becklin et al.
 50 2012; Peiffer et al. 2013). Even studies where soil type was considered to have the strongest

effect on structuring the root microbiota, differences in bacterial community composition between genotypes was still detected (Bulgarelli et al. 2012; Lundberg et al. 2012).

The root environment varies greatly among plant species; these differences may lead to the selection of distinct bacterial communities. Plants can differ in terms of both root lifespan (Roumet et al. 2006), root architecture (Hodge et al. 2009), root surface structure and components and patterns of root exudation (Bais et al. 2006). Root exudates are known to provide a food source for the microbes (Farrar et al. 2003), instigators of symbiotic associations (such as mycorrhizal infection or nodule formation) (Bais et al. 2004), and defend the plant against pathogens (Doornbos et al. 2011). All these plant characteristics could contribute to shaping root systems of different plants into local habitats and potentially distinct niches for microbial colonizers.

The role of intra-species variation among root associated microbial communities has been overlooked, but might represent a significant proportion of variation in natural systems (Bell et al. 2013). Since we know that natural populations exhibit variation in root exudation patterns and root morphology, one would expect there to be variation among individual plants in their root microbiota as well (Micallef et al. 2009). But variation among plants might also be driven by environmental heterogeneity because we know that small-scale environmental heterogeneity exists in soil systems. Is this variation static across plant taxa, or do different taxa exhibit more variation than others? If plant genetics are determining bacterial community composition, then certainly, populations with low genetic diversity (i.e. asexually reproducing, or metapopulations), would be expected to have less variation than sexually reproducing populations with high levels of gene flow. Because we sampled a plant species known to reproduce clonally through stolons and apomixis (*P. aurantiaca*), we also examined whether individuals within that species had less dispersion in their microbial community composition than individuals within the other two plant species.

The majority of studies characterizing bacterial communities in the root microbiota have been conducted with model plants in artificial greenhouse settings or agricultural contexts (Marschner & Yang 2001; Garbeva et al. 2004; Micallef et al. 2009; Manter et al. 2010; Doornbos et al. 2011; Lundberg et al. 2012) where the study of genetically modified plants have been especially informing when it comes to understanding slight differences between plant genotypes (van Overbeek & van Elsas 2008; Weinert et al. 2009; Inceoğlu et al. 2010). While these studies are crucial for understanding the mechanistic basis of plant:microbe interactions, they do not reflect how natural environmental conditions contribute to variation in bacterial community composition across individual plants, particularly in complex environments where a wide diversity of plants and biota are interacting.

In this study, we explored variation in bacterial community composition between individual root systems of neighboring plants in a common field in order to determine how much variation exists within and between plant taxa. We sampled the root microbiota of three plant species growing within 10 meters from each other in a field and asked – are bacterial root communities distinct among plant species growing in a common location? And – do certain plant species contain more intra-species variance in bacterial communities than others?

Methods

Field site and target plant

Field site description

Samples were collected in August, 2011, from a subalpine meadow near Chute Lake, British Columbia, Canada (49.698859N,-119.533133W). The sampling area has not been used for agriculture or forestry but is in proximity to a forestry road as well as a camp site. Since it also contains a high number of invasive plant species it could therefore be considered disturbed site. The dominant soil at the site is a sandy loam and the site is classified under the biogeoclimatic

100 zone Interior Douglas Fir, dry warm (IDFdw) (Biogeoclimatic Ecosystem Classification (BEC)
101 and Ecology Research program of the British Columbia). The dominant vegetation at the field
102 site consists of Orange hawkweed (*Pilosella aurantiaca* (L.) F.W. Schultz & Schultz-Bip), Hairy
103 vetch (*Vicia villosa*, Roth), Oxeye daisy (*Leucanthemum vulgare*, Lam.), Wild strawberry
104 (*Fragaria virginiana*, Duchesne) Timothy (*Phleum pratense*, L.) and Alsike clover (*Trifolium*
105 *hybridum*, L.) growing homogenously across the field.

106 *Target plant*

107 Our target plant was *P. aurantiaca* (formerly known as *Hieracium aurantiacum*), which is native
108 to Europe and invasive in North America. Genetic diversity within *P. aurantiaca* has previously
109 been examined across 48 locations in North America, and results showed that there were only
110 three genotypes, of which two were found only in isolated locations (one in Alaska and one in
111 Oregon) (Loomis & Fishman 2009). By choosing to work with a plant expressing this low
112 diversity in wild populations, we hoped to minimize genetic variance within the population that
113 we sampled.

114 To clarify the role of host identity and intra-species variance in bacterial root microbiota, we
115 additionally sampled two of the co-occurring plant species, *L. vulgare* and *T. hybridum* that were
116 in the same developmental stage (flowering) as *P. aurantiaca*.

117 Experimental design

118 Root systems of *P. aurantiaca* were collected one meter apart along two 10 m transects (n=20).
119 Additional samples of *T. hybridum* (n=10) and *L. vulgare* (n=10) were collected where present
120 along the transects, several of which were growing within centimeters of *P. aurantiaca* samples.

Each root system was rinsed from surrounding rhizospheric soil in de-ionized water in order to separate it from roots of neighboring plants. Root systems were then cut up in pieces and a subsample of root tissue, representative of the whole root system, including young fresh roots as well as older root tissues (with no exclusion of nodules in *Thyridum*), was collected and further used for classification of bacterial community composition. Since no further treatment was performed in order to remove rhizoplane microbes, we assume that the communities extracted could be of either endophytic or epiphytic origin.

Bacterial community analysis

Amplification and sequencing of target gene

DNA from all collected plant tissues (0.25g/sample) was extracted using a PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., USA) according to the manufacturer's protocol. Microbial diversity and the relative abundances of individual taxa were assessed by barcoded pyrosequencing of a portion of the 16S rRNA gene. Each DNA sample was amplified in triplicate through PCR reactions using the protocol described in Fierer et al. (2008) except with a different primer pair. The forward primer contained the 454 Life Sciences primer B sequence, the bacterial primer 799f (Chelius & Triplett 2001) and a two-base linker sequence ('AG'). The reverse primer contained the 454 Life Sciences primer A sequence, a unique 12 bp error-correcting Golay barcode (Fierer et al. 2008), a 'GT' linker sequence, and the 'universal' bacterial primer 1115r (Reysenbach & Pace 1995). The targeted gene region has shown to be appropriate for accurate taxonomic classification of bacterial sequences and the primers are designed to exclude chloroplasts from plant tissues in the samples (Redford et al. 2010). Amplicons were visualized via gel electrophoresis purified and quantified. Amplicons from all samples were then combined in equimolar ratios into a single tube. Samples were sequenced at Engencore (University of South Carolina) on a Roche GS-FLX sequencer running the Titanium chemistry.

Processing raw sequence data

All sequences were de-multiplexed and further analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) toolkit (Caporaso et al. 2010). Operational Taxonomic Units (OTUs) were defined at the level of $\geq 97\%$ similarity and the taxonomy assigned by comparing a representative sequence from each OTU to the Greengenes database (February 14th 2012 version) (DeSantis et al. 2006) using the Basic Local Alignment Search Tool (BLAST) classifier (Altschul et al. 1990). In order to correct for differences in the number of sequences analyzed per sample, a randomly selected subset of 400 sequences per root sample was used to compare relative differences in taxonomic diversity. Only samples from which we obtained a minimum of 400 bacterial sequences per sample or more were considered in the study, eliminating 3 samples from the study (one *P. aurantiaca* and two *L. vulgare*). Though 400 sequences cannot fully capture the rare biosphere, it allowed us to compare samples while still maintaining as many samples as possible. It has previously been shown that studies of bacterial communities show similar results even at a lower rarefaction (Hamady & Knight 2009; Kuczynski et al. 2010). In fact, re-analyzing our data set with a higher rarefaction limit showed the same general trends but drastically lowered our number of samples available to analyze.

Statistics

Differences in community composition between samples were calculated using phylogenetic metric (UniFrac) where weighted UniFrac shows an emphasis on the more abundant taxa in samples and un-weighted UniFrac treats all taxa the same (Lozupone et al. 2007; Hamady et al. 2010). As a comparison, we also included a taxonomic metric (Bray-Curtis distance) to explore whether dissimilarity patterns were the same in terms of presence/absence of taxa. Before calculating Bray-Curtis distances, all relative abundances were log-transformed. 2-D scatterplots

of Principal Coordinates Analysis (PCoA) generated in PRIMER-E (Clarke & Gorley, 2006) were used to visualize the greatest amount of variability in the pair-wise distances between samples.

We tested for variance among host plants in their root microbiota using a 2-way PerMANOVA (Anderson 2005) with host species and transect as factors and weighted and un-weighted UniFrac as well as Bray Curtis as our dissimilarity metrics. All analyses were permuted 9999 times. Since it has been shown that PerMANOVA is not robust when sampling efforts are un-equal (Anderson & Walsh 2013), we subsampled 8 samples from each species which were used for both PerMANOVA and PermDISP analyses.

Variability in community composition within each of the three species was analyzed through PermDISP (Anderson 2004) (9999 permutations), creating a centroid for each species and measuring the average spread of samples belonging to that species from the centroid. A large spread (high average) would indicate a high variability in community composition among individuals within the species (Anderson 2004).

Results

Variation between host species

When comparing the phylogenetic overlap between bacterial root microbiota (UniFrac) across three different species of plant hosts growing in a common field, bacterial communities from samples of the same plant species were significantly more similar to each other than to bacterial communities sampled from plants of the two other species (Table 1). This was true for both weighted (Pseudo-F=8.54 p=0.0001) and un-weighted UniFrac (Pseudo-F=1.66 p=0.0001) as well as Bray Curtis dissimilarities (Pseudo-F=2.27 p=0.0001) (Table 1). These patterns were also evident from the principal coordinates analyses which showed little overlap between samples of different plant species (Fig.1).

Variation within host species

Plant species differed in how much variance there was among bacterial communities of individual root samples (Fig.1). There was a significant difference between plant species in the amount of compositional dissimilarity of bacterial taxa between individual plants (Bray Curtis: $F=9.56$ $p=0.02$). That is, the amount of dispersion of individual plants from the centroid differed among plant species. In this case, *P. aurantiaca* exhibited the most variance among plant individuals, while *T. hybridum* showed the least (Table 2). This was not the case when the same data was analyzed using phylogenetic measures, since we could not detect any significant difference in dispersion among plant taxa (UniFrac: weighted $F=2.57$ $p=0.15$; un-weighted $F=2.56$ $p=0.63$) (Table 2).

Relative abundance of taxa across hosts

A total number of 4384 unique OTUs were analyzed within the rarefied data set. A taxonomic summary, showing the average abundance of bacterial phyla in *P. aurantiaca*, *T. hybridum* and *L. vulgare* samples, illustrates the compositional differences between root systems of different plant species (Fig.2). In *P. aurantiaca*, the most abundant phylum was *Betaproteobacteria* which made up, on average, 29% out of all sequences found in *P. aurantiaca* samples, followed by *Bacteroidetes* (19%), *Alphaproteobacteria* (16%) and *Actinobacteria* (12%). In *T. hybridum*, *Betaproteobacteria* made up, on average, 51% of the all bacterial sequences found in the species, followed by *Alphaproteobacteria* (21%) and *Bacteroidetes* (16%). Bacterial communities in *L. vulgare* samples were, similarly to *T. hybridum*, dominated by *Betaproteobacteria* (50%), followed by *Bacteroidetes* (18%) and *Alphaproteobacteria* (12%). A table showing the relative distribution of the 14 most abundant OTUs across all samples as well as their relative abundance within samples of the different species is given in Table 3.

215 A closer examination of the relative abundances of all *Betaproteobacteria* found in samples
216 showed that while *Burkholderiales* was the predominant order across all three species, *T.*
217 *hybridum* and *L. vulgare* samples were heavily dominated by bacteria of the family
218 *Oxalobacteriaceae* – especially bacteria of the genus *Herbaspirillum* (11% of the total bacterial
219 community in *T. hybridum* and 18% in *L. vulgare*) (Fig.3).

220 Discussion

221 Host specificity

222 Our study shows that root bacterial communities vary significantly between plants belonging to
223 three different species, growing in close proximity to each other in natural plant communities.
224 These results support previous work showing bacterial host plant specificity in roots of
225 agricultural crops (Marschner & Yang 2001; Wieland et al. 2001; Haichar et al. 2008) and wild
226 grass species (Kuske & Ticknor 2002; Osanai et al. 2012).

227 Although all plant species investigated in this study (*P. aurantiaca*, *T. hybridum* and *L.*
228 *vulgare*), are perennial, there are significant morphological differences between the species. For
229 example, *P. aurantiaca* and *L. vulgare* (both belonging to the family *Asteraceae*) have creeping
230 root stocks and produce fibrous root systems whereas *T. hybridum* (family *Fabaceae*) grows a
231 branching tap root system that is known to form nodules with nitrogen fixing bacteria. This
232 variation in root morphology could contribute to the differences in abundance and composition of
233 bacteria in our results. For example, roots that penetrate deeper soil may encounter different
234 microbes than those in shallow layers (Fierer et al. 2003). Similarly, the thickness and/or texture
235 of the root surface (i.e. woody, fibrous) may be more or less penetrable to colonizing bacteria.

236 Part of the variance seen in bacterial community composition between the three plant
237 species could also be caused by species-specific root exudation patterns. For example, several
238 members of the *Asteraceae* family are known to produce allelochemicals that could affect the

239 bacterial community as well as surrounding plants (Alford et al. 2009). However, these
240 differences are difficult to assess in wild plant communities, especially when roots of different
241 plant species grow in close proximity to each other with entangled root systems. In our study, the
242 roots grew so intimately that exudation from one plant species could have influenced root
243 systems of neighboring plants.

244 *Individual variation in root microbiota*

245 We know that genetic differences between plants, even at the genotype level, can affect the
246 composition of the root microbiota (Bailey et al. 2005; Schweitzer et al. 2008; Peiffer et al.
247 2013). Thus, we would expect variation in bacterial community composition among individuals
248 within a population of plants, even when they are growing in a common environment, due to
249 genetic variation in the population in terms of root traits and exudation chemistry, among other
250 factors. Though there is a potential for clonality in *T. hybridum*, we still predicted less individual
251 variance in the root microbiota among individuals from *P. aurantiaca* (thought to consist of
252 mainly one genotype across all of North America (Loomis & Fishman 2009)) than within the two
253 co-occurring out-crossing plant species with presumed higher genetic diversity (*L. vulgare* and *T.*
254 *hybridum*).

255 Though our data show a significant difference in compositional turnover within different
256 plant species, it rejects the hypothesis that *P. aurantiaca* had the most similar root communities
257 across individuals. Comparing the average dispersion of bacterial community composition for the
258 three plant species, there was no indication that *P. aurantiaca* had a smaller dispersion than the
259 two other plant species (Table 2). Instead, it shows that *P. aurantiaca* had the highest variation
260 within a species comparing dispersion based on taxonomic differences (Table 2). The fact that we
261 could not detect any differences in dispersion when using phylogenetic metrics suggests that

individual root systems differ more in terms of which taxa are present or absent than how related they are, or that there is little phylogenetic conservatism at the individual level.

Overall, this study shows that the extent of individual variation seen in root microbiota varies between species, but that a plant species thought to be more genetically homogenous does not necessarily host more homogeneous root communities. It also indicates that individual variation in bacterial community composition in root systems is determined, not only by plant genetics, but also by the surrounding environment and potentially, events throughout the plant's life that could affect root colonization (Alekklett & Hart 2013).

Bacterial community composition

Similar patterns of bacterial community composition to what we found in our plants, growing in a subalpine meadow in Canada, have been reported in rhizosphere samples of other studies. For example, roots tissues of the plant species that we sampled were mainly dominated by *Betaproteobacteria*, (Fig. 2), especially members of the order *Burkholderiales* and the family *Oxalobacteriaceae*, which represented as much as 32% of the total bacterial community in *L. vulgare* (Fig. 3). Seed- and root-colonizing populations of *Oxalobacteriaceae* have previously shown to be responsive to plant species (Green et al. 2007), supporting our data of plant species hosting distinct bacterial communities. Dominance by these taxa in root systems has also been reported in other studies. For example, roots of *Arabidopsis thaliana*, examined at the same taxonomic resolution by Lundberg et al. (2012), were dominated by *Betaproteobacteria* and *Oxalobacteriaceae* both in samples of rhizosphere soil as well as in the endophytic root compartment.

Other studies have found *Actinobacteria* to dominate in root tissues of plants (e.g. Ottesen et al. 2013), especially in communities of the endophytic compartment (Bodenhause et al. 2013). In our study, *Actinobacteria* represented at most 12% out of the total bacterial community

286 in the plant species that we sampled (Fig. 2) and was mainly found in samples of *P. aurantiaca*
287 that, in general, were less dominated by beta-proteobacteria.

288 The dominance of sequences belonging to the genus *Herbaspirillum* was further
289 emphasized when we examined the fourteen most abundant OTUs across all samples (Table 3).
290 *Herbaspirillum* spp. are known to colonize apoplastic or intracellular spaces of plant tissues and
291 several species have shown the ability to fix nitrogen (Schmid et al. 2006). While it is believed
292 that this nitrogen fixing ability could be beneficial to their plant host, it has also been documented
293 that certain *Herbaspirillum* strains are mild pathogens and a causative agent of “mottled stripe
294 disease” in crops such as sugar-cane (Schmid et al. 2006). Besides *Herbaspirillum*, we also saw
295 high abundances of sequences belonging to *Limnohabitans* and *Cytophaga* (Table 3), two genera
296 more commonly associated with bacterial communities in fresh water (Kirchman 2002; Simek et
297 al. 2010) as well as the species *Methylobium petroleiphilum*, a recognized methylophilum (Kane et
298 al. 2007) and *Janthinobacterium lividum*, known to thrive in soils (Shivaji et al. 1991) and
299 produce antibiotics (Johnson et al. 1990). The high presence of these groups in our samples could
300 be due to the inclusion of epiphytic members of the root microbiota, where bacteria associated
301 with water films and soil particles of the root surface would be expected.

302 In comparison, Bodenhausen and colleagues (2013) found that a *Flavobacterium* of the
303 phylum *Bacteroidetes* stood out as the single most abundant OTU in endophytic root samples,
304 making up 10.15% of the total community. Though bacteria of the phylum *Bacteroidetes*
305 represented a significant part of the community in root samples of the three plant species sampled
306 in our study (Fig.2), they were by no means the most dominant taxonomic group in any of the
307 species (Table 3).

308 As the genus *Trifolium* are known to be hosts of nitrogen fixing bacteria that form nodules
309 in their roots, *T. hybridum* samples were expected to host larger populations of
310 *Alphaproteobacteria*, specifically belonging to the order *Rhizobiales* which is a common

symbiont of legumes (Masson-Boivin et al. 2009). This expected pattern was not evident in our results though. The only OTU belonging to the order *Rhizobiales* detected in notable abundances in our study was a *Bradyrhizobium* taxa which made up 1% of the collective community of *T. hybridum* samples and 1.5% in *P. aurantiaca* samples (Table 3). Instead, it was evident that the *T. hybridum* community was dominated by the family *Oxalobacteraceae* (23.48%) (Fig.3) and specifically one OTU of the genus *Herbaspirillum* (10.75%) (Table 3), which is mainly known to colonize roots of non-leguminous plants, and have nitrogen fixing properties (Baldani et al.1997). What stands out though is that this group of bacteria was even more predominant in *L. vulgare* samples, where *Oxalobacteraceae* made up 32.47% of the community and the same *Herbaspirillum* OTU represented 18.26% of the total community.

Variance in relative abundances of bacterial taxa across plant species

We observed differences in the evenness of bacterial taxa across host plants. While the roots of *L. vulgare* and *T. hybridum* seemed dominated by a few select groups of microbes, samples of *P. aurantiaca* supported communities with abundances more evenly distributed among bacterial taxa (Fig. 2; Table 3). Though few studies have looked specifically at variance in bacterial evenness between plant species, it could be an important source of variation. For example, dominance of single taxon may indicate specialized plant/bacterial associations whereas high evenness in community composition could reflect generalist associations among plants and bacteria. Alternatively, differences in evenness may result from microbial interactions within the plant, not driven by the plant but microbial competition for plant resources.

Conclusions

In this study, we showed that plant identity plays a major role in explaining the variation seen in root microbiota both between and within plant species growing under natural conditions. Further

studies across a larger set of wild plant species as well as more detailed investigations of the effect of plant genetics versus plant phenotypic traits on bacterial community assembly could help resolve the relative contribution of host identity at an individual level in shaping the root microbiota. It would also allow us to draw further conclusions as to whether plants more related to each other actually host more similar bacterial communities across plant species and families.

The results of our study speak of how intimately related bacterial communities are with their host plants. Root systems of wild plants are never alone; they are constantly surrounded by the roots of other plants, entangled in the soil, competing for resources and space. Yet, our results show that bacterial communities associated with roots of plants growing in a common field are distinct between plant species.

Ultimately, we are not able to tell exactly why these three plant species have such distinct bacterial root communities, but further studies linking metabolomics of wild plants with bacterial community composition would be useful for better understanding how plants affect bacterial community assembly.

Acknowledgements

We would like to thank Monika Gorzelak for helping out with sampling efforts in the field and KA would like to acknowledge the support of UBC Okanagan Internal Research Grant Program.

References

- Aleklett K, Hart M. 2013. The root microbiota—a fingerprint in the soil? *Plant and Soil* 370: 671–686. doi:10.1007/s11104-013-1647-7.
- Alford ÉR, Vivanco JM, Paschke MW. 2009. The effects of flavonoid allelochemicals from knapweeds on legume-rhizobia candidates for restoration. *Restoration Ecology* 17: 506–514. doi:10.1111/j.1526-100X.2008.00405.x.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–10. doi:10.1016/S0022-2836(05)80360-2.

- 359 Anderson MJ. 2004. PERMDISP: a FORTRAN computer program for permutational analysis of
360 multivariate dispersions (for any two-factor ANOVA design) using permutation tests.
361 Department of Statistics, University of Auckland, New Zealand
- 362 Anderson MJ. 2005. PERMANOVA: a FORTRAN computer program for permutational
363 multivariate analysis of variance. Department of Statistics, University of Auckland, New
364 Zealand.
- 365 Anderson MJ, Walsh DCI. 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of
366 heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monographs*
367 83: 557–574.
- 368 Bailey JK, Deckert R, Schweitzer JA, Rehill BJ, Lindroth RL, Gehring C, Whitham TG. 2005.
369 Host plant genetics affect hidden ecological players: links among *Populus*, condensed
370 tannins, and fungal endophyte infection. *Canadian Journal of Botany* 83: 356–361.
371 doi:10.1139/b05-008.
- 372 Baldani J, Caruso L, Baldani V, Goi S, Döbereiner J. 1997. Recent advances in BNF with non-
373 legume plants. *Soil Biology and Biochemistry* 29: 911–922.
- 374 Bais HP, Park S-W, Weir TL, Callaway RM, Vivanco JM. 2004. How plants communicate using
375 the underground information superhighway. *Trends in Plant Science* 9: 26–32.
376 doi:10.1016/j.tplants.2003.11.008.
- 377 Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. 2006. The role of root exudates in
378 rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*
379 57: 233–66. doi:10.1146/annurev.arplant.57.032905.105159.
- 380 Becklin KM, Hertweck KL, Jumpponen A. 2012. Host identity impacts rhizosphere fungal
381 communities associated with three alpine plant species. *Microbial Ecology* 63: 682–93.
382 doi:10.1007/s00248-011-9968-7.
- 383 Bell C, Carrillo Y, Boot CM, Rocca JD, Pendall E, Wallenstein MD. 2013. Rhizosphere
384 stoichiometry: are C : N : P ratios of plants, soils, and enzymes conserved at the plant
385 species-level? *New Phytologist*: 1–13. doi:10.1111/nph.12531.
- 386 Berg G, Smalla K. 2009. Plant species and soil type cooperatively shape the structure and
387 function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology* 68: 1–
388 13. doi:10.1111/j.1574-6941.2009.00654.x.
- 389 Bodenhausen N, Horton MW, Bergelson J. 2013. Bacterial communities associated with the
390 leaves and the roots of *Arabidopsis thaliana*. *PloS one* 8: e56329.
391 doi:10.1371/journal.pone.0056329.
- 392 Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, Rauf
393 P, Huettel B, Reinhardt R, Schmelzer E, Peplies J, Gloeckner FO, Amann R, Eickhorst T,
394 Schulze-Lefert P. 2012. Revealing structure and assembly cues for *Arabidopsis* root-
395 inhabiting bacterial microbiota. *Nature* 488: 91–5. doi:10.1038/nature11336.

- 396 Bulgarelli D, Schlaeppli K, Spaepen S, Ver Loren van Themaat E, Schulze-Lefert P. 2013.
397 Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant*
398 *Biology* 64: 807–38. doi:10.1146/annurev-arplant-050312-120106.
- 399 Caporaso J, Kuczynski GJ, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña
400 AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE,
401 Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ,
402 Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis
403 of high-throughput community sequencing data. *Nature Methods* 7: 335–6.
404 doi:10.1038/nmeth.f.303.
- 405 Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM. 2013. Root
406 exudation of phytochemicals in Arabidopsis follows specific patterns that are
407 developmentally programmed and correlate with soil microbial functions. *PloS one* 8:
408 e55731. doi:10.1371/journal.pone.0055731.
- 409 Chelius MK, Triplett EW. 2001. The diversity of archaea and bacteria in association with the
410 roots of *Zea mays* L. *Microbial Ecology* 41: 252–263. doi:10.1007/s002480000087.
- 411 Clarke KR, Gorley RN. 2006. PRIMER v6: User Manual/Tutorial. Plymouth Marine Laboratory,
412 Plymouth, UK.
- 413 Costa R, Götz M, Mrotzek N, Lottmann J, Berg G and Smalla K. 2006. Effects of site and plant
414 species on rhizosphere community structure as revealed by molecular analysis of microbial
415 guilds. *FEMS Microbiology Ecology* 56: 236–49. doi:10.1111/j.1574-6941.2005.00026.x.
- 416 DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P,
417 Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and
418 workbench compatible with ARB. *Applied and Environmental Microbiology* 72: 5069–72.
419 doi:10.1128/AEM.03006-05.
- 420 Doornbos RF, van Loon LC, Bakker PAHM. 2011. Impact of root exudates and plant defense
421 signaling on bacterial communities in the rhizosphere. A review. *Agronomy for Sustainable*
422 *Development* 32: 227–243. doi:10.1007/s13593-011-0028-y.
- 423 Farrar J, Hawes M, Jones D, Lindow S. 2003. How roots control the flux of carbon to the
424 rhizosphere. *Ecology* 84: 827–837.
- 425 Fierer N, Hamady M, Lauber CL, Knight R. 2008. The influence of sex, handedness, and
426 washing on the diversity of hand surface bacteria. *Proceedings of the Natural Academy of*
427 *Science USA* 105: 17994–9. doi:10.1073/pnas.0807920105.
- 428 Fierer N, Schimel JP, Holden PA. 2003. Variations in microbial community composition through
429 two soil depth profiles. *Soil Biology and Biochemistry* 35: 167–176. doi:10.1016/S0038-
430 0717(02)00251-1.
- 431 Garbeva P, van Veen JA and van Elsas JD. 2004. Microbial diversity in soil: selection microbial
432 populations by plant and soil type and implications for disease suppressiveness. *Annual*
433 *review of phytopathology* 42: 243–70. doi:10.1146/annurev.phyto.42.012604.135455.

- 434 Gittel, NR, Castro HF, Kerley M, Yang Z, Pelletier DA, Podar M, Karpinets T, Uberbacher E,
435 Tuskan GA, Vilgalys R, Doktycz MJ, Schadt CW. 2011. Distinct microbial communities
436 within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil
437 types. *Applied and Environmental Microbiology* 77: 5934–44. doi:10.1128/AEM.05255-11.
- 438 Green SJ, Michel FC, Hadar Y, Minz D. 2007. Contrasting patterns of seed and root colonization
439 by bacteria from the genus *Chryseobacterium* and from the family Oxalobacteraceae. *The*
440 *ISME Journal* 1: 291–9. doi:10.1038/ismej.2007.33.
- 441 Haichar FEZ, Marol C, Berge O, Rangel-Castro JI, Prosser JI, Balesdent J, Heulin T, Achouak W.
442 2008. Plant host habitat and root exudates shape soil bacterial community structure. *The*
443 *ISME journal* 2: 1221–30. doi:10.1038/ismej.2008.80.
- 444 Hamady M, Knight R. 2009. Microbial community profiling for human microbiome projects:
445 Tools, techniques, and challenges. *Genome research*, 19, 1141–52.
- 446 Hamady M, Lozupone C, R. Knight. 2010. Fast UniFrac: facilitating high-throughput
447 phylogenetic analyses of microbial communities including analysis of pyrosequencing and
448 PhyloChip data. *The ISME Journal* 4: 17–27. doi:10.1038/ismej.2009.97.
- 449 Hartmann A, Schmid M, van Tuinen D, Berg G. 2008. Plant-driven selection of microbes. *Plant*
450 *and Soil* 321: 235–257. doi:10.1007/s11104-008-9814-y.
- 451 Hodge A, Berta G, Doussan C, Merchan F, Crespi M. 2009. Plant root growth, architecture and
452 function. *Plant and Soil* 321: 153–187. doi:10.1007/s11104-009-9929-9.
- 453 Inceoglu O, Salles JF, van Overbeek L, van Elsas JD. 2010. Effects of plant genotype and growth
454 stage on the betaproteobacterial communities associated with different potato cultivars in
455 two fields. *Applied and Environmental Microbiology*, 76, 3675–84.
- 456 Kane SR, Chakicherla AY, Chain PSG, Schmidt R, Shin MW, Legler TC, Scow KM, Larimer
457 FW, Lucas SM, Richardson PM, Hristova KR. 2007. Whole-genome analysis of the methyl
458 tert-butyl ether-degrading beta-proteobacterium *Methylibium petroleiphilum* PM1. *Journal*
459 *of Bacteriology* 189: 1931–45. doi:10.1128/JB.01259-06.
- 460 Kirchman DL. 2002. The ecology of Cytophaga-Flavobacteria in aquatic environments. *FEMS*
461 *Microbiology Ecology* 39: 91–100. doi:10.1111/j.1574-6941.2002.tb00910.x.
- 462 Kuczynski J, Liu Z, Lozupone C, McDonald D, Fierer N, Knight R. 2010. Microbial community
463 resemblance methods differ in their ability to detect biologically relevant patterns. *Nature*
464 *methods* 7: 813–9.
- 465 Kuske C, Ticknor L. 2002. Comparison of soil bacterial communities in rhizospheres of three
466 plant species and the interspaces in an arid grassland. *Applied and Environmental*
467 *Microbiology* 68: 1854–1863. doi:10.1128/AEM.68.4.1854–1863.2002.
- 468 Loomis ES, Fishman L. 2009. A continent wide clone: population genetic variation of the
469 invasive plant *Hieracium aurantiacum* (Orange Hawkweed; Asteraceae) in North America.
470 *International Journal of Plant Science* 170: 759–765. doi:10.1086/599241.

- 471 Lozupone CA, Hamady M, Kelley ST, Knight R. 2007. Quantitative and qualitative beta diversity
472 measures lead to different insights into factors that structure microbial communities. *Applied*
473 *and Environmental Microbiology* 73: 1576–85. doi:10.1128/AEM.01996-06.
- 474 Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J,
475 Engelbrektson A, Kunin V, del Rio TG, Edgar RC, Eickhorst T, Ley RE, Hugenholtz P,
476 Tringe SG, Dangl JL. 2012. Defining the core Arabidopsis thaliana root microbiome. *Nature*
477 488: 86–90. doi:10.1038/nature11237.
- 478 Manter DK, Delgado JA, Holm DG, Stong RA. 2010. Pyrosequencing reveals a highly diverse
479 and cultivar-specific bacterial endophyte community in potato roots. *Microbial Ecology* 60:
480 157–66. doi:10.1007/s00248-010-9658-x.
- 481 Marschner P, Grierson PF, Rengel Z. 2005. Microbial community composition and functioning in
482 the rhizosphere of three Banksia species in native woodland in Western Australia. *Applied*
483 *Soil Ecology* 28: 191–201. doi:10.1016/j.apsoil.2004.09.001.
- 484 Marschner P, Yang C. 2001. Soil and plant specific effects on bacterial community composition
485 in the rhizosphere. *Soil Biology and Biochemistry* 33: 1437–1445.
- 486 Masson-Boivin C, Giraud E, Perret X, Batut J. 2009. Establishing nitrogen-fixing symbiosis with
487 legumes: how many rhizobium recipes? *Trends in Microbiology* 17: 458–66.
- 488 Micallef SA, Channer S, Shiaris MP, Colón-Carmona A. 2009. Plant age and genotype impact the
489 progression of bacterial community succession in the Arabidopsis rhizosphere. *Plant*
490 *signaling and behavior* 4: 777–80. doi:10.1093/jxb/erp053.
- 491 Osanai Y, Bougoure DS, Hayden HL, Hovenden MJ. 2012. Co-occurring grass species differ in
492 their associated microbial community composition in a temperate native grassland. *Plant*
493 *and Soil* 368: 419–431. doi:10.1007/s11104-012-1529-4.
- 494 Ottesen AR, González Peña A, White JR, Pettengill JB, Li C, Allard S, Rideout S, Allard M, Hill
495 T, Evans P, Strain E, Musser S, Knight R, Brown E. 2013. Baseline survey of the anatomical
496 microbial ecology of an important food plant: Solanum lycopersicum (tomato). *BMC*
497 *Microbiology* 13: 114. doi:10.1186/1471-2180-13-114.
- 498 van Overbeek L, van Elsas JD. 2008. Effects of plant genotype and growth stage on the structure
499 of bacterial communities associated with potato (Solanum tuberosum L.). *FEMS*
500 *Microbiology Ecology* 64: 283–96. doi:10.1111/j.1574-6941.2008.00469.x.
- 501 Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL, Buckler ES, Ley RE. 2013. Diversity
502 and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of*
503 *the Natural Academy of Science USA* 110: 6548–53. doi:10.1073/pnas.1302837110.
- 504 Redford, A. J., Bowers RM, Knight R, Y. Linhart and N. Fierer. 2010. The ecology of the
505 phyllosphere: geographic and phylogenetic variability in the distribution of bacteria on tree
506 leaves. *Environmental microbiology* 12: 2885–93. doi:10.1111/j.1462-2920.2010.02258.x.

- 507 de Ridder-Duine AS, Kowalchuk GA, Klein Gunnewiek PJA, Smant W, van Veen JA, de Boer W.
508 2005. Rhizosphere bacterial community composition in natural stands of *Carex arenaria*
509 (sand sedge) is determined by bulk soil community composition. *Soil Biology and*
510 *Biochemistry* 37: 349–357. doi:10.1016/j.soilbio.2004.08.005.
- 511 Roumet C, Urcelay C., Díaz S. 2006. Suites of root traits differ between annual and perennial
512 species growing in the field. *The New Phytologist* 170: 357–68. doi:10.1111/j.1469-
513 8137.2006.01667.x.
- 514 Schmid M, Baldani JIVO, Hartmann A. 2006. The Prokaryotes. Pages 141–150 (M. Dworkin, S.
515 Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, Eds.). 141–150, New York, NY.
- 516 Schweitzer JA, Bailey JK, Fischer DG, LeRoy CJ, Lonsdorf EV, Whitham TG, Hart SC. 2008.
517 Plant–soil–microorganism interactions: heritable relationship between plant genotype and
518 associated soil microorganisms. *Ecology* 89: 773–781. doi:10.1890/07-0337.1.
- 519 Shivaji S, Ray M, Kumar G, Reddy G, Saisree L, Wynn-Williams D. 1991. Identification of
520 *Janthinobacterium lividum* from the soils of the islands of Scotia Ridge and from Antarctic
521 peninsula. *Polar biology* 11: 267–271.
- 522 Simek K, Kasalicky V, Jezbera J, Jezberová J, Hejzlar J, Hahn MW. 2010. Broad habitat range of
523 the phylogenetically narrow R-BT065 cluster, representing a core group of the
524 Betaproteobacterial genus *Limnohabitans*. *Applied and Environmental Microbiology* 76:
525 631–9. doi:10.1128/AEM.02203-09.
- 526 Singh, B. K., S. Munro, J. M. Potts and P. Millard. 2007. Influence of grass species and soil type
527 on rhizosphere microbial community structure in grassland soils. *Applied Soil Ecology* 36:
528 147–155. doi:10.1016/j.apsoil.2007.01.004.
- 529 Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, Roskot N. 2001. Bulk and
530 rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis :
531 plant-dependent enrichment and seasonal shifts revealed. *Applied and Environmental*
532 *Microbiology* 67: 4742–4751. doi:10.1128/AEM.67.10.4742.
- 533 Weinert N, Meincke R, Gottwald C, Heuer H, Gomes NCM, Schlöter M, Berg G, Smalla K.
534 2009. Rhizosphere communities of genetically modified zeaxanthin-accumulating potato
535 plants and their parent cultivar differ less than those of different potato cultivars. *Applied*
536 *and Environmental Microbiology* 75: 3859-3865.
- 537 Wieland G, Neumann R, Backhaus H. 2001. Variation of microbial communities in soil,
538 rhizosphere, and rhizoplane in response to crop species, soil type, and crop development.
539 *Applied and Environmental Microbiology* 67: 5849–5854. doi:10.1128/AEM.67.12.5849–
540 5854.2001.

Factor	Diversity metric	Pseudo-F	P (perm)
<i>Species</i>	Weighted UniFrac	6.15	0.0001
	Un-weighted UniFrac	1.43	0.0001
	Bray Curtis	1.88	0.0001
<i>Transect</i>	Weighted UniFrac	1.22	0.23
	Un-weighted UniFrac	1.06	0.22
	Bray Curtis	1.07	0.24
<i>SpXTr</i>	Weighted UniFrac	1.54	0.09
	Un-weighted UniFrac	1.03	0.28
	Bray Curtis	1.06	0.20

541 *Table 1. PerMANOVA results, comparing bacterial community resemblance between plant*
542 *species and transects using different diversity metrics.*

Diversity metric	F	P (perm)	Species	Average	SE
Weighted UniFrac	3.10	0.11	<i>P.</i>	9.96 E ⁻²	4.28 E ⁻³
			<i>aurantiaca</i>		
			<i>T. hybridum</i>	8.57 E ⁻²	5.70 E ⁻³
			<i>L. vulgare</i>	0.12	1.29 E ⁻²
Un-weighted UniFrac	1.04	0.37	<i>P.</i>	0.47	3.39 E ⁻³
			<i>aurantiaca</i>		
			<i>T. hybridum</i>	0.46	5.42 E ⁻³
			<i>L. vulgare</i>	0.47	7.25 E ⁻³
Bray Curtis	9.30	0.005	<i>P.</i>	51.43	0.73
			<i>aurantiaca</i>		
			<i>T. hybridum</i>	47.13	0.44
			<i>L. vulgare</i>	51.05	1.05

543 Table 2. PermDISP results showing the average spread from centroid and standard error (SE) for
544 samples of each species. The PERMANOVA (P(perm)) values are assessing whether there is a
545 significant difference between species in sample dispersion, using different diversity metrics.

546 Table 3 was submitted separately as a PDF due to formatting issues upon submission

547 *Table 3. The core root microbiota represented by the fourteen OTUs with the highest abundances across all samples*
548 *calculated as the average percent out of the 400 sequences recorded for each sample, across all species (Total %).*
549 *As a comparison, data is also included for what percentage (on average) the fourteen OTUs make up within the*
550 *bacterial communities of the three plant species examined (P. aurantiaca, T. hybridum and L. vulgare).*

551 Fig.1 Principal coordinates analysis plot illustrating the phylogenetic overlap in root prokaryotic
552 community composition among samples from three different plant species. Phylogenetic overlap
553 between communities was assessed using weighted UniFrac. Community composition was
554 significantly different among plant species ($P < 0.001$; PerMANOVA).

555 Fig. 2 Comparison of the average bacterial community composition and relative abundances, at
556 the phylum level (for *Proteobacteria* also divided into class) in root samples from three different
557 plant species. Results show a strong dominance of sequences belonging to *Betaproteobacteria* in
558 all three plant species, but especially in *T. hybridum* (51%) and *L. vulgare* (50%). The phyla
559 representing less than 1% out of the total community were grouped as “Other” and consisted of:
560 NKB19, *Nitrospirae*, PAUC34f, *Cyanobacteria*, *Elusimicrobia*, *Fibrobacteres*, *Chlamydiae*, SC4,
561 *Spirochaetes* and *Thermi*. Sequences not matching the database were recorded as “No blast hit”.

562 Fig.3 Average relative abundances of *Betaproteobacteria* families and *Oxalobacteraceae* genera
563 found in root samples of the three plant species. Values are given as the percentage of sequences
564 belonging to a certain taxa out of the total average bacterial community for each of the three plant
565 species (rarefied at 400 sequences/sample). The heat map is colour coded from blue (low
566 abundance) to red (high abundance).

Figure 1(on next page)

Principal coordinates analysis plot illustrating the phylogenetic overlap in root prokaryotic community composition among samples from three different plant species.

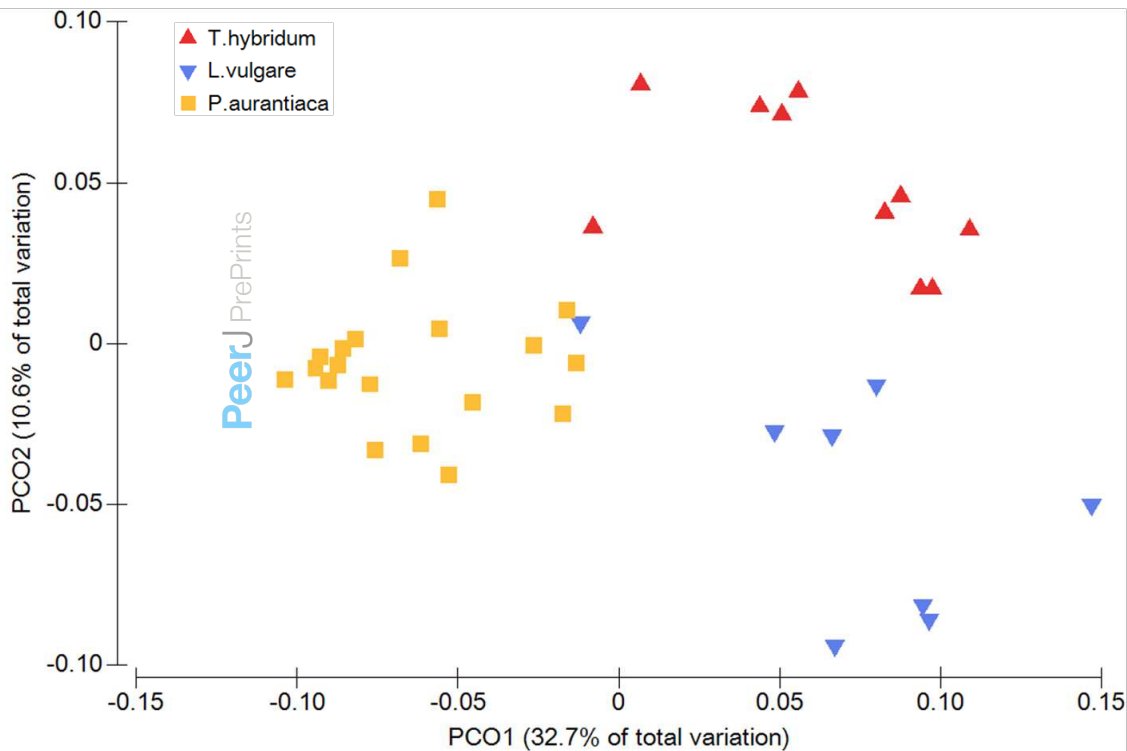
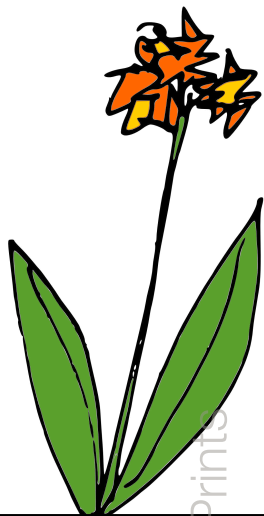
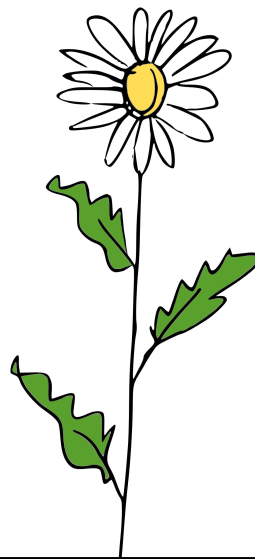


Figure 2 (on next page)

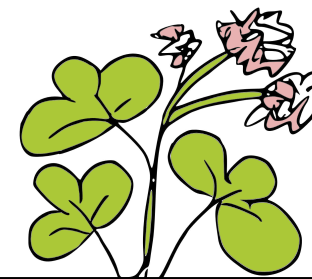
Comparison of the average bacterial community composition and relative abundances, at the phylum level (for *Proteobacteria* also divided into class) in root samples from three different plant species.



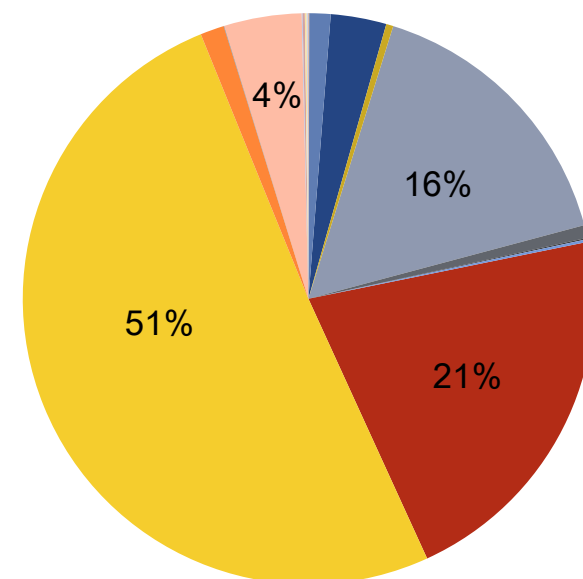
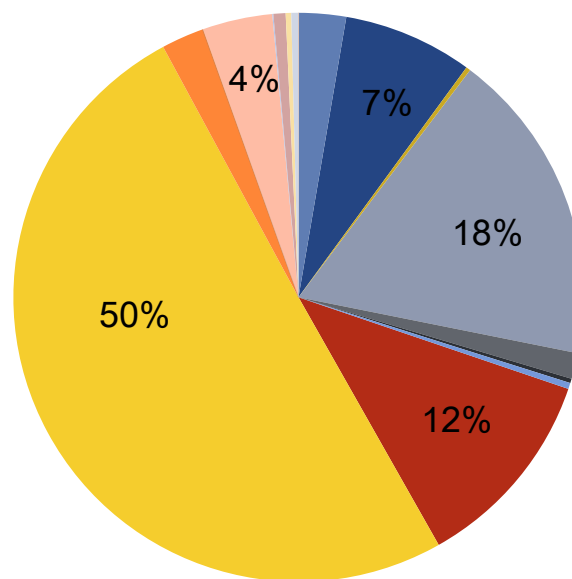
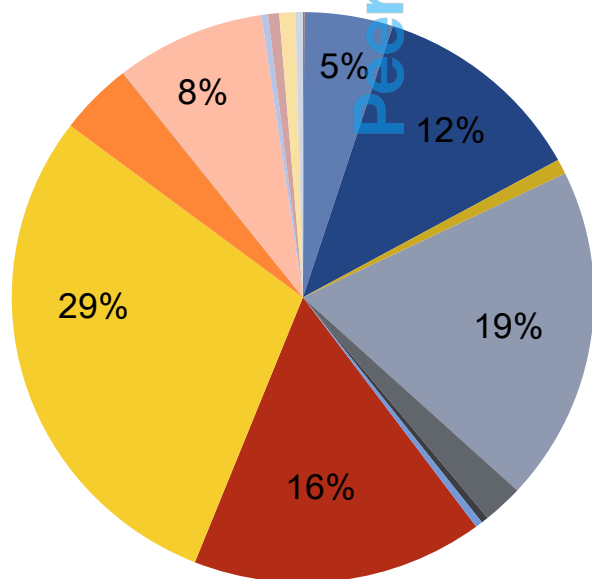
P.aurantiaca



L.vulgare



T.hybridum



- | | | | | |
|-------------------|--------------------|-------------------------|-----------------------|---------|
| ■ No blast hit | ■ Bacteroidetes | ■ Alphaproteobacteria | ■ Gammaproteobacteria | ■ Other |
| ■ Acidobacteria | ■ Chloroflexi | ■ Betaproteobacteria | ■ SC3 | |
| ■ Actinobacteria | ■ Firmicutes | ■ Deltaproteobacteria | ■ TM7 | |
| ■ Armatimonadetes | ■ Gemmatimonadetes | ■ Epsilonproteobacteria | ■ Verrucomicrobia | |

Figure 3 (on next page)

Relative abundance of selected phyla

Average relative abundances of *Betaproteobacteria* families and *Oxalobacteraceae* genera found in root samples of the three plant species. Values are given as the percentage of sequences belonging to a certain taxa out of the total average bacterial community for each of the three plant species (rarefied at 400 sequences/sample). The heat map is colour coded from blue (low abundance) to red (high abundance).

Taxon	<i>P.aurantiaca</i>	<i>T.hybridum</i>	<i>L.vulgare</i>
Unknown Betaproteobacteria	2.33	1.43	1.50
Burkholderiales	9.92	13.65	7.19
Burkholderiales_Alcaligenaceae	0.32	0.53	0.31
Burkholderiales_Burkholderiaceae	0.57	0.63	0.31
Burkholderiales_Comamonadaceae	2.67	9.78	5.88
Burkholderiales_Oxalobacteraceae	9.28	23.48	32.47
Gallionellales_Gallionellaceae	0.08	0.00	0.09
Hydrogenophilales_Hydrogenophilaceae	0.01	0.05	0.00
Methylophilales	0.05	0.03	0.03
Methylophilales_Methylophilaceae	1.37	0.70	0.88
Rhodocyclales	0.76	0.08	0.44
Rhodocyclales_Rhodocyclaceae	1.87	0.35	1.22
Total percentage beta-proteobacteria	29%	51%	50%



	<i>P.aurantiaca</i>	<i>T.hybridum</i>	<i>L.vulgare</i>
Oxalobacteraceae_unknown	3.39	6.93	8.94
Oxalobacteraceae_Collimonas	0.38	0.88	0.72
Oxalobacteraceae_Herbaspirillum	3.21	10.75	18.28
Oxalobacteraceae_Janthinobacterium	1.17	2.13	2.66
Oxalobacteraceae_Massilia	0.61	1.50	1.13
Oxalobacteraceae_Oxalobacter	0.50	1.30	0.75

Table 1 (on next page)

Core root microbiota

The core root microbiota represented by the fourteen OTUs with the highest abundances across all samples calculated as the average percent out of the 400 sequences recorded for each sample, across all species (Total %). As a comparison, data is also included for what percentage (on average) the fourteen OTUs make up within the bacterial communities of the three plant species examined (*P. aurantiaca*, *T. hybridum* and *L. vulgare*).

# OTU ID	Total %	<i>P. aurantiaca</i> %	<i>T. hybridum</i> %	<i>L. vulgare</i> %	Phylum	Class	Order	Family	Genus	Species
1537	7.6	2.8	9.4	16.8	Proteo- bacteria	Beta- proteobacteria	Burk- holderiales	Oxalo- bacteraceae	Herba- spirillum	
19032	3.1	3.2	3.9	2.2	Proteo- bacteria	Beta- proteobacteria	Burk- holderiales			
22328	2.7	1.0	5.1	3.8	Proteo- bacteria	Beta- proteobacteria	Burk- holderiales	Coma- monadaceae	Limno- habitans	
30435	1.5	1.7	1.3	1.2	Proteo- bacteria	Beta- proteobacteria	Burk- holderiales		Methy- libium	<i>Methylibium petroleiphilum</i>
4453	1.3	0.8	1.7	2.1	Proteo- bacteria	Beta- proteobacteria	Burk- holderiales	Oxalo- bacteraceae	Janthino- bacterium	<i>Janthinobacterium lividum</i>
1231	1.2	1.9	0.5	0.4	Bacteroi- detes	Sphingo- bacteria	Sphingo- bacteriales	Flexi- bacteraceae	Cytophaga	
22285	1.0	1.5	1.0	0.4	Proteo- bacteria	Alpha- proteobacteria	Rhizobiales	Brady- rhizobiaceae	Brady- rhizobium	
20009	1.0	1.5	0.2	0.6	Actino- bacteria	Actinobacteria	Actino- mycetales	Thermomon- osporaceae	Actino- corallia	<i>Actinocorallia longicatena</i>
25072	0.9	1.0	1.0	0.7	Proteo- bacteria	Gamma- proteobacteria	Xantho- monadales	Xantho- monadaceae	Rhodano- bacter	<i>Rhodanobacter lindaniclasticus</i>
1340	0.9	1.0	1.0	0.6	Bacteroi- detes	Sphingo- bacteria	Sphingo- bacteriales	Sphingo- bacteriaceae	Sphingo- bacterium	<i>Sphingobacterium faecium</i>
5213	0.8	0.6	0.9	1.3	Bacteroi- detes	Sphingo- bacteria	Sphingobact- eriales	Sphingo- bacteriaceae		
29492	0.8	1.3	0.1	0.6	Proteo- bacteria	Gamma- proteobacteria	Chro- matiales	Sino- bacteraceae		
26917	0.8	1.1	0.3	0.7	Chloroflexi	Chloroflexi	Roseiflexales	Kouleo- thrixaceae	Kouleo- thrix	
1184	0.8	0.4	1.3	1.1	Bacteroi- detes	Flavobacteria	Flavo- bacteriales	Flavo- bacteriaceae	Chryseo- bacterium	