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Shared and host-specific microbiome diversity and functioning of grapevine and accompanying weed plants

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Summary

Weeds and crop plants select their microbiota from the same pool of soil microorganisms, however, the ecology of weed microbiomes is poorly understood. We analysed the microbiomes associated with roots and rhizospheres of grapevine and four weed species (*Lamium amplexicaule* L., *Veronica arvensis* L., *Lepidium draba* L. and *Stellaria media* L.) growing in proximity in the same vineyard using 16S rRNA gene sequencing. We also isolated and characterized 500 rhizobacteria and root endophytes from *L. draba* and grapevine. Microbiome data analysis revealed that all plants hosted significantly different microbiomes in the rhizosphere as well as in root compartment, however, differences were more pronounced in the root compartment. The shared microbiome of grapevine and the four weed species contained 145 OTUs (54.2%) in the rhizosphere, but only nine OTUs (13.2%) in the root compartment. Seven OTUs (12.3%) were shared in all plants and compartments. Approximately 56% of the major OTUs (>1%) showed more than 98% identity to bacteria isolated in this study. Moreover, weed-associated bacteria generally showed a higher species richness in the rhizosphere, whereas the root-associated bacteria were more diverse in the perennial plants grapevine and *L. draba*. Overall, weed isolates showed more plant growth-promoting characteristics compared with grapevine isolates.

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Introduction

Plants host highly diverse microbial communities, which interact with their host in a variety of ways ranging from mutualism to commensal or pathogenic forms (Newton *et al.*, 2010; Hardoim *et al.*, 2015). Such interactions play key roles for soil quality, plant productivity and plant health by, for example, mineralizing soil organic matter, stimulating plant defence mechanisms and/or inhibiting phytopathogens (Lugtenberg and Kamilova, 2009; Compant *et al.*, 2010; Bhattacharyya and Jha, 2012). The rhizosphere is influenced by plant roots and root exudates and hosts an enormous diversity of microorganisms (Mendes *et al.*, 2013). In addition, the root endosphere is colonized by microorganisms spending at least part of their life cycle inside plant roots (Hardoim *et al.*, 2015), and root microbiota have been found to be different than those of bulk and rhizosphere soils (Haichar *et al.*, 2008; Gottel *et al.*, 2011; Lundberg *et al.*, 2012). Although root-associated microbiota have been investigated for a long time, there is still little agreement on how these communities are shaped and what factors regulate their composition (Bulgarelli *et al.*, 2012; Aleklett *et al.*, 2015).

Plants can re-shape their root microbiota by modulating the soil environment in the root zone through the exudation of sugars, organic and amino acids that serve as nutrients and signalling molecules for microorganisms of surrounding soil (Bais *et al.*, 2006; Chaparro *et al.*, 2013). As different plant species and varieties are characterized by distinct root exudation patterns, the plant exerts a pronounced effect on root microbiota (Micallef *et al.*, 2009). In addition to the plant genotype, the presence or absence of competitor plants may influence microbial activity and biomass (Melo *et al.*, 2014). Together with the plant species, the soil type is a major driver of microbiota in the root environment (Berg and Smalla, 2009).

Weeds are known to be good competitors in agricultural soils (Brenchley, 1920; Lorenzi, 2008). They efficiently establish in agricultural fields and their interaction with soil microbiota may result in either better weed growth or impairing growth of their competitor plants (Klironomos, 2002; reviewed by Trognitz *et al.*, 2016). Weeds behave like invasive plants in various natural

ecosystems, and weed establishment is likely based on the same mechanisms. Regarding the interaction with soil microbiota, it was shown that the invasive plant *Centaurea maculosa* Lam. alters the rhizosphere soil microbial community structure, and thus obtains a competitive advantage over native plant species (Callaway *et al*., 2004). Furthermore, Massenssini *et al*. (2014) demonstrated that weeds seem to have positive feedback interactions with soil microbiota, while crops may show neutral or negative feedback interactions. In another study, Santos *et al*. (2012) investigated eight weed and two crop species and reported a greater dependence of weeds on soil microorganisms than crops. Despite these few studies, there is still a lack of knowledge on weed-microbe interactions and differences to crop-microbe interactions, especially when they grow in close vicinity, as it is the case in agricultural soils.

In vineyards, many weed types occur, such as the annual weeds *Lamium amplexicaule* L., *Veronica arvensis* L., *Stellaria media* L. and the perennial weed *Lepidium draba* L. They emerge during autumn and early winter, which can subsequently compromise vineyard productivity by many ways if not controlled. The aim of this study was to elaborate a better understanding on the structure and functioning of weed microbiota as compared with those associated with grapevine. Therefore, we investigated in detail weed- and grapevine-associated microbiomes using next generation sequencing of 16S rRNA genes as well as by isolating and characterizing root- and rhizosphere-associated bacterial strains.

Results

Cultivation-independent analysis of rhizosphere and root microbiomes

Sequencing of the V5-V6 region of the 16S rRNA gene produced 4 847 374 high-quality merged paired-end reads, which corresponds to an average of 32 316 ± 21 215 reads per sample, with an average merged paired-end read length of 367 bp. The rhizosphere samples gave a total of 2 489 489 reads, with an average of 33 193 ± 17 463 ($n = 75$) sequences per sample. This corresponded to an average of 532 ± 23 observed OTUs per sample. The root endophytic dataset yielded a total of 2 357 885 reads, with an average of 31 438 ± 24 614 ($n = 75$) reads and 366 ± 95 OTUs per sample. Prior to further analysis, read numbers were rarefied to 1252 for each sample corresponding to a total of 6791 OTUs. Three replicates from the root samples (*L. amplexicaule* and *Vitis* spp.) with less than 1252 reads were eliminated during rarefaction to capture sufficient diversity by maintaining adequate sequencing depth.

Variance in relative abundance of microbial taxa across host species

Differences in the abundances of bacterial taxa were observed among grapevine and weeds, also among different weed species. In addition to the plant species, the compartment (rhizosphere vs roots) affected the abundance of individual taxa. Generally, in the root compartment the plant species effect was stronger (Fig. 1). Overall, each microbial community was classified at each major taxonomic level, corresponding to 38 phyla, 121 classes, 234 orders, 396 families and 673 genera. The three major phyla representing 88.9% of the microbiome were *Actinobacteria* (40.8%), *Proteobacteria* (32.9%) and *Bacteroidetes* (15%). In the rhizosphere samples, the most abundant phylum was *Actinobacteria* ranging from 47.2% (*Vitis* spp.) to 52.1% (*S. media*) abundance followed by *Proteobacteria* ranging from 21.6% (*Vitis* spp.) to 25.8% (*L. amplexicaule*) and *Bacteroidetes* ranging from 4.8% (*L. draba*) to 13.1% (*Vitis* spp.) abundance. However, in the root compartment *Proteobacteria* were the most abundant phylum ranging from 31.5% (*S. media*) to 47.8% (*L. amplexicaule*) abundance followed by *Actinobacteria* ranging from 28.7% (*V. arvensis*) to 36.4% (*Vitis* spp.) and *Bacteroidetes* ranging from 15% (*L. amplexicaule*) to 35.5% (*S. media*) abundance (Fig. 1). Among others, the archaeal phylum *Crenarchaeota* accounted for 0.2% and 0.1% of total microbial abundance in the rhizosphere and root compartment respectively. Fifty-one per cent of the total reads were identified at the genus level. At genus level, the overall top three genera were *Flavobacterium* (5.4%), *Arthrobacter* (4.6%) and *Sphingomonas* (2.6%). In the rhizosphere the most dominant genera were *Arthrobacter*, *Flavobacterium* and *Skermanella*, whereas in roots *Flavobacterium*, *Pedobacter* and *Sphingomonas* dominated (Supporting Information Fig. S1).

Plant specificity of rhizosphere and root microbiomes

Alpha-diversity characteristics were estimated using the observed OTUs and the Simpson's index. Both, rhizosphere and root-associated microbiomes of all plant species showed significantly different numbers of observed OTUs. Simpson index values of root microbiomes were significantly different across different plant species, whereas in the rhizosphere significant differences were not observed (Fig. 2A). In the rhizosphere of weeds, higher diversity values were observed as compared with the grapevine rhizosphere. Root microbiomes of perennial plants (*L. draba* and grapevine) showed higher diversity indices as compared with annual plants (*L. amplexicaule*, *V. arvensis*, *S. media*). Sampling points also significantly affected the alpha-diversity (particularly the observed

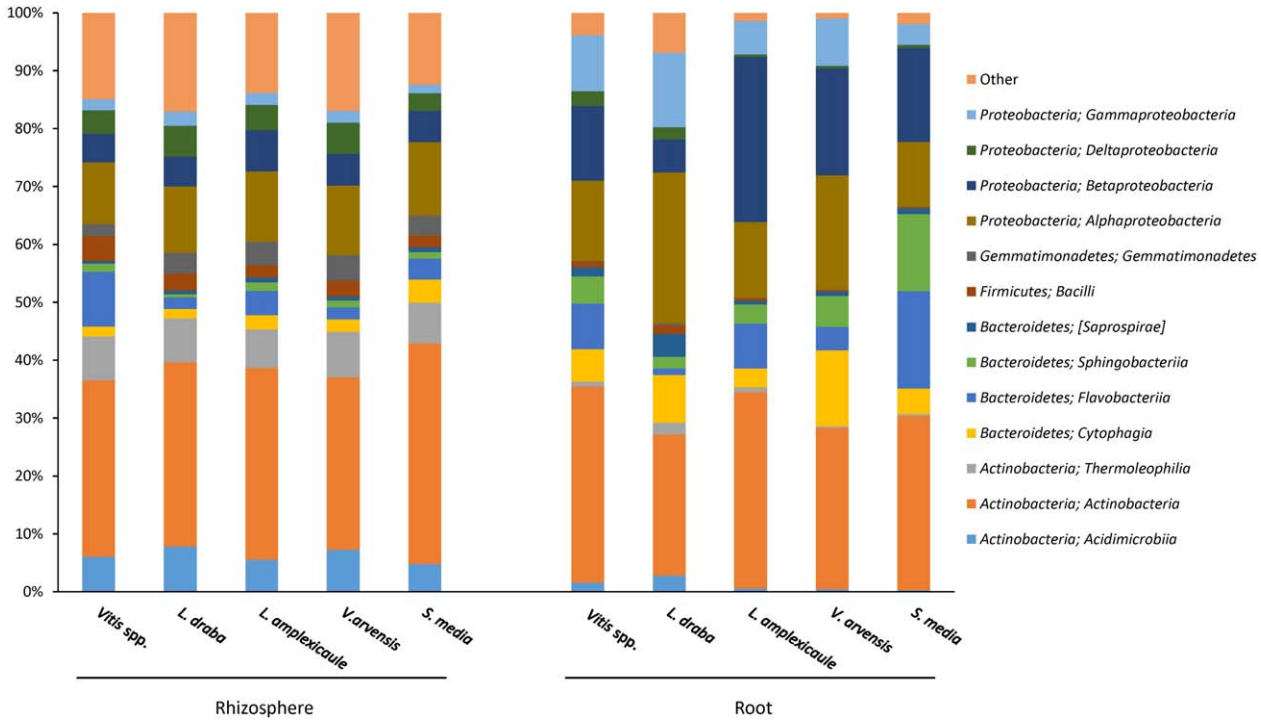

Fig. 1. Rhizosphere and root bacterial community composition at the class level of grapevine and weeds representing OTUs showing more than 1% relative abundance of all reads. Classes representing less than 1% out of total reads were grouped as 'Other'. [Colour figure can be viewed at wileyonlinelibrary.com]

OTUs) of rhizosphere microbiomes, but differences were not significant for root microbiomes (Table 1).

Unsupervised exploratory ordination offered by PCoA (Supporting Information Fig. S2) and its constrained counterpart represented by CAP (Fig. 2B) revealed highly significant differences in beta-diversity of the microbiome. The host plant was responsible for the biggest portion of the variation in microbiome diversity. Moreover, Bray–Curtis values statistically differed between the plants both in rhizosphere as well as in root compartment. Overall, plant species played an important role in the diversification of the associated microbiome. Furthermore, the sampling points slightly affected beta-diversity, especially in the rhizosphere, but differences were not significant between sampling points when pairwise comparisons between sampling points were performed using MANOVAs (Table 2).

Linear discriminant analysis (LDA) (Kruskal–Wallis *p*-value: 0.01, Wilcoxon *p*-value: 0.05, LDA > 2) was used to visualize OTUs responsible for the beta-diversity patterns. 174 and 108 bacterial clades at all taxonomic levels were differentially abundant in the rhizospheres or root compartment, respectively, of the different plant species (Supporting Information Table S2A and B). In the rhizosphere, LDA detected the highest number of bacterial clades in *S. media* (96) followed by *V. arvensis* (32), *L. draba* (29), *L. amplexicaule* (10) and *Vitis* spp. (7). In root samples a strikingly high number of differentially abundant OTUs was observed for the perennial plant species *L. draba* (83), followed by *Vitis* spp. (18), while for annual plant species lower numbers of differentially abundant OTUs (*S. media* = 3, *V. arvensis* = 4, *L. amplexicaule* = 0) were observed.

Shared microbiome of grapevine and weed plants

To identify shared microbiomes of grapevine and weed plants, OTUs occurring in at least 2 out of 3 replicates and 3 out of 5 sampling points were considered. Despite the significant differences in microbiomes associated with different plant species, rhizosphere samples of the five plant species tested shared 145 OTUs, which contributed to 52.4% of the total microbial abundance in the rhizosphere. In contrast, root microbiomes shared only nine OTUs, which corresponds to 13.2% of total microbial abundance in the root compartment (Fig. 3A and B). Interestingly, we found seven abundant (0.5–4.3%) OTUs representing 12.3% of the total microbial community shared among all plant species in both compartments (rhizosphere and root) (Fig. 4A and B). This shared microbiome consisted of OTUs belonging to *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Gemmatimonadetes*, *Nitrospirae* in rhizosphere samples, and *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* in root samples. At family level, the shared rhizosphere microbiome was dominated by

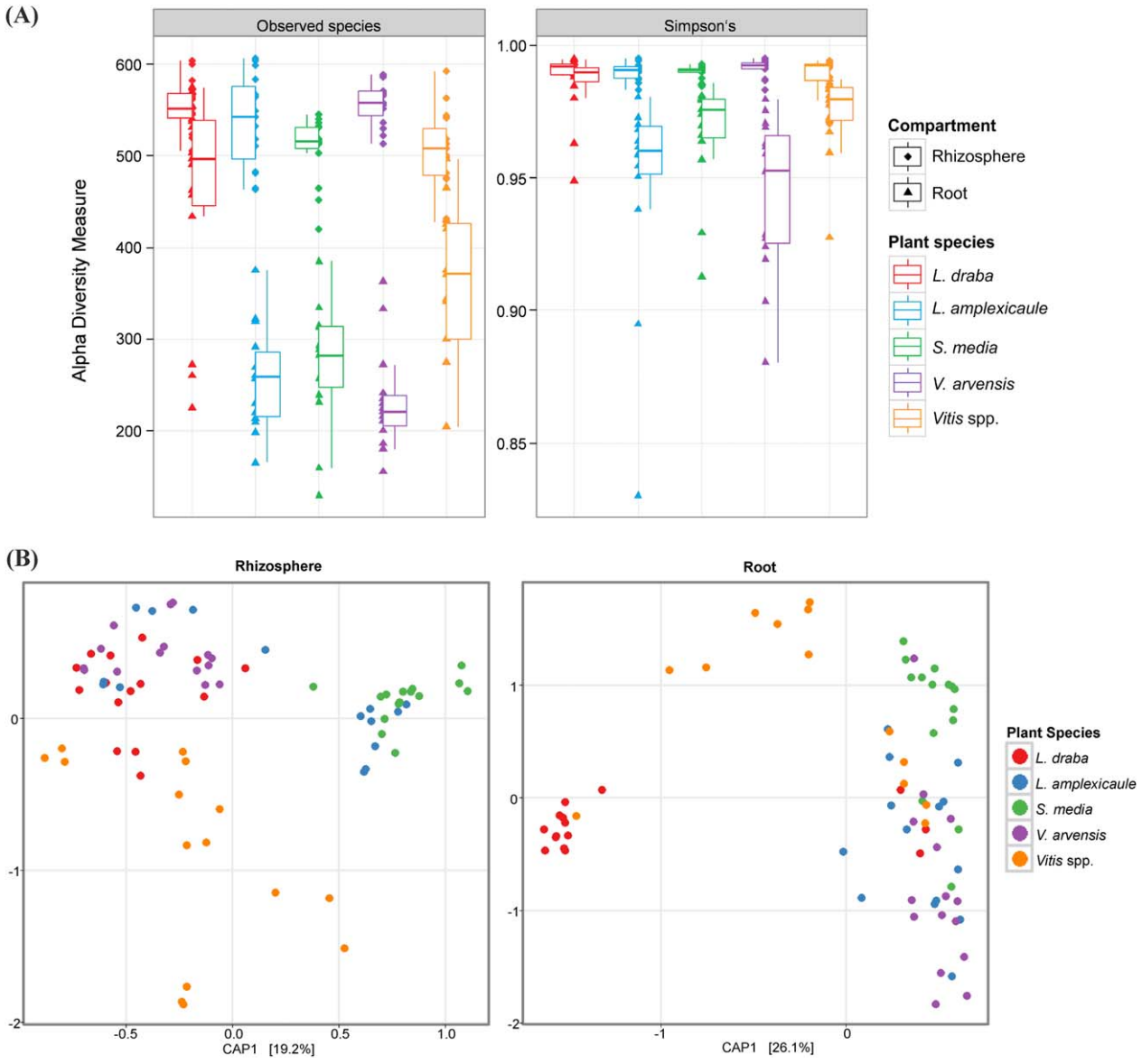

Fig. 2. A. Distribution of alpha diversity within plant and compartment as measured by observed species and Simpson's index using PERMANOVA. Diversity differences were compared between all pairwise groups using permutational *t*-tests shown in Table 1.
B. Constrained analysis of principal coordinates (CAP) of Bray–Curtis dissimilarities based on 16S rRNA gene V5–V6 regions split into the rhizosphere and root plant compartment and constrained to the plant grouping factor. Pairwise comparisons using permutational MANOVAs on a distance matrix are shown in Table 2. [Colour figure can be viewed at wileyonlinelibrary.com]

Micrococcaceae (11–13.5%) and *Nocardioidaceae* (2.6–5.1%), whereas in the root compartment *Microbacteriaceae* (1.9–9.9%) and *Comamonadaceae* (2.9–9.8%) dominated. We observed significantly different abundances of shared taxa between plant species at the family as well as at the OTU level (Fig. 4B and Supporting Information Fig. S3).

Shared OTUs identified in the strain collection

We compared the findings of the cultivation-independent analysis with isolated bacteria from the same samples. Shared OTUs sequences from the molecular study were aligned to sequences obtained from isolated strains, using a 98% similarity threshold. All shared OTUs (7 OTUs shared across all plants and compartments) were represented in strains obtained from these samples. Interestingly the three most abundant shared OTUs assigned to *Arthrobacter oxidans*, *Flavobacterium succinicans* and *Variovorax*, showing a total abundance (in all plants and compartments) of 4.3%, 2.5% and 1.7%, matched 11, 21 and 7 strains respectively (Figs 4B and 5D). However, the three above-mentioned genera varied in

Table 1. Alpha-diversity pairwise comparisons among plant species and sampling points.

	Observed		Simpson	
Plant species	Rhizosphere***	Root***	Rhizosphere	Root ***
L. draba vs *L. amplexicaule*	0.1961	0.00025***	0.56	0.001**
L. draba vs *S. media*	0.0010**	0.00025***	0.67	0.011*
L. draba vs *V. arvensis*	0.5227	0.00025***	0.56	0.001**
L. draba vs *Vitis* spp.	0.0028**	0.03062*	0.56	0.072
L. amplexicaule vs *S. media*	0.1961	0.3905	0.56	0.105
L. amplexicaule vs *V. arvensis*	0.1262	0.16178	0.54	0.921
L. amplexicaule vs *Vitis* spp.	0.1961	0.00120**	0.67	0.046*
S. media vs *V. arvensis*	0.0010**	0.03062*	0.54	0.037*
S. media vs *Vitis* spp.	0.9527	0.00383**	0.56	0.523
V. arvensis vs *Vitis* spp.	0.0010**	0.00025***	0.54	0.011*
Sampling points	Rhizosphere***	Root	Rhizosphere*	Root
A vs B	0.012*	0.96	0.031*	0.75
A vs C	0.122	0.96	0.185	0.74
A vs D	0.122	0.88	0.185	0.74
A vs E	0.617	0.88	0.57	0.74
B vs C	0.16	0.96	0.185	0.75
B vs D	0.176	0.88	0.062	0.75
B vs E	0.002**	0.88	0.005**	0.74
C vs D	0.919	0.88	0.984	0.86
C vs E	0.012*	0.88	0.185	0.86
D vs E	0.012*	0.58	0.138	0.96

Note: Significant values are indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (p value adjustment method: fdr).

their abundance and species (OTU) diversity in the different plant species and compartments (Fig. 5).

Root-associated core families of bacteria shared with other studies

The grapevine and weeds belonging to different plant families shared a set of microbial taxa. This raised the question if bacterial taxa are shared among a wide range of plant species growing in different conditions. We therefore compared the shared root microbiome from our study with those reported previously in different studies. Abundant shared root taxa at family level from our study substantially overlap with the reported core set of *Arabidopsis* (Bulgarelli *et al*., 2012; Lundberg *et al*., 2012; Bodenhausen *et al*., 2013) (11 out of a total of 14 families detected), sugarcane (Yeoh *et al*., 2015) (11 out of a total of 14 families detected), grapevine (Zarraonaindia *et al*., 2015) (3 out of a total of 14 families detected) and wild plant species (*P. aurantiaca*, *L. vulgare*, *T. hybridum*) growing in natural conditions (Aleklett *et al*., 2015) (6 out of a total of 14 families detected). *Hyphomicrobiaceae* were found as core member of the root microbiome in all eight studies reported here, while *Comamonadaceae* and *Oxalobacteraceae* were observed as core taxa of the root microbiome in seven different studies (Table 3).

Plant species-specific OTUs

Every plant species hosted unique OTUs in the rhizosphere as well as in association with roots (Fig. 3A and B). Overall, most of the weed species hosted higher numbers of specific OTUs than grapevine (23/23 with grapevine, 49/174 with *L. draba*, 25/6 with *L. amplexicaule*, 32/28 with *V. arvensis* and 61/34 with *S. media* in rhizosphere/roots, respectively), which all had low (1.6–3.1%) relative abundance in the rhizosphere. However, in the root compartment the relative abundance ranged between 0.9 (*L. amplexicaule*) and 35% (*L. draba*) (Supporting Information Table S3A and B). OTUs that were typical for grapevine assigned particularly to *Pedobacte*r (0.4%), *Solirubrobacteraceae* (0.18%) and *Rhizobiales* (0.13%), while the most abundant weed species-specific OTUs were assigned to *Mycobacterium* (1.6%) in *L. draba*, *Hymenobacter* (1.0%) *in L. amplexicaule*, *Pedobacter* (1.8%) in *S. media* and *Rhizobiaceae* (0.2%) in *V. arvensis* (Supporting Information Fig. S4).

Diversity and functional characteristics of isolates

Cultivation-independent analysis showed that *L. draba* hosts a unique microbiome in comparison with the other weed species. Therefore, *L. draba* and *Vitis* spp. rhizosphere and root samples were selected for the isolation and characterization of bacteria. From the randomly picked bacterial colonies, 125 from the rhizosphere and roots of each plant, in total 500 bacterial isolates, were analysed.

Identification of isolates. The distribution and abundance of bacteria varied according to the type of samples (Fig. 6A). Five hundred bacterial isolates were assigned to

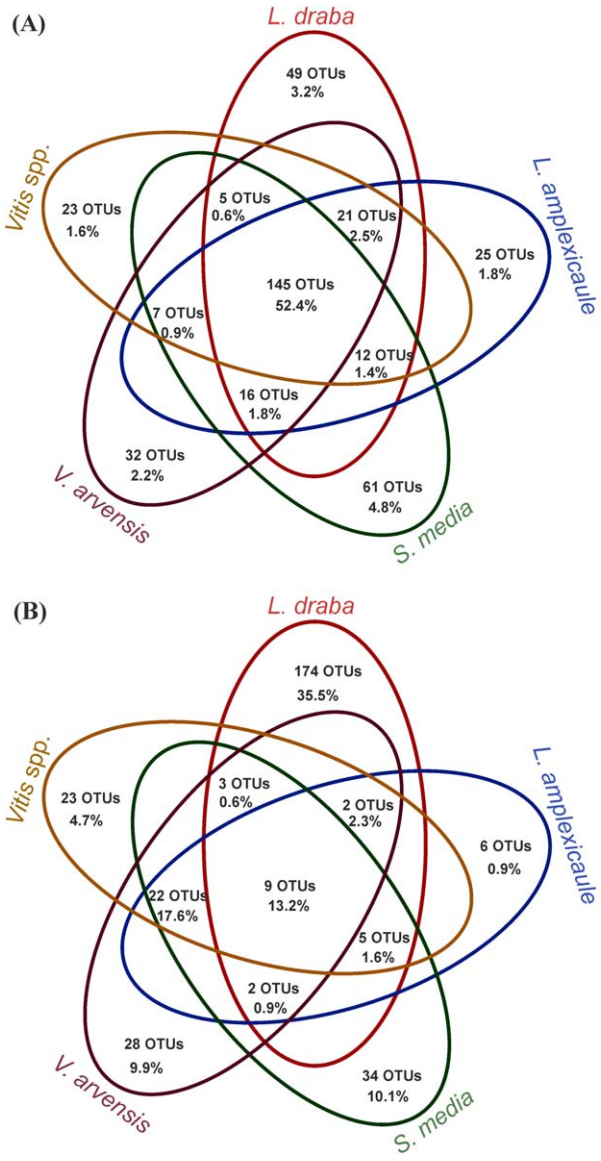

Fig. 3. Shared phylotypes associated with five different plant species. Venn diagrams show absolute numbers of operational taxonomic units (OTUs) and percent abundance in (A) rhizosphere and (B) root samples. [Colour figure can be viewed at wileyonlinelibrary.com]

seven different classes (*Alpha*-, *Beta*- and *Gammaproteobacteria*, *Actinobacteria*, *Sphingobacteria*, *Flavobacteria* and *Bacilli*) and 35 genera (Supporting Information Fig. S5). *Pseudomonas* was the most prevalent genus in the isolate collection, accounting for 35% of total isolates, followed by *Arthrobacter* (28%), *Bacillus* (6%), *Flavobacterium* (5%), *Rhizobium* (3%), *Microbacterium* (3%) and *Variovorax* (3%). Grapevine roots hosted the highest number of culturable genera (23), followed by *L. draba* rhizosphere (14), *L. draba* root (13) and grapevine rhizosphere (10). In contrast, by cultivation-independent analysis the rhizospheres of both plants hosted more genera (grapevine 453 genera, *L. draba* 445 genera) than roots (*L. draba* 420 genera, grapevine 376 genera). In grapevine roots, among the isolates the genera *Pseudomonas* (14.4%) and *Flavobacterium* (14.4%) were the most abundant, but unlike in other samples the occurrence of *Arthrobacter* (1.6%) was low. *L. draba* root samples contained the highest abundance of *Pseudomonas* (60%), *Bacillus* (10.4%) and *Arthrobacter* (9.6%). In the rhizosphere, *Arthrobacter* (*Vitis* spp., 50.4%; *L. draba*, 51.2%) and *Pseudomonas* (*Vitis* spp. 38.4%; *L. draba*, 26.4%) were the most abundant genera.

Shared and unique taxa among plant compartments. Some of the genera – *Flavobacterium*, *Arthrobacter*, *Paenibacillus*, *Pseudomonas* and *Bacillus* – were present in all sample types, although with varying abundances (Supporting Information Fig. S6A). Some other genera were sample type-specific, such as twelve genera (*Nocardia*, *Pseudoxanthomonas*, *Brevundimonas*, *Sphingomonas*, *Agrobacterium*, *Afipia*, *Neorhizobium*, *Methylopila*, *Kaistobacter*, *Bradyrhizobium*, *Caulobacter*, *Bosea)* in grapevine roots, three genera (*Nocardioides*, *Pedobacter*, *Chryseobacterium*) in the grapevine rhizosphere, two genera (*Methylobacterium*; *Pantoea*) in *L. draba* roots and five genera (*Moraxella*, *Clavibacter*, *Mycetocola*, *Agrococcus* and *Agromyces*) in the *L. draba* rhizosphere. Generally, around 50–60% of the genera identified in rhizosphere samples were also found in roots. At OTU level of isolates, 5 OTUs assigned to *Pseudomonas* were shared among all samples while OTUs belonging to *Arthrobacter*, *Flavobacterium* and *Variovorax* were found in 75% of all samples (Supporting Information Fig. S6B).

Functional characteristics of isolates. A range of bacterial features known to contribute to plant growth promotion, stress tolerance or biocontrol was tested. The results are summarized in Supporting Information Fig. S5. The most common traits were phosphate solubilization (found in 32% of all isolates), followed by indole acetic acid production (30%), siderophore production 27%, ACC deaminase (11%), hydrogen cyanide (7%) and antifungal activity against *Cylindrocarpon destructans* (5%).

A high number of *L. draba*-associated isolates produced hydrogen cyanide (75% of positive isolates), siderophores (60%) and IAA (60%) and solubilized phosphate (55%), whereas many bacteria isolated from grapevine showed ACC deaminase production (85%) and antifungal activity (62%) against *C. destructans* (Fig. 6B). Rhizosphere soils hosted mostly bacteria with the ability to produce hydrogen cyanide, antifungal compounds and siderophores and to solubilize phosphate, whereas a high number of root endophytes showed IAA and ACC deaminase production.

Only *Pseudomonas* spp. isolates were able to produce hydrogen cyanide (21% of *Pseudomonas* isolates). Strains belonging to the genera *Arthrobacter* (42 out of 141)

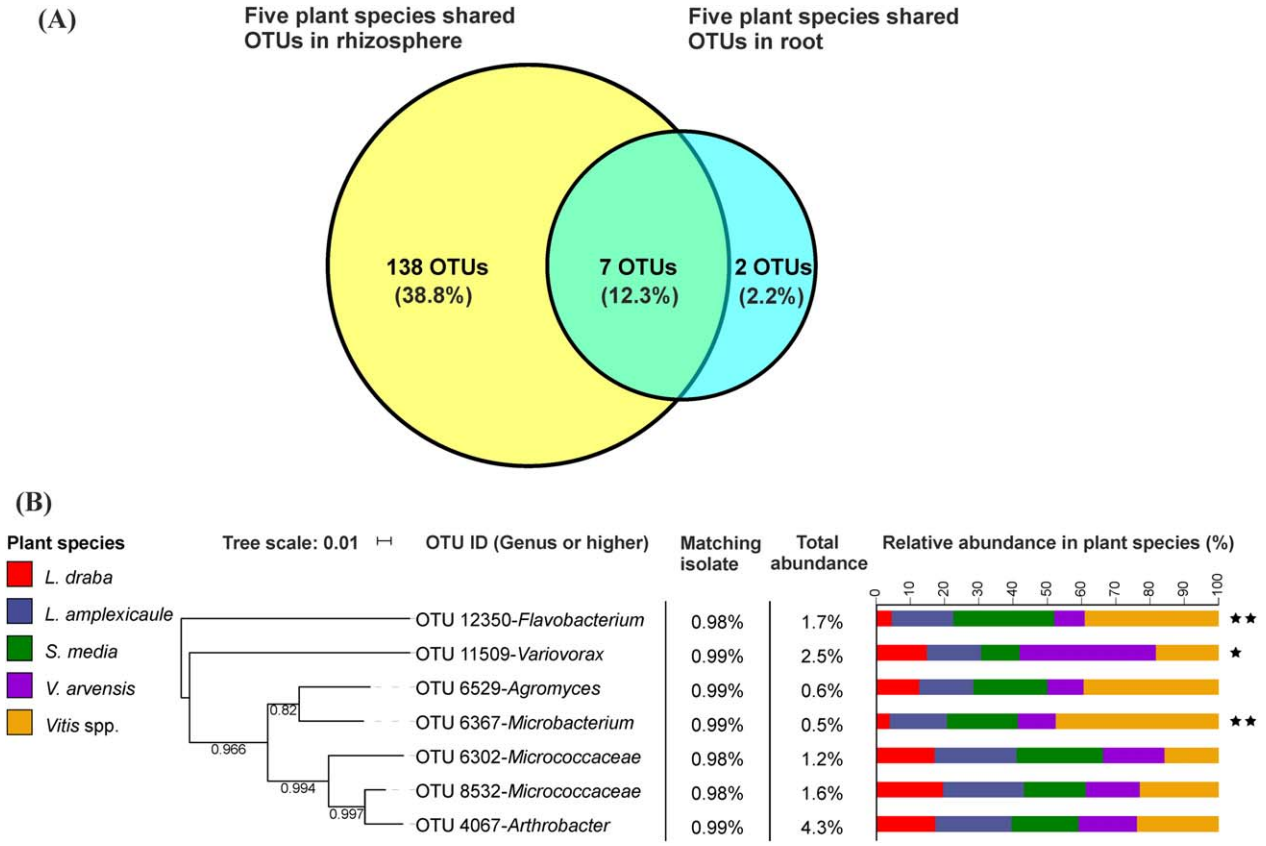

Fig. 4. A. Overlaps between rhizosphere shared OTUs and root shared OTUs of all plant species.
B. Phylogenetic tree of the 7 OTUs conserved in all plant species and both compartments. Permutation ANOVA was used to test the effect of the 'plant species' on the abundance of the shared phylotypes. Significant values are indicated as $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (p value adjustment method: fdr). [Colour figure can be viewed at wileyonlinelibrary.com]

Microbacterium (2 out of 14), *Plantibacter* (1 out of 3), *Rhodococcus* (1 out of 4), *Flavobacterium* (3 out of 27), *Bacillus* (13 out of 30), *Agrobacterium* (2 out of 11), *Variovorax* (1 out of 13) and *Pseudomonas* (70 out of 174) were able to produce siderophores (Table 4). Few members of sixteen genera were able to solubilize phosphate, some strains belonging to nineteen genera were able to produce IAA, and twelve genera comprised strains with ACC deaminase activity. Few strains belonging to ten genera showed antifungal activity against *C. destructans*.

Discussion

Our study revealed that the four weed species and grapevine growing in the same field host significantly different microbiomes in the rhizosphere and particularly in association with roots. Overall, differences were more pronounced between the perennial and annual plants than between the three annual weeds. These results are consistent with a large body of literature showing microbial host plant specificity in roots of different crops (Haichar *et al.*, 2008; Winston *et al.*, 2014) and wild species growing under natural conditions (Osanai *et al.*, 2013; Aleklett *et al.*, 2015).

We generally observed a higher richness of bacteria associated with weeds, which may be due to a greater dependence of weeds on beneficial plant-microbe interactions and a more efficient association with microorganisms than crop plants (Massenssini *et al.*, 2014). Modern crop plants may have lost the ability to recruit some of microbial species that are still associated with weeds. The lower species richness of microbiomes we found in association with roots of annual weeds might be due to the smaller timespan available for the build-up of a microbial community as compared with microbiota associated with perennial plants. The extensive and thick, root system of the perennial weed *L. draba*, which can account for up to 75% of the total plant biomass, may additionally promote bacterial diversity. Its foliage dies back during herbicide application, freezing temperature or drought but roots survive (DiTomaso *et al.*, 2013), and its eradication is considered to be extremely difficult (Miller *et al.*, 1994). Plant species investigated in this study show different root morphologies (Supporting Information Table S1), which are likely to

Table 2. Beta-diversity pairwise comparisons among plant species and sampling points.

Factors	Compartment	MS	Pseudo-*F*	R^2	*p*
Plant	Rhizosphere	0.274982	5.7066	0.23683	0.0002***
	Root	1.2397	7.5431	0.30395	0.0002***
Sampling point	Rhizosphere	0.09103	1.8891	0.0784	0.0019**
	Root	0.16435	1.524	0.06141	0.0372*
Plant species			Sampling points		
Groups of plants	*p* (Rhizosphere)	*P* (Root)	Sampling points	*p* (Rhizosphere)	*P* (Root)
L. draba vs *L. amplexicaule*	0.00050***	0.00020***	A vs B	0.236	0.81
L. draba vs *S. media*	0.00025***	0.00020***	A vs C	0.071	0.81
L. draba vs *V. arvensis*	0.00025***	0.00020***	A vs D	0.068	0.81
L. draba vs *Vitis* spp.	0.00050***	0.00067***	A vs E	0.24	0.22
L. amplexicaule vs *S. media*	0.00040***	0.00062***	B vs C	0.07	0.81
L. amplexicaule vs *V. arvensis*	0.00050***	0.01590*	B vs D	0.093	0.81
L. amplexicaule vs *Vitis* spp.	0.00440**	0.00043***	B vs E	0.08	0.2
S. media vs *V. arvensis*	0.00025***	0.00043***	C vs D	0.039*	0.81
S. media vs *Vitis* spp.	0.00025***	0.00020***	C vs E	0.237	0.22
V. arvensis vs *Vitis* spp.	0.00067***	0.00020***	D vs E	0.151	0.2

Note: Significant values are indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (*p* value adjustment method: fdr).

influence resource uptake and the formation of an interaction with microbial communities (Bardgett *et al*., 2014). Furthermore, plant growth stage (Rasche *et al*., 2006; Micallef *et al*., 2009), species-specific root exudation patterns (Dennis *et al*., 2010), input of litter with unique chemical properties (Ashton *et al*., 2005), the release of secondary compounds (Callaway and Aschehoug, 2000) and production of allelochemicals (Alford *et al*., 2009) could also contribute to the observed variances in bacterial community composition.

Many bacterial taxa, i.e. phyla, classes, genera and OTUs, were conserved across different plant compartments and plant species, although the relative abundance of the common taxa varied across the five plant species. This result is consistent with findings from recent studies in which the microbiomes of different plant species or cultivars showed both specific microbial taxa and a conserved core microbiome (Aleklett *et al*., 2015; Bulgarelli *et al*., 2015; Coleman-Derr *et al*., 2016). The major phyla detected in our study do not differ considerably from those found in previous studies examining microbiomes associated with diverse plant hosts (Lundberg *et al*., 2012; Bulgarelli *et al*., 2015; Edwards *et al*., 2015). In our study, the majority of microbiome members (88.9%) are representatives of *Actinobacteria*, *Proteobacteria* and *Bacteroidetes*. Recently, it has been reported that wheat root-derived carbon was predominantly assimilated by *Proteobacteria* and *Actinobacteria* (Ai *et al*., 2015). It was also shown that plant-derived carbon decomposition favours faster growing copiotrophs such as *Proteobacteria* and *Bacteroidetes*, while slower growing *Acidobacteria* oligotrophs declined under these conditions (Fierer *et al*., 2007). Similarly, the higher presence of *Proteobacteria* and lower abundance of *Acidobacteria* in the root-associated samples as compared with the surrounding soil has been observed in different crops (Edwards *et al*., 2015; Zarraonaindia *et al*., 2015; Coleman-Derr *et al*., 2016). We also found an overlap of taxa at the family level when comparing our results with those obtained previously in association with grapevine, *Arabidopsis*, sugarcane and wild growing plants (Bodenhausen *et al*., 2013; Schlaeppi *et al*., 2014; Aleklett *et al*., 2015; Yeoh *et al*., 2015; Zarraonaindia *et al*., 2015). The shared plant root microbiome raises the interesting question if a number of bacterial families have a long association with plants (Schlaeppi *et al*., 2014) since the division of monocots and dicots by at least 150 million years ago (Chaw *et al*., 2004).

We found three dominant genera, i.e. *Arthrobacter*, *Variovorax* and *Flavobacterium*, and some representatives were members of the shared microbiome. Overall, the OTUs of these genera were encountered in all plant species and both compartments, but varied in their diversity and abundance. *Arthrobacter* was generally more abundant in the rhizosphere than in roots, whereas *Variovorax* was more abundant in root samples as compared with the rhizosphere. Both genera showed low species (OTU) diversity, whereas *Flavobacterium* was characterized by very high diversity. Although we could culture several representatives of the major OTUs of these three genera, many of these strains were exclusively found in one plant species. The overall ubiquitous presence of *Arthrobacter* strains could be due to their extraordinary traits such as enormous nutritional versatility, high resistance to desiccation and starvation (Hagedorn and Holt, 1975; Cacciari and Lippi, 1987) as the sampling field was characterized by high salinity and low water content. Our results are consistent with the findings of Kobayashi *et al*. (2015), who found *Arthrobacter nicotinovorans* to be frequently

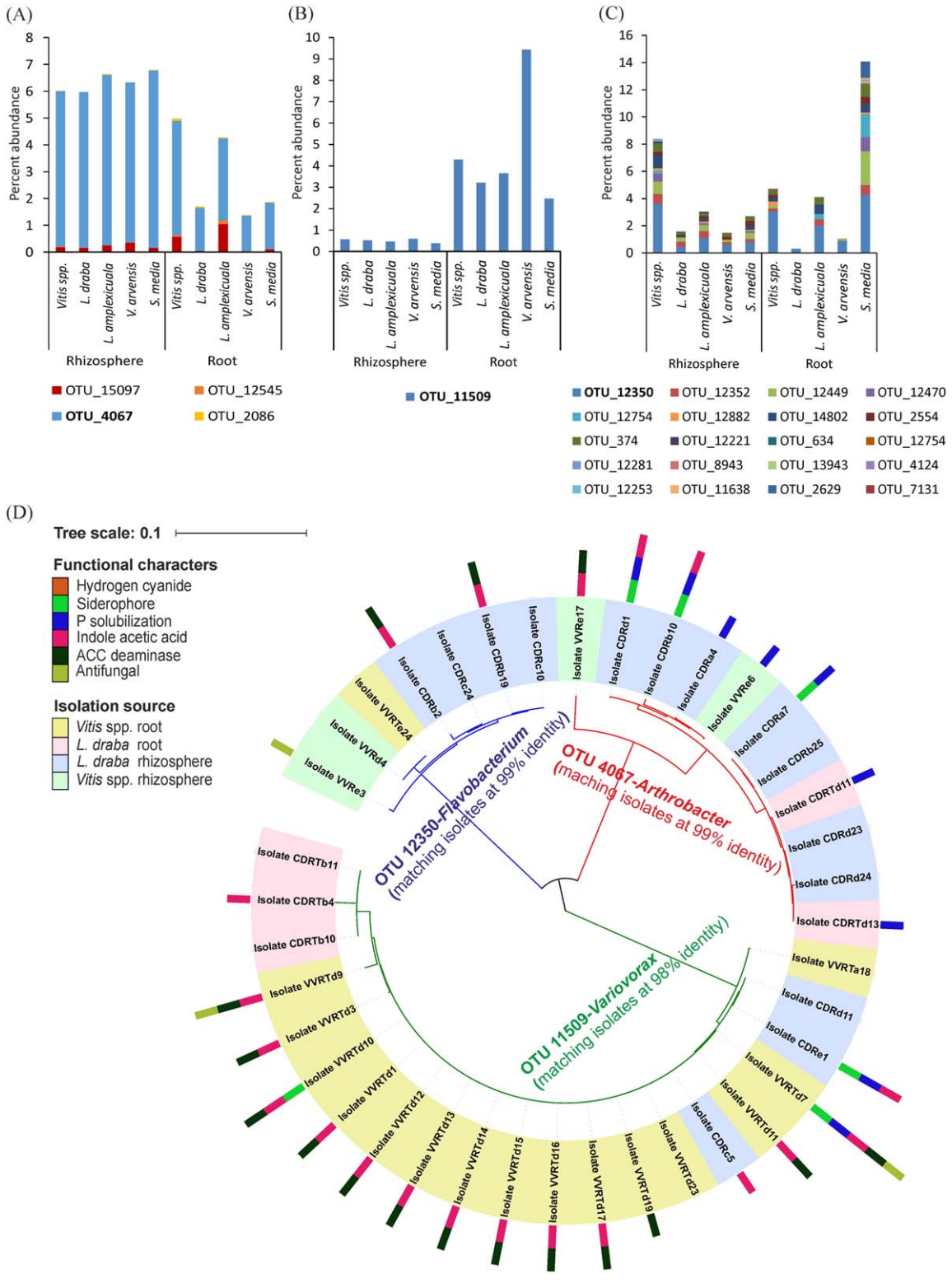

Fig. 5. Dominant and prevalent OTUs (present in at least 2/3 replicates and 3/5 sampling points, OTUs shown in bold letters present across all the samples) of the genera (A) *Arthrobacter*, (B) *Variovorax*, (C) *Flavobacterium* in the various host plant species and (D) A neighbor-joining (NJ) tree showing representative isolates that matched three most abundant shared OTUs from high-throughput sequencing data. The tree was generated based on 800 bp 16S rRNA gene sequences using MEGA 6.0 with bootstrap testing 1000 replicates and annotated using iTOL. The corresponding OTUs to which isolates align are shown. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 3. Root-associated bacterial families reported as core members in previous studies.

Present study	*Vitis* spp.	*A. thaliana*	*S. officinarum*	*P. aurantiaca*, *L. Vulgare* and *T. hybridum*
Microbacteriaceae		✓[c]	✓	✓
Micrococcaceae	✓	✓[b,c]		
Nocardioidaceae		✓[b]		
Streptomycetaceae		✓[a,b,c]	✓	
Chitinophagaceae		✓[c]	✓	
Cytophagaceae			✓	
Flavobacteriaceae		✓[a,b,c]	✓	✓
Sphingobacteriaceae			✓	✓
Hyphomicrobiaceae	✓	✓[b]	✓	✓
Sphingomonadaceae		✓[b,c]	✓	
Comamonadaceae		✓[a,b,c,d]	✓	✓
Oxalobacteraceae		✓[a,b,c,d]	✓	✓
Methylophilaceae				
Pseudomonadaceae	✓	✓[a,b,c]	✓	

Note: ✓corresponds to bacterial families if present where *Vitis* spp. bacterial families are based on Zarraonaindia *et al*. (2015); *A. thaliana* based on Bulgarelli *et al*. (2012)[a], Lundberg *et al*. (2012)[b], Bodenhausen *et al*. (2013)[c] and Schlaeppi *et al*. (2014)[d]; *S. officinarum* are based on Yeoh *et al*. (2015) and *P. aurantiaca*, *L. vulgare*, *T. hybridum* are based on Aleklett *et al*. (2015).

associated with roots and tubers of different potato cultivars. *Arthrobacter* species are known to be involved in the biodegradation of organic matter (Cacciari and Lippi, 1987) and may produce antibiotics (Kamigiri *et al*., 1996). *Flavobacteria* have been reported to colonize the rhizoplane as well as the phyllosphere of many plant species (Kolton *et al*., 2011; 2013; Bodenhausen *et al*., 2013), suggesting that they may play a pivotal role in these environments especially by mineralizing poorly degradable macromolecules (Nagata, 2008). Some *Flavobacterium* strains are known for their biocontrol activities (Sang *et al*., 2008; Sang and Kim, 2012). Members of *Variovorax* have been frequently found in association with plants and some strains have shown plant growth-promoting activities (Allison *et al*., 1995). In brief, these findings suggest the presence of a set of conserved forces that structure microbial communities in the rhizosphere and in association with roots across a wide range of host species.

Although a lower diversity was recovered by cultivation compared with community sequencing, approximately 56% of the most abundant OTUs (>1% of the total microbial community) were recovered by cultivation-dependent approach when we used a 16S rRNA gene identity threshold of 98%. This culture-independent recovery estimate is in the range of 54–65%, which was reported by Bai *et al*. (2015) in *Arabidopsis* regarding the recovery of the top 100 OTUs. Also the three most abundant genera, *Arthrobacter*, *Flavobacterium* and *Variovorax*, were recovered by cultivation. Moreover, *Arthrobacter* with 134 strains, *Flavobacterium* with 21 strains and *Variovorax* with three strains were found to be abundant while these genera were represented by 26, 100 and 3 OTUs, respectively, in the cultivation-independent analysis. The most abundant shared OTUs of these genera, OTU 4067 (*Arthrobacter*) OTU 11509, (*Variovorax*) and OTU 12350 (*Flavobacterium*), matched with many isolated strains (with 98–99% sequence identity) having characteristics related to plant growth promotion, stress tolerance and biocontrol (Fig. 5D). It can be tenuous to generalize the role of any of these representative isolates in relation to the whole bacterial community since the alignment of sequences of isolated strains to sequences of OTUs spans only a small region (350–360 bp) of the 16S rRNA gene. Furthermore, a comparison at the strain level would be of advantage, which requires the analysis of a phylogenetic marker allowing discrimination at the strain or sub-species level. Generally, comparison of cultivation-dependent data to those obtained by high-throughput sequencing may help to screen for useful isolates based on their estimated abundance and prevalence in the overall bacterial community. The use of sub-species markers allowing higher resolution would enable a more precise correlation.

Regarding plant growth-promoting characteristics *L. draba* and grapevine isolates showed different features. *L. draba* hosted a high number of strains with the ability to produce HCN (all pseudomonads) and IAA, whereas grapevine showed a high number of isolates able to show ACC deaminase activity and the ability to antagonize the grapevine fungal pathogen *C. destructans*. Grapevine may have favoured or selected bacteria with antagonistic activities against the grapevine pathogen. Weed species usually thrive in adverse conditions and have been found as a rich source of plant growth-promoting traits (Sturz *et al*., 2001; Arun *et al*., 2012). Sturz *et al*. (2001) reported also a higher number of plant growth-promoting bacteria associated with weeds than with crop plants.

In conclusion, we found distinct microbiomes associated with grapevine and weeds with major differences between annual and perennial plants. Generally, weeds have the tendency to host a higher microbial diversity but weeds

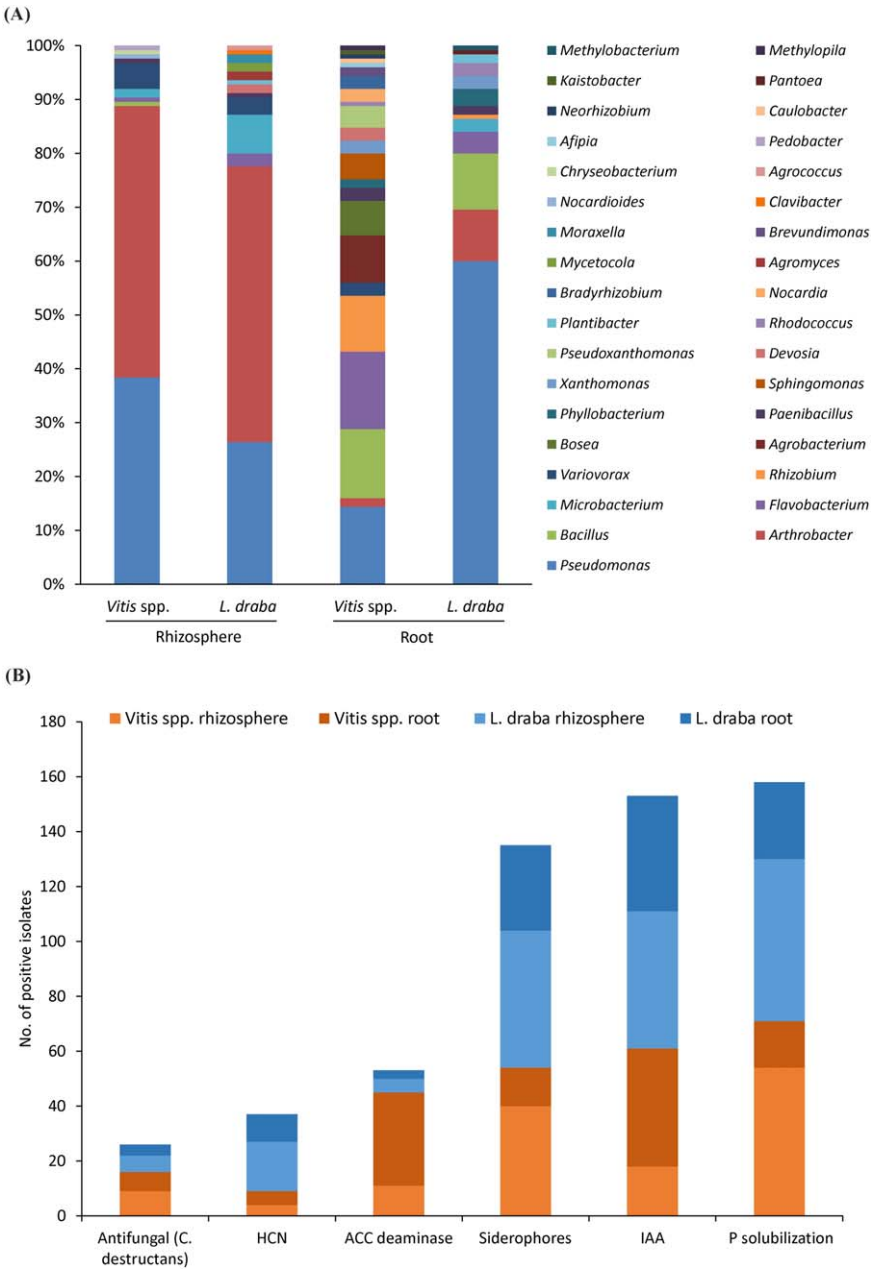

Fig. 6. A. Genera of bacterial isolates obtained from rhizosphere and roots of *Vitis* spp. and *L. draba*.
B. Distribution of tentative plant growth-promoting characteristics of bacterial isolates among plants and compartments. [Colour figure can be viewed at wileyonlinelibrary.com]

and grapevine could not be distinguished based on their microbiome structures. Major microbial players were conserved across different weed species and grapevine in addition to host- and compartment-specific taxa. The observation that host specificity at the strain level is extremely high indicates that we need more analyses at the intraspecific level to better understand the functional consequences of host-specific plant-microbiome interactions. Further research is needed to determine, whether weeds influence the build-up of grapevine microbiomes resulting in different functioning. This understanding would provide the basis for designing new strategies for weed control.

Experimental procedures

Sampling of roots and rhizosphere soil

Grapevine cultivated on sandy soil, located in Illmitz in the vicinity of the lake Neusiedl, Austria (47°46′30.9″N 16°47′43.5″E), was sampled in April 2013. Roots and rhizosphere soil samples of *Vitis* spp. (*Vitis vinifera* L. cv. Zweigelt clone GU4 grafted on the rootstock Kober 5BB (*Vitis berlandieri* Planch. x *Vitis riparia* Michx.)) plants and four different types of weeds *L. amplexicaule* L., *V. arvensis* L., *L. draba* L. L. and *S. media* were taken from five independent points (3 meters apart) within the vineyard (plant characteristics and vegetation stages are shown in Supporting Information Table S1). Briefly, for grapevine, soil was carefully removed to

Table 4. Plant growth-promoting traits of bacterial strains isolated from weed and grapevine.

Genus	Total isolates tested	Hydrogen cyanide	Siderophore	Phosphate solubilization	Indole acetic acid	ACC deaminase	Antifungal activity
Arthrobacter	141	0	42	54	42	4	8
Agromyces	2	0	0	0	2	0	0
Clavibacter	1	0	0	0	0	0	0
Microbacterium	14	0	2	2	3	0	3
Mycetocola	2	0	0	0	1	0	0
Plantibacter	3	0	1	1	0	0	0
Nocardia	3	0	0	0	0	0	0
Nocardioides	1	0	0	0	0	0	0
Rhodococcus	4	0	1	1	3	0	0
Agrococcus	1	0	0	0	0	0	0
Flavobacterium	27	0	3	2	17	15	3
Chryseobacterium	1	0	0	1	0	1	0
Pedobacter	1	0	0	0	0	0	0
Bacillus	30	0	13	4	8	1	0
Devosia	5	0	0	0	1	0	0
Rhizobium	14	0	0	1	3	2	0
Agrobacterium	11	0	2	2	5	6	1
Afipia	1	0	0	0	0	0	0
Caulobacter	1	0	0	0	0	0	0
Paenibacillus	7	0	0	0	0	0	0
Neorhizobium	1	0	0	0	0	0	0
Brevundimonas	2	0	0	1	1	2	1
Bosea	8	0	0	0	2	1	0
Phyllobacterium	6	0	0	1	4	0	1
Variovorax	13	0	1	2	3	2	3
Pantoea	1	0	0	0	0	0	0
Moraxella	2	0	0	1	1	0	0
Bradyrhizobium	3	0	0	0	0	1	0
Kaistobacter	1	0	0	0	1	0	0
Methylopila	1	0	0	0	0	0	1
Methylobacterium	1	0	0	0	0	0	0
Sphingomonas	6	0	0	0	1	0	0
Pseudoxanthomonas	5	0	0	1	1	1	1
Pseudomonas	174	37	70	83	53	17	4
Xanthomonas	6	0	0	1	1	0	0
Total	500	37	135	158	153	53	26

locate roots, and roots as well as rhizosphere soil were sampled close to the stem at depths of 10–12 cm. Remaining roots were covered with the same soil, while weed roots were collected by uprooting the plant. Five samples were collected per plant from each sampling point. Loosely attached soil was removed from the root system, and rhizosphere soil was separated by agitating the roots and delicately separating the soil manually. Samples were divided in two parts to be used for cultivation-dependent and -independent analysis.

Microbial community DNA isolation

Rhizosphere soil DNA was extracted from four different weed species and grapevine samples using FastDNA® SPIN Kit for Soil (BIO101, Vista, CA) according to manufacturer's protocol. For surface sterilization of root samples, 5 g of root were treated with 70% ethanol for 5 min, followed by an immersion in 2.5% sodium hypochlorite solution for 15 min (Compant *et al.*, 2011). Surface sterilization was checked by plating three times 100 μl of the last washing solution on R2A medium (Difco, Detroit, MI), no bacterial growth was observed after five days of incubation at 28°C. For the isolation of root (endophyte) DNA, 100 mg root material was pre-chilled with liquid nitrogen in 2-ml Safe-Lock tubes (Greiner Bio-One, Germany) and homogenized by the use of a ball mill MM301 mixer (Retsch GmbH & Co., Germany) at 30 Hz for 2 min using a single steel ball (5 mm diameter). Then, 310 μl of sodium chloride-Tris-EDTA (STE) buffer, 440 μl lysis buffer (0.2 M TRIS-HC1, 0.05 M EDTA, 2 M NaC1, 2% CTAB w/v, pH 7.5) and 150 μl sarkosyl was added and incubated at 65°C on a water bath with occasionally mixing (van der Beek *et al.*, 1992). After cooling for 5 min, 900 μl chloroform/isoamylalcohol (24:1) was added, mixed and centrifuged at 9500×*g* for 10 min. Supernatants were transferred to 2 ml Eppendorf tubes, and DNA was precipitated by adding an equal volume of cold isopropanol. Tubes were centrifuged at 18 500×*g* for 10 min to pellet DNA, then DNA pellets were washed with 70% ethanol, dissolved in 100 μl TE buffer after air drying and stored at −20°C for subsequent use.

Paired-end library preparation and MiSeq sequencing

A two-step tailed PCR approach was employed to construct the paired-end libraries following protocol for 16S rRNA gene sequencing: 16S rRNA gene amplicon libraries were prepared according to the instructions for the Illumina MiSeq system provided by Illumina (part no. 15044223 Rev. B) with some modifications. First, the target region was amplified by PCR using primers 799-forward (Chelius and Triplett, 2001) and 1175-reverse (Bonder *et al.*, 2012) with the Illumina adaptor. PCR amplifications were carried out with 25 cycles in a 25 μl reaction volume containing 5 μl of KAPA HiFi fidelity buffer, 0.75 μl of 10 mM dNTP mix, 0.75 μl of each 799-forward and 1175-reverse primers, 1 μl (20 ng) template DNA and 0.5 μl of 1 U/μl KAPA HIFI polymerase (KAPA Biosystems, Wilmington, MA, USA) and 11.25 μl PCR grade water. The thermal cycle (BIOMETRA) profile after an initial 3 min denaturation step at 95°C was as follows: denaturation at 98°C for 30 s; annealing at 55°C for 30 s and extension at 72°C for 30 s with the final extension at the same temperature for 5 min. In the case of root DNA, amplicons were subjected to agarose gel electrophoresis and bacterial bands were excised to eliminate mitochondrial amplicons. AMPure XP beads were used to purify the 16S rRNA V5 and V6 amplicon and get rid of free primers and primer dimers.

In a second PCR, the cleaned PCR products from the first run were used as a template and amplified using Index 1 (N701–N712) and Index 2 Primers (S517–S508) from the Nextera XT Index kit (Illumina, San Diego, CA, USA). This PCR was carried out with 8 cycles of a 50 μl reaction volume containing 5 μl (10 ng) DNA template, 5 μl (10 μM), of each forward and reverse Index primer, 25 μl 2× KAPA HiFi HotStart Ready Mix and 10 μl PCR grade water. Different combinations of indices (12 different Index 1 adapters N701–N712 and 8 different Index 2 adapters S517–S508) were used for different templates for a massive parallel sequencing using the MiSeq platform. PCR was performed on a thermal cycler (BIOMETRA) using the following program: 95°C for 3 min, 8 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s and final extension 72°C for 5 min. PCR products were cleaned using AMPure XP beads before quantification.

The final library concentration was quantified by qPCR using KAPA Library Quantification Kits for Illumina sequencing platforms and library insert size of app. 550 bp was validated by using an Agilent 2100 Bioanalyzer. The final library was diluted to 4 nM by using 10 mM Tris pH 8.5 and 5 μl aliquots were pooled from each library with unique indices. Pooled libraries were validated using the Agilent 2100 Bioanalyzer.

Pooled libraries (4 nM) were denatured using fresh prepared 0.2 N NaOH and further diluted to 20 pM using prechilled HT1 buffer (provided by the Illumina MiSeq Reagent Kit v3for 2 × 300 bp PE). A 20% phiX DNA spike (4 pM) was added as control to improve the data quality of low diversity samples and the combined library was incubated at 96°C for 2 min on a thermomixer R (Eppendorf). After incubation the library was immediately placed on ice-water bath (3 parts ice and 1 part water) for 5 min. A final concentration of 4 pM denatured DNA was sequenced on the MiSeq instrument (Illumina, San Diego, CA) using the MiSeq Reagent v3 Kit (part number MS-102-3003) with paired end, 2 × 300 bp cycle run and applying GenerateFASTQ Miseq reporter workflow.

16S rRNA gene sequence processing

MiSeq raw data quality was checked in FASTQC (Andrews, 2010) and reads were screened for PhiX contamination using Bowtie 2.2.6 (Langmead and Salzberg, 2012). A Bayesian clustering for error correction (Nikolenko *et al.*, 2013; Schirmer *et al.*, 2015) was applied before merging the paired-end reads using PEAR 0.9.6 ($p < 0.001$) (Zhang *et al.*, 2014). Forward and reverse primers were then stripped from merged reads employing Cutadapt 1.8.3 (Martin, 2011) and quality filtering performed in USEARCH v8.0.1517 (Edgar, 2013). Filtered reads were labelled according to the sample name of origin and combined in QIIME (Caporaso *et al.*, 2010). Sequences were de-replicated, sorted and clustered at 97% of similarity using VSEARCH 1.1.1 (Rognes, 2015). Chimeras were checked adopting both a *de novo* and a reference based approach, as routine of the above-mentioned tool. The RDP classifier training set v15 (09/2015) was used as a reference database. METAXA2 (Bengtsson-Palme *et al.*, 2015) was used to target the extraction and to verify the 16S V7-V9 region of the representative sequences. An optimal global alignment was applied afterwards in VSEARCH and a BIOM table generated. Taxonomy assignment was performed employing the naïve Bayesian RDP classifier v.2.10.2 (Wang *et al.*, 2007) with a minimum confidence of 0.6 and queried against the Greengenes database (McDonald *et al.*, 2011) (08/2013). Sequence data are available at NCBI SRA database under the accession SRP077301, BioProject number PRJNA326072 and GenBank under the accession numbers KX503822–KX504321.

16S rRNA gene-based microbial community analysis and statistics

An OTU-based analysis was performed in QIIME to calculate the richness and diversity after multiple rarefactions. The observed OTUs were counted and the diversity within each individual sample was estimated using the Simpson's diversity index. Richness and diversity values were compared between plants, compartments and sampling points by means of permutation ANOVA and permutational pairwise comparisons in the RVAideMemoire R package (Hervé, 2016). The resulting *p* values were adjusted by False Discovery Rate (FDR). Richness and diversity value boxplots were then plotted using ggplot2 package in R (Wickham and Chang, 2016).

A data-driven adaptive method for selecting normalization scale quantile was conducted on the BIOM table and data normalized by scaling counts by the *n*th percentile of each sample's non-zero count distribution in the metagenomeSeq Bioconductor package (Paulson *et al.*, 2013; McMurdie *et al.*, 2014). The resulting normalized BIOM table was used for the beta-diversity analysis. Multivariate analysis of community structure and diversity was performed according to the recommendations by Anderson and Willis (2003): (1) unconstrained ordination offered by Principal Coordinate Analysis (PCoA), (2) constrained multidimensional scaling using Constrained Analysis of Principal Coordinates (CAP) as re-implemented in the vegan R package (Oksanen *et al.*, 2016), (3) permutation test for assessing the significance of the constraints and permutational multivariate analysis of variance (PERMANOVA)

and (4) individuation and correlation of OTUs responsible for shaping the diversity structure.

In more detail, the differences between bacterial communities were investigated using the Bray–Curtis dissimilarity distance and the ordination methods applied to the same distance matrices. All the ordination analyses were computed and CAP plotted in phyloseq (McMurdie and Holmes, 2013) (see above, points 1 and 2). The significance of the treatment grouping factor used as constraint in the CAP was assessed via the permutation test in the vegan R package. The null hypothesis of no differences between *a priori* defined groups was investigated recurring to the PERMANOVA (Anderson, 2001) approach, implemented in vegan as the ADONIS function and applied to the Bray–Curtis dissimilarity distances.

Permutational pairwise comparisons between plants, compartments and sampling points were carried out in the RVAideMemoire R package and *p* values adjusted by FDR (point 3). A linear discriminant analysis (LDA) effect size (LEfSe) method was applied for differential OTU abundance calculation among the treatments and *p* values corrected by FDR (point 4). Shared microbiome was calculated using QIIME and Venn diagrams generated using VennDIS software (Ignatchenko *et al*., 2015). All evolutionary analyses were conducted in MEGA6 (Tamura *et al*., 2013). The domain architectures and taxa colours were added to the final tree using iTOL (Letunic and Bork, 2011).

Isolation of bacteria

Rhizosphere bacteria and root endophytes associated with *Vitis* spp. and *L. draba* L. were isolated according to the protocol reported by (Compant *et al*., 2011). Briefly, 5 g rhizosphere soils were suspended in 10 ml sterile 0.85% NaCl solution, homogenized by vortexing for 60 s at high speed and then soil suspensions were used for the isolation of bacteria. For the isolation of endophytes root surfaces were sterilized as described above for DNA isolation. Surface sterilization was checked by plating three times 100 μl of the last washing solution on R2A medium (Difco, Detroit, MI) and no bacterial growth was observed after five days of incubation at 28°C. Root samples were macerated with 10 ml sterile 0.85% NaCl using sterile mortar and pestle and further homogenized by vortexing for 60 s at high speed. Microbial suspensions from each sample were ten-fold diluted with sterile 0.85% NaCl solution and then 100 μl of each dilution were plated on R2A medium in triplicates. Plates were incubated at 28°C for 5 days, then bacterial numbers were counted and numbers of colony-forming units (log10 CFU) per gram were calculated. In total, 500 isolates were picked by randomly selecting 125 isolates from each rhizosphere and root compartment of *L. draba* and *Vitis* spp. Selected isolates were additionally purified by repeated streaking on R2A medium and stored at −80°C in sterile broth solution amended with 30% glycerol.

Isolation of DNA from bacterial isolates and PCR amplification of 16S rRNA genes

DNA was extracted from 500 isolates using the InstaGene matrix (BIO-RAD) following the manufacturer's instructions. For the identification of bacteria, 16S rRNA genes were amplified using 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) (Weisburg *et al*., 1991) and 1520R (5′AAGGAGGTGATCCA GCCGCA-3′) (Edwards *et al*., 1989) primers. After treatment with the InstaGene matrix six μl were used as template DNA in a 25 μl PCR reaction mix containing 1 × PCR reaction buffer (Invitrogen), 2.5 mM $MgCl_2$, 0.2 μM of each primer, 0.2 mM of each deoxynucleoside triphosphate and 1 U FIREPol DNA polymerase (Solis BioDyne). Amplifications were performed with 30 cycles of denaturation at 95°C for 45 s, annealing at 54°C for 60 s and elongation at 72°C for 90 s. PCR products were subsequently sequenced using the 1520R primer (by LGC-Genomics) and the results were analysed using BLAST on NCBI to identify the bacteria.

Functional characteristics

All 500 isolates were tested *in vitro* for various functional characteristics, i.e. the production of ACC deaminase, HCN, IAA and siderophores, phosphate solubilization and antifungal activity against *C. destructans*.

Hydrogen cyanide (HCN) production. HCN production was determined using 96-well microtiter plate–based method described by (Takos *et al*., 2010). The cyanide-sensitive paper developed by (Feigl and Anger, 1966) was used, which was prepared by soaking Whatman 3MM paper (GE Healthcare, Chalfont St Giles, UK) in a 5 g l^{-1} chloroform solution of copper ethylacetoacetate (Sigma-Aldrich) and 4,4′-methylene-bis(*N,N*-dimethylaniline) (Sigma-Aldrich). In the case of HCN production the colour of the filter paper changes from white to blue after 24 h incubation at 28°C. HCN production was confirmed by inoculating King's B agar plates (King *et al*., 1954) amended with 4.4 g l^{-1} glycine (Lorck, 1948). Filter paper (Whatman no. 1) saturated with picrate solution (2% Na_2CO_3 in 0.5% picric acid) was placed in the lid of a petri dish plate inoculated with bacterial isolates. The plates were incubated at 28°C for 5 days. HCN production was assessed by the colour change of yellow filter paper to reddish brown.

Indole-3-acetic acid (IAA) production. Auxin production by bacterial isolates in the presence of L-tryptophan (L-TRP) was determined colorimetrically and expressed as IAA equivalents (Sarwar *et al*., 1992). 72 h old bacterial cells grown (20°C at 180 rpm) in tryptic soy broth (Merck KGaA, Darmstadt, Germany) supplemented with 0.02% L-TRP solution were harvested by centrifugation (10 000 g for 10 min). Supernatants (120 μl) were mixed with 80 μl Salkowski's reagent (12 g l^{-1} $FeCl_3$ in 429 ml l^{-1} H_2SO_4). Mixtures were incubated at room temperature in the dark for 30 min for colour development and samples were read (in triplicates) at 530 nm absorbance by using 96-well microtiter plate in multi-mode microplate reader (BioTek Instruments, Winooski, VT). The level of auxin production was determined using standard curves for IAA (Sigma-Aldrich) prepared from serial dilutions of 0–100 μg ml^{-1}.

Siderophore production. Bacterial isolates were assayed for siderophore production on Chrome azurol sulphonate (CAS) agar medium described by Schwyn and Neilands (1987). Bacterial isolates were spot inoculated on chrome azurol sulphonate agar. Plates were incubated at 28°C for 4 days. A

size of yellow-orange haloes around bacterial colonies indicated siderophore activity.

Phosphate solubilization. Bacterial strains were evaluated for their ability to solubilize inorganic phosphate. For qualitative measurement of phosphate solubilization, National Botanical Research Institute's phosphate growth medium containing 0.1 g l^{-1} bromophenol blue (designated as NBRI-PBP) was used (Mehta and Nautiyal, 2001). Bacterial strains were cultured and a loop full of each culture was placed on the plates, and plates were incubated at 28°C for 7 days to observe halo zones around bacterial colonies.

ACC-deaminase activity. Plates containing minimal medium (Dworkin and Foster, 1958) were supplemented with ammonium chloride (2 g l^{-1}) (serving as control plates) or 3 mM 1-aminocyclopropane-1-carboxylic acid (ACC) (Penrose and Glick, 2003). A loop of three days old bacterial culture was spotted on these plates and incubated for 2–5 days at 28°C. Bacteria showing good growth on ACC supplemented medium plates and capable of utilizing ACC as a nitrogen source were scored as having ACC deaminase activity.

Antifungal assay. For the production of bioactive molecules, a medium consisting of half of nutrient agar and half PDA (14 g nutrient agar, 12 g of PDA, 7 g of agar, pH = 5.10) was used. The fungus *C. destructans* (a causal organism of black foot disease in grapevine, kindly provided by Andrea Campisano, Fondazione Mach, Italy) was placed in the center of the half PDA plate and bacteria were spot inoculated on four corners of petri dishes. Petri dishes without bacterial inoculation served as controls. Plates were incubated at 28°C for 14 days. The results of plates were visualized 14 days post inoculation.

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References

Ai, C., Liang, G., Sun, J., Wang, X., He, P., Zhou, W., and He, X. (2015) Reduced dependence of rhizosphere microbiome on plant-derived carbon in 32-year long-term inorganic and organic fertilized soils. *Soil Biol Biochem* **80:** 70–78.

Aleklett, K., Leff, J.W., Fierer, N., and Hart, M. (2015) Wild plant species growing closely connected in a subalpine meadow host distinct root-associated bacterial communities. *PeerJ* **3:** e804.

Alford, ÉR., Vivanco, J.M., and Paschke, M.W. (2009) The effects of flavonoid allelochemicals from knapweeds on legume–rhizobia candidates for restoration. *Restor Ecol* **17:** 506–514.

Allison, N., Turner, J.E., and Wait, R. (1995) Degradation of homovanillate by a strain of *Variovorax paradoxus* via ring hydroxylation. *FEMS Microbiol Lett* **134:** 213–219.

Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Aust Ecol* **26:** 32–46.

Anderson, M.J., and Willis, T.J. (2003) Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* **84:** 511–525.

Andrews, S. (2010) *FastQC: A Quality Control Tool for High Throughput Sequence Data*. Available at http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Arun, B., Gopinath, B., and Sharma, S. (2012) Plant growth promoting potential of bacteria isolated on N free media from rhizosphere of *Cassia occidentalis*. *World J Microbiol Biotechnol* **28:** 2849–2857.

Ashton, I.W., Hyatt, L.A., Howe, K.M., Gurevitch, J., and Lerdau, M.T. (2005) Invasive species accelerate decomposition and litter nitrogen loss in a mixed deciduous forest. *Ecol Appl* **15:** 1263–1272.

Bai, Y., Müller, D.B., Srinivas, G., Garrido-Oter, R., Potthoff, E., Rott, M., *et al.* (2015) Functional overlap of the *Arabidopsis* leaf and root microbiota. *Nature* **528:** 364–369.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., and Vivanco, J.M. (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu Rev Plant Biol* **57:** 233–266.

Bardgett, R.D., Mommer, L., and Vries, FT. D. (2014) Going underground: root traits as drivers of ecosystem processes. *Trends Ecol Evol* **29:** 692–699.

van der Beek, J.G., Verkerk, R., Zabel, P., and Lindhout, P. (1992) Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: Cf9 (resistance to *Cladosporium fulvum*) on chromosome 1. *Theor Appl Genet* **84:** 106–112.

Bengtsson-Palme, J., Hartmann, M., Eriksson, K.M., Pal, C., Thorell, K., Larsson, D.G.J., and Nilsson, R.H. (2015) Metaxa2: improved identification and taxonomic classification of small and large subunit rRNA in metagenomic data. *Mol Ecol Resour* **15:** 1403–1414.

Berg, G., and Smalla, K. (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol Ecol* **68:** 1–13.

Bhattacharyya, P.N., and Jha, D.K. (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J Microbiol Biotechnol* **28:** 1327–1350.

Bodenhausen, N., Horton, M.W., and Bergelson, J. (2013) Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. *PLoS One* **8:** e56329.

Bonder, M.J., Abeln, S., Zaura, E., and Brandt, B.W. (2012) Comparing clustering and pre-processing in taxonomy analysis. *Bioinformatics* **28:** 2891–2897.

Brenchley, W.E. (1920) *Weeds of Farm Land Longmans*. New York, NY: Green and Co.

Bulgarelli, D., Garrido-Oter, R., Münch, P.C., Weiman, A., Dröge, J., Pan, Y., *et al.* (2015) Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host Microbe* **17:** 392–403.

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., *et al.* (2012) Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* **488:** 91–95.

Cacciari, I., and Lippi, D. (1987) Arthrobacters: successful arid soil bacteria: a review. *Arid Soil Res Rehab* **1:** 1–30.

Callaway, R.M., and Aschehoug, E.T. (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* **290:** 521–523.

Callaway, R.M., Thelen, G.C., Barth, S., Ramsey, P.W., and Gannon, J.E. (2004) Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. *Ecology* **85:** 1062–1071.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., *et al*. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Meth* **7:** 335–336.

Chaparro, J.M., Badri, D.V., Bakker, M.G., Sugiyama, A., Manter, D.K., and Vivanco, J.M. (2013) Root exudation of phytochemicals in *Arabidopsis* follows specific patterns that are developmentally programmed and correlate with soil microbial functions. *PLoS One* **8:** e55731.

Chaw, S.M., Chang, C.C., Chen, H.L., and Li, W.H. (2004) Dating the monocot–dicot divergence and the origin of core eudicots using whole chloroplast genomes. *J Mol Evol* **58:** 424–441.

Chelius, M.K., and Triplett, E.W. (2001) The diversity of Archaea and bacteria in association with the roots of *Zea mays* L. *Microb Ecol* **41:** 252–263.

Coleman-Derr, D., Desgarennes, D., Fonseca-Garcia, C., Gross, S., Clingenpeel, S., Woyke, T., *et al*. (2016) Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. *New Phytol* **209:** 798–811.

Compant, S., Clément, C., and Sessitsch, A. (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol Biochem* **42:** 669–678.

Compant, S., Mitter, B., Colli-Mull, J.G., Gangl, H., and Sessitsch, A. (2011) Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. *Microb Ecol* **62:** 188–197.

Dennis, P.G., Miller, A.J., and Hirsch, P.R. (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiol Ecol* **72:** 313–327.

DiTomaso, J.M., Kyser, G.B., Oneto, S.R., Wilson, R.G., Orloff, S.B., Anderson, L.W., *et al*. (2013) Weed Control in Natural Areas in the Western United States University of California Weed Research and Information Center. Davis, CA. 544 pp. http://wric.ucdavis.edu/information/natural%20areas/wr_A/Atriplex.pdf

Dworkin, M., and Foster, J. (1958) Experiments with some microorganisms which utilize ethane and hydrogen. *J Bacteriol* **75:** 592–603.

Edgar, R.C. (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Meth* **10:** 996–998.

Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N.K., Bhatnagar, S., *et al*. (2015) Structure, variation, and assembly of the root-associated microbiomes of rice. *Proc Natl Acad Sci USA* **112:** E911–E920.

Edwards, U., Rogall, T., Blöcker, H., Emde, M., and Böttger, E.C. (1989) Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res* **17:** 7843–7853.

Feigl, F., and Anger, V. (1966) Replacement of benzidine by copper ethylacetoacetate and tetra base as spot-test reagent for hydrogen cyanide and cyanogen. *Analyst* **91:** 282–284.

Fierer, N., Bradford, M.A., and Jackson, R.B. (2007) Toward an ecological classification of soil bacteria. *Ecology* **88:** 1354–1364.

Gottel, N.R., Castro, H.F., Kerley, M., Yang, Z., Pelletier, D.A., Podar, M., *et al*. (2011) Distinct microbial communities within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil types. *Appl Environ Microbiol* **77:** 5934–5944.

Hagedorn, C., and Holt, J.G. (1975) A nutritional and taxonomic survey of *Arthrobacter* soil isolates. *Can J Microbiol* **21:** 353–361.

Haichar, F., el. Z., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., *et al*. (2008) Plant host habitat and root exudates shape soil bacterial community structure. *ISME J* **2:** 1221–1230.

Hardoim, P.R., van Overbeek, L.S., Berg, G., Pirttila, A.M., Compant, S., Campisano, A., *et al*. (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol Mol Biol Rev* **79:** 293–320.

Hervé, M. (2016) *RVAideMemoire: Diverse Basic Statistical and Graphical Functions*. Available at https://cran.r-project.org/web/packages/RVAideMemoire/index.html.

Ignatchenko, V., Ignatchenko, A., Sinha, A., Boutros, P.C., and Kislinger, T. (2015) VennDIS: a JavaFX-based Venn and Euler diagram software to generate publication quality figures. *Proteomics* **15:** 1239–1244.

Kamigiri, K., Tokunaga, T., Shibazaki, M., Setiawan, B., Rantiatmodjo, R.M., Morioka, M., and Suzuki, K. (1996) YM-30059, a novel quinolone antibiotic produced by *Arthrobacter* sp. *J Antibiot* **49:** 823–825.

King, E.O., Ward, M.K., and Raney, D.E. (1954) Two simple media for the demonstration of pyocyanin and fluorescein. *J Lab Clin Med* **44:** 301–307.

Klironomos, J.N. (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* **417:** 67–70.

Kobayashi, A., Kobayashi, Y.O., Someya, N., and Ikeda, S. (2015) Community analysis of root- and tuber-associated bacteria in field-grown potato plants harboring different resistance levels against common scab. *Microbes Environ* **30:** 301–309.

Kolton, M., Meller Harel, Y., Pasternak, Z., Graber, E.R., Elad, Y., and Cytryn, E. (2011) Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. *Appl Environ Microbiol* **77:** 4924–4930.

Kolton, M., Sela, N., Elad, Y., Cytryn, E., and Robinson, D.A. (2013) Comparative genomic analysis indicates that niche adaptation of terrestrial *Flavobacteria* is strongly linked to plant glycan metabolism. *PLoS One* **8:** e76704.

Langmead, B., and Salzberg, S.L. (2012) Fast gapped-read alignment with Bowtie 2. *Nat Methods* **9:** 357–359.

Letunic, I., and Bork, P. (2011) Interactive tree of life v2: online annotation and display of phylogenetic trees made easy. *Nucleic Acids Res* **39:** W475–W478.

Lorck, H. (1948) Production of hydrocyanic acid by bacteria. *Physiol Plant* **1:** 142–146.

Lorenzi, H. (2008) *Plantas Daninhas do Brazil: Terrestres, Aquáticas, Parasitas e Toxicas*. 4th ed. Nova Odessa, SP: Intituto Plantarum.

Lugtenberg, B., and Kamilova, F. (2009) Plant-growth-promoting rhizobacteria. *Annu Rev Microbiol* **63:** 541–556.

Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S., *et al*. (2012) Defining the core *Arabidopsis thaliana* root microbiome. *Nature* **488:** 86–90.

Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J* **17:** 10–12.

Massenssini, A.M., Bonduki, V.H.A., Tótola, M.R., Ferreira, F.A., and Costa, M.D. (2014) Arbuscular mycorrhizal associations and occurrence of dark septate endophytes in the roots of Brazilian weed plants. *Mycorrhiza* **24:** 153–159.

McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., *et al*. (2011) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* **6:** 610–618.

McMurdie, P.J., and Holmes, S. (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* **8:** e61217.

McMurdie, P.J., Holmes, S., and McHardy, A.C. (2014) Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Comput Biol* **10:** e1003531.

Mehta, S., and Nautiyal, C.S. (2001) An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Curr Microbiol* **43:** 51–56.

Melo, C., Fialho, C., Faria, A., Neto, M., Saraiva, D., Costa, M., *et al*. (2014) Microbial activity of soil cultivated with corn in association with weeds under different fertility management systems. *Chil J Agric Res* **74:** 477–484.

Mendes, R., Garbeva, P., and Raaijmakers, J.M. (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol Rev* **37:** 634–663.

Micallef, S.A., Channer, S., Shiaris, M.P., and Colón-Carmona, A. (2009) Plant age and genotype impact the progression of bacterial community succession in the *Arabidopsis* rhizosphere. *Plant Signal Behav* **4:** 777–780.

Miller, R.F., Svejcar, T.J., Rose, J.A., and McInnis, M.L. (1994) Plant development, water relations, and carbon allocation of heart-podded hoary cress. *Agron J* **86:** 487–491.

Nagata, T. (2008) Organic matter–bacteria interactions in seawater. In *Microbial Ecology of the Oceans*. Kirchman, D.L. (ed). Hoboken, NJ: John Wiley & Sons, pp. 207–241.

Newton, A.C., Fitt, B.D.L., Atkins, S.D., Walters, D.R., and Daniell, T.J. (2010) Pathogenesis, parasitism and mutualism in the trophic space of microbe–plant interactions. *Trends Microbiol* **18:** 365–373.

Nikolenko, S.I., Korobeynikov, A.I., and Alekseyev, M.A. (2013) BayesHammer: Bayesian clustering for error correction in single-cell sequencing. *BMC Genomics* **14:** Suppl 1: S7.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., and Minchin, P.R. (2016) *Vegan: Community Ecology Package*. Available at https://cran.r-project.org/web/packages/vegan/index.html.

Osanai, Y., Bougoure, D.S., Hayden, H.L., and Hovenden, M.J. (2013) Co-occurring grass species differ in their associated microbial community composition in a temperate native grassland. *Plant Soil* **368:** 419–431.

Paulson, J.N., Stine, O.C., Bravo, H.C., and Pop, M. (2013) Differential abundance analysis for microbial marker-gene surveys. *Nat Meth* **10:** 1200–1202.

Penrose, D.M., and Glick, B.R. (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol Plant* **118:** 10–15.

Rasche, F., Hodl, V., Poll, C., Kandeler, E., Gerzabek, M.H., van Elsas, J.D., and Sessitsch, A. (2006) Rhizosphere bacteria affected by transgenic potatoes with antibacterial activities compared with the effects of soil, wild-type potatoes, vegetation stage and pathogen exposure. *FEMS Microbiol Ecol* **56:** 219–235.

Rognes, T. (2015) *VSEARCH: Versatile Open-Source Tool for Metagenomics*. Available at https://github.com/torognes/vsearch.

Sang, M.K., Chun, S.C., and Kim, K.D. (2008) Biological control of *Phytophthora* blight of pepper by antagonistic rhizobacteria selected from a sequential screening procedure. *Biol Control* **46:** 424–433.

Sang, M.K., and Kim, K.D. (2012) The volatile-producing *Flavobacterium johnsoniae* strain GSE09 shows biocontrol activity against *Phytophthora capsici* in pepper. *J Appl Microbiol* **113:** 383–398.

dos Santos, EA., Ferreira, L.R., Costa, M.D., dos Santos, J.B., Silva, M.de.C.S.da, and Aspiazu, I. (2012) The effects of soil fumigation on the growth and mineral nutrition of weeds and crops. *Acta Sci Agron* **34:** 207–212.

Sarwar, M., Arshad, M., Martens, D.A., and Frankenberger, W.T. (1992) Tryptophan-dependent biosynthesis of auxins in soil. *Plant Soil* **147:** 207–215.

Schirmer, M., Ijaz, U.Z., D'amore, R., Hall, N., Sloan, W.T., and Quince, C. (2015) Insight into biases and sequencing errors for amplicon sequencing with the Illumina MiSeq platform. *Nucleic Acids Res* **43:** e37.

Schlaeppi, K., Dombrowski, N., Oter, R.G., Ver Loren van Themaat, E., and Schulze-Lefert, P. (2014) Quantitative divergence of the bacterial root microbiota in *Arabidopsis thaliana* relatives. *Proc Natl Acad Sci USA* **111:** 585–592.

Schwyn, B., and Neilands, J.B. (1987) Universal chemical assay for the detection and determination of siderophores. *Anal Biochem* **160:** 47–56.

Sturz, A.V., Matheson, B.G., Arsenault, W., Kimpinski, J., and Christie, B.R. (2001) Weeds as a source of plant growth promoting rhizobacteria in agricultural soils. *Can J Microbiol* **47:** 1013–1024.

Takos, A., Lai, D., Mikkelsen, L., Abou Hachem, M., Shelton, D., Motawia, M.S., *et al*. (2010) Genetic screening identifies cyanogenesis-deficient mutants of *Lotus japonicus* and reveals enzymatic specificity in hydroxynitrile glucoside metabolism. *Plant Cell* **22:** 1605–1619.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* **30:** 2725–2729.

Trognitz, F., Hackl, E., Widhalm, S., and Sessitsch, A. (2016) The role of plant-microbiome interactions in weed establishment and control. *FEMS Microbiol Ecol*. Doi: 10.1093/femsec/fiw138

Wang, Q., Garrity, G.M., Tiedje, J.M., and Cole, J.R. (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* **73:** 5261–5267.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. (1991) 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173:** 697–703.

Wickham, H., and Chang, W. (2016) *W. ggplot2: An Implementation of the Grammar of Graphics*. Available at https://cran.r-project.org/web/packages/ggplot2/index.html.

Winston, M.E., Hampton-Marcell, J., Zarraonaindia, I., Owens, S.M., Moreau, C.S., Gilbert, J.A., *et al.* (2014) Understanding cultivar-specificity and soil determinants of the *Cannabis* microbiome. *PLoS One* **9:** e99641.

Yeoh, Y.K., Paungfoo-Lonhienne, C., Dennis, P.G., Robinson, N., Ragan, M.A., Schmidt, S., and Hugenholtz, P. (2015) The core root microbiome of sugarcanes cultivated under varying nitrogen fertilizer application. *Environ Microbiol* **18:** 1338–1351.

Zarraonaindia, I., Owens, S.M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., *et al.* (2015) The soil microbiome influences grapevine-associated microbiota. *mbio* **6:** e02527–14.

Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. (2014) PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* **30:** 614–620.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Rhizosphere and root bacterial community composition at the genus level of grapevine and weeds representing OTUs showing more than 1% relative abundance of all reads. Data labels show the number of OTUs in the representative genera.

Fig. S2. PCoA on the 16S rRNA gene V5–V6 regions split into the rhizosphere and root plant compartment and coloured by plant grouping factor.

Fig. S3. Bar graph shows shared OTUs at family level with relative abundance $\geq$1%. Permutation ANOVA was used to test the effect of 'plant species' on the abundance of the shared taxa. Significant values are indicated as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (p value adjustment method: fdr).

Fig. S4. A Neighbor-joining (NJ) tree of plant-specific OTUs based on 16S rRNA gene sequences using MEGA 6.0 with 1000 bootstraps, annotated and coloured according to the plant species using iTOL. The bar graph indicates percent abundance of OTUs in the respective plant. OTUs showing $\geq$1% abundance in respective plant are indicated in bold letters.

Fig. S5. Phylogenetic analysis of 500 bacteria isolated from roots and rhizosphere of *L. draba* and *Vitis* spp. based on 16S rRNA sequences: A Neighbor-joining (NJ) tree was generated based on 800-bp 16S rRNA sequences using MEGA 6.0 with bootstrap testing 1000 replicates and annotated using iTOL. Bacterial taxa are labelled at major branch, isolates are coloured according to the source of isolation and coloured bars indicate functional traits associate with isolates.

Fig. S6. Shared and unique culturable isolates among plants and compartments **(A)** genus, **(B)** OTU (OTUs were clustered at 97% identity).

Table S1. Characteristics of target plants and plant development stages at the time of sampling.

Table S2. Linear discriminant analysis (ANOVA p-value: 0.01, Wilcoxon p-value: 0.05, LDA $>$ 2) Effect Size (LEfSe) of bacterial OTUs in rhizosphere and root compartment of five plant species (Supplementary data 2_Table_S2A-S2B).

Table S3. Plant-specific OTUs in the rhizosphere and root compartment of target plants (Supplementary data 2_Table_S3A-S3B).

Table S4. Soil physical and chemical properties.