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Diversity analysis and function prediction of rhizo- and endophytic bacterial communities of *Senecio vulgaris* L. (Asteraceae) in an invasive range

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Because increasing evidence has confirmed the importance of plant-associated bacteria for plant growth and productivity, it is believed that interactions between bacteria and alien plants play an important role in plant invasions. However, the diversity of bacterial communities associated with invasive plants is poorly understood. Therefore, we investigated the diversity of rhizo- and endophytic bacteria associated with the invasive annual plant *Senecio vulgaris* L (Asteraceae) based on bacterial 16S rRNA gene data obtained from 57 samples of four *S. vulgaris* populations in a subtropical mountainous area in central China. Significant differences in diversity were observed between plant compartments. Rhizosphere harbored much more bacterial OTUs and showed higher alpha diversity than the leaf and root endosphere. Bacterial community composition differed substantially between compartments and locations in relative abundance profiles, especially at phyla and family level. However, the top five phyla (Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria and Acidobacteria) comprised more than 90% of abundance in all the bacterial communities. And similar endophytic communities with a shared core set of bacteria were observed from different *S. vulgaris* populations. According to the function prediction based on the identification and abundance information of the OTU, bacteria characterized as plant pathogens, as well as those involved in ureolysis and nitrate reduction, were rich in endophytic communities. This study reveals the microbiomes and their putative function in the invasive *S. vulgaris* plants and is also the first step for future studies on the role of interactions between bacteria and alien plants in plant invasions.

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2 # Diversity analysis and function prediction of rhizo- and endophytic bacterial communities of *Senecio vulgaris* L. (Asteraceae) in an invasive range

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21 **Abstract**

22 Because increasing evidence has confirmed the importance of plant-associated bacteria for plant
23 growth and productivity, it is believed that interactions between bacteria and alien plants play an
24 important role in plant invasions. However, the diversity of bacterial communities associated
25 with invasive plants is poorly understood. Therefore, we investigated the diversity of rhizo- and
26 endophytic bacteria associated with the invasive annual plant *Senecio vulgaris* L (Asteraceae)
27 based on bacterial 16S rRNA gene data obtained from 57 samples of four *S. vulgaris* populations
28 in a subtropical mountainous area in central China.

29 Significant differences in diversity were observed between plant compartments：rhizosphere
30 harbored much more bacterial OTUs and showed higher alpha diversity than the leaf and root
31 endosphere. Bacterial community composition differed substantially between compartments and
32 populations in relative abundance profiles, especially at phyla and family level. However, the top
33 five phyla (Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria and Acidobacteria)
34 comprised more than 90% of abundance in all the bacterial communities. And similar endophytic
35 communities with a shared core set of bacteria were observed from different *S. vulgaris*
36 populations. According to the function prediction based on the identification and abundance
37 information of the OTU, bacteria characterized as plant pathogens, as well as those involved in
38 ureolysis and nitrate reduction, were rich in endophytic communities.

39 This study reveals the bacteria and their putative function in the invasive *S. vulgaris* plants and is
40 also the first step for future studies on the role of interactions between bacteria and alien plants in
41 plant invasions.

42 **Key words: invasive plant, bacterial community, plant-microbe interactions, endophytic**
43 **bacteria, 16S rRNA gene**

44 **Introduction**

45 With the development of globalization, the spread and outbreak of invasive species is occurring
46 more frequently. Invasive plants can displace native species, destroy the structure and function of
47 local plant communities, and further influence various animals or microbes inhabiting local
48 communities, leading to decreased local or regional biodiversity and ultimately, unbalanced local
49 ecosystems and loss of ecological function (Pysek et al., 2010; Blackburn et al., 2011). People
50 worry that constant expansion of invasive plants reduces the uniqueness of local flora and even
51 leads to global homogenization of species composition (Orians & Ward, 2010). To
52 fundamentally control exotic plant invasion, it is essential to understand the mechanism of exotic
53 plant invasion; accordingly, this topic has become one of the core studies of invasion ecology.

54 Some studies have shown that plants already have genetic characteristics in favor of invasion,
55 known as the preadaptation hypothesis, which was supported by the observation of more biomass
56 and higher root-stem ratios when compared with non-invasive plant species in the same genus
57 under the same conditions (Van Kleunen et al., 2011). Hypotheses such as the Natural Enemies
58 Release Hypothesis (ERH) (Keane & Crawley, 2002), Evolution of Increased Competition
59 Ability (EICA) (Blossey & Notzold, 1995), Shifting Defense Hypothesis (Müller-Schärer et al.,
60 2004; Joshi & Vrieling, 2005) and New Weapon Hypothesis (Callaway et al., 2008) explain the
61 invasion mechanism based on the relationship between plants and aspects of their biotic
62 environments, such as natural enemies or competitors.

63 However, plants can also form mutualistic symbiotic relationships with other organisms. Land
64 plants are colonized by microbiota in the rhizosphere, phyllosphere, and endophytic
65 compartment (within the leaves and roots) (Rodriguez et al., 2008; Bulgarelli et al., 2012;
66 Lundberg et al., 2012). It is well known that arbuscular mycorrhizal fungi (AMF) and root
67 nodule bacteria form mutualistic symbioses with plants (Hardoim et al., 2015). Moreover, it was
68 recently recognized that bacteria other than rhizobia are beneficial to plants. Such plant growth-
69 promoting bacteria (PGPB) or plant growth-promoting rhizobacteria (PGPR) can stimulate plant
70 growth, increase yield, reduce pathogen infection, and reduce biotic or abiotic stress without
71 conferring pathogenicity (Compant et al., 2010; Pieterse et al., 2014). PGPR can produce
72 growth-promoting substances such as IAA, GA3, zeatin, and ABA (Perrig et al., 2007). Many
73 nitrogen-fixing bacteria in addition to *Rhizobium* species have been identified from plants (Gaby
74 & Buckley, 2011).

75 Endophytic microbiome, which live within the tissues and organs of plants but do not cause plant
76 infections (Rodriguez et al., 2009). Some PGPB are endophytic microbes that can enhance the
77 tolerance of host plants to stressful environments, promote plant growth and improve plant
78 protection (Bulgarelli et al., 2013). Moreover, unlike PGPR, endophytic PGPB can be
79 propagated to the next generation of plants by seeds (Truyens et al., 2015). Accordingly, it can
80 be inferred that endophytic bacteria can establish long-term symbiotic relationships with host
81 plants and have an evolutionary impact on the adaptation of plant populations.

82 In recent years, several studies have suggested that endophytic bacteria play an important role in
83 plant-invasion mechanisms. *Sorghum halepense*, an invasive plant that thrives on grassland with
84 few nitrogen sources, contains endogenous nitrogen-fixing bacteria, which have improved the
85 availability of resources in the soil (Rout & Chrzanowski, 2009; Rout et al., 2013). The effects of

86 rhizo- and endophytic bacteria on the invasion of exotic plants are species-specific and vary
87 across environmental conditions (Long et al., 2008; Rout & Callaway, 2012; Dai et al., 2016). As
88 people have done for fungal diversity in invasive plants (Shipunov et al., 2008; Mei et al., 2014),
89 it is equally important to explore the diversity of bacteria associated with invasive plants to
90 understand the plant-bacterial interactions that occur in the plant-invasion mechanism,

91 *Senecio vulgari*s (Asteraceae), an annual or biennial herb*,* is treated as a weed in the United
92 Kingdom, Western Europe, North America, Australia and New Zealand (Paul & Ayres, 1987;
93 Müller-Schärer & Frantzen, 1996; Vitousek et al., 1996; Frantzen & Hatcher, 1997; Robinson et
94 al., 2003; Figueroa et al., 2007). *Senecio vulgaris* are small plants with short life cycles and a
95 high self-crossing rate that can produce large numbers of seeds, which can germinate under the
96 right conditions at any time; therefore, its ability to spread is very strong (Robinson et al., 2003).
97 This species was introduced into northeast China in the 19th century, and it is now widely
98 distributed and included in The Checklist of the Invasive Plants in China (Ndihokubwayo et al.,
99 2016; Zhu et al., 2016; Cheng et al., 2017). *Senecio vulgaris* grows well in ambient habitats, such
100 as gardens, lawns and arable land, while it survives in stressful habitats such as roadside areas
101 and waste facilities (Robinson et al., 2003). Bacteria might help *S. vulgaris* resist heavy metals as
102 well as to acquire nitrogen and phosphate in contaminated and oligotrophic environments.

103 In this study, we collected rhizosphere soil and plant samples of *S. vulgaris* populations from
104 four sites in the Shennongjia Forestry District, Hubei Province, China. We made the following
105 hypotheses: (1) plant compartments and sampling locations determine the diversity and function
106 of rhizosphere and endophytic bacterial communities associated with *S. vulgaris* plants; (2)
107 endophytic bacteria communities from different sites share core operational taxonomic units
108 (OTUs); and (3) rhizosphere and endophytic bacteria have the potential to be beneficial to host
109 plants. To test these hypotheses, we examined bacterial communities in the rhizosphere and leaf
110 and root endospheres of *S. vulgaris* populations using Illumina amplicon sequencing targeting
111 the bacterial 16S rRNA gene region and through subsequent analyses. We also explored the
112 functions of the OTUs, especially some of the top core endophytic bacterial OTUs of *S. vulgaris*
113 plants based on the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database (Louca
114 et al., 2016) and by review of previous studies.

115

116 Materials and Methods

117 Sample collection and processing

118 We aimed to examine bacterial communities in the rhizosphere, leaf and root endosphere of *S.*
119 *vulgaris* plants in four locations. Five quadrats were set in three locations and four quadrats were
120 set in the fourth location. Thus, nineteen quadrats were set in our experiment. From each quadrat,
121 we collected one rhizosphere, one root and one leaf endosphere sample. In total, we analyzed 57
122 samples.

123 All samples were collected in April of 2016 in Shennongjia Forestry District, Hubei Province
124 (Figure 1). In Shennongjia, the annual temperature is 12°C, annual precipitation ranges from 800
125 to 2500 mm, and the elevation ranges from 398 to 3105 m above sea level. In March and April

126 2016, the daily minimum temperature in Shennongjia was often below 10°C (Figure S1). The
127 vertical vegetation spectrum along sampling sites consisted of mixed deciduous and evergreen
128 broad-leaved forest (1000–1700 m) and deciduous forest (1600–2100 m).

129 We sampled the *S. vulgaris* population in a waste disposal facility and a roadside area. At each
130 sampling point, we set four or five square quadrats with an area of 1 m×1 m. The distance
131 between each quadrat was greater than 5 m. In each quadrat, more than three healthy *S. vulgaris*
132 plants were gently pulled out of the ground, and soil around the roots was shaken off. We then
133 put these plants into a sterile plastic bag, which was subsequently sealed and stored at 4°C until
134 return to the laboratory, at which time the samples were treated immediately. All plants from one
135 quadrat were polled as one sample.

136 We put the roots of *S. vulgaris* from one quadrat into a 50 ml centrifuge tube, after which they
137 were rinsed with sterile water and centrifuged for 5 min at 2000 g. The supernatant was then
138 discarded, while the rhizosphere soil was stored at -80°C until DNA extraction. Healthy and
139 undamaged leaves and roots were randomly selected, washed with ultrapure water, soaked and
140 oscillated for 1 min with 70% alcohol, then washed for 1 or 5 min with 1% sodium hypochlorite
141 solution (leaves for 1 min and roots for 5 min), and finally rinsed 4 times with sterile water.
142 Next, 0.1 mL of the final wash was spread on trypticase soy agar (TSA) plates to check for
143 contamination (Siciliano & Germida, 1999).

144 Plant tissue was macerated with a sterile pestle and mortar with liquid nitrogen and 0.25–0.3 g of
145 finely ground material of soil or plant tissue were used for DNA extraction. We extracted DNA
146 with the MOBIO Power Soil DNA Isolation Kit (MO-BIO, Carlsbad, CA, USA) according to the
147 manufacturer's protocols.

148 **PCR amplification and next-generation sequencing**

149 We used 16S rRNA gene amplicons to determine the diversity of the bacterial communities in
150 each of the samples. For polymerase chain reaction (PCR), we used primers 799F (5'-
151 AACMGGATTAGATACCCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3'), which
152 were designed to specifically amplify the V5, V6, and V7 hypervariable regions of the 16S
153 rRNA gene of bacterial DNA while excluding amplification of chloroplast DNA from plants as
154 suggest in some previous studies (Chelius & Triplett, 2001; Bulgarelli et al., 2012; Bodenhausen
155 et al., 2013; Beckers et al., 2016). PCR reactions were conducted with a Phusion® High-Fidelity
156 PCR Master Mix (New England Biolabs). Briefly, the same volume of 1× loading buffer
157 (contained SYB green) was mixed with PCR products, then electrophoresed on 2% agarose gel
158 for detection. Samples with a bright main strip between 400–450 bp were chosen for further
159 experiments. PCR products mixed in equidensity ratios were purified with a Qiagen Gel
160 Extraction Kit (Qiagen, Germany) and sequencing libraries were generated using a TruSeq®
161 DNA PCR-Free Sample Preparation Kit (Illumina, USA) following the manufacturer's
162 recommendations. In addition, index codes were added to the libraries. The library quality was
163 assessed using a Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100
164 system. Finally, the library was sequenced on an IlluminaHiSeq2500 platform and 250 bp
165 paired-end reads were generated. Sequencing was conducted at Novogene Bioinformatics
166 Technology Co., Ltd. (Beijing, China).

167 **Sequence data treatment**

168 Paired-end reads were assigned to samples based on their unique barcode, truncated by cutting
169 off the barcode and primer sequence and then merged using FLASH (V1.2.7,
170 http://ccb.jhu.edu/software/FLASH/). Quality filtering of the raw tags was performed under
171 specific filtering conditions to obtain high-quality clean tags according to the QIIME (V1.7.0,
172 http://qiime.org/index.html) quality-controlled process. The tags were compared with those in a
173 reference database (Gold Database, http://drive5.com/uchime/uchime_download.html) using the
174 UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html) to detect
175 chimera sequences, which were removed to yield the effective tags.

176 Sequence analyses were performed with the Uparse software (Uparse v7.0.1001,
177 http://drive5.com/uparse/), and sequences with ≥97% similarity were assigned to the same OTU.
178 Representative sequences for each OTU were then screened for further annotation. For each
179 representative sequence, the GreenGene Database (http://greengenes.lbl.gov/cgi-bin/nph-
180 index.cgi) was employed based on the RDP classifier (Version 2.2,
181 http://sourceforge.net/projects/rdp-classifier/) algorithm to annotate taxonomic information.

182 To investigate the phylogenetic relationships of different OTUs and the differences in the
183 dominant species among samples (groups), multiple sequence alignment was conducted using
184 the MUSCLE software (Version 3.8.31, http://www.drive5.com/muscle/). OTUs abundance
185 information were normalized using a standard sequence number corresponding to the sample
186 with the lowest number of sequences.

187 **Selection of core bacterial OTUs in the endosphere**

188 The core OTUs were manually selected based on the average relative abundance and the relative
189 frequency of each OTU per compartment. We first ranked the OTUs from highest relative
190 abundance to lowest, then selected a certain number of top OTUs that collectively comprised
191 about 80% of the total abundance of the bacterial community. This is similar to the Pareto
192 concept (the 80–20 rule) applied in microbiological community analysis as suggested by Werner
193 et al. (2011). After their identification, we plotted average relative abundance and frequency of
194 the core OTUs across each sample type.

195 **Bacterial function prediction**

196 Based on the identification and abundance information of the OTU, we predicted its
197 metabolically and ecologically relevant functions using the FAPROTAX database and quantified
198 every functional groups (Louca et al., 2016). We then illustrated the metabolic structure of the
199 bacterial communities using a heatmap based on the standard and average data of the relative
200 abundance of OTUs associated with each function group annotated by FAPROTAX for each of
201 the 12 sampling groups (3 plant compartments × 4 sampling locations).

202 **Statistical analyses**

203 Analyses of alpha and beta diversity were performed based on the output normalized data. We
204 calculated the Shannon diversity (H') index using the BiodiversityR, while Venn diagrams were

205 plotted with the 'venn.diagram' function of the VennDiagram package. Differences in the
206 bacterial alpha diversities between compartments and locations were compared by two-way
207 ANOVA using the 'aov' function. Multiple comparisons of means between compartments were
208 accomplished using Tukey Contrasts. Nonmetric multidimensional scaling (NMDS) was
209 performed using the 'Mass' and 'vegan' packages. Permutational ANOVAs (PERMANOVAs)
210 were conducted with the 'adonis' function in the 'vegan' package as described by Desgarennes et
211 al. (2014). All analyses were conducted using R v.2.15.2 (R Foundation for Statistical
212 Computing; available at http://www.R-project.org).

213 Results

214 Alpha-diversity of bacterial communities

215 Of 3,046,898 high-quality reads that we obtained, we used the 2,620,319 sequences that
216 remained after removing OTUs not classified as bacteria or matching chloroplasts, mitochondrial
217 or Viridiplantae for further analysis. The average length of the sequences was 375 nt. Because of
218 contamination from chloroplasts, less sequences were obtained from leaf samples than from root
219 and soil samples. However, all samples showed high-coverage (>10,000 usable reads); therefore,
220 we used all samples (Table S1). In total, 554,085 reads were annotated to 34 bacterial phyla,
221 518,579 reads were annotated to 275 bacterial family and 165,219 reads annotated to 246 species
222 (Table S2).

223 The majority of bacterial OTUs identified in the leaf and root endosphere were also present in
224 the rhizosphere. Moreover, 289 OTUs were detected solely in the aboveground tissues, which
225 was a considerably small number and only 6.6% of all identified OTUs. Additionally, only 160
226 and 69 OTUs were exclusively observed in leaves and roots, representing 12.4% and 4.6% of the
227 leaf and root communities (Figure 2a). The percentage of OTUs shared between locations was
228 33%, 17% and 9% for rhizosphere, root and leaf samples, respectively (Figure 2b–d).

229 The levels of microbial diversity differed significantly among compartments. Alpha diversity
230 measured by the Shannon (H') index was affected by compartments, but not by locations.
231 Specifically, H' decreased significantly from the soil to the root and leaf endospheres (Figure 3;
232 Table 1–2).

233 Bacterial community composition

234 Across all samples, we detected a total of 34 distinct bacterial phyla, among which the top ten
235 phyla comprised an average of > 98% bacteria abundance in all samples, and the top five
236 (Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria and Acidobacteria) comprised an
237 average of > 90% of the bacterial abundance (Figure 4). Bacterial community composition
238 differed substantially between compartments and locations in relative abundance profiles at the
239 phylum level (Table 1), and the same pattern was found at the OTU level as well (Figure 5).
240 Samples from different compartments differed from one another in relation to the relative
241 abundance of the five dominant phyla; specifically, rhizosphere bacterial communities were
242 enriched for Acidobacteria; root endosphere samples had lowest abundance of Actinobacteria
243 and leaf endosphere samples had highest abundance of Firmicutes, but depleted levels of

244 Proteobacteria and Bacteroidetes (Figure 4; Table 2).

245 The bacterial community composition differed significantly between compartments at the family
246 level. Rhizosphere bacterial communities had higher abundances of Flavobacteriaceae and
247 Comamonadaceae, while Oxalobacteraceae and Pseudomonadaceae were most abundant in the
248 root endosphere and Caulobacteraceae and Pseudomonadaceae were enriched in the leaf
249 endosphere (Figure 6).

250 **Core bacterial OTUs in root and leaf endospheres**

251 From the 1,284 OTUs in leaf endosphere, we identified 36 OTUs with > 0.70 relative frequency
252 as core OTUs that collectively comprised about 80.28% of the leaf endophytic bacterial
253 communities. The endosphere bacterial communities were dominated by a few bacterial phyla or
254 orders including Alpha-, Beta-, Gammaproteobacteria, Actinobacteria, Firmicutes (Bacilli) and
255 Bacteroidetes (Flavobacteria, Table 3).The top five OTUs in the leaf endosphere were
256 *Brevundimonas diminuta* (Alphaproteobacteria), *Exiguobacterium sibiricum* (Bacilli),
257 *Pseudomonas* sp. (OTU7, Gammaproteobacteria), OTU6 (Alcaligenaceae, Betaproteobacteria),
258 and *Pseudomonas viridiflava* (Gammaproteobacteria, Figure 7a; Table S3).

259 Similarly, from the 1,543 OTUs, we identified 30 OTUs as core root endophytic bacteria, the
260 four most abundant being OTU3 (Oxalobacteraceae, Betaproteobacteria), *Pseudomonas* sp.
261 (OTU7), *Pseudomonas viridiflava* and *Duganella* sp. (OTU15, Betaproteobacteria, Figure 7b;
262 Table S4). With the exception of three OTUs, all core root endophytic bacteria were present with
263 =1.00 relative frequency, and these OTUs collectively comprised about 79.62% of the root
264 endophytic bacterial communities.

265 **Bacterial function prediction**

266 In this study, 63 function groups were represented, indicating that any one of these groups was
267 associated with at least one OTU identified from the samples. Overall, 1,269 of 4,902 OTUs
268 (25.89%) were assigned to at least one function group, while 3,633 (74.11%) could not be
269 assigned to any group. Additionally, several OTUs were assigned to multiple functional groups.

270 We found that the metabolic functional structure of bacterial communities was quite different
271 among samples from different plant compartments. Moreover, samples from the same plant
272 compartments showed similar metabolic functional structures ((Figure 8). Samples from
273 rhizosphere soil were distinct in that they contained abundant OTUs involved in nitrogen
274 metabolic pathways, plastic degradation, and arsenate detoxification (Figure S2b), while root
275 endosphere samples were more closely related to nitrogen and methanol (or methylal) metabolic
276 pathways (Figure S2c). Interestingly, leaf samples differed from others in that they contained
277 OTUs related to animal parasites, plants and human pathogens (Figure S2d).

278 Twenty-eight of the 60 core OTUs were functionally annotated, among which 22 were annotated
279 by FAPROTAX and six according to previous studies. Quite a few OTUs were predicated being
280 associated with the ability to reduce nitrate and ureolysis, while a few were classified as plant or
281 human pathogens, and two might have been able to conduct methanol oxidation (Table S5-6).

282 **Discussion**

283 **Difference between plant compartments and sampling locations**

284 We determined that bacterial communities associated with *S. vulgaris* were primarily influenced
285 by plant compartments, where the alpha diversity was significantly decreased in the root and leaf
286 endospheres compared with the rhizosphere soil (Figure 3; Table 1–2). These findings were
287 consistent with observations from many plants such as *Agave* species (Coleman-Derr et al.,
288 2015), rice (Edwards et al., 2015) and poplar trees (Beckers et al., 2016). Our study and others
289 provided evidence that soil is a potential reservoir for endophytic bacteria. Microbial diversity
290 declines sequentially from the rhizosphere to roots and leaves, which suggests increasingly
291 stronger competition among microorganisms as the habitat becomes more tightly defined (Müller
292 et al., 2016).

293 The rhizosphere bacteria and those in the root and leaf endospheres were clearly distinct from
294 one another. Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes dominated the
295 rhizosphere and endosphere of *S. vulgaris* plants. However, the relative abundance of
296 Proteobacteria and Firmicutes increased, while that of Acidobacteria decreased from the
297 rhizosphere to the endosphere. These findings are consistent with observations from other plants
298 such as rice (Edwards et al., 2015), maize (Liu et al., 2017), grapes (Zarraonaindia et al., 2015),
299 agave (Coleman-Derr et al., 2015), *Brassica stricta* (Wagner et al., 2016), *Oxyria digyna*, and
300 *Saxifraga oppositifolia* (Kumar et al., 2017). Taken together, these results indicate that there may
301 be some factors that shape the structure of endophytic bacteria acting in different environments
302 and host species. Bulgarelli et al. (2012) suggested that such factors included the
303 physicochemical properties of plant cell walls and metabolites from active plant cells. Moreover,
304 Bulgarelli et al. (2013) put forward a two-step selection model in which rhizodeposition and
305 convergent host genotype-dependent selection drives the community shift in the rhizosphere and
306 endophyte microbiota differentiation. Obviously, this plant selection process can explain the
307 differentiation between the bacteriome in the endosphere and in soil.

308 We also found that bacterial communities associated with *S. vulgaris* were influenced by the
309 sampling locations. This kind of influence lies in the difference between climate and soil
310 physiochemical properties between locations. Moreover, *S. vulgaris* plants often differed
311 between locations, which could also affect bacterial communities. Recent studies have
312 demonstrated that plant host-specific traits, including broad morphological characteristics
313 (Kembel et al., 2011) and specific genetic pathways and gene products (Horton et al., 2014;
314 Lebeis et al., 2015), can have significant effects on microbiome composition and diversity.

315 **Core bacterial OTUs in root and leaf endospheres**

316 When only the profile of the endophytic bacterial OTUs was considered, there were great
317 differences between locations (Figure 2). However, when the abundance of the OTUs was
318 considered, *S. vulgaris* plants from different locations were found to share the same core OTUs
319 in the leaf and root endospheres. These core OTUs accounted for much less than 20% of the total
320 OTUs, but 80% of the abundance of the endophytic bacterial communities. These findings
321 demonstrated that the core endophytic bacteriome was consistent across hosts of the same
322 species grown in different locations, as has been observed in *Arabidopsis* (Bulgarelli et al., 2012;

323 Lundberg et al., 2012), grapes (Samad et al., 2017) and some other plant species (Kumar et al.,
324 2017).

325 The dominating phyla or order, including Alpha-, Beta-, and Gammaproteobacteria,
326 Actinobacteria, Firmicutes (Bacilli) and Bacteroidetes (Flavobacteria) also tend to dominate the
327 endophytic bacteriomes of other plants reviewed by Hardoim et al.(2015), Müller et al. (2016)
328 and Finkel et al. (2017).

329 The core OTUs in leaves belonged to 19 families (Table S3), while those in roots belonged to 10
330 families (Table S4). We compared these dominant families with those reported in previous
331 studies. Dominant families in *S. vulgaris* roots substantially overlapped with those reported as a
332 core set of *Arabidopsis thaliana*, *Salicornia europaea* and *Helianthus annuus*: Oxalobacteraceae
333 and Flavobacteriaceae were found as core members of the root microbiome in six studies, while
334 Comamonadaceae were observed as core taxa of the root microbiome in seven different studies.
335 In relation to leaf endophytic bacteria, *A. thaliana* shared the abundant leaf taxa at the family
336 level, while *Sequoia sempervirens* and *Sequoia dendrongiganteum* shared few leaf taxa with *S.*
337 *vulgaris* (Table 4). The comparison indicated that although the host effect on the structure of
338 endophytic bacteria communities was strong, taxa similarity could be observed at the phylum,
339 order or even the family level.

340 In leaf and root bacterial communities of *S. vulgaris*, there were several dominant genera;
341 namely, *Brevundimonas*, *Pseudomonas*, *Exiguobacterium*, *Sphingomonas*, *Flavobacterium*,
342 *Rhizobium*, *Massilia*, and *Duganella*. Among these, *Pseudomonas* and *Rhizobium* have been
343 thoroughly investigated as plant-associated genera. *Pseudomonas* are known to occupy
344 numerous ecological niches, including the rhizospheres and endospheres of many plants. For
345 instance, 21 *Pseudomonas* strains were isolated from the roots of *Populus deltoides* (Jun et al.,
346 2015), and 12 *Pseudomonas* strains showed promising growth-promoting effects when applied to
347 lettuce in the field (Cipriano et al., 2016). *Massilia* and *Duganella* are Burkholderiales, which are
348 well known for their biodegradative capacities and antagonistic properties toward multiple soil-
349 borne fungal pathogens (Benítez & Gardener, 2009; Chebotar et al., 2015). Finally, the genus
350 *Flavobacterium* comprises a significant fraction of endophytic microbiomes in a broad range of
351 plant species, indicating a specialized capacity to proliferate in plant environments and
352 suggesting a role in plant function (Kolton et al., 2016).

353 We also identified some cold-resistant bacteria as core bacterial OTUs in root and leaf
354 endospheres of *S. vulgaris*. These bacteria included *Sphingomonas aerolata*, *Sphingomonasfaeni*,
355 *Exiguobacterium sibiricum* and OTU 3. Isolates of two *Sphingomonas* species (*S. aerolata* and *S.*
356 *faeni*) showed psychrotolerant traits (Busse et al., 2003). *Exiguobacterium sibiricum* is one of 14
357 known *Exiguobacterium* spp. (Vishnivetskaya et al., 2009). Strains of this species isolated from
358 the Siberian permafrost could grow well at low temperature (e.g., 4°C) and had remarkable
359 tolerance to repeated freeze-thawing cycles (Vishnivetskaya et al., 2007). OTU3
360 (Oxalobacteraceae), which may have been from members of the *Duganella*, *Rugamonas* or
361 *Janthinobacterium* genus, was highly abundant in root samples (Figure 7b; Table S4).
362 *Janthinobacterium lividum* was observed in the endosphere of two native perennial plants,
363 *Oxyria digyna* and *Saxifraga oppositifolia*, in three Arcto-Alpine regions (Kumar et al., 2017).
364 *Janthinobacterium* spp. were reported to be thriving in extreme cold, dry, and high solar
365 ultraviolet (UV) radiation environments and to manifest strong antimicrobial activity (Koo et al.,

366 2016)(and references inthere). When our plants were collected in April of 2016, in Shennogjia,
367 we found that *S. vulgaris* was one of the weeds that emerges in early spring, and that the daily
368 minimum temperature was often below 10°C (Figure S1). Therefore, it is not surprising that the
369 cold-resistant bacteria are present in the endosphere of *S. vulgaris* plants in this region, and it is
370 possible that they could facilitate host growth under cold conditions.

371 **Bacterial function prediction**

372 Corresponding to the structural differences between plant compartments, bacterial communities
373 from different compartments also differed relative to functional grouping. This functional
374 fraction based on the plant microenvironment has also been observed in other plants, including
375 *Espeletia* species in an Andean high-mountain ecosystem (Ruiz-Pérez et al., 2016). Similar to
376 PICRUST (Langille et al., 2013) and Geochip (He et al., 2010), FAPROTAX classifies bacterial
377 function based on metabolomic traits. Moreover, FAPROTAX adds annotations according to the
378 ecological relationship between bacteria and eukaryotes (plants, animal and humans). Thus, we
379 may see that more human, animal and plant pathogens were harbored in the *S. vulgaris* leaf
380 endosphere than that in the other compartments (Fig 7, Table S5–6). However, care should be
381 taken when drawing this conclusion because the properties of pathogenicity may depend on
382 many factors, including plant and microbial genotype, microbial numbers, and quorum sensing
383 or environmental conditions (Hardoim et al., 2015).

384 There were abundant OTUs involved in nitrogen metabolic pathways, including ureolysis and
385 nitrate reduction. Six endophytic bacteria belonging to four genera (*Pseudomonas,*
386 *Flavobacterium, Rhizobium* and *Xanthomonas*) isolated from burley tobacco had strong abilities
387 to reduce nitrate and nitrite, and they are also observed in the *S. vulgaris* endospheres. These
388 endophytic bacteria can be used to reduce tobacco-specific nitrosamines (TSNA), which are
389 carcinogens found in the tobacco plant (Zhu et al., 2004). The six endophytic bacteria may have
390 a close affinity to bacteria involved in nitrogen metabolic pathways, and we may isolate
391 endophytic bacteria from these four genera and investigate whether they were related to nitrogen
392 metabolic pathways in future studies.

393 The FAPROTAX annotates the dominant endophytes *B. diminuta* and *R. leguminosarum* as plant
394 pathogens; however, some studies offer evidence suggesting that they may also be beneficial to
395 host plants. Singh *et al.* (2016) applied *B. diminuta* to rice and found it helped reduce arsenic
396 accumulation, and that it produced IAA to obtain soluble phosphate and promote the growth of
397 rice. Moreover, *R. leguminosarum* biovar. Phaseoli isolated from sludge-treated soil was found
398 to form root nodules in white clover (*Trifolium repens*) (Chaudri et al., 1992; Chaudri et al.,
399 1993). Purchase et al. (1997) found that *R. leguminosarum* were resistant to heavy metals,
400 especially to cadmium, and that they could effectively conduct nitrogen fixation. In addition,
401 Chabot et al. (1996) showed that *R. leguminosarum* promoted the growth of maize and lettuce
402 via phosphate solubilization.

403 When studying the plant bacteriome, it is important to know whether a certain bacterium has
404 plant growth-promoting traits (PGPT), such as the ability to produce indole acetic acid (IAA),
405 hydrogen cyanide, siderophore, and ACC deaminase, the ability to fix nitrogen or solubilize
406 phosphate, and antifungal activity. Because large culture collections are available for controlled
407 experimentation, the function of plant-associated bacteria is becoming more accessible, and it is

408 anticipated that databases focusing on the PGPT diversity of plant bacteria will soon be
409 available.

410 Conclusions

411 Bacterial 16S rRNA gene data obtained from rhizosphere soil and root and leaf endosphere
412 samples in four *S. vulgaris* populations in a subtropical mountainous area revealed significant
413 structural and functional differences between bacterial communities from different plant
414 compartments and populations. However, similar endophytic communities formed from a shared
415 core set of bacteria were acquired, despite a distance of over 100 km and an elevation range of
416 1,200–1,800 m. As expected, we observed heavy metal-resistant, phosphate-solubilizing and
417 nitrogen-fixing bacteria, such as *B. diminuta* and *R. leguminosarum*, in *S. vulgaris* at relatively
418 high abundance. However, the presence of cold-resistant bacteria was unexpected. The presence
419 of these kind of bacteria might be important to the ability of *S. vulgaris* to adapt to harsh
420 environments. Future studies should be conducted to isolate these endophytes in *S. vulgaris*
421 plants and test their function *in vitro* and *in vivo*.

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Figure 1 (on next page)

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The map of four sampling locations in Shennongjia, Hubei Province, China

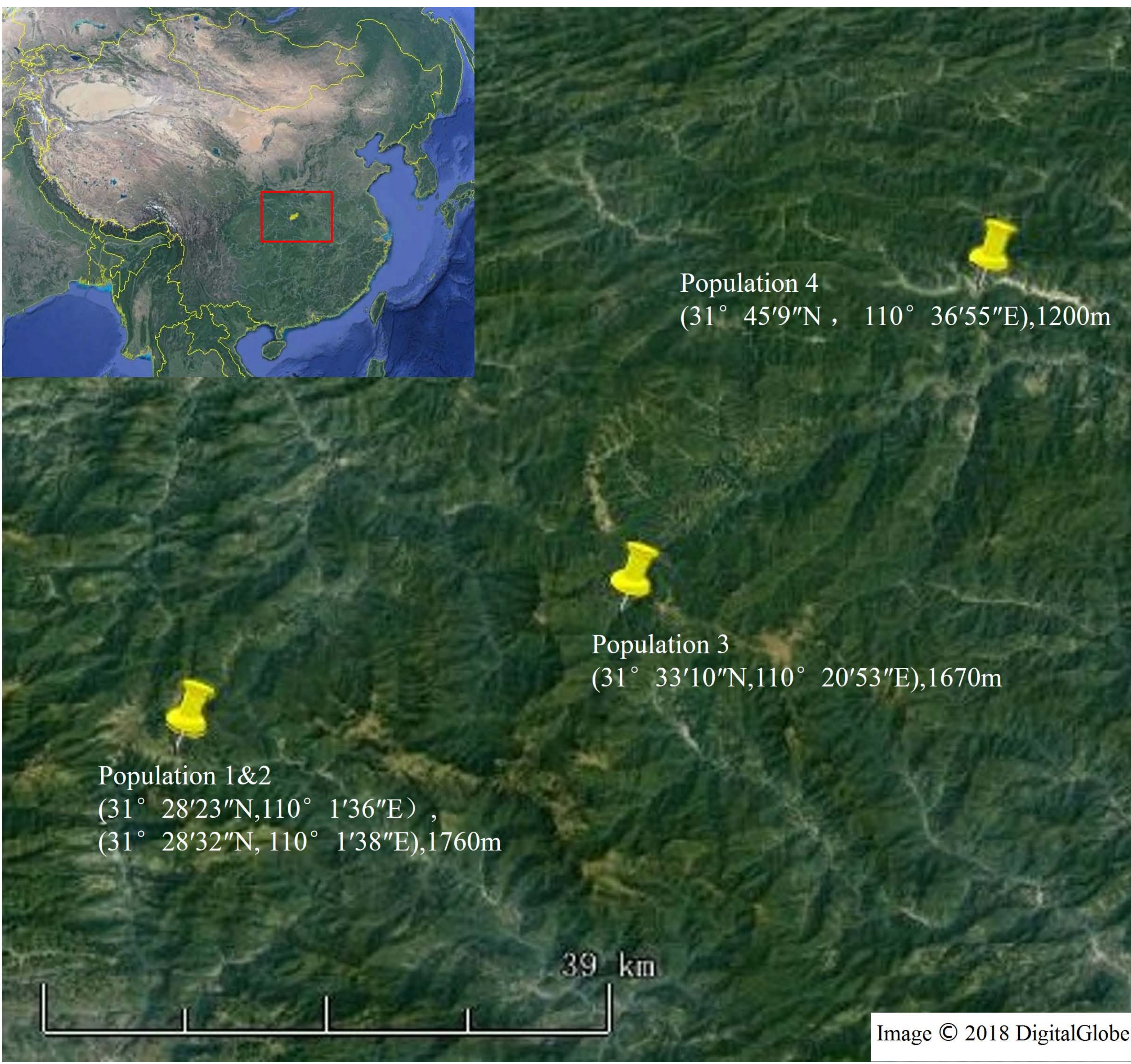
Population 4
(31° 45′9″N， 110° 36′55″E),1200m
Population 3
(31° 33′10″N,110° 20′53″E),1670m
Population 1&2
(31° 28′23″N,110° 1′36″E）,
(31° 28′32″N, 110° 1′38″E),1760m
39 km
Image © 2018 DigitalGlobe

Figure 2
(on next page)

Venn diagrams of shared OTUs (number of OTUs) across three compartments of *Senecio vulgaris* plants and four sampling locations.

L=leaf endosphere, R=root endosphere, RS=rhizosphere; 1–4 represent the four sampling locations.

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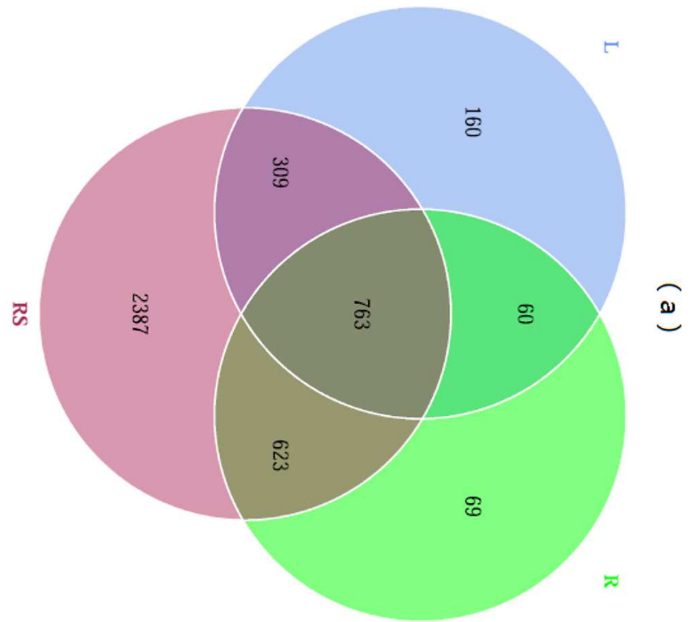

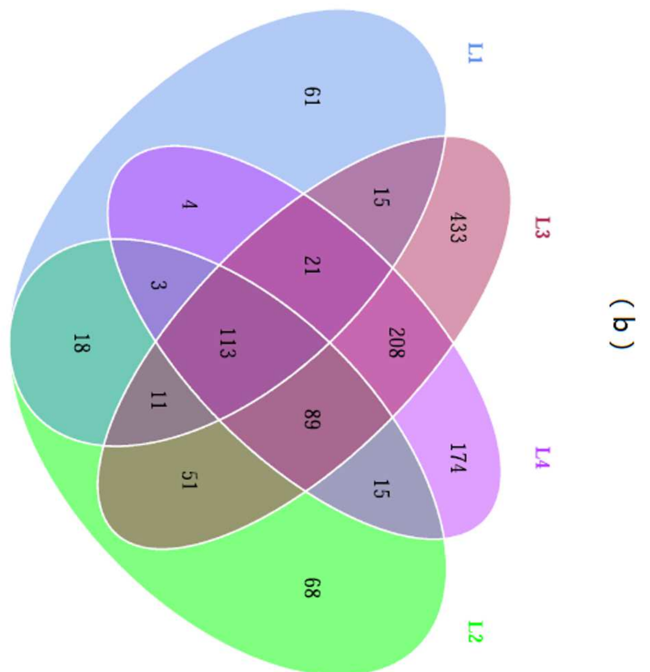

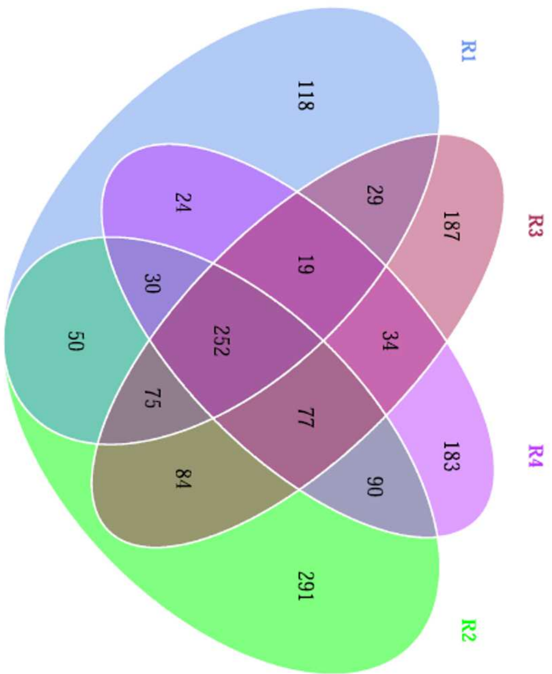

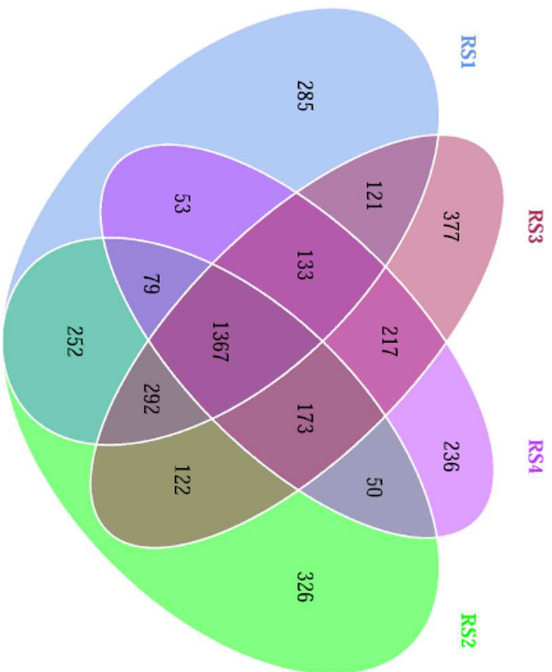

Figure 3 (on next page)

Estimated Shannon index (H') in the bacterial communities of each compartment of *Senecio vulgaris* plants and sampling location

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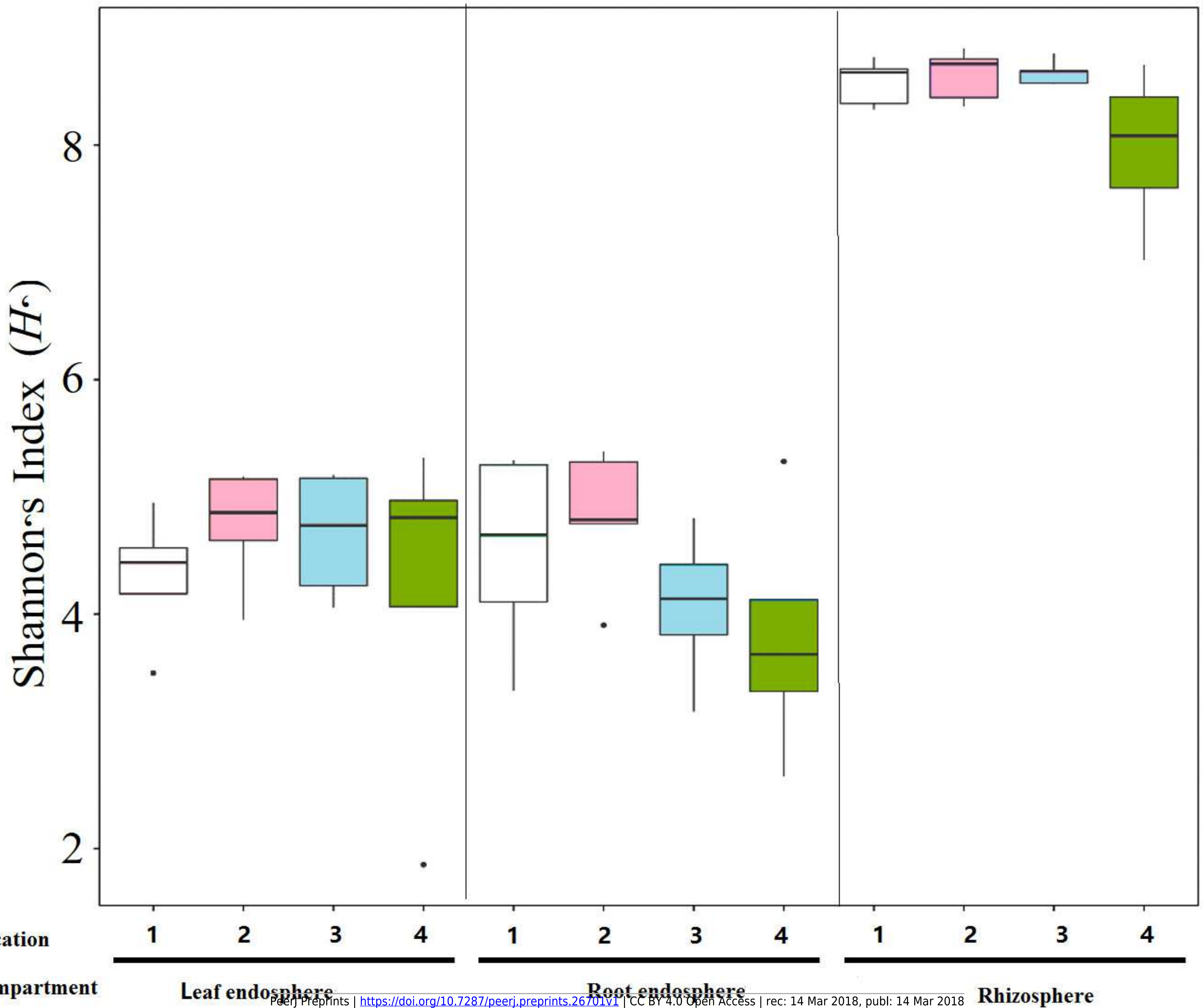

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Figure 4 (on next page)

Phylum-level relative abundance plots of the bacterial communities associated with each compartment of *Senecio vulgaris* plants and sampling location.

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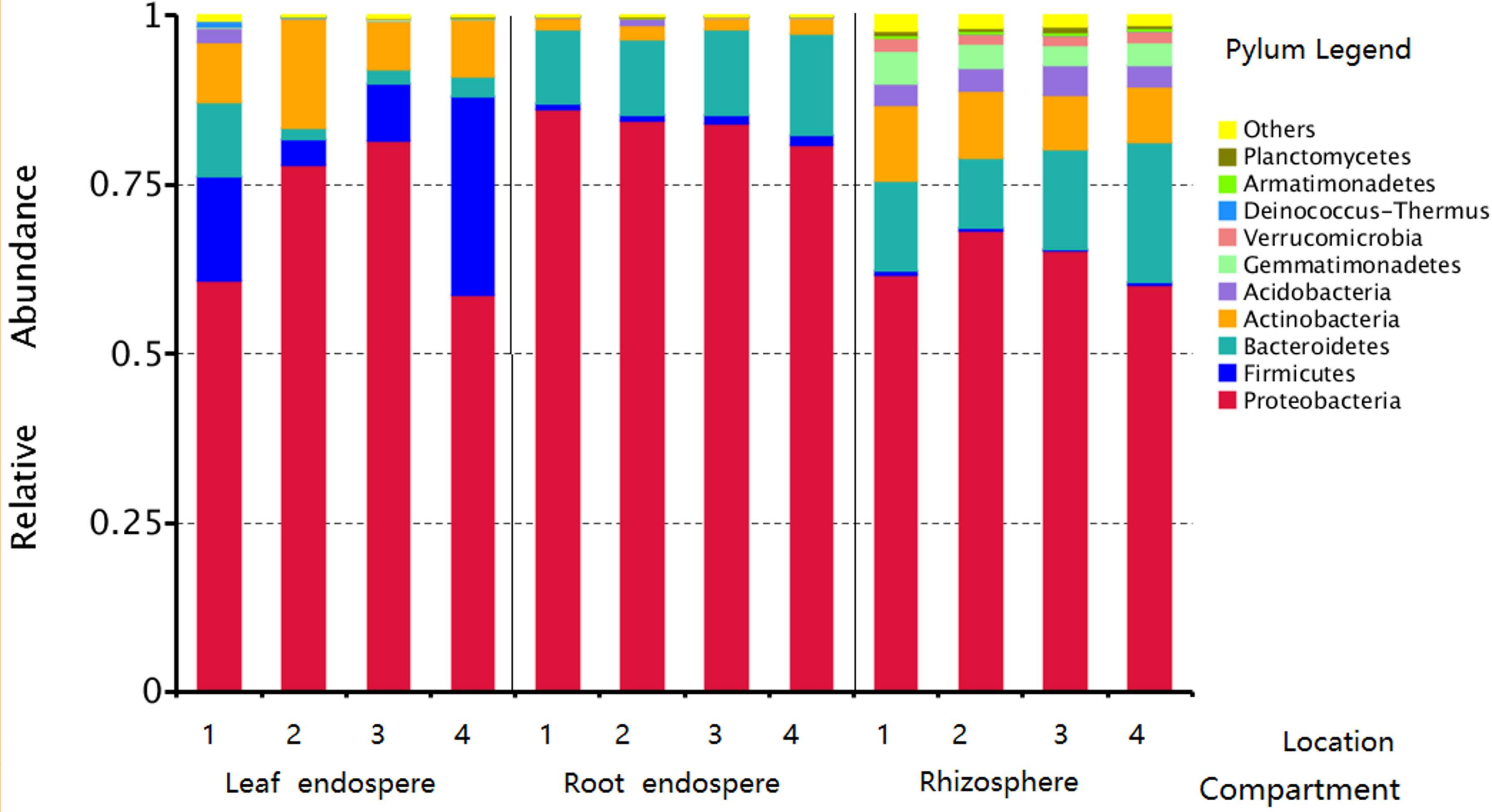

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Figure 5 (on next page)

Nonmetric multidimensional scaling (NMDS) plots for Bray–Curtis distances of the bacterial communities associated with each compartment of *Senecio vulgaris* plants and sampling location.

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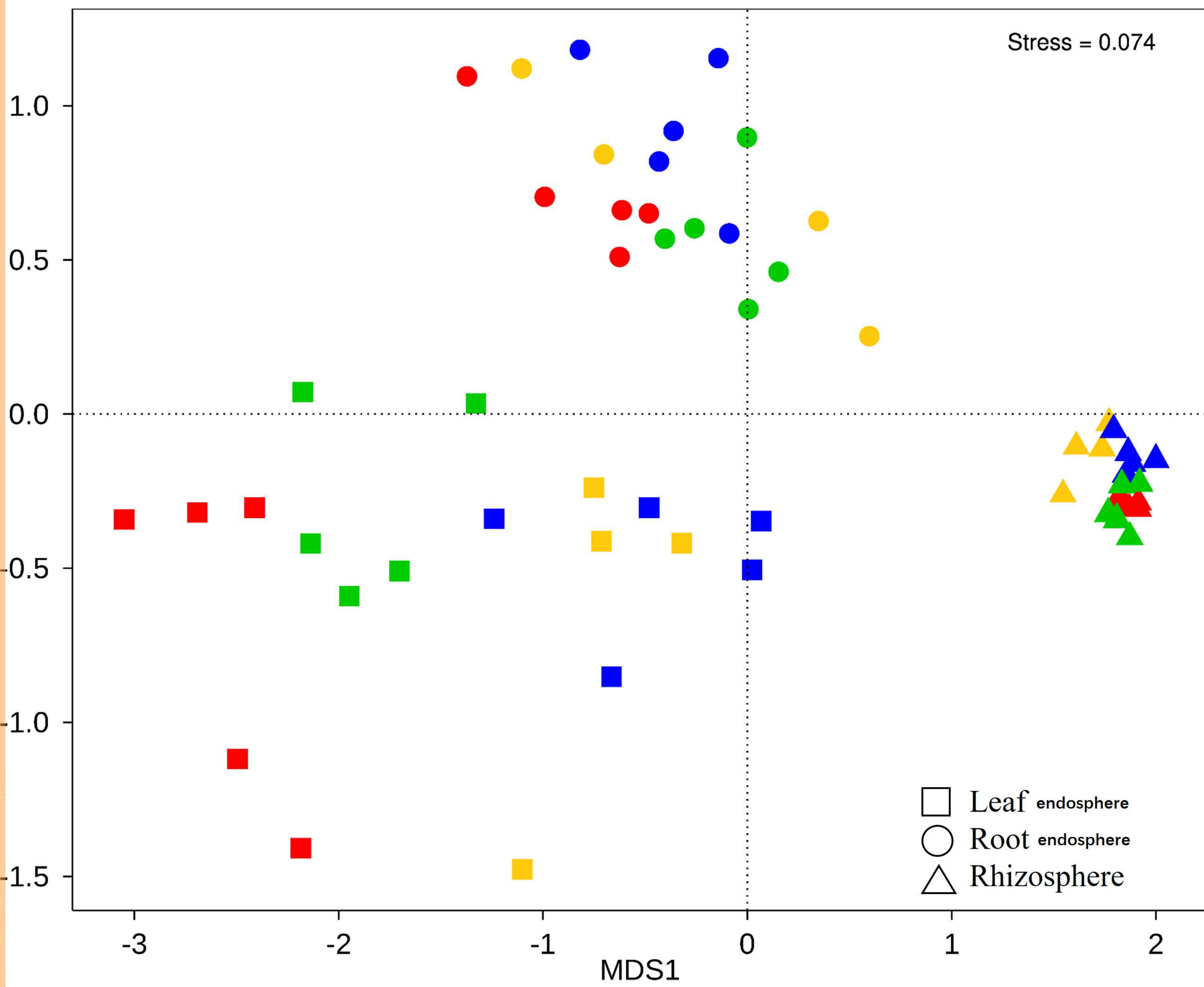

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Figure 6 (on next page)

Family-level relative abundance plots of bacterial communities associated with each compartment of *Senecio vulgaris* plants and sampling locations

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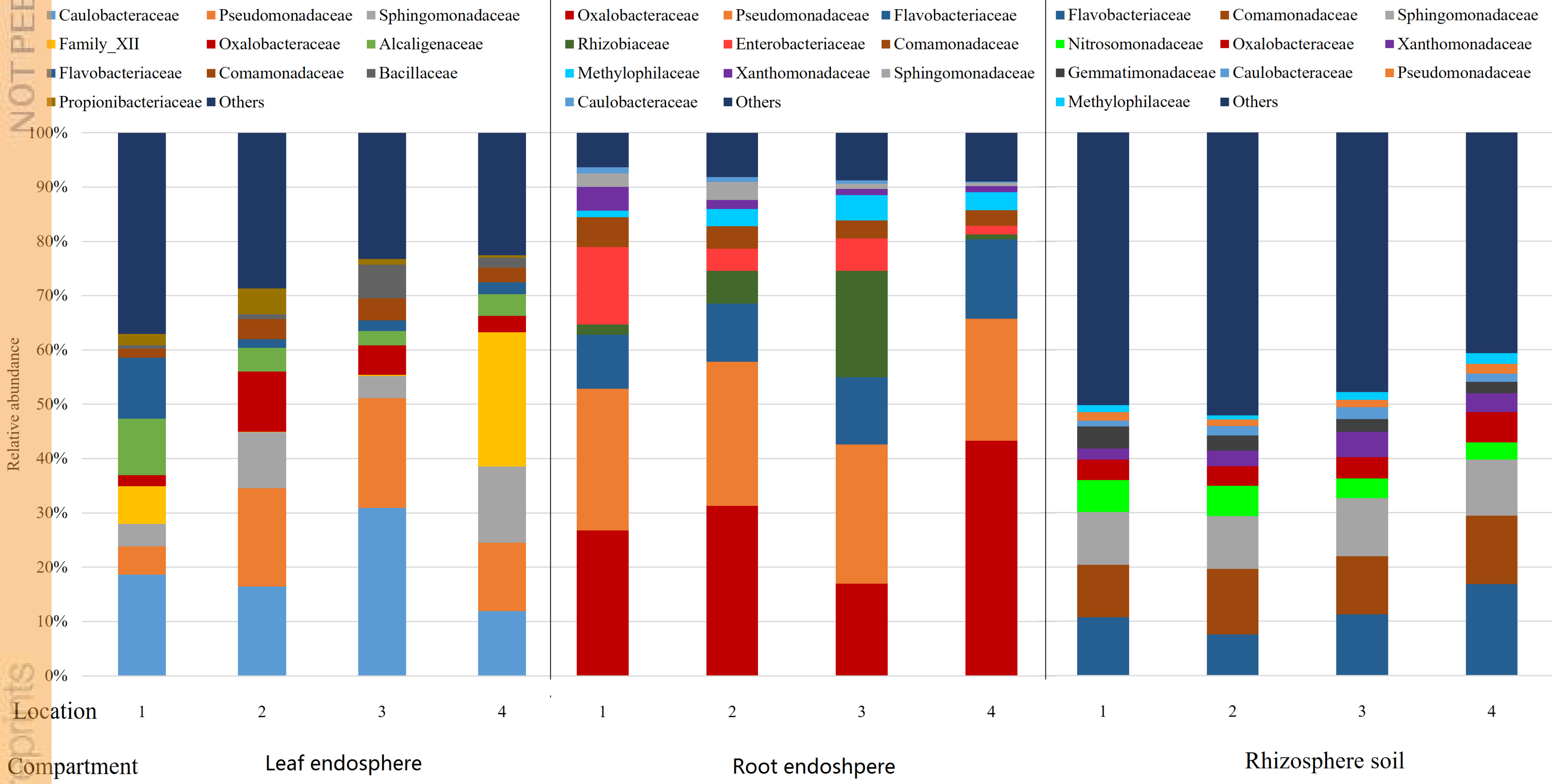

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Figure 7 (on next page)

Relative frequency versus relative abundance of core bacterial operational taxonomic units (OTUs) in the root and leaf endospheres of *Senecio vulgaris* plants

OTUs: 1=Brevundimonas diminuta, 2=Exiguobacterium sibiricum, 3=Pseudomonas spp., 4=an undefined species from Alcaligenaceae, 5=Pseudomonas viridiflava, 6=an undefined species from Oxalobacteraceae, and 7=Duganella spp.

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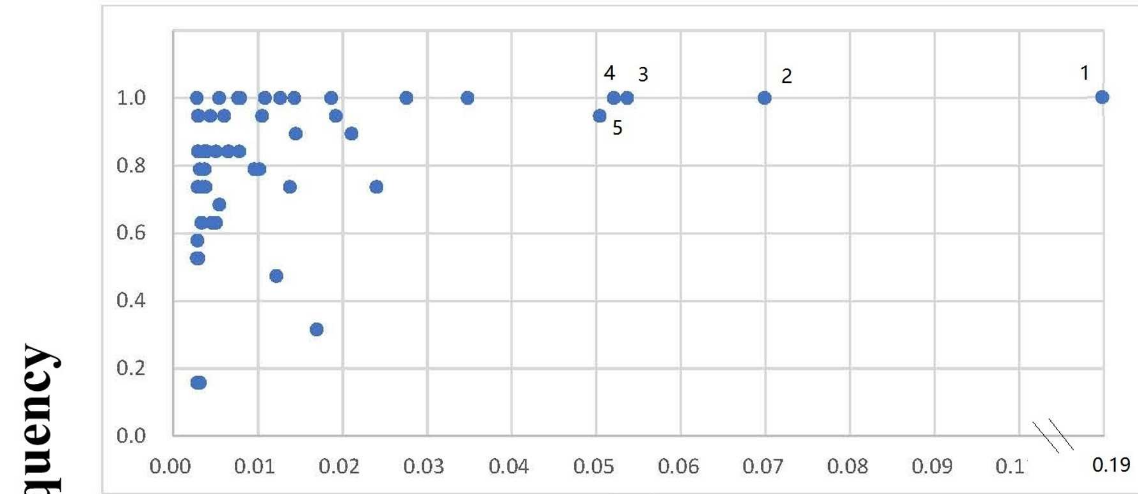
(a) Leaf endosphere



Relative frequency

(b) Root endosphere

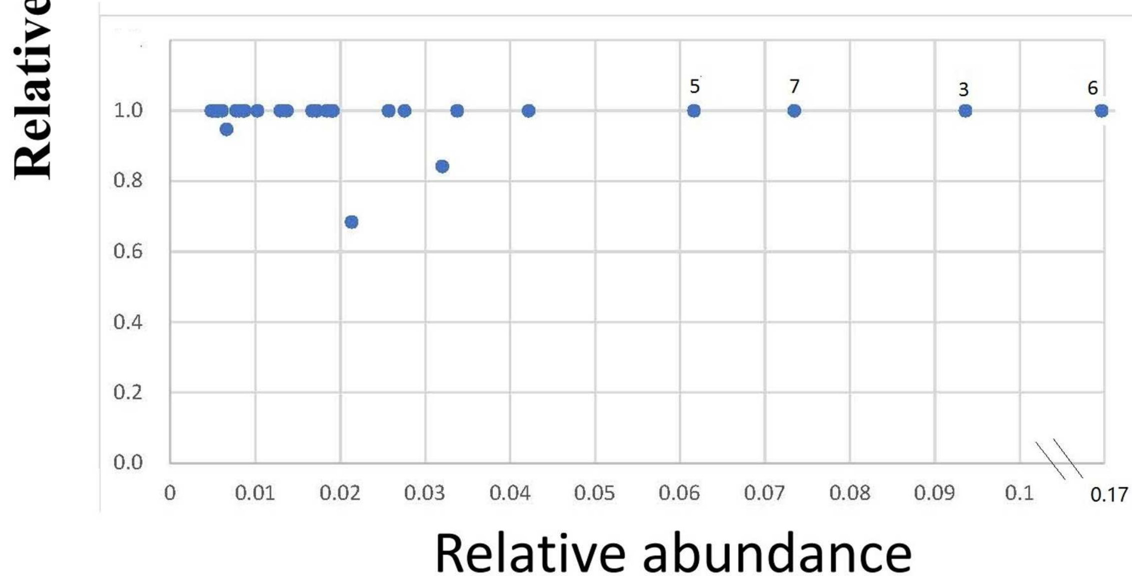


Figure 8(on next page)

Functional community structure of bacterial communities associated with each compartment of *Senecio vulgaris* plants and sampling locations

L = leaf endosphere, R=root endosphere, RS=rhizosphere; 1-4 represent the four sampling locations.

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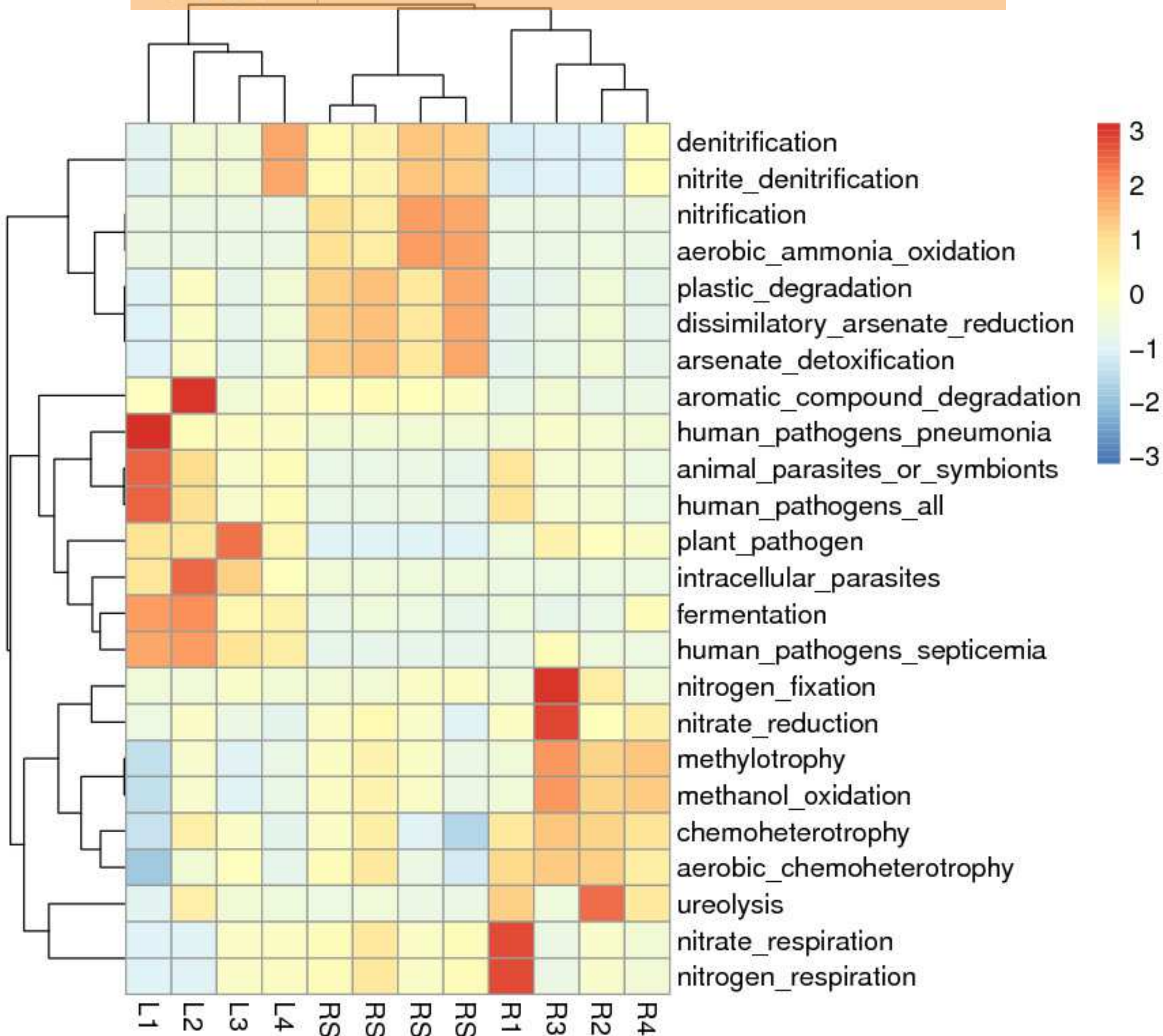

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Table 1 (on next page)

Effect of plant compartments and sampling locations on diversity and structure of bacterial communities in rhizo- and (leaf or root) endosphere of *Senecio vulgaris* plants

***P <0:001; **P <0:01; *P <0:05.

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(a) Two – way ANOVA test (Shannon index as independent variable)				
Factor	Sum.Sq	Df	F.value	P.value
Location	3.65	3	2.47	0.07*
Compartment	206.06	2	209.40	0.001 ***
Location: Compartment	1.47	6	0.50	0.81
Residuals	22.14	45		
(b) Permutational ANOVAs (relative abundance of the top 10 phyla as independent variable)				
Location	0.13	3	2.28	0.016*
Compartment	0.77	2	19.76	0.001***
Location: Compartment	0.20	6	1.74	0.022*
Residual	0.88	45		

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Table 2 (on next page)

Results of the multiple comparisons of diversity and relative abundance of the top five phyla of bacterial community from different compartments of *Senecio vulgaris* plants

$^{***}P < 0{:}001$; $^{**}P < 0{:}01$; $^{*}P < 0{:}05$

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(a) Shannon index			
Comparison pair	Estimate	Std. Error	t. value
Root endosphere-Leaf endosphere	-0.17	0.23	-0.72
Rhizosphere -Leaf endosphere	3.95	0.23	17.12 ***
Rhizosphere - Root endosphere	4.11	0.23	17.85 ***
(b) Acidobacteria			
Root endosphere-Leaf endosphere	0.00	0.00	0.67
Rhizosphere -Leaf endosphere	0.03	0.00	11.78 ***
Rhizosphere - Root endosphere	0.03	0.00	11.26 ***
(c) Actinobacteria			
Root endosphere-Leaf endosphere	-0.07	0.01	-5.71***
Rhizosphere -Leaf endosphere	0.01	0.01	0.43
Rhizosphere - Root endosphere	0.07	0.01	6.23***
(d) Bacteroidetes			
Root endosphere-Leaf endosphere	0.09	0.02	5.18***
Rhizosphere -Leaf endosphere	0.09	0.02	5.46 ***
Rhizosphere - Root endosphere	0.01	0.02	0.35
(e) Firmicutes			
Root endosphere-Leaf endosphere	-0.12	0.03	-3.55**
Rhizosphere -Leaf endosphere	-0.13	0.03	-3.74**
Rhizosphere - Root endosphere	-0.01	0.03	-0.19
(f) Proteobacteria			
Root endosphere-Leaf endosphere	0.14	0.04	3.41 **
Rhizosphere -Leaf endosphere	-0.06	0.04	-1.57
Rhizosphere - Root endosphere	-0.20	0.04	-4.98***

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Table 3 (on next page)

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The bacterial taxa dominating in the endosphere of *Senecio vulgaris* plants *

*This table summarized the taxa information of the core OTUs in endosphere of *S.vulgaris* plants, details of the core OTUs can be seen in Table S2-3; /=unidentified taxa

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Phylum	Class	Order	Family	Genus
In leaves and roots				
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Chryseobacterium
				Flavobacterium
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	/
		Pseudomonadales	Pseudomonadaceae	Pseudomonas
Only in leaves				
Actinobacteria	/	Corynebacteriales	Corynebacteriaceae	Corynebacterium
			Mycobacteriaceae	Mycobacterium
		Micrococcales	Brevibacteriaceae	Brevibacterium
			Micrococcaceae	Kocuria
		Propionibacteriales	Propionibacteriaceae	Propionibacterium
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus
			Family_XII	Exiguobacterium
			Staphylococcaceae	Staphylococcus
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas
		Rhizobiales	Bradyrhizobiaceae	Bosea
				Bradyrhizobium
			Rhizobiaceae	Ensifer
	Betaproteobacteria	Burkholderiales	Alcaligenaceae	/
			Comamonadaceae	Pelomonas
				Variovorax
			Oxalobacteraceae	/
				Duganella
				Massilia
	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter
Only in roots				
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium
		Sphingomonadales	Sphingomonadaceae	Sphingobium
	Betaproteobacteria	Burkholderiales	Comamonadaceae	Acidovorax
		Methylophilales	Methylophilaceae	Methylophilus
				Methylotenera
	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas

Table 4(on next page)

Dominant bacterial families in the root and leaf endosphere of *Senecio vulgaris* plants reported as core members in previous studies

[a] Dominant family in root endosphere of *S. vulgaris*, [b]Dominant family in leaf endosphere of *S. vulgaris* (Figure 6). √ corresponds to bacterial families present as core members. *Arabidopsis thaliana,* Barely and Rice are based on Müller *et al.* and references inthere (2016); *Vitis* spp*.* based on Samad *et al.* (2017); *Oxyria digyna* and *Saxifraga oppositifolia* based on Kumar *et al.* (2017); *Populus tremula,* and *Populus alba* based on Beckers *et al.* (2016); *Salicornia europaea* based on *Zhao et al.* (2016); *Helianthus annuus* based on Leff *et al.* (2016); *Sequoia sempervirens* and *Sequoia dendrongiganteum* based on Carrell & Frank (2015).

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Famliy	Root endosphere								Leaf endosphere		
	A. thaliana	Barely	Rice	*Vitis* spp.	*O. digyna, S. oppositifolia*	*P. tremula, P. alba*	*S. europaea*	*H. annuus*	*A. thaliana*	*P. tremula, P. alba*	*S. sempervirens, S. giganteum*
Caulobacteraceae[a, b]	√					√		√	√		
Pseudomonadaceae[a, b]	√			√	√		√	√	√	√	
Sphingomonadaceae[a, b]	√					√	√	√	√	√	
Oxalobacteraceae[a, b]	√	√		√	√	√		√	√	√	
Flavobacteriaceae[a, b]	√	√		√	√		√	√	√		
Comamonadaceae[a, b]	√	√	√	√		√	√	√	√		
Rhizobiaceae[a]	√	√	√				√	√	√	√	
Enterobacteriaceae[a]							√	√	√		√
Methylophilaceae[a]	√							√			
Xanthomonadaceae[a]	√						√	√	√		
Alcaligenaceae[b]	√								√	√	
Family_XII[b]											
Bacillaceae[b]	√								√		
Propionibacteriaceae[b]											

2

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