Tree phyllosphere bacterial communities: comparing intra-individual, intraspecific and interspecific variation

Isabelle Laforest-Lapointe, Christian Messier, Steven W. Kembel

Background. Many studies of microbial community structure on tree leaves (the phyllosphere) have assumed that intra-individual variation in phyllosphere microbial community structure is negligible, and use single samples of leaves to represent individual tree and species-level microbiome structure. In this context, our goal was to characterize the relative importance of intra-individual, intra-specific and interspecific variation of phyllosphere epiphytic bacterial communities in a natural temperate forest in Quebec, Canada.

Methods. We targeted five dominant tree species including angiosperms and gymnosperms: *Acer saccharum*, *Acer rubrum*, *Betula papyrifera*, *Abies balsamea* and *Picea glauca*. For randomly selected trees of each species, we sampled microbial communities at six distinct canopy locations: bottom-canopy (1-2m height), the four cardinal points of mid-canopy (2-4m height), and the top-canopy (4-6m height). We also collected bottom-canopy leaves from two additional trees from each species.

Results. Based on analysis of bacterial community structure measured via sequencing of the bacterial 16S gene, we demonstrate that host species identity is the main driver (R^2=65%) of leaf bacterial community structure while the effect of canopy location is not significant. However, after accounting for the variation explained by host species identity, canopy location explains 70% of the remaining variation in bacterial community structure.

Discussion. Our results suggest that for interspecific studies of phyllosphere bacteria, individual samples from consistent positions within the tree canopy can be used to accurately quantify interspecific variation in phyllosphere microbiome structure. However, for intraspecific studies, it may be necessary to account for variation in phyllosphere microbiome structure within individual trees and host species.

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The authors declare that the experiment comply with the current laws of the country in which the experiment was performed. The authors declare that they have no conflict of interest.

ABSTRACT

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Key words: Phyllosphere/bacteria/plant-bacteria interaction/microbiome/temperate forest/intra-individual-variation/interspecific-variation.

INTRODUCTION

The phyllosphere microbiome represents the communities of microorganisms including bacteria, archaea, and eukaryotes such as fungi that are associated with plant leaves (Inácio *et al.* 2002; Lindow & Brandl 2003). Phyllosphere microbes influence host fitness through a variety of mechanisms such as plant hormone production and protection from pathogen colonization (Lindow & Leveau 2002; Rasche *et al.* 2006). As a result of their effect on host plant fitness, leaf microorganisms can influence plant population dynamics and community diversity (Clay & Holah 1999; Bradley *et al.* 2008) as well as ecosystem functions including nutrient and carbon cycling (van der Heijden *et al. 2008*; McGuire & Treseder 2010; Allison & Treseder 2011). In order to understand the structure and function of phyllosphere microbial communities, studies typically assume that a single sample of leaves from a plant canopy is representative of the phyllosphere community of the entire tree or the entire host species. In this study our aim was to test this assumption by quantifying the relative importance of intra-individual versus intra-specific and inter-specific variation in the structure of temperate tree phyllosphere communities.

Host genetic factors (Bodenhausen et al. 2014) and taxonomic identity (Redford *et al.* 2010; Kembel *et al.* 2014) are important drivers of phyllosphere bacterial community structure. Most studies of phyllosphere communities across different host species have assumed within-plant and within-species variation in phyllosphere community structure to be negligible, and use a single sample to represent the microbiome of each host plant (but see Redford *et al.* 2010 and Leff *et al.* 2015). In tree phyllosphere studies, samples are usually taken from shade leaves either at the bottom of the canopy or at mid-canopy height near the trunk. However, the technique to sample phyllosphere communities vary between studies, ranging from studies that sampled

leaves from a specific canopy location (i.e. Kembel *et al.* 2014; Kembel & Müller 2014) to taking multiple leaves from around the canopy at the same height (i.e. Redford & Fierer 2009; Redford et al. 2010; Jackson & Denney 2011). However, Leff *et al.* 2015 demonstrated for a single tree species (*Ginkgo biloba*) that there is intra-individual variation in phyllosphere community structure within the canopy of a single tree. The relative importance of this within-individual variation versus inter-individual and inter-specific variation, and the degree to which a sample of leaves from a canopy are representative of the microbiome of an individual or a species, is not well understood.

In the past, many studies of phyllosphere microbes focused on leaf-associated fungal communities because of their pathogenic effects (Suda *et al.* 2009) or because of the importance of beneficial plant-fungi interactions (Osono 2006; Arnold & Lutzoni 2007; Rodriguez & Redman 2007). However, phyllosphere bacterial communities also play key functional roles in terrestrial ecosystems including nitrogen fixation and nitrification (Murty 1983; Papen *et al.* 2002) as well as and methane and methanol degradation (Iguchi *et al.* 2012). Most phyllosphere studies have focused on model plant host species (Bodenhausen *et al.* 2013; Reisberg *et al.* 2013; Maignien *et* al. 2014) or on agricultural plants (Kadivar & Stapleton 2003; Balint-Kurti *et al.* 2010; Knief *et al.* 2012). More recently, with the discovery of the ecological importance of phyllosphere microbes, more effort has been put into exploring the structure of phyllosphere communities of tree species in tropical (Lambais *et al.* 2006, 2014; Kim *et al.* 2012; Kembel *et al.* 2014; Kembel & Müller 2014), temperate (Jumpponen & Jones 2009; Redford & Fierer 2009; Redford *et al.* 2010; Jackson & Denney 2011) and Mediterranean forests (Penuelas *et al.* 2012), along altitudinal gradients (Cordier *et al.* 2012ab), and in deserts (Finkel *et al.* 2011, 2012).

A multitude of factors could influence microbial community structure on leaves within a tree canopy. Leaf position in the canopy defines the degree of exposure to ultraviolet radiation and wind and therefore community structure could change depending on the position of the leaves sampled. Exposure to ultraviolet radiation can increase the diversity of the leaf microbial community (Kadivar & Sapleton 2003) by permitting the presence of phototrophic or light tolerant bacteria in the leaf microbiome. This phenomenon could also be caused by leaf morphological and ecophysiological attributes associated with high light availability (thicker leaves, lower specific leaf area, lower water content, higher total chlorophyll, higher photosynthetic activity rate; Lichtenthaler *et al.* 2007). Increased exposure to wind creates drier conditions that can influence leaf epiphytic bacterial communities by inhibiting the growth of particular groups while favoring other groups better adapted to these conditions (Medina-Martìnez *et. al* 2015). Wind exposure could reduce leaf moisture and induce a stomata closure (Grace *et al.* 1975), which could impact the diffusion of nutrients and reduce the size of microbial aggregates (Leveau & Lindow 2001; Miller *et al.* 2001).

In this study, we aim to (1) compare the intra-individual, intra-specific and interspecific variation of bacterial phyllosphere communities; (2) characterize the composition of epiphytic phyllosphere bacterial communities at different canopy locations for five tree species; and (3) make practical recommendations for the sampling of tree phyllosphere bacterial communities. We hypothesized that (1) the magnitude of intra-individual and intra-specific variation will be smaller than interspecific variation, (2) that leaves from the top of the canopy (light leaves) will harbor distinctive bacterial assemblages with higher diversity than mid-canopy samples because

of the exposure to radiation, increased immigration rates, and ecophysiological and morphological characteristics of light leaves.

MATERIALS AND METHODS

Study Site & Host-Tree Species

The study site is located in a natural temperate forest in Gatineau (45°44'50"N; 75°17'57"W), Quebec, Canada. This site is characterized by a cold and humid continental climate with temperate summer. Five tree species (Table 1) common to temperate forests and dominant in the canopy at this site were sampled to provide representatives of both angiosperms and gymnosperms: *Abies balsamea* (Balsam fir)*, Acer rubrum* (Red maple)*, Acer saccharum* (Sugar maple)*, Betula papyrifera* (Paper birch) and *Picea glauca (*White spruce).

Bacterial community collection

We sampled phyllosphere communities from trees on August 29, 2013. Sampling was carried out one week after the last rainfall event. We defined three strata within the canopy: bottom-canopy (1-2m height), mid-canopy (2-4m height), and top-canopy (4-6m height). For a randomly chosen tree from each species, we clipped 50–100 g of leaves at the four cardinal points at mid-canopy height, plus a single sample at bottom-canopy and top-canopy heights, into sterile roll bags with surface-sterilized shears. We also sampled bottom-canopy leaves from two other randomly chosen trees from each species. For bacterial community collection and amplification we used the protocols described by Kembel *et al.* (2014). We collected microbial communities from the leaf surface by agitating the samples in a diluted Redford buffer solution. We resuspended cells in 500 µL of PowerSoil bead solution (MoBio, Carlsbad, California). We extracted DNA from

146 isolated cells using the PowerSoil kit according to the manufacturer's instructions and stored at -
147 80°C.
148
149 **DNA library preparation and sequencing**
150 We used a two-step PCR approach to prepare amplicon libraries for the high-throughput Illumina
151 sequencing platform. The use of combinatorial primers for paired-end Illumina sequencing of
152 amplicons reduced the number of primers while maintaining the diversity of unique identifiers
153 (Gloor *et al.* 2010). First, we amplified the V5–V6 region of the bacterial 16S rRNA gene using
154 chloroplast-excluding primers in order to eliminate contamination by host plant DNA (16S
155 primers 799F-1115R (Redford *et al.* 2010; Chelius & Triplett 2001)) following protocols
156 described by Kembel et al. (2014). We cleaned the resulting product using MoBio UltraClean
157 PCR cleanup kit. We isolated a ~445-bp fragment by electrophoresis in a 2% agarose gel, and
158 recovered DNA with the MoBio GelSpin kit. We prepared multiplexed 16S libraries by mixing
159 equimolar concentrations of DNA, and sequenced the DNA library using Illumina MiSeq 250-bp
160 paired-end sequencing at Genome Quebec.
161
162 We processed the raw sequence data with PEAR (Zhang *et al.* 2014) and QIIME
163 (Caporaso *et al.* 2010) software to merge paired-end sequences to a single sequence of length of
164 350 bp, eliminate low quality sequences (mean quality score < 30 or with any series of 5 bases
165 with a quality score < 30), and de-multiplex sequences into samples. We eliminated chimeric
166 sequences using the Uclust and Usearch algorithms (Edgar 2010). Then, we binned the
167 remaining sequences into operational taxonomic units (OTUs) at a 97% sequence similarity
168 cutoff using the Uclust algorithm (Edgar 2010) and determined the taxonomic identity of each

169 OTU using the BLAST algorithm (Greengenes reference set) as implemented in QIIME
170 (Caporaso *et al.* 2010). The number of sequences per sample ranged from 6 232 to 50 007. From
171 these 512 819 sequences, we rarefied each sample to 6 000 sequences. We included the resulting
172 180 000 sequences in all subsequent analyses.
173
174 **Statistical analyses**
175 We created a database excluding OTUs represented fewer than 3 times to minimize the presence
176 of spurious OTUs caused by PCR and sequencing errors (Acinas *et al.* 2005). We identified the
177 OTUs that were present on all samples to define the "core microbiome" (Shade & Handelsman,
178 2012). Then we tested for significant associations between bacterial taxa and host species, and
179 canopy location using the Linear Discriminant Analysis Effect Size (LEfSe) algorithm (Segata *et*
180 *al.* 2011). This analysis allows the recognition of significant individual host-microbe associations
181 and evaluates the strength of associations between organisms from different groups (Segata *et al.*
182 2011).
183
184 We performed analyses with the ape (Paradis *et al.* 2004), ggplot2 (Wickham 2009),
185 picante (Kembel *et al.* 2010), and vegan (Oksanen *et al.* 2007) packages in R (R Development
186 Core Team 2013). We quantified the taxonomic and phylogenetic variation in bacterial
187 community structure among samples with respectively the Bray-Curtis dissimilarity and the
188 weighted UniFrac index (an abundance-weighted measure of the phylogenetic differentiation
189 among bacterial communities; Lozupone *et al.* 2006). To illustrate patterns of bacterial
190 community structure, we performed a nonmetric multidimensional scaling (NMDS) ordination of
191 Bray–Curtis dissimilarity and weighted UniFrac distance among all samples. We performed

192 three permutational tests of multivariate homogeneity of group dispersions (Levene's test for
193 variances' homogeneity multivariate equivalent; Anderson 2006, Anderson *et al.* 2006): one to
194 test if variance in intra-individual canopy bacterial communities was equal between species; a
195 second to compare per species intra-individual and inter-individual (intraspecific); and finally a
196 third to test if there was more variation at the intra-individual level than at the interspecific level.
197 We identified relationships between bacterial community structure, host species identity, and
198 sample canopy location by conducting a permutational multivariate analysis of variance
199 (PERMANOVA, Anderson 2001) on the community matrix. To decompose the total variation in
200 the community matrix between the host species identity and canopy location, we performed a
201 partial redundancy analysis (RDA; Legendre & Legendre 1998). This technique measures the
202 amount of variation that can be attributed exclusively to each set of explanatory variables.
203
204 **RESULTS**
205 **Sequences, OTUs and taxonomy**
206 High-throughput Illumina sequencing of the bacterial 16S rRNA gene (Claesson *et al.* 2010)
207 identified 3 752 bacterial operational taxonomic units (OTUs, sequences binned at 97%
208 similarity) in the phyllosphere of five temperate tree species, an average of 982 ± 67 OTUs
209 (mean ± SE) per tree sampled. Most of these bacterial taxa were abundant, with only 1.3% of
210 OTUs occurring on a single tree and 1.4% of OTUs occurring on all trees. The OTUs present on
211 all samples represent the "core microbiome": the microbial taxa shared among multiple
212 communities sampled from the same habitat (Shade & Handelsman, 2012). In this study, the core
213 microbiome consisted of 51 OTUs (Table 1) representing 57.6% of all sequences, of which 75%
214 were *Alphaproteobacteria*, 8% *Cytophagia*, 7% *Betaproteobacteria*, 3% *Acidobacteria*, 3%

215 *Gammaproteobacteria* and 2% *Actinobacteria*. The most abundant order was *Rhizobiales* (46%)
216 from which 73% of sequences were assigned to the family *Methylocystaceae*. While there was
217 some variation in the most abundant classes both across the five tree species and among canopy
218 locations (Figure 1 and 2), the class *Alphaproteobacteria* was always the dominant taxon, with
219 relative abundances ranging from 33% on *P. glauca* to 87% on *B. papyrifera* (Figure 1a).
220
221 **Intra-individual vs. Inter-Individual and Interspecific variation**
222 There was a clear clustering of the community composition of samples based on the species from
223 which they were collected (non-metric multidimensional scaling (NMDS) based on Bray-Curtis
224 distances among samples (Figure 3)). Host species identity is a significant driver of tree
225 phyllosphere community structure explaining 65% of the taxonomic (Bray-Curtis dissimilarities)
226 and 64% of the phylogenetic (weighted UniFrac distances) variation in bacterial community
227 structure (PERMANOVA; Table 2). The first permutational multivariate test of variance
228 homogeneity (an analogue of Levene's test of homogeneity of variances) indicated that among
229 host species, only *B. papyrifera* and *P. glauca* showed significantly distinct ($P<0.05$) variance in
230 community structure (Figure 4). The second test of the homogeneity of variance between per
231 species intra-individual and inter-individual variation indicated no significant differences in
232 variation (all $P>0.05$). The third test of the homogeneity of variance between intra-individual vs.
233 interspecific samples was also significant, showing there was a greater variation ($P<0.005$) in
234 leaf bacterial communities between host species than within the canopy of an individual host.
235
236 Because host species identity is a strong driver of leaf bacterial community structure, we
237 tested whether canopy position had an effect on community structure after accounting for the

238 variation explained by host species, using a partial redundancy analysis (RDA) on bacterial
239 community structure constrained by host species identity. The RDA showed that when
240 differences in bacterial community structure driven by host species identity were accounted for,
241 sample canopy location explained 70% of the remaining variation in community structure.
242
243 The alpha-diversity of leaf bacterial community differed significantly across host species
244 identity but not across canopy locations. Post-hoc Tukey honestly significant differences tests
245 confirmed that Shannon alpha-diversity is higher on conifer species (5.0 ± standard error (SE) of
246 0.08 for *A. balsamea* and 5.3 ± SE 0.07 for *P. glauca*) than on angiosperm species (3.8 ± SE 0.12
247 for *A. rubrum*, 4.1 ± SE 0.11 for *A. saccharum* and 3.7 ± SE 0.16 for *B. papyrifera*), a fact also
248 confirmed by the multiple bacterial taxonomic classes present on conifer species but not on
249 angiosperm species (Figure 2a-e).
250
251 **Bacterial Indicator Species**
252 The LEfSe analysis successfully identified indicator taxonomic groups associated with different
253 host species, but not across different canopy locations (Table 3). The conifers, *A. balsamea* and
254 *P. glauca*, had the highest number of associated bacterial indicator taxa (46 and 188
255 respectively). The most differentially abundant bio-indicators of *A. balsamea* were the
256 *Frankiaceae* family and multiple taxonomic levels of the phylum *Acidobacteria*: *Acidobacteria*,
257 *Acidobacteriales* and *Acidobacteriaceae*. For *P. glauca*, the strongest bioindicators were
258 multiple taxa from the *Bacteroidetes* phylum (*Cytophagia*, *Cytophagales*, *Cytophagaceae*,
259 *Spirosoma* and *Saprospirae*, *Saprospirales*, *Chitinophagaceae*), and from the *Actinobacteria*,
260 *Chloroflexi*, and *Deltaproteobacteria*. In contrast, *B. papyrifera* showed an overrepresentation of

24 bacterial taxa including the phylum *Proteobacteria*, the class *Alphaproteobacteria* and several of its orders (*Rhodospiralles*, *Rickettsiales*, *Caulobacterales*). Finally, the two *Acer* species (*A. rubrum and A. saccharum*) were associated with 19 and 32 indicators respectively, including the order *Rhizobiales*: *A. rubrum* being associated with the family *Methylocystaceae* and *A. saccharum* with the order *Methylobacteriaceae*.

DISCUSSION

In this study, we demonstrate for multiple host species that while there is intra-individual variation in phyllosphere bacterial community structure, this intra-individual variation is not significant when compared to the magnitude of interspecific variation, confirming our first hypothesis. This finding agrees with past studies of tropical (Kim *et al.* 2012; Kembel *et al.* 2014; Lambais *et al.* 2014) and temperate trees (Redford et al. 2010) that found host species identity to be the strongest driver of leaf bacterial community structure. Previous studies have quantified intra-individual and intraspecific variation in phyllosphere bacterial community structure, but these studies mixed leaves from within tree canopies without quantifying intra-individual variation (Redford *et al.* 2010) or explored intra-individual variation for a single host species (Leff *et al.* 2015). Our results show that after taking host species identity into account, there exist detectable differences in microbial community structure within tree canopies, at least in natural forest settings. However, the magnitude of this variation is much smaller than interspecific variation.

In terms of the taxonomic composition of the tree phyllosphere, each tree species can be characterized by a particular combination of most abundant classes across all canopy locations,

284 consistent with other studies of the phyllosphere microbiome (Redford *et al.* 2010; Kim *et al.*
285 2012; Kembel *et al.* 2014). *B. papyrifera*, a shade intolerant species (Krajina *et al.* 1982; Burns
286 & Honkala 1990) exposed to sunlight in the upper part of the forest canopy, exhibits the smallest
287 alpha diversity with a dominance of *Alphaproteobacteria* (Figure 2e) and also the smallest
288 amount of intra-individual variation (Figure 4). In contrast, both conifer host species, fully
289 located below deciduous canopy, exhibited the highest diversity in their community structure.
290 While ultraviolet radiation could be driving the observed differences in leaf alpha diversity
291 across species, our results provide no evidence of a significant and consistent difference in the
292 alpha-diversity of top-canopy samples nor for any canopy location. As shown by the multivariate
293 test of homogeneity of variance, the intra-individual variation in phyllosphere community
294 structure is not different from the variation observed at the inter-individual level. However,
295 because of the known impact of ultraviolet radiation on leaf bacterial community (Kadivar &
296 Sapleton 2003), leaf samples should not include top canopy leaves. Future phyllosphere studies
297 characterizing the relative influence of potential key factor such as random colonization via
298 vectors such as the wind (Copeland et al. 2015) or animals (Scheffers et al. 2013), competition
299 between bacterial populations (Vorholt 2012); or intra-individual variation in leaf functional
300 traits (Hunter et al. 2010; Reisberg et al. 2012) are needed to understand the dynamics driving
301 intra-individual variability in bacterial community structure.
302
303 In conclusion, our results demonstrate that while there exists intra-individual and intra-
304 specific variation in phyllosphere community structure, the magnitude of this variation is small
305 and statistically insignificant when compared to the magnitude of interspecific variation in
306 phyllosphere communities. When designing a study of tree phyllosphere bacterial communities,

307 if quantifying interspecific variation is the goal then samples from a consistent location within
308 the tree canopy for individual trees are sufficient to quantify the majority of the variation in
309 community structure. However, for studies of a single host species, there can be significant intra-
310 individual variation in phyllosphere community structure, and this should be taken into account
311 when designing a sampling plan by explicitly sampling at different positions within the canopy,
312 or by pooling samples from different canopy positions in order to obtain robust estimates of the
313 overall community composition for individual trees.
314

AVAILABILITY OF THE DATA AND MATERIALS

The datasets supporting the conclusions of this article are available on Figshare at:
https://figshare.com/s/45794d675d912b83a2f2 (code in R),
https://figshare.com/s/bd133cfbd2ca0cf8f449 (OTU table),
https://figshare.com/s/be5c5ed6b224667e8fd1 (barcodes),
https://figshare.com/s/d1703c191b2c0f4f54db (metadata) and
https://figshare.com/s/b93099c2ea930c5d6553 (16S rRNA sequences).

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CONFLICT OF INTEREST

The authors declare that the experiment comply with the current laws of the country in which the experiment was performed. The authors declare that they have no conflict of interest.

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518

519 **FIGURE LEGENDS**

520

521 **FIGURE 1**. Relative abundance of sequences from bacterial classes in the phyllosphere
522 microbiome of temperate tree species in a Quebec forest. a) average relative abundance of
523 bacterial classes across all samples per species; and b) average relative abundance of bacterial
524 classes for OTUs that were unique to each species. (ABBA for *Abies balsamea*; ACRU for *Acer*
525 *rubrum*; ACSA for *Acer saccharum*; BEPA for *Betula papyrifera*; and PIGL for *Picea glauca*).
526 **FIGURE 2.** Relative abundance of bacterial classes in the phyllosphere at six canopy locations
527 (B:Bottom, E:East, N:North, W:West,S:South and T:Top) for five temperate tree species in
528 Quebec forest. a) *Abies balsamea*; b) *Picea glauca*; c) *Acer rubrum*; d) *Acer saccharum*; and e)
529 *Betula papyrifera*.
530 **FIGURE 3.** Non-metric multidimensional scaling (NMDS) ordination of within-individual
531 variation in bacterial community structure across 40 phyllosphere samples from Quebec
532 temperate forest trees. Ordination based on Bray-Curtis dissimilarities among samples. Symbols
533 indicate sample position in the tree canopy; colours indicate by host species identity (ABBA for
534 *Abies balsamea*; ACRU for *Acer rubrum*; ACSA for *Acer saccharum*; BEPA for *Betula*
535 *papyrifera*; and PIGL for *Picea glauca*).
536 **FIGURE 4.** Permutation test for homogeneity of multivariate dispersions in leaf bacterial
537 communities between intra-individual samples. Symbols indicate sample position in the tree
538 canopy; colours indicate host species identity (ABBA for *Abies balsamea*; ACRU for *Acer*
539 *rubrum*; ACSA for *Acer saccharum*; BEPA for *Betula papyrifera*; and PIGL for *Picea glauca*).

TABLE LEGENDS

TABLE 1. Taxonomy and relative abundance of the 51 OTUs constituting the tree phyllosphere bacterial core microbiome in Quebec temperate forest (present in all samples).

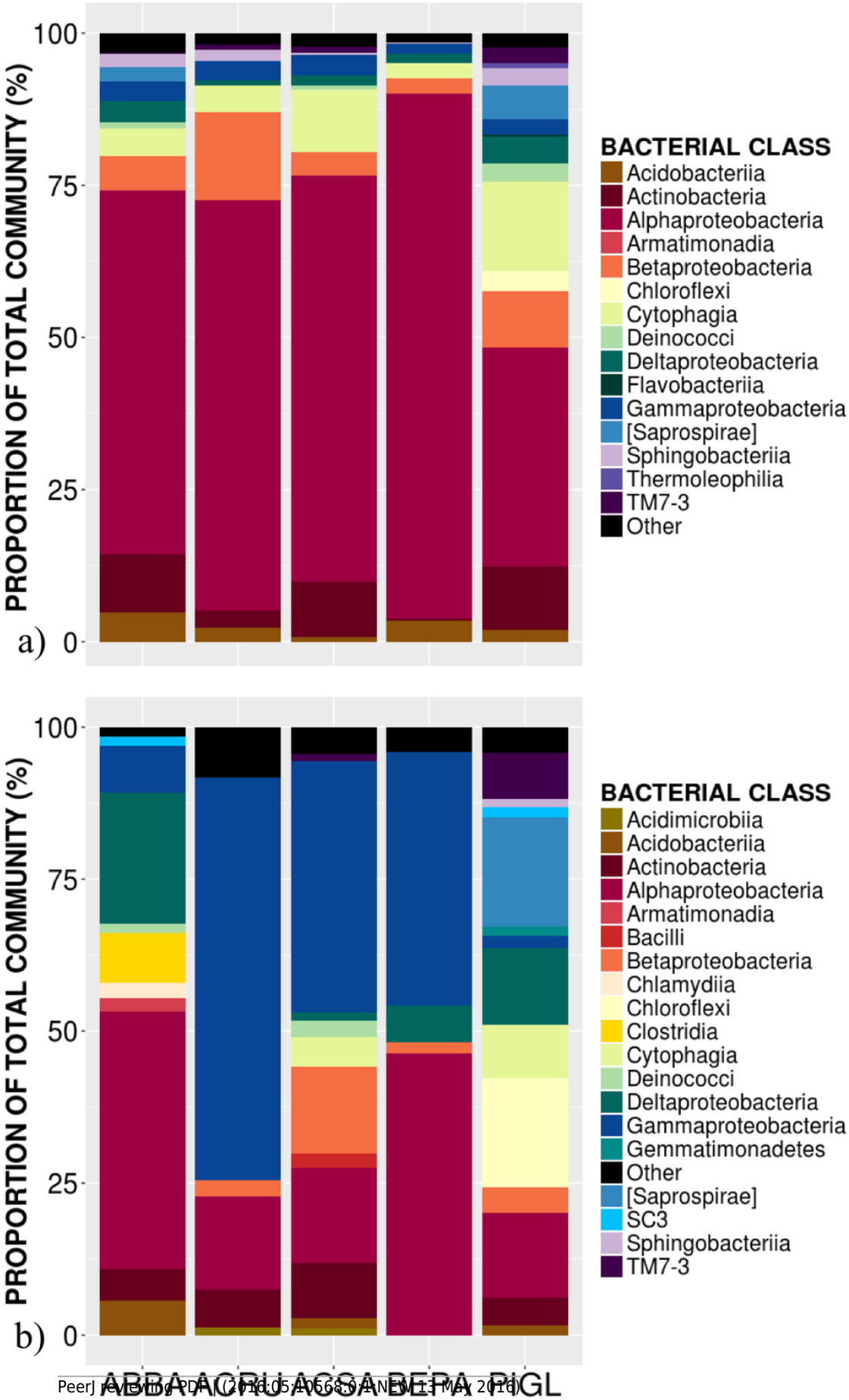
TABLE 2. Variation in phyllosphere bacterial community structure explained by host species identity and sample location within the tree canopy (PERMANOVA on Bray-Curtis dissimilarities and weighted UniFrac distances). Significance levels for each variable are indicated by symbols: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, $P > 0.05$.

TABLE 3. Bacterial taxa identified as bio-indicators of different host species in Quebec temperate forests. The top five bio-indicators from the LEfSe analysis are shown for each species. Significance are given by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, $P > 0.05$.

1

Relative abundance of sequences from bacterial classes in the phyllosphere microbiome of temperate tree species in a Quebec forest.

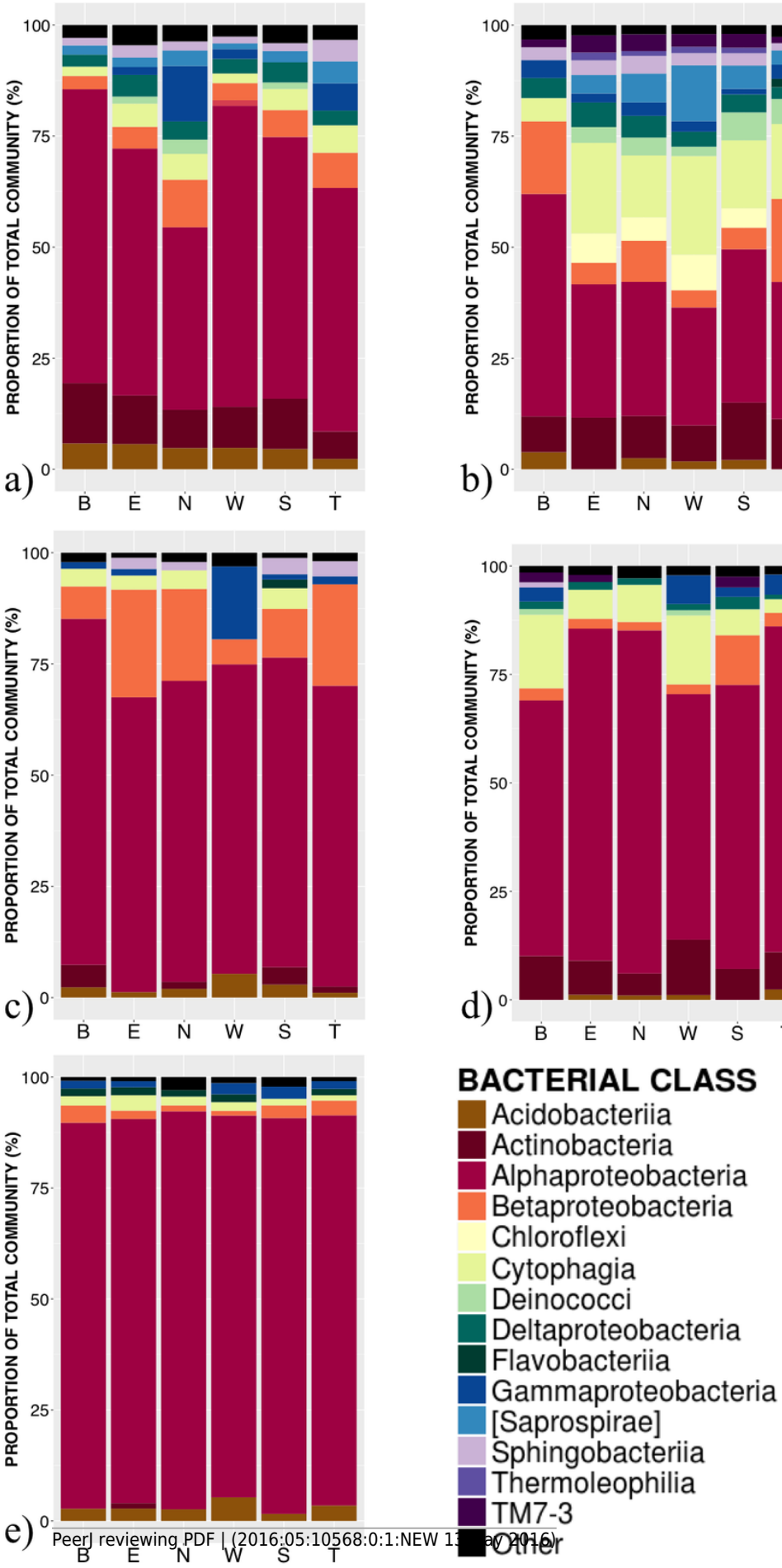
a) average relative abundance of bacterial classes across all samples per species; and b) average relative abundance of bacterial classes for OTUs that were unique to each species. (ABBA for *Abies balsamea*; ACRU for *Acer rubrum*; ACSA for *Acer saccharum*; BEPA for *Betula papyrifera*; and PIGL for *Picea glauca*).


a)
b)
PROPORTION OF TOTAL COMMUNITY (%)
100
75
50
25
0
BACTERIAL CLASS
Acidobacteriia
Actinobacteria
Alphaproteobacteria
Armatimonadia
Betaproteobacteria
Chloroflexi
Cytophagia
Deinococci
Deltaproteobacteria
Flavobacteriia
Gammaproteobacteria
[Saprospirae]
Sphingobacteriia
Thermoleophilia
TM7-3
Other
BACTERIAL CLASS
Acidimicrobiia
Acidobacteriia
Actinobacteria
Alphaproteobacteria
Armatimonadia
Bacilli
Betaproteobacteria
Chlamydiia
Chloroflexi
Clostridia
Cytophagia
Deinococci
Deltaproteobacteria
Gammaproteobacteria
Gemmatimonadetes
Other
[Saprospirae]
SC3
Sphingobacteriia
TM7-3

2

Relative abundance of bacterial classes in the phyllosphere at six canopy locations for five temperate tree species in Quebec forest.

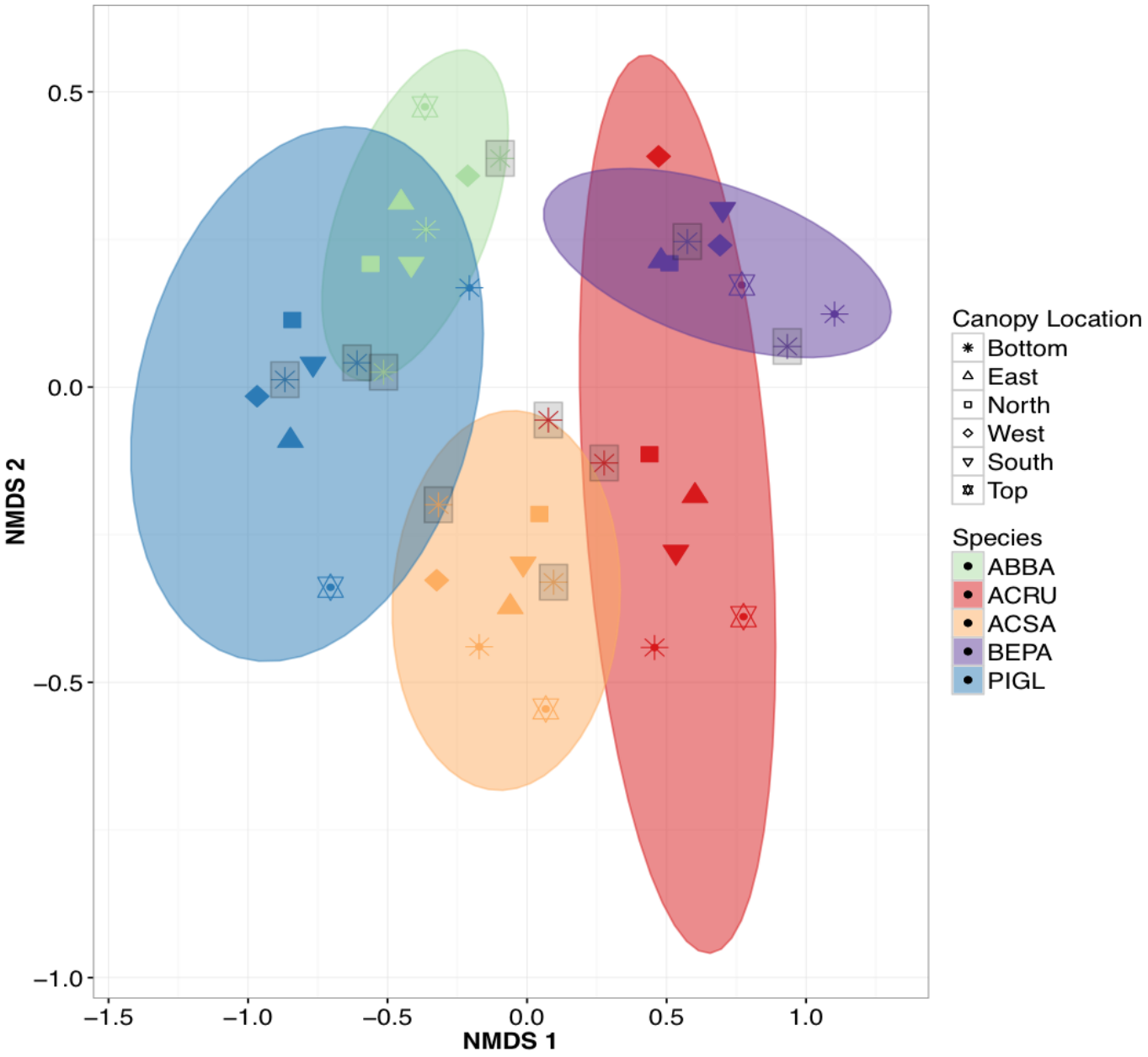
a) *Abies balsamea*; b) *Picea glauca*; c) *Acer rubrum*; d) *Acer saccharum*; and e) *Betula papyrifera*. Canopy locations are abbreviated as: B:Bottom, E:East, N:North, W:West,S:South and T:Top.


a)
b)
c)
d)
e)
PROPORTION OF TOTAL COMMUNITY (%)
100
75
50
25
0
B
E
N
W
S
T
BACTERIAL CLASS
Acidobacteriia
Actinobacteria
Alphaproteobacteria
Betaproteobacteria
Chloroflexi
Cytophagia
Deinococci
Deltaproteobacteria
Flavobacteriia
Gammaproteobacteria
[Saprospirae]
Sphingobacteriia
Thermoleophilia
TM7-3
Other

3

Non-metric multidimensional scaling (NMDS) ordination of within-individual variation in bacterial community structure across 40 phyllosphere samples from Quebec temperate forest trees.

Ordination based on Bray-Curtis dissimilarities among samples. Symbols indicate sample position in the tree canopy; colours indicate by host species identity (ABBA for *Abies balsamea*; ACRU for *Acer rubrum*; ACSA for *Acer saccharum*; BEPA for *Betula papyrifera*; and PIGL for *Picea glauca*).


0.5
0.0
−0.5
−1.0
NMDS 2
−1.5
−1.0
−0.5
0.0
0.5
1.0
NMDS 1
Canopy Location
Bottom
East
North
West
South
Top
Species
ABBA
ACRU
ACSA
BEPA
PIGL

4

Permutation test for homogeneity of multivariate dispersions in leaf bacterial communities between intra-individual samples.

Symbols indicate sample position in the tree canopy; colours indicate host species identity (ABBA for *Abies balsamea*; ACRU for *Acer rubrum*; ACSA for *Acer saccharum*; BEPA for *Betula papyrifera*; and PIGL for *Picea glauca*).

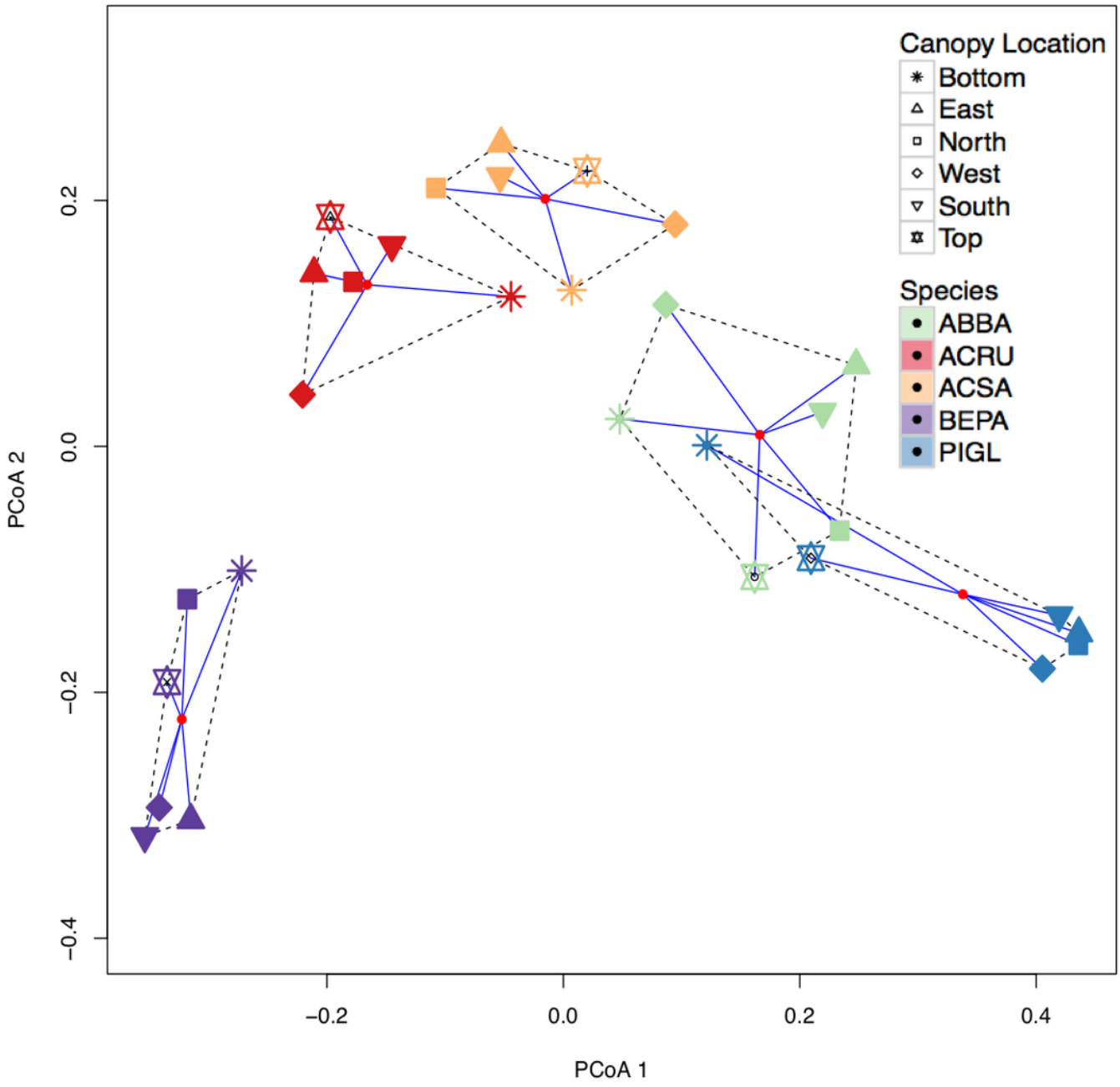

Canopy Location
Bottom
East
North
West
South
Top
Species
ABBA
ACRU
ACSA
BEPA
PIGL
PCoA 2
PCoA 1
0.2
0.0
−0.2
−0.4
−0.2
0.0
0.2
0.4

Table 1(on next page)

Taxonomy and relative abundance of the 51 OTUs constituting the tree phyllosphere bacterial core microbiome in Quebec temperate forest (present in all samples).

CLASS	ORDER	FAMILY	GENERA	SPECIES	%
Acidobacteriia	Acidobacteriales	Acidobacteriaceae	Bryocella	elongata	0.28
			4 NAs		1.52
Actinobacteria	Actinomycetales	Frankiaceae	NA		0.89
		Microbacteriaceae	Frondihabitans	cladoniiphilus	0.36
Cytophagia	Cytophagales	Cytophagaceae	Hymenobacter	2 NAs	4.68
Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	daejeonensis	0.30
			NA		0.17
Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA		0.61
	Rhizobiales	Beijerinckiaceae	Beijerinckia	4 NAs	5.07
		Methylobacteriaceae	Methylobacterium	2 NAs	1.83
		Methylocystaceae	9 NAs		19.83
	Rhodospirillales	Acetobacteraceae	6 NAs		8.58
		Rhodospirillaceae	NA		0.05
	Rickettsiales	NA	NA		0.10
		Rickettsiaceae	Rickettsia	NA	0.70
	Sphingomonadales	Sphingomonadaceae	Sphingomonas	6 NAs	5.15
				wittichii	0.11
				wittichii	1.18
				wittichii	0.18
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	2 NAs		4.08
Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	NA	0.18
	Myxococcales	Cystobacterineae	NA		0.22
Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Erwinia	NA	0.55
	Pseudomonadales	Pseudomonadaceae	Pseudomonas	fragi	0.99

1

Table 2(on next page)

Variation in phyllosphere bacterial community structure explained by host species identity and sample location within the tree canopy.

PERMANOVA on Bray-Curtis dissimilarities and weighted UniFrac distances. Significance levels for each variable are indicated by symbols: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, $P > 0.05$.

Variable	Bray-Curtis Dissimilarities	UniFrac Weighted Distance
Species	65 %***	64 %***
Canopy Location	NS	NS
Species * Canopy Location	NS	NS
Total R^2	65 %	64 %

1

Table 3(on next page)

Bacterial taxa identified as bio-indicators of different host species in Quebec temperate forests.

The top five bio-indicators from the LEfSe analysis are shown for each species. Significance are given by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, $P > 0.05$.

HOST SPECIES IDENTITY	BACTERIAL TAXA	EFFECT SIZE
Abies balsamea	Actinobacteria.Actinobacteria.Actinomycetales.Frankiaceae	4.34***
	Acidobacteria	4.30***
	Acidobacteria.Acidobacteriia.Acidobacteriales.Acidobacteriaceae	4.27***
	Acidobacteria.Acidobacteriia.Acidobacteriales	4.27***
	Acidobacteria.Acidobacteriia	4.27***
Acer rubrum	Proteobacteria.Alphaproteobacteria.Rhizobiales.Methylocystaceae	5.13***
	Proteobacteria.Betaproteobacteria	4.79***
	Proteobacteria.Betaproteobacteria.Burkholderiales	4.79***
	Proteobacteria.Betaproteobacteria.Burkholderiales.Oxalobacteraceae	4.77***
	Proteobacteria.Alphaproteobacteria.Rickettsiales.Rickettsiaceae	3.81***
Acer saccharum	Proteobacteria.Alphaproteobacteria.Rhizobiales	5.18***
	Bacteroidetes.Cytophagia.Cytophagales.Cytophagaceae.Hymenobacter	4.48***
	Proteobacteria.Alphaproteobacteria.Rhizobiales.Beijerinckiaceae	4.47***
	Proteobacteria.Alphaproteobacteria.Rhizobiales.Beijerinckiaceae.Beijerinckia	4.47***
	Actinobacteria.Actinobacteria.Actinomycetales.Microbacteriaceae	4.33***
Betula papyrifera	Proteobacteria.Alphaproteobacteria	5.39***
	Proteobacteria	5.28***
	Proteobacteria.Alphaproteobacteria.Rhodospirillales	5.26***
	Proteobacteria.Alphaproteobacteria.Rhodospirillales.Acetobacteraceae	5.25***
	Proteobacteria.Alphaproteobacteria.Rickettsiales	4.13***
Picea glauca	Bacteroidetes	4.97***
	Bacteroidetes.Cytophagia.Cytophagales	4.74***
	Bacteroidetes.Cytophagia	4.74***
	Actinobacteria	4.73***
	Bacteroidetes.Cytophagia.Cytophagales.Cytophagaceae	4.73***

1