Tree Phyllosphere Bacterial Communities: exploring the magnitude of intra- and inter-individual variation among host species

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Background. The diversity and composition of the microbial community of tree leaves (the phyllosphere) varies among trees and host species and along spatial, temporal, and environmental gradients. Phyllosphere community variation within the canopy of an individual tree exists but the importance of this variation relative to among-tree and among-species variation is poorly understood. Sampling techniques employed for phyllosphere studies include picking leaves from one canopy location to mixing randomly selected leaves from throughout the canopy. In this context, our goal was to characterize the relative importance of intra-individual variation in phyllosphere communities across multiple species, and compare this variation to inter-individual and interspecific variation of phyllosphere epiphytic bacterial communities in a natural temperate forest in Quebec, Canada.

Methods. We targeted five dominant temperate forest tree species including angiosperms and gymnosperms: *Acer saccharum*, *Acer rubrum*, *Betula papyrifera*, *Abies balsamea* and *Picea glauca*. For one randomly selected tree 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 five additional trees from each species.

Results. Based on analysis of bacterial community structure measured via Illumina sequencing of the bacterial 16S gene, we demonstrate that 65% of the intra-individual variation in leaf bacterial community structure could be attributed to the effect of inter-individual and inter-specific differences while the effect of canopy location was not significant. In comparison, host species identity explains 47% of inter-individual and inter-specific variation in leaf bacterial community structure followed by individual identity (32%) and canopy location (6%).

Discussion. Our results suggest that individual samples from consistent positions within the tree canopy from multiple individuals per species can be used to accurately quantify variation in phyllosphere bacterial community structure. However, the considerable amount of intra-individual variation within a tree canopy ask for a better understanding of how changes in leaf characteristics and local abiotic conditions drive spatial variation in the phyllosphere microbiome.

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ABSTRACT

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49 **Discussion.** Our results suggest that individual samples from consistent positions within the tree
50 canopy from multiple individuals per species can be used to accurately quantify variation in
51 phyllosphere bacterial community structure. However, the considerable amount of intra-
52 individual variation within a tree canopy ask for a better understanding of how changes in leaf
53 characteristics and local abiotic conditions drive spatial variation in the phyllosphere
54 microbiome.

INTRODUCTION

The phyllosphere microbiota 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 (Innerebner *et al.* 2011; Ritpitakphong *et al.* 2016). 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 water (Rodriguez *et al.* 2009) and nutrient cycling (van der Heijden *et al*. 2008; McGuire & Treseder 2010; Allison & Treseder 2011). Tree microbial phyllosphere communities have been studied 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). In order to understand the structure and function of phyllosphere microbial communities, studies typically either assume that a single sample of leaves from a plant canopy is representative of the phyllosphere community of the entire tree or host species (Lambais *et al.* 2006; Kim *et al.* 2012; Kembel *et al.* 2014), or control for spatial structure in phyllosphere community structure by mixing leaves from multiple canopy locations (Redford & Fierer 2009; Redford *et al.* 2010; Jumpponen & Jones 2009, 2010; Finkel *et al.* 2011, 2012; Cordier *et al.* 2012ab). In this study our aim was to quantify the relative importance of intra-individual versus inter-individual and inter-specific variation in the structure of temperate tree phyllosphere communities across multiple host species.

78 Host genetic factors (Bodenhausen *et al.* 2014; Horton *et al.* 2014) and taxonomic
79 identity (Redford *et al.* 2010; Kembel *et al.* 2014) are important drivers of phyllosphere bacterial
80 community structure. Most studies of phyllosphere communities across different host species
81 have assumed within-plant and within-species variation in phyllosphere community structure to
82 be negligible, and looked passed intra-individual and inter-individual variation (but see Redford
83 *et al.* 2010 and Leff *et al.* 2015). In tree phyllosphere studies, samples are usually taken from
84 shade leaves either at the bottom of the canopy or at mid-canopy height near the trunk. However,
85 the technique to sample phyllosphere communities vary between studies, ranging from studies
86 that sampled leaves from a specific canopy location (i.e. Kembel *et al.* 2014; Kembel & Müller
87 2014) to taking multiple leaves from around the canopy at the same height (i.e. Redford & Fierer
88 2009; Redford et al. 2010; Jackson & Denney 2011). However, Leff *et al.* 2015 demonstrated for
89 a single tree species (*Ginkgo biloba*) that there is intra-individual variation in phyllosphere
90 community structure within the canopy of a single tree. The relative importance of this within-
91 individual variation versus inter-individual and inter-specific variation, and the degree to which a
92 sample of leaves from a canopy are representative of the microbiome of an individual or a
93 species, is not well understood.
94
95 A multitude of factors could influence microbial community structure on leaves within a
96 tree canopy. Leaf position in the canopy defines the degree of exposure to ultraviolet radiation
97 and wind and therefore community structure could change depending on the position of the
98 leaves sampled. Exposure to ultraviolet radiation has been shown to increase the diversity of the
99 maize leaf microbial community (Kadivar & Sapleton 2003) and anoxygenic phototropic
100 bacteria have been detected in the phyllosphere of *Tamarix nilotica* (Atamna-Ismaeel *et al.*

2012). 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). Variation in atmosphere conditions within the canopy (i.e. increased exposure to wind and gas exchange levels) modifies local leaf humidity conditions potentially influencing leaf epiphytic bacterial communities by inhibiting or favoring the growth of particular groups (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, inter-individual and interspecific variation of phyllosphere bacterial 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 variation will be smaller than inter-individual and interspecific variation, (2) that canopy location will be a significant driver of phyllosphere bacterial community structure because of variation in abiotic conditions (e.g. radiation, wind), and changes in ecophysiological and morphological leaf characteristics.

MATERIALS AND METHODS

Study Site & Host-Tree Species

123 The two study sites are located in a natural temperate forest stand in Gatineau (45°44'50"N;
124 75°17'57"W) and Sutton (45°6'46"N; 72°32'28"W) Quebec, Canada. These sites are
125 characterized by a cold and humid continental climate with temperate summer. A total of six
126 individuals (three at each site) from each of five tree species common to temperate forests and
127 dominant in the canopy were sampled to provide representatives of both angiosperms and
128 gymnosperms: *Abies balsamea* (Balsam fir)*, Acer rubrum* (Red maple)*, Acer saccharum* (Sugar
129 maple)*, Betula papyrifera* (Paper birch) and *Picea glauca (*White spruce).
130

131 **Bacterial community collection**

132 We sampled phyllosphere communities from trees on August 29, 2013 as part of another
133 experiment (Laforest-Lapointe *et al.* 2016). Sampling was carried out one week after the last
134 rainfall event. We defined three strata within the canopy: bottom-canopy (1-2m height), mid-
135 canopy (2-4m height), and top-canopy (4-6m height). 30 individuals were randomly selected by
136 picking random geographic coordinates and finding the closest individual at this location. For the
137 first tree sampled from each species, we clipped 50-100 g of leaves at the four cardinal points at
138 mid-canopy height, plus a single sample at bottom-canopy and top-canopy heights, into sterile
139 roll bags with surface-sterilized shears. We also sampled bottom-canopy leaves from two other
140 randomly chosen trees from each species. For bacterial community collection and amplification
141 we used the protocols described by Kembel *et al.* (2014). We collected microbial communities
142 from the leaf surface by five minutes of horizontal mechanical agitation of the samples in a
143 diluted Redford buffer solution. We resuspended cells in 500 µL of PowerSoil bead solution
144 (MoBio, Carlsbad, California). We extracted DNA from isolated cells using the PowerSoil kit
145 according to the manufacturer's instructions and stored at -80°C.

146

147 **DNA library preparation and sequencing**

148 We used a two-step PCR approach to prepare amplicon libraries for the high-throughput Illumina
149 sequencing platform. The use of combinatorial primers for paired-end Illumina sequencing of
150 amplicons reduced the number of primers while maintaining the diversity of unique identifiers
151 (Gloor *et al.* 2010). First, we amplified the V5–V6 region of the bacterial 16S rRNA gene using
152 chloroplast-excluding primers in order to eliminate contamination by host plant DNA (16S
153 primers 799F-1115R (Redford *et al.* 2010; Chelius & Triplett 2001)) following protocols
154 described by Kembel et al. (2014). We cleaned the resulting product using MoBio UltraClean
155 PCR cleanup kit. We isolated a ~445-bp fragment by electrophoresis in a 2% agarose gel, and
156 recovered DNA with the MoBio GelSpin kit. We prepared multiplexed 16S libraries by mixing
157 equimolar concentrations of DNA, and sequenced the DNA library using Illumina MiSeq 250-bp
158 paired-end sequencing at Genome Quebec.

159

160 We processed the raw sequence data with PEAR (Zhang *et al.* 2014) and QIIME
161 (Caporaso *et al.* 2010) software to merge paired-end sequences to a single sequence of length of
162 350 bp, eliminate low quality sequences (mean quality score < 30 or with any series of 5 bases
163 with a quality score < 30), and de-multiplex sequences into samples. We eliminated chimeric
164 sequences using the Uclust and Usearch algorithms (Edgar 2010). Then, we binned the
165 remaining sequences into operational taxonomic units (OTUs) at a 97% sequence similarity
166 cutoff using the Uclust algorithm (Edgar 2010) and determined the taxonomic identity of each
167 OTU using the BLAST algorithm (Greengenes reference set) as implemented in QIIME
168 (Caporaso *et al.* 2010). The number of sequences per sample ranged from 6 256 to 75 412. From

169 these 1 499 777 sequences, we rarefied each sample to 5 000 sequences and repeated analyses on
170 100 random rarefactions. Re-analysis did not quantitatively change results and so we report only
171 the result of the analysis of a single random rarefaction. We included the resulting 275 000
172 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), picante (Kembel *et al.* 2010), and
185 vegan (Oksanen *et al.* 2007) packages in R (R Development Core Team 2013) and ggplot2
186 (Wickham 2009) for data visualization. We quantified the taxonomic variation in bacterial
187 community structure among samples with respectively the Bray-Curtis dissimilarity. To illustrate
188 patterns of bacterial community structure, we performed a nonmetric multidimensional scaling
189 (NMDS) ordination of Bray–Curtis dissimilarity. We identified relationships between bacterial
190 community structure, host species identity, and sample canopy location by conducting a
191 permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) on the

192 community matrix. We employed a blocking randomization to account for the non-independence
193 of observations among sites. To decompose the total variation in the community matrix
194 explained by host species identity and canopy location, we performed a partial redundancy
195 analysis (RDA; Legendre & Legendre 1998). This technique measures the amount of variation
196 that can be attributed exclusively to each set of explanatory variables. We performed three
197 permutational tests of multivariate homogeneity of group dispersions (Levene's test for
198 variances' homogeneity multivariate equivalent; Anderson 2006, Anderson *et al.* 2006): one to
199 test if variance in intra-individual canopy bacterial communities was equal between individuals
200 (30 samples from five trees sampled at six canopy locations); a second to compare interspecific
201 variation between species (30 bottom-canopy samples from 30 different trees); and finally a third
202 to test per-species intra- and inter-individual variation (all 55 samples). We estimated
203 phyllosphere bacterial alpha diversity using the Shannon index calculated from OTU relative
204 abundances for each community. We performed an analysis of variance (ANOVA) and
205 subsequent post-hoc Tukey's tests to compare differences in diversity across species. The
206 authors declare that the experiment comply with the current laws of the country in which the
207 experiment was performed.
208
209 **RESULTS**
210 **Sequences, OTUs and taxonomy**
211 High-throughput Illumina sequencing of the bacterial 16S rRNA gene (Claesson *et al.* 2010)
212 identified 5 005 bacterial operational taxonomic units (OTUs, sequences binned at 97%
213 similarity) in the phyllosphere of five temperate tree species, an average of 1055 ± 57 OTUs
214 (mean ± SE) per tree sampled. Most of these bacterial taxa were relatively common across

samples, with only 3.4% of OTUs occurring on a single tree and 0.8% of OTUs occurring on all trees. The OTUs present on all samples represent the "core microbiome": the microbial taxa shared among multiple communities sampled from the same habitat (Shade & Handelsman, 2012). In this study, the core microbiome consisted of 42 OTUs (Table 1) representing 61% of all sequences, of which 72% were *Alphaproteobacteria*, 9% *Cytophagia*, 7.8% *Betaproteobacteria*, 5% *Acidobacteria*, 2% *Gammaproteobacteria* and 2% *Actinobacteria*. The most abundant order was *Rhizobiales* (49%) from which 77% of sequences were assigned to the family *Methylocystaceae*. While there was some variation in the most abundant classes both across the five tree species and among canopy locations (Figure 1 and 2), the class *Alphaproteobacteria* was always the dominant taxon, with relative abundances ranging from 42% on *P. glauca* to 84% on *B. papyrifera* (Figure 1).

Intra-individual vs. Inter-Individual and Interspecific variation

Host species identity and individual identity effects could not be distinguished statistically due to the fact that analyses of intra-individual variation were based on a single individual per species. This host species/individual effect explained 65% of variation in phyllosphere bacterial taxonomic community structure while the impact of canopy location was not statistically significant (PERMANOVA on Bray-Curtis dissimilarities; Table 2). We then tested whether canopy position had an effect on community structure after accounting for the variation explained by host species/individual using a partial redundancy analysis (RDA) on bacterial community structure constrained by host species identity. The RDA showed that when differences in bacterial community structure driven by host species identity were accounted for, sample canopy location explained 22% of the remaining variation in community structure. In

238 comparison, in the dataset with 30 different individuals, host species identity explained only 47%
239 of variation in phyllosphere bacterial community structure (PERMANOVA on Bray-Curtis
240 dissimilarities; Table 2). When considering intra-individual and inter-individual samples, host
241 species identity (R^2=47%) was the strongest driver of variation in phyllosphere bacterial
242 community structure closely followed by individual identity (R^2=32%) and finally by canopy
243 location (R^2=6%; PERMANOVA on Bray-Curtis dissimilarities; Table 2). Community
244 composition of samples clustered based both on the individual (Figure 3a) and species (Figure
245 3b) from which they were collected (non-metric multidimensional scaling (NMDS) based on
246 Bray-Curtis distances among samples).
247
248 The first permutational multivariate test of variance homogeneity (an analogue of
249 Levene's test of homogeneity of variances) on intra-individual phyllosphere communities
250 indicated a significant difference between *P.glauca* and *B. papyrifera* (Tukey's post hoc test;
251 P=0.03). The second test of the homogeneity of inter-individual variance between host species
252 showed that *P. glauca*'s variance in community structure (mean distance to centroid = 0.34) was
253 higher than *A. saccharum* (0.25; P<0.01) and *A. rubrum* (0.26; P<0.05) while all other
254 comparisons were not significant. Finally, the third test between per species intra-individual and
255 inter-individual variation indicated one significant difference in variation for *B. papyrifera*
256 (P=0.005; Figure 4).
257
258 The alpha-diversity of leaf bacterial community differed significantly across host species
259 identity but not across canopy locations. Post-hoc Tukey honestly significant differences tests
260 confirmed that Shannon alpha-diversity is higher on conifer species (4.9 ± standard error (SE) of

0.04 for *A. balsamea* and 5.3 ± SE 0.04 for *P. glauca*) than on angiosperm species (3.7 ± SE 0.06 for *A. rubrum*, 4.1 ± SE 0.05 for *A. saccharum* and 3.6 ± SE 0.09 for *B. papyrifera*).

Bacterial Indicator Taxa

The LEfSe analysis successfully identified indicator taxonomic groups associated with different host species, but not across different canopy locations (Table 3). The conifers, *A. balsamea* and *P. glauca*, had the highest number of associated bacterial indicator taxa (46 and 188 respectively). The strongest bio-indicators of *A. balsamea* were the *Frankiaceae* family and multiple taxonomic levels of the phylum *Acidobacteria*: *Acidobacteria*, *Acidobacteriales* and *Acidobacteriaceae*. For *P. glauca*, the strongest bioindicators were multiple taxa from the *Bacteroidetes* phylum (*Cytophagia*, *Cytophagales*, *Cytophagaceae*, *Spirosoma* and *Saprospirae*, *Saprospirales*, *Chitinophagaceae*), and from the *Actinobacteria*, *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 there is a significant amount of intra-individual variation in phyllosphere bacterial community structure (Figure 3a). While the mean distance to centroid is always smaller for intra- than for inter-individual variation (Figure 4), this

284 distance was only statistically significant for *B. papyrifera.* This result therefore provides partial
285 support for our first hypothesis, stating that magnitude of intra-individual variation would be
286 smaller than inter-individual and interspecific variation. When analyzing all samples, we found
287 host species identity to be a stronger determinant of phyllosphere bacterial community structure
288 than individual identity (Table 2). However, this result could be biased by the fact that we
289 sampled a single individual for multiple canopy location. The importance of host species identity
290 as a driver of phyllosphere community structure agrees with past studies of tropical (Kim *et al.*
291 2012; Kembel *et al.* 2014; Lambais *et al.* 2014) and temperate trees (Redford et al. 2010).
292 Previous studies have quantified intra- and inter-individual variation in phyllosphere bacterial
293 community structure, but these studies mixed leaves from within tree canopies without
294 quantifying intra-individual variation (Redford *et al.* 2010) or explored intra-individual variation
295 for a single host species (Leff *et al.* 2015). Our results show that after taking host species identity
296 into account, there exist detectable differences in microbial community structure within tree
297 canopies, at least in natural forest settings.
298
299 In terms of the taxonomic composition of the tree phyllosphere, each tree species can be
300 characterized by a particular combination of most abundant classes across all canopy locations,
301 consistent with other studies of the phyllosphere microbiome (Redford *et al.* 2010; Kembel *et al.*
302 2014; Laforest-Lapointe *et al.* 2016). Amongst the potential mechanisms that could explain host
303 species selective power on their phyllosphere bacterial communities, ecological strategies could
304 play a role by impacting leaf abiotic conditions. *B. papyrifera*, a shade intolerant species (Krajina
305 *et al.* 1982; Burns & Honkala 1990) exposed to sunlight in the upper part of the forest canopy,
306 exhibited the smallest alpha diversity with a dominance of *Alphaproteobacteria* (Figure 2e) and

307 also the smallest amount of intra-individual variation (Figure 4). In contrast, both conifer host
308 species, growing below a deciduous canopy, exhibited the highest diversity in their community
309 structure. While ultraviolet radiation could be driving the observed differences in leaf alpha
310 diversity across species, our results provide no evidence of a significant and consistent difference
311 in the alpha-diversity among canopy locations. However, because we sampled only one
312 individual per species, canopy location effects remain to be quantified across multiple
313 individuals of the same species. As shown by the multivariate test of homogeneity of variance,
314 the intra-individual variation in phyllosphere community structure is not different from the
315 variation observed at the inter-individual level. Future phyllosphere studies characterizing the
316 relative influence of potential key factor such as random colonization via vectors such as the
317 atmospheric air flow (Barberán *et al.* 2014) or animals (Scheffers et al. 2013), competition
318 between bacterial populations (Vorholt 2012); or intra-individual variation in leaf functional
319 traits (Hunter et al. 2010; Reisberg et al. 2012) are needed to understand the dynamics driving
320 intra-individual variability in bacterial community structure.
321
322 In conclusion, our results demonstrate that there exists considerable intra-individual
323 variation in phyllosphere community structure, and that the magnitude of this variation is smaller
324 but not statistically different from the magnitude of inter-individual variation. When designing a
325 study of tree phyllosphere bacterial communities, if quantifying interspecific variation is the goal
326 then samples from a consistent location within the tree canopy for individual trees are sufficient
327 to quantify the majority of the variation in community structure. However, future studies and
328 especially studies focusing on a single host species should acknowledge that there can be
329 significant intra-individual variation in phyllosphere community structure, and sampling plans

330 should explicitly select leaves at different positions within the canopy to describe spatial
331 structure of the overall community composition for individual trees.

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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511

512 **FIGURE LEGENDS**

513

514 **FIGURE 1**. Relative abundance of sequences from bacterial taxonomic classes in the
515 phyllosphere microbiome of temperate tree species in a Quebec forest. (ABBA: *Abies balsamea*;
516 ACRU: *Acer rubrum*; ACSA: *Acer saccharum*; BEPA: *Betula papyrifera*; PIGL: *Picea glauca*).
517 **FIGURE 2.** Relative abundance of bacterial classes in the phyllosphere at six canopy locations
518 (B:Bottom, E:East, N:North, W:West, S:South T:Top) for one individual of the five temperate
519 tree species under study. a) *Abies balsamea*; b) *Picea glauca*; c) *Acer rubrum*; d) *Acer*
520 *saccharum*; and e) *Betula papyrifera*.
521 **FIGURE 3.** Non-metric multidimensional scaling (NMDS) ordination of within-individual
522 variation in bacterial community structure across 55 phyllosphere samples from Quebec
523 temperate forest trees. Ellipses indicate 1 standard deviation confidence interval around of a)
524 intra-individual samples and b) inter-individual samples. Gray boxes indicate the 30 samples that
525 came from individuals sampled at six different canopy locations. The other 25 samples came
526 from 5 more individuals per host species. Symbols indicate sample position in the tree canopy;
527 colours indicate by host species identity (green: *Abies balsamea*; red: *Acer rubrum*; orange: *Acer*
528 *saccharum*; purple: *Betula papyrifera*; blue: *Picea glauca*). Stress value was 0.16.
529 **FIGURE 4.** Permutation test for homogeneity of multivariate dispersions in leaf bacterial
530 communities between per species intra- and inter-individual samples. Colours indicate host
531 species identity (green for *Abies balsamea*; red for *Acer rubrum*; orange for *Acer saccharum*;
532 purple for *Betula papyrifera*; and blue for *Picea glauca*); shading indicate intra- (pale color) and
533 inter-individual (dark color) variance respectively.

534 **TABLE LEGENDS**

535

536 **TABLE 1.** Taxonomy and relative abundance of the 42 OTUs constituting the tree phyllosphere
537 bacterial core microbiome in Quebec temperate forests (present in all 55 samples).

538

539 **TABLE 2.** Variation in phyllosphere bacterial community structure explained by various drivers:
540 host species identity, sample location within the tree canopy and individual identity.
541 PERMANOVA on Bray-Curtis dissimilarities.

542

543 **TABLE 3.** Bacterial taxa identified as bio-indicators of different host species in Quebec
544 temperate forests. The LEfSe analysis was performed on 30 samples: 6 individuals per species.
545 Only the top five bio-indicators are shown. Significance are given by: * $P < 0.05$; ** $P < 0.01$;
546 *** $P < 0.001$; NS, $P > 0.05$.

547

Figure 1

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

(ABBA: *Abies balsamea*; ACRU: *Acer rubrum*; ACSA: *Acer saccharum*; BEPA: *Betula papyrifera*; PIGL: *Picea glauca*).

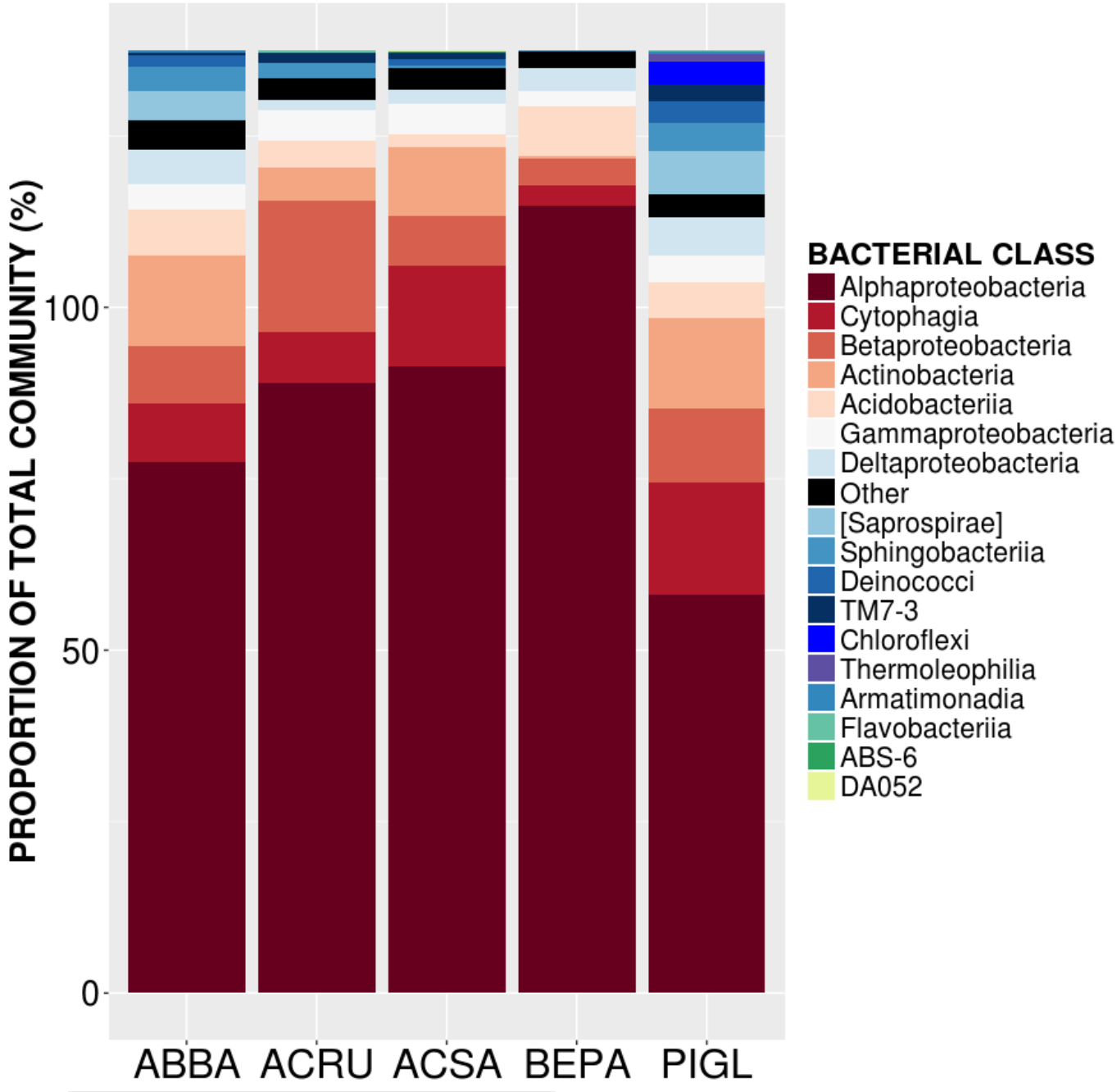


Figure 2

Relative abundance of bacterial classes in the phyllosphere at 6 canopy locations (B:Bottom, E:East, N:North, W:West, S:South T:Top) for one individual of the five temperate tree species under study.

a) *Abies balsamea*; b) *Picea glauca*; c) *Acer rubrum*; d) *Acer saccharum*; and e) *Betula papyrifera*.

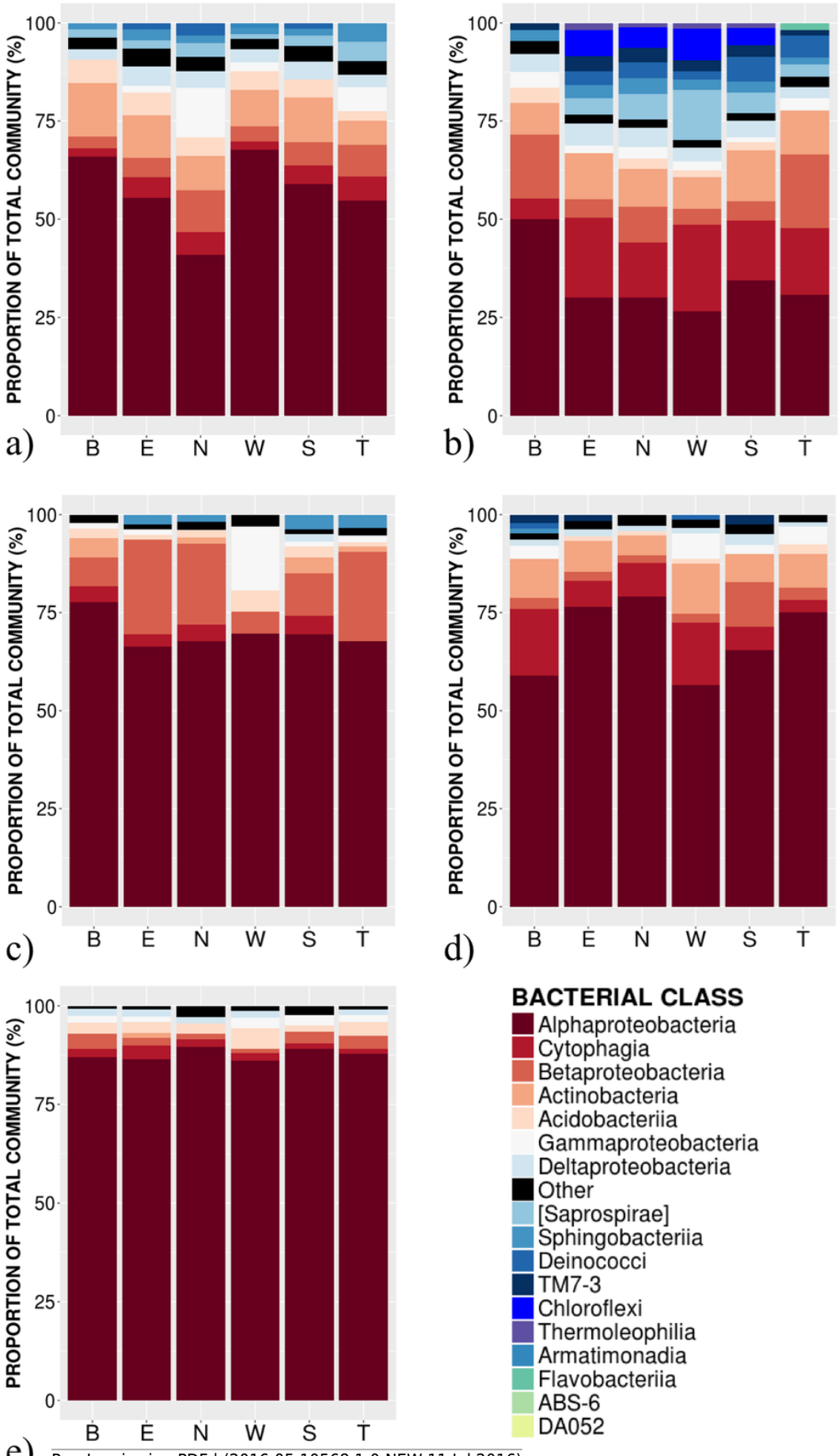
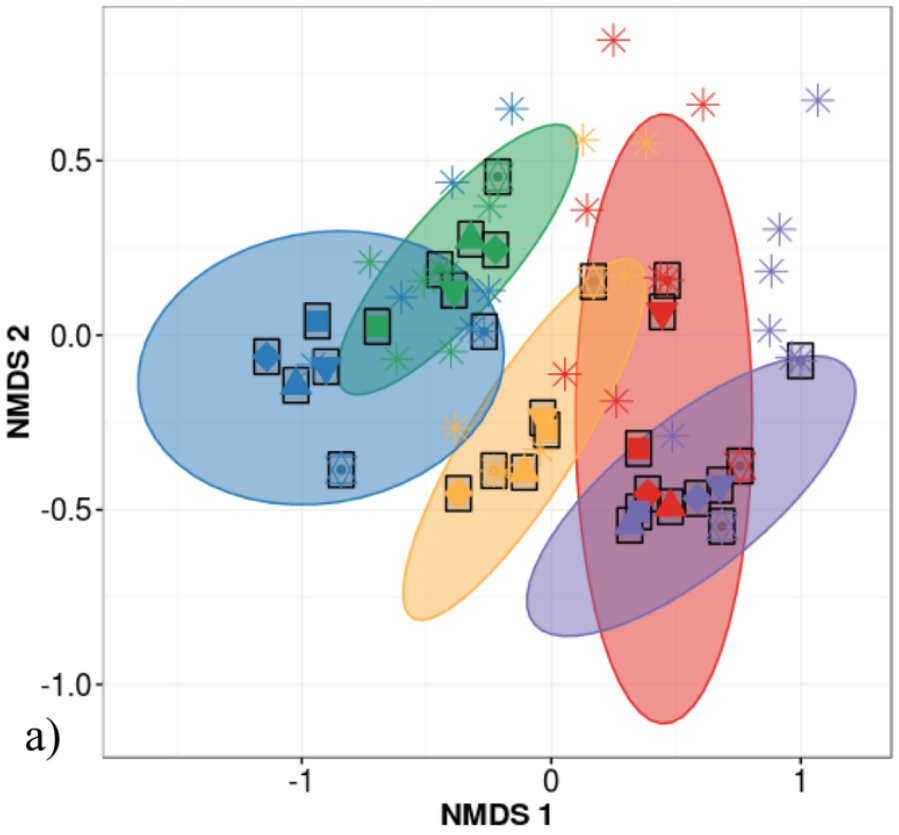
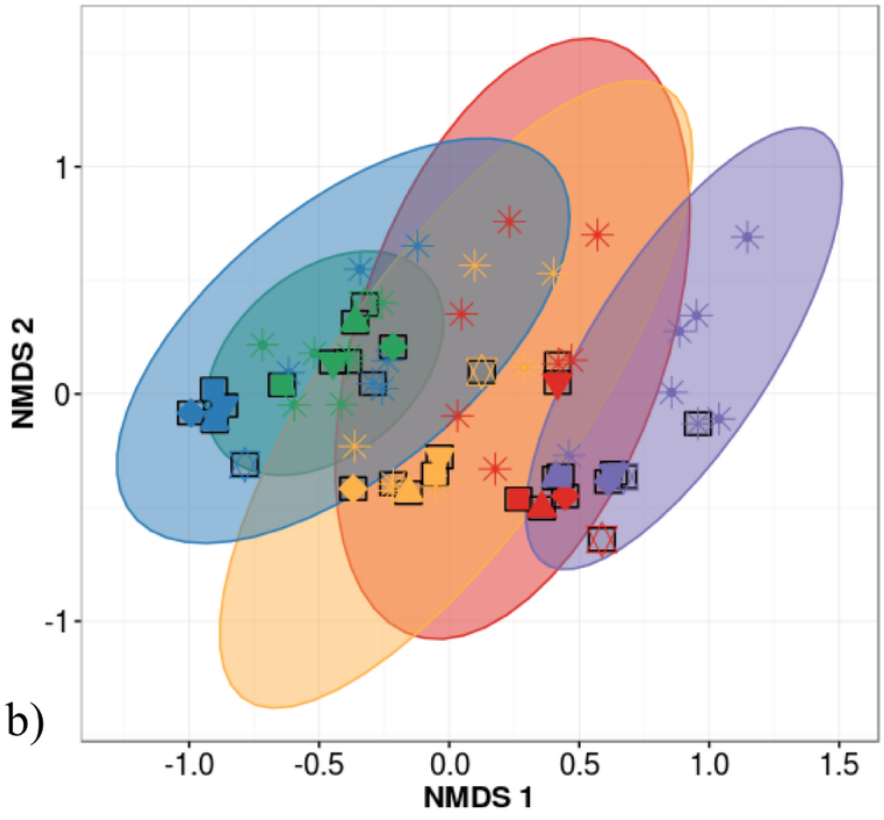

PROPORTION OF TOTAL COMMUNITY (%)
100
75
50
25
0
B
E
N
W
S
T
a)
b)
c)
d)
e)
BACTERIAL CLASS
Alphaproteobacteria
Cytophagia
Betaproteobacteria
Actinobacteria
Acidobacteriia
Gammaproteobacteria
Deltaproteobacteria
Other
[Saprospirae]
Sphingobacteriia
Deinococci
TM7-3
Chloroflexi
Thermoleophilia
Armatimonadia
Flavobacteriia
ABS-6
DA052

Figure 3

Non-metric multidimensional scaling (NMDS) ordination of within-individual variation in bacterial community structure across 55 phyllosphere samples from Quebec temperate forest trees.

Stress amounted to 0.16. Ellipses indicate 1 standard deviation confidence interval around of a) intra-individual samples and b) inter-individual samples. Gray boxes indicate the 30 samples that came from individuals sampled at six different canopy locations. The other 25 samples came from 5 more individuals per host species. Symbols indicate sample position in the tree canopy; colours indicate by host species identity (green: *Abies balsamea*; red: *Acer rubrum*; orange: *Acer saccharum*; purple: *Betula papyrifera*; blue: *Picea glauca*).


0.5
0.0
-0.5
-1.0
NMDS 2
a)
-1
0
1
NMDS 1


1
0
-1
NMDS 2
b)
-1.0
-0.5
0.0
0.5
1.0
1.5
NMDS 1

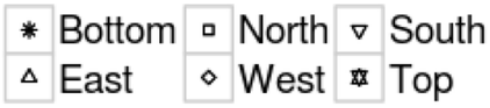

Bottom
North
South
East
West
Top

Figure 4

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

Colours indicate host species identity (green for *Abies balsamea*; red for *Acer rubrum*; orange for *Acer saccharum*; purple for *Betula papyrifera*; and blue for *Picea glauca*); shading indicate intra- (pale color) and inter-individual (dark color) variance respectively.

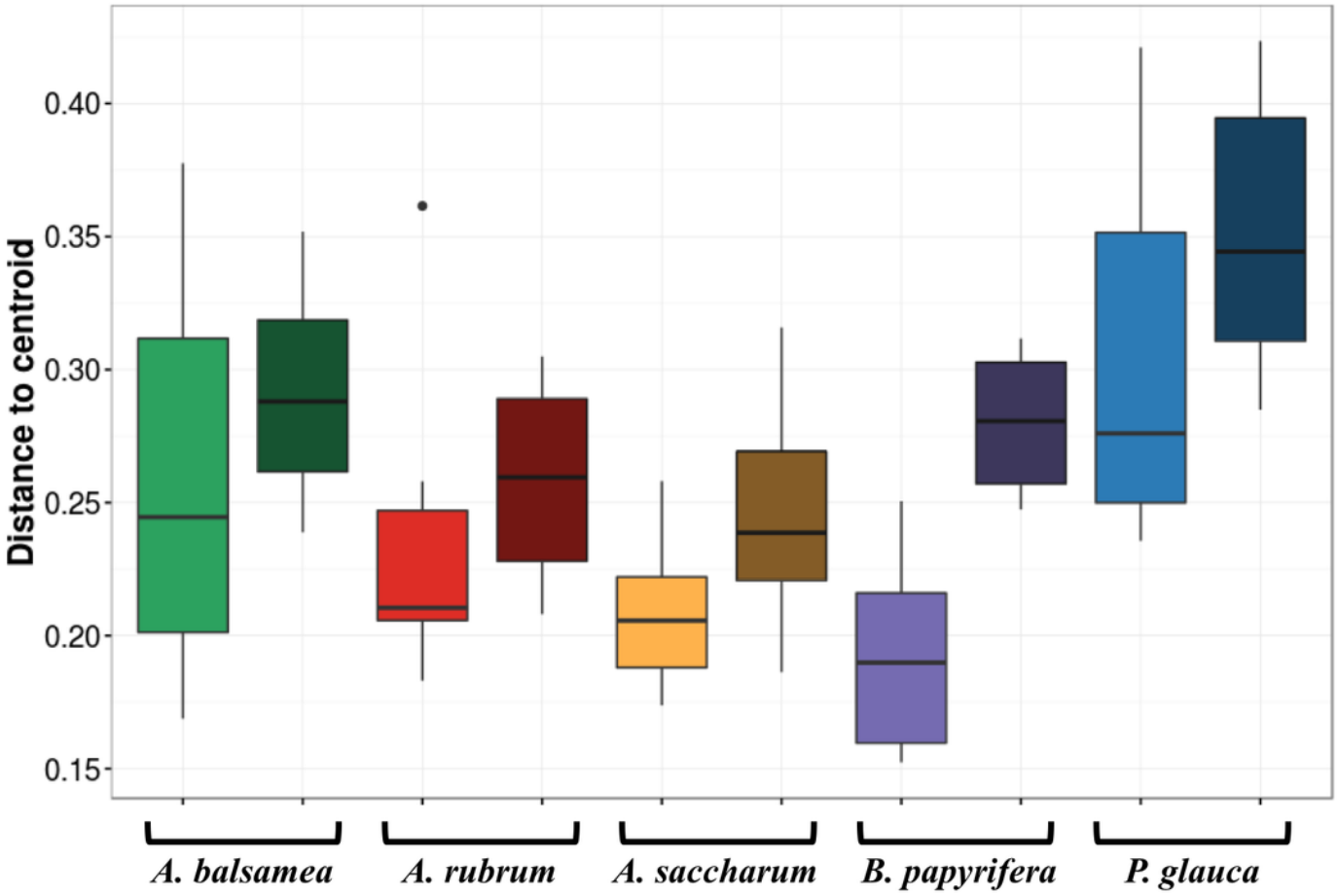


Table 1(on next page)

Taxonomy and relative abundance of the 42 OTUs constituting the tree phyllosphere bacterial core microbiome in Quebec temperate forests (present in all 55 samples).

CLASS	ORDER	FAMILY	GENERA	SPECIES	%
Acidobacteriia	Acidobacteriales	Acidobacteriaceae	Bryocella	elongata	0.5
			4 NAs		4.8
Actinobacteria	Actinomycetales	Frankiaceae	NA		1.3
		Microbacteriaceae	Frondihabitans	cladoniiphilus	0.5
Cytophagia	Cytophagales	Cytophagaceae	Hymenobacter	2 NAs	9.0
Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	daejeonensis	0.5
			NA		0.2
Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA		1.5
	Rhizobiales	Beijerinckiaceae	Beijerinckia	2 NAs	8.9
		Methylobacteriaceae	Methylobacterium	2 NAs	2.3
		Methylocystaceae	7 NAs		38.1
	Rhodospirillales	Acetobacteraceae	6 NAs		11.2
	Rickettsiales	NA	NA		0.10
		Rickettsiaceae	Rickettsia	NA	0.6
	Sphingomonadales	Sphingomonadaceae	Sphingomonas	6 NAs	7.9
				wittichii	1.7
				wittichii	0.1
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	2 NAs		7.8
Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	NA	0.2
	Myxococcales	Cystobacterineae	NA		0.7
Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Erwinia	NA	0.7
	Pseudomonadales	Pseudomonadaceae	Pseudomonas	fragi	1.3

1

Table 2(on next page)

Variation in phyllosphere bacterial community structure explained by various drivers: host species identity, sample location within the tree canopy and individual identity.

PERMANOVA on Bray-Curtis dissimilarities.

Dataset	Scope	Nb samples	Nb ind./species	Variables R^2 (%)		
				Canopy location	Host species identity	Individual identity
#1	Intra-individual	30	1	8*	**65****	
#2	Inter-individual and interspecific	30	6	*na*	**47**	*na*
#3	Intra- and inter-individual, and interspecific	60	6	**6**	**47**	**32*****

*The effect of canopy location was not significant after accounting for individual identity.
**Host species identity and individual identity are confounded as there were no replicates per species.
***Individual identity was nested in host species identity. *na*: non applicable.

1

Table 3(on next page)

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

The LEfSe analysis was performed on 30 samples: 6 individuals per species. Only the top five bio-indicators are shown. 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