Title: Drivers of phyllosphere microbial functional diversity in a neotropical forest

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Abstract

Background

The phyllosphere is an important microbial habitat but our understanding of how plant hosts drive the composition of their associated leaf microbial communities and whether taxonomic associations between plants and phyllosphere microbes represent adaptive matching remains limited. In this study we quantify bacterial functional diversity in the phyllosphere of 17 tree species in a diverse neotropical forest using metagenomic shotgun sequencing. We ask how hosts drive the functional composition of phyllosphere communities and their turnover across tree species, using host functional traits and phylogeny. We compare functional predictions inferred from 16S gene sequencing with functions estimated from metagenomic shotgun sequencing.

Results

Neotropical tree phyllosphere communities are dominated by functions related to the metabolism of carbohydrates, amino acids and energy acquisition, along with environmental signalling pathways involved in membrane transport. While most functional variation was observed within communities, there is non-random assembly of microbial functions across host species possessing different leaf traits. Metabolic functions related to biosynthesis and degradation of secondary compounds, along with signal transduction and cell-cell adhesion were particularly important in driving the match between microbial functions and host traits. These microbial functions were also evolutionarily conserved across the host phylogeny. Functional predictions inferred from 16S gene sequences were weakly correlated with functional

annotations from the same samples through metagenomic shotgun sequencing, especially for finer-scale functional annotations.

Conclusions

Functional profiling based on metagenomic shotgun sequencing offers evidence for the presence of a core functional microbiome across phyllosphere communities of neotropical trees. While functional turnover across phyllosphere communities is relatively small, the association between microbial functions and leaf trait gradients among host species supports a significant role for plant hosts as selective filters on phyllosphere community assembly. This interpretation is supported by the presence of phylogenetic signal for the microbial traits driving inter-community variation across the host phylogeny. Our comparison of functional annotations derived from 16S genes versus metagenomic shotgun sequencing suggests caution in using functions inferred from 16S genes for studying ecological dynamics in phyllosphere communities. Taken together, our results suggest that there is adaptive matching between phyllosphere microbes and their plant hosts.

Keywords

Microbial communities, Phyllosphere, Functional traits, Host-symbiont matching, Metagenomic shotgun sequencing

Background

The phyllosphere – the aerial surfaces of plants including leaves – is a widespread microbial habitat that hosts a diversity of microorganisms that play key roles in plant ecology and evolution [1]. Phyllosphere microbes play key roles in plant health [2, 3] and human health [4], and can influence ecosystem function [5]. At a broad taxonomic scale, phyllosphere bacterial communities are consistently dominated by taxa including Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria [6], indicating that plants also influence the composition of their microbial partners. A key goal of phyllosphere microbial ecology research has been to identify the adaptive basis of such relationships between plants and associated microbes.

Comparative studies of the taxonomic composition of phyllosphere microbial communities across plant hosts have demonstrated the importance of host identity as a key driver of variation in phyllosphere microbial taxonomic diversity. At fine taxonomic scales, the composition of these communities varies predictably across host plant species [7–9] and across genotypes within host plant species [10, 11]. Plants and associated bacteria also show cophylogenetic associations, with clades of plants and bacteria consistently occurring together [9, 12, 13], suggesting close adaptive associations between plants and their phyllosphere microbes.

Determining whether plant-microbe associations in the phyllosphere have an adaptive basis will require establishing how both plant and microbial functions are related across a range of host species. Plant functional traits – measures of morphology and physiology that capture key axes of variation in plant life history and ecology [14] – have been targeted as a potential proxy

for explaining microbial community turnover among plant species. These traits determine the potential for nutrient, metabolite and secondary compound leaching from the plant, which should largely determine the quality of a leaf as a habitat for phyllosphere microbes [15]. In support of this hypothesis, plant functional traits such as leaf mass per area, leaf elemental composition, and growth rate are correlated with phyllosphere microbial community turnover both within [16] and among plant species [12, 17–20].

Several studies have reciprocally identified the broad-scale microbial functional categories and adaptations that epiphytic microbes possess for living on plants [e.g. 16–19]. Functions including the biosynthesis of osmoprotectants such as trehalose and betaine and the production of extracellular polysaccharides are enriched in the phyllosphere and are thought to provide key adaptations to life on leaf surfaces by allowing microbes to attach to the leaf surface and by providing resistance to environmental stresses and plant defenses [25, 26]. However, studies of microbial functions in the phyllosphere have largely been based on comparison of one or a few host plant species. How microbial functions map onto variation in host plant functions in diverse natural communities thus remains largely unknown. As a result, it is not clear whether plant microbiomes exhibit the pattern of taxonomic turnover but functional homogeneity across hosts that has been observed in some animal microbiomes [27] or if a turnover in microbial functions can also be observed across functionally different tree species.

In this study, we quantified the functional repertoire of microbial communities on leaves of multiple tree species in a neotropical forest in Panama using metagenomic shotgun sequencing. We asked which microbial functions are abundant in the phyllosphere, and how these functions are linked to the taxonomy and functional traits of plant hosts. Our central hypothesis was that the plant-microbe taxonomic associations previously observed in this forest

105 [12, 28] should be driven by adaptive matches between microbes and host plants, leading to
106 several key predictions. First, we predicted that microbial functions should vary among host
107 plant species and be correlated with the functional traits of the hosts. Second, we predicted that
108 cophylogenetic associations between trees and microbes should lead to phylogenetic signal in
109 microbial functions present on different plant hosts. Third, we predicted that microbial functions
110 present on leaves should be filtered by the host, since conditions on the leaves of different host
111 plants create a selection pressure on the functions of microbes able to persist on those leaves.
112 Given the increasing interest in using metagenomic predictions of functional genes from
113 metabarcoding data in assessing functional diversity in microbial communities, we lastly aimed
114 to compare the performance of functional predictions from 16S sequencing performed on the
115 same samples in retrieving patterns of functional variation observed in our metagenomic shotgun
116 sequencing dataset [see also 29].

117

118 **Results**

119 *Metagenomic shotgun sequencing characterization of phyllosphere microbial functions*

120 Overall, we detected 4587 different functional genes across all samples based on
121 annotation of metagenomic shotgun sequencing of tropical tree phyllosphere communities.
122 Functions related to metabolism were the most abundant overall in our dataset, making up 45%
123 of all functionally annotated sequences (Fig. 1). The principal metabolic functions in the
124 phyllosphere were related to metabolism of amino acids (e.g. amino acid related enzymes),
125 nucleotides (e.g. purine and pyrimidine metabolism), carbohydrates (e.g. pyruvate, glyoxylate
126 and dicarboxylate metabolism), and energy (e.g. oxidative phosphorylation & TCA cycle) (Fig.

1). Groups of functional genes related to environmental and genetic information processing also had a high relative abundance, mainly membrane transport (e.g. transporters), translation (e.g. aminoacyl-tRNA biosynthesis), and signal transduction (e.g. two-component systems).

Variation in phyllosphere functions and taxa among versus within samples

The bacterial functions present on tree leaves were remarkably consistent among different samples. The vast majority of functional variation occurred within samples (>97%), with a very small contribution of functional turnover among samples (<3%) to total functional diversity, regardless of the functional level under study. Most taxonomic diversity was also observed within samples, with a contribution of beta-diversity increasing from 1 to 4.4% of total diversity with a refinement of the taxonomic scale utilized (Table 1). The principal component analysis of bacterial community functional composition indicated that metabolic functions related to biosynthesis and degradation of secondary compounds and antibiotics, as well as functions related to signal transduction and cell-cell adhesion were the most strongly varying among hosts (Fig. 2; Supp. Tab. 1, Additional File 1). We detected 17 Tier 3 functions that exhibited a significantly non-random phylogenetic signal with respect to the host phylogeny ($P<0.05$; Fig. 3). These functions were mostly involved in the metabolism of terpenoids and polyketides, signal transduction and cellular processes.

Associations between microbial and plant traits and host filtering

Many of the plant traits displayed some level of correlation with the principal axes of microbial functional community composition. Among these, morphological leaf traits (e.g. leaf area, leaf mass per area) were most strongly associated with the first two axes of microbial functional variation. Leaf elemental concentrations of copper, aluminum and manganese were

149 also strongly correlated with these first dimensions. The plant trait gradients explained altogether
150 ~17% of variation in functional composition among microbial communities. Around half of the
151 microbial Tier 3 functions were significantly more abundant or less abundant than expected by
152 chance in their community, based on a null model keeping both the total abundance of a trait and
153 the number of traits in a community constant (Table 2). The filtering signal was slightly stronger
154 for the microbial taxa than for the microbial functions (Table 2).

155 *Comparison of functional annotations based on metagenomic shotgun versus 16S sequencing*

156 Prediction of the functional content of phyllosphere microbial communities from their
157 16S rRNA genes using Tax4Fun yielded a higher diversity of functional genes (6429 genes)
158 across all samples compared to predictions from direct annotation of metagenomic shotgun
159 sequence data for the same samples (4587 genes). Most (~95%) functional genes were detected
160 in both the metagenomic and 16S datasets. The relative abundances of individual genes covaried
161 between the two datasets, with an increasing coherence of functional annotations at broader
162 levels of functional classification (Fig. 4). When testing correlation between metagenomic and
163 16S annotations within Tier 2 functional categories, we observed generally strong associations
164 (median R^2 = 0.85) between relative abundances of functions in the two datasets, though the
165 slope of the relationship was often deviant from the 1:1 line (Supp. Fig. 1, Additional File 2). A
166 constrained analysis of principal coordinates analysis revealed that genes related to
167 environmental information processing functions, especially membrane transport and signal
168 transduction, as well as functions related to cellular processes such as bacterial motility proteins
169 and quorum sensing were especially more represented in the 16S dataset, while metabolism
170 functions including nucleotide metabolism and energy metabolism as well as genetic information
171 processing functions (transcription and translation) were more represented in the metagenomic

dataset (Supp. Tab. 2, Additional File 1). The type of functional prediction used had an influence on the classification of samples in ordination space. While the classification of samples was consistent among functional levels for each analysis separately, ordinations were incoherent between datasets at all levels (m^2 similar to that expected by chance) (Supp. Fig. 2, Additional File 2).

Discussion

The functional composition of tree phyllosphere microbial communities in a tropical forest in Panama is largely consistent with those reported in the literature, regardless of the type of plant studied, suggesting the presence of a core functional microbiome in phyllosphere microbial systems. Core functional microbiomes in host-associated systems have also been reported for other hosts. Our study supports findings of an important role for the metabolism of carbohydrates and amino acids in bacterial survival in the phyllosphere [18, 46, 47] that is consistent with the abundance of these compounds in leaf leachates and photosynthates. The main mechanism of energy acquisition from these compounds appeared to be the TCA (citric acid) cycle, as reported in experimental studies of bacterial colonization of the phyllosphere [47]. Membrane transporters were also reported to be an important component of the epiphytic microbe functional repertoire, maximising the ability to monopolize otherwise limiting resources [48]. The abundance of signal transduction functional pathways, involved in the rapid sensing and response to environmental change, would lastly be coherent with the high variability in conditions of humidity, light and temperature in that microbial habitat [25].

The low functional variability in microbiomes observed among tree species represents a further line of evidence supporting the presence of a core phyllosphere functional microbiome.

194 This low variability, observed even at fine functional levels, could be the consequence of
195 essentially similar constraints imposed by the generally harsh leaf environment on its microbial
196 communities, regardless of the specific physiological traits of the host plant species. This low
197 functional turnover among communities was also associated with a low taxonomic turnover,
198 contrasting with reports from phyllosphere-associated temperate systems where species identity
199 was a strong driver of taxonomic composition of the microbial communities [8]. These results
200 could be explained by a finer-scale partitioning of taxa among neotropical than temperate tree
201 species, or a greater overlap in species functional types limiting strong associations between
202 microbial taxa and their hosts. Such differences should be further investigated.

203 Despite the high levels of convergence in microbial functions among the phyllospheres of
204 different tree species, several lines of evidence support a role for plant species taxonomic and
205 functional identity in driving microbial community assembly. Tree traits explained a notable
206 portion of the functional turnover among microbial communities. Traits correlated with
207 microbial functional turnover (e.g. leaf area, leaf mass per area) are mostly part of the leaf
208 economics spectrum [49], a functional strategy scheme describing photosynthetic resource-use
209 efficiency in plants, which is coherent with what we know of phyllosphere microbial physiology.
210 The ability of a tree to be conservative of its resources and generate thicker and better protected
211 leaves (i.e. high leaf mass per area) is likely to limit the leaching of nutrients from the leaf to the
212 phyllosphere, in turn constituting a filter on resource-use strategies in microbes. The high
213 correlation of leaf mass per area with turnover in microbial communities is coherent with a
214 previously described role for cuticle characteristics in determining functional turnover among
215 leaf microbial communities [16, 20]. The high correlation of aluminum and copper
216 concentrations in leaves with microbial functional variation may be explained by their role as

antibiotics. The predominance of two-component systems associated with high aluminum and copper concentrations suggests that the ability to sense and quickly respond to fluxes in these elements at the cell surface might constitute an efficient stress-response to deal with these conditions [50]. This type of plant trait gradient is analog to the leaf chemical gradient described by Yadav and colleagues [51], who reported variation in leaf colonization by phyllosphere microbes on different tree species as a function of their total leaf phenolics content. Taken together, these interpretations are concordant with the importance of energy metabolism, secondary metabolites and antibiotics production as well as environmental sensing in driving functional turnover of microbes among tree species.

Other lines of evidence support the idea that the plant host plays a selective role on microbial community assembly, such as the detection of bacterial traits that are non-randomly structured in the plant phylogeny. While this pattern might arise from the filtering of microbes on phylogenetically structured selective plant traits or from co-evolution of the two partners, it is regardless indicative of an influence of the host on the functional make-up of bacterial phyllosphere communities. Interestingly, the set of pathways that are important in driving functional turnover among communities belong to the same functional categories as the ones that are phylogenetically structured among plant hosts, supporting the proposed match between these bacterial functions and their host's functional and taxonomic identity. The fact that the relative abundance of a large set of functions was different within communities than that expected by chance given their relative abundance across samples, also supports a role for individual tree species in structuring the functional composition of their phyllosphere bacterial communities. The higher filtering of most microbial taxa relative to microbial functions suggests a role for unmeasured trait variation in driving functional turnover among communities.

The relatively small but significant contribution of functional turnover among microbial communities to the total functional diversity observed across samples suggests that the functions that are of importance in driving the distribution of bacteria across different host trees are actually relatively few compared to those enabling the bacteria to pass the overall "phyllosphere filter" that is needed to survive in the phyllosphere habitat. It remains unknown whether the majority of functional pathways that do not vary among trees are actually important for the ecology of the microbes, or if that trait variation is adaptively neutral within communities. It is also possible that some pathways important for microbial adaptations to leaf physiological gradients are not yet functionally described and are part of the large number of sequences that could not be functionally annotated. Ongoing efforts to better characterize gene functions will help improve the precision of ecological inferences in environmental metagenomes.

The functional predictions generated from annotation of inferred functions from 16S sequences were broadly comparable to those obtained through shotgun metagenomic sequencing at broad functional levels (e.g. Tier 2 functions), but at finer levels (Tier 3 and functional genes) there were numerous discrepancies in the relative abundance of functions inferred using these different approaches. The categories of functions for which we observed the largest discrepancies between the two approaches to functional annotations overlapped with those described for aquatic bacterial communities by Staley and colleagues [29] (i.e. membrane transport, translation). These results point to consistent biases in predictions of metagenomic functions from 16S sequences across ecosystems and warrant caution in interpreting ecological dynamics from inferred functions, especially when Tier 3 or functional gene abundances are being inferred.

Conclusions

In conclusion, we have identified a core functional microbiome in the phyllosphere of neotropical trees. While most functional variation was observed within individual microbial communities, we reveal a functional matching between the traits of microbes and the traits of plants across 17 tree species, emphasizing the role for energy metabolism, secondary metabolites and antibiotic production as well as environmental sensing in mediating bacterial adaptation to leaf trait gradients in the canopy. Our identification of the adaptive drivers of phyllosphere microbial community composition in this neotropical ecosystem represents a good starting point for identifying the types of microbial traits that could be routinely studied by phyllosphere microbial ecologists to address global questions on the ecological and evolutionary dynamics of phyllosphere microbes. Empirical testing of the fitness consequences of variation in those traits will represent an important next step in understanding adaptive processes in the phyllosphere.

Methods

Microbial DNA collection, extraction and sequencing

Microbial communities were collected from the leaves of 24 individual trees from 17 tree species (1-2 samples per species) in the tropical lowland rainforest of Barro Colorado Island, Panama, in December 2010. These samples were selected from a larger pool of samples [12, 28] for which we had sufficient quantities of high-quality DNA, selecting host species to maximize the phylogenetic and functional diversity of hosts. Methodological details of sample collection are described by Kembel et al. 2014 [12]. Briefly, 50-100g of fresh leaves were collected from the subcanopy of one tree of each species. Microbial cells were then washed from each leaf

sample using phosphate buffer [1 M Tris•HCl, 0.5 M Na EDTA, and 1.2% CTAB] and collected by centrifuging at 4,000 × g for 20 min. DNA was extracted using MoBio PowerSoil DNA extraction kits and samples stored at -80°C for future analyses. We quantified DNA concentrations and sequenced both extraction negative controls and PCR negative controls for these samples as part of previously published analyses of bacterial 16S and fungal 28S amplicon sequencing of these samples [12, 28]; none of the negative control samples contained measurable concentrations of DNA and upon sequencing they contained fewer DNA sequences than the minimum cut-off for inclusion in analyses As a result, they were all excluded from subsequent analyses in previously published studies and the present study. To quantify the metagenomic structure of each microbial community, we constructed a paired-end metagenomic shotgun library including a random sample of the whole community DNA composition using an Illumina Nextera XT® kit (Illumina reference FC-131-1024). These libraries were then sequenced using Illumina MiSeq paired-end 2 x 250 base pair sequencing (V2 kit, Illumina reference MS-102-2003). Analyses were performed on these 24 samples unless stated otherwise. Results were not influenced by including replicates of the same species (see tests below).

Microbial taxonomy and functional trait annotation

Metagenomic shotgun sequencing yielded 14,642,408 reads in total. We trimmed sequences to remove Illumina adapters and truncate end-bases with a quality score less than 20, and removed sequences shorter than 25bp, leaving 14,634,072 trimmed and quality-controlled reads. Taxonomic annotation of all sequences in each microbial community was performed to restrict functional analyses only to bacterial sequences. We annotated metagenomic reads using Kaiju, which annotates taxonomic identity of reads by comparing sequenced reads to the microbial subset of the NCBI BLAST non-redundant protein database [30]. Out of the 7,317,036

sequences, we were able to annotate taxonomy to at least the taxonomic level of kingdom for 2,138,885 sequences, of which 2,100,491 sequences were from Bacteria, representing 29% of total sequences. Of these Bacterial sequences, 1,902,749 were annotated to at least the phylum level, representing 26% of total sequences. Analysis of taxonomic composition was carried out on this subset of sequences annotated to at least the bacterial Phylum level. We rarefied all samples to 20,100 randomly chosen sequences per sample for taxonomic composition analyses, resulting in a total of 482,400 sequences for taxonomic analyses.

Functional annotation of microbial sequences was performed via protein homology searches using the KEGG annotation framework [31, 32] via the software COGNIZER [33]. Analyses resulted in the identification of functional genes and categories for 873,082 sequences representing 12% of sequences. In total, of the 7,317,036 bacterial sequences that were obtained from the metagenomic sequencing of all samples, 722,936 sequences were taxonomically annotated as bacteria and had a functional annotation. We lastly classified each of these sequences into functional categories, defined by the BRITE functional hierarchy manually curated for the KEGG annotation system based on published literature [32]. This hierarchy contains four different levels, which were designed as Tier 1, Tier 2, Tier 3 and functional genes, ranging from the more general to the more specific functional assignment (see [29]). Most analyses were performed at the Tier 3 level, in the intent of reaching a balance between the complexity of the data and its interpretability. In a few instances, Tier 3 categories were perfectly correlated across samples so we removed the duplicates from the dataset (Supp. Tab. 3, Additional File 1).

Inference of microbial functions from 16S gene sequences

Several methods have been proposed to estimate the functional composition of microbial communities based on estimation of functions from 16S gene sequences [e.g. 25–27]. In order to compare estimates of functional composition based on direct sequencing and annotation of metagenomic shotgun sequencing data versus estimation of functions based on 16S sequences, we analyzed previously published 16S gene sequence data for each sample [12]. The randomly rarefied set of 4000 16S sequences per sample described by Kembel et al. [12] were analyzed using Tax4Fun [35] which provided an inferred estimate of the relative abundance of microbial functions in each sample.

Plant functional traits and phylogeny

We obtained measurements of plant functional traits for all plant species from a dataset collected previously on Barro Colorado Island [37]. This trait database initially included 21 whole-plant and leaf traits, but we reduced these traits to a subset of 12 traits with limited overlap in functional significance [38]. This reduced set of traits included height at maturity, sapling growth rate and sapling mortality rate as whole-plant resource-use traits, leaf area and leaf dry matter content as leaf structural traits, and a suite of leaf elemental chemistry traits including concentration of aluminum, calcium, copper, magnesium, phosphorus, zinc and nitrogen content. A phylogenetic hypothesis for host plant species was obtained by grafting tree species onto a dated megatree of angiosperms provided by Zanne et al. [39] using Phylomatic v.3 [40].

Variation in phyllosphere functions among versus within samples

We determined the contributions of within- and among-sample variation in function of total functional variation among metagenomic samples using additive diversity partitioning

352 implemented in the R package entropart [41]. The proportions of alpha and beta diversity were
353 calculated as a ration of the portion of alpha (or beta) diversity on total diversity. Analyses were
354 performed at three levels of functional aggregation (Tier 1 to Tier 3). We tested whether the
355 presence of two samples rather than one for some of the sampled species would affect this
356 diversity partitioning by subsampling the dataset to include all possible combinations of samples
357 totally a single sample per species (n=128) and rerunning the analyses. This subsampling did not
358 affect our results (Supp. Fig. 3, Additional File 2), such that we kept the 24 samples in the
359 subsequent analyses. We then compared sources of turnover for functions and taxonomy
360 between samples by performing the same analysis from the taxonomically annotated
361 metagenomic sequences, defined at levels from phylum to species.

362 *Associations between microbial and plant traits*

363 We performed a principal component analysis (PCA) of functional trait matrices and
364 identified the functions contributing most to variation along the first axes of variation using R
365 package FactoMineR [42]. We fitted the plant traits onto this ordination to identify correlations
366 between bacterial traits driving the PCA and the plant traits. We evaluated the influence of tree
367 species replicates in our samples on these results and did not uncover important differences in
368 the main drivers of functional differences among samples when excluding these duplicates such
369 that all 24 samples were kept in this analysis.

370 We quantified the phylogenetic signal in associations between microbial functions and
371 host plant phylogeny using function *multiphylosignal* from R package Picante [43] to calculate
372 Blomberg's K and an associated P-value, which quantifies whether a microbial function exhibits
373 stronger phylogenetic signal than expected by chance. We selected a single random sample per

374 host species for those host species with more than one sample prior to calculating phylogenetic
375 signal. We repeated this for different random subsamples and it did not qualitatively change the
376 results so we report phylogenetic signal for a representative random subsample.

377 *Host filtering of microbial functions and taxa*

378 The degree of host filtering on microbial communities was assessed by comparing the
379 occurrence of traits in observed communities to those obtained from 999 randomizations of
380 community trait matrices. Host filtering was detected as an over- or under-representation of the
381 given trait in individual communities. Randomizations were generated by permutations of the
382 trait matrix preserving row and column totals. For each site and bacterial trait combination, we
383 compare the observed frequency of the trait to the random values to assess whether it was lower
384 or higher than expected by chance. To compare the strength of functional vs. taxonomic filtering,
385 we applied the same procedure to the taxonomic datasets defined at each of six taxonomic levels,
386 from the phylum to the species.

387 *Comparison of functional annotations based on shotgun metagenomic versus 16S sequencing*

388 We compared functional annotations obtained through shotgun metagenomic sequencing
389 with those obtained from functional predictions made from 16S sequencing on the same samples.
390 Since one sample was an outlier in the 16S functional predictions so we removed it in both
391 datasets prior to analyses, resulting in 23 samples total.

392 We first compared the relative abundances of individual functional pathways across the
393 two datasets by performing a correlation analysis between their average abundance across all
394 samples. The functional pathways the furthest from the 1:1 relationship would be the most over-
395 or under- represented in either method. We then determined the functional pathways which were

the most important in differentiating between the metagenomic and the 16S functional annotations by performing a Constrained Analysis of Principal Coordinates (CAP) analysis on Bray-Curtis distances. We next tested whether such differences would have an importance in the ecological classification of samples by performing a Procrustes analysis on the functional tables of each dataset [44], and tested the degree of similarity in the relationships among sites calculated for each dataset using a permutation approach. The degree of similarity is described by the m^2 term, representing the sum of the squared deviations between sample positions in one dataset vs. the other. A m^2 statistic smaller than expected by chance indicates that the two datasets are more similar than expected by chance [45]. Finally, we generated a diversity partitioning of the 16S functional predictions as described above for the metagenomic functional annotations to determine the impact of the 16S prediction approach in the description of biodiversity within and across samples.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

415 The datasets generated and/or analysed during the current study are available in a MG-RAST
416 repository: https://www.mg-rast.org/linkin.cgi?project=mgp91848

417 The scripts used to perform analyses for the current study are available in a GitHub repository:
418 https://github.com/glajoie1/panama_metagenome

419 *Competing interests*

420 The authors declare that they have no competing interests.

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424 *Authors' contributions*

425 GL and SK conceptualized the study. SK collected the data. RM and SK curated the data. GL
426 and SK analyzed the data and wrote the manuscript. All authors revised and accepted the final
427 manuscript.

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430

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Additional Files

Additional File 1 (.docx)

Supplementary Tables. This additional file contains 3 supplementary tables, referred to in the main text.

Additional File 2 (.docx)

Supplementary Figures. This additional file contains 3 supplementary figures, referred to in the main text.

Additional File 3 (.newick)

Host phylogeny. A phylogenetic hypothesis for host plant species obtained by grafting tree species from the study site onto a dated megatree of angiosperms (see Methods for details).

Tables

Table 1. Functional and taxonomic additive diversity partitioning of bacterial communities across 24 tree phyllosphere samples.

	Functional						Taxonomic					
	Metagenomic			16S								
	Tier 2	Tier 3	Functional gene	Tier 2	Tier 3	Functional gene	Phylum	Class	Order	Family	Genus	Species
Alpha diversity (%)	100.0	99.8	97.2	99.7	99.6	99.5	99.0	99.0	99.0	98.8	98.2	95.6
Beta diversity (%)	0.0	0.2	2.8	0.3	0.4	0.5	1.0	1.0	1.0	1.2	1.8	4.4

Diversity partitioning was calculated for both the metagenomic dataset and the 16S functional predictions. The percentage of alpha diversity was calculated as the amount of alpha entropy divided by the amount of total entropy across all communities. The percentage of beta diversity was calculated as 1 minus the percentage of alpha diversity.

578 **Table 2.** Occurrences of Tier 3 functions and taxa across 24 tree phyllosphere samples.

	Number of combinations higher than expected by chance	% of total	Number of combinations lower than expected by chance	% of total	Total number of combinations
Functions					
Tier 3 functions	1553	25	1570	25	6192
Taxa					
Phylum	495	25	510	25	2016
Class	493	29	556	33	1704
Order	1124	29	1310	34	3888
Family	2403	27	2804	32	8808
Genus	10010	25	11575	29	40608
Species	47492	20	45628	19	240288

579

580 Occurrences of Tier 3 functions and taxa that are more or less abundant than would be expected by chance (α=0.05) across all

581 combinations of functions or taxa per site.

582 **Figure legends**

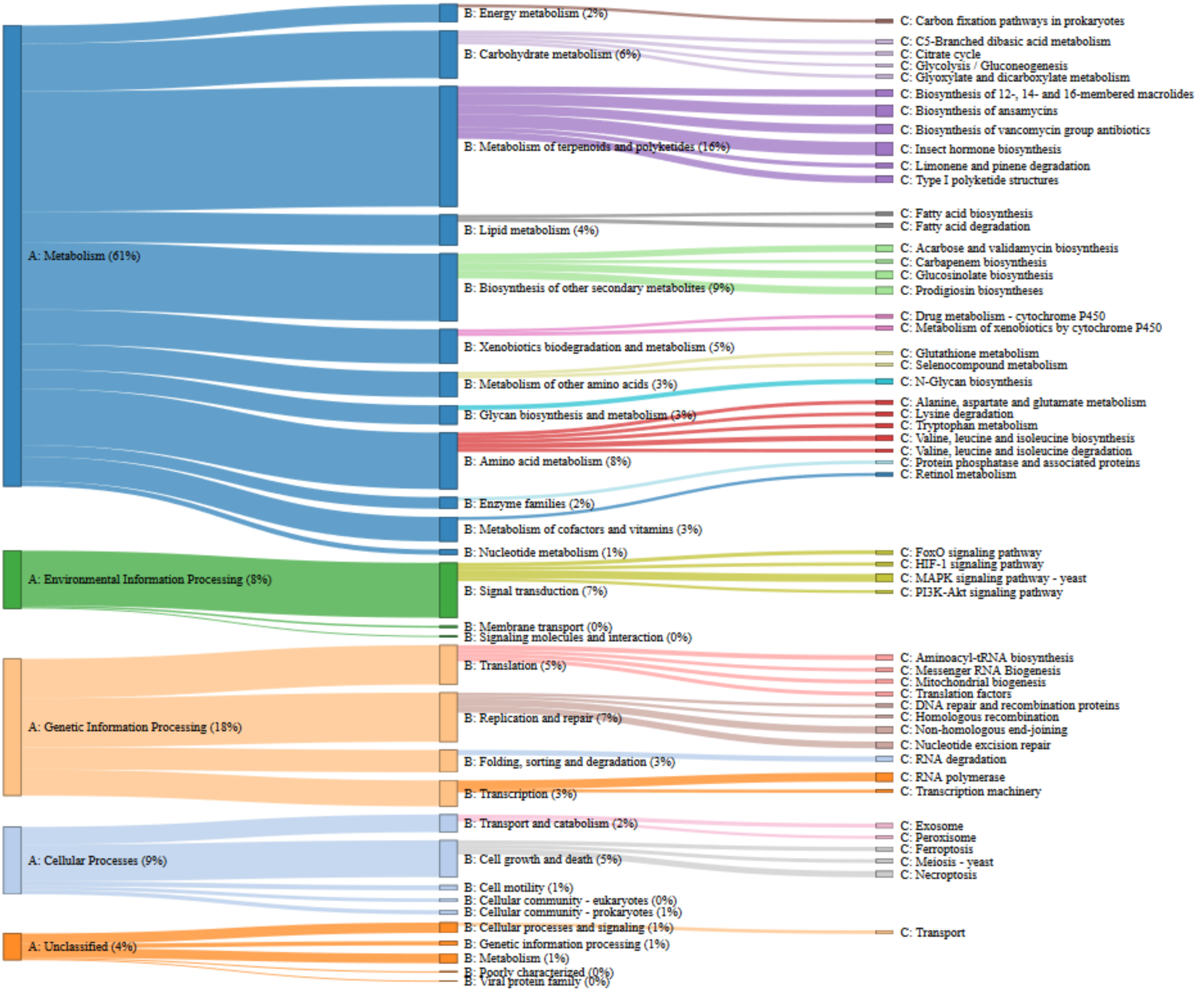
583 **Figure 1.** Relative abundance of the most abundant functional pathways detected across 24 tree
584 phyllosphere samples in a neotropical forest in Panama. Functional pathways are classified using
585 the KEGG functional hierarchy [32].

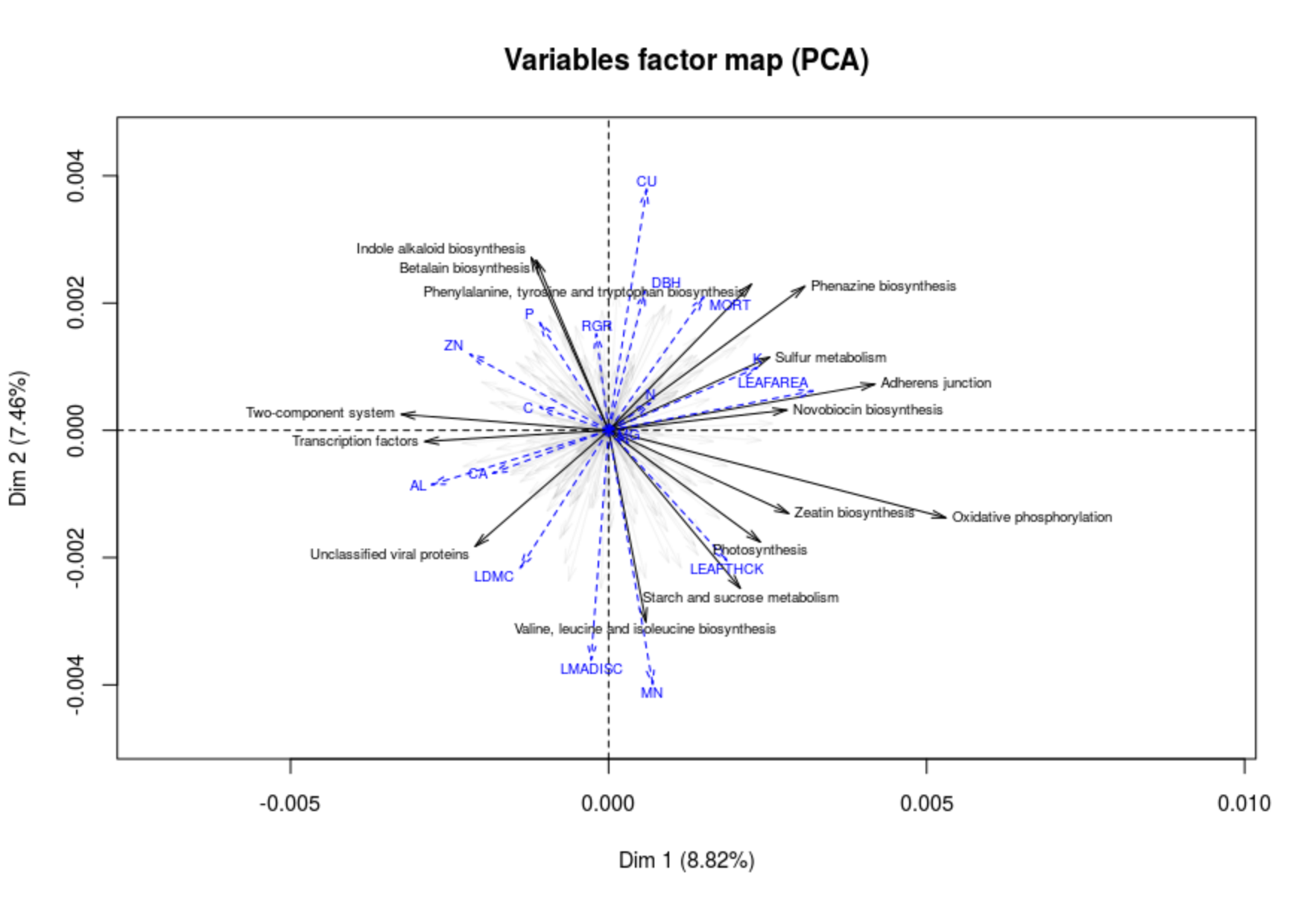
586 **Figure 2.** Principal components analysis (PCA) of microbial functional composition from the
587 phyllosphere of neotropical trees. The 20 Tier 3 functions contributing the most to variation
588 among samples are indicated as black arrow. Plant traits were fitted onto the PCA in a
589 configuration that would maximize correlation with the PCA axes and are represented as blue
590 dashed lines. Plant trait abbreviations are the following: Aluminum (AL), Calcium (CA),
591 Carbon (C), Copper (CU), Diameter at breast height (DBH), Leaf area (LEAFAREA), Leaf dry
592 matter content (LDMC), Leaf mass per area (LMA), Leaf thickness (LEAFTHICK), Manganese
593 (MN), Mortality (MORT), Nitrogen (N), Phosphorus (P), Potassium (K), Relative growth rate
594 (RGR), Zinc (ZN).

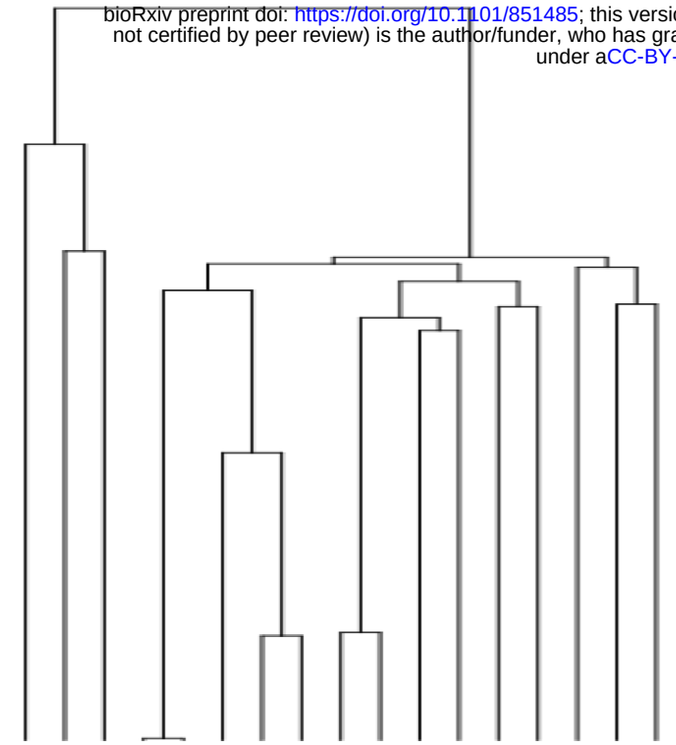
595 **Figure 3.** Distribution of microbial functions with respect to plant phylogeny. Distributions are
596 shown for the subset of Tier 3 microbial functions with significant phylogenetic signal according
597 to the K statistic test ($P<0.05$). Symbol size indicates the scaled relative abundance of microbial
598 functions for each host species.

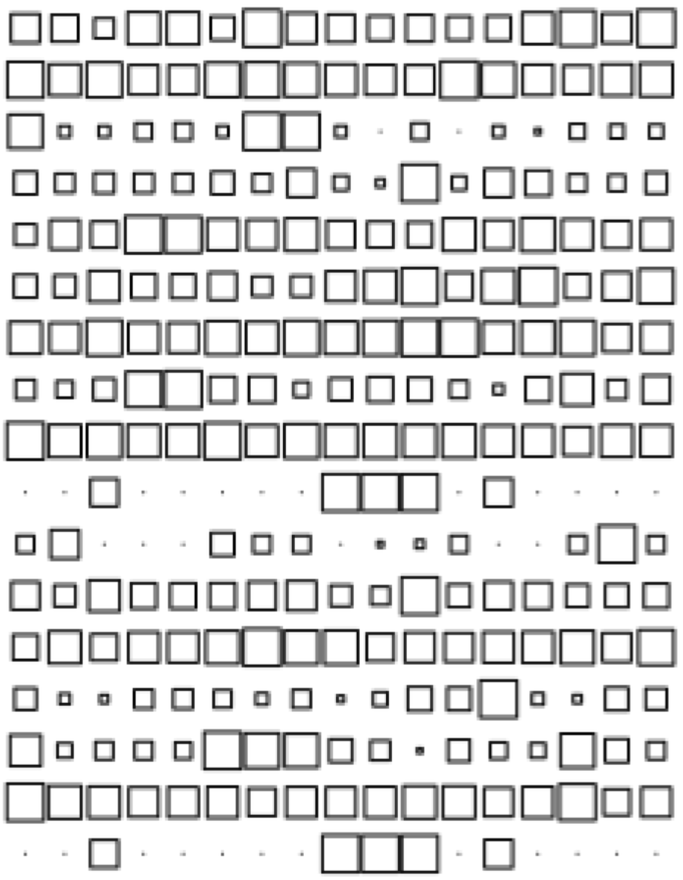
599 **Figure 4.** Log-transformed relative abundance of functions detected in the metagenomic
600 annotations and the 16S functional predictions across 24 tree phyllosphere samples in a
601 neotropical forest in Panama. Relative abundances were evaluated at each of 3 functional
602 classification levels. The red line represents a 1:1 relationship between the relative abundances of
603 functions observed at each site, such that points below the red line represent occurrences of

functions over-represented in the metagenomic dataset, and those above the red line occurrences of functions over-represented in the 16S dataset.


A: Metabolism (61%)
A: Environmental Information Processing (8%)
A: Genetic Information Processing (18%)
A: Cellular Processes (9%)
A: Unclassified (4%)
B: Energy metabolism (2%)
B: Carbohydrate metabolism (6%)
B: Metabolism of terpenoids and polyketides (16%)
B: Lipid metabolism (4%)
B: Biosynthesis of other secondary metabolites (9%)
B: Xenobiotics biodegradation and metabolism (5%)
B: Metabolism of other amino acids (3%)
B: Glycan biosynthesis and metabolism (3%)
B: Amino acid metabolism (8%)
B: Enzyme families (2%)
B: Metabolism of cofactors and vitamins (3%)
B: Nucleotide metabolism (1%)
B: Signal transduction (7%)
B: Membrane transport (0%)
B: Signaling molecules and interaction (0%)
B: Translation (5%)
B: Replication and repair (7%)
B: Folding, sorting and degradation (3%)
B: Transcription (3%)
B: Transport and catabolism (2%)
B: Cell growth and death (5%)
B: Cell motility (1%)
B: Cellular community - eukaryotes (0%)
B: Cellular community - prokaryotes (1%)
B: Cellular processes and signaling (1%)
B: Genetic information processing (1%)
B: Metabolism (1%)
B: Poorly characterized (0%)
B: Viral protein family (0%)
C: Carbon fixation pathways in prokaryotes
C: C5-Branched dibasic acid metabolism
C: Citrate cycle
C: Glycolysis / Gluconeogenesis
C: Glyoxylate and dicarboxylate metabolism
C: Biosynthesis of 12-, 14- and 16-membered macrolides
C: Biosynthesis of ansamycins
C: Biosynthesis of vancomycin group antibiotics
C: Insect hormone biosynthesis
C: Limonene and pinene degradation
C: Type I polyketide structures
C: Fatty acid biosynthesis
C: Fatty acid degradation
C: Acarbose and validamycin biosynthesis
C: Carbapenem biosynthesis
C: Glucosinolate biosynthesis
C: Prodigiosin biosyntheses
C: Drug metabolism - cytochrome P450
C: Metabolism of xenobiotics by cytochrome P450
C: Glutathione metabolism
C: Selenocompound metabolism
C: N-Glycan biosynthesis
C: Alanine, aspartate and glutamate metabolism
C: Lysine degradation
C: Tryptophan metabolism
C: Valine, leucine and isoleucine biosynthesis
C: Valine, leucine and isoleucine degradation
C: Protein phosphatase and associated proteins
C: Retinol metabolism
C: FoxO signaling pathway
C: HIF-1 signaling pathway
C: MAPK signaling pathway - yeast
C: PI3K-Akt signaling pathway
C: Aminoacyl-tRNA biosynthesis
C: Messenger RNA Biogenesis
C: Mitochondrial biogenesis
C: Translation factors
C: DNA repair and recombination proteins
C: Homologous recombination
C: Non-homologous end-joining
C: Nucleotide excision repair
C: RNA degradation
C: RNA polymerase
C: Transcription machinery
C: Exosome
C: Peroxisome
C: Ferroptosis
C: Meiosis - yeast
C: Necroptosis
C: Transport

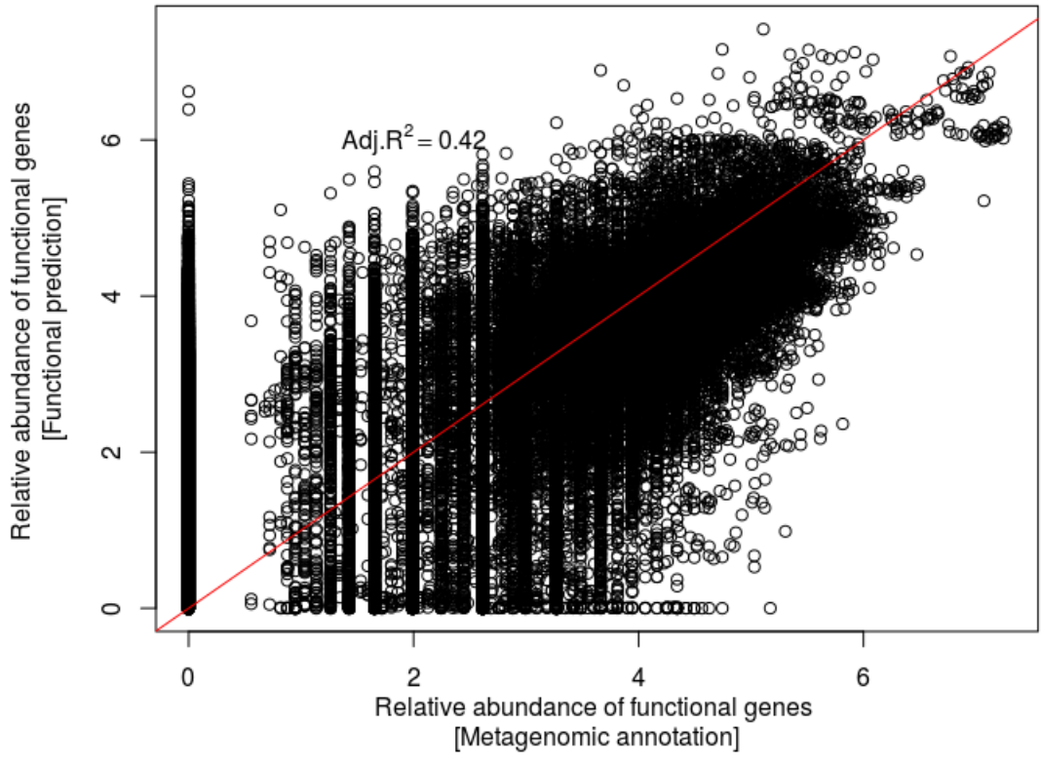

Variables factor map (PCA)
Dim 2 (7.46%)
Dim 1 (8.82%)
0.004
0.002
0.000
-0.002
-0.004
-0.005
0.000
0.005
0.010
CU
Indole alkaloid biosynthesis
Betalain biosynthesis
Phenylalanine, tyrosine and tryptophan biosynthesis
DBH
MORT
Phenazine biosynthesis
P
RGR
ZN
K
Sulfur metabolism
LEAFAREA
Adherens junction
N
C
Novobiocin biosynthesis
Two-component system
Transcription factors
AL
CA
Zeatin biosynthesis
Oxidative phosphorylation
Unclassified viral proteins
Photosynthesis
LDMC
LEAFTHCK
Starch and sucrose metabolism
Valine, leucine and isoleucine biosynthesis
LMADISC
MN

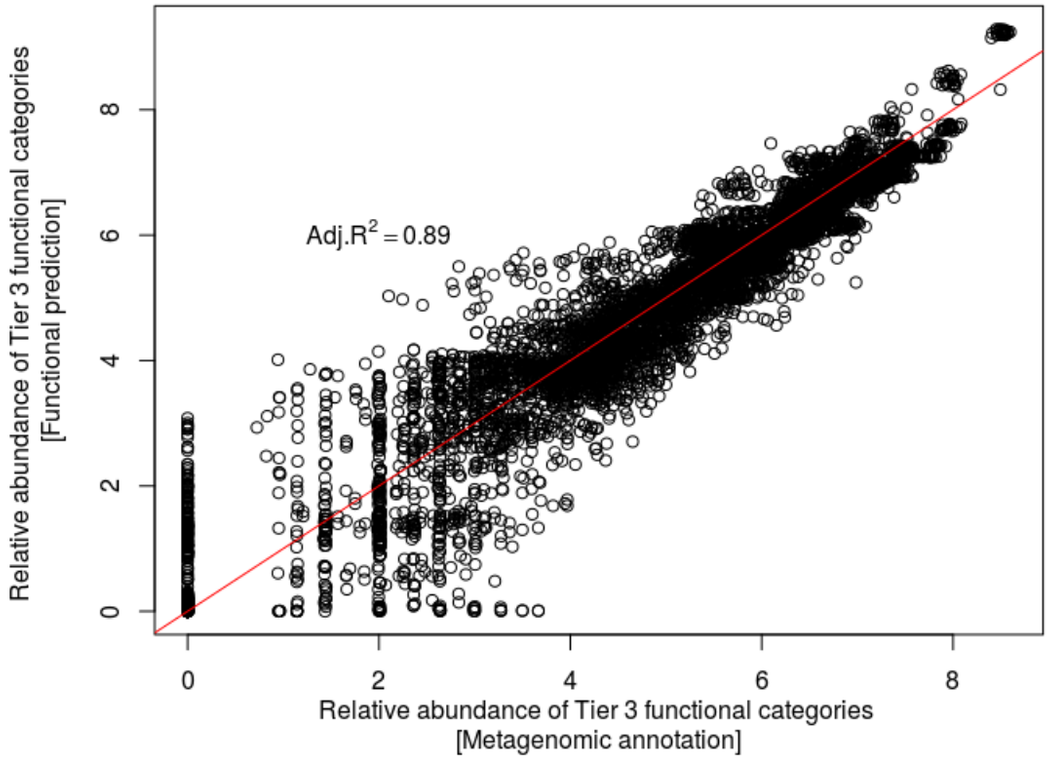


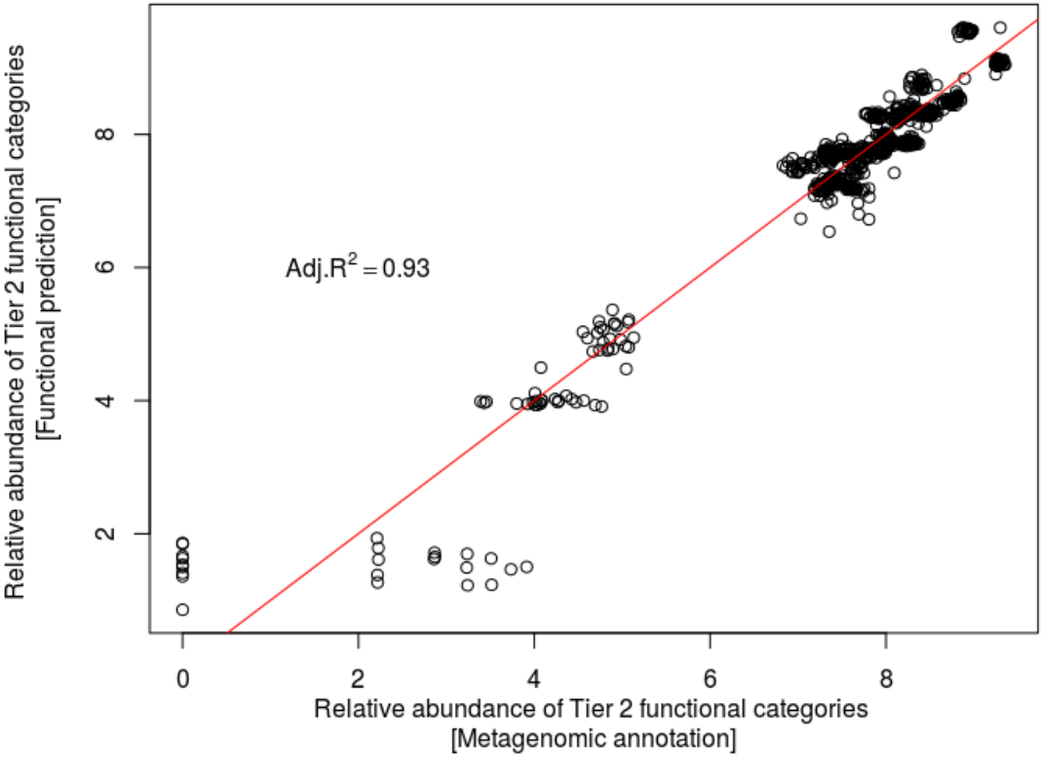


Bacterial toxins
beta Alanine metabolism
Betalain biosynthesis
Biosynthesis of siderophore group nonribosomal peptides
Biosynthesis of unsaturated fatty acids
Ferroptosis
Lysine degradation
Lysosome
Messenger RNA Biogenesis
Notch signaling pathway
Phospholipase D signaling pathway
Polyketide biosynthesis proteins
Protein folding and associated processing
Protein processing in endoplasmic reticulum
Ribosome biogenesis in eukaryotes
Ribosome
Wnt signaling pathway

Piper cordulatum
Ocotea oblonga
Desmopsis panamensis
Eugenia oerstediana
Eugenia nesiotica
Protium tenuifolium
Trichilia tuberculata
Guarea guidonia
Hirtella triandra
Licania hypoleuca
Garcinia intermedia
Rinorea sylvatica
Sorocea affinis
Inga marginata
Heisteria concinna
Pouteria stipitata
Faramea occidentalis


Adj.R^2 = 0.42
Relative abundance of functional genes [Functional prediction]
Relative abundance of functional genes [Metagenomic annotation]
0
2
4
6


Adj.R^2 = 0.89
Relative abundance of Tier 3 functional categories [Functional prediction]
Relative abundance of Tier 3 functional categories [Metagenomic annotation]
0
2
4
6
8


Adj.R^2 = 0.93
Relative abundance of Tier 2 functional categories [Functional prediction]
Relative abundance of Tier 2 functional categories [Metagenomic annotation]
2
4
6
8