Cross-continental biogeography of the common bean rhizosphere microbiome reveals a persistent core membership

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Keywords: plant-microbiome, Phaseolus vulgaris, plant health, neutral model, co-occurrence network, rhizobia, fertilizer, abundance-occupancy, 16S rRNA, ITS, microbial ecology, soil, hub taxa, connector taxa, plant genotype, sustainable agriculture

Significance statement

Ongoing and magnifying consequences of environmental change present a complex challenge for plants, food security and sustainable agriculture. One mechanism by which plants may promote long-term stress tolerance is via beneficial interactions with the microbiome. However, there are thousands of microbiome members in the rhizosphere, and it is difficult to know which engage with the plant. We applied biogeography and assembly concepts from ecology to discover a core rhizosphere microbiome for the globally important legume, common bean. The core microbiome was not influenced by genotype, local soil properties or agricultural management, and was cosmopolitan among beans grown in North and South America. The insights and core approach provided here advance goals to manage microbiomes to support plant health and resilience.

Abstract

Plants recruit soil microbes that provide nutrients, promote growth and protect against pathogens. However, the full potential of microbial communities for supporting plant health and agriculture is unrealized, in part because rhizosphere members key for plant health are difficult to prioritize. Microbes that ubiquitously associate with a plant species across large spatial scales and varied soil conditions provide a practical starting point for discovering beneficial members. Here, we quantified the structures of bacterial/archaeal and fungal communities in the common bean rhizosphere (*Phaseolus vulgaris*), and assessed its core membership across two evolutionarily-distinct genotypes. Beans were grown in field conditions across five major growing regions in the United States. We discovered a conserved core microbiome of 271 bacterial, archaeal and fungal taxa that were consistently associated with the common bean, despite different soil types, management, climates and genotypes. Neutral models of abundance-occupancy relationships and co-occurrence networks suggest that these core taxa are in intimate relationships with the plant,

rather than important members of the local soil microbiome. We expanded our study to leverage rhizosphere samples inclusive of eight additional common bean genotypes that were grown in Colombian soils. Surprisingly, there were 48 persistent bacterial taxa that were detected in all samples, inclusive of U.S. and Colombian-grown beans. Many of the core taxa were yet-uncultured and affiliated with Proteobacteria; these taxa are prime targets for functional investigation in support of sustainable common bean agriculture. More generally, our approach provides insights into microbial taxa that can be prioritized towards translational studies of plant-microbiome. management.

Introduction

Current agricultural practices rely on unsustainable levels of agrochemicals that can harm the environment. Agriculture requires more efficient use of available resources, and the naturally-occurring, soil-dwelling microbiota offers potential to contribute to the responsible intensification of agriculture. Selection and breeding of plants for their beneficial associations with microbiota has promise to deliver a new generation of microbe-improved plants (1–7). The ideal outcome of such efforts would achieve a balance of sustainable agriculture with food security. To achieve this, we must understand the relationships between plant genomes and microbiomes. To date, several studies have investigated the influence of plant genotype on the plant microbiome (8–11). Though there is not complete agreement among these studies, they generally suggest that plant genotype has a limited influence on the microbiome relative to the local environmental conditions, especially when grown in field conditions (12–15) (as opposed to greenhouse or controlled environments (9, 10)). There is clearly more work needed to advance goals of microbe-improved plants.

Common bean (*Phaseolus vulgaris* L.) is the most important food legume grown worldwide, and especially for developing economies in South America, Africa and Asia (16). The origin of common bean is nowadays central Mexico, and from there it spread to central and south America around 165,000 years ago (17). This resulted in the development of two major eco-geographically distinct common bean gene pools with partial reproductive isolation (18–20). The Mesoamerican gene pool was distributed from northern Mexico to Colombia and the Andean gene pool ranged from southern Peru to northwestern Argentina. Since 8,000 years ago, each pool was separately and selectively bred, leading to further diversification between them (21–23). Because of pre-existing genetic differences in each gene pool followed by divergent breeding history, common bean presently offers a distinctive model for understanding crop evolution and plant domestication on plant-microbiome interactions.

The objective of this study was to quantify the influences of genotype and cross-continental biogeography on the common bean root microbiome, inclusive of bacteria, archaea and fungi. We also aimed to identify core microbial lineages that have cosmopolitan associations with common bean and therefore potential as targets towards microbe-improved plant breeding. With the cooperation of U.S. common bean breeding programs, we executed a first-of-its kind study of two divergent bean genotypes grown under field conditions in five major North American bean growing regions. We additionally performed a meta-analysis of the bacterial microbiome with bean genotypes grown in South America (9). Surprisingly and excitingly, we discovered a core bean rhizosphere microbiome of a handful of members that persistently and consistently associated with this nutritionally, agronomically, and economically important crop. This core was discovered in spite of apparent rhizosphere microbiome divergences that were attributable to differences in local soil conditions and management. We did not detect an influence of plant genotype, suggesting that this core membership supersedes it.

Results and Discussion

Study design, sequencing summary, and alpha diversity

To assess the influence of plant genotype and environment on the common bean rhizosphere microbiome, we designed a biogeography study of two divergent bean genotypes (from Mesoamerican and Andean gene pools (24, 25)), both grown in the field at five research and extension farms that represent major U.S. bean production regions (**Table 1**) (26). From each growing location, triplicate bean plants grown in three to four plots were harvested at flowering (mid to late July).

We sequenced 39 rhizosphere (attached to the root, see methods) and root-associated soil samples using 16S rRNA gene and ITS1 region, respectively to assess microbiome community structures. We assessed the bacterial and archaeal communities using 16S rRNA gene amplicon sequencing, and the fungal communities using ITS1 amplicon sequencing. After clustering reads to OTUs at 97% similarity threshold and filtering chloroplast and mitochondria, there was 31,255 to 506,166 16S rRNA reads per sample and 22,716 to 252,810 ITS reads. We rarefied samples to 31,255 reads for 16S rRNA gene amplicons and to 22,716 for ITS. With these thresholds, we achieved richness asymptotes for both datasets, suggesting that sequencing efforts were sufficient to capture comparative dynamics and diversity (**SI Appendix, Fig. S1**). The total richness observed at this rarefaction depth was 1,505 fungal and 23,872 bacterial and archaeal OTUs.

As reported in other rhizosphere studies, the total fungal diversity was lower than bacterial /archaeal diversity in the rhizosphere of the common bean (27–29). Richness varied by growing location (ANOVA, F value=12.4, p-value<0.0001 and F value=13.1, p-value<0.0001 for 16S rRNA and ITS data, respectively, **SI Appendix, Fig. S2**).

Beta diversity: the impact of biogeography and plant genotype

There were differences across growing locations in edaphic soil properties (e.g. pH, nitrogen species, organic matter) as well as in management practices (e.g. fertilization, irrigation, crop rotation) and climate (**Table 1**). Growing location, pH and fertilization explained differences in microbial community structure for bacteria/archaea and fungi, but plant genotype did not (**Fig. 1AB**, **SI Appendix, Dataset S1**). Fungal and bacterial/archaeal communities had synchronous biogeographic patterns (Procrustes m12 squared: 0.2137, R^2=0.8867 and *p*-value=0.001), supporting that both communities are shaped by local edaphic factors. An effect of growing location on beta-diversity has been previously reported for plant- and soil- associated microbiomes (11, 13, 14, 30).

We selected two distinct bean genotypes with divergent evolutionary and breeding histories to assess the impact of plant genotype on the root microbiota, expecting that any signal would be maximized between these lineages. Briefly, these two genotypes belong to the two major lineages of common bean that diverged and bottlenecked ~165,000 years ago, and represent the Mesoamerican (Eclipse) and Andean (CELRK) gene pools (17). There was a weak interaction between plant genotype and growing location in explaining fungal community structure (PERMANOVA, R^2=0.0377, *p*-value=0.001) (**SI Appendix, Dataset S1**), but no effect of plant genotype alone on the bacterial and archaeal communities, even when controlling for growing location. Similarly, plant genotype had a weak but statistically detectable effect on bacterial and archaeal richness (*p*-value=0.019) but no measurable effect on fungal richness (*p*-value=0.95). (**SI Appendix, Fig. S2CD**). An analysis of differential taxon abundances across genotypes (DESeq2 (31)) detected 20 taxa out of 21,881 total that were distinguishing between CELRK and Eclipse, but their adjusted *p*-value were marginally significant (> 0.02; **SI Appendix, Dataset S2**). The absence of a robust genotype effect on the microbiome is in contrast to a previous study on common bean (9). However, several studies have reported a relatively weak to no influence of

genotype more generally on the rhizosphere communities of other plants grown in the field (14, 30, 32). The study that observed a genotype effect on the bean root microbiota included wild, domesticated and landrace common beans of Mesoamerican origin that were grown in a greenhouse experiment using Colombian field soil (9). The authors attributed those bean genotype differences to differences in root architecture, which often has a different phenotype in greenhouse pots than in the field (33). We summarize that, in this study and in agreement with other field studies, plant genotype has a minor to no measurable impact on rhizosphere microbiome.

A core rhizosphere microbiome across U.S. bean growing regions

The common bean rhizosphere microbiome included the major expected lineages for both bacteria and fungi (**SI Appendix, Fig. S3AB**), in agreement with other plant rhizosphere studies (10, 15, 34–37). Together with previous studies, these data provide more evidence that root-associated microbial taxa are phylogenetically and functionally conserved (38). Proteobacteria, Acidobacteria, Bacteroidetes, and Actinobacteria collectively comprised on average 73.5% of the bacteria/archaeal community, Ascomycota dominated the fungal community with a mean total relative abundance of 53% with notable sample-to-sample variance (range from 16.5% to 84.5%).

Despite differences in the rhizosphere microbiomes across growing locations, we noticed a large number of OTUs that were shared for the bacterial/archaeal communities (2,173 taxa, mean 31.5%, range 29.5% to 34.7%). There was a smaller but notable overlap for the fungal communities (70 taxa or mean 4.5%, range from 0.9% to 17.9%; **SI Appendix, Fig. S3CD**). These data suggested that, despite measured edaphic differences across growing locations and strong biogeographic signal, the common bean rhizosphere recruited many similar taxa that could be functionally important for the bean. Therefore, we explored occupancy-abundance distributions of taxa ((39, 40) and references therein) to infer the core bean microbiome of taxa with an occupancy

of 1 (i.e., found in all samples and across all growing locations; **Fig. 2AB**). Among bacteria and archaea, 258 phylogenetically diverse taxa were cosmopolitan in the dataset (**Fig. 2C**), including numerous and abundant Proteobacteria (117 OTUs) with a dominant taxon classified as *Arthrobacter* sp. (FM209319.1.1474, mean relative abundance of 1.43%). The bacterial/archaeal core also contained taxa of interest for potential plant benefits (e.g. *Sphingomonas*, *Rhizobium*, *Bacillus, Streptomyces*), as well as some genera that can be associated with disease (e.g. *Ralstonia*). There were 13 taxa in the fungal core (**Fig. 2D**), and these were largely composed of Ascomycota (10 OTUs), with dominating taxon OTU823 from the *Phaeosphaeriaceae* family (mean relative abundance 10.1%). Notably, taxa that were specific to either bean genotype were relatively rare and inconsistently detected (**Fig. 2**, orange and black points). Together, these results suggest that common bean, regardless of the genotype, consistently recruits particular taxa.

The neutral expectation of abundance-occupancy distributions is that very abundant taxa will have high occupancy, while rare taxa will have low (40–43). Taxa that deviate from the neutral expectation are more strongly influenced by deterministic factors, like environmental conditions, than by stochastic factors, like drift and dispersal. The neutral model fit (41, 42) of the abundance-occupancy distribution (solid line, **Fig. 2AB**) identified several taxa that had frequencies either above or below the 95% confidence intervals of the model (dashed lines). Specifically, 13.7% of the bacterial/archaeal and 30.4% of fungal taxa, deviated from the neutral expectation (**SI Appendix, Dataset S3**). One hundred and seventy-one core taxa were predicted above the neutral model partition; these deterministically-selected taxa are prime candidates for follow-up studies of their interactions with the host plant. Overall, the bacteria/archaea community had better fit to the neutral expectation than fungal (R^2 of 0.74 and 0.34, and migration rates (m) of 0.301 and 0.003, respectively), suggesting that dispersal was relatively less limiting for the bacteria/archaea than for

the fungi. This finding agrees with other work suggesting that fungi are more sensitive to local climate or more dispersal limited than bacteria (44–47).

A cross-continental core rhizosphere microbiome for common bean

We wanted to better understand if these U.S. core taxa were associated with the bean rhizosphere across a larger geographical scale, which would suggest the potential for selective plant recruitment and cosmopolitan distribution of core taxa. Therefore, we compared our U.S. data to a recently published study of rhizosphere bacteria and archaea from common beans grown in Colombian soil (9). The Colombian study offered a key contrast because it included eight divergent bean lineages, including wild (n=2), landrace (n=1), and cultivated genotypes (n=5), grown in soil from a different continent that has starkly different climate and management from the U.S. growing regions. To enable direct comparison, we re-analyzed raw reads and compared the datasets by matching to either the same taxon identifiers when clustered to SILVA database, or 100% identity by BLAST to *de novo* clustered reads (see Materials and Methods). Surprisingly, 39.6% (3,359 OTUs) of rhizosphere taxa from the Colombian-grown beans were also shared with the U.S. dataset (**Fig. 3AB**). Both datasets included taxa that were highly represented in the other: 62% of U.S. core (159 out of 258) were found also in Colombia, and 51% of Colombian core (433 out of 848) were shared with the U.S. (**Fig. 3B**). Core taxa were again defined stringently with an occupancy of 1, and 48 taxa were found across all samples, inclusive of both datasets. These core taxa were composed of many Proteobacteria, with *Rhizobiales* showing the most consistent relative abundance between the studies (**Fig. 3C,** e.g. 0.187% and 0.138% in Colombia and U.S. dataset, respectively). None of the cross-continental core taxa were universally detected in very high abundance, and all but two OTUs (a U.S. *Arthrobacter* sp. and a Colombian *Austrofundulus limnaeus* with mean relative abundances of 1.43% and 1.01%, respectively) would be classified as

rare by a typical mean relative abundance threshold below 1%, hinting to a potential role of rare taxa in providing key functions. Notably, only 48% of these cross-continental shared core taxa have genus classification, suggesting that most of them are under-described in their functional potential and interactions with plants.

We used a 97% sequence identity to conservatively define the core membership, but we wanted to explore whether there was more resolved underlying taxonomic structure in the core microbiome members. Therefore, we also analyzed reads associated with the global core taxa with the UNOISE3 pipeline (48) to generate predicted biological sequences (zero-radius OTUs – ZOTUs with100% sequence identity) and provide the maximal possible biological resolution. We found that the global core taxa consisted of 422 ZOTUs, and that there was a range of 2 to 35 ZOTUs identified within each OTU (**SI Appendix, Fig. S4**). With one exception (HQ597858.1.1508), all of core OTUs contained at least one ZOTUs that also had an occupancy of 1. In addition, all of the ZOTUs with an occupancy of 1 were also the most abundant ZOTU within each OTU (**SI Appendix, Fig. S4**). This result is important because it suggests that the same members constitute the core, even with increased taxonomic resolution. In summary, these results show that common bean can associate with a core set of rhizosphere microbiome members at taxon and ecotype levels, across diverse bean genotypes and across continents.

Inter-kingdom network analysis of the bean rhizosphere microbiome

Returning to the U.S. dataset that included both bacterial and fungal data, we wanted to better understand the potential interactions of core taxa. We were particularly interested in fungal-bacterial co-occurrences because of reports of their potential benefits for the plant (49–51). We applied a co-occurrence network analysis to explore these patterns (52). We merged rarified 16S and ITS datasets, filtered the datasets to include taxa with occupancy greater than or equal to 50%,

and considered only interactions significant at *p*-value<0.05 and LA score greater or equal to 0.88. The resulting network included 572 taxa (nodes) and 1,857 statistically significant interactions (edges) structured among 52 modules. Most of the modules were relatively small, with only six including more than 10 nodes (**Fig. 4**). The network was scale-free and had small-world characteristics as indicated by the node degree distribution fitting to the power law model (R^2= 0.993), and also had significant deviation of the modularity, length and clustering coefficients from those calculated from random network (same number of nodes and edges), respectively (**SI Appendix, Dataset S4**).

The topological role of each taxon within the network was determined by the relationship between their within-module (Zi) and among-module (Pi) connectivity scores (**Fig. 4A**) (53). Based on this, the majority of taxa were peripheral (potentially, specialists; 563 nodes). There were also 3 connectors and 6 module hubs, but no network hubs. Indeed, supporting the conclusions of the beta-diversity analysis (**Fig. 1**), there was a strong geographic signal in the largest four modules, and these were comprised mostly of bacterial-bacterial (rather than bacterial-fungal or fungal-fungal) hypothesized interactions (**Fig. 4B**). Interestingly, cross-kingdom interactions constituted only a small fraction of all co-occurrences (n=168; bacteria-fungi=156, archaea-bacteria=6 and archaea-fungi=6).

The analysis identified 26 co-occurrences between core taxa. However, we were surprised to find that only two bacterial core and no fungal core taxa were also classified as network hubs (taxa that connect to many other taxa within a modules) or connectors (taxa that connect across modules). As exceptions, core *Chitinophagaceae* taxon FR749720.1.1492 was a module hub node and a *Nitrobacter* sp. GDHX01215817.4.1477 was a connector. However, the lack of overlap between core and hub/connector taxa is important because hubs and connectors are often hypothesized to play important roles for the plant (50, 54, 55). Our results, inclusive of a dataset of

divergent plant genotypes and broad biogeography, suggest that while hub and connector taxa may be important for the maintenance of the root microbiome, these taxa are not consistently detected in the common bean rhizosphere and, by deduction, could not be of universal importance for the host plant. Our study cannot speak to the potential for functional redundancy among hub or connector taxa, which could ultimately suggest a functional core among phylogenetically diverse taxa (56). However, it is expected that more resolved taxonomy also will reveal functional redundancy at the sub-OTU level among core members. Taken together, these results suggest that core taxa likely are important for the plant, while hub and connector taxa are important for the integrity of the soil microbial community and its responses to the local environment.

Signatures of fertilizer on the rhizosphere microbiome

As a legume, common bean can form symbiotic relationships with nitrogen-fixing bacteria (mainly, *Rhizobium* sp.). However, as compared to other legumes of agricultural importance (e.g., soy, chickpeas), common bean is inefficient in that a relatively lower percentage of its total nitrogen can be attributed to that fixed by symbionts (57). As a consequence, many U.S. growers provide nitrogen fertilizer to common bean crops. Not only is nitrogen fertilizer an expense to the grower, but its use also contributes to global emissions of nitrous oxide, a powerful greenhouse gas (58). It is of interest to decrease use of nitrogen fertilizer in agriculture, but especially in legumes that are capable of acquiring nitrogen through their symbiotic relationships with bacteria.

Our study included research farms that used different fertilizer types, including conventional synthetic fertilizer, organic application, and no added fertilizer. Fertilizer type explained 35% variance of the differences in the microbiome communities across growing locations (**SI Appendix, Dataset S1**). Additionally, changes in bacterial, archaeal and fungal communities were more strongly correlated with nitrate concentrations than with total nitrogen or

ammonium concentrations (**SI Appendix, Dataset S1**). Therefore, we wanted to explore the U.S. dataset for rhizosphere taxa that were indicative of fertilizer type, which would be suggestive of conditional plant preference or competitive advantage and could ultimately provide hints as to which taxa are most sensitive to changes in nitrogen management. We assessed differentially abundant taxa between each fertilizer pair (p-value < 0.001; **SI Appendix, Figure S5**), and also applied indicator species analysis to identify taxa that were characteristic of particular fertilizer types (**Fig. 5**) (59). In summary, synthetic fertilizer enriched for 198 taxa, organic for 95 taxa and no fertilization for 66 taxa (**Fig. 5**). Organic fertilization proportionally enriched for taxa belonging to the Gammaproteobacteria relative to other treatments, whereas synthetic fertilization was associated with their depletion, in agreement with previous studies (60, 61). In contrast, Alphaproteobacteria were enriched in soils without fertilization, but were detected only in relatively low abundance (**Fig. 5**). Interestingly, some taxa with potential to fix atmospheric nitrogen (i.e. *Rhizobium*, *Bradyrhizobium*, *Burkholderia-Paraburkolderia*) were more abundant in the fertilized sites (**Fig. 5**). This is surprising considering the importance of these lineages for plants in unfertilized soils. *Rhizobium* sp., which have specific symbiotic interactions with common bean (62, 63), were well represented in all growing locations and not statistically affected by fertilization type or plant genotype, but instead by soil conditions and management (ANOVA, p-value=0.019) (**Fig. 5**). Anecdotally, we observed substantial nodulation only on plant root sampled at Saginaw Valley, MI, the only location where irrigation was not available (**Table 1**).

A caveat to these analyses is that there was not cross-farm replication of each fertilizer type, and so these results should be considered a first observation towards understanding the selective consequences of fertilizer type on the bean rhizosphere microbiome. Broadly, these results provide first insights as to taxa that could be targeted towards microbiome management to decrease reliance on nitrogen fertilizer.

Prospectus

Legumes like common bean that form symbiotic relationships with nitrogen fixing bacteria are a crucial component of sustainable agriculture. Growing common bean can both improve soil fertility (62, 64) and contribute to food security, and its production has reached more than 31 million metric tons worldwide (http://www.fao.org/faostat/, accessing on July 12, 2019)). However, common bean yield estimates, production areas, and even nutritional value, are predicted to decrease in this century due to the impacts of global change (65–67). Thus, breeding or other improvements to common bean is needed to make this important crop resistant to stressors associated with climate change while increasing yields to meet higher demands. To date, only traditional plant breeding approaches have been used for the common bean, exploiting the genetic diversity of the plant, but the latest knowledge of the plant microbiome in supporting host well-being has opened new avenues for breeding the next generation of crops with plant microbiome manipulation (68).

Here, we show that common bean genotype has marginal effect on the rhizosphere microbiome grown in field conditions. We describe for the first time the common bean core microbiome at continental and cross-continental scales. A majority of the 48 bacterial taxa cosmopolitan in the common bean rhizosphere are under-described or largely unknown, as only 23 out of 48 have genus classification. But, due to their ubiquitous association with the bean, we hypothesize that these taxa provide functions that are crucial for common bean health. A next step is to understand functions associated with these taxa and to determine if and how they contribute to plant health and productivity under different growth conditions, such as drought or with particular management strategies, as well as over plant development (32, 69). Additionally, targeting persistent microbiota that are part of a native assemblage for management and manipulation can be advantageous, as these are expected to be both host and environment adapted and thus may be

more persistent *in situ* than external or non-native biocontrols (70). In conclusion, this work provides insights and approaches for prioritizing plant-microbiome members that will advance goals in microbiome management and microbe-improved crops.

Material and Methods

Sampling and soil physicochemical analysis

We selected two common bean cultivars with very distinct genotypes: Eclipse (24) with Mesoamerican origin and California Early Light Red Kidney (CELRK) and old kidney bean landrace with Andean origin (25). The plants were grown at 5 different locations on research extension farms: Saginaw Valley, Montcalm county (both in Michigan), Scott Bluff county (Nebraska), Fort Collins (Colorado) and Othello (Washington), each representing an important U.S. common bean growing region (26). Both bean genotypes were grown in the same locations except in Michigan, where CELRK was grown at Saginaw Valley and Eclipse at Montcalm county. Plants ($n \geq 3$) were harvested at flowering from replicated plots ($n \geq 3$) at each location. The flowering time point was selected because we wanted to analyze mature microbial communities as influenced by the plant and not by plant development. Plant roots with attached soil were packed into bags, stored on ice and shipped immediately to Michigan State University for processing.

From each sample, root attached soil was collected and used as rhizosphere soil and remaining soil was collected and used as bulk soil. Both soils were sieved through 4 mm sieves to remove any organic, plant debris and larger soil minerals before soil analysis and DNA isolation.

Soil analysis was done at the Michigan State Soil and Plant Nutrient Laboratory and included following parameters: pH, phosphorous (P ppm), potassium (K ppm), calcium (Ca ppm), magnesium (Mg ppm), organic matter content (OM%), nitrate (NO_3^- ppm), ammonium (NH_4^+ ppm)

and total nitrogen (%). Soil analysis was done on bulk soil samples pooled by plant genotype and plot.

DNA isolation and sequencing

DNA was isolated using DNeasy PowerSoil kit (QIAGEN, US) by following the manufacturer's recommendations. Quality and quantity of isolated DNA was checked with Qubit 2.0 fluorometer using HS dsDNA Assay kit (ThermoFisher, US). Presence of 16S rRNA gene was confirmed by PCR using the V4 16S rRNA specific sequencing primer set 515f and 806r (71) and then visualizing the PCR products by gel electrophoresis. Because samples from Nebraska were sent in bags by plot and genotype, we pooled all other samples by plot and did not sequence separately by plant. All 16S rRNA samples were prepared by the Michigan State Genomics Core Research Support Facility. Their standard protocol included 16S rRNA gene PCR amplification and library preparation. The ITS samples were amplified in our lab using the primer pair ITS1f and ITS2 (72) with index adapters as instructed by the Genomics Core (https://rtsf.natsci.msu.edu/genomics/sample-requirements/illumina-sequencing-sample-requirements/, June 2019), The Genomics Core used the Illumina TruSeq Nano DNA library preparation kit for both 16S and ITS libraries. Paired-end, 250-bp reads were generated on an Illumina MiSeq platform using v2 Standard 500 cycle kit, and the Genomics Core provided standard Illumina quality control, adaptor, barcode trimming, and sample demultiplexing.

16S rRNA and ITS amplicon processing

For both datasets, raw reads were merged, quality filtered, dereplicated, and clustered into 97% identity operational taxonomic units (OTUs) using the UPARSE pipeline version 11 (73). Reads not matching to the SILVA database were used for de-novo clustering at 97% identity.

Reference picked and de-novo reads were combined before taxonomy was assigned. Taxonomic annotations for 16S rRNA OTU representative sequences were assigned in the QIIME 1.19 environment (74) using SILVA rRNA database release v128 (75). ITS OTU representative sequences were taxonomically annotated using the CONSTAX tool (76) with the UNITE database (version 7.2) (77). OTUs with unassigned taxonomy at the domain level and OTUs annotated as mitochondria or chloroplasts were removed.

Statistical analysis

Alpha and beta diversity analyses were performed to datasets subsampled to the minimum observed quality filtered reads per sample (31,255 for 16S rRNA and 22,716 for ITS). Due to low read counts one sample (SVERC1) was removed from the 16S rRNA dataset. We report richness as total number of OTUs clustered at 97% sequence identity. Differences among groups (plant genotype, growing location, fertilization, pH) were assessed using analysis of variance (ANOVA), with a Tukey *post hoc* test for multiple comparisons. We used the *protest* function in the vegan package in R (78) to test for synchrony between bacterial/archaeal and fungal communities.

To quantify the variation in community composition, we calculated pairwise Bray-Curtis distances. Permutational multivariate analysis (PERMANOVA) using 1,000 random permutations was used to test hypothesis of beta diversity using *adonis* function in the vegan package in R (78).

Differential abundance and indicator species analysis

We used DESeq2 (31) to determine bacterial and archaeal taxa with differential abundance across plant genotypes and fertilization types. Thresholds for calling taxa as differentially abundant between selected groups was set at p-value of 0.05 and 0.001 or lower for plant genotype and

fertilization types, respectively. Taxa that were differentially abundant between fertilization types were then used for indicator species analysis using *labdsv* package in R (79).

Defining continental and cross-continental core rhizosphere microbiota of common bean

For cross-continental core, we first downloaded and processed raw reads from a study that investigated the influence of common bean genotype on the rhizosphere microbiome composition and included many Mesoamerican genotypes but used only one soil type, greenhouse conditions for growth of the plants, and focused only on the bacterial and archaeal communities (9) (NCBI BioProject ID PRJEB19467). We processed the raw sequences as described above, and despite differences in our pipeline and the originally published pipeline, we generated very similar amount of OTUs (12,209 instead of 12,293 (9)). Because the studies used different sequencing primers, we identified the identical OTUs by matching the IDs as given by the SILVA database v128 (75) for all reference clustered OTUs. For the *de-novo* clustered OTUs, we used BLAST (80) to identify 100% matches between the two datasets, across the overlapping V4 region of reads from both studies. As before, we calculated occupancy of every taxon in the rhizosphere datasets and identified those with occupancy of 1. Taxa with the same OTU IDs or 100% BLAST match were designated as the cross-continental core rhizosphere microbiota of the common bean.

Neutral model and distance-decay analysis

To assess the importance of neutral process in the assembly of common bean rhizosphere communities we applied the Sloan neutral model (81). This model predicts the relationship between the frequency with which taxa occur in a set of local communities (occupancy) and their mean abundance across a broader metacommunity. The prediction is that rare taxa will be lost from individual hosts due to ecological drift, but abundant taxa will be more widespread in a

metacommunity due to higher chance of dispersal and thus be randomly sampled by individual hosts.

The occupancy of OTUs and their mean relative abundances across the metacommunity were fitted to the model, using the R code described by Burns et al. (42). We used the following parameters of neutral model: 95% confidence intervals, the goodness of fit of the neutral model (R^2), and the estimated migration rate (m).

Co-occurrence network analysis

Global network properties were calculated using the Molecular Ecological Network Analysis Pipeline (MENAP) (52). The majority was set to 0.5, missing data were kept blank, read counts were converted by the logarithm, and the Pearson correlation coefficient was used as the similarity measure. The results were then filtered by keeping pairwise correlations with absolute LA values greater or equal to 0.88. To determine the modularity of the network we created 100 random networks within MENAP using the same number of nodes and edges as the complete potentially active network. The general properties of the inferred networks were analyzed using NetworkAnalyzer in Cytoscape (82, 83).

The connectivity of each node in the network was calculated using within-module connectivity (Zi) and among-module connectivity (Pi) scores which defined the topological role of each node (taxon) (52, 53). We classified our nodes as per the four categories describing node topology: *network hubs* (highly connected nodes within the entire network, Zi > 2.5 and Pi > 0.62), *module hubs* (highly connected nodes within modules, Zi > 2.5), *connectors* (nodes that connect modules, Pi > 0.62), and *peripheral* nodes (nodes connected in modules with few outside connections, Zi < 2.5 and Pi < 0.62) (53).

Cytoscape v.3.5.1 (82) was used for visualization of significant co-occurrences and editing the appearance of nodes size, shape and color based on the number of connections (degrees), taxonomic affiliation and module, respectively.

Acknowledgments

This work was supported by the Plant Resilience Institute at Michigan State University. We are grateful to Dr. James Kelly, Dr. Phillip Miklas, Dr. James J. Heitholt, Dr. Carlos Urrea Florez, Dr. Mark A. Brick, Dr. Juan M. Osorno, Dr. Thomas H Smith and other supporting staff at the research extension farms for their partnership in this study. We would also like to thank the Michigan State Genomics Core Research and Technology Support Facility and the Institute for Cyber-Enabled Research High Performance Computing Center for excellent support and service.

Footnotes

Author contributions: N.S. and A.S. designed research, analyzed data and wrote the paper; and N.S. performed research.

The authors declare no conflict of interest.

Data deposition: The raw sequence data have been deposited in the NCBI Sequence Read Archive (BioProject accession no. PRJNA524532). All read processing steps, bioinformatic workflows, and custom scripts are available on GitHub (https://github.com/ShadeLab/PAPER_Stopnisek_2019_BeanBiogeography).

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Figure Legends

Fig. 1: Growing location drives bacterial/archaeal (A) and fungal (B) microbiome structure of the common bean rhizosphere. The principal coordinate analysis (PCoA) is based on Bray-Curtis distances. Growing location is indicated by color and plant genotype is indicated by shape shapes (diamond=CELRK, circle= Eclipse, square=bulk soil). The strength of statistically significant (*p-value* < 0.01) explanatory variables are shown as the length of fitted vectors.

Fig. 2: Abundance-occupancy distributions were used to identify core members of the rhizosphere microbiome for bacteria/archaea (A) and fungi (B). Taxa exclusive to a genotype are indicated in orange (CELRK) or black (Eclipse), and taxa shared across both genotypes are white. The solid line represents the fit of the neutral model, and the dashed line is 95% confidence around the model prediction. Taxa with an occupancy of 1 (i.e., detected in all samples) were considered members of the core. Relative abundance of these taxa is represented as boxplots, grouped by order and number of taxa therein (C, D). Panels C and D are color-coded by phyla.

Fig. 3: A cross-continental core rhizosphere microbiome. There were 3361 shared bacterial/archaeal taxa across U.S. and Colombia rhizosphere samples, suggesting highly similar recruitment across continental scales (A). Forty-eight taxa were detected in all samples of both datasets, as depicted by the Venn diagram (B), and included many Proteobacteria (C). Relative abundance of the 48 U.S.-Colombia core taxa is represented as boxplots (left panel), grouped by order and dataset (Colombia/U.S.). Number of taxa per order is represented as bars (right panel). Boxplots and bars are color-coded by phylum.

Fig. 4: Network co-occurrence analysis shows that core rhizosphere microbiome members are predominantly classified as peripheral taxa that are weakly connected, and generally clustering by the growing location. The network depicts only clusters of modules that were connected by more than 6 nodes (A). Nodes shape is representing domain associations (archaeal, bacterial or fungal) and node size is proportional to its total number of connecting edges (A). The four largest modules, are generally reflective of community biogeography and distinguished by color (A). The within- (Zi) and among- (Pi) module connectivity plot was used to identify module (Pi<0.62, Zi>0.2.5) or network hub taxa (Pi>0.62, Zi>2.5), as well as connector (Pi>0.62, Zi<2.5) and peripheral taxa (B). The density plots surrounding the Zi-Pi plot represent core (green) and non-core taxa (black) (B). The relative abundances of taxa within each module is represented in box plots (C).

Fig. 5: Effects of fertilization on the rhizosphere microbiome of the common bean. Proteobacterial taxa with significant deviation in relative abundance between fertilization treatments were determined by the indicator species analysis (*indval* (84)). Ninety-five taxa were indicators of organic fertilizer (manure, urea), 66 were indicators of no fertilizer, and 198 of synthetic fertilizer (A). Presence-absence of indicator taxa is represented in the panel B, with symbol size indicating their numbers if present (B) and their relative abundance in panel C. Number of indicator taxa per taxonomic affiliation is reported in the brackets (C).

Table 1: Description of geographical, managemental and soil properties of the common bean growing locations included in the study.

Sample same	Growing location	Elevation	Bean genotype	Weather (T, precipitation)*	Rotation history	Fertilization (per acre)	Irrigation	pH	Nitrogen (%)	Organic matter (%)
SVREC	Saginaw Valley Research and Extension Center, MI, US	190 m	CELRK	24.5ºC, 5.7 mm	Common bean, wheat, maize,	Synthetic (400lbs of 15N-5 P-13K)	None	7.7 (± 0.1)	0.13 (± 0.00)	2.33 (± 0.06)
MRC	Montcalm Research Center, MI, US	280 m	Eclipse	24ºC, 7.4 mm	Common bean, maize, potato	Synthetic (200lbs of 19N-0P-19K)	Yes	5.9 (± 0.4)	0.10 (± 0.01)	2.13 (± 0.06)
NE	Scottsbluff, NE, US	1200 m	CELRK and Eclipse	27ºC, 2.18 mm	Common bean, maize, common bean	None	Yes	7.9 (± 0.1)	0.07 (± 0.01)	1.39 (± 0.18)
CO	ARDEC, Fort Collins, CO, US	1536 m	CELRK and Eclipse	31ºC, 1.4 mm **	Common bean, maize, barley	Organic (220lbs urea (2016), 3 tons manure (2015))	Yes	8.1 (± 0.2)	0.11 (± 0.00)	1.7 (± 0.15)
WA	WSU, Othello, WA, US	320 m	CELRK and Eclipse	26.3ºC, 0.2 mm	Common bean, maize, wheat	Synthetic (40lbs N/20lbs P/10lbs ZN2O5)	Yes	6.1 (± 0.4)	0.08 (± 0.01)	1.78 (± 0.10)

* Temperature (as max air temperature) and precipitation are averaged for the period between May 1st and July 31st 2017.
** Data available only for the period between Jun 28th and Jul 31st 2017.

Fig.1

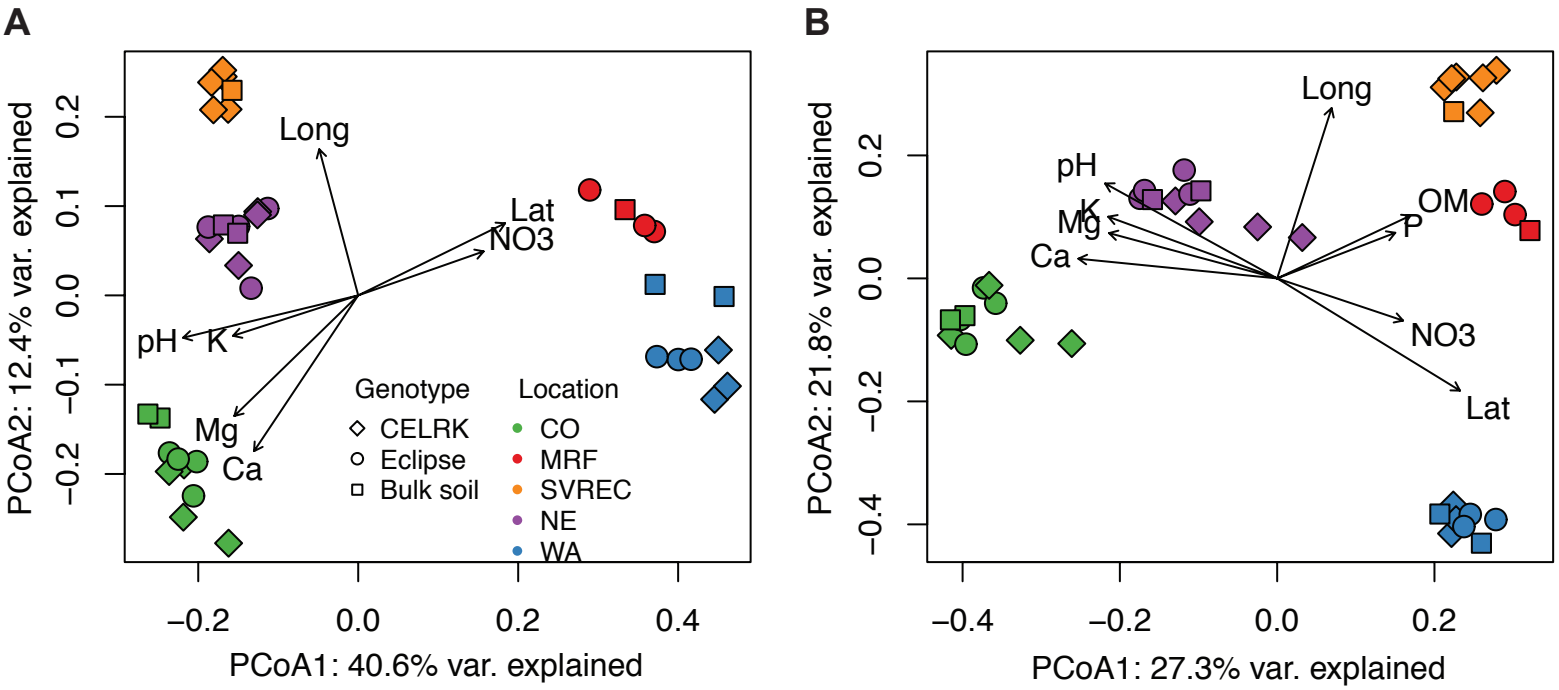

A
Long
Lat
NO3
pH
K
Mg
Ca
Genotype
CELRK
Eclipse
Bulk soil
Location
CO
MRF
SVREC
NE
WA
PCoA2: 12.4% var. explained
PCoA1: 40.6% var. explained
B
Long
pH
K
Mg
Ca
OM
P
NO3
Lat
PCoA2: 21.8% var. explained
PCoA1: 27.3% var. explained

Fig. 2

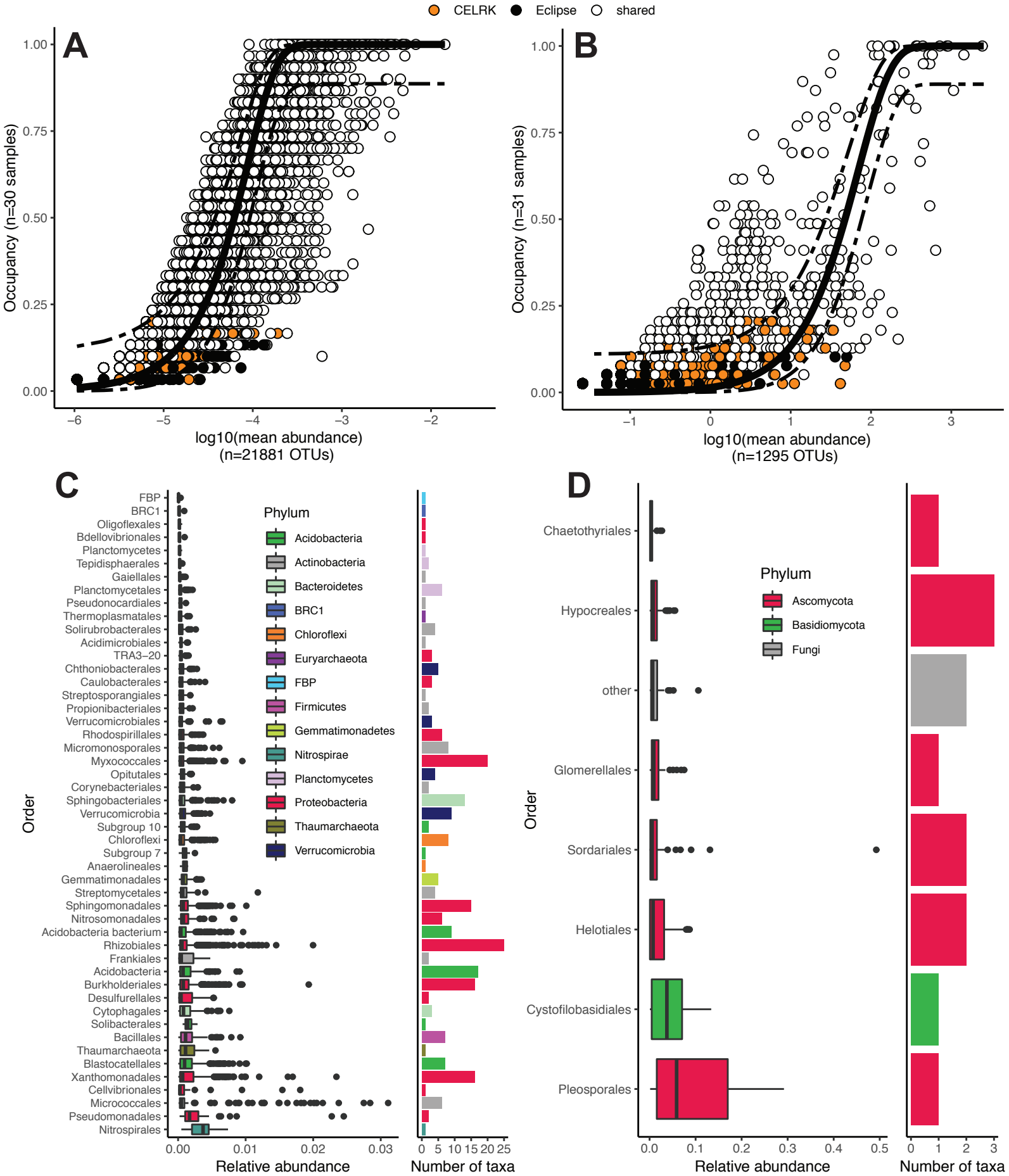

CELRK
Eclipse
shared
A
Occupancy (n=30 samples)
log10(mean abundance)
(n=21881 OTUs)
B
Occupancy (n=31 samples)
log10(mean abundance)
(n=1295 OTUs)
C
Order
Phylum
Acidobacteria
Actinobacteria
Bacteroidetes
BRC1
Chloroflexi
Euryarchaeota
FBP
Firmicutes
Gemmatimonadetes
Nitrospirae
Planctomycetes
Proteobacteria
Thaumarchaeota
Verrucomicrobia
Relative abundance
Number of taxa
D
Order
Phylum
Ascomycota
Basidiomycota
Fungi
Chaetothyriales
Hypocreales
other
Glomerellales
Sordariales
Helotiales
Cystofilobasidiales
Pleosporales
Relative abundance
Number of taxa

Fig. 3

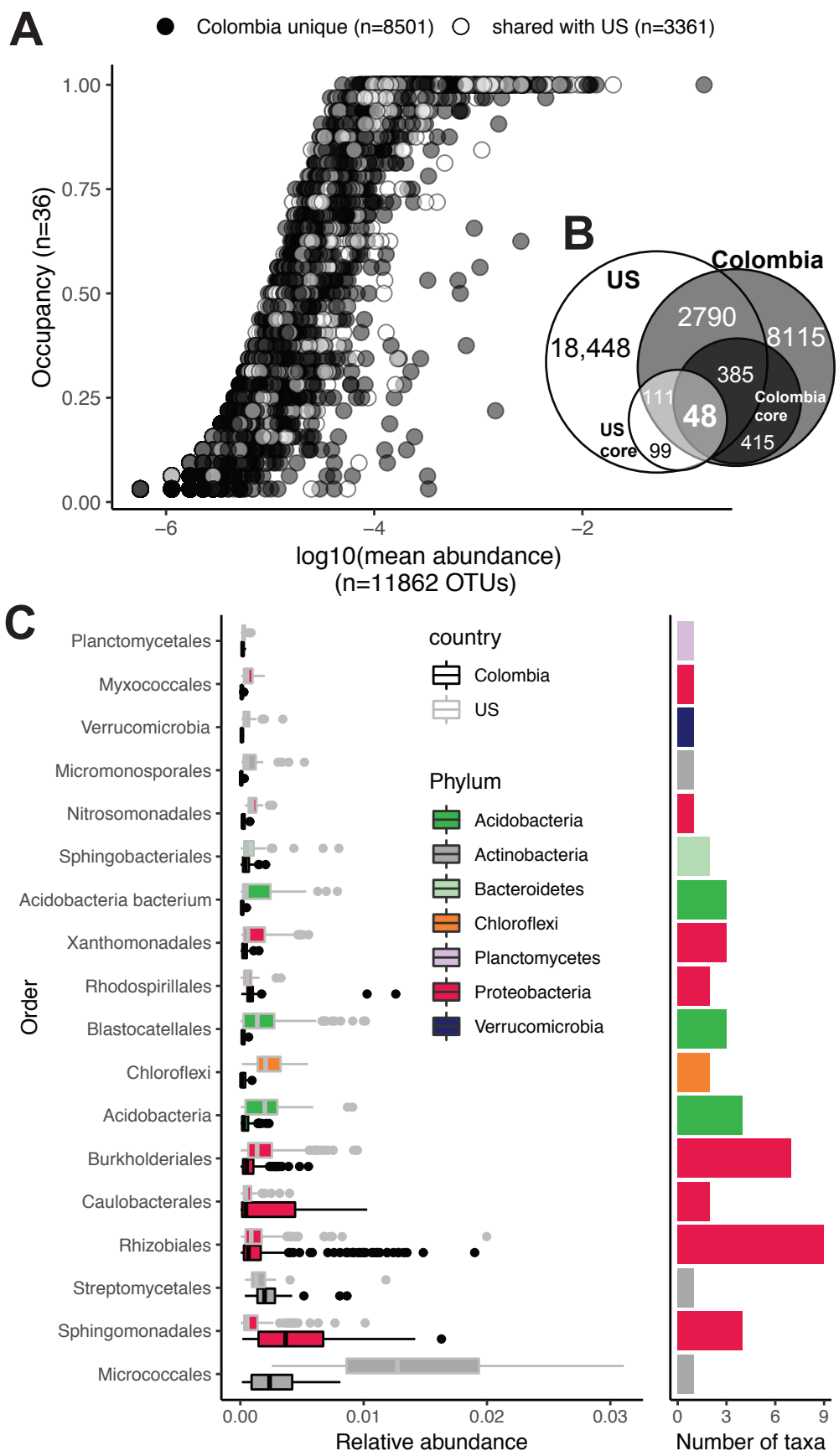

A
Colombia unique (n=8501)
shared with US (n=3361)
Occupancy (n=36)
1.00
0.75
0.50
0.25
0.00
−6
−4
−2
log10(mean abundance)
(n=11862 OTUs)
B
Colombia
US
2790
8115
18,448
385
111
48
Colombia core
US core
99
415
C
Planctomycetales
Myxococcales
Verrucomicrobia
Micromonosporales
Nitrosomonadales
Sphingobacteriales
Acidobacteria bacterium
Xanthomonadales
Rhodospirillales
Blastocatellales
Chloroflexi
Acidobacteria
Burkholderiales
Caulobacterales
Rhizobiales
Streptomycetales
Sphingomonadales
Micrococcales
Order
country
Colombia
US
Phylum
Acidobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Planctomycetes
Proteobacteria
Verrucomicrobia
0.00
0.01
0.02
0.03
Relative abundance
0
3
6
9
Number of taxa

Fig. 4

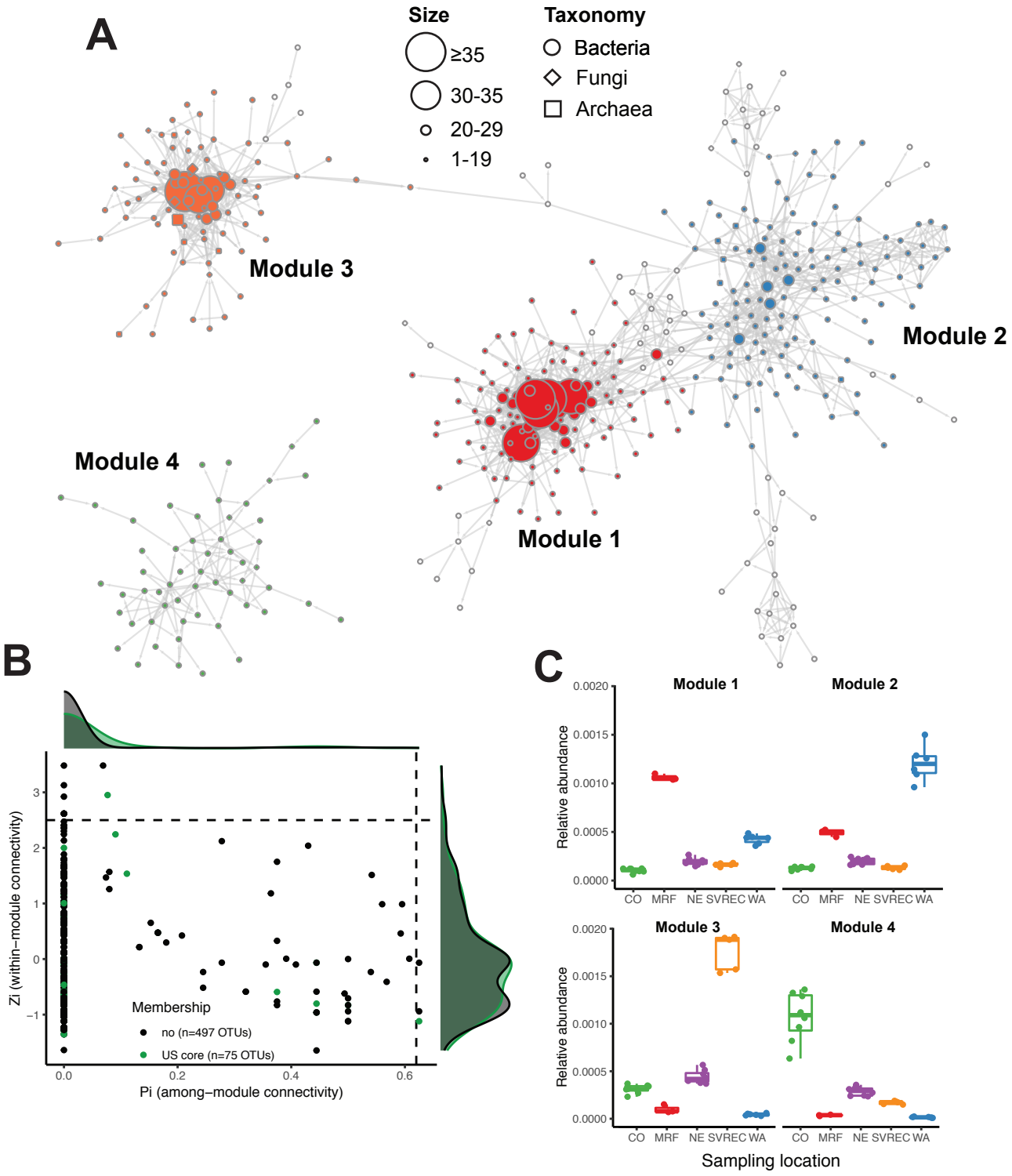
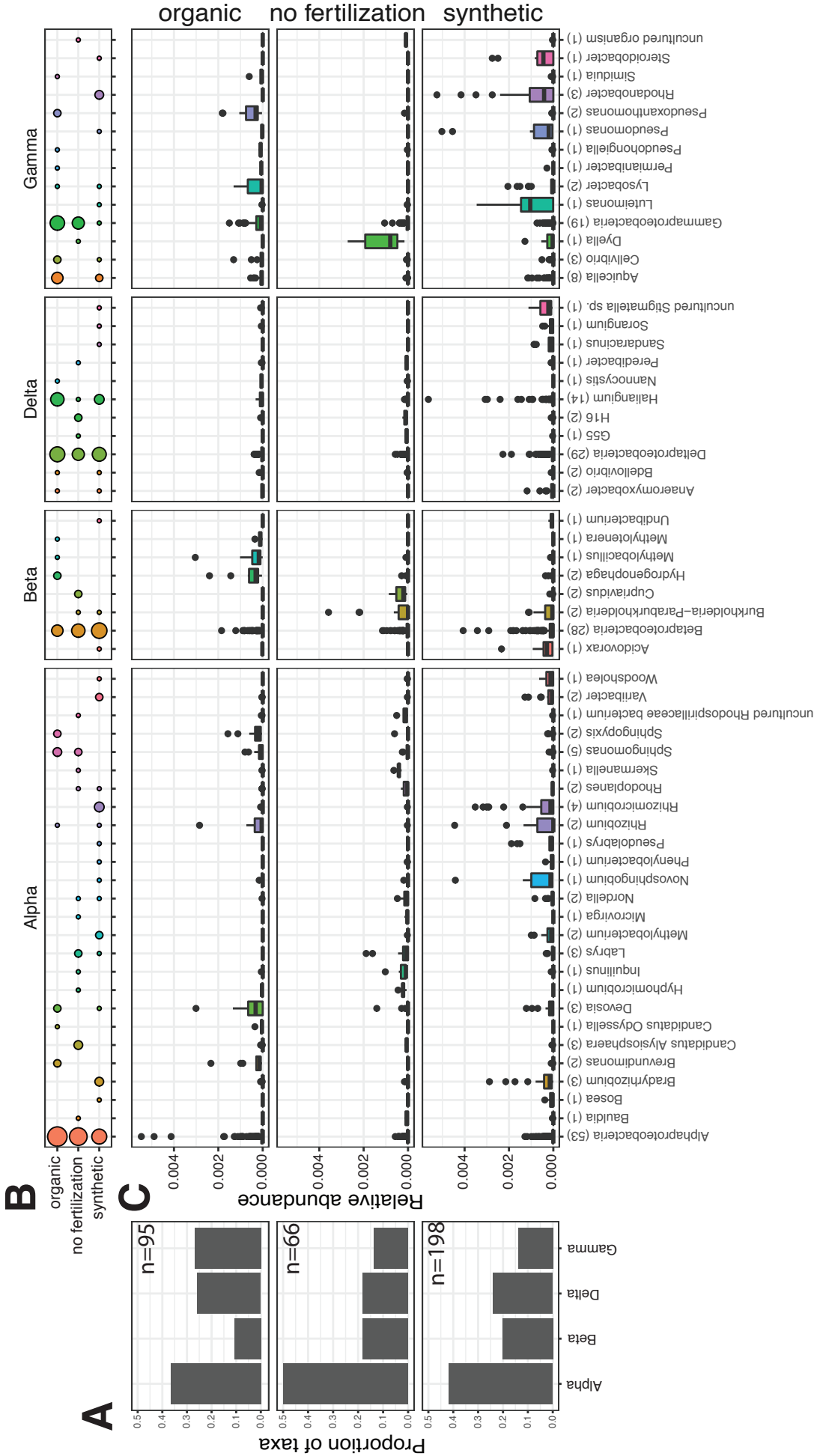

A
Size
≥35
30-35
20-29
1-19
Taxonomy
Bacteria
Fungi
Archaea
Module 3
Module 2
Module 4
Module 1
B
Zi (within−module connectivity)
Pi (among−module connectivity)
Membership
no (n=497 OTUs)
US core (n=75 OTUs)
C
Module 1
Module 2
Module 3
Module 4
Relative abundance
CO
MRF
NE
SVREC
WA
Sampling location

Fig. 5


A
Proportion of taxa
n=95
n=66
n=198
Alpha
Beta
Delta
Gamma
B
organic
no fertilization
synthetic
C
Relative abundance
organic
no fertilization
synthetic
Alphaproteobacteria (53)
Baulda (1)
Bosea (1)
Bradyrhizobium (3)
Brevundimonas (2)
Candidatus Alysiosphaera (3)
Candidatus Odyssella (1)
Devosia (3)
Hyphomicrobium (1)
Inquilinus (1)
Labrys (3)
Methylobacterium (2)
Microvirga (1)
Nordella (2)
Novosphingobium (1)
Phenylobacterium (1)
Pseudolabrys (1)
Rhizobium (2)
Rhizomicrobium (4)
Rhodoplanes (2)
Skermanella (1)
Sphingomonas (5)
Sphingopyxis (2)
uncultured Rhodospirillaceae bacterium (1)
Varibacter (2)
Woodsholea (1)
Acidovorax (1)
Betaproteobacteria (28)
Burkholderia–Paraburkholderia (2)
Cupriavidus (2)
Hydrogenophaga (2)
Methylobacillus (1)
Methylotenera (1)
Undibacterium (1)
Anaeromyxobacter (2)
Bdellovibrio (2)
Deltaproteobacteria (29)
G55 (1)
H16 (2)
Haliangium (14)
Nannocystis (1)
Peredibacter (1)
Sandaracinus (1)
Sorangium (1)
uncultured Stigmatella sp. (1)
Aquicella (8)
Cellvibrio (3)
Dyella (1)
Gammaproteobacteria (19)
Lutelimonas (1)
Lysobacter (2)
Permianibacter (1)
Pseudohongiella (1)
Pseudomonas (1)
Pseudoxanthomonas (2)
Rhodanobacter (3)
Simiduia (1)
Steroidobacter (1)
uncultured organism (1)