

# Abiotic and biotic drivers of endosymbiont community assembly in *Jatropha curcas*

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**Abstract.** While the endosymbiotic communities recruited by plants are known to vary among host species and across environmental gradients, the drivers of community assembly remain poorly understood. We tested the hypothesis that establishment of an endosymbiotic bacterial community is driven primarily by the influence of the abiotic environment on biotic interactions between plant and soil bacteria. We planted sterile *Jatropha curcas* seedlings at three field sites in Panama and in a greenhouse with soil from those sites. After allowing sufficient time for endosymbiont colonization, we sequenced bacterial 16S rRNA to study the endophytic bacterial community in root and leaf tissue. We compared the communities between field and greenhouse plants and examined associations among the endosymbionts, the soil microbial community, and local abiotic factors. We found that endosymbiont richness and community composition varied between the greenhouse and field, despite plants being grown in the same soil. Plants in each field site harbored a distinct bacterial community, determined by soil microbes and select environmental variables, particularly major plant nutrients. *Jatropha curcas* can harbor a wide variety of endosymbiotic communities, and the composition of these communities is a product of the local environment. Fertility and agricultural practices may determine the fate of plant symbionts and therefore plant properties modulated through those symbionts.

**Key words:** endophytes; neotropics; Panama; prokaryote diversity; soil fertility; symbiosis.

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## INTRODUCTION

A central goal in community ecology research has been to unravel the processes that define community structure. A well-supported model implicates environmental filtering as defining where organisms establish, where abiotic conditions and local biotic interactions act as a filter to limit the survival and persistence of select species within a specific area or habitat (Kraft et al. 2015). We know much more about community assembly via environmental filtering in plants

than microorganisms (Lebrija-Trejos et al. 2010, Siefert et al. 2013, Laliberté et al. 2014), but current understanding of plant–microorganism interactions has opened up the question of how plant endosymbiotic communities assemble (Saunders and Kohn 2009, Winston et al. 2014, Edwards et al. 2015, Campisano et al. 2017). This question is relevant because plant endosymbionts can have high levels of diversity with profound effects on plant phenotype and host community dynamics (Partida-Martínez and Heil 2011, Wagner et al. 2014).

Transmission of endosymbionts occurs in multiple ways. The entry of first microorganisms into a plant begins when it is still a seed, with the source being the maternal tissue (vertical transmission). Vertically transmitted microbes, however, have relatively little influence on the mature plant microbiome (Shade et al. 2017). The bulk of the plant microbiome is horizontally transmitted and originates in the surrounding soil and leaf litter (Christian et al. 2015, Hardoim et al. 2015, Zarraonaindia et al. 2015). Prior to filtering, the local species pool (the soil microbial diversity) constrains the endophytic community by limiting the reservoir of bacterial symbionts with which a plant can associate (Berendsen et al. 2012).

Biotic filtering influences the community composition of endosymbionts through host selectivity. The chemical environment in the rhizosphere, which is influenced by plant exudates and microbial symbionts, differs from that in the bulk soil and therefore acts as a biotic filter on the community of bacteria within this zone (Berendsen et al. 2012). The internal chemistry of the host plant, of both roots and shoots, acts as an additional biotic filter driving endosymbiont community differences among different host tissues (Bonito et al. 2014, Hardoim et al. 2015). Shoot tissue also tends to have less diversity of microbial symbionts than the roots and rhizosphere, indicating a tissue-specific filtering within the individual as microbes colonize the entire host (Romero et al. 2014). Rhizosphere and endosphere communities can be influenced by significant interactions of host identity and soil type, particularly when different plant hosts secrete unique root exudates (Berg and Smalla 2009, Bonito et al. 2014, Winston et al. 2014, Lareen et al. 2016). Interactions with disease can also shape the endosymbiont community, enriching it with disease antagonists (Li et al. 2019). As there are many elements of biotic filtering, it can have a strong influence in driving community assembly. However, the strength of these biotic filters can also be affected by the abiotic environment and the surrounding soil community.

As soil contains the source of the majority of endosymbionts, the endosymbiont community should correlate strongly with the soil microbial community and soil properties. The diversity of soil bacteria is greatest at neutral pH, but at the

micro-habitat scale vegetation type, soil moisture, nutrients, carbon, and clay content may also correlate with bacterial community structure (Fierer and Jackson 2006, Naveed et al. 2016). Environmental stresses can directly or indirectly select for divergent symbiont communities. For example, pulses of heat to soil can directly increase the diversity of soil bacteria and significantly change bacterial community composition and ecosystem functioning within the rhizosphere (van der Voort et al. 2016). Abiotic stressors causing the plant host to alter its root exudate composition can create host-mediated changes in rhizosphere bacterial composition, as has been observed with drought and altered nutrient levels (Yang and Crowley 2000, Bakker et al. 2013). In soil and plant surfaces, microbial community assembly has been found to be determined by the nutrient availability, specifically the carbon source available for microbial metabolism (Goldford et al. 2018). Such determinism suggests that limiting resources act as abiotic environmental filters by promoting competition. Some of the endosymbionts selected from the rhizosphere then can affect plant productivity, influence the ability of plants to respond to environmental stresses, and mediate intensity of selection on the plant within a community (Compant et al. 2010, Marasco et al. 2012, Wagner et al. 2014). Understanding how and when different environmental stresses act as abiotic filters on the endophytic community may provide insight into how plant hosts can differentially use the soil microbial community to respond to these stresses.

We tested the overarching hypothesis that assembly of the endosymbiont community is driven primarily by the abiotic environment's influence on biotic interactions between plant and soil bacteria. We approached this hypothesis using *Jatropha curcas* (Euphorbiaceae), a key biodiesel crop that is cultivated globally. Specifically, we tested two hypotheses about *jatropha's* endosymbiont community composition. (1) If abiotic filtering due to the local environment drives biotic filtering of endosymbionts, then *jatropha* grown under the same conditions will recruit similar endosymbiotic communities despite differences in soil type and microbiota. Furthermore, *jatropha* under different environmental conditions will recruit distinct endosymbiotic communities

in response to the abiotic environment and local soil microbiota. (2) If abiotic filtering drives biotic filtering, then specific abiotic variables will play a larger role than soil microbial community composition in assembling the endosymbiont community. We tested these hypotheses through four experiments. First, we analyzed soil microbial communities across different sites in Panama where *jatropha* is native and examined the effect of application of agricultural lime on soil chemistry and the microbial community. Second, we tested soil effects on the assembly of the plant endophytic community under the same abiotic conditions using a common greenhouse environment. Third, we used field experiments to quantify the assembly of plant endophytic communities in different sites with different abiotic environments. Finally, we developed a model to partition the variation in the endophytic community with respect to soil microbiota and local abiotic environmental variables. *Jatropha* is an economically important crop cultivated in a wide array of environments with profound resilience to stress, including limited nutrient levels and drought (Sapeta et al. 2013, Edrisi et al. 2015). Extracts from its leaves, roots, latex, bark, and seed all demonstrate strong levels of antimicrobial activity (Abdelgadir and Van Staden 2013), meaning that the endosymbiotic community may be highly specialized. It is hypothesized that *jatropha* is able to tolerate severe abiotic stresses through the acquisition of plant growth-promoting bacteria (Mohanty et al. 2017) which makes *jatropha* an excellent host to study the effects of abiotic and biotic filtering of endosymbionts.

## MATERIALS AND METHODS

We worked at the Smithsonian Tropical Research Institute (STRI) in Panama, including the Gamboa greenhouse facilities and Naos Molecular Laboratory. We chose three field sites in the Azuero Peninsula on the Pacific coast of central Panama and set up two study plots per site. These sites lay roughly evenly along Highway 2, which served as a transect of 125-km distance and runs along the eastern coast of the peninsula. The region is characterized as dry tropical forest and experiences an extremely arid season from January through April, where weeks may pass without rain. The area is mainly utilized by farmers for cattle pasture which is dominated by grass and shade trees (Griscom et al. 2011). The southernmost two plots within Los Asientos are located on a small-scale ranch where the plots were established in open pasture near a living fence of *jatropha*. The central site, El Ejido, contained two plots within a 3-yr-old *jatropha* plantation. The northernmost two plots were within a 3-yr-old *jatropha* plantation in the town of Divisa. These sites experience varying amounts of rain and soil nutrients, particularly in key plant nutrients such as phosphorus and potassium, and with higher rainfall at Divisa and Los Asientos than in El Ejido (Table 1; Appendix S1: Table S1).

### *Characterization of the soil microbial community and the effect of agricultural lime*

In late May 2015, six plots in three sites were cleared of above-ground vegetation with machetes. Plots were  $6 \times 8 \text{ m}^2$  and further

Table 1. Environmental conditions at field sites, for both limed and un-limed areas.

Site	Divisa	+ CaCO <sub>3</sub>	El Ejido	+ CaCO <sub>3</sub>	Los Asientos	+ CaCO <sub>3</sub>
Description	Jatropha plantation		Jatropha plantation		Cattle pasture	
Mean monthly rainfall June–August (mm)	197.5 ± 24.9		108.7 ± 10.6		218.8 ± 11.2	
Mean soil pH	6.2 ± 0.8	7.6 ± 0.3	6.4 ± 0.3	7.8 ± 0.2	6.7 ± 0.0	7.6 ± 0.2
P (mg/kg)	39.4 ± 8.2	38.6 ± 4.9	6.6 ± 1.5	6.23 ± 0.1	2.4 ± 0.6	3.8 ± 2.0
K (mg/kg)	221.7 ± 238.1	130.1 ± 109.1	158.5 ± 18.2	150.7 ± 23.9	46.9 ± 12.3	39.7 ± 5.7
Mg (mg/kg)	254.1 ± 4.0	243.7 ± 15.7	1716.8 ± 53.1	1486.5 ± 2.0	515.1 ± 279.3	445.8 ± 244.2
Ca (mg/kg)	2159.6 ± 952.0	2267.8 ± 582.2	1621.5 ± 33.7	4573.2 ± 42.7	1813.6 ± 1113.5	5116.7 ± 1095.2
Al (mg/kg)	433.24 ± 69.1	441.46 ± 106.4	562.1 ± 49.1	157.5 ± 101.3	768.8 ± 1.1	240.4 ± 58.1
B (mg/kg)	0.185 ± 0.01	0.16 ± 0.06	0.305 ± 0.08	0.34 ± 0.07	0.185 ± 0.1	0.185 ± 0.1

divided into twelve equal subplots of 2 m<sup>2</sup>. We sampled soil from each subplot to a depth of 10 cm, pooled the soil into a single sample per plot, and air-dried the soil for pH analysis. Following the sampling, we applied 1 kg of agricultural lime to half of the subplots (6) within each plot at random. We spread the lime out in a circle of 1 m diameter, with the center of each circle being the center of the subplot and mixed the lime with the top two inches of soil. Four weeks later, we re-sampled the soil to ensure that the pH had been raised by at least one unit. Soil samples from each subplot were pooled by treatment (limed and un-limed), grouped by plot (12 samples total from the 6 plots), and air-dried. These samples were analyzed for pH in a 1:2 soil:deionized water ratio using a glass electrode and concentrations of cation and available P by extraction in Mehlich-3 solution, with detection by ICP spectrometry (Turner and Romero 2009; Table 1; Appendix S1: Table S1). After jatropha seedlings had been established in each subplot (see *Field experiment* section), we spade-sampled bulk soil 1 m away from each plant. We pooled the six limed and six un-limed bulk soil samples from each subplot and carried the samples to the lab, where they were kept at –20°C until DNA extraction. We extracted DNA and amplified the V5–V6 region of 16S rRNA to characterize the bacterial community (See *Molecular methods*).

#### *Endophyte community assembly with similar abiotic filtering pressures—greenhouse experiment*

In 2014, we conducted a greenhouse experiment in Gamboa, Panama, using jatropha seeds collected from a stock of native jatropha trees in Divisa. We tested the hypothesis that when grown in one location under the same ambient conditions with soil from different locales, jatropha seedlings with harbor similar richness and community composition of endosymbionts. We surface sterilized the seeds in a 10% Clorox solution for 5 min and followed with two washes of sterile DI water. We collected soil adjacent to the plots at Los Asientos, El Ejido, and Divisa, which had already been characterized for bacterial diversity (see *Molecular Methods*). We autoclaved half of the soil sample twice for 1-h cycles at 151°C at 51 psi to sterilize it. We then transplanted the sterile seedlings into individual 1-L

pots with 90% sterile vermiculite and 10% inoculum soil (live or sterile). Each treatment (soil origin × sterile/live) consisted of 20 plants (120 plants total). After 2 weeks, we harvested one root and one leaf from six plants in each treatment combination for 16S bacterial community profiling. We surface sterilized the tissue for 2 min in 70% ethanol, 1 min in 10% Clorox, and 1 min in 70% ethanol, and then rinsed the tissue with sterile DI water. Once sterilized, we froze the tissue at –70°C until extracting DNA.

#### *Endophyte community assembly with different abiotic filtering pressures—field experiment*

To test the hypothesis that jatropha will recruit distinct communities when grown under different environmental conditions, we collected seeds from the same stand of jatropha trees used for the greenhouse experiment in Divisa, Panama, in August of 2014. We surface sterilized the seeds in a 10% Clorox solution for 5 min and with two washes of sterile DI water. We started sterile seedlings in an incubation chamber in Gamboa and allowed them to grow for two weeks. The seedlings were then maintained in a greenhouse for one week to harden for transplantation. Each seedling had two cotyledons and two leaves at the time of transplant.

Between June 16 and 18, 2015, we transplanted the seedlings into the center of each subplot described above ( $n = 12$  subplots per plot; see *Characterization of the soil microbial community*). The plots were surrounded by barbed wire fences to protect the seedlings from grazing cattle. Seedlings were encircled by a plastic cup coated in FluOn to deter leaf-cutting ants. The seedlings were monitored for 10 d, and they were watered every 2–3 d if it did not rain. Any seedlings that died within the first 10 days were replanted. The seedlings were then left to grow unmonitored for 10 weeks. After 10 weeks, we returned to the plots and re-measured each of the plants. One leaf and root (larger root with multiple fine roots attached) sample from each plant in each subplot was collected and placed in a sterile bag on ice. From one plot in El Ejido (plot 4) and one plot in Divisa (plot 6), we kept the sampled roots in sterile aluminum foil, and placed the sample in a liquid nitrogen tank. These roots from plots 4 and 6 were kept at –80°C and all other tissues were kept at –20°C.



Before DNA was extracted, all leaf and root samples were surface sterilized for 2 min in 70% ethanol, 1 min in 10% Clorox, and 1 min in 70% ethanol, and then rinsed with sterile DI water.

### *Molecular methods*

We used MoBio Power Soil DNA isolation kits for DNA extraction of soil, and we amplified the 16S rRNA region using 515F and 806R primers (V4 region; Caporaso et al. 2011) for the preliminary soil analysis (soil collected May 2014), and 799F and 1115R (V5–V6 region; Hanshew et al. 2013, Kembel et al. 2014) primers for the final soil collected in May 2015 for comparison with endosymbiont community. We selected these primers because they were specific for isolating microbial 16S rRNA from plant tissue, thus allowing us to compare the soil community with the plant endosphere community (Hanshew et al. 2013, Kembel et al. 2014). We used MoBio PowerPlantPro DNA isolation kits for all roots and leaves except the roots from plots 4 and 6, for which MoBio PowerPlant RNA isolation kits were used to isolate DNA and RNA (RNA not included here). We conducted all amplifications in triplicates. The PCR triplicates were subsequently pooled and normalized with SequelPrep Normalization Plates. We then pooled the libraries and sequenced them on an Illumina MiSeq. All downstream analyses were conducted with sequences obtained from the 799F and 1115R primers.

### *Data analysis*

We used QIIME 1.9.1 (Compant et al. 2010) to demultiplex and quality filter the samples set at the phred default (15). We picked OTUs in an open reference manner using UCLUST based on 97% identity, suppressing step 4 due to the high volume of samples. We assigned taxonomy with the May 2013 version of GreenGenes 3\_5 reference sequences (McDonald et al. 2012) and filtered out mitochondrial and chloroplast sequences prior to subsequent analyses. We rarefied each group of samples using the highest number of sequences possible for each analysis, which was always 7067 or higher. All alpha diversity metrics were created with Chao 1 and conducted with OTUs. We compared alpha diversity with nonparametric t-tests, ANOVAs, and two-way ANOVAs if we included a factor, such as site

or soil origin. We used Kruskal-Wallis tests to make diversity comparisons when the data were non-normal. Beta diversity/community composition was measured using the unweighted unifracs dissimilarity measure and Principle Coordinates Analyses (PCoA) plots were made using the `beta_diversity_through_plots.py` function within the QIIME pipeline. Differences in beta diversity were measured with PERMANOVAs through the `adonis` function in the *vegan* package (Oksanen et al. 2016) with 999 permutations.

### *Model selection for importance of abiotic and biotic factors on community assembly*

We conducted a db-RDA in the R package *vegan* (Oksanen et al. 2016) with the 16S data set of roots and leaves (separately) from all of the plants in the field. From the PCoA, we extracted the values from each principle coordinates axis for each sample, and used these values for the db-RDA analysis (Appendix S1: Table S1). To find the variables which best explained the endosymbiont bacterial communities (in unweighted unifracs dissimilarity matrix format), we used the `ordistep` function to select from the variables measured from the environment as well as the principle coordinates axes values (PCs) associated with the soil microbial community. These PCs were extracted from the beta diversity analyses of the soils at each site (unweighted unifracs measures) from the analysis using 799F and 1115R primers (Appendix S1: Table S1). To differentiate the effect of the soil microbiota (using the PCs) and the local environmental variables (soil pH, Mehlich analysis results, and local rainfall for June–August), we alternately constrained the model for environmental variables or soil microbiota to partition the variance. All analysis code is available on GitHub at <https://github.com/VanBaelLab/MigheIl-2018-jatropha>.

## RESULTS

### *Overall summary*

After filtering out mitochondrial and chloroplast 16S sequences and samples with low sequencing success (<7067 sequences), our 279 samples from soil ( $n = 14$ ), roots ( $n = 98$ ), and leaves ( $n = 101$ ) of field and greenhouse seedlings contained 13.9 million sequences, which

clustered into 23,998 OTUs of bacteria and archaea (henceforth microbial taxa).

***The soil communities varied by site, with shifts in community composition which corresponded to application of lime***

Lime application increased soil pH by at least 1 unit across plots (Table 1) and we observed corresponding shifts in soil bacterial community composition along PCoA axis 2 (Fig. 1). The soil bacterial community was equally rich at each site using the Chao-1 alpha diversity measure (Fig. 2). Community composition (measured by unweighted unifracs) was significantly different by site (PERMANOVA: Pseudo  $F_{2,11} = 2.22$ ,  $P < 0.001$ ; Fig. 1). We also found variation in pH and soil composition across these sites, particularly major plant nutrients (Table 1; Appendix S1: Table S1).

***Live soil successfully inoculated plants with an endosymbiont community when compared to a sterile soil control***

In the greenhouse, live soil produced a significantly richer endosymbiont community that was distinct from that of plants grown in sterile soil.

For the roots, the community composition was significantly different among sterile ( $n = 18$  samples) and nonsterile ( $n = 14$ ) treatments (PERMANOVA: Pseudo  $F_{1,31} = 7.69$ ,  $P < 0.001$ ). Live treatments had significantly higher endosymbiont OTU richness measured with Chao1 and a nonparametric  $t$ -test (Live:  $1375 \pm 246$ ; Sterile:  $871 \pm 93$ ;  $t = 7.72$ ,  $P < 0.001$ ). When analyzing only leaves, we found differences in community composition between live ( $n = 17$ ) and sterile ( $n = 17$ ) treatments (PERMANOVA: Pseudo  $F_{1,33} = 2.19$ ,  $P = 0.007$ ). Live treatments also had significantly higher endosymbiont species richness measured with Chao1 and a nonparametric  $t$ -test (Live:  $884 \pm 194$ ; Sterile:  $649 \pm 142$ ;  $t = 3.91$ ,  $P < 0.001$ ). Therefore, we conclude that using live soil was a successful way to inoculate plants with bacterial endosymbionts, and we conducted future analyses with only plants in live soil.

***Biotic filtering occurred within all host plants, with changes in richness and community composition from soil to roots to leaves***

Diversity in all of the host plants (greenhouse and field) varied by isolation source (sample

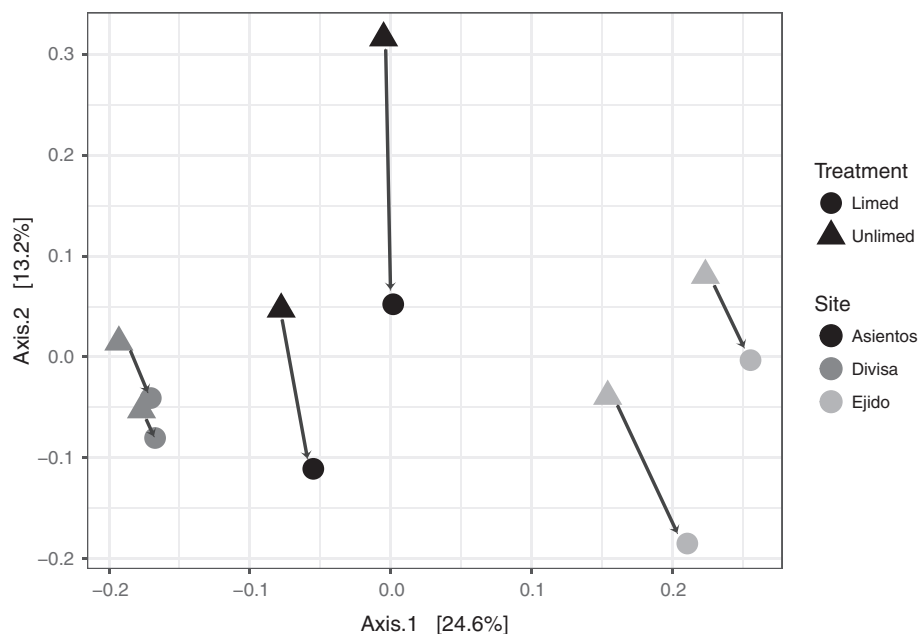


Fig. 1. Principal Coordinates Analysis of beta diversity of soil using the unweighted unifracs measure. Microbial community composition varied significantly with site. Within each site, we observed an effect of application of agricultural lime along Axis 2. The arrows represent the shift within each plot.

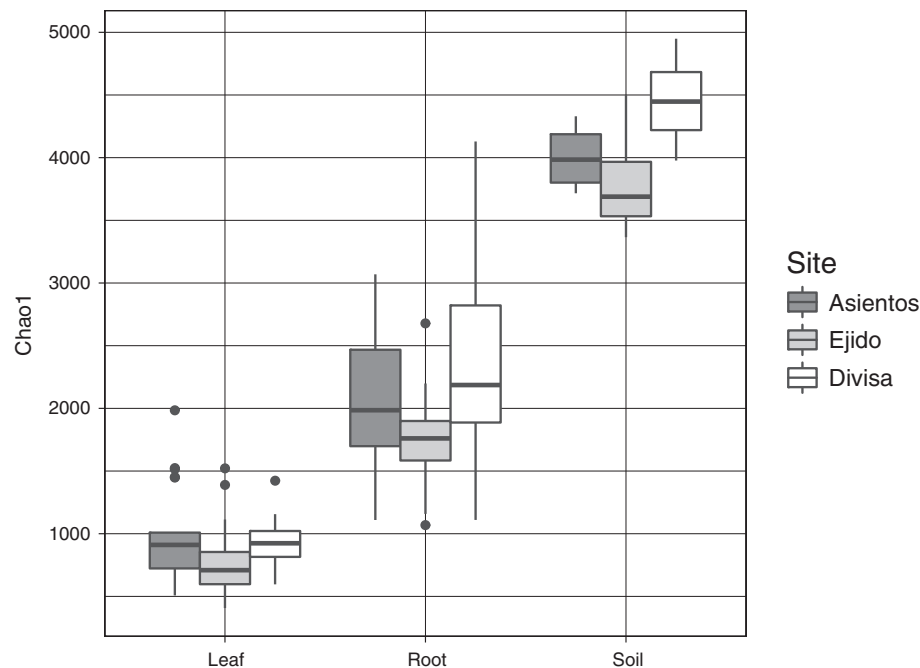


Fig. 2. OTU richness (Chao 1) was significantly different among soil, roots, and leaves (Kruskal–Wallis = 117.5,  $P < 0.001$ ) and among sites (Kruskal–Wallis = 6.1,  $P < 0.047$ ). This demonstrates a decrease in diversity of the microbial community as it is recruited from the soil and colonizes host tissue and suggests that soils play a role in determining alpha diversity.

type), decreasing from soil to roots to leaves (Fig. 2). OTU richness (Chao 1) was significantly different among isolate sources (Kruskal–Wallis = 117.5,  $P < 0.001$ ) and among sites (Kruskal–Wallis = 6.1,  $P < 0.047$ ). This demonstrates a decrease in diversity of the microbial community as it is recruited from the soil and colonizes host tissue. The Principal Coordinate Analysis (PCoA) also showed soil, roots, and leaves as distinct microbial habitats indicating shifts in community composition as well (PERMANOVA Pseudo  $F_{2,157} = 24.119$ ,  $P < 0.001$ ). For this reason, we constrained subsequent analyses by isolation source (soil, roots, and leaves).

***Abiotic conditions strongly influenced the biotic filtering of endosymbionts as seen through community differences among field sites and between field sites and the greenhouse***

Roots in the field setting tended to have greater endosymbiont richness than roots in the greenhouse, but leaves tended to have lower endosymbiont richness in the field versus

greenhouse. Roots harbored greater diversity of endosymbionts in the field than in the greenhouse ( $F_{1,38} = 9.12$ ,  $P = 0.005$ ) but soil origin did not influence the alpha diversity ( $F_{2,38} = 2.96$ ,  $P = 0.063$ ). There was no significant interaction of location and soil origin ( $F_{2,28} = 2.05$ ,  $P = 0.143$ ). Leaf microbial diversity was distinct by location (field or greenhouse;  $F_{1,34} = 41.92$ ,  $P < 0.001$ ), with leaves harboring greater diversity in the greenhouse than in the field (Fig. 3). Leaf community diversity also differed with soil origin ( $F_{2,34} = 3.82$ ,  $P = 0.032$ ). A significant interaction existed between the location and soil origin ( $F_{2,34} = 3.87$ ,  $P = 0.031$ ), which was most likely driven by the high diversity of endophytes in greenhouse leaves grown from Los Asientos soil (Fig. 3). These differences in richness indicate differences in abiotic pressures can alter the diversity of endosymbiotic communities.

Root bacterial community composition significantly varied among sites in the field (PERMANOVA: Pseudo  $F_{2,62} = 6.79$ ,  $P < 0.001$ ; Fig. 4). When looking at this diversity through taxa via a

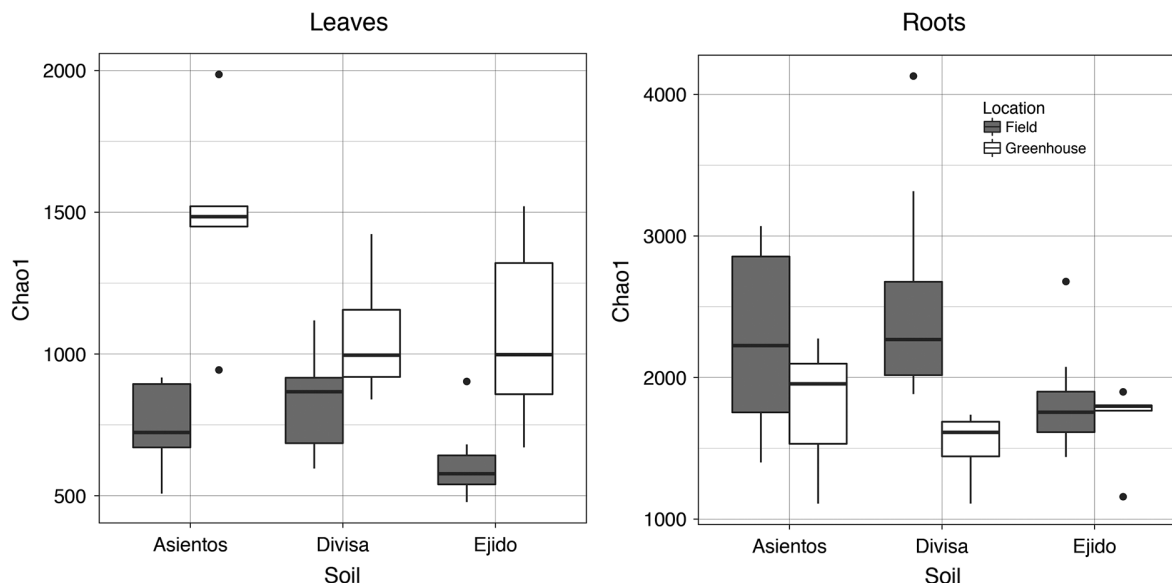


Fig. 3. Comparison of community richness (Chao1) between greenhouse and field by soil type for roots (right) and leaves (left). Roots tended to harbor more diverse endosymbionts in the field, while leaves tended to harbor more diverse endosymbionts in the greenhouse.

Kruskal-Wallis test, fifteen phyla were significantly different among the three sites (Bonferroni  $P < 0.05$ ). Eight of these (Acidobacteria, Actinobacteria, Armatimonadetes, Chloroflexi, Firmicutes, Gemmatimonadetes, Proteobacteria, and Verrucomicrobia) were in the ten most abundant phyla found in the data set (Appendix S1: Fig. S1). The sites also had different microbial communities within leaf tissue (PERMANOVA: Pseudo  $F_{2,51} = 2.47$ ,  $P < 0.001$ ; Fig. 4). However, these differences were not reflected through taxa at the phyla level (Appendix S1: Fig. S1). In the PCoA ordination, we saw that the endosymbiont communities in roots from the field demonstrated a stronger separation by site than in the leaves (Fig. 4). Furthermore, the community composition in the roots was more similar to the soil than the leaves (Fig. 4). When looking at the roots, Divisa was the most similar in community composition to the soil, while El Ejido had root communities that were the most distinct from the soil.

Plants in the field setting generally acquired a different community of endosymbionts than plants in the greenhouse, despite growing in the same soil. Particularly, the roots in the field contained a shift toward greater representation by

the TM\_7, Actinobacteria, Chloroflexi, and Gemmatimonadetes (Kruskal–Wallis tests, Bonferroni  $P < 0.05$ ). Leaves in the greenhouse shifted to represent greater richness in Acidobacteria, Chlamydiae, and Spirochetes (Kruskal–Wallis tests, Bonferroni  $P < 0.05$ ). These shifts are also observed to be within the top ten most abundant phyla of either the roots or the leaves, although some abundances were small in comparison to hyper-abundant phyla within these tissues (Appendix S1: Fig. S2). The community composition of leaf and root endosymbionts was distinct by soil origin and by location (field or greenhouse) when controlling for tissue (root or leaf; Fig. 5). This indicates the importance of local abiotic conditions in the biotic filtering of endosymbionts, with select orders of bacteria becoming established in either roots or leaves depending on the abiotic conditions.

#### *Application of agricultural lime partially altered the community composition of endophytic bacteria within roots*

Overall, we detected no consistent pattern within the roots of plants grown in subplots treated with agricultural lime. In roots, we saw shifts in community composition of root endosymbionts



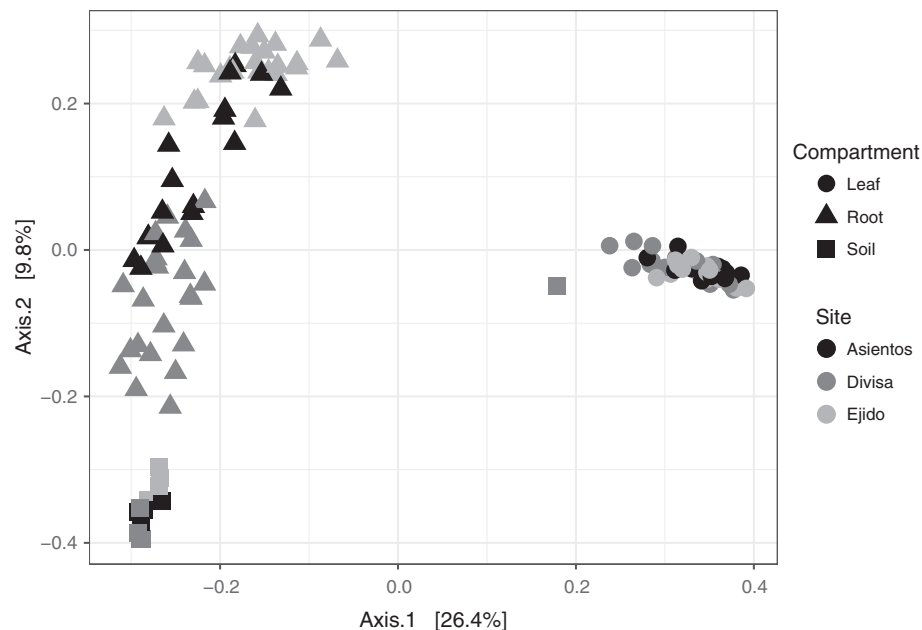


Fig. 4. Principle coordinates ordination of bacterial communities from roots, leaves, and soil from three field sites based on an unweighted unifrac dissimilarity matrix. Soil, roots, and leaves contained significantly distinct bacterial communities (PERMANOVA Pseudo  $F_{2, 126} = 23.83$ ,  $P < 0.001$ ). Numbers in parentheses represent the percent of variation explained by each PCoA axis.

in subplots that received applications of agricultural lime in three of the six plots. This occurred for both plots in Los Asientos (PERMANOVA: Pseudo  $F_{1,6} = 1.47$ ,  $P = 0.039$  and Pseudo  $F_{1,9} = 2.36$ ,  $P = 0.008$ ) and one plot in El Ejido (PERMANOVA: Pseudo  $F_{1,11} = 1.76$ ,  $P = 0.005$ ). In leaves, we saw a shift in community composition of leaf endosymbionts in limed subplots for one El Ejido plot (PERMANOVA: Pseudo  $F_{1,10} = 1.30$ ,  $P = 0.007$ ).

***Endosymbiont community variation was explained primarily by abiotic environmental variables and secondly by soil microbial composition***

The model for root endosymbionts selected the variables K, P, Rainfall, Mg, Ca, Al, and the soil microbial principle coordinates axes 8, 9, 10, and 11 as significant explanatory variables which explained a significant amount of variation, 33.32%, in the model ( $F_{10,52} = 2.60$ ,  $P < 0.001$ ; Table 2). When we alternatively constrained the model (using the capscale function) for the local environmental measures and the soil PCs that were selected previously, we found that the environment explained 24.24% of the local variation

( $F_{4,52} = 1.77$ ,  $P < 0.001$ ), the soil PCs explained 11.21% ( $F_{6,52} = 2.87$ ,  $P < 0.001$ ), and 2.16% was explained jointly. These analyses demonstrate that under field conditions, environmental measures and the soil microbial community measures both contributed significantly to the model, in total 33%, and explained the endosymbiont community composition of roots.

Using the same environmental inputs, we constructed a model to explain the variation within leaf microbial communities. This model explained a significant amount of variation, 13.46% ( $F_{5,46} = 1.43$ ,  $P < 0.001$ ; Table 3). In contrast to the roots, boron (B) was the only environmental variable explaining leaf community composition, and the rest of the variation was explained by the soil PCs 4, 6, 10, and 11.

## DISCUSSION

We found that endosymbiont richness and community composition were strongly influenced by the environmental conditions associated with each site as well as the soil bacterial community. *Jatropha* plants in the field and

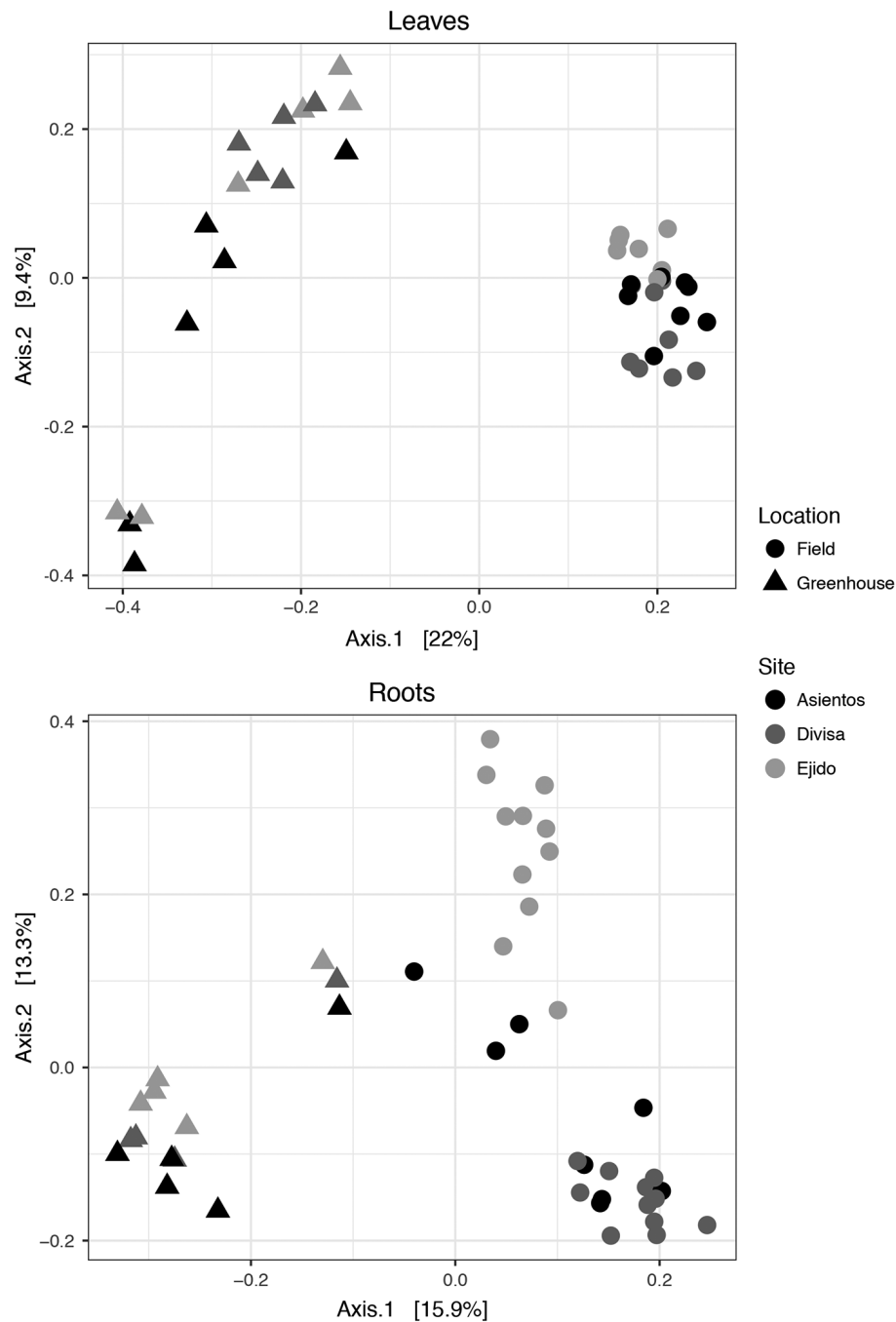


Fig. 5. Comparison of 16S community of leaves (left) and roots (right) in the greenhouse and the field. Microbial community composition was distinct by experiment location (greenhouse and field), as well as by the soil origin that the plants were grown in.

greenhouse had significantly different symbiont richness and community composition when controlling for soil origin and soil microbial diversity,

indicating that the environmental conditions of the host plant play a role in endosymbiont filtering from the soil community. Furthermore, a

Table 2. ANOVA of the root db-RDA model.

Characteristic	df	Sum of squares	F	P
K	1,52	0.4633	2.5325	0.002
P	1,52	0.414	2.2631	0.004
Mg	1,52	0.3432	1.8762	0.006
Rainfall	1,52	0.3448	1.8848	0.009
PC11	1,52	0.3546	1.9383	0.01
PC8	1,52	0.3355	1.8339	0.018
PC10	1,52	0.2794	1.5271	0.037
Ca	1,52	0.2568	1.4038	0.078
Al	1,52	0.2621	1.4327	0.069
PC9	1,52	0.219	1.1973	0.173
Residual	52	9.5112		

similar microbial community emerged in roots when plants were grown in different soils under the same ambient environment. This contrasted with our observations in the field, where site was a strong driver of divergence in community composition of roots and leaves. This work supports previous studies finding that the cultivation site in the field can have a stronger effect than the soil microbial community in influencing the plant endosymbiont community (Edwards et al. 2015) and that soil type and plant tissue can strongly influence the community assembly of endosymbionts under controlled conditions (Bulgarelli et al. 2012, Hardoim et al. 2012, Winston et al. 2014). In fact, there is evidence that host genetics can shape how the host microbiome responds to the local environment (Wagner et al. 2016). Therefore, the local environment is extremely important in filtering endosymbionts from the local species pool and allowing them to become established in host tissue.

Through model selection on the abiotic factors and measures of the soil microbial pool, we found that soil nutrients and rainfall can significantly affect the recruited endosymbiont community, and that soil community structure may also influence the assembly of the endosymbionts. This supports our hypothesis that abiotic factors drive the biotic filtering of endosymbionts and corroborates our *in vivo* hypothesis that the abiotic environment is the major environmental filter on the endosymbiont community. Abiotic and biotic factors within a plant's local environment are known to alter its root exudate composition and concentration, thereby altering the soil microorganisms that are attracted to the rhizosphere (Chaparro et al. 2012, Sasse et al. 2018).

Table 3. ANOVA results of the leaf db-RDA model.

Characteristic	df	Sum of squares	F	P
PC6	1,46	0.338	1.6143	0.003
B	1,46	0.3075	1.4685	0.009
PC11	1,46	0.2814	1.3438	0.024
PC4	1,46	0.2725	1.3015	0.044
PC10	1,46	0.257	1.2272	0.066
Residual	46	9.632		

We surmise that the abiotic factors of soil nutrients and rainfall may have altered the exudate composition within *Jatropha*'s rhizosphere, leading to altered carbon sources for bacteria and biotic filtering of certain bacterial groups. It is curious that models selected higher-level soil principle components to explain the endosymbionts, as each of those components explained less than 10% of the soil microbial community. It is possible that some uncommon members of the soil microbial community corresponded to those components and influenced the final endophytic community structure. It is interesting to note that pH was not a significant predictor of endosymbiotic community composition, and its effects may have been weaker than nutrient effects. This may be because all plots were between pH 6.0 and 8.0, the pH at which soil bacterial diversity is expected to be the highest (Lauber et al. 2009). Another possibility is that we did not allow sufficient time for the lime to incorporate into the soil or cause substantial turnover in the soil species pool. Agriculturalists commonly manipulate their crop soil through amendments with agricultural lime, fertilizers, and irrigation. Such manipulations are known to alter the soil microbial community (Kennedy et al. 2004, Xue et al. 2010, Frenk et al. 2014). Here, we further show that such changes to soil chemistry can alter the microbiome of host plants. As these changes can affect host growth rates and nutrient cycling, this alteration of the microbiome should be a focus of future research.

When looking specifically at the taxa, we found profound differences at the phyla level in the roots of the different field locations, and some differences in the leaves and roots between field and greenhouse locations. In the field, different sites had significantly different abundances within the root for over 15 phyla. Many of those phyla, specifically Proteobacteria, are

often major components of endophytic communities with many common groups that are associated with plant growth promotion (Santoyo et al. 2016). We also found that our OTU richness within roots varied significantly with respect to site, and this is most likely due to a decrease in alpha diversity within the roots of the jatropha at our most arid site, El Ejido. It is likely that certain taxa, such as Proteobacteria, do not establish in high abundance, while others, such as Actinobacteria, establish much more readily within jatropha under extreme conditions, such as drought. In the leaves, we found a decreased richness of bacteria, with the community being dominated by fewer phyla than in the roots (Appendix S1: Figs. S1, S2). Although this pattern has been observed in previous studies (Zarraonaindia et al. 2015, Wagner et al. 2016), future work should focus on why jatropha leaves exclude certain bacterial taxa. These taxa should be analyzed in the future as potential plant growth promoters or as possible opportunistic antagonists.

We have demonstrated that the endosymbiont community of jatropha is a function of ambient environments, soil nutrients, agricultural practices such as liming, and soil microbial structure. While plants have been observed as singular organisms in the past, a novel idea is taking hold in ecology and evolution that plant fitness is a product of the plant holobiont (i.e., the plant host and the sum of its microbial symbionts; Vandenkoornhuyse et al. 2015). As certain bacterial taxa have abilities to respond to environmental stresses in plants (limiting drought stress and providing nutrients; Bulgarelli et al. 2013), it would be advantageous for the holobiont to be enriched in these helpful bacteria and limited in less-helpful bacteria. In this study, we find that nutrients in the soil and rainfall are significant predictors of the plant endosymbiont community. It appears that when hosts are under less-stressful conditions (increased rain and nutrients, as in Divisa), plants recruit a broad array of bacteria from the soil, with greater species richness and a community more similar to bulk soil. When plants are in harsher environments like El Ejido (e.g., lower rainfall and soil nutrients), their community is more specialized, with a lower diversity of endosymbionts. However, if stress on the plant decreases or changes the

composition of its photosynthate concentration in the roots and rhizosphere, competition between bacteria may favor only those which can efficiently scavenge nutrients (Bulgarelli et al. 2013). Likewise, a stressed plant may become more susceptible to disease. Interestingly, when nutrients were added in the form of agricultural lime in plots within Los Asientos and El Ejido, we were able to detect a response in the plant microbiome through altered endophytic composition. While this pattern is inconsistent across all plots and sites, we believe that the endosymbiotic community was responding to abiotic changes in the soil, and this response may have been stronger if the lime was given a longer time to assimilate into the soil. Future work should aim to determine if these communities specialized for abiotic conditions are enriched in helpful bacteria, strong competitors, or opportunistic antagonists.

While this work can inform plant biology and agriculture, we should be cautious when generalizing these results to other plant species and life stages. Endosymbiont communities can change as plants age and the patterns we see may not hold true as the plants mature into saplings. Future work should follow plants over time, and aim to measure more environmental variables, such as nitrogen and clay content. By analyzing life stage and more soil edaphic factors, we could build a more reliable model for predicting the endosymbiont community. In the neotropics, drought stress is temporal, with wet and dry seasons having tremendous impact on plant community structure (Engelbrecht et al. 2007, Condit et al. 2013). As this study was conducted in the wet season, we may only have captured an endosymbiont community in a rainy environment. Endosymbiont communities and general patterns observed between sites may be different when all plants are highly drought stressed in the dry season. However, early establishment of some endosymbionts may influence the future establishment of other microbial colonists, as it is known that competitive endophytes have a strong influence over future community interactions (Gaiero et al. 2013). Therefore, the snapshot captured by this study could explain patterns of symbiont communities observed in these plants in the future.

More evidence is accumulating that contradicts the old adage of microbial ecology that

“everything is everywhere,” while reinforcing the role of the environment in determining community composition (Hughes Martiny et al. 2006). Here, we provide further support for the importance of environmental filtering, but also show the importance the pool of available microbial symbionts in determining the assembly of an endosymbiont community, which can be integral in mediating local community interactions. Other studies have found that environmental filtering of endosymbionts can lead to functional convergence of microbial communities across similar environments, and functional variation is often reflective of taxonomic variation at the community level (Turnbaugh et al. 2009, Louca et al. 2016). As dissimilar environments in the field led to distinct communities, and similar environments in the greenhouse shaped similar communities, we expect to see the endosymbiont community shift in functionality between environments as well. Unlike community profiling with 16S amplicons, metagenomics/transcriptomics studies would allow us to test for functional genes in root tissue. With whole metagenome analyses of the rhizosphere or bacterial metagenome of the endosphere, we would be able to test whether these different communities across sites are in fact associated with distinct plant growth promotion genes, or if these communities have unique gene compositions. This would demonstrate the extent to which the plant holobiont (1) responds to environmental stresses through the recruitment of helpful bacteria, (2) carries beneficial genes, or (3) becomes susceptible to invasion by antagonists or opportunists. Nonetheless, such drastically different communities assembled in plant hosts under variable environmental conditions will likely have profound effects on plant host physiology and, ultimately, productivity. These effects could change how productive *Jatropha* is under a variety of circumstances and could explain ecosystem processes in agro-ecosystems.

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