Bacterial communities of *Aphis gossypii* and *Myzus persicae* (Hemiptera: Aphididae) from pepper crops (*Capsicum* sp.)

Jenny Johana Gallo-Franco\(^1,2\), Diana Nataly Duque-Gamboa\(^1,2\) & Nelson Toro-Perea\(^1,2\)

Insects harbor a wide variety of microorganisms that form complex and changing communities and play an important role in the biology and evolution of their hosts. Aphids have been used as model organisms to study microorganism-insect interactions. Almost all aphids are infected with the obligate endosymbiont *Buchnera aphidicola* and can host different bacteria that allow them to acquire traits of agronomic importance, such as resistance to high temperatures and/or defense against natural enemies. However, the bacterial communities of most aphid species remain poorly characterized. In this study, we used high-throughput DNA sequencing to characterize the bacterial communities of *Aphis gossypii* and *Myzus persicae* from two cultivable pepper species, *Capsicum frutescens* (Tabasco variety) and *C. annuum* (Cayenne variety), in four localities of southwestern Colombia. In addition, we evaluated the dynamics of *A. gossypii*-associated microorganisms on a seasonal basis. Our results show that the bacterial communities of *A. gossypii* and *M. persicae* are dominated by the primary endosymbiont *B. aphidicola*, while the presence of the facultative symbiont *Arsenophonus* sp. was only detected in one *A. gossypii* population from cayenne pepper. In addition to these two known symbionts, eight bacterial OTUs were identified that presented a frequency of 1% or more in at least one of the analyzed populations. The results show that the bacterial communities of aphids associated with pepper crops appear to be structured according to the host aphid species and the geographical location, while no differences were observed in the diversity of bacteria between host plants. Finally, the diversity and abundance of the *A. gossypii* bacterial community was variable among the four sampling points evaluated over the year and showed a relation with the aphid's population dynamics. This study represents the first approach to the knowledge of the bacterial community present in chili pepper aphids from Colombia. Nevertheless, more in-depth studies, including replicates, are required to confirm the patterns observed in the microbial communities of aphids from pepper crops.

Insects are associated with various microorganisms, many of which are capable of significantly affecting different aspects of their biology\(^1\). Through long periods of co-evolution, microorganisms and insects have developed complex interactions, ranging from pathogenesis to an obligate mutualism\(^2\). Although a great deal of attention has been devoted to pathogenic bacteria, it is likely that most insect species harbor a microbial community that even outnumbers their own cells, which mainly consists of non-pathogenic and free-living bacteria\(^3\).

Aphids (Hemiptera: Aphididae) are a diversified group of specialized insects that feed on the sap of plants and have been recognized as model organisms for studying microorganism-insect relationships\(^4\). Almost all aphids present a primary endosymbiont, *Buchnera aphidicola*, which is transmitted in a stable manner from mother to offspring and is essential to compensate for the nutritional deficiencies of these sap-sucking insects\(^5\). This endosymbiont has been shown to have a co-speciation pattern with aphids, the result of an ancestral infection and its vertical transmission along the host lineage\(^5\).

---

\(^1\)Biology department (Departamento de biología), Universidad del Valle, Street 13 No. 100-00, 760032, Cali, Colombia. \(^2\)Centre for Bioinformatics and Photonics-CIBioFi, Universidad del Valle, Street 13 # 100-00, Building 320 No. 1069, 760032, Cali, Colombia. Correspondence and requests for materials should be addressed to J.J.G.-F. (email: jenny.gallo@correounivalle.edu.co)
The cotton aphid, *Aphis gossypii* (Glover 1877), is considered one of the most destructive aphid species in the world. Although this insect has a cosmopolitan distribution, it is particularly abundant in the tropics and attacks at least 64 species of plants of economic importance, primarily of the families Cucurbitaceae, Malvaceae and Solanaceae, *Myzus persicae* (Sulzer 1776), known as the green peach aphid, is a cosmopolitan aphid that infests several plants of economic importance, primarily dicots. It has been reported that this aphid can transmit more than 100 viral diseases to approximately 30 different plant families. Severe infestations of this pest can cause chlorosis, necrosis, wilting, stunted growth, flower and fruit abortion, leaf distortion and defoliation, among other symptoms. These two aphid species, especially *A. gossypii*, are considered representative pests of pepper crops, causing several different production constraints. The cultivation of this Solanaceae is extremely important to the horticultural production of Colombia, because its growth rate is higher than the average of other crops, with a 13% rate per year. Three varieties of pepper, *Capsicum frutescens* (Tabasco var.), *C. annum* (Cayenne var.) and *C. chinense* (Habanero var.), are the most cultivated for domestic consumption and for export purposes.

Despite the economic importance of *A. gossypii* and *M. persicae* worldwide, the diversity of their microorganism communities has remained poorly characterized, and no studies have identified factors that may affect these communities. In this study, we performed mass sequencing of the 16S rRNA gene using an Illumina platform to evaluate the effects of the aphid species, host plant, geographical location and the time of year on the structuring of bacterial communities in aphids associated with pepper crops in the Colombian southwest.

**Results**

**Field sampling.** Twelve aphid samples, each consisting of a pool of 15 individuals, collected from crops of *C. frutescens* (Tabasco var.) and *C. annum* (Cayenne var.) in five localities of southwestern Colombia, were subjected to 16S rRNA Illumina MiSeq sequencing, covering variable regions V3 and V4 (Table 1, Fig. 1). Eight samples of *A. gossypii* and *M. persicae* were used to compare diversity among localities, aphid species and host plants, and four samples collected in the Yotoco locality were used to evaluate seasonal dynamics of the *A. gossypii* bacterial community.

### Table 1. Aphid sampling locations for pepper crops in southwestern Colombia.

| Location | Latitude/Longitude | Sample code | Collection date | Aphid species | Pepper variety |
|----------|--------------------|-------------|----------------|---------------|---------------|
| Toro     | N 4.60600; W 76.08300 | Ag_T1       | Feb-2017       | *A. gossypii* | Tabasco (C. frutescens) |
|          |                    | Ag_T2       | Feb-2017       | *A. gossypii* | Cayenne (C. annum)   |
|          |                    | Mp_T2       | Feb-2017       | *M. persicae* | Cayenne (C. annum)   |
| Voto     | N 3.70500; W 76.43000 | Ag_V1       | Feb-2017       | *A. gossypii* | Tabasco (C. frutescens) |
|          |                    | Ag_V2       | Feb-2017       | *A. gossypii* | Cayenne (C. annum)   |
| Dagua    | N 3.75300; W 76.65600 | Ag_D1       | Feb-2017       | *A. gossypii* | Tabasco (C. frutescens) |
| Bolivar  | N 4.30000; W 76.20800 | Ag_B2       | Feb-2017       | *A. gossypii* | Cayenne (C. annum)   |
|          |                    | Mp_B2       | Feb-2017       | *M. persicae* | Cayenne (C. annum)   |
| Yotoco   | N 3.82526; W 76.39697 | Ag_T1_Sep   | Sep-2016       | *A. gossypii* | Tabasco (C. frutescens) |
|          |                    | Ag_T1_Dic   | Dec-2016       | *A. gossypii* | Tabasco (C. frutescens) |
|          |                    | Ag_T1_Mar   | Mar-2017       | *A. gossypii* | Tabasco (C. frutescens) |
|          |                    | Ag_T1_Jun   | Jun-2017       | *A. gossypii* | Tabasco (C. frutescens) |

In addition to *Buchnera*, aphids can present a series of secondary or facultative symbionts that are inherited vertically but can also be transferred horizontally within and between host species. Although these symbionts are not necessary for the survival of the host, they appear to replicate only in association with the host and can confer characteristics of agronomic, ecological and evolutionary importance to insects under certain environmental conditions. For example, these bacteria can increase host resistance to pathogens and natural enemies. The presence of certain species of bacteria seems to confer resistance to high temperatures, which could affect the range and variability of climates that a host organism tolerates and determine its range of geographical distribution. These microorganisms can also be involved in determining the host plant that is attacked by aphids and can facilitate the transmission of viruses, an effect of the symbiont-insect interaction that is of great relevance for agronomic management due to the role that aphids can play as virus vectors for different host plants. The ability of facultative symbionts to introduce new hereditary traits in their hosts can affect both the abundance of symbionts and the success and evolution of aphids.

Several molecular characterization studies have assessed the diversity of microorganisms in the pea aphid, *Acrithosiphon pisum*, focusing on a limited range of symbionts in geographical regions such as Europe, Japan and the United States. Studying the diversity of microorganisms based only on the search for known symbionts, limits our knowledge about the possible interactions between members of microbial communities and limits the possibility of finding new symbionts, especially in non-model aphid species. High-throughput sequencing technologies are now replacing traditional PCR for the investigation of microbial communities. These sequencing technologies have become an efficient tool to characterize the diversity and structure of bacterial communities in aphids because they do not rely on prior knowledge about the diversity of the communities investigated, allowing for a better understanding of the roles of microorganisms in these insects and the importance of these symbiotic associations, specially in non-model aphids.
Sequence analysis and taxonomic assignment. After filtering for sequence quality and size, between 15392 and 16721 reads were recovered for eight samples of *A. gossypii* and *M. persicae* (Table 2). The rarefaction curves tended toward saturation (Fig. S1), and the coverage value of the sequencing data, with a dissimilarity value of 0.03, was greater than 99% in all the samples (Table 2). These results suggest that the depth of sequencing used was adequate to detect most of the bacterial diversity of *A. gossypii* and *M. persicae* from pepper crops.

The obtained reads were distributed into 454 OTUs with an identity level of 97%. These OTUs included 22 phyla, with Proteobacteria being the most dominant phylum, with a relative abundance of 93.83% (Average values across all samples, Fig. S2). The dominant class of the samples was Gammaproteobacteria with a relative abundance of 91.3% (Fig. S3). At the family level, Enterobacteriaceae was the dominant family, with a relative abundance of 87.4% (Fig. S4). The relative abundances of the representative OTUs of the bacterial communities of *A. gossypii* and *M. persicae* (>1% relative frequency in at least one sample) are shown in Table 3. The values of relative abundances obtained correspond only to approximate proportions of the bacterial OTUs in each sample evaluated.
The Enterobacteriaceae reported in
A
two bacteria were not associated with any known symbiont (Fig. 2). The genetic distance (p-distance) between
A
Phylogenetic reconstruction. According to the Greengenes database, the taxonomic assignment agrees
A
Two known endosymbionts, B. aphidicola and Arsenophonus sp., were observed, of which B. aphidicola was
don in the bacterial communities of both aphid species and was observed in all samples, as well as an OTU belonging to an unreported genus of the family Enterobacteriaceae. The highest relative abundance of B. aphidicola was observed in the MpT2 and MpB2 samples of M. persicae, and the lowest relative abundance was observed in the AgD1 sample of A. gossypii (Table 3). In contrast, Arsenophonus sp. was only observed in the AgT2 sample, with a relative abundance of 2.01% (Table 3).

Comparative analysis of bacterial communities in aphids in localities and in host plants. The samples that presented the lowest diversity indices were AgT2 (A. gossypii) and MpT2 (M. persicae), both from the Toro locality (Table 2), while the highest diversity indices were observed for the samples from A. gossypii, AgD1 and AgV1, in the localities of Dagua and Vives, respectively (Table 2). In those localities (Toro and Vives) where aphids were collected from the two varieties of pepper, the bacterial community associated with the Tabasco variety was the most diverse according to all measured diversity indices. In the localities of Toro and Bolívar, where both A. gossypii and M. persicae were collected from the Cayenne variety, A. gossypii presented the most diverse bacterial community (Table 2).

Although the diversity of bacteria seems to be greater in aphids collected from the Tabasco pepper variety (Table 2), the Principal Coordinates Analysis (PCoA), performed to determine the structure of the bacterial communities, did not show a clear clustering by host plant (Fig. 3). In the PCoA, Toro was separated from the other localities, and the Cayenne and Tabasco samples in this locality are distant from each other compared with the other localities (Fig. 3). Equally, the AMOVA (Analysis of Molecular Variance) showed that there are no significant differences in the microbial community among aphids from Cayenne and Tabasco (Fs = 1.306, p = 0.215).

The PCoA performed among aphid species showed two groups that differentiated between the M. persicae and A. gossypii samples, especially when the weighted UniFrac index was used (Fig. 4). This result shows that the bacterial communities are structured according to the host aphid. A microbial community composition different from the other localities was still observed in the locality of Toro (Fig. 4).

Seasonal dynamics of the A. gossypii bacterial community. The temporal dynamics analysis of the A. gossypii bacterial community was carried out using one sample for each of the four different times point of the year we studied, for which more than 14000 sequences of the 16S rRNA gene were obtained (Table 4). The rarefaction curves tended toward saturation (Fig. S5), and the coverage value of the sequencing data, with a dissimilarity

| Taxon                        | Relative frequencies (%) | Tabasco | Cayenne |
|------------------------------|-------------------------|---------|---------|
|                             | Ag_T1 | Ag_V1 | Ag_D1 | Ag_T2 | Ag_V2 | Ag_B2 | Mp_T2 | Mp_B2 |
| Buchnera aphidicola         | 89.91 | 86.93 | 70.27 | 93.48 | 78.41 | 83.71 | 98.21 | 95.24 |
| Pseudomonas sp.             | 0.29  | 0     | 17.48 | 1.40  | 1.82  | 0     | 0.53  |       |
| Enterobacteriaceae          | 0.60  | 0.33  | 0.27  | 0.59  | 0.40  | 0.28  | 1.05  | 0.40  |
| Arsenophonus sp.            | 0     | 0     | 2.01  | 0     | 0     | 0     | 0     | 0     |
| ACK_M1                       | 0.03  | 0.77  | 0.31  | 0     | 1.02  | 1.08  | 0     | 0.05  |
| Pseudomonadaceae            | 1.87  | 0.96  | 0.24  | 3.51  | 0     | 0     | 0.23  |       |
| Bacteroidetes                | 0.11  | 0.87  | 0.67  | 0     | 0.71  | 1.40  | 0     | 0.06  |
| Pelagibacteraceae           | 0.17  | 0.72  | 0.50  | 0     | 0.46  | 0.97  | 0.06  | 0.09  |
| Selenomonas sp.             | 1.05  | 0.23  | 0.42  | 0.31  | 0.23  | 0.31  | 0     | 0.33  |
| Sphingomonas sp.            | 0.08  | 0     | 0.15  | 0.18  | 2.47  | 0     | 0.09  | 0.08  |

Table 3. Relative frequencies of representative OTUs (>1% in at least one sample) of the A. gossypii and M. persicae bacterial communities. aTaxonomic assignment to phylum. bTaxonomic assignment to family.
value of 0.03, was above 99% (Table 4), indicating that the sampling of the microbial communities was adequate to identify most of the bacterial diversity of *A. gossypii* in the Yotoco experimental plot.

From the 16S rRNA gene sequences, a maximum of 731 OTUs were identified, with 97% identity. These OTUs were assigned to 24 phyla, among which the Proteobacteria phylum predominated with a relative abundance of 88.4% (Fig. S6) and 61 classes, with the class Gammaproteobacteria being predominant with a relative abundance of 84.9%. We identified 139 families, among which Enterobacteriaceae was the most abundant with a relative abundance of 84.6%. The relative abundances of the bacteria most representative of the bacterial community (>1% of relative frequency in at least one sample) are shown in Table 5. The dominant bacteria in all the samples was the primary endosymbiont *B. aphidicola*, with relative frequencies between 38.4 and 98.7%. The lowest relative abundance of *B. aphidicola* was observed in the sample taken in December 2016, and the highest relative abundance was observed in June 2017 (Table 5).

The samples from the month of December presented the highest diversity index and the highest number of bacterial OTUs. The other three samplings presented diversity indices similar to each other but lower than that observed in the sample from December. When we compare this seasonal variation in the *A. gossypii* bacterial community with that of *M. persicae* from pepper crops (the names of the sequences from our work end in Gallo_Franco and are colored by genus) and related sequences obtained from GenBank (the accession number of each sequence is shown at the end of the name). The phylogenetic inference was made using maximum likelihood and the GTR model.

**Figure 2.** Phylogenetic analysis of the representative bacterial OTUs (>1%) associated with *A. gossypii* and *M. persicae* from pepper crops (the names of the sequences from our work end in Gallo_Franco and are colored by genus) and related sequences obtained from GenBank (the accession number of each sequence is shown at the end of the name). The phylogenetic inference was made using maximum likelihood and the GTR model.
community with the average rainfall and temperature data in the Yotoco locality, no relationship was observed between bacterial diversity and environmental conditions (Fig. 5).

**Discussion**

In this study, the microbial diversity of *A. gossypii* and *M. persicae* from pepper crops of southwestern Colombia was characterized. These two aphid species are of global economic importance, and little is known regarding their interactions with microorganisms, which have proven to be key elements in the evolution and ecology of insects.

The results showed that the bacterial communities of *A. gossypii* and *M. persicae* are dominated by the endosymbiont *B. aphidicola*. The dominant presence of the primary endosymbiont in both aphid species is in agreement with the obligate mutualistic relationship that has been reported between these insects and their primary endosymbiont. This relationship is crucial, since aphids are dependent on this microorganism for the production of the essential amino acids, vitamins and sterols that are necessary for their normal development and reproduction. Similarly, aphids provide nutrients for *B. aphidicola*, including non-essential amino acids and...
carbohydrates that are abundant in the aphid diet, which is based on the phloem of plants, or that are produced by the aphids themselves35–37.

In addition to the primary endosymbiont *Buchnera aphidicola*, we found the secondary endosymbiont *Arsenophonus* sp. in *A. gossypii* specimens collected from the Cayenne pepper variety from Toro locality. Former studies have found that natural populations of *A. gossypii* can be associated with six genera of secondary or facultative endosymbionts (*Hamiltonella*, *Arsenophonus*, *Regiella*, *Rickettsia*, *Serratia* and *Wolbachia*)24,38–44, while natural populations of *M. persicae* with three genera of symbionts (*Regiella*, *Serratia* and *Hamiltonella*)27. However, despite the biological importance that these endosymbionts may have, they are only partially distributed in the populations of some aphid species and are even completely absent in others24,26,27. The absence of different secondary symbionts in our samples of aphids, may be because maintaining a secondary symbiont, despite being a benefit, can infer a fitness cost to the host45, which suggests that the persistence of a symbiont is determined by the balance between cost and benefit46. Fukatsu et al.47, proposed a simple model to explain the presence or absence of a symbiont in natural populations, in which the infection frequency of an endosymbiont in a host population is determined mainly by three parameters: fidelity of vertical transmission, fitness effect on the host and frequency of horizontal transmission. These patterns of variation of secondary symbionts in natural populations are consistent with previous studies realized in *A. gossypii* populations. Najar-Rodríguez et al.41, evaluated the bacterial diversity of *A. gossypii* in localities of Australia and Japan, and reported the presence of the endosymbiont *Arsenophonus* in some of the evaluated populations. In Chinese populations of *A. gossypii*, Zhao et al.24 report the presence of the symbionts *Arsenophonus* and *Hamiltonella* in all the analyzed populations, nevertheless the other three symbionts were not detected in their study.

Unlike what was believed a few years ago, when *Arsenophonus* was considered an absent endosymbiont in aphids4,48, recent studies have shown that it is a bacterium widely distributed in various insect species, including those of the family Aphididae49. Jousselin et al.44, in a study of 86 aphid species, reported an *Arsenophorus*

| Taxon                  | Relative frequencies (%) |
|------------------------|--------------------------|
|                        | Ag_Sep_16 | Ag_Dic_16 | Ag_Mar_17 | Ag_Jun_17 |
| *Buchnera aphidicola*  | 96.40     | 38.38     | 97.97     | 98.67     |
| *Enterobacteriaceae*   | 1.18      | 0.16      | 1.13      | 1.06      |
| *ACK-M1*               | 0         | 4.36      | 0         | 0         |
| *Bacteroidetes*        | 0         | 3.25      | 0         | 0         |
| *Pelagibacteraceae*    | 0         | 2.14      | 0         | 0         |
| *Cerasicoccaceae*      | 0         | 2.15      | 0         | 0         |
| *Bacteria*             | 0         | 1.50      | 0         | 0         |
| *Selenomonas sp.*      | 0         | 1.09      | 0         | 0         |
| *Chitinophagaceae*     | 0         | 2.30      | 0         | 0         |
| *Comamonadaceae*       | 0         | 1.94      | 0         | 0         |
| *Limnohabitans sp.*    | 0         | 1.64      | 0         | 0         |
| *Flavicola sp.*        | 0         | 1.59      | 0         | 0         |
| *Allobaculum sp.*      | 0         | 1.09      | 0         | 0         |
| *Biodococcus sp.*      | 1.08      | 0         | 0         | 0         |

Table 5. Seasonal relative frequencies of the representative OTUs (>1% in at least one sample) of the bacterial community of *A. gossypii* in an experimental plot of Yotoco. *Taxonomic assignment to filum. *Taxonomic assignment to family.

Figure 5. Variation in the diversity of the *Aphis gossypii* bacterial community in four temporal sampling points compared to fluctuations in temperature and precipitation in an experimental plot in the locality of Yotoco (Data taken from the Cenicaña-Yotoco weather station). The gray bars represent the alpha diversity present of the four samplings made throughout the year 2017.
infection incidence of 7%. These researchers reported an especially high incidence of *Arsenophonus* in the *Aphis* genus, more than 31% of the species were infected, but absence of *Arsenophonus* in the *Myzus* genus. Our results confirm these findings because *Arsenophonus* sp. was only detected in *A. gossypii* in populations where both *A. gossypii* and *M. persicae* coexisted on the same crop. We report the presence of *Arsenophonus* sp. in populations of *A. gossypii* infesting *C. annuum* plants (Cayenne var.), which had not been reported by Jousselin *et al.* in their study.

*Arsenophonus* sp. can have different effects on its hosts, including obligate mutualism in blood-sucking insects50, improving the performance of whiteflies31, or through facultative mutualism by protecting psyllids against parasitoid attacks52. Although the effect of *Arsenophonus* on aphids is still not fully understood, some studies have shown, particularly for the genus *Aphis*, that *Arsenophonus* could be involved in important aspects of aphid ecology. Wagner *et al.* observed that *Arsenophonus* appears to be involved in the food specialization that the polyphagous aphid *Aphis craccivora* has developed on one of its host plants. Wulf & White observed that the presence of *Arsenophonus* in individual *A. glycines* aphids improved the performance of this insect on aphid-resistant soybean plants.

In addition to the symbionts *B. aphidicola* and *Arsenophorus* sp., eight bacterial OTUs were observed at a frequency of 1% or more in at least one sample. After *B. aphidicola*, *Pseudomonas* sp. presented the highest frequency in the locality of Dagua (17.48%) and was observed at a frequency of higher than 1% in two additional samples. The OTU of the genus *Pseudomonas* observed in this study was phylogenetically grouped with the species *Pseudomonas fulva* reported in GenBank. *P. fulva* has been reported as a symbiotic bacterium in *Hypothenemus hampei* (Coleoptera: Curculionidae), one of the primary pests of coffee. By using caffeine from plants to produce nitrogen, this bacterium allows the coffee borers to survive in coffee plants55. Microorganisms of the genus *Pseudomonas* have also been reported as common members of the bacterial community of insects55,56 and it has been suggested that these bacteria have superficial associations with insects, for example, on the surface of the body or, if in the gut, then close to gut orifices. Likewise, *Pseudomonas* and *Sphingomonas*, another genus reported in our study, have previously been described in associations with phloem-feeding insects, in low abundances17,23,37,58. Conversely, *Selenomonas* genus, that was found in all of our samples, it is a group of bacteria uncommon in insects. However, this bacterial genus has been reported as part of the intestinal microbiota of the tick, *Amblyomma maculatum*19.

Several studies of non-model aphid species have shown that there are bacteria of the family Enterobacteriaceae that have not been described but that may be important in the ecology of aphids22,42. For example, Guidolin & Cóscoli suggest that an unknown genus of this family, which they named Cluster B, can play a key role in helping *Aphis citricus* to exploit less suitable host plants, complementing the contributions of *B. aphidicola*. In our study, the presence of a bacterial OTU belonging to the Enterobacteriaceae family was also reported in all the samples of *A. gossypii* and *M. persicae*. According to the phylogenetic tree made with sequences of the genebank and genus of known symbionts, this bacterium is closely related to the obligate symbiont *Buchnera*, as well as another genus of the family Enterobacteriaceae reported for the aphid *A. glycines*.

The bacterial OTUs found in this study, including the bacterium of the family Enterobacteriaceae, may be playing an important role in the aphid biology that has not yet been reported due to the lack of studies of non-model aphid species. To evaluate this, it is necessary to perform laboratory-based experiments that compare the response of aphids, in terms of performance, in presence or absence of these bacteria, evaluate the location of the symbionts inside the aphids, and report as common members of the bacterial community of insects. The possibility that some of these bacteria could be contaminants. Although heritability cannot be discarded, it is likely that this microbes participate in opportunistic associations with these insects, perhaps as gut associates, pathogens, or they could be contaminants from soil, plants or human management. Jousselin *et al.* report that contaminants accounted for a small proportion of the bacteria identified in their samples, but, the removal of sequences accounting for <1% of the reads in aphid samples would eliminate most of the sequences from contaminants present at low frequency in the aphid samples.

The bacterial communities of *A. gossypii* and *M. persicae* seem to be structured according to the species of host aphid. The association between the aphid and its bacterial community is affected by a large number of abiotic and biotic factors and may involve the immune system, nutrition, reproduction, communication and many other host systems. According to Henry *et al.*, there is a relationship between the life history traits of the aphids and the cooperative relationships they establish with the symbionts, which may determine the presence of symbionts in certain aphid species or in certain populations within a species. Recent studies have even highlighted that the microbe is involved in the ability of a given host to transmit a pathogen.

According to our results, geography can be another factor that influence the composition and frequency of bacteria associated with insects88. In our study, the locality of Toro showed a microbial community that was differentiated from the other localities and was the only one that presented the endosymbiont *Arsenophonus*. Natural populations of aphids may experience different selection pressures according to geographical location. For example, agricultural management practices, the dynamics of natural enemies and environmental conditions can affect the diversity and frequency of associated bacteria. Suchida *et al.* observed a geographiccline in the distribution and frequency of secondary endosymbionts, in the pea aphid, *A. pisum*, which in turn may be related to the host plant species, temperature and precipitation. In the psyllid species *Glycaspis brimblecombei*, significant geographic variation of the endosymbiont *Arsenophonus* was also reported. It is necessary to carry out a greater number of samplings or replicates in each of the localities evaluated in this study to confirm the existing geographical variation, as well as, the prevalence of the *Arsenophonus* symbiont in the locality of Toro.

According to the AMOVA and PCoA results, there are no significant differences between the *A. gossypii* microbial communities from the two evaluated pepper species (Cayenne and Tabasco). However, the PCoA shows that the populations of *A. gossypii* from the Tabasco and Cayenne varieties in Toro are separated by the composition of their bacterial communities and the symbiont *Arsenophonus* sp. was only detected in the Cayenne
variety. In addition, in the Viges and Toro localities, where both pepper varieties were sampled, and thereby could be compared, simultaneously, it was found the three indices of diversity were higher in the Tabasco variety. These pieces of evidence of possible differences between bacterial communities of Cayenne and Tabasco, can be the starting point for further studies that test the effects of the host plant in aphids.

The bacterial community of *A. gossypii* showed fluctuations in diversity and frequency between the different seasons of sampling, suggesting that the microbial community of aphids is dynamic over time. When comparing our results with reports of population dynamics of *A. gossypii* by Melo & Manzano 

The tool was used to verify that the primers sequences were present on the genome of the most common symbionts obtained on a MiSeq Illumina platform generating 300-bp paired reads. Additionally, the NCBI Primer-BLAST 

Data analysis. Sequence analysis and taxonomic assignments. Twenty-thousand sequences per sample were processed using the program *Mother* (v 1.39.5) according to the protocol described in the *MiSeq Standard Operating Procedure*. The maximum length of the allowed sequences was 465 bp. We used the *Uchime* algorithm implemented in *Mother* to detect chimeric sequences (sequences resulting from

**Materials and Methods**

**Sampling of aphids.** Aphid sampling was carried out for crops of *C. frutescens* Linneo (Tabasco var.) and *C. annuum* Linneo (Cayenne var.), located in five localities of southwestern Colombia (Table 1, Fig. 1). Colonies of aphids were collected systematically, taking into account the edges and center of each plot. Each sampling point was separated by at least 20 m to minimize the probability of collecting the offspring of the same mother. To guarantee that the aphids were free of parasitoids, they were collected alive and brought to the laboratory where they were reared under controlled conditions in an environmental chamber (SANYO-MLR-351H) with a photoperiod of 12 hours of light and 12 hours of darkness at a temperature of 25 °C for fifteen days. Captive-born nymphs were excluded from the study. Next, from the colonies of aphids collected in the field, groups of 15 adult apterus surviving aphids were formed from each of the plots sampled (each individual from a different colony) to form a single sample, which was preserved in 96% ethanol at −20 °C until the extraction of DNA. In those localities where *A. gossypii* and *M. persicae* were observed, 15 aphids of each species were collected. The morphological identification of the aphids was carried out using the key from Blackman & Eastop.

In the locality of Yotoco (Table 1, Fig. 1), the dynamics of the bacterial communities associated with the aphid species *A. gossypii* were evaluated on a seasonal basis. For this analysis, four samplings were carried out in an insecticide-free experimental plot between September 2016 and June 2017 with intervals of three months (Table 1). The date and the number of samples taken were chosen considering the average temperature and precipitation recorded by IDEAM (the Institute of Hydrology, Meteorology and Environmental Studies) for 2014 and 2015 (http://www.ideam.gov.co/web/tiempo-y-clima/climatologico-mensual, visited in November 2016). The two peaks of maximum precipitation and minimum temperature occurred during the months of March-April and October-November, and the two peaks of minimum precipitation and maximum temperature occurred during the months of December-January and June-July.

DNA extraction, amplification and sequencing of the 16S rRNA gene. Prior to DNA extraction, aphid samples were washed for 5 min in 70% ethanol and rinsed three times with sterile water to remove surface contamination. DNA was extracted from surface sterilized aphids (15 per sample) using a commercial DNAeasy® Blood & Tissue kit (QIAGEN) kit, following the manufacturer’s protocol. The amplification of the 16S rRNA gene was carried out using the universal primers Bakt_341F (CCTACGGGNGGCWGCAG) and Bakt_805R (GACTACHVGGGTATCTAATCC)69, which amplify the V3 and V4 hypervariable regions. The sequencing was performed using the MiSeq Illumina platform generating 300-bp paired reads. Additionally, the NCBI Primer-BLAST tool was used to verify that the primers sequences were present on the genome of the most common symbionts in aphids (*S. symbiotica, H. defensa* and *R. insecticola*). The entire amplification and sequencing process was performed by Macrogen Corp. (http://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html).

Data analysis. Sequence analysis and taxonomic assignments. Twenty-thousand sequences per sample were processed using the program *Mother* (v 1.39.5) according to the protocol described in the *MiSeq Standard Operating Procedure*. The maximum length of the allowed sequences was 465 bp. We used the *Uchime* algorithm implemented in *Mother* to detect chimeric sequences (sequences resulting from
the recombination of two different taxa sequences due to jumping events in the PCR). This procedure was carried out for each sample and the identified chimeric sequences were excluded from the data set. The operational taxonomic units (OTUs) were defined at a level of 97% similarity, and a taxonomic assignment was subsequently made using the Greengenes reference taxonomy database as a reference and a minimum confidence value of 0.8 (https://www.mothur.org/wiki/Greengenes-formatted_databases).

**Phylogenetic reconstruction.** Representative sequences of OTUs with frequencies equal to or greater than 1% in the different samples were selected, as this threshold allowed us to reduce the probability of processing sequences from contaminating bacteria. Subsequently, these sequences were used to search for symbiotic and free-living bacteria at the databases using the BLASTn tool at NCBI web interface and the database Nucleotides Collection (nr/nt). A phylogenetic analysis of the whole set of sequences was carried out using the maximum likelihood method, which also included GenBank sequences. The alignment was made using the ClustalW algorithm in MEGA. Phylogenetic reconstruction was achieved using the RAxML-HPC BlackBox program (8.2.8) through the CIPRES platform with automatic bootstrapping and the other criteria applied by default.

**Analysis of bacterial communities among aphids, localities and host plants.** From the taxonomic allocation file and their respective absolute frequencies, the relative frequencies were calculated and the OTUs were selected with a representation of at least 1% in some of the samples. Rarefaction curves were generated and diversity descriptors were calculated, including Simpson's index, the Shannon index and the Chao index using Mothur, to quantify the diversity and dominance of bacteria by aphid species, locality, host plant and season. To explore differences in the structure of the bacterial communities between the aphid species and between the host plants (in the latter comparison, only the A. gossypii samples were included, since they have greater representation in both host plants), a PCoA was realized using Mothur with the weighted (includes relative abundance of each OTU) and unweighted (only absence or presence of each OTU) UniFrac distances. UniFrac is a measure of distance that is used to calculate differences between bacterial communities based on phylogenetic information. Finally, an AMOVA was carried out in Mothur to evaluate if there were significant differences between the bacterial communities of the A. gossypii aphid, taking into account the host plant.

**References**

1. Bahndorf, S., Alemu, T., Alemneh, T. & Nielsen L. J. The Microbiome of Animals: Implications for Conservation Biology. International Journal of Genomics. Article ID 5304028 (2016).
2. Jurkevitch, E. Riding the Trojan horse: Combating pest insects with their own symbionts. Microbial Biotechnology. 4, 620–627 (2011).
3. Dillon, R. J. & Dillon, V. M. The gut bacteria of insects: Nonpathogenic interactions. Annual Review of Entomology. 49, 71–92 (2004).
4. Oliver, K. M., Degnan, P. H., Burke, G. R. & Moran, N. A. Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. Annual Review of Entomology. 55, 247–266 (2010).
5. Chichester (2000).
6. Oliver, K. M. & Moran, N. A. Defensive symbionts in aphids and other insects. In Defensive Mutualism in Microbial Symbiosis, ed. White, J. F. & Torres, M. S. 129–47 (2009).
7. Tsuchida, T., Koga, R., Shibao, H., Matsumoto, T. & Fukatsu, T. Plant virus epidemiology. Ed by Plumb, R. T. & Thresh, J. M. Oxford: Blackwell Scientific Publications. 115–132 (2003).
8. Blackman, R. L. & Eastop, V. F. Aphids on the World’s Crops: An Identification and Information Guide. John Wiley and Sons, Chichester (2000).
9. Tsuchida, T., Koga, R., Shibao, H., Matsumoto, T. & Fukatsu, T. Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, Acyrthosiphon pisum. Molecular Ecology. 11(10), 2123–2135 (2002).
10. Tsuchida, T., Kogal, R., Meng, X. Y., Matsumoto, T. & Fukatsu, T. Characterization of a Facultative Endosymbiotic Bacterium of the Pea Aphid Acyrthosiphon pisum. Microbial ecology. 49, 126–133 (2005).
11. Gauthier, J. P., Outreman, Y., Mieuzet, L. & Simon, J. C. Bacterial communities associated with host-adapted populations of pea aphids revealed by deep sequencing of 16S ribosomal DNA. Plos One. 10, e0120664 (2015).
12. Smith, A. H. et al. Patterns, causes and consequences of defensive microbiome dynamics across multiple scales. Molecular ecology. 25(5), 1135–1149 (2015).
13. Williams, S. J. & Yoosop, S. From bacterial to microbial ecosystems (metagenomics). Methods of molecular biology. 804, 35–55 (2012).
14. Degnan, P. H. & Ochman, H. Illumina-based analysis of microbial community diversity. The ISME Journal. 6, 183–194 (2012).
15. Phelan, J. A., Koga, R., Fukatsu, T. & Godfray, H. C. J. Insect symbiont facilitates vector acquisition, retention, and transmission of plant virus. Science. 337, 1367 (2013).
16. Eastop, V. F. The biology of the principle virus vectors. In: Plant virus epidemiology. Ed by Plumb, R. T. & Thresh, J. M. Oxford: Blackwell Scientific Publications. 115–132 (2003).
17. Blackman, R. L. & Eastop, V. F. Aphids on the World’s Crops: An Identification and Information Guide. John Wiley and Sons, Chichester (2000).
18. Tsuchida, T., Koga, R., Shibao, H., Matsumoto, T. & Fukatsu, T. Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, Acyrthosiphon pisum. Molecular Ecology. 11(10), 2123–2135 (2002).
19. Tsuchida, T., Kogal, R., Meng, X. Y., Matsumoto, T. & Fukatsu, T. Characterization of a Facultative Endosymbiotic Bacterium of the Pea Aphid Acyrthosiphon pisum. Microbial ecology. 49, 126–133 (2005).
20. Gauthier, J. P., Outreman, Y., Mieuzet, L. & Simon, J. C. Bacterial communities associated with host-adapted populations of pea aphids revealed by deep sequencing of 16S ribosomal DNA. Plos One. 10, e0120664 (2015).
21. Smith, A. H. et al. Patterns, causes and consequences of defensive microbiome dynamics across multiple scales. Molecular ecology. 25(5), 1135–1149 (2015).
22. Williams, S. J. & Yoosop, S. From bacterial to microbial ecosystems (metagenomics). Methods of molecular biology. 804, 35–55 (2012).
23. Degnan, P. H. & Ochman, H. Illumina-based analysis of microbial community diversity. The ISME Journal. 6, 183–194 (2012).
24. Phelan, J. A., Koga, R., Fukatsu, T. & Godfray, H. C. J. Insect symbiont facilitates vector acquisition, retention, and transmission of plant virus. Science. 337, 1367 (2013).
25. Blackman, R. L. & Eastop, V. F. Aphids on the World’s Crops: An Identification and Information Guide. John Wiley and Sons, Chichester (2000).
Bansal, R., Mian, M. A. R. & Michel, A. P. Microbiome diversity of Aphis glycines with extensive superinfection in native and invasive populations. *Environmental Microbiology Reports*. 6, 57–69 (2014).

Jing, X. et al. The bacterial communities in plant phloem-sap-feeding insects. *Molecular Ecology*. 23, 1433–1444 (2014).

Zhao, Y. et al. Bacterial communities of the cotton aphid *Aphis gossypii* associated with Bt cotton in northern China. *Scientific Reports*. 6, 22958 (2016).

Weintraub, P. C. Integrated control of pests in tropical and subtropical sweet pepper production. *Pest Management Science*. 63, 753–760 (2007).

Carletto, J. et al. Ecological specialization of the aphid *Aphis gossypii* Glover on cultivated host plants. *Molecular Ecology*. 18, 2198–2212 (2009).

Barbagallo, S., Cocuzza, G., Cravedi, P. & Komazaki, S. IPM case studies: deciduous fruit trees. In: Aphids as crop pests. ed. by Emden, H. F. & Harrington, R. Wallingford. UK: CAB, 651–661, http://www.cabi.org/cabkoobsebook/20073204012 (2007).

Black, L. L., Green, S. K., Hartman, G. L. & Paulus, J. M. Pepper diseases: A field guide. Asian Vegetable Research and Development Centre publication. 91–101 (1991).

Corporación colombiana internacional. AJ COLOMBIANO: Grandes oportunidades de mercado. Portafolio 2007. pp. 7.

Frago, E., Dicke, M. & Godfray, H. C. J. Insect symbionts as hidden players in insect-plant interactions. Trends in Ecology & Evolution. 27, 705–711 (2012).

Morton, N. A., Dunbar, H. E. & Wilcox, J. L. Regulation of transcription in a reduced bacterial genome: nutrient-provisioning genes of the obligate symbiont *Buchnera aphidicola*. *Journal of Bacteriology*. 187, 4229–4237 (2005).

Morton, N. A. & Degnan, P. H. Functional genomics of *Buchnera* and the ecology of aphid hosts. *Molecular Ecology*. 5, 1251–1261 (2006).

Hansen, A. K. & Moran, N. A. Aphid genome expression reveals host-symbiont cooperation in the production of amino acids. *Proceedings of the National Academy of Sciences USA*. 108, 2849–2854 (2011).

Russell, J., Latorre, A., Sabater-Muñoz, B., Moya, A. & Moran, N. A. Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea. *Molecular Ecology*. 12, 1061–1075 (2003).

Carletto, J., Gueguen, F., Fleury, F. & Vanlengerbe-Masutti, F. Screening the bacterial endosymbiotic community of sap-feeding insects by terminal-restriction fragment length polymorphism analysis. *Entomologia Experimentalis et Applicata*. 129, 228–238 (2010).

Desneux, N., Barta, R. J., Hoelmer, K. A., Hopper, K. R. & Heimpel, G. E. Multifaceted determinants of host specificity in an aphid parasitoid. *Oecologia*. 160, 387–398 (2009).

Najar-Rodriguez, A. J., McCraw, E. A., Mensah, R. K., Pittman, G. W. & Walter, G. H. The microbial flora of *Aphis gossypii* Patterns across host plants and geographical space. *Journal of invertebrate pathology*. 100, 123–126 (2009).

Augustinos, A. A. et al. Detection and characterization of *Wolbachia* infections in natural populations of aphids: Is the hidden diversity fully unraveled? *Plos One*. 6, e28695 (2011).

Jones, R., Bressan, A., Greenwell, A. M. & Fierer, N. Bacterial Communities of Two Parthenogenetic Aphid Species Cocolonizing Two Host Plants across the Hawaiian Islands. *Applied and environmental microbiology*. 77(23), 8345–8349 (2011).

Jousselin, E., Coeer d’acier, A., Vanlengerbe-Masutti, F. & Duron, O. Evolution and diversity of *Arsenophonus* endosymbionts in aphids. *Molecular Ecology*. 22, 260–270 (2013).

Vorburgh, C. & Gouskov, A. Only helpful when required: a longevity cost of harbouring defensive symbionts. *Journal of Evolutionary Biology*. 24, 1611–1617 (2011).

Oliver, K. M., Smith, A. H. & Russell, J. A. Defensive symbiosis in the real world - advancing ecological studies of heritable, protective bacteria in aphids and beyond. *Functional Ecology*. 28, 341–355 (2014).

Fukatsu, T., Nikoh, N., Kawai, R. & Koga, B. The secondary endosymbiotic bacterium of the pea aphid *Acyrthosiphon pisum* (Insecta: Homoptera). *Applied and Environmental Microbiology*. 66, 2748–2758 (2000).

Wille, B. D. & Hartman, G. L. Two species of symbiotic bacteria present in the soybean aphid (Hemiptera: Aphididae). *Environmental Entomology*. 38, 110–115 (2009).

Duron, O., Wilkes, T. E. & Hurst, G. D. Interspecific transmission of a male-killing bacterium on an ecological timescale. *Ecology Letters*. 13, 1139–1148 (2010).

Dale, C., Beeton, M., Harbison, C., Jones, T. & Pontes, M. Isolation, pure culture, and characterization of “Candidatus Arsenophonus arthropodicus,” an intracellular secondary symbiont from the hibbiscosid fly *Pseudolynchia canariensis*. Applied. *Environmental Microbiology*. 72, 2997–3004 (2006).

Raina, H. S. et al. Elimination of Arsenophonus and decrease in the bacterial symbionts diversity by antibiotic treatment leads to increase in fitness of whirledly, *Bemisia tabaci*. *Infection, Genetics and Evolution*. 32, 224–230 (2015).

Hansen, A., Jeong, G., Paine, T. & Stouthamer, R. Frequency of secondary symbiont infection in an invasive psyllid relates to parasitism pressure on a geographic scale in California. *Applied Environmental Microbiology*. 73, 7531–7535 (2007).

Wagner, S. M. et al. Facultative endosymbionts mediate dietary breadth in a polyphagous herbivore. *Functional Ecology*. 29, 1402–1410 (2015).

Wulff, J. A. & White, J. A. The Endosymbiont *Arsenophonus* Provides a General Benefit to Soybean Aphid (Hemiptera: Aphididae) Regardless of Host Plant Resistance (Rag). *Environmental Entomology*. 44(3), 574–81 (2015).

Ceja-Navarro, J. A. et al. Gut microbiota mediate caffeine detoxification in the primary insect pest of coffee. *Nature communications*. 6, 7618 (2015).

Zahner, V., Lucarotti, C. J. & McIntosh, D. Application of 16S rDNA-DGGE and plate culture to characterization of bacterial communities associated with the sawfly, *Acantholyda erythrocephala* (Hymenoptera, Pamphiliidae). *Current Microbiol*. 57(6), 564–569 (2008).

Clark, E. L., Daniell, T. J., Wishart, J., Hubbard, S. F. & Karley, A. J. How conserved are the bacterial communities associated with aphids? A detailed assessment of the *Brevicoryne brassicae* (Hemiptera: Aphididae) using 16S rDNA. *Environmental Entomology*. 41, 1386–1397 (2012).

Singh, S. T. et al. Diversity and phylogenetic analysis of endosymbiotic bacteria from field caught *Bemisia tabaci* from different locations of North India based on 16S rDNA library screening. *Infection, Genetics and Evolution*. 12, 411–419 (2012).

Budachetri, K. et al. An Insight Into the Microbiome of the *Amblyomma maculatum* (Acari: Ixodidae). *Journal of Medical Entomology*. 51(1), 119–129 (2014).

Guidolin, A. S. & Cósfolí, F. L. Symbiont Diversity of *Aphis* (Toxoptera) *citricidus* (Hemiptera: Aphididae) as Influenced by Host Plants. *Microbial Ecology*. 73, 201–210 (2017).

Jousselin, E. et al. Assessment of a 16S RNA amplicon illumina sequencing procedure for studying the microbiome of a symbiont-rich aphid genus. *Molecular ecology*. 16, 628–640 (2016).

Staubach, F., Baines, J. F., Kunzel, S., Bik, E. M. & Petrov, D. A. Host species and environmental effects on bacterial communities associated with *Drosophila* in the laboratory and in the natural environment. *Plos one*. 8(8), e70749 (2013).

Weiss, B. & Aksoy, S. Microbiome influences on insect host vector competence. *Trends in Parasitology*. 27(11), 514–522 (2011).

Oliver, K. M., Campos, J., Moran, N. A. & Hunter, M. S. Population dynamics of defensive symbionts in aphids. *Proceedings of the Royal Society of London B*. 275, 293–299 (2008).

Hodgson, F., Obertović, Z., Brown, C. & Lawrenson, R. PSA testing in general practice. *Journal of Primary Health Care*. 4(3), 199–204 (2012).
66. Melo, C.I & Manzano, M.R. Influencia de insectos depredadores en poblaciones de Aphis gossypii (Glover) (Hemiptera: Aphididae) en Capsicum frutescens L. Poster presentado en el 44 congreso de la sociedad colombiana de entomología. Bogotá, Colombia (2017, Julio).
67. Salter, S. J. et al. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biology. 12, 87 (2014).
68. Glassing, A., Dowd, E. S., Galandius, S., Davis, B. & Chiodini, R. J. Inherent bacterial DNA contamination of extraction and sequencing reagents may affect interpretation of microbiota in low bacterial biomass samples. Gut Pathogens. 8, 24 (2016).
69. Herlemann, D. P. R. et al. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. The ISME Journal. 5, 1571–1579 (2011).
70. Schloss, P. D. et al. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Applied. Environmental Microbiology. 75(23), 7537–7541 (2009).
71. Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K. & Schloss, P. D. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology. 79(17), 5112–5120 (2013).
72. Kozich, J., Westcott, S., Baxter, N. & Highlander, S. MiSeq SOP. University of Michigan, United States. Visited in noviembre de 2017, https://mothur.org/wiki/MiSeq_SOP (2013).
73. Edgar, R. C., Haas, B. L., Clemente, J. C., Quince, C. & Knight, R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 27, 2194–2200 (2011).
74. Edgar, R. C. Muscle: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research. 32(5), 1792–1797 (2004).
75. Kumar, S., Stecher, G. & Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution. 33, 1870–1874 (2016).
76. Stamatakis, A. RAxML-VI-HPC: Maximum Likelihood-based Phylogenetic Analyses with Thousands of Taxa and Mixed Models. Bioinformatics 22(21), 2688–2690 (2006).
77. Miller, M. A., Pfeiffer, W., & Schwartz, T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees in Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA. Pag. 1–8 14 Nov. 2010.
78. Lozupone, C. & Knight, R. UniFrac: a New Phylogenetic Method for Comparing Microbial Communities. Applied. Environmental Microbiology. 71(12), 8228–8235 (2005).
79. Excoffier, L., Smouse, P. E. & Quattro, J. M. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 131, 479–491 (1992).
80. Anderson, M. J. A new method for non-parametric multivariate analysis of variance. Austral Ecology. 26, 32–46 (2001).
81. Martin, A. P. Phylogenetic approaches for describing and comparing the diversity of microbial communities. Applied Environmental Microbiology. 68, 3673–3682 (2002).

Acknowledgements
We acknowledge financial support from the Colombian Science, Technology and Innovation Fund-General Royalties System (Fondo CTeI-Sistema General de Regalías, contract BPIN 2013000100007) and Centre for Bioinformatics and Photonics—CIBioFi. We thank the research group Interacción Planta - Microorganismo - Ambiente (IPMA) from the Universidad Nacional-Palmira for their support in the collection and identification of samples. We thank to Hugo Restrepo y Cía. S.A for the support during the field phase. We thank to the Network and Distributed Systems Laboratory, from Universidad del Valle for their support in the bioinformatic analysis and to the Posgrado en Ciencias-Biología from Universidad del Valle for their academic assistance. We are grateful to N. Rivera, R. Viáfara for his technical assistance and contributions to the data analysis. We are grateful to M. Peñuela for a critical reading of the manuscript and contributions to the data analysis.

Author Contributions
J.J.G. and D.N.D. designed the experiments and collected the samples. J.J.G and N.T. performed the experiments and analyzed the data. J.J.G. wrote the manuscript. All of the authors reviewed the manuscript.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-019-42232-8.

Competing Interests: The authors declare no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2019