**Research Article**

*Vanilla* aerial and terrestrial roots host rich communities of orchid mycorrhizal and ectomycorrhizal fungi

Lynnaun J. A. N. Johnson¹,² | Ma. del Carmen A. Gónzalez-Chávez³ | Rogelio Carrillo-González³ | Andrea Porras-Alfaro⁴ | Gregory M. Mueller¹,²

¹Negaunee Institute for Plant Conservation Science and Action, Chicago Botanic Garden, Glencoe, IL, USA
²Program in Plant Biology and Conservation, Northwestern University, Evanston, IL, USA
³Programa de Edafología, Colegio de Postgraduados, Montecillo, Mexico State, Mexico
⁴Department of Biological Sciences, Western Illinois University, Macomb, IL, USA

**Correspondence**
Lynnaun J. A. N. Johnson, Negaunee Institute for Plant Conservation Science and Action, Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, IL 60022, USA.
Emails: Lynnaun_Johnson@rush.edu; lynnaunjohnson2018@u.northwestern.edu

**Funding information**
SAGARPA-CONACYT-SNITT

---

**Social Impact Statement**

*Vanilla planifolia* is the source of the spice vanilla. This study is part of an international initiative to study the biology, including mycorrhizal fungi and cultivation practices of vanilla to improve its production in Mexico. The study focused on documenting mycorrhizal fungal diversity in vanilla. It also provided preliminary data on differences in mycorrhizal fungal communities associated with cultivation practices. A richer mycorrhizal community was observed in vanilla growing in a wild natural farm compared with those from a highly managed farm. Our research provides insights for sustainable vanilla production that can benefit Mexican farming communities.

**Summary**

- Relatively little is known regarding differences in root symbionts (i.e., mycorrhizae) between epiphytic and terrestrial orchids. We characterized the mycorrhizal fungal communities of aerial and terrestrial roots of the orchid, *Vanilla planifolia*, from four Mexican farms representing different management systems.
- Amplicon sequencing identified 40 putative mycorrhizal fungi based on ITS sequence data, these included traditional orchid mycorrhizal fungi such as *Ceratobasidium*, and *Thanatephorus* in the order Cantharellales as well as *Serendipitaceae* in the order Sebacinales, and species of several genera traditionally considered as ectomycorrhizal fungi. Mycorrhizal fungal communities were similar in aerial and terrestrial roots, but differed in read abundances.
- Plants growing in wild-natural conditions hosted a richer, but not statistically different, community of mycorrhizal fungi in comparison with plants in the highly managed farm. Soil characteristics including texture, organic matter, N, P, and K do not explain differences between fungal communities at these farms.
- This is one of the first reports of a diverse community of fungi traditionally considered to form ectomycorrhizas in association with aerial orchid roots. Further research is needed to understand the functional role of these putative mycorrhizal fungi in the ecology of *V. planifolia*, and if ectomycorrhizal fungi commonly occur in other hemiepiphytic and epiphytic orchids.
1 | INTRODUCTION

Orchids are the largest flowering plant family in the world with an estimated 27,800 species (Chase et al., 2015; Christenhusz & Byng, 2016). The family occurs worldwide, and the majority (69%) are adapted to grow as epiphytes (Zotz, 2013). Although pollinators are essential drivers of orchid diversity (Cazzonello & Widmer, 2005), researchers have argued that fungi are also involved in fostering orchid diversity and their distribution patterns (Otero & Flanagan, 2006). All wild orchids require orchid mycorrhizal fungi (OMF) to germinate and in some cases, continue to rely on fungi as adults (Gebauer et al., 2016; Rasmussen et al., 2015; Yoder et al., 2000). Understanding the relationship between orchid diversity and OMF has received some attention (Roche et al., 2010; Shefferson et al., 2007; Taylor et al., 2004; Waterman et al., 2011), but more information is needed to elucidate fundamental aspects of OMF diversity, including potential differences in OMF composition and abundance between epiphytic and terrestrial orchids.

Orchid mycorrhizal fungi are facultative biotrophs (Smith & Read, 2010). They are a polyphyletic group of mainly Basidiomycota fungi belonging to the form genus *Rhizoctonia*, a group consisting of species of Ceratobasidiaceae, Serendipitaceae, and Tulasnellaceae (Dearnaley et al., 2012; Weiß et al., 2016). In general, mature roots of both epiphytic and terrestrial orchids have been reported to associate with OMF with varying degrees of specificity. Furthermore, some terrestrial orchids that are non-photosynthetically frequently associate with ectomycorrhizal (ECM) fungi (Smith & Read, 2010). These orchids are considered mycoheterotrophic where the orchid is chlorophyllous and uses carbon derived from ECM fungal associations with other photosynthetic hosts (McKendrick et al., 2000; Taylor & Bruns, 1997). So far, ECM fungi that have been found to colonize orchid roots based on the review by Dearnaley et al. (2012) includes Ascomycota [e.g., *Tuber* (Selosse et al., 2004)] and Basidiomycota fungi [e.g., *Inocybe*, *Russula*, and *Sclerotera* (Bidartondo et al., 2004; González-Chávez et al., 2018; Roy et al., 2009; Selosse et al., 2004; Taylor & Bruns, 1997)]. Epiphytic orchid associations with ECM fungi have rarely been reported (Kartzinel et al., 2013), although roots of terrestrial orchids that are photosynthetic have been observed in shaded forests to also associate with ECM fungi (González-Chávez et al., 2018; Julou et al., 2005).

Several differences between the root morphology and ecology of epiphytic versus terrestrial orchids are likely to influence the mycorrhizal fungal community. For instance, roots of epiphytic orchids have a hard velamen, an external, hygroscopic tissue layer around the cortex, whereas the roots of terrestrial orchids have a spongy velamen (Stern & Judd, 1999). Epiphytic orchids are adapted to microhabitats that are water-stressed, nutrient-poor, and have a high irradiance when compared to co-occurring terrestrial orchids (Benzing, 2008). Microscopy studies of epiphytic roots have shown OMF colonization where the root adheres to the surface of the host tree bark (Porras & Bayman, 2003; Smith & Read, 2010). While a recent study from Xing et al., (2019) showed modularity in epiphytic and terrestrial OMF communities, Martos et al., (2012) showed high nestedness of mycorrhizal fungi associating with epiphytic orchids compared to terrestrial orchids. Martos et al., (2012) hypothesized that epiphytic orchids adapted to stressful environments are predisposed to associate with mycorrhizal fungi that facilitate water and nutrient access compared to terrestrial orchids. Further studies to determine these differences have yet to be investigated.

The traditional reliance on culture-based methods has limited the characterization of fungal communities of orchid roots because many fungi are either unculturable or very slow-growing (Allen et al., 2003; Arnold et al., 2007). This includes the vast majority of ECM fungi. The use of environmental sequencing (i.e., DNA isolated and sequenced directly from an environmental sample) is an improved alternative to culture-based methods for determining mycorrhizal fungi (Edwards et al., 2015; Lundberg et al., 2012; Manter et al., 2010). Recent studies of epiphytic and terrestrial orchids in temperate and tropical habitats have revealed the usefulness of environmental sequencing, specifically amplicon sequencing, to characterize OMF (Cevallos et al., 2017; Herrera et al., 2019; Oja et al., 2015).

To assess differences in mycorrhizal fungal communities (OMF and ECM fungi) between epiphytic (i.e., aerial) and terrestrial roots, we compared fungal communities of the aerial and terrestrial roots of the orchid *Vanilla planifolia* Jacks. ex Andrews employing amplicon sequencing. Morphologically the aerial roots of *V. planifolia* are chlorophyllous (Díez et al., 2017), while its terrestrial roots are chlorophyllous (Stern & Judd, 1999). In previous studies, *Rhizoctonia*-like fungi such as *Ceratobasidium, Thanatephorus*, and *Tulasnella* were observed in both root types of *V. planifolia* (Bayman et al., 2011; Porras & Bayman, 2003; Porras-Alfaro & Bayman, 2007) and a recent study by González-Chávez et al., (2018) found *Sclerotera*, an ECM fungus, forming pelotons in the terrestrial roots of *V. planifolia*.

This study was part of a broader interdisciplinary effort to understand the biotic and abiotic factors that influence the production of *V. planifolia*, an economically valuable crop in Mexico sold worldwide for its aroma and flavor (Havkin-Frenkel & Belanger, 2018; Herrera-Cabrera, 2016). In 2008/2009 the annual production in Mexico was 150 tons of green vanilla resulting in 20 tons of cured vanilla (Havkin-Frenkel & Belanger, 2018). Typically, *V. planifolia* crops are a monoculture with terrestrial roots continually grown in soil while aerial roots are rooted in supports (“tutors”) of live or dead host trees or inorganic materials (e.g., concrete). We examined the diversity of mycorrhizal fungi associated with *V. planifolia* aerial and terrestrial roots. As the four farms available to us differed in cultivation practices, our study also served as a pilot project for investigating how cultivation practices in Mexico...
influence-associated fungal communities. We hypothesized that terrestrial roots of *V. planifolia* have a distinct and more diverse mycorrhizal fungal community compared to its aerial roots. We also hypothesized that *V. planifolia* of a highly managed farm have lower species richness of mycorrhizal fungi than a wild natural farm.

### 2 MATERIALS AND METHODS

#### 2.1 Sampling sites and sample collections

Aerial and terrestrial roots were randomly sampled from four *V. planifolia* farms in Mexico during March and April 2014 (during anthesis) (Table 1). Three of the farms were in the state of Veracruz, and the fourth farm was in the state of Puebla. Farms were in 1 de Mayo, Papantla de Olarte Ocampo, Veracruz (20° 17′ 719″ N, 97° 15′ 909″ W); 20 Soles, Papantla de Olarte, Veracruz (20° 25′ 1.57″N, 97° 18′ 8.04″ W); Puntilla Aldama, San Rafael, Veracruz (20° 10′ 45.58″ N, 96° 54′ 13.69″ W), and at Pantepec, Puebla (20° 30′ 18″ N, 97° 53′ 22″ W). Each vanilla farm was characterized into one of three general categories based on their farming systems (i.e., cultivation practices): (a) wild natural, (b) traditional, and (c) highly managed (see Figure S1 and Table 1). The wild natural farm of 1 de Mayo grew *V. planifolia* as a monoculture with no other crops interspersed (González-Chávez et al., 2018). One aerial and one terrestrial root each from five healthy *V. planifolia* plants at the four different farms were collected for a total of 40 root samples. Only aerial roots that adhered to tutors were collected (Table 1). The length of each root sample was approximately 5 mm. Each aerial and terrestrial root sample was immediately stored in cetyltrimethylammonium bromide (CTAB) buffer and then placed on ice (U'Ren et al., 2014). Roots were kept at 4°C until surface sterilization and DNA extraction. Mycorrhizal colonization was confirmed by observing the roots under a microscope.

#### 2.2 Amplicon library preparations

Root samples were first surface sterilized with 70% ethanol and 50% Clorox® (=2.6% sodium hypochlorite) following the methods of Bayman et al. (1997) before DNA extraction to reduce contamination. We did not remove the velamen on either aerial and terrestrial roots as a pilot study using amplicon sequencing revealed no significant differences between the mycorrhizal communities of terrestrial roots with or without velamen (Johnson et al. unpublished). Genomic DNA was extracted with a DNeasy Plant Mini Kit (Qiagen Inc.) following instructions from the manufacturer. The presence of genomic DNA was visualized using electrophoresis gels.

Amplicon libraries for the Internal Transcribed Spacer (ITS) region 2 were generated using a three-step PCR approach for paired-end sequencing on an Illumina miSeq (Table S1). PCR was performed using primers ITS86f (5′- GTGAATCATCGAATCTTTGAA-3′) (Turenne et al., 1999) and ITS4 (5′- TCCTCGGCTTATTGATATGC-3′) (White et al., 1990). Polymerase chain reaction was performed in 25 µl reactions and contained: 1 µl of each primer (forward and reverse) (5 µM), 1 µl of genomic DNA (5 mM final concentration), 9.5 µl of PCR-grade water, and 12.5 µl of 2X My Taq Master Mix (Bioline). The thermal cycler conditions were as follows: 2 min at 94°C followed by 32 cycles at 94°C for 45 s, then annealing at 59°C for 45 s, an extension for 1 min at 72°C, and a final extension at 72°C for 5 min. These PCR products were then cleaned with a concentration of 0.8x AMPure XP beads (Beckman Coulter).

Next, for the second round of PCRs, primers ITS86F-adpt and ITS4-adpt (see Table S1) were used in 25 µl reactions to amplify the PCR products generated from the first-round of PCR. This second PCR step used the same reagents and quantities as the first PCR; however, this PCR step was reduced to 25 cycles. Gel electrophoresis was used to visualize successful PCR products. These PCR were cleaned with a concentration of 0.8x AMPure XP beads.

For the third-round of PCRs, index adapters (Nextera XT Index Kit v2 Set B, 96 indices, 384 samples, Illumina) were ligated onto the amplicons generated from the second-round of PCRs. The third round used the same reagents and quantities as the first and second rounds for 8 cycles. PCR products were then purified with a concentration of 1.0x AMPure XP beads then quantified using a Qubit 2.0 Fluorometer (Life Technologies, Burlington, ON, Canada).
with the Qubit dsDNA HS kit (Invitrogen). The PCR products were pooled into equimolar concentrations for a final amplicon library. The approximate bp lengths of the final amplicon library was determined using a Bioanalyzer - Agilent 2.100 (Agilent Technologies). The sequencing of the final amplicon library was completed on an Illumina miSeq for paired-end sequencing (2 × 250 bp) at the Field Museum. For the miSeq run, 40% PhiX (Control v3, Illumina, Inc.) was spiked at the same equimolar concentration as the final amplicon library. The sequences generated from this study were submitted to NCBI's Sequence Read Archive under the BioProject PRJNA540935.

### 2.3 Data processing and statistical analyses

Paired-end reads were processed using the Pipits pipeline (version 2.2) (Gweon et al., 2015). First, forward and reverse sequences were joined (VSEARCH) (Rognes et al., 2016), and quality filtered with FASTQ_QUALITY_FILTER (FASTX-toolkit, http://hannonlab.cshl.edu) (Gordon & Hannon, 2010) using the default settings of Pipits. During this quality filtering step global singletons were removed. Fungal ITS 2 reads were then detected and retained using ITSx (Bengtsson-Palme et al., 2013) that used HMMER3 (Mistry et al., 2013), whereas chimeras were removed with VSEARCH (Rognes et al., 2016). Lastly, reads were clustered at 95% sequence similarity into Operational Taxonomic Units (OTUs) and, taxonomy was assigned with the RDP classifier (Wang et al., 2007) that relied on the UNITE fungal database (Abarenkov et al., 2010; Nilsson et al., 2019). The OTU clustering of 95% sequence similarity is the recommended OTU clustering for reads generated with primers ITS86F and ITS4 (Waud et al., 2014). The default setting for a confidence threshold of 85% for RDP classifier was used in Pipits to assign taxonomy. Finally, to assess the functions of OTUs, OTUs were assigned to guilds using the program FUNGuild v1.0 (Nguyen et al., 2016). FUNGuild assignments did not characterize all putative fungae were assigned to guilds using the program FUNGuild v1.0 (Nguyen et al., 2016). The sequencing of the final amplicon library was completed on an Illumina miSeq for paired-end sequencing (2 × 250 bp) at the Field Museum. For the miSeq run, 40% PhiX (Control v3, Illumina, Inc.) was spiked at the same equimolar concentration as the final amplicon library. The sequences generated from this study were submitted to NCBI's Sequence Read Archive under the BioProject PRJNA540935.

### 2.4 Soil physiochemical analysis

Soil samples from the four V. planifolia farms were collected at the base of tutors adjacent to growing V. planifolia (n = 20, 5 samples per farm, see Table S2). Briefly, the following soil characteristics were analyzed: pH and electrical conductivity (EC) in a 1:2.5 soil:solution ratio (Rowell, 2014), exchangeable K after extraction (Chapman, 1965), organic matter by wet digestion (Nelson & Sommers, 1983), soil particle by Boyoucos procedure (Day, 1965), total nitrogen after soil mineralization (Bremer, 1965), extractable P by the procedure of Olsen et al. (1954), and micronutrients by DTPA extraction procedure (Lindsay & Norvell, 1978). Principal component analysis (PCA) was used to investigate the differences between soil characteristics of each farm. Analyses were generated for PCA using the R package Factoextra (Kassambara & Mundt, 2017).

### 3 RESULTS

Analyses of V. planifolia roots (i.e., 18 aerial and 19 terrestrial) from four Mexican farms yielded 656,918 quality reads (i.e., aerial: 355,299 reads and terrestrial: 301,619 reads). 834 OTUs were resolved after clustering reads at 95% sequence similarity. OTUs were mostly Ascomycota (55%) and Basidiomycota (22%), with the remaining 23% classified as “unknown” at the phylum level. The most dominant class for Ascomycota was Sordariomycetes with 27% of the total reads followed by Dothidiomycetes with 12% of the total reads. For Basidiomycota, Agaricomycetes accounted for 16% of the total reads while the other classes totaled 6%. For the total reads, no single OTU had read abundances greater than 15% for either root type. For example, a dominant Fusarium OTU accounted for 8% of the total reads in terrestrial roots and 2% of the aerial roots.

Most OTUs (60%) could not be assigned to trophic modes with the program FUNGuild (Table S3). Of the remaining 40%, saprotrophs comprised 12%, symbiotrophs 5%, pathotrophs 6.5%, and multiple guild associations 16.5% (e.g., pathotroph-symbiotroph) (Table S3). FUNGuild is not effective at assigning trophic modes of genera that have multiple trophic modes (e.g., Ceratobasidium spp. are assigned as pathogens in FUNGuild but are well-documented symbionts in orchids). The review article of Dearnaley et al. (2012) was used to further resolve trophic modes to determine putative OM. We assigned 40 OTUs, 9.8% of total reads, as putative mycorrhizal fungi of which some OTUs were previously assigned to multiple guilds and ECM fungi by FUNGuild (Table S4 and Figure 1). Mycorrhizal fungi included OM species in families Ceratobasidiaceae and Serendipitaceae as well as ECM fungi such as Inocybe, Russula, Sclerotermia, Tomentella, and Tuber. We also detected saprobic fungi such as Marasmius, Mycena, and Gymnoporus that we characterized as putative OM for this study based on Dearnaley et al. (2012). Using microscopy, we detected fungal pelotons in all examined aerial and terrestrial root samples of V. planifolia.

The PCoA ordination of total fungal communities revealed distinct clusters for aerial and terrestrial roots in the ordination...
space (Figure 2). These differences were further highlighted with a PERMANOVA revealing that differences were significant between aerial and terrestrial root fungal communities ($F_{1, 35} = 3.65$, $r^2 = 0.09$, $p < .05$). Furthermore, rarefaction curves revealed that aerial roots had a greater OTU richness in comparison to terrestrial roots (Figure S2, and also see Figure S3).

Most mycorrhizal fungi of aerial roots (86%) were present in the terrestrial roots (Figure S4a) and, unlike the situation observed for the total fungal community, putative mycorrhizal fungi were more abundant in terrestrial roots compared to aerial roots (Figure 1). Mycorrhizal fungi of aerial roots comprised 2% of the total reads, whereas the terrestrial root community made up 19% of the total reads. Moreover, we detected lower read abundances for mycorrhizal fungi in most aerial root samples (Figure 1).

Ceratobasidiaceae were the dominant mycorrhizal fungi in *V. planifolia* roots (both aerial and terrestrial) (Figures 1 and 3). We further refined the identification of Ceratobasidiaceae OTUs using the species hypothesis function of UNITE (Nilsson et al., 2019) and identified several of the OTUs as *Thanatephorus* species. Other putative mycorrhizal fungi such as *Inocybe* (OTU 131) and *Tuber* (OTU 181) were frequent among terrestrial roots from all farms while others such as *Gymnopus* (Omphalotaceae), *Marasmius* (Marasmiaceae), *Mycena* (Tricholomataceae), *Russula* (Russulaceae), *Scleroderma* (Sclerodermataceae), and *Thelephora* (Thelephoraceae) were rare (Figure 1). Some of the rare mycorrhizal fungi such as *Serendipita* sp. (OTU 225), *Scleroderma* (OTU 53), and *Tuber* (OTU 181) were present in both terrestrial and aerial roots at multiple farms (Figure 4). Moreover, the relative number of reads for all putative mycorrhizal fungi did not show a dominant OTU among either root type or farms (Figure 4).

Comparisons of putative mycorrhizal fungal communities among farming systems revealed some differences (Figure 1 and Figure S5). For instance, rare taxa such as *Mycena* and *Marasmius* (non-traditional OMF) were not found in root samples taken from the highly managed farm (Pantepec) and terrestrial roots of the wild natural farm (1 de Mayo) had increased read abundances for *Inocybe* OTU 131 which was rare in roots at the highly managed farm (Figure S5). Conversely, *Gymnopus* was unique to the highly managed farm (Figure S6), whereas other putative mycorrhizal fungi such as *Russula* and *Inocybe* OTUs were present in roots from both the highly managed and wild natural farms. Differences in the presence and absence of rare taxa such as *Mycena*, *Marasmius*, and *Scleroderma* OTUs between the two traditional farms (the intermediate cultivation practices) were also observed (Figure S6). Lastly, the dominant OMF, Ceratobasidiaceae OTU 88 and Ceratobasidiaceae OTU 93, were present among all sites but were most abundant in the terrestrial roots of the two traditional farms (Figure S6).

Soil characteristics of the highly managed and wild natural farms were similar to each other but differed from both traditional farms (Table S2). PCA results showed that pH, EC, organic matter, and Fe were significant for distinguishing farms (Figure S7). Potential drivers...
FIGURE 2  Principal coordinate analysis of fungal community composition in all root samples (aerial = ○, and terrestrial = Δ) using Bray-Curtis dissimilarity on abundance data (i.e., CSS normalized abundance data). The 95% confidence ellipses show 1 standard deviation around the aerial and terrestrial centroids.
of the clustering seen in ordination space is likely due to similar pH at the highly managed and wild natural farms, an increase of Fe at the traditional farm at Puntilla, and increased organic matter at the traditional farm 20 Soles (Figure S7).

**4 | DISCUSSION**

Using amplicon sequencing, we made in-depth comparisons between the fungal communities of the morphologically distinct aerial and terrestrial roots of *V. planifolia*. Aerial roots had greater total fungal OTU richness with 692 OTUs compared to 342 OTUs in terrestrial roots (Figure S4a,c). However, we did not detect differences in mycorrhizal fungal communities (OMF and ECM fungi), as aerial and terrestrial roots shared 86% of their OTUs (Figure S4b).

We detected most of the major traditional OMF genera, that is, *Ceratobasidium*, *Thanatephorus*, and *Sebacina*. However, we did not detect *Tulasnella*, which was reported from *V. planifolia* by Porras-Alfaro and Bayman, (2007) and many other orchids (Currah et al., 1997; Suárez et al., 2006; Zettler et al., 2017). Although the primers we employed were optimized to detect OMF including *Tulasnella* OTUs (Waud et al., 2014), Tedersoo et al. (2015) noted *Tulasnella* sequences having mismatches with these primers, and this could potentially limit their efficiency to recover *Tulasnella* species.

A diversity of fungal guilds in addition to potential mycorrhizal fungi were detected in *V. planifolia* roots. For instance, we repeatedly observed a *Fusarium* OTU within roots of healthy *V. planifolia*.
plants. Researchers have routinely reported *Fusarium* species from roots of *V. planifolia* as pathogens (Havkin-Frenkel & Belanger, 2018; Koyyappurath et al., 2016). However, a recent study has shown that *Fusarium oxysporum* may function as an OMF when associating with the terrestrial orchid *Bletilla striata* (Jiang et al., 2019). We did not resolve the species identity of the *Fusarium* OTUs recovered in our study, and its ecological guild remains unknown. The other principal fungal pathogen of *V. planifolia*, *Colletotrichum* (Havkin-Frenkel & Belanger, 2018) was rarely detected in our study with <1% of the total reads.

Similar mycorrhizal fungal communities in aerial and terrestrial roots suggest that root morphology and physiology does not constrain the fungal symbiotic diversity for either root type in *V. planifolia*. Putative mycorrhizal fungi in aerial roots attached to organic tutors likely encounter more favorable conditions for growth than those at the highly managed farm (Pantepec) where the aerial roots were attached to concrete tutors and exposed to more stressful conditions. The bark and wood of tutors at the semi-natural and traditional sites may serve as sources of nutrients which are lacking at the highly managed farm (Pantepec). Fungi in aerial roots of *V. planifolia* may have different ecologies/play different roles depending on their microhabitat.

While we did not detect a difference in richness of mycorrhizal fungi between root types, we did identify differences in read abundance (Figure S6) which has been used as a proxy for species abundance (Amend et al., 2010). The low read abundance for aerial

---

**Figure 4** Relative number of reads of putative mycorrhizal fungal OTUs characterized from *V. planifolia* roots across four different farms. Pantepec (highly managed farm), 20 Soles (traditional farm), Puntilla (traditional farm), and 1 de Mayo (wild natural farm).
roots compared to terrestrial roots is consistent with the findings of Porras-Alfaro and Bayman (2007) who observed fewer pelotons in aerial roots than terrestrial roots of V. planifolia. Other microscopy studies also reported aerial roots sampled from epiphytic orchids being colonized at lower rates than terrestrial roots and that colonization was restricted to the root surface adjacent to their substrata (i.e., host tree bark) (Bermudes & Benzing, 1989; Hadley & Williamson, 1972; Lesica & Antibus, 1990; Porras & Bayman, 2003). Lower colonization may be due to structural barriers (e.g., passage cells) in aerial roots limiting mycorrhizal fungal colonization as proposed by Chomicki et al. (2014). The lower nutrient availability in the aerial substrate may also influence lower fungal colonization.

We observed greater diversity of fungi traditionally viewed as forming ECM (Nguyen et al., 2016) than previously reported in aerial roots. ECM fungi are rarely obtained in culture which may explain the lack of detection in traditionally culture-based studies of OMF communities (Johnston et al., 2017). The occurrence of a diverse ECM fungal community in both aerial and terrestrial roots of V. planifolia is suggestive that these fungi may be common in other photosynthetic orchids growing in tropical habitats, but little data are available. Orchids may be predisposed to forming associations with ECM fungi. For instance, mycoheterotrophic orchids are well-known examples of orchids that associate ing associations with ECM fungi. For instance, mycoheterotrophic orchids being colonized at lower rates than terrestrial roots and that colonization was restricted to the root surface adjacent to their substrata (i.e., host tree bark) (Bermudes & Benzing, 1989; Hadley & Williamson, 1972; Lesica & Antibus, 1990; Porras & Bayman, 2003). Lower colonization may be due to structural barriers (e.g., passage cells) in aerial roots limiting mycorrhizal fungal colonization as proposed by Chomicki et al. (2014). The lower nutrient availability in the aerial substrate may also influence lower fungal colonization.

We observed greater diversity of fungi traditionally viewed as forming ECM (Nguyen et al., 2016) than previously reported in aerial roots. ECM fungi are rarely obtained in culture which may explain the lack of detection in traditionally culture-based studies of OMF communities (Johnston et al., 2017). The occurrence of a diverse ECM fungal community in both aerial and terrestrial roots of V. planifolia is suggestive that these fungi may be common in other photosynthetic orchids growing in tropical habitats, but little data are available. Orchids may be predisposed to forming associations with ECM fungi. For instance, mycoheterotrophic orchids are well-known examples of orchids that associate ing associations with ECM fungi. For instance, mycoheterotrophic orchids being colonized at lower rates than terrestrial roots and that colonization was restricted to the root surface adjacent to their substrata (i.e., host tree bark) (Bermudes & Benzing, 1989; Hadley & Williamson, 1972; Lesica & Antibus, 1990; Porras & Bayman, 2003). Lower colonization may be due to structural barriers (e.g., passage cells) in aerial roots limiting mycorrhizal fungal colonization as proposed by Chomicki et al. (2014). The lower nutrient availability in the aerial substrate may also influence lower fungal colonization.

We observed greater diversity of fungi traditionally viewed as forming ECM (Nguyen et al., 2016) than previously reported in aerial roots. ECM fungi are rarely obtained in culture which may explain the lack of detection in traditionally culture-based studies of OMF communities (Johnston et al., 2017). The occurrence of a diverse ECM fungal community in both aerial and terrestrial roots of V. planifolia is suggestive that these fungi may be common in other photosynthetic orchids growing in tropical habitats, but little data are available. Orchids may be predisposed to forming associations with ECM fungi. For instance, mycoheterotrophic orchids are well-known examples of orchids that associate ing associations with ECM fungi. For instance, mycoheterotrophic orchids being colonized at lower rates than terrestrial roots and that colonization was restricted to the root surface adjacent to their substrata (i.e., host tree bark) (Bermudes & Benzing, 1989; Hadley & Williamson, 1972; Lesica & Antibus, 1990; Porras & Bayman, 2003). Lower colonization may be due to structural barriers (e.g., passage cells) in aerial roots limiting mycorrhizal fungal colonization as proposed by Chomicki et al. (2014). The lower nutrient availability in the aerial substrate may also influence lower fungal colonization.

We observed greater diversity of fungi traditionally viewed as forming ECM (Nguyen et al., 2016) than previously reported in aerial roots. ECM fungi are rarely obtained in culture which may explain the lack of detection in traditionally culture-based studies of OMF communities (Johnston et al., 2017). The occurrence of a diverse ECM fungal community in both aerial and terrestrial roots of V. planifolia is suggestive that these fungi may be common in other photosynthetic orchids growing in tropical habitats, but little data are available. Orchids may be predisposed to forming associations with ECM fungi. For instance, mycoheterotrophic orchids are well-known examples of orchids that associate ing associations with ECM fungi. For instance, mycoheterotrophic orchids being colonized at lower rates than terrestrial roots and that colonization was restricted to the root surface adjacent to their substrata (i.e., host tree bark) (Bermudes & Benzing, 1989; Hadley & Williamson, 1972; Lesica & Antibus, 1990; Porras & Bayman, 2003). Lower colonization may be due to structural barriers (e.g., passage cells) in aerial roots limiting mycorrhizal fungal colonization as proposed by Chomicki et al. (2014). The lower nutrient availability in the aerial substrate may also influence lower fungal colonization.

The source of ECM fungi in V. planifolia aerial roots is unclear. ECM fungi in terrestrial roots of V. planifolia likely originate through mycelial growth through soil. Wind-dispersed spores, hyphae growing along tutors, systemic colonization, or some combination of these may explain the occurrence of ECM fungi in V. planifolia aerial roots. A recent study by Schneider-Maunoury et al. (2020) revealed that some ECM fungi are widely dispersed in terrestrial plants as endophytes. Further work is needed to elucidate their function and the potential for peloton formation.

Additional research is needed to understand the function of ECM fungi in adult orchid roots for improving the management of V. planifolia farms, and more broadly, for enhancing ex situ orchid conservation. The presence of OMF and ECM fungi in the roots of V. planifolia expands the work of previous investigations by Porras-Alfaro and Bayman (2007) and González-Chávez et al., (2018), Gebauer et al. (2016) proposed that most orchids, including putative fully autotrophic orchids, are likely partial mycoheterotrophs in adult stages. Tropical orchids may benefit from forming associations with a more diverse community of ECM fungi than previously reported due to low light conditions and limited nutrient access in some substrata. Investigating the complete root mycobiota is needed for understanding the diversity and potential role fungi play in driving orchid diversity and adaptation.

This study also provided preliminary insights on possible influences of different cultivation practices of V. planifolia on mycorrhizal fungi. We observed lower OTU richness of mycorrhizal fungi at the highly managed farm (growing on concrete tutors) than at the wild natural farm (growing on living trees), and differences in fungal taxa among each of the farms. Soil characteristics at the highly managed and the wild natural farms were similar, suggesting that differences in mycorrhizal communities were not associated with differences in soil characteristics. Other aspects of vanilla farming are likely driving the differences in observed mycorrhizal communities. Studies have documented positive impacts of diverse mycorrhizal communities on plant fitness (Smith & Read, 2010). Our study provides support for the need for additional studies on the impact of cultivation practices on the mycobiome of vanilla.

ACKNOWLEDGMENTS

The financial support for this project was provided by funding awarded to Ma. del Carmen A. González-Chávez from SAGARPA-CONACYT-SNITT, Mexico as part of Subproject 03, project 2012-04-190442. Additional funding was received from the Program in Plant Biology and Conservation, a joint program between Northwestern University and the Chicago Botanic Garden awarded to Lynnaun Johnson for research and travel funds. We also acknowledge the research support from the Chicago Botanic Garden and The Negaunee Foundation. Lastly, we gratefully acknowledge the Mexican vanilla growers for access to their farms. This material is based upon work supported by the National Science Foundation (Porras-Alfaro). Any opinion, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

AUTHORS’ CONTRIBUTIONS

L.J.A.N.J. produced sequence data, and performed analysis and writing of the manuscript. L.J.A.N.J., M.G.C., R.C.G., and A.P.A. designed the experiment and collected samples from field sites. All authors contributed to data interpretation and manuscript editing. M.G.C. and G.M.M. secured funding for fieldwork and sample processing.

DATA AVAILABILITY STATEMENT

Sequences were deposited in NCBI’s Sequence Read Archive under the BioProject PRJNA540935.

ORCID

Lynnaun J. A. N. Johnson https://orcid.org/0000-0002-6296-4329
orchids in an Andean cloud forest. Mycological Research, 110(11), 1257-1270. https://doi.org/10.1016/j.mycres.2006.08.004

Taylor, D. L., & Bruns, T. D. (1997). Independent, specialized invasions of ectomycorrhizal mutualism by two nonphotosynthetic orchids. Proceedings of the National Academy of Sciences, 94(9), 4510-4515. https://doi.org/10.1073/pnas.94.9.4510

Taylor, D. L., Bruns, T. D., & Hodges, S. A. (2004). Evidence for mycorrhizal races in a cheating orchid. Proceedings of the Royal Society of London. Series B: Biological Sciences, 271(1534), 35-43.

Tedersoo, L., Anslan, S., Bahram, M., Põlme, S., Rõõm, T., Liiv, I., Kõljalg, U., Kisand, V., Nilsson, H., Hildebrand, F., Bork, P., & Abarenkov, K. (2015). Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. MycoKeys, 10, 1-43. https://doi.org/10.3897/mycokeys.10.4852

Turetta, C. Y., Sanche, S. E., Hoban, D. J., Karlowsky, J. A., & Kabani, A. M. (1999). Rapid identification of fungi by using the ITS2 genetic region and an automated fluorescent capillary electrophoresis system. Journal of Clinical Microbiology, 37(6), 1846-1851. https://doi.org/10.1128/JCM.37.6.1846-1851.1999

U'Ren, J. M., Riddle, J. M., Monacell, J. T., Carbone, I., Miadlikowska, J., & Arnold, A. E. (2014). Tissue storage and primer selection influence pyrosequencing-based inferences of diversity and community composition of endolithic and endophytic fungi. Molecular Ecology Resources, 14(5), n/a. https://doi.org/10.1111/1755-0998.12252

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology, 73(16), 5261-5267.

Warcup, J. H. (1985). Rhizanthella gardneri (orchidaceae), its Rhizoctonia endophyte and close association with Melaleuca uncinata (Myrtaceae) in Western Australia. New Phytologist, 99(2), 273–280. https://doi.org/10.1111/j.1469-8137.1985.tb03655.x

Waterman, R. J., Bidartondo, M. I., Stolberg, J., Combs, J. K., Gebauer, G., Saloainen, V., Barraclough, T. G., & Pauw, A. (2011). The effects of above- and belowground mutualisms on orchid speciation and coexistence. The American Naturalist, 177(2), E54-E68. https://doi.org/10.1086/657955

Waud, M., Busschaert, P., Ruyters, S., Jacquemyn, H., & Lieveens, B. (2014). Impact of primer choice on characterization of orchid mycorrhizal communities using 454 pyrosequencing. Molecular Ecology Resources, 14(4), 679-699. https://doi.org/10.1111/1755-0998.12229

Weiß, M., Waller, F., Zuccaro, A., & Selosse, M. A. (2016). Sebacinales - one thousand and one interactions with land plants. New Phytologist, 211(1), 20-40. https://doi.org/10.1111/nph.13977

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols. New York, NY: Elsevier, 315–322.

Xing, X., Jacquemyn, H., Gai, X., Gao, Y., Liu, Q., Zhao, Z., & Guo, S. (2019). The impact of life form on the architecture of orchid mycorrhizal networks in tropical forest. Oikos, 128(9), 1254–1264. https://doi.org/10.1111/oik.06363

Yagame, T., Orihara, T., Selosse, M.-A., Yamato, M., & Iwase, K. (2012). Mixotrophy of Platanthera minor, an orchid associated with ectomyorrhiza-forming Ceratobasidiaceae fungi. New Phytologist, 193(1), 178-187. https://doi.org/10.1111/j.1469-8137.2011.03896.x

Yagame, T., Yamato, M., Suzuki, A., & Iwase, K. (2008). Ceratobasidiaceae mycorrhizal fungi isolated from nonphotosynthetic orchid Chamaegastrodia sikokiana. Mycorrhiza, 18(2), 97-101. https://doi.org/10.1007/s00572-007-0155-0

Yoder, J. A., Zettler, L. W., & Stewart, S. L. (2000). Water requirements of terrestrial and epiphytic orchid seeds and seedlings, and evidence for water uptake by means of mycotrophy. Plant Science, 156(2), 145-150. https://doi.org/10.1016/S0168-9452(00)00246-6

Zettler, L. W., Rajaovelona, L., Yokoya, K., Kendon, J. P., Stice, A. L., Wood, A. E., & Sarasan, V. (2017). Techniques for the collection, transportation, and isolation of orchid endophytes from afar: A case study from Madagascar. Botanical Studies, 58(1), 54. https://doi.org/10.1186/s40529-017-0209-3

Zotz, G. (2013). The systematic distribution of vascular epiphytes - a critical update. Botanical Journal of the Linnean Society, 171(3), 453–481. https://doi.org/10.1111/bot.12010

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Johnson LJAN, Gónzalez-Chávez M, Carrillo-González R, Porras-Alfaro A, Mueller GM. Vanilla aerial and terrestrial roots host rich communities of orchid mycorrhizal and ectomycorrhizal fungi. Plants, People, Planet. 2020;00:1-12. https://doi.org/10.1002/ppp3.10171