Genetic identification of potential nodulating bacteria and nodule-associated bacteria (NAB) within root nodules of Inga punctata trees in a Costa Rican Cloud Forest

William Eaton

weaton@pace.edu

Pace University https://orcid.org/0000-0002-6596-0331

Debra Hamilton

Wen Chen

Alexander Lemenze

Patricia Patricia Soteropoulos

Research Article

Keywords: Root nodule bacteria, nodule-associated bacteria, Inga punctata root nodules, Plant growth-promoting bacteria, Bradyrhizobium diazoefficiens, DNA analysis of root nodule bacteria

Posted Date: December 20th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3767521/v1

Abstract

Background and Aims

Leguminous tree root nodules have N-fixing, nodulating bacteria important for soil C and N cycle recovery following forest disturbance, and nodule-associated bacteria (NAB), which often provide plant growth promoting (PGP) activities. However, composition and function of these microbiomes within tropical leguminous forest trees is understudied.

Methods

Root nodules were collected from 1, 2, 13-year-old and old growth *Inga punctata* trees within a tropical cloud forest. Nodule DNA was extracted, from which bacterial taxa were identified through Illumina DNA sequencing methods.

Results

Bradyrhizobium diazoefficiens DNA sequences represented 63% to 86% and NAB sequences represented 7% to 12% of the total bacterial taxa in the nodules. Collectively, 26 of the NAB taxa were identified with 9 PGP activities. The 1 year-old nodules had the greatest percentages of PGP NAB taxa, which decreased with tree age, though the percentage of total NAB DNA increased with tree age, as did the NAB community complexity.

Conclusions

Bradyrhizobium diazoefficiens appears to be the root nodule-forming bacteria, while the NAB genera with known PGP activities appear to provide important functions that benefit both the nodule microbiome and *I. punctata*. The greater percentage of PGP NAB in the youngest nodules suggests their importance in early growth and development of *I. punctata* and its root nodules, the great presence of NAB DNA in all nodules suggests the importance of NAB throughout the lifespan of *I. punctata,* and the increasing NAB community complexity with tree age suggests the microbiome undergoes parallel succession with the tree.

Introduction

Leguminous trees are ecologically important species in Central and South American tropical forests and are considered critical in tropical forest recovery processes following deforestation and other disturbances (Lojka et al. 2010; Macedo et al. 2008; Orwa et al. 2009; Piotto 2008). Specifically, the leguminous tree root nodule N-fixing bacterial activities provide the principal pathways by which damaged tropical lands recuperate the soil N and C lost following deforestation and subsequent agricultural use (Amazonas et al. 2011; Chazdon et al. 2016; Davidson et al. 2007; Gehring et al. 2005; Groppo et al. 2015; Lojka et al. 2010; Macedo et al. 2008; Pan et al. 2011; Poorter et al. 2016), yet too little is known of the root nodule microbiome of these tropical leguminous trees. Some work has determined

the taxonomic identity of root nodule-forming bacteria of tropical agricultural plants, such as mung bean, soybean, cow pea, etc. (for example, Chidebe et al. 2018; Favero et al. 2021; Lopez et al. 2020; Mayhood and Mirza 2021; Ramírez et al. 2019; Soe et al. 2012) and also of some tropical forest trees (for example, daSilva et al. 2014; Freitas et al. 2016; Leblanc et al. 2005; Parker and Lunk, 2000; Tian et al. 2015). However, there is a large knowledge gap in the identification of the root nodule microbiome in these ecologically important tropical forest leguminous trees.

It has been recognized that the root nodule bacteria are not alone in the nodule microbiome as entire communities of bacteria, or nodule-associated bacteria (NAB), have been found within root nodules, many of which are known to serve as plant growth promoting (PGP) bacteria (Etesami 2022; Martínez-Hildalgo and Hirsch 2017; Olanrewaju et al. 2017; Singh et al. 2023; Velázquez et al. 2014). These PGP NAB genera are thought to enhance both the root nodule bacterial and plant growth (Busby et al. 2015; Dudeja et al. 2012; Martínez-Hildalgo and Hirsch 2017; Selvakumar et al. 2013; Singh et al. 2023; Velázquez et al. 2014). Yet here too, there is far too little known of the NAB taxonomic composition in tropical forest leguminous tree nodules.

Species of the genus *Inga* are ecologically important leguminous forest trees commonly found throughout the tropical Americas that are thought to enhance the quantity and quality of the soil organic C and N and stimulate C sequestration and long-term N accumulation (Eaton and Hamilton 2022; Leblanc et al. 2005; Lojka et al. 2008, 2010). These characteristics, along with *Inga*'s ability to thrive in the high acid soils of the tropical Americas have resulted in use of different *Inga* species in tropical reforestation practices in Central and South America (Lojka et al. 2008 and 2010). Some primary root nodule-forming bacteria have been identified in several *Inga* species (Aguiar et al. 2020; DaSilva et al. 2014; Freitas et al. 2016; Hernández-Oaxaca et al. 2023; Leblanc et al. 2005), and some species of *Inga* have been shown to influence the soil ecosystems in Costa Rican forests. For example, in a Northern Zone Costa Rican Forest, *I. edulis* facilitated development of a soil microbiome that enhanced soil NH_4^+ levels and was associated with switching the dominance of 10 functionally redundant N-cycle bacterial taxa in response to disturbance (Eaton et al. 2020). In the Cloud Forest of Monteverde, *I. punctata* was found to enhance the taxonomic complexity of its tree-soil N-fixer and Lignin Degrader communities, accumulate tree soil C and N, and support land recovery (Eaton and Hamilton 2022). To our knowledge, there has been no work conducted to identify the NAB community composition in any *Inga* species, nor to identify the root nodule-forming bacteria in *I. punctata*. Characterizing the taxonomic composition of the *Inga* root nodule microbiome could help explain how the NAB community influences tree development and growth, if the community composition or activity changes with disturbances such as deforestation or a changing climate, and the potential that such changes might have on tree development and growth, or tropical forest soil ecosystem recovery after disturbance.

In the current study, we examined root nodules from 1 year-old, 2 year-old, 13 year-old and old growth *I. punctata* trees all within the same reforestation zone in order to: 1) identify the genus of the primary nodulating bacteria of *I. punctata*; 2) determine the earliest age that root nodules could be seen in *I. punctata*; 3) determine if the primary nodulating bacterial taxa differed by tree age; 4) characterize the

taxonomic composition of the NAB community within the nodules; 5) determine if there were differences in the NAB composition by tree age; 6) determine if the complexity of the NAB community increased with tree age; and 7) determine if the potential plant growth promoting activities associated with the NAB taxa differed with tree age.

Materials and methods

Study Site

The *Inga punctata* trees for this study were located in the nursery, restoration sites, and 60 + year old secondary forest within the Finca Rodríguez Ecological Reserve (FRER), located in Monteverde, Costa Rica (10° 18' 55.3" N, 84° 50' 29.8" W), at an elevation of between 1250–1280 m asl. This site is within the Premontane Wet Life Zone (Holdridge and Poveda 1975), which is typified as evergreen forest with few deciduous species and moderate epiphyte load (Haber 2000), and annual precipitation of 2000–2900 mm (LaVal 2020). The different tree ages used were 1 year-old trees germinated from seeds at the nursery in plastic bags with local soil, and had not been planted yet (IP1 year samples), 2 year-old trees that were planted as seedlings (IP2 year samples) and had been in the forest for about 1 year, 13 year-old trees also planted as seedlings in the forest (IP13 year samples), and adult trees within the old secondary forest (IPOG).

Root Nodule Collection and Preparation

Five trees within the FRER were chosen from each of the four age categories for the study. Tree roots were exposed *in situ* and examined for nodules, with 10 nodules being collected from each of the five trees per age group, pooled by each individual tree, and stored in sterile plastic bags for no more than 5 days at 5^{o}C. Nodule surface sterilization occurred by rinsing nodules 5xs in sterile water with scrubbing using a sterile soft bristle brush for 1 minute, with water changes each time. Nodules were then rinsed 5xs in 70% ethanol with scrubbing for 1 minute, with changing of the ethanol with each wash. The nodules were washed 5x's in 4% sodium hypochlorite, with scrubbing, and changing of the solution with each wash, then with 3xs washes in hydrogen peroxide with changing of hydrogen peroxide solution each time. Then the nodules were rinsed 5xs in 70% ethanol with changing of the ethanol, and finally being rinsed 5xs in sterile distilled water, with changing of the water each time. After surface sterilization, the nodules were diced with sterile razor blades, mixed with 150 µL sterile water and crushed with sterile forceps, and mixed to generate a macerated root nodule sample for each tree, generating 5 nodule DNA samples per tree.

Root Nodule DNA Extraction, Sequencing, and Bioinformatics

The DNA was individually extracted from each 150 µL of macerated root nodule solution using the DNeasy PowerSoil Pro Kit (Qiagen LLC, MD). A NanoDrop 1000 spectrophotometer (ThermoFisher Scientifc, Waltham, MA) was used to determine the concentration and purity (A260/A280 ratio) of extracted root nodule DNA prior to downstream analyses. All bacterial PCR, DNA sequencing, and

taxonomic identification methods have been described in detail by McGee et al. (2019) and Eaton and Hamilton (2022). All resulting DNA sequence data have been submitted to the NCBI-Sequence Read Archive (SRA) repository with SRA Project Code PRJNA1019742. The number of times a specific DNA-identified genus appeared in a sample was converted to mean proportion of the sequences (MPS) per sample, as per Weiss et al. (2017).

Nodule-associated bacterial genera previously shown to have with plant growth promoting (PGP) activity were identified within the DNA sequence datasets for each tree root nodule sampled. To do this, we used 16 articles to identify what appear to be some of the more common PGP capabilities within root nodules that were also linked to specific bacterial genera within the nodules. These capabilities were for Protease, Lipase, or Cellulase activity, PO_4^{3-} solubilization, CO_2/C1 fixation, IAA (the growth hormone indole-3-acetic acid) production, or Siderophore production (Table S1). As well, bacterial genera known to have the additional PGP capability for N-fixation or ammonium oxidation (AMO) were identified using the KEGG database (https://www.genome.jp/kegg/), the Ribosomal Database Project (RDP) "Hierarchy Browser" (http://rdp.cme.msu.edu/hierarchy/), the FAPROTAX database (www.zoology.ubc.ca/louca/FAPROTAX/lib/php/index.php), lists from the International Committee for Systematics of Prokaryotes, Subcommittee on the Taxonomy of Rhizobia and Agrobacteria http://edzna.ccg.unam.mx/rhizobial-taxonomy/, the current taxonomy of rhizobia by Weir (2016) https://www.rhizobia.co.nz/taxonomy/rhizobia, and other literature sources (Table S2).

Data analysis

Differences in MPS of Root Nodule and Nodule-Associated Bacterial (NAB) Taxa

The MPS values of all root nodule genera analyzed to identify the dominant N-Fixing root nodule bacteria as the proposed as nodulating bacteria. All NAB genera with MPS values > 0.1% were also identified and the number of NAB genera represented in 1, 2, 3, or all 4 of the tree age nodules was determined and compared. The MPS values of the proposed nodulating bacteria and the NAB genera were analyzed for mean differences in MPS between the four different tree age nodules by the Kruskal – Wallis non-parametric test, using SPSS (v. 26). The number of NAB genera proposed to have any of the 9 PGP activities (mentioned above) were identified for each tree age nodule sample, ranked by MPS values, and with the percent presence of each NAB PGP-linked activity determined for the nodules in each tree age.

The MPS of the NAB genera were transformed using the 4th root method to account for dominant and rare taxa, as recommended by Anderson et al. (2008), converted into Bray-Curtis similarity matrices in PRIMER-E (v6) to quantify the differences between all pairs of samples (Clarke and Warwick 2001). These matrices were analyzed using the multivariate ANOSIM routine in the PRIMER-E package (Anderson et al. 2008; Clarke 1993) to show differences in NAB community compositions, with the number of permutations set to 9,999 (Clarke 1993; Clarke and Gorley 2006). ANOSIM provided Global R and *p* values as the "main" test results and pairwise R and *p* values for comparisons between the different years (Clarke and Warwick 2001). The strength of any NAB compositional differences between

nodule groups was determined using the multivariate Canonical Analysis of Principal Coordinates (CAP) ordination method (Clarke and Gorley 2006; Clarke and Warwick 2001) in the PRIMER-E/PERMANOVA + software package, with the number of permutations for the CAP analysis set to 9,999, and the program allowed to select the number of PCO axes (m) that would provide the best separation between groups. The resulting CAP axis canonical correlation squared (R^2) provided an approximation of the strength of any differences observed between NAB community compositions, with strong differences indicated by CAP axis R^2 values > 0.7, moderate differences are indicated by R^2 > 0.5 to 0.69, and weak differences represented by R^2 values < 0.5 (Anderson and Willis 2003).

Differences in Indicators of NAB Community Compositional Complexity

Microbial communities in older, more established habitats (such as the older tree age root nodules) are thought to demonstrate greater community complexity as indicated by greater taxonomic richness and diversity, with greater levels of taxonomic stability in community compositions indicating greater levels of taxonomic constancy (Allison and Martiny 2008; Eaton and Hamilton 2022; Griffiths and Philipott 2013; Grimm and Wissel 1997; Louca and Doebeli 2016; Louca et al. 2016; Shade et al. 2012; Tilman et al. 2006). Several metrics were used to approximate the complexity of the NAB communities. Margalef's Richness index *d* and Shannon's Diversity *H* index of the different NAB MPS levels were calculated in PRIMER-E. The percent taxonomic similarity of the NAB community compositions was determined by the multivariate SIMPER routine (Similarity Percentage) in PRIMER-E v.6 which showed the similarity in taxonomic composition between the different replicate root nodule samples from each tree age. Lastly, the *S* (or constancy) index was determined for the MPS, the Richness and the Diversity values, with a greater *S* value indicating a more constant compositional makeup of a community, which is likely to occur in an older and more stable or environment (Griffths and Philipott 2013; Grimm and Wissel 1997; Shade et al. 2012; Tilman et al. 2006; van Rujiven and Berendse 2007).

Differences In NAB Genera With Specific PGP Activities. The literature mentioned in the Methods section were used to identify the NAB genera with Protease, Lipase, Cellulase, PO_4^{3-} solubilization, CO_2/C1 fixation, IAA (the growth hormone indole-3-acetic acid) production, Siderophore production, N-fixation, and/or AMO capabilities. The total MPS of all NAB genera with MPS values > 0.1% and with the capacity to conduct any of the different PGP functions within each of the tree age nodules were determined and compared.

Results

Differences in MPS of Root Nodule and Nodule-Associated Bacterial (NAB) Taxa

The results indicated that *B. diazoefficiens* (formerly *B. japonicum*) is the likely root nodule-forming, N-fixing bacteria in *I. punctata* nodules as it's DNA was present in all tree age root nodules as the dominant taxa (Figures 1a, b, and c; Table 1), beginning in the youngest tree nodules (IP1yr). The MPS values of *B. diazoefficiens* significantly decreased (Figure 2a) from the IP1yr (85.71%) to that in the IP2yr nodules

(63.16%) and the IP13yr nodules (68.22%), and significantly increased in the IPOG nodules (78.2%). While there were also 7 other bacterial genera with the potential for nodulation (Table 2), their MPS values were only between 0.11% and 1.11%, as compared to the large MPS values of *B. diazoefficiens*.

There were 26 NAB genera collectively found in the root nodules with MPS levels between 0.1% and 2.49% (Table 1). The total MPS values for the NAB communities (Table 1, Figure 2b) were similar in the IP1yr, IP2yr, and IP13yr nodules but increased somewhat with age (from ~7.4% to ~8.4% and to ~8.0%, respectively), and were far greater in the IPOG nodules (~12.2%). Differences were found in the presence and composition of the 26 NAB genera between the 4 different tree ages (Table 1 and Table 3). There were 12 of the 26 NAB genera found in the IP1yr nodules, 11 in the IP2yr nodules, 15 in the IP13yr nodules, and 17 in the IPOG nodules. The IP1yr nodules had 1 unique NAB genus, IP2yr nodules had 2 unique NAB genera, the IP13yr nodules had 4 unique genera, and the IPOG nodules had 5 unique genera. As well, 12 genera were found in only 1 age of root nodules, 4 were found in 2 ages of root nodules, 3 genera were found in 3 ages of nodules, and 6 genera were found in all 4 types of root nodules and would be the most common NAB genera.

The multivariate analyses also demonstrated differences in the composition of the NAB communities in the nodules between the different ages of trees. The ANOSIM results (Table 4) showed the IP1yr and IP2yr NAB community compositions were different (p = 0.050), the IPOG community composition was different from that in the IP1yr and IP2yr NAB communities (p = 0.028 and 0.048), but IPOG and IP13yr NAB community were not different (p = 0.214), and the IP2yr and IP13yr were not different (p = 0.357). The CAP results (Table 2, Figure 3) supported those from the ANOSIM analysis as moderate differences were found between the IP1yr and IP2yr and the IP2yr and IPOG communities (R^2 = 0.606), strong differences were found between the IP1yr and IP13yr and the IP1yr and IPOG communities (R^2 = 0.748), while there were only weak differences found between the IP2yr and IP13yr and the IP13yr and IPOG NAB communities (R^2 = 0.244).

Differences in Indicators of NAB Community Compositional Complexity

There was evidence for increasing taxonomic complexity of the NAB communities with tree age (Table 5). The SIMPER results showed the taxonomic similarity of the NAB communities increased from the IP1yr nodules (26.17%) to that in the IP2yr and IP13yr nodules which were similar (53.85% and 50.01%, respectively), and increased again in the IPOG nodules (79.65%). Consistent with this, the *S* indices of the NAB community MPS values increased from that in the IP1yr nodules (8.73) to that in the IP2yr and IP13yr root nodules which were similar (13.65 and 13.94) but increased to a far greater level in the IPOG nodules (21.42). The Margalef's Richness *d* index was relatively constant for the NAB communities in the IP1yr, IP2yr and IP13yr nodules (7.04, 7.31, and 7.95, respectively) but increased significantly in the IPOG nodules (9.33). As well, the *S* indices for Richness were constant in the IP1yr and IP2yr (5,28 and 5.71), increased slightly in the IP13yr nodules (6.31), then showed a great increase in the IPOG nodules (24.7). The Shannon's Diversity "*H*" index was about the same in the IP1yr, IP2yr and IP13yr nodules (2.37, 2.86, and 2.85, respectively), and increased in the IPOG nodules (3.5). However, the *S* index for the Diversity *H*

values moderately increased from the NAB community in the IP1yr nodules (6.22) to that in the IP2yr nodules (7.94) and again to that in the IP13yr nodules (8.91), but was far greater for the NAB community in the IPOG nodules than in the other three tree age nodules (24.69)

Differences in NAB Genera with Specific PGP Activities

About 17% to 77% of the NAB genera found within the root nodules have previously been shown to perform the 9 plant growth promoting (PGP) activities of interest. Although the number of NAB genera in the nodules increased with tree age (Table 1), there was a decreasing percentage of the NAB genera with potential PGP activities also occurring with increasing tree age (Table 6). There were about 56% of all IP1yr nodule NAB genera associated with PGP activities, which decreased to about 41-42% in the IP2yr and IP13yr nodules and then again to about 38% in the IPOG nodules. Six of the 9 PGP activities were associated with about 54% to 77% of the IP1yr nodule NAB genera, 3 of 9 PGP activities were associated with about 58% and 67% of the IP2yr NAB genera, 3 of the 9 activities were associated with about 50% and 69% of the IP13yr NAB genera, and even though the IPOG nodules had the greatest percent of NAB genera, only 2 of the 9 activities were associated with only 50% and 61% of these IPOG NAB genera. The greatest decrease in potential PGP activity capabilities occurred between the IP1yr and IP2yr nodules as the percent of the NAB genera associated with Protease, Lipase, Cellulase, PO_4^{3-}Solubilization, and CO_2/C1 Fixation activities, and IAA and Siderophore production decreased an average 33.29%. The next greatest decrease occurred in the NAB genera between the IP13yr and IPOG nodules as the genera associated with Protease, Lipase, Cellulase, PO43- Solubilization, and CO2/C1 Fixation activities, and IAA production decreased an average of 14.33%, while the NAB genera with Siderophore production activity increased 25%. Interestingly, the NAB genera with N-Fixing or AMO capabilities remained relatively similar in the nodules across the tree ages, with fluctuations of 3% only to 12%.

Discussion

This study was conducted to determine the taxonomic identity of the root nodulating bacteria in *I. punctata*, to determine how early nodules are visible in young trees, to characterize the NAB community within the root nodules, and to determine if this NAB community composition changed and became more complex over time within the root nodules of the different tree ages. Members of the genus *Inga* are ecologically important leguminous trees commonly found throughout the tropical Americas (Hartshorn et al. 1994; Holdridge and Poveda 1976; Orwa et al. 2009), thought to be important as forage resources, in enhancing accumulation and sequestration of soil N and C, and stimulating above ground biomass development (Eaton and Hamilton 2022; Lawrence et al. 1995; Leblanc et al. 2005; Lojka et al. 2005, 2008, 2010). Earlier work identified different species of *Bradyrhizobium* in the root nodules of some members of the genus *Inga*. Leblanc et al. (2005) found bacteria closely related to *B. japonicum* and *B. liaoningense* associated with *I. edulis* root nodules in South America. Freitas et al. (2016) successfully inoculated I. *edulis* with B. *japonicum*. daSilva et al. (2014) found *B. ingae* in root nodules of *I. laurina*, Aguiar et al. (2020) found *B. diazoefficiens* in root nodules of *I. striata*, and Hernández-Oaxaca et al. (2023) found *B. rifense* and *B centrolobii* in nodules of *I. vera.* However, to our knowledge, this current

study was the first to examine the root nodules of *I. punctata* for identification of the nodulating bacteria and to characterize the NAB community composition.

Bradyrhizobium diazoefficiens as the Nodulating Bacteria

The results suggest that *B. diazoefficiens* is both the dominant N-fixing bacteria and the likely root nodulating bacteria for *I. punctata* as its DNA was found in all root nodules of this tree at MPS values from about 63–86%. In addition, none of the NAB genera were present in any of the nodules at MPS levels > 2.5%, none of the 7 other NAB genera identified in the literature as nodulating bacteria had MPS values > 2.3%, and the total MPS for all NAB genera within any of the nodules ranged from 7.41–12.2%. Root nodules from the youngest trees in this study (IP1 year) had the greatest MPS of *B. diazoefficiens* at 85.71%, which decreased to about 63% and 68% in the IP2 year and IP13 year trees and increased again to 78.2% in the IPOG root nodules. This may suggest a greater role of this bacteria in nodule formation and need for N-fixation within the youngest trees.

Bradyrhizobium diazoefficiens was previously known as a strain of *B. japonicum* but was recognized as a new species within the genus in 2013 (Delamuta et al. 2013). Prior to 2013, there are some articles identifying *B. japonicum* in root nodules of tropical forest trees (e.g., Lafay and Burdon 2007; Li et al. 2008; Parker and Lunk 2000). Since then, there have also been some identifications of *B. diazoefficiens* in many tropical agricultural plants such as mung bean, soybean, and cow pea (e.g., Chidebe et al. 2018; Jaiswal and Dakora 2019; Lopez et al. 2020; Naamala et al. 2016; Ramírez et al. 2019), and also in some *Inga* species (Aguiar et al. 2020; daSilva et al. 2014; Freitas et al. 2016; Parker 2015), but not in *I. punctata*.

Increasing NAB Abundance and Community Complexity

Although not as abundant as *B. diazoefficiens*, the NAB genera appeared to be important in the root nodule microbiomes based on their MPS levels, common presence in nodules from all tree age, and their known potential PGP activities. The number of NAB genera in the root nodules increased with tree age from 12 and 11 genera in the nodules of 1 and 2 year old trees to 15 genera in the nodules of the 13 year old tree nodules and 17 in the old growth tree nodules. The MPS of the total NAB in the nodules also increased with tree age from about 7% in the IP1 year nodules to about 8% in the IP2 year and IP13 year nodules, and to about 12% in the IPOG nodules. It also appears that the NAB root nodule communities are undergoing successional development with increased taxonomic community complexity occurring as the trees age. This was evident based on the greater richness and diversity of the NAB community, the greater constancy (*S* index) of these two metrics, greater constancy of their MPS values, and the greater taxonomic similarity of the NAB community in the nodules as the trees age (i.e., SIMPER analyses). All of these are indicators of an increasing complexity of the NAB community occurring with tree age, which would be expected to occur for any soil microbial community undergoing successional development within a habitat (Allison and Martiny 2008; Eaton and Hamilton 2022; Griffiths and Philipott 2013; Grimm and Wissel 1997; Louca and Doebeli 2016; Louca et al. 2016; Shade et al. 2012; Tilman et al. 2006; van Rujiven and Berendse 2007). This suggests an interesting picture of an endophytic microbiome within *I.*

punctata that undergoes classic community compositional succession in parallel with tree age that follows the patterns typically associated with other communities advancing successionally.

Potential NAB PGP Activities for Nodule and Plant Growth Enhancement

Although the specific PGP activities associated with the NAB genera examined were not definitively proven to be occurring at the time of this study, previous evidence in the literature for these PGP activities to commonly be associated with these genera allow for proposing potential roles of these different bacterial genera in nodule activity and plant growth. These speculations are based on some of the known functions of the PGP activities, and we believe warrant further study.

The percentage of the NAB genera with the potential for N-Fixing ability was large in all nodules at about 61–69% of the total NAB genera. This suggests the importance of the production of NH_4^+ in the nodules throughout the lifespan of *I. punctata*. This is consistent with the known preference for NH_4^+ over NO_3^- for nodule development and activity by *Inga* (Fisher 1995; Justino et al. 2017; Omena-Garcia et al. 2015). This also suggests that these N-fixing NAB genera and *B. diazoefficiens* may be enhancing the activity of the nodules in which they are found. *Bradyrhizobium diazoefficiens* is also thought to be one of the only members of the genus that performs denitrification using NO_3^- as an electron acceptor in this anaerobic process for energy transformation reactions under microoxic to anaerobic conditions (Bedmar et al. 2013; Fernandez et al. 2019; Salas et al. 2021), which is the case within the root nodules. Consistent with this, the MPS levels of NAB genera with AMO capability, and therefore the ability to produce NO_3^-, was between 38–44%, suggesting these NAB would support the ability of *B. diazoefficiens* to perform denitrification via NO_3^- reduction under low oxygen conditions.

There were several PGP activities associated with different bacterial genera that were more common in the younger tree root nodules, suggesting these NAB genera may be facilitating nodule activity and plant growth beginning as early as 1 year of age. The greater percent of NAB genera with the potential for protease, lipase, or cellulase activity occurring within the IP1 year nodules suggests the young nodules require more decomposition of organic C for biosynthesis during the early stages of growth and development of the nodules, and perhaps to assist in nodule-based enhancement of young plant growth. The greater levels of potential IAA-producing NAB genera in the IP1 year root nodules suggests they may be enhancing plant growth as bacterial production of IAA has been shown to be important for regulating plant physiological activity genes associated with enhanced plant growth (Fu et al. 2015). The greater percentage of the NAB genera with potential for siderophore production in the IP1 year nodules suggests these PGP bacteria may be enhancing young plant growth through increasing the availability of iron needed for oxidoreduction mechanisms involving such iron-containing structures used in electron transport, and in TCA cyclic activity, and amino acid and nucleic acid synthesis used in plant growth (Gupta et al. 2019; Sarode et al. 2013; Scavino and Pedraza 2013). Lastly, the far greater percent of NAB genera with potential PO_4^{3-} solubilization capability in the IP1 year root nodules also suggests the NAB community is enhancing plant growth starting at least 1 year of age, as PO_4^3 solubilization has been

shown to increase phytohormone production and N Fixation activities in order to enhance legume plant development (Khan et al. 2013).

Unique *Bradyrhizobium diazoefficiens* Metabolic Capabilities

Bradyrhizobium diazoefficiens is one of the rhizobial species that can grow both, as a free-living and symbiotic N-fixing bacteria (Salas et al. 2021), but also is one of the only rhizobia that can grow under anaerobic or microoxic conditions using complete reduction of $NO3^-$ via denitrification processes (Bedmar et al. 2013; Fernandez et al. 2019; Salas et al. 2021) and has been shown to denitrify NO_3^- both when growing as a free-living or symbiotic root nodular N-fixer (Bedmar et al. 2013). There is an interesting potential link between the ability of *B. diazoefficiens* to grow under anaerobic or microoxic conditions growth and the PGP activity of CO_2/C1 fixation pathways associated with some of the NAB genera within the root nodules and. About 41% of the PGP bacteria within the *I. punctata* root nodules have been shown to have the potential to fix 1-C compounds via CO_2/C1 fixation pathways, which could be used for production of reduced forms of C for assimilatory processes. However, it is also known that *B. diazoefficiens* can form poly-β-hydroxybutyrate (PHB) for use as a mechanism for energy storage under anaerobic or microoxic conditions, which would help the bacteria to maintain its N-Fixing ability under such conditions (Nishihata et al. 2018). The synthesis of PHB requires acetyl CoA, which is synthesized by a special CO_2/C1 fixation pathway (Wu et al. 2022), which is a possible activity of some of the PGP bacterial genera within all the root nodules. These connections are certainly speculative, but it is interesting that *B. diazoefficiens* has the unique ability to grow under microoxic to anaerobic conditions, and can use anaerobic denitrification for energy transformation reactions, while some NAB genera produce the NO_3^- that is needed for denitrification, conducts CO_2/C1 fixation that could be used for production of PBH to assist with energy storage under low oxygen levels, and that life within the root nodules is at least partially microoxic. Thus, it is possible there may be potential links between the NAB community metabolic functions and the ability of *B. diazoefficiens* to grow within the root nodule under microoxic or anaerobic conditions, which would be beneficial as N-fixation is essentially an anaerobic process. This is an interesting area for focus of future work.

Summary

This study indicates that *B. diazoefficiens* is most likely the root nodulating, N-fixing bacteria of *I. punctata*, and that the unique characteristics and activities of this bacteria along with the potential PGP activities of the NAB communities in the tree root nodules appear important for the growth and development of the nodule and *I. punctata*. As the greatest percentage of the NAB with PGP activity was found within the IP1 year nodules, it suggests that the NAB community is especially critical for early phases of growth, development and health of the root nodules and the tree itself, the continued large presence of these NAB PGP taxa within the nodules of all ages of the tree indicates their importance for the growth and development of *I. punctata* throughout its' lifespan. It is also noteworthy that as *I. punctata* trees age, the NAB community appears to be undergoing successional development, resulting in NAB taxonomic communities that are becoming more complex, which is consistent with what has been

observed in more advanced microbial communities that are following the normal successional processes seen in soil bacterial communities. However, currently, it is not known why the NAB MPS levels are increasing but the potential for PGP activity is decreasing in the nodules with tree age. This should be studied.

Some important questions remain concerning the primary root nodule bacteria and NAB communities within *Inga* root nodules. Specifically: 1) Are there differences in the primary root nodule bacteria and the NAB community genera from different species of *Inga* within the same life zone in the Monteverde cloud forest region? 2) Are there differences in the primary root nodule bacteria and NAB community genera among species of *Inga* when they are found in multiple life zones within the Monteverde cloud forest regions? 3) How does the composition of these genera compare to those found within root nodules from other non-*Inga* leguminous trees in the same forests? 4) Are there seasonal-based differences in NAB community compositions, distributions, or abundances in any of these tree root nodules between wet and dry season, and can any such differences be used as indicators of increasing influence of a changing climate or extreme climate events in the region? 5) Is there a correlation between distances to an *Inga* species and composition of non-*Inga* legume NAB composition? 6) What is the role of non-PGP NAB genera and why do these increase in MPS with tree age? Answers to these questions have implications for forest restoration in the region, as they could inform strategies on the incorporation of different species of legumes in replanting of deforested areas to enhance recovery. Also, these answers could provide information concerning potential indicators of the effects of a changing climate in the region if certain legumes and NAB compositions occur under either wetter, dryer, or warmer conditions. This could be a useful tool for easy taxonomic monitoring of the influence of a changing climate, extreme weather events, and other disturbances to the soil ecosystem in the region.

Declarations

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

DNA data is available as stated in the Materials and methods section. Any other data will be made available upon request.

Declarations

Funding This work was supported by internal funding from Office of the Provost and Office for Undergraduate Research at Pace University, NYC, and also by the Monteverde Institute Research Department.

Conflicts of Interest The authors have no relevant financial or non-financial conflicts of interest to declare.

Availability of Data The generated DNA sequence data is publicly available at the NCBI-Sequence Read Archive (SRA) repository with SRA Project Code PRJNA1019742. All other data is available from the corresponding author by request.

Author Contributions William D. Eaton and Debra A. Hamilton developed the study conception, design, and sampling methods. Data collection was performed by William D. Eaton and Wen Chen. DNA sequencing was performed by Alexander Lemenze and Patricia Soteropoulos. Data analysis was conducted by William D. Eaton. Drafts of the manuscript were written by William D. Eaton, with edits made by all authors. All authors read and approved the final manuscript.

Ethics Approval Not applicable, as no humans or other animals were used in this work.

Consent to participate All authors have consented to participate in this work and subsequent publication

Consent for publication All authors agree to have all data published.

References

1. Aguiar LM, Souza MF, de Laia ML et al (2020) Metagenomic analysis reveals mechanisms of atrazine biodegradation promoted by tree species. Environ Pollut 267:115636. 10.1016/j.envpol.2020.115636
2. Allison SD, Martiny JBH (2008) Colloquium paper: resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci USA 105:11512–11519
3. Amazonas NT, Martinelli LA, Piccolo MDC, Rodrigues RR (2011) Nitrogen dynamics during ecosystem development in tropical forest restoration. For Ecol Manag 262:1551–1557
4. Andam CP, Parker MA (2008) Origins of Bradyrhizobium nodule symbionts from two legume trees in the Philippines. J Biogeogr 35:1030–1039
5. Anderson MJ, Willis TJ (2003) Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84:511–525
6. Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA + for PRIMER: guide to software and statistical methods PRIMER-E Ltd. Plymouth UK
7. Aserse AA, Räsänen LA, Aseffa F, Hailemariam A, Lindström K (2013) Diversity and sporadic symbionts and nonsymbiotic endophytic bacteria isolated from nodules of woody, shrub, and food legumes in Ethiopia. Appl Microbiol Biotechnol 97:10117–10134
8. Bedmar EJ, Bueno E, Correa D et al (2013) Ecology of denitrification in soils and plant-associated bacteria. In: Rodelas B, González-López J (eds) Beneficial plant-microbial interactions: Ecology and Applications. CRC Press, Boca Raton, FL, pp 164–182

9. Busby RR, Rodriguez G, Gebhart DL, Yannarell AC (2016) *Lespedeza* species harbor greater non-rhizobial bacterial diversity in root nodules compared to the coexisting invader, *L. cuneata*. Plant Soil 401:427–436
10. Caporaso JG, Lauber CL, Walters WA et al (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. PNAS 108:4516–4522
11. Chazdon RL, Broadbent EN, Rozendaal DMA et al (2016) Carbon sequestration potential of second-growth forest regeneration in the Latin American tropics. Sci Adv 2:e1501639–e1501639
12. Chidebe IN, Jaiswal SK, Dakora FD (2018) Distribution and phylogeny of microsymbionts associated with cowpea (Vigna unguiculata) nodulation in three agroecological regions of Mozambique. Appl Environ Microbiol 84:e01712–e01717
13. Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18:117–143
14. Clarke KR, Gorley RN (2006) PRIMER v6: User manual. PRIMER-E Ltd Plymouth UK
15. Clarke KR, Warwick RM (2001) A further biodiversity index applicable to species lists: variation in taxonomic distinctness. Mar Ecol Prog Ser 216:265–278
16. da Silva K, de Meyer SE, Rouws LFM et al (2014) *Bradyrhizobium inga* sp. nov., isolated from effective nodules of Inga laurina grown in Cerrado soil. Int J Syst Evol Microbiol 64:3395–3401
17. Davidson EA, de Carvalho CJR, Figueira AM et al (2007) Recuperation of nitrogen cycling in Amazonian forests following agricultural abandonment. Nature 447:995–998
18. Delamuta JRM, Ribeiro RA, Ormeño-Orrillo E et al (2013) Polyphasic evidence supporting the reclassification of *Bradyrhizobium japonicum* group Ia strains as *Bradyrhizobium diazoefficiens* sp. nov. Int J Syst Evol Microbiol (IJSEM) 63:3342–3351
19. Dudeja SS, Giri R, Saini R et al (2012) Interaction of endophytic microbes with legumes. J Basic Microbiol 52:248–260
20. Eaton WD, Hamilton DA (2022) Enhanced carbon, nitrogen and associated bacterial community compositional complexity, stability, evenness, and differences within the tree-soils of *Inga punctata* along an age gradient of planted trees in reforestation plots. Plant Soil 484:327–346
21. Eaton WD, McGee KM, Hoke E, Lemenze A, Hajibabaei M (2020) Influence of Two Important Leguminous Trees on Their Soil Microbiomes and Nitrogen Cycle Activities in a Primary and Recovering Secondary Forest in the Northern Zone of Costa Rica. Soil Syst 4:65. https://doi.org/10.3390/soilsystems4040065
22. Etesami H (2022) Root Nodules of Legumes: A suitable ecological niche for isolating non-rhizobial bacteria with biotechnological potential in agriculture. Curr Res Biotech 4:78–86
23. Favero VO, Carvalho RH, Motta VM et al (2021) *Bradyrhizobium* as the only rhizobial inhabitant of mung bean (vigna radiata) nodules in tropical soils: a strategy based on microbiome for improving biological nitrogen fixation using bio-products. Front Plant Sci 11:602645

24. Fernández N, Cabrera JJ, Varadarajan AR et al (2019) An integrated systems approach unveils new aspects of microoxia-mediated regulation in *Bradyrhizobium diazoefficiens*. Front Microbiol 10:924
25. Fisher RF (1995) Amelioration of Degraded Rain Forest Soils by Plantations of Native Trees. Soil Sci Soc Amer J 59:544549
26. Freitas J, Mônico AZ, Lírio EJ et al (2016) Inoculation of *Bradyrhizobium japonicum* (Kirchner, 1896) Jordan (1982) (Bradyrhizobiaceae) on *Inga edulis* Mart.(Leguminosae, Mimosoideae). Greener J Agric Sci 6:087–092
27. Fu SF, Wei JY, Chen HW et al (2015) Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. Plant Signal Behav 10:e1048052
28. Gehring C, Vlek PLG, de Souza LAG, Denich M (2005) Biological nitrogen fixation in secondary regrowth and mature rainforest of central Amazonia. Agric Ecosyst Environ 111237–111252. https://doi.org/10.1016/j.agee.2005.06.009
29. Goncalves J (2015) Mineral nitrogen associated changes in growth and xylem-N compounds in Amazonian legume tree. J Plant Nutr 4:584–595
30. Griffiths BS, Philippot L (2013) Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol Rev 37:112–129
31. Grimm V, Wissel C (1997) Babel, or the ecological stability discussions: an inventory and analysis of terminology and a guide for avoiding confusion. Oecologia 109:323–334
32. Groppo JD, Lins SRM, Camargo PB et al (2015) Changes in soil carbon, nitrogen, and phosphorus due to land-use changes in Brazil. Biogeosci 12:4765–4780
33. Gupta P, Rani R, Usmani Z, Chandra A, Kumar V, Singh DP (2019) 69–76
34. Haber W (2000) An introduction to cloud forest trees. In Nadkarni N and Wheelwright NT (eds) Monteverde: Ecology and Conservation of a Tropical Cloud Forest Bowdoin Scholars' Bookshelf. Book 1 http://digitalcommons.bowdoin.edu/scholars-bookshelf/1
35. Hartshorn GS, Hammel B, McDade LA et al (1994) Vegetation types and floristic patterns. La Selva: ecology and natural history of a neotropical rain forest. The University of Chicago Press, Chicago, IL
36. Hernández-Oaxaca D, Claro K, Rogel MA, Rosenblueth M et al (2023) Novel symbiovars ingae, lysilomae and lysilomaefficiens in Bradyrhizobia from tree-legume nodules. Syst Appl Microbiol 46:126433
37. Holdridge LR, Poveda LJ (1975) Árboles de Costa Rica. Centro Cientı´fico Tropical, San Jose´, Costa Rica, 546 pp
38. The International Committee for Systematics of Prokaryotes, Subcommittee on the Taxonomy of Rhizobia and Agrobacteria (ICSP) (2020) http://edzna.ccg.unam.mx/rhizobial-taxonomy/
39. Jaiswal SK, Dakora FD (2019) Widespread distribution of highly adapted *Bradyrhizobium* species nodulating diverse legumes in Africa. Front Microbiol 10:310
40. Justino G, Omena-Garcia R, Santos A et al (2017) dos Nitrogen use strategies of nodulated amazonian legume: *Inga edulis*. J Trop For Sci 29:1–9

41. Khan MS, Ahmad E, Zaidi A, Oves M (2013) Functional aspect of phosphate-solubilizing bacteria: Importance in crop production. In: Maheshwari DK, Saraf M, Aeron A (eds) Bacteria in Agrobiology: Crop Productivity. Springer, Berlin/Heidelberg, Germany, pp 237–263
42. LaVal R (2020) Datos casa LaVal [Online]. Available at: https://monteverde-institute.org/mvi-digital-collections.html
43. Lafay B, Burdon JJ (2007) Molecular diversity of legume root-nodule bacteria in Kakadu National Park, Northern Territory, Australia. PLoS ONE 2:e277
44. Lawrence A, Pennington TD, Hands MR, Zuniga RA (1995) In: Evans DO, Szott LT (eds) *Inga*: high diversity in the neotropics. In Nitrogen Fixing Trees for Acid Soils. pp. Nitrogen Fixing Tree Research Reports, Special Issue, pp 130–141
45. Leblanc HA, McGraw RL, Nygren P, Roux CL (2005) Neotropical legume tree *Inga edulis* forms N_2-fixing symbiosis with fast-growing *Bradyrhizobium* strains. Plant Soil 275:123–133
46. Li JH, Wang ET, Chen WF, Chen WX (2008) Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang province of China. Soil Biol Biochem 40:238–246
47. Lojka B, Preininger D, Lojkova J et al (2005) Biomass growth and farmer knowledge of *Inga edulis* in Peruvian Amazon. Agricultura Trop Et Subtropica 38:44–51
48. Lojka B, Lojkova J, Banout J et al (2008) Performance of an improved fallow system in the Peruvian Amazon— modelling approach. Agrofor Syst 72:27–39
49. Lojka B, Dumas L, Preininger D, Polesny Z et al (2010) The use and integration of *Inga edulis* in agroforestry systems in the amazon – review article. Agricultura Trop Et Subtropica 43:352–359
50. Lopez BDO, dos Teixeira AFS, Michel DC et al (2021) Genetic and symbiotic characterization of rhizobia nodulating legumes in a mining area in Southeast Brazil. Sci Agric 79:e20200238
51. Louca S, Doebeli M (2016) Transient dynamics of competitive exclusion in microbial communities. Environ Microbiol 18:1863–1874
52. Louca S, Jacques SMS, Pires APF et al (2016) High taxonomic variability despite stable functional structure across microbial communities. Nat Ecol Evol 1:0015
53. Macedo MO, Resende AS, Garcia PC et al (2008) Changes in soil C and N stocks and nutrient dynamics 13 years after recovery of degraded land using leguminous nitrogen-fixer trees. For Ecol Manag 255:1516–1524
54. Martínez-Hidalgo P, Hirsch AM (2017) The Nodule Microbiome: N_2 -Fixing Rhizobia Do Not Live Alone. Phytobiomes J 1:70–82
55. Mayhood P, Mirza BS (2021) Soybean root nodule and rhizosphere microbiome: distribution of rhizobial and nonrhizobial endophytes. Appl Environ Microbiol 87:e02884–e02820
56. McGee KM, Eaton WD, Porter TM, Shokralla S, Hajibabaei M (2019) Soil microbiomes associated with two dominant Costa Rican tree species, and implications for remediation: A case study from a Costa Rican conservation area. Appl Soil Ecol 137:139–153

57. Naamala J, Jaiswal SK, Dakora FD (2016) Microsymbiont diversity and phylogeny of native bradyrhizobia associated with soybean (*Glycine max* L. Merr.) nodulation in South African soils. Syst Appl Microbiol 39:336–344
58. Nishihata S, Kondo T, Tanaka K et al (2018) *Bradyrhizobium diazoefficiens* USDA110 PhaR functions for pleiotropic regulation of cellular processes besides PHB accumulation. BMC Microbiol 18:156
59. Olanrewaju OS, Glick BR, Babalola OO (2017) Mechanisms of action of plant growth promoting bacteria. World J Microbiol Biotechnol 33:197. https://doi.org/10.1007/s11274-017-2364-9
60. Omena-Garcia RP, Justino GC, Ferreira de Araujo VB et al (2015) Mineral nitrogen associated changes in growth and xylem-N compounds in Amazonian legume tree. J Plant Nutr 4:584–595
61. Orwa C, Mutua A, Kindt R et al (2009) Agrolforestry tree Database: a tree reference and selection guide. version 4.0. World Agroforestry Centre Kenya
62. Pan Y, Birdsey RA, Fang J et al (2011) A large and persistent carbon sink in the world's forests. Science 333:988–993
63. Parker MA (2015) The spread of *Bradyrhizobium* lineages across host legume clades: from *Abarema to Zygia*. Microb Ecol 69:630–640
64. Parker MA, Lunk A (2000) Relationships of bradyrhizobia from *Platypodium* and *Machaerium* (Papilionoideae: tribe Dalbergieae) on Barro Colorado Island, Panama. Int J Syst Evol Microbiol 50:1179–1186
65. Piotto D (2008) A meta-analysis comparing tree growth in monocultures and mixed plantations. For Ecol Manage 255:781–786
66. Poorter L, Craven D, Jakovac CC et al (2021) Multidimensional tropical forest recovery. Science 374:1370–1376
67. Powers JS, Marín-Spiotta E (2017) Ecosystem Processes and Biogeochemical Cycles in Secondary Tropical Forest Succession. Ann Rev Ecol Evol Syst 48:497–519
68. Ramírez MDA, España M, Aguirre C et al (2019) *Burkholderia* and *Paraburkholderia* are predominant soybean rhizobial genera in Venezuelan soils in different climatic and topographical regions. Microbes Environ 34:43–58
69. Salas A, Cabrera JJ, Jiménez-Leiva A et al (2021) Bacterial nitric oxide metabolism: recent insights in rhizobia. Adv Microb Phys 78:259–315
70. Sarode P, Rane M, Kadam M, Chincholkar S (2013) Role of microbial siderophores in improving crop productivity in wheat. In: Maheshwari DK, Saraf M, Aeron A (eds) Bacteria in Agrobiology: Crop Productivity. Springer, Berlin/Heidelberg, Germany, pp 287–308
71. Scavino AF, Pedraza RO (2013) The role of siderophores in plant growth-promoting bacteria. In: Bacteria in Agrobiology: Crop Productivity. Maheshwari, D.K., Saraf, M., Aeron, A., Eds.; Springer: Berlin/Heidelberg, Germany, pp. 265–285
72. Selvakumar G, Panneerselvam P, Ganeshamurthy AN (2013) Legume root nodule associated bacteria. In: Aurora NK (ed) Plant microbe symbiosis: fundamentals and advances. Springer, New

Delhi, pp 215–232

73. Shade A, Peter H, Allison SD et al (2012) Fundamentals of microbial community resistance and resilience. Front Microbiol 3:417
74. Singh A, Yadav VK, Chundawat RS et al (2023) Enhancing plant growth promoting rhizobacterial activities through consortium exposure: A review. Front Bioeng Biotechnol 11:1099999. 10.3389/fbioe.2023.1099999
75. Soe KM, Bhromsiri A, Karladee D, Yamakawa T (2012) Effects of endophytic actinomycetes and *Bradyrhizobium japonicum* strains on growth, nodulation, nitrogen fixation and seed weight of different soybean varieties. Soil Sci Plant Nutr 58:319–325
76. Sundquist A, Bigdeli S, Jalili R et al (2007) Bacterial flora-typing with targeted, chip-based pyrosequencing. BMC Microbiol 7:108–111
77. Tian R, Parker M, Seshadri R et al (2015) High-quality permanent draft genome sequence of *Bradyrhizobium* sp. Ai1a-2; a microsymbiont of *Andira inermis* discovered in Costa Rica. Stand Gen Sci 10:33
78. Tilman D, Reich PB, Knops JM (2006) Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441:629–632 van Ruijven J, Berendse F (2007) Effects of Diversity on the Temporal Stability of Plant Populations. Oikos 116:1323–1330
79. Velázquez E, Martínez-Hidalgo P, Carro L et al (2014) Nodular endophytes: an untapped diversity. In: González MBR, Gonzalez-López J (eds) Beneficial plant-microbial interactions: ecology and applications. CRC Press, Boca Raton, pp 215–236
80. Weir BS (2016) The current taxonomy of rhizobia. NZ Rhizobia website. https://www.rhizobia.co.nz/taxonomy/rhizobia
81. Weiss S, Xu ZZ, Peddada S et al (2017) Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 5:27
82. Wu C, Lo J, Urban C, Gao X et al (2022) Acetyl-CoA synthesis through a bicyclic carbon-fixing pathway in gas-fermenting bacteria. Nat Synth 1:615–625

Tables

Tables 1-6 is available in the Supplementary Files section.

Figures

Figure 1a)

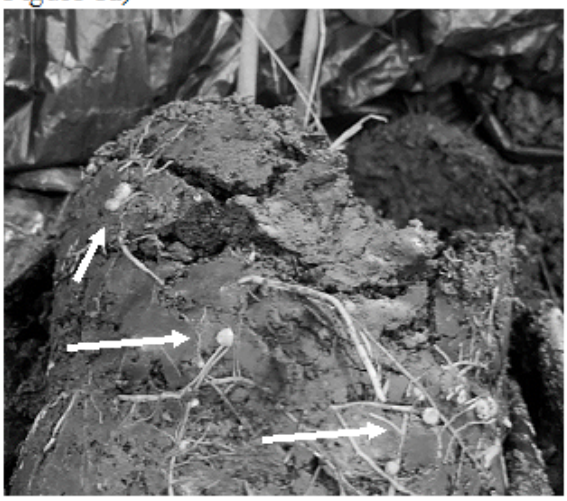

Figure 1b)

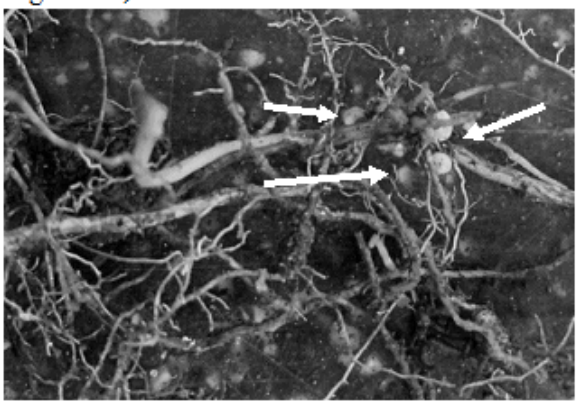

Figure 1c)

Figure 1

Nodules on the roots of *Inga punctata*. 1a) Nodules on the roots of 1 year-old *I. punctata*

trees; 1b) Nodules on the roots of 13 year-old *I. punctata* trees. 1c) Large nodule on the root of old growth *I. punctata* trees

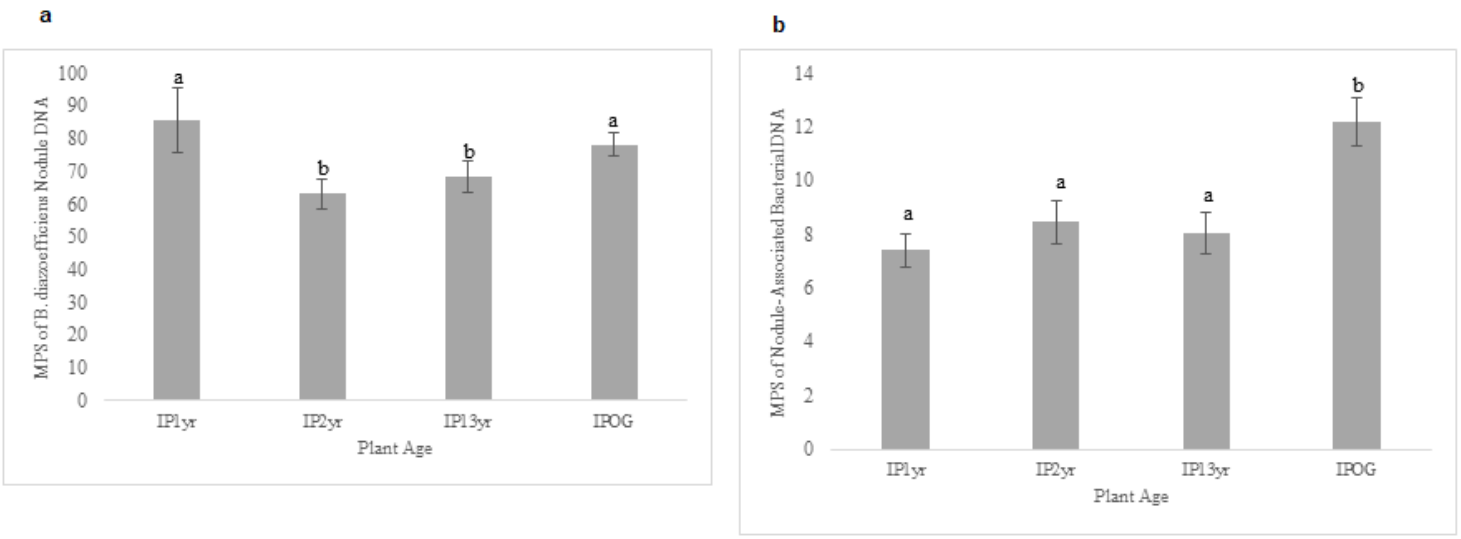

Figure 2

Bar charts of MPS of *Bradyrhizobium diazoefficiens*DNA and nodule associated (NAB) DNA within root nodules assessed. (2a). Bar charts of the MPS of *Bradyrhizobium diazoefficiens* DNA within root nodules of 1 year-old (IP1yr), 2 year-old IP2yr), 13 year-old (IP13yr), and old growth (IPOG) *Inga punctata*. Different letters represent differences in mean values < 0.05 as determined by Kruskal – Wallis analyses. (2b). Bar charts of the total MPS of Nodule-Associated Bacterial (NAB) DNA within root nodules of 1 year-old (IP1yr), 2 year-old IP2yr), 13 year-old (IP13yr), and old growth (IPOG) *Inga punctata*. Different letters represent differences in mean values < 0.05 as determined by Kruskal – Wallis analyses.

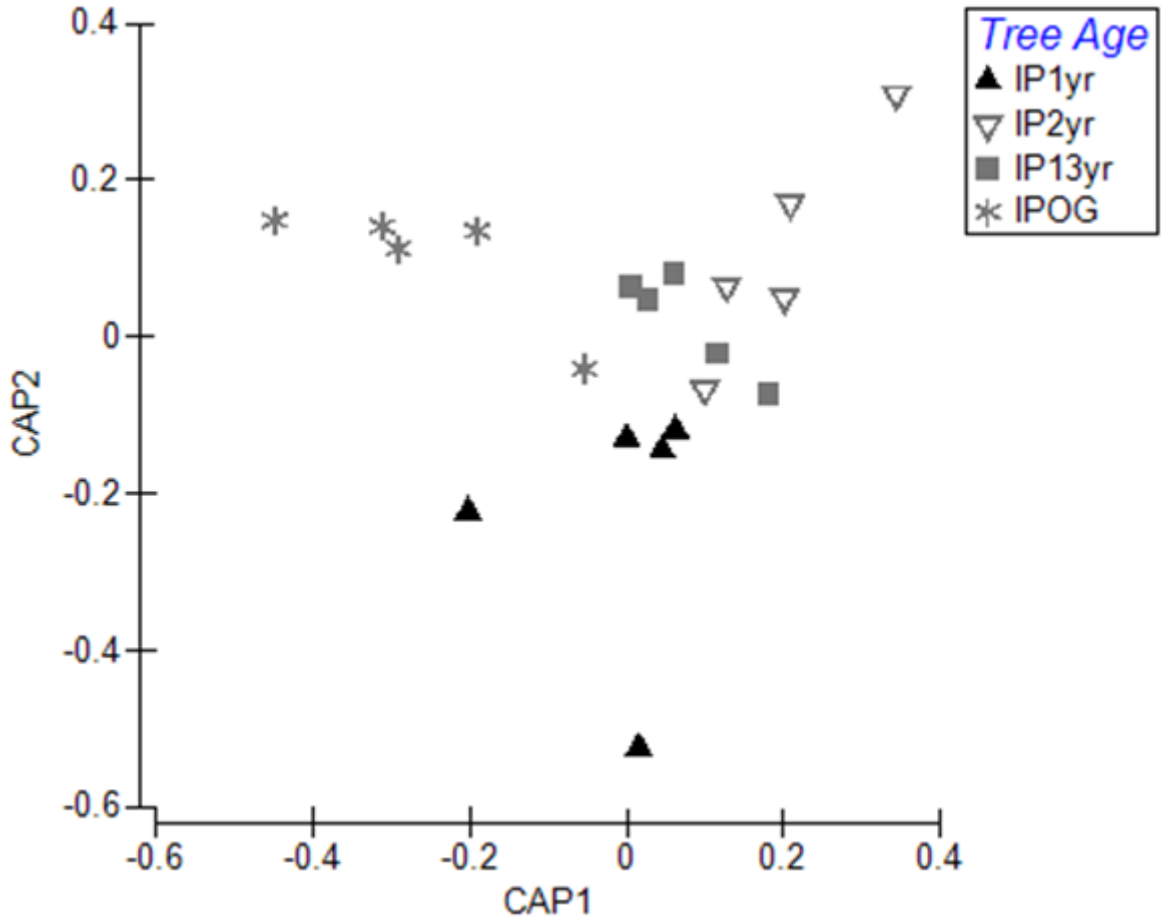

Figure 3

Visualization of the Canonical Analysis of Principal Coordinates (CAP) model (p = 0.035) demonstrating the differences in the community composition of the nodule-associated bacteria within the root nodules of 1 year-old (IP1yr), 2 year-old IP2yr), 13 year-old (IP13yr), and old growth (IPOG) *Inga punctata*.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PlantandsoilarticleTableS1.docx
- PlantandSoilarticleTableS2.docx
- Table16.docx