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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

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Abstract: *Inga edulis* and *Pentaclethra macroloba* are dominant N-fixing forest trees in Costa Rica, likely important for recovery of soil N and C after deforestation, yet little is known of their soil microbiomes nor how land use impacts them. Soils from both trees in a primary and secondary forest were assessed for N-cycle metrics and DNA sequence-based composition of total bacterial, potential N-fixing bacterial, and potential ammonium oxidizing bacterial genera. The compositions of the functional groups of bacteria, but not their total relative abundance of DNA, were different across the soils. The *P. macroloba* soils had greater NO_3^- levels and richness of both functional groups, while *I. edulis* soils had greater NH_4^+ levels, consistent with its NH_4^+ preference for root nodule development. The bacterial communities were different by habitat, as secondary forest *I. edulis* microbiomes were less rich, more dominant, possibly more affected by the disturbance, or reached equilibrium status quicker than the richer, less dominant *P. macroloba* microbiomes, which may be developing slower along with secondary forest succession, or were less affected by the disturbance. Functional redundancy and switching of 10 N-cycle bacterial genera was evident between the primary and secondary forest soils, likely to maintain stable levels of N-cycle activity following disturbance. In summary, the two tree soil microbiomes are different, land use differentially affects them, and, thus, both tree species should be used during forest regeneration strategies in this region.

Keywords: plant microbiomes; microbial ecology; tropical soils; leguminous trees

1. Introduction

Deforestation for agricultural uses has long been occurring in tropical regions such as the Northern Zone of Costa [1,2], resulting in diminished rates and levels of activity of soil nitrogen (N) and carbon (C) cycle dynamics and related soil ecosystem conditions, and the colonization of scrub growth or invasive species rather than development healthy secondary forests [1]. The N-fixing soil microbe activities represent the principal pathway by which deforested tropical areas recuperate the soil N and C [3–6] that are depleted during deforestation and subsequent agricultural uses [7–10], and are

also in high demand for the rapidly growing vegetation during reforestation [11]. To remediate these disturbed soils and forested areas, secondary forest regeneration following different types of land use damage is becoming common in these regions [2,12–14]. It has also been recognized that leguminous trees and their soil microbiomes provide the principal pathways for soil N and C recuperation, and belowground biomass development during tropical forest restoration [3–6,15–20]. This recognition has led to the use of N-fixing trees as an important component in tropical forest regeneration and restoration strategies [3,16,18,19,21].

Currently, little is known of the ecological relationships between these leguminous trees, their soil ecosystems, or their soil N-cycle-associated microbiome communities. It is also unclear how land management or other disturbances and restoration activities impact the overall soil ecosystem conditions, the composition or recovery of the soil N-cycle microbial communities, or even how these impacts might influence rates of regrowth of tropical forests. This information could provide insights useful in maximizing the efficacy of tropical forest and soil restoration and recovery plans [22–27]. For example, some suggest that N-fixing trees appear to interfere with tropical tree regrowth trajectories, while others suggest they appear to enhance tropical tree regrowth trajectories [7,28–30]. Certainly, these observed differences in forest regrowth trajectories could be linked to inhibition by N-fixing trees, or covarying components like stand age and N-fixing tree species composition [4,11,31,32], or past land-use histories [1,15,31,33–36]. However, it is also possible that specific leguminous tree species are differentially influencing the soil microbiome community compositions such that they result in altered soil C and N cycle dynamics, sequestration and distribution, and rates of soil biomass development, all of which could certainly affect both the soil recovery and forest regrowth patterns [10,37,38]. For example, it is known that bacterial N-fixation is important for production of ammonium (NH_4^+) and stimulation of the ammonium oxidizing bacteria (AMO) that produce nitrate (NO_3^-), and that both NH_4^+ and NO_3^- are critical for healthy forest and soil recovery. However, if different leguminous tree species have dissimilar soil microbiome community compositions, their N-cycle activities could be differentially regulated, resulting in variability in N cycle dynamics that could ultimately impact forest regrowth trajectories. For example, it is well known that N-fixing activities in different bacterial species are facultative and feedback-regulated, which results in variations of N-fixation rates not necessarily proportional to the abundance or species of either N-fixing tree or N-fixing bacteria within a reforested area [39,40].

Clearly, such differential regulation and activities of the N-cycle associated with the soil microbiome can alter the soil ecosystem and, thus, have an impact on tree regrowth patterns. However, as there is currently insufficient knowledge about the ecological relationships between different leguminous tree species soil N cycle-linked microbiome communities and associated N-cycle components, either between tree species or along restoration gradients following land disturbance or management practices, a study was conducted on the N-fixing and AMO bacterial communities and N-cycle components in the soils of two species of leguminous trees in a primary forest and a regenerating secondary forest in the Northern Zone of Costa Rica. *Pentaclethra macroloba* (Willd.) Kuntze (Fabaceae) and *Inga edulis* (Sw.) Willd. (Fabaceae) are two common leguminous tree species that are early colonizers and dominant later successional stage trees, thought to be important for N resource inputs in the Northern Zone forests of Costa Rica, [19,21,41–43] and as such have been used in tropical reforestation practices, although *I. edulis* has been more commonly used [19,21,42]. The current study was the first to characterize the compositions of the total bacterial assemblage and N-cycle-associated bacterial microbiomes and the N components within soils of these two tree species and to identify whether any differences in these metrics could be found between the different species or between the species under different land use histories. Thus, we examined the differences in the composition of the general bacterial, N-fixing, and AMO bacterial soil communities and N components from soils of *I. edulis* and *P. macroloba* trees within a recovering 23-year old secondary forest compared to a primary forest within the Northern Zone of Costa Rica. The goals were to determine: (a) if differences existed between the two plant species soil microbiomes; (b) if these microbiomes showed evidence of being differentially

influenced by the land management practice used; and (c) if there was evidence that one species might better serve for reforestation purposes. To address the goals, two questions were asked:

1. Are there differences in composition of the communities of total bacterial genera and bacterial genera associated with potential N-Fixing or AMO activities between soils of *P. macroloba* and *I. edulis* that were driven by Tree Species or by Land Management (Primary vs. Secondary Forest)?
2. Are there differences in total N (TN), NO_3^-, NH_4^+, NO_3^-/NH_4^+, NO_3^-/TN, and/or NH_4^+/TN by soil comparisons, and can any of these metrics best predict/explain the differences in the bacterial communities?

2. Methods

2.1. Site Description, Tree Identification, and Soil Sample Collection

This study was conducted in primary and secondary Uplands Forests within the Maquenque National Wildlife Refuge, Costa Rica (MNWR; 10°27′05.7′′ N, 84°16′24.32′′ W [2]), where mean annual temperature is 27 °C, mean annual rainfall is 4300 mm, and the dominant soil type in these Upland Forests is oxisols [41]. The *I. edulis* and *P. macroloba* trees used were from Upland Primary Forest sites not previously harvested, and Upland Secondary Forest sites that were part of the same primary Uplands forests, but cleared in 1982, grazed for 10 years, then abandoned and allowed to naturally regenerate since 1992. The different forest sites used were 100 m to 1 km apart, with the same oxisol soil types, no significant differences in pH, soil texture structure, % water holding capacity, density, or topography, and with slopes of 0% to 10% [26,27].

Six trees of each species were identified from the Primary Forest sites and six of each species from the Secondary Forest sites that were at least 50 m apart, with no overlapping canopy cover. All trees chosen were considered to be actively growing adult trees, with diameter at breast height (DBH) values between about 35% to 50% of the maximum for the species in the region (for *P. macroloba* = 24–47 cm, for *I. edulis* = 10–19 cm), which ensured active root nodule functionality based on previous studies [44–47]. Tree size (i.e., DBH) is commonly used as a proxy for tree age in this region of Costa Rica, as annual tree rings are inconsistently and minimally evident, at best, due to there being no strong dry season—wet season moisture variations [48,49]. The use of tree size correlated with the previous literature on the two species allowed us to approximate a relatively equal tree age for the tree soils.

The Tree Protection Zone or TPZ method [26], recently used in these same forests [26], was used to identify soil sample locations around tree bases as it better represents the critical root area associated with tree health and vigor, while the drip-line method often underestimates this important area [50,51]. Four soil cores (7.5 cm wide × 15 cm deep × 1.25 cm thick) per tree were aseptically collected (70% ethanol disinfection of gloves and tools between trees) at approximately 15 cm depth, at the 4 cardinal points around the tree bases, and at 10% of the calculated TPZ, after which the 4 cores were pooled into a single tree soil sample, in June 2015. All soil samples were passed through sterilized 4-mm sieve at field moist conditions prior to any analysis.

2.2. Soil Nitrogen Properties

Standing pools of soil N-cycle metrics were chosen as potential indicators of microbial group activity. Standing pools of nutrients are thought to represent more long-term effects on the soil biota due to land management compared to fluxes, which tend to represent rapid nutrient turnover rates of nutrients that are more associated with short-term changes in the soil biota and do not as effectively reflect land management-induced changes [7,50,52,53]. About 200 g of each soil sample were analyzed at the Center for Tropical Agriculture Research and Education (CATIÉ) Laboratories in Turrialba, Costa Rica for TN levels by the Kjeldahl method, and NH_4^+ and NO_3^- levels from 2M KCl extracts, spectrophotometrically [54]. The levels of NO_3^-, and ratios of NO_3^-/NH_4^+, and NO_3^-/TN levels were used as indicators of potential ammonium oxidizing activity, while NH_4^+ and NH_4^+/TN

levels were used as indicators of soil NH_4^+ production, such as from N-fixation or organic N compound decomposition.

2.3. DNA Extraction, Sequencing, and Bioinformatics

Total soil community environmental DNA (eDNA) was extracted from three 0.33g replicate sub-samples for a total of 1g for each soil sample using the MoBio PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). The concentration and purity (A_{260}/A_{280} ratio) of soil eDNA were then determined using a NanoDrop 1000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). All the amplification and sequencing methods have been previously described in detail [26,55]. Briefly, the different soil sample eDNA extracts were used for a 2-step PCR amplification, targeting the v3 and v4 of 16S ribosomal RNA gene region for bacteria and archaea [56]. All generated soil amplicons were sequenced in several Illumina MiSeq runs using a V3 MiSeq sequencing kit (FC-131-1002 and MS-102-3003). The subsequent 16S Illumina-generated sequences were processed using semiautomated pipelines, producing operational taxonomic units (OTUs). These OTUs were processed and taxonomically assigned from Phylum to Genus using the Ribosomal Database Project (RDP) classifier v2.12 [57] for bacteria using a standard QIIME protocol [56] with in-house Python data management scripts. Input reads were processed through QIIME's open reference clustering with standard parameters and 97% similarity to the GreenGenes database for bacterial analysis and assigned to known taxonomy where possible using the UCLUST algorithm. The identified soil bacterial DNA-based OTUs were organized taxonomically down to the genus rank.

This process resulted in 6,796,607 bacterial DNA sequences that were successfully identified at the phylum (24 phyla), class (36 classes), order (78 orders) and family (147 families) levels, with 1,123,988 of these sequences clearly identified to the level of 296 genera (hereafter called total bacterial genera). For this project, we chose to analyze the differences in the composition of the various soil bacterial communities at the genus level in the soil microbiome by Tree Species and by Land Management. The variable library sizes for each soil sample per habitat were normalized by converting each specific sequence (or OTU) read number to the mean proportion of the sequences (MPS) per sample [58], as the number of sequences from each within each soil sample, divided by the total number of sequences per that sample.

In microbial ecology studies, one can determine the relative amount of a functional gene in an eDNA sample through qPCR studies based on amplification of that functional gene sequence, but this does not concretely connect that specific sequence of the functional gene and its abundance to a specific microbial taxon, as identification of the gene is based on the amplification of that gene DNA sequence while identification of the taxon is generally based on amplification of variable regions of microbial rRNA. However, reference-based associations between certain microbial groups and functional genes have been used to connect functional genes with specific microbial genera or species [59,60]. Such efforts have resulted in valuable databases that, while not exhaustive nor said to be 100% accurate, provide a means of linking specific microbial taxa with potential functional activities. We took an analogous approach [61], using these same databases (www.genome.jp/kegg/; http://rdp.cme.msu.edu/hierarchy/; www.zoology.ubc.ca/louca/FAPROTAX/lib/php/index.php) and additional literature sources (references presented in Table S1) to suggest that specific bacterial genera have a strong possibility of having the potential for the functional group activities of nitrogen fixation or ammonium oxidation. These groups were called N-Fix_{pot} and AMO_{pot}, respectively; there were 41 potential N-Fixers bacterial genera (N-Fix_{pot}), and 16 potential AMO bacterial genera (AMO_{pot}) identified (Table S1). We only included genera that were clearly considered to commonly perform these functions. This method is only meant as a preliminary, exploratory tool to suggest the possibility for the representation of the different functional groups in the soils. Although this is not meant as a definitive characterization of the functions of the different genera, we believe this approach can provide a useful preliminary determination of the effect that various treatments might have on microbial community

composition associated with different functions. The MPS values of the N-Fix$_{pot}$ and AMO$_{pot}$ groups were also determined.

2.4. Data Analysis

All tree soil data were analyzed by two groupings as predictor variables: Tree Species (*P. macroloba* vs. *I. edulis*), and Land Management (Primary or Secondary Forest). To address Question 1, several approaches were used. Initially, the total MPS values for the N-Fix$_{pot}$ and AMO$_{pot}$ bacterial communities were summed and compared between the different Tree Species and between Land Management practices (Primary vs. Secondary Forest) using the Mann–Whitney test for significance. In addition, the MPS values for the total bacterial, the N-Fix$_{pot}$ and AMO$_{pot}$ bacterial communities were 4th root transformed to account for dominant and rare tax [62]. The MPS transformed DNA data was then converted into Bray–Curtis dissimilarity matrices using PRIMER-E v6 and its' add-on PERMANOVA [62,63]. A series of 2-factorial main and pairwise permutational multivariate analysis of variance (PERMANOVA) tests were conducted on the Bray–Curtis matrices from the three different bacterial groups of genera using PRIMER-E v. 6 and PERMANOVA+, with Tree Species alone (*P. macroloba* vs. *I. edulis*), and Land Management (Primary Forest and Secondary Forest) as the factors to determine if either Tree Species or Land Management were drivers of any differences found in the bacterial community compositions. As suggested [62,63], all main and pairwise PERMANOVA tests were based on 9999 unrestricted permutations. A discriminant Canonical Analysis of the Principal Coordinates (CAP; [64]) was also performed on the Bray–Curtis matrices that showed a significant difference between them by PERMANOVA, following PERMANOVA+ guidelines, to provide a rigorous assessment of the strength of the differences of the microbial community compositions between the soils. Strong differences in microbiome compositions between soil comparisons are indicated by CAP axis squared canonical correlations are represented by R^2 values > 0.7, moderate differences by R^2 values ≥ 0.5 to 0.69, and weak differences by R^2 values < 0.5 [64].

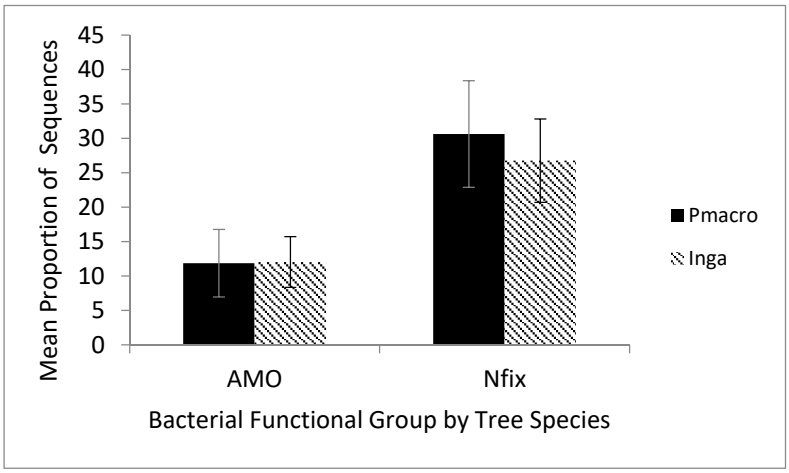
The Bray–Curtis matrices in Primer-E v6 were also used to calculate Pielou's evenness (J = H'/ln[S]), and the Margalef's richness (d = (S − 1)/Log(N)) of the total bacterial genera, the N-Fix$_{pot}$ and the AMO$_{pot}$ bacterial genera communities. The Margalef's method compensates for the effects of sample size, while other measurements of richness do not, such as Shannon's index or simple OTU count, both of which can increase simply with sample size. The strength of the differences in the richness and evenness across the different tree soil comparisons were determined using a one-way ANOVA followed by Tukey's HSD or Dunnett's T3 post-hoc analyses or independent samples t-test in SPSS (v.25, Armonk, NY, USA). Prior to ANOVA, the Levene's test was performed in SPSS to determine homogeneity of the variances of the data, and the Shapiro–Wilk's test was performed in SPSS to determine normality of all the data, and whether Tukey's HSD or Dunnett's T3 post-hoc test should be used. Lastly, Mann–Whitney analyses of the MPS values were conducted in SPSS on the N-Fix$_{pot}$ and AMO$_{pot}$ genera with a MPS values of >0.5 in at least one of the soil types analyzed to identify differences in specific genera between the different soil groups.

To address Question 2, all N-cycle data were examined for mean differences between the three tree soil comparisons by one-way ANOVA followed by Tukey's HSD or Dunnett's T3 post-hoc tests, as appropriate using SPSS (v.25, Armonk, NY, USA). In addition, a Distance-Based Linear Model (DistLM) permutation test in PERMANOVA+ was used to determine if there were soil N-cycle metrics or ratios that were significant predictors of the differences in multivariate patterns observed between the different tree soil bacterial community comparisons. We used the previously mentioned MPS data Bray–Curtis matrices as response variables, and log (x + 1) transformed N-cycle data as predictor variables for the DistLM analyses, along with a step-wise selection process, along with an AICc (Akaike's Information Criterion Corrected) selection criterion and 9999 permutations. The AICc criterion is the appropriate method to use when the number of samples/the number of predictor variables is <40 [62], as was the case in this study.

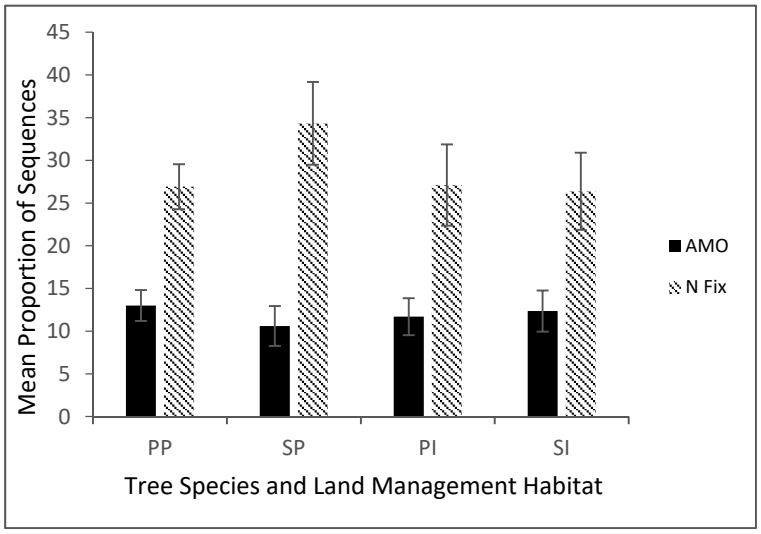
3. Results

3.1. Differences in Bacterial Community MPS, Richness, Evenness, and Specific Genera

Although there were no differences observed in the total MPS values of the N-Fix$_{pot}$ or AMO$_{pot}$ genera between the soils of the two tree species (Figure 1a), differences in richness and evenness of the bacterial communities were observed (Table 2). The richness of the total bacterial, N-Fix$_{pot}$, and AMO$_{pot}$ communities was greater in the *P. macroloba* than *I. edulis* soils (T-Test, $F_{(2,22)}$ range = 2.18 to 3.62, p range ≤ 0.01 to 0.03), and the evenness of the total bacterial and N-Fix$_{pot}$ (but not the AMO$_{pot}$) community was less in the *P. macroloba* than *I. edulis* soils (T-Test, $F_{(2,22)}$ range = 6.03 and 7.12, p value ≤ 0.01 and 0.05, respectively).



(**a**)



(**b**)

Figure 1. The total mean proportion of DNA sequences, or MPS, (±std. dev.) of the potential Nitrogen-Fixation (N Fix) and potential Ammonium Oxidizing (AMO) bacterial genera from soils of 12 *Pentaclethra macroloba* and 12 *Inga edulis* trees: (**a**) by Tree Species (Pmacro vs. Inga), (**b**) by Tree Species and Land Management Habitat (6 trees from each of PP = Primary Forest *P. macroloba*, SP = Secondary Forest *P. macroloba*, PI = Primary Forest *I. edulis*, SI = Secondary Forest *I. edulis*).

There were differences in the overall MPS values of both the N-Fix$_{pot}$ and AMO$_{pot}$ communities in the *P. macroloba* soils between the Primary and Secondary forests (i.e., intraspecies differences) but

not in the *I. edulis* soils (Figure 1b). Specifically, MPS values of the N-Fix$_{pot}$ community were greater in the Secondary (34.3% ± 4.5%) than in the Primary (26.9% ± 2.6%) *P. macroloba* soils ($p = 0.008$) and the MPS of the AMO$_{pot}$ communities were greater in the Primary (13.0% ± 1.8%) than Secondary (10.4% ± 2.3%) *P. macroloba* soils ($p = 0.05$). The only interspecies differences in the overall MPS of the two bacterial groups observed was that the MPS of the N-Fix$_{pot}$ communities were greater in the Secondary *P. macroloba* (34.3% ± 4.5%) than Secondary *I. edulis* (26.4% ± 4.5%) soils ($p = 0.015$). The Primary and Secondary Forest soils also had differences in the richness and evenness of the total bacterial and N-Fix$_{pot}$ bacterial (but not the AMO$_{pot}$) communities (Table 1, ANOVA, F(2, 22) range = 4.164 to 11.795, p range ≤ 0.01 to 0.02). Specifically, intertree species differences in both habitats were evident as the richness of the total bacterial and N-Fixpot communities were greater and the evenness less in both the Primary Forest and Secondary Forest *P. macroloba* compared to the Primary and Secondary Forest *I. edulis* soils (ANOVA. p range ≤ 0.01 to 0.05).

Table 1. A comparison of the mean (±std. dev.) of the richness and evenness of the communities of total bacterial, potential Nitrogen-Fixation (N-Fixation), and potential Ammonium Oxidizing (AMO) bacterial genera in soils from *Pentaclethra macroloba* and *Inga edulis* by Tree Species (Pm vs. Ie), showing significance by t-test and p values parenthetically; followed by a comparison of the richness and evenness of the bacterial communities by Land Management (PP = Primary Forest *P. macroloba*, SP = Secondary Forest *P. macroloba*, PI = Primary Forest *I. edulis*, SI = Secondary Forest *I. edulis*), showing differences by ANOVA and post-*hoc* test p values, shown parenthetically. Significantly different samples are highlighted in **bold**. N.D = no difference.

Comparisons	Bacterial Richness	Bacterial Evenness	N-Fixation Richness	N-Fixation Evenness	AMO Richness	AMO Evenness
P. macroloba	25.32 (±3.47)	0.33 (±0.02)	5.72 (±0.88)	0.31 (±0.02)	2.94 (±0.44)	0.53 (±0.05)
I. edulis	14.55 (±6.4)	0.44 (±0.04)	4.19 (±1.21)	0.44 (±0.04)	2.48 (±0.63)	0.52 (±0.08)
Sig. Differences	**Pm > Ie**	**Pm < Ie**	**Pm > Ie**	**Pm < Ie**	**Pm > Ie**	N.D
	(3.62, <0.01)	**(6.03, <0.01)**	**(2.51, <0.01)**	**(7.12, 0.05)**	**(2.18, 0.03)**	(0.26, 0.80)
PP	26.21 (±3.10)	0.34 (±0.02)	7.96 (±0.93)	0.79 (±0.01)	2.87 (±0.35)	0.51 (±0.05)
SP	24.43 (±3.86)	0.32 (±0.02)	7.13 (±0.92)	0.76 (±0.01)	3.01 (±0.54)	0.56 (±0.04)
PI	15.24 (±7.06)	0.44 (±0.02)	5.71 (±1.22)	0.90 (±0.01)	2.35 (±0.77)	0.55 (±0.07)
SI	13.86 (±6.25)	0.46 (±0.04)	5.94 (±1.49)	0.92 (±0.02)	2.41 (±0.42)	0.49 (±0.08)
Sig. Differences	**PP > PI (p <0.01)**	**PP < PI, (p = 0.05)**	**PP > PI, (p = 0.01)**	**PP < PI (p = 0.05)**	N.D.	N.D.
	SP > SI (p = 0.01)	**SP < SI, (p = 0.04)**	**SP > SI (p = 0.05)**	**SP < SI (p < 0.01)**	(p = 0.26–0.99)	(p = 0.19–0.46)

There were 10 genera with MPS values > 0.5% that were significantly different between the soil groups studied (Table 2). The Mann–Whitney analyses by Tree Species showed the MPS values for the N-Fix$_{pot}$ and AMO$_{pot}$ genera *Burkholderia* and *Bacillus* were greater in the *P. macroloba* soils ($p \leq 0.01$ and 0.05, respectively), while the MPS of the AMO$_{pot}$ genera *Nitrospira* were greater in the *I. edulis* soils ($p = 0.03$). Intertree species differences by habitat were observed as the MPS values of *Burkholderia* were greater in the Primary Forest *P. macroloba* than *I. edulis* soils ($p \leq 0.01$); the MPS of *Burkholderia*, *Bacillus*, and the N-Fix$_{pot}$ genera *Rhodoplanes* were greater in the Secondary Forest *P. macroloba* than *I. edulis* soils ($p \leq 0.01$, 0.05, and 0.04, respectively). Intratree species differences between habitats were observed as the MPS values of *Bacillus* and the N-Fix$_{pot}$ genera *Clostridium* and *Geobacter* were greater in the Secondary than Primary Forest *P. macroloba* soils ($p = 0.01$, <0.01, and 0.05, respectively); those of N-Fix$_{pot}$ genera *Rhizobium*, *Beijerinckia*, and the AMO$_{pot}$ genera *Nitrospira*, *Sphingomonas*, and *Comamonas*, were greater in the Primary than Secondary *P. macroloba* soils ($p \leq 0.01$, 0.02, 0.05, <0.01, respectively).

Table 2. Mann–Whitney analyses potential Nitrogen-Fixation and potential Ammonium Oxidizing bacterial genera with MPS values > 0.5% in soils from *Pentaclethra macroloba* and *Inga edulis* by Tree Species (Pmacro vs. Inga) and by Land Management (PP = Primary Forest *P. macroloba*, SP = Secondary Forest *P. macroloba*, PI = Primary Forest *I. edulis*, SI = Secondary Forest *I. edulis*.

Significant Differences in MPS of Nitrogen-Fixation Bacterial Genera		
Genus	**Pattern Observed**	***p* Values**
Rhodoplanes	SP > SI	0.04
Burkholderia	Pmacro > Inga; PP > PI; SP > SI	All < 0.01
Bacillus	Pmacro > Inga; SP > PP; SP > SI;	0.05; 0.01; 0.05
Clostridium	SP > PP	<0.01
Geobacter	SP > PP	0.05
Rhizobium	PP > SP	0.02
Beijerinckia	PP > SP	0.05
Significant Differences in MPS of Ammonium Oxidizing Bacterial Genera		
Genus	**Pattern Observed**	***p* Values**
Nitrospira	Inga > Pmacro; PP > SP	0.03; <0.01
Burkholderia	Pmacro > Inga; PP > PI	<0.01;0.05
Bacillus	Pmacro > Inga; SP > PP	0.05; 0.05
Sphingomonas	PP > SP	0.01
Comamonas	PP > SP	0.01

3.2. Multivariate Analyses of the Bacterial Community Compositions

Significant differences were found in the composition of the total bacterial, N-Fix$_{pot}$, and AMO$_{pot}$ communities between the different Tree Species soils (Table 3. PERMANOVA, Pseudo-F$_{(2,22)}$ range = 3.716 to 10.853, p range = all < 0.01, respectively). There were strong differences found in the compositions of the total bacterial communities (CAP R_2 = 0.809, $p < 0.001$) and moderate differences in the N-Fix$_{pot}$ and AMO$_{pot}$ bacterial communities (CAP R_2 = 0.591, and 0.514, $p < 0.001$, and 0.004, respectively) between the two Tree Species soils (Table 4). At the Land Management level of analysis, there were differences observed in the bacterial community compositions between the Primary and Secondary Forest soils (Table 3. PERMANOVA, Pseudo-F$_{(2,22)}$ RANGE = 2.579 to 6.982, p range = all < 0.01). There were intratree species differences found in the bacterial communities between the *P. macroloba* (but not the *I. edulis*) Primary and Secondary Forest soils (Table 4). The *P. macroloba* Primary and Secondary Forest soils had strong and significant differences in the compositions of the total bacterial and AMO$_{pot}$ communities (CAP R^2 = 0.917 and 0.850, p = 0.015 and < 0.001, respectively; and PERMANOVA, Pseudo-F$_{(2,22)}$ = 5.295 and 4.533, p both < 0.01, respectively) and moderate differences in the N-Fix$_{pot}$ communities (CAP R^2 = 0.529, p = 0.002; PERMANOVA, Pseudo-F$_{(2,22)}$ = 2.205, $p < 0.01$). There were intertree species differences observed within the two different habitats (Table 3) as the total bacterial, N-Fix$_{pot}$, and AMO$_{pot}$ community compositions were significantly different, although weakly, between the Primary Forest *P. macroloba* and *I. edulis* tree soils (CAP R^2 range = 0.312 to 0.468, p range ≤ 0.001 to 0.002; PERMANOVA, Pseudo-F$_{(2,22)}$ range = 1.847 to 2.347, p range = 0.01 to 0.05), and moderately to strongly different between the Secondary Forest *P. macroloba* and *I. edulis* tree soils (CAP R^2 range = 0.505 to 0.867, p range = 0.001 to 0.052; PERMANOVA, Pseudo-F$_{(2,22)}$ range = 2.108 to 4.335, p range ≤ 0.01 to 0.03).

Table 3. Community composition differences in total bacterial, potential Nitrogen-Fixing, and potential Ammonium Oxidizing (AMO) genera in *Pentaclethra macroloba* and *Inga edulis* soils by Tree Species (*P. macroloba* vs. *I. edulis*), and by Tree Species and Land Management (PP = Primary Forest *P. macroloba*, SP = Secondary Forest *P. macroloba*, PI = Primary Forest *I. edulis*, SI = Secondary Forest *I. edulis*) by PERMANOVA Main and Pairwise Tests and Canonical Analysis of Principal Coordinates (CAP). Significant differences are highlighted in **bold**.

PERMANOVA and CAP Results of Total Bacterial Community				
Main PERMANOVA Test Results			Main CAP Test Results	
	Pseudo-F	*p* value	CAP R^2	*p* value
By Tree Spp	4.415	**<0.01**	0.809	**<0.001**
By Land Management	2.692	**<0.01**	0.955	**0.015**
Pairwise PERMANOVA Test Results			Pairwise CAP Test Results	
Land Management	Pseudo-F	*p* value	CAP R^2	*p* Value
Pairwise Test: PP to SP	5.295	**<0.01**	0.917	**0.015**
Pairwise Test: PI to SI	0.743	0.71	not done	not done
Pairwise Test: PP to PI	1.847	**0.04**	0.312	**<0.001**
Pairwise Test: SP to SI	4.335	**0.02**	0.867	**0.038**
PERMANOVA and CAP Results of Potential Nitrogen-Fixing Community				
Main PERMANOVA Test Results			Main CAP Test Results	
	Pseudo-F	*p* value	CAP R^2	*p* value
By Tree Spp	3.716	**<0.01**	0.591	**<0.001**
By Land Management	2.579	**<0.01**	0.505	**0.052**
Pairwise PERMANOVA Test Results			Pairwise CAP Test Results	
Land Management	Pseudo-F	*p* value	CAP R^2	*p* value
Pairwise Test: PP to SP	2.205	**<0.01**	0.529	**0.002**
Pairwise Test: PI to SI	0.399	0.89	not done	not done
Pairwise Test: PP to PI	2.347	**0.01**	0.349	**0.002**
Pairwise Test: SP to SI	2.108	**0.03**	0.505	**0.052**
PERMANOVA and CAP Results of AMO Community				
Main PERMANOVA Test Results			Main CAP Test Results	
	Pseudo-F	*p* value	CAP R^2	*p* value
By Tree Spp	10.853	**<0.01**	0.514	**0.004**
By Land Management	6.982	**<0.01**	0.779	**0.001**
Pairwise PERMANOVA Test Results			Pairwise CAP Test Results	
Land Management	Pseudo-F	*p* value	CAP R^2	*p* value
Pairwise Test: PP to SP	4.533	**<0.01**	0.85	**<0.001**
Pairwise Test: PI to SI	0.321	0.84	not done	not done
Pairwise Test: PP to PI	2.211	**0.05**	0.468	**<0.001**
Pairwise Test: SP to SI	4.111	**<0.01**	0.779	**0.001**

Table 4. Differences in mean values of the N-cycle metrics between *Pentaclethra macroloba* and *Inga edulis* soils within an Uplands Forested area in the Maquenque National Wildlife Refuge in the Northern Zone of Costa Rica. Comparisons were made by Tree Species alone (i.e., *P. macroloba* vs. *I. edulis*), and by Land Management (PP = Primary Forest *P. macroloba*, SP = Secondary Forest *P. macroloba*, PI = Primary Forest *I. edulis*, SI = Secondary Forest *I. edulis*). Significant differences in mean values by ANOVA and post-hoc test are highlighted in **bold**, and marked with ** The F-stat and *p* value are presented along with the direction of the trends of the level in value.

(**a**)

	P. macroloba	***I. edulis***	**Comparison**
TN(μg/g)	48.08 ± 5.79	46.83 ± 5.04	no difference
NO_3^- (μg/g) **	46.99 ± 11.43	31.61 ± 6.54	***P. macro* > *I. edulis*: F = 4.04, $p < 0.01$**
NH_4^+ (μg/g) **	4.97 ± 2.47	7.75 ± 2.71	***I. edulis* > *P. macro*: F = 2.11, $p = 0.05$**
NO_3^-/NH_4^+ **	8.87 ± 3.15	5.89 ± 3.96	***P. macro* > *I. edulis*: F = 2.05, $p = 0.05$**
NO_3^-/TN **	0.97 ± 0.19	0.68 ± 0.18	***P. macro* > *I. edulis*: F = 3.83, $p < 0.01$**
NH_4^+/TN **	0.11 ± 0.02	0.17 ± 0.04	***I. edulis* > *P. macro*: F = 2.94, $p = 0.015$**

(**b**)

	PP	PI	SP	SI	Comparisons
TN(μg/g)	48.33 ± 7.84	46.17 ± 4.92	47.83 ± 3.49	47.5 ± 5.54	No differences (p values > 0.91)
NO_3^- (μg/g) **	**41.53 ± 6.93**	**33.04 ± 4.96**	**52.45 ± 8.58**	**29.19 ± 7.45**	**PP > PI, $p = 0.035$; SP > SI, $p < 0.01$**
NH_4^+ (μg/g)	4.57 ± 1.68	5.38 ± 2.46	7.28 ± 2.49	8.22 ± 2.29	No differences (p values > 0.16)
NO_3^-/NH_4^+ **	**9.95 ± 2.23**	**7.31 ± 2.11**	**7.85 ± 2.36**	**3.84 ± 1.39**	**PP > PI, $p = 0.06$; SP > SI, $p = 0.04$**
NO_3^-/TN **	**0.85 ± 0.17**	**0.75 ± 0.16**	**1.09 ± 0.13**	**0.62 ± 0.18**	**SP > PP, $p = 0.06$; SP > SI, $p = 0.04$**
NH_4^+/TN	0.10 ± 0.04	0.13 ± 0.05	0.14 ± 0.05	0.18 ± 0.17	No differences (p values > 0.205)

3.3. Differences in N-Metrics

The patterns of the N-metrics levels were different between the Tree Species and the Land Management (Table 4). The *P. macroloba* tree soils had greater levels of NO_3^-, NO_3^-/NH_4^+, and NO_3^-/TN ($F_{(2,22)}$ range = 2.05 to 4.04, p range ≤ 0.01 to 0.05), and the *I. edulis* soils had greater levels of NH_4^+ and NH_4^+/TN ($F_{(2,22)}$ = 2.11 and 2.94, p = 0.05 and 0.015, respectively). ANOVA post-hoc analyses by Land Management showed that the levels of NO_3^- and NO_3^-/NH_4^+ were greater in the *P. macroloba* than *I. edulis* Primary Forest soils (p = 0.035 and 0.06, respectively), and the levels of NO_3^-, NO_3^-/NH_4^+, and NO_3^-/TN were greater in the *P. macroloba* than *I. edulis* secondary Forest soils (p = 0.01, 0.04, and <0.01, respectively). There were no differences in the NH_4^+ and NH_4^+/TN levels between habitats, although these were generally greater, but not statistically, in the different *I. edulis*-related samples.

3.4. Distance Based Linear Modeling

The DistLM analyses (Table 5) by Tree Species showed that NO_3^- levels best predicted the differences in composition of the total bacterial and N-Fix_{pot} communities, explaining 20.85% and 18.09% of the differences, respectively, and NO_3^-, NO_3^-/NH_4^+ best predicted the differences in the composition of the AMO_{pot} community, explaining 14.72% of the differences in group composition. The DistLM analyses by Land Management showed that NO_3^- and NO_3^-/TN levels best predicted the compositional differences in total bacterial and N-Fix_{pot} communities, explaining 22.75% and 18.43% of the differences, respectively, and that NO_3^- and NO_3^-/NH_4^+ best predicted the differences in AMO_{poy} community composition, explaining 21.4% of the differences in the composition of this group.

Table 5. The distance-based linear modeling (DistLM) sequential tests of the Nitrogen-cycle metrics that best predicted and explained the variations in composition of the total bacterial, potential Nitrogen-Fixation (N-Fix_{pot}), and potential Ammonium Oxidizing (AMO_{pot}) genera in soils from *Pentaclethra macroloba* and *Inga edulis* by Tree Species and by Tree Species and Land Management.

Tree Soil Comparisons	Best Predictors	AICc	Pseudo-F	*p* Value	Cuml. Prop.
Tree Species					
Total Bacterial Genera	NO_3^-	167.6	5.794	0.014	20.85
N-Fix_{pot} Genera	NO_3^-	186.3	3.11	0.035	18.09
$AMO_{potential}$ Genera	NO_3^-, NO_3^-/NH_4^+	104.4	2.541	0.018	14.72
Land Management					
Total Bacterial Genera	NO_3^-, NO_3^-/TN	167.1	6.477	0.011	22.75
N-$Fix_{potnetial}$ Genera	NO_3^-, NO_3^-/TN	119.2	2.443	0.025	18.43
$AMO_{potnetial}$ Genera	NO_3^-, NO_3^-/NH_4^+	136.3	5.971	0.006	21.41

4. Discussion

In this study, we observed significant differences in the soil microbiome communities between *P. macroloba* and *I. edulis* and also found inter- and intratree species differences in soil microbiomes when they were compared by the effect of the local land use practice of conversion of forest-to-pasture-to-secondary forest. Our conclusions are that: (a) *P. macroloba* and *I. edulis* stimulate the development of different soil microbiome assemblages; (b) differences in these microbiome are also driven by the different the Land Management practice of Secondary Forest development used in this region; (c) *I. edulis* soils had a less rich but more evenly distributed total bacterial soil microbiome that may have been more impacted by the land use practice or may be developing more slowly than that of *P. macroloba* soils; (d) the *P. macroloba* soil microbiome had a richer and less evenly distributed total bacterial microbiome, than *I. edulis*, suggesting it may have been less impacted by the land use practice or may be developing more quickly with the vegetation changes during secondary forest succession; (e) there was greater generation of the indicators of ammonium oxidation along with an increased richness of the Nit-Fix_{pot} and AMO_{pot} communities associated with the *P.macroloba* soil microbiomes compared to those of *I. edulis*, suggesting *P. macroloba* soil microbiomes may be more important for ammonium oxidizing activity; (f) there was greater generation of the indicators of NH_4^+ production in the *I. edulis* soil microbiomes compared to those of *P. macroloba*, suggesting that the *I.edulis* soil microbiomes may be more important for ammonium production activity in these forests; (g) the land management-driven changes in the soil NO_3^-, NH_4^+, NO_3^-/NH_4^+, NO_3^-/TN were closely associated with changes in the soil microbiomes; moreover, the levels of NO_3^-, NO_3^-/NH_4^+, and NO_3^-/TN were found to be predictive of these changes in the soil microbiomes; and (h) the impacts of the land management may be stimulating functional redundancy and taxonomic switching of functional group genera associated with both tree species. These findings indicate that *P. macroloba* should at minimum be included with *I. edulis* in future restoration activities in the region, as it promotes a different microbiome and is either less impacted or recovers quicker than the *I. edulis* soil microbiomes.

4.1. Differences between Tree Species

The Tree Species-driven differences observed in the composition, richness, and evenness of the total bacterial, AMO_{pot}, and N-Fix_{pot} bacterial microbiome communities were best predicted by the differences in levels of NO_3^-, NO_3^-/NH_4^+, or NO_3^-/TN in the different soils using the DistLM modeling assessment. Moreover, the differences found in the in bacterial communities between the tree soil microbiomes, the greater levels of NO_3^--related metrics, and the reduced levels of NH_4^+-related metrics found in the *P. macroloba* soils, and the opposite found in the *I. edulis* soils, suggests *P. macroloba* soil microbiomes are more critical for producing NO_3^-, and the *I. edulis* microbiomes more critical for producing NH_4^+ into these forest soil ecosystems. These findings build on previous studies that have

shown *I. edulis* trees enhance the quantity and quality of soil inorganic N, organic C and N, stimulate C-sequestration and long-term N accumulation, and above ground biomass development in tropical areas more than other non-*Inga* species [19,42,65,66] and that *I. edulis* nitrogen-fixing root nodule development and activity is stimulated by the presence of NH_4^+ and more inhibited by NO_3^- [67–69]. These previous and current findings suggest *I. edulis* may be stimulating development of a bacterial community that is stable, less rich well-fitted for its niche, and more efficiently producing NH_4^+ in comparison to that of *P. macroloba*, and, thus, may be enhancing the success of nodulation and subsequent growth for *I. edulis* in these forests. At this point, whether these relationships between plant and soil microbiome are occurring in a "cause and effect" manner is not known, but these observations suggest a logical hypothesis that should be further studied. It would also be of interest to determine if *P. macroloba* root nodules are stimulated by NO_3^-, in contrast to those of *I. edulis*.

Much less is known about the influence of *P. macroloba* on soil ecosystems, although it has been suggested that the activity of the root nodular N-fixing microbes associated with the tree represent an important mechanism for N availability in soils in this region of Costa Rica [65,70]. In these same forests, McGee et al. [26] showed that *P. macroloba* soil microbiomes were different and associated with greater levels of production of both NH_4^+ and C-biomass activities than those of *Dipteryx panamensis*, which is another member of the Fabaceae family and a dominant canopy tree in the region and also suggested that *P. macroloba* would be a critically important tree for remediation of damaged soils in the region.

4.2. Differences in Land Use Legacy Effects

Legacy effects of land management have been shown to influence composition and succession of microbial communities in damaged habitats [71,72]. Our study suggests legacy effects from the forest-to-pasture-to-secondary forest land management practice common to this region of Costa Rica are influencing the differences in composition and succession of the two tree species soil microbiomes between the Primary and Secondary Forests. There were differences in the composition, along with greater richness and reduced evenness of the total bacterial and $N\text{-}Fix_{pot}$ communities, and the greater richness of the AMO_{pot} community, within the both Primary and Secondary Forest soils of *P. macroloba*, compared to *I. edulis* tree soils. This could suggest that *P. macroloba* soil microbiomes are either less affected by the specific land management practice used, or recovering quicker during recovery, along the successional vegetation changes occurring in the developing Secondary Forests, when compared to the *I. edulis* soil microbiomes. Conversely, the legacy effects of this land management practice could also be resulting in *I. edulis* soil microbiome communities that are either more impacted by the damage or are recovering more slowly than those of *P. macroloba*, resulting in a less rich, more evenly distributed group of bacteria that is changing more slowly with the vegetation successional gradient, which could be providing more longer-term soil ecosystem changes. These would be the expected patterns for bacterial communities differentially impacted by habitat damage or recovering from the damage at different rates [71–73] during the processes of competitive exclusion [74].

Thus, these ecological patterns suggest that either *P. macroloba* soil microbial communities are further along the competitive exclusion processes and have stabilized more than in *I. edulis* soils or that *P. macroloba* soil microbiomes are less affected by the land management practice implemented. Although these ideas may be speculative interpretations based on the data, they provide the first evidence that this land management practice commonly used in the Northern Zone of Costa Rica may influence the soil microbiomes of *P. macroloba* differently than *I. edulis*. Regardless of the reasons or causes of the response, these findings support the idea that *P. macroloba* should be included with *I. edulis* in future restoration practices the *P. macroloba* microbiome may be less affected by the land management disturbance, or the *P. macroloba* microbiome recovers more quickly from the damage. However, our data also show that the *I. edulis* soil microbiomes appear to be very important for the production of NH_4^+ that may actually enhance growth of that tree. These findings support the use of both trees in remediation of damage.

4.3. Evidence of Functional Redundancy and Taxonomic Switching

Functional redundancy is considered as the number of taxa within community guilds performing the same functions [69,75], which could be represented by multiple microbial genera in communities within the same niches, with high degrees of flexibility and resilience, and with similar metabolic capabilities that are phylogenetically conserved [75–80]. The switching of the taxa within a niche might serve as predictors of the influences that environmental changes have on microbial communities [77,79,81]. Our study suggests functional redundancy and taxonomic switching may be occurring across the different tree soil treatments, with the changes in functional group genera occurring in response to land management. There were differences in MPS values of certain N-Fix$_{pot}$ and AMO$_{pot}$ genera across the tree soil comparisons, even though there were no differences in total MPS values of either functional group across the comparisons. This suggests these genera may be part of a larger group of functionally redundant bacteria critical for N-cycle activities such that some genera may be replaced by others following land disturbances or successional changes in the vegetation community, but the overall abundance and activity of the entire functional group remains at a steady state. This may be ecologically important for maintaining steady state metabolic activities in these soils during these environmental disturbances/vegetational changes. Future studies are planned to measure the actual functional group metabolic activities, simultaneous with the assessment of the MPS values and diversity of the 7 N-Fix$_{pot}$ genera and 5 AMO$_{pot}$ genera found to "switch" in these tree soil comparisons.

5. Conclusions

This project showed that *P. macroloba* and *I. edulis* tree soil microbiomes and N-cycle metrics were different, and that each of the tree soil systems responded differently to the previous forest-to-pasture-to-secondary forest conversion. The *I. edulis* soil microbiome communities may be more affected by the land disturbances or may be reaching a steady state equilibrium in composition faster than the *P. macrobloba* soil microbiomes, which may be more resilient to the land management used or are responding slower to the disturbance and in concert with changes in vegetation that occur during forest succession. However, both of the tree species microbiomes appear to be beneficial to soil ecosystem recovery. Functional redundancy and taxonomic switching are also occurring in the soils, differentially by tree species, in response to the land disturbance. It appears that the *I. edulis* soil microbiomes may be developing an environment that facilitates the growth of *I. edulis* and development of its root nodules through the production of greater amounts of NH_4^+. Thus, the soil microbiomes of these two N-fixing tree species are different and are differentially influenced by land management. Both trees should be planted together during reforestation in this region as they would likely provide a more rapid initial microbiome response, followed by a slower developing microbiome response that allows successional vegetation changes occurring during secondary forest regeneration.

Nucleotide Sequence Accession Numbers

The DNA sequence data were submitted to the NCBI Sequence Read Archive (SRA) SUB6799852, the NCBI BioProject PRJNA599579.

Supplementary Materials: The following are available online at http://www.mdpi.com/2571-8789/4/4/0065/s1, Table S1: Genera identified as potentially within one or more of bacterial functional groups Nitrogen-Fixing or Ammonium Oxidizing, using the scientific articles listed, in addition to the databases mentioned in the text.

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References

1. Leopold, A.C.; Andrus, R.; Finkeldey, A.; Knowles, D. Attempting restoration of wet tropical forests in Costa Rica. *For. Ecol. Manag.* **2001**, *142*, 243–249.
2. Chassot, O.; Monge, G. Connectivity conservation of the Great Green Macaw's landscape in Costa Rica and Nicaragua (1994–2012). *Parks* **2012**, *18*, 61–70.
3. Carpenter, F.L.; Nichols, J.D.; Pratt, R.T.; Young, K.C. Methods of facilitating reforestation of tropical degraded land with the native timber tree, Terminalia amazonia. *For. Ecol. Manag.* **2004**, *202*, 281–291.
4. Gehring, C.; Vlek, P.L.G.; De Souza, L.A.G.; Denich, M. Biological nitrogen fixation in secondary regrowth and mature rainforest of central Amazonia. *Agric. Ecosyst. Environ.* **2005**, *111*, 237–252.
5. Pan, Y.; Birdsey, R.A.; Fang, J.; Houghton, R.; Kauppi, P.E.; Kurz, W.A.; Phillips, O.L.; Shvidenko, A.; Lewis, S.L.; Canadell, J.G.; et al. A Large and Persistent Carbon Sink in the World's Forests. *Science* **2011**, *333*, 988–993.
6. Chazdon, R.L.; Broadbent, E.N.; Rozendaal, D.M.A.; Bongers, F.; Zambrano, A.M.A.; Aide, T.M.; Balvanera, P.; Becknell, J.M.; Boukili, V.; Brancalion, P.H.S.; et al. Carbon sequestration potential of second-growth forest regeneration in the Latin American tropics. *Sci. Adv.* **2016**, *2*, e1501639.
7. Davidson, E.A.; De Carvalho, C.J.R.; Figueira, A.M.; Ishida, F.Y.; Ometto, J.P.H.B.; Nardoto, G.B.; Sabá, R.T.; Hayashi, S.N.; Leal, E.C.; Vieira, I.C.G.; et al. Recuperation of nitrogen cycling in Amazonian forests following agricultural abandonment. *Nature* **2007**, *447*, 995–998.
8. Amazonas, N.T.; Martinelli, L.A.; Piccolo, M.d.C.; Rodrigues, R.R. Nitrogen dynamics during ecosystem development in tropical forest restoration. *For. Ecol. Manag.* **2011**, *262*, 1551–1557.
9. Groppo, J.D.; Lins, S.R.M.; Camargo, P.B.; Assad, E.D.; Pinto, H.S.; Martins, S.C.; Salgado, P.R.; Evangelista, B.; Vasconcellos, E.; Sano, E.E.; et al. Changes in soil carbon, nitrogen, and phosphorus due to land- use changes in Brazil. *Biogeosciences* **2015**, *12*, 4765–4780.
10. Powers, J.S.; Marín-Spiotta, E. Ecosystem Processes and Biogeochemical Cycles in Secondary Tropical Forest Succession. *Annu. Rev. Ecol. Evol. Syst.* **2017**, *48*, 497–519.
11. Batterman, S.A.; Hedin, L.O.; Van Breugel, M.; Ransijn, J.; Craven, D.J.; Hall, J.S. Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature* **2013**, *502*, 224–227.
12. Schelhas, J.; Sánchez-Azofeifa, G.A. Post-Frontier Forest Change Adjacent to Braulio Carrillo National Park, Costa Rica. *Hum. Ecol.* **2006**, *34*, 407–431.
13. Chazdon, R.L. Beyond deforestation: Restoring forests and ecosystem services on degraded lands. *Science* **2008**, *320*, 1458–1460. [PubMed]
14. Aide, T.M.; Clark, M.L.; Ricardo Grau, H.; Lopez-Carr, D.; Levy, M.A.; Redo, D.; Bonilla-Moheno, M.; Riner, G.; Andrade-Nunez, M.J.; Muniz, M. Deforestation and reforestation of Latin America and the Caribbean (2001–2010). *Biotropica* **2013**, *45*, 262–271.
15. Guariguata, M.R.; Ostertag, R. Neotropical secondary forest succession: Changes in structural and functional characteristics. *For. Ecol. Manag.* **2001**, *148*, 185–206.
16. Piotto, D.; Montagnini, F.; Ugalde, L.; Kanninen, M. Performance of forest plantations in small and medium-sized farms in the Atlantic lowlands of Costa Rica. *For. Ecol. Manag.* **2003**, *175*, 195–204.
17. Nichols, J.D.; Carpenter, F.L. Interplanting Inga edulis yields nitrogen benefits to Terminalia amazonia. *For. Ecol. Manag.* **2006**, *233*, 344–351.
18. Macedo, M.O.; Resende, A.S.; Garcia, P.C.; Boddey, R.M.; Jantalia, C.P.; Urquiaga, S.; Campello, E.F.C.; Franco, A.A. Changes in soil C and N stocks and nutrient dynamics 13 years after recovery of degraded land using leguminous nitrogen-fixing trees. *For. Ecol. Manag.* **2008**, *255*, 1516–1524.
19. Lojka, B.; Dumas, L.; Preininger, D.; Polesny, Z.; Banout, J. The use and integration of Inga edulis in agroforestry systems in the amazon—Review article. *Agric. Trop. Subtrop.* **2010**, *43*, 352–359.

20. Poorter, L.; Bongers, F.; Aide, T.M.; Zambrano, A.M.A.; Balvanera, P.; Becknell, J.M.; Boukili, V.; Brancalion, P.H.S.; Broadbent, E.N.; Chazdon, R.L.; et al. Biomass resilience of Neotropical secondary forests. *Nature* **2016**, *530*, 211–214.
21. Orwa, C.; Mutua, A.; Kindt, R.; Jamnadass, R.; Anthony, S. *Agroforestry Tree Database: A Tree Reference and Selection Guide Version 4.0*; World Agroforestry Centre: Nairobi, Kenya, 2009.
22. Berg, G.; Smalla, K. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere: Plant species, soil type and rhizosphere communities. *FEMS Microbiol. Ecol.* **2009**, *68*, 1–13.
23. Kardol, P.; Wardle, D.A. How understanding aboveground-belowground linkages can assist restoration ecology. *Trends Ecol. Evol.* **2010**, *25*, 670–679.
24. Wagg, C.; Jansa, J.; Schmid, B.; Van der Heijden, M.G.A. Belowground biodiversity effects of plant symbionts support aboveground productivity: Biodiversity effects of soil symbionts. *Ecol. Lett.* **2011**, *14*, 1001–1009.
25. Mendes, M.S.; Latawiec, A.E.; Sansevero, J.B.B.; Crouzeilles, R.; Moraes, L.F.D.; Castro, A.; Alves-Pinto, H.N.; Brancalion, P.H.S.; Rodrigues, R.R.; Chazdon, R.L.; et al. Look down-there is a gap-the need to include soil data in Atlantic Forest restoration. *Restor. Ecol.* **2018**, *27*, 361–370.
26. McGee, K.M.; Eaton, W.D.; Porter, T.M.; Shokralla, S.; Hajibabaei, M. 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.* **2019**, *137*, 139–153.
27. Eaton, W.D.; McGee, K.M.; Donnelly, R.; Lemenze, A.; Karas, O.; Hajibabaei, M. Differences in the soil microbial community and carbon-use efficiency following development of *Vochysia guatemalensis* tree plantations in unproductive pastures in Costa Rica. *Restor. Ecol.* **2019**, *27*, 1263–1273.
28. Nasto, M.K.; Alvarez-Clare, S.; Lekberg, Y.; Sullivan, B.W.; Townsend, A.R.; Cleveland, C.C. Interactions among nitrogen fixation and soil phosphorus acquisition strategies in lowland tropical rain forests. *Ecol. Lett.* **2014**, *17*, 1282–1289. [PubMed]
29. Menge, D.N.L.; Chazdon, R.L. Higher survival drives the success of nitrogen-fixing trees through succession in Costa Rican rainforests. *New Phytol.* **2016**, *209*, 965–977.
30. Taylor, B.N.; Chazdon, R.L.; Bachelot, B.; Menge, D.N.L. Nitrogen-fixing trees inhibit growth of regenerating Costa Rican rainforests. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 8817–8822. [PubMed]
31. Bürgi, M.; Östlund, L.; Mladenoff, D.J. Legacy Effects of Human Land Use: Ecosystems as Time-Lagged Systems. *Ecosystems* **2016**, *20*, 94–103.
32. Arroyo-Rodríguez, V.; Melo, F.P.L.; Martínez-Ramos, M.; Bongers, F.; Chazdon, R.L.; Meave, J.A.; Norden, N.; Santos, B.A.; Leal, I.R.; Tabarelli, M. Multiple successional pathways in human-modified tropical landscapes: New insights from forest succession, forest fragmentation and landscape ecology research: Multiple successional pathways. *Biol. Rev.* **2017**, *92*, 326–340.
33. Chaparro, J.M.; Badri, D.V.; Vivanco, J.M. Rhizosphere microbiome assemblage is affected by plant development. *ISME J.* **2014**, *8*, 790–803.
34. Norden, N.; Angarita, H.A.; Bongers, F.; Martínez-Ramos, M.; Granzow-de la Cerda, I.; van Breugel, M.; Lebrija-Trejos, E.; Meave, J.A.; Vandermeer, J.; Williamson, G.B.; et al. Successional dynamics in Neotropical forests are as uncertain as they are predictable. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 8013–8018.
35. Hogan, J.A.; Zimmerman, J.K.; Thompson, J.; Nytch, C.J.; Uriarte, M. The interaction of land-use legacies and hurricane disturbance in subtropical wet forest: Twenty-one years of change. *Ecosphere* **2016**, *7*, e01405. [CrossRef]
36. Ziter, C.; Graves, R.A.; Turner, M.G. How do land-use legacies affect ecosystem services in United States cultural landscapes? *Landsc. Ecol.* **2017**, *32*, 1–14.
37. Potthast, K.; Hamer, U.; Makeschin, F. Land-use change in a tropical mountain rainforest region of southern Ecuador affects soil microorganisms and nutrient cycling. *Biogeochemistry* **2012**, *111*, 151–167.
38. Tischer, A.; Potthast, K.; Hamer, U. Land-use and soil depth affect resource and microbial stoichiometry in a tropical mountain rainforest region of southern Ecuador. *Oecologia* **2014**, *175*, 375–393.
39. Barron, A.R.; Purves, D.W.; Hedin, L.O. Facultative nitrogen fixation by canopy legumes in a lowland tropical forest. *Oecologia* **2011**, *165*, 511–520.
40. Gei, M.; Rozendaal, D.M.A.; Poorter, L.; Bongers, F.; Sprent, J.I.; Garner, M.D.; Aide, T.M.; Andrade, J.L.; Balvanera, P.; Becknell, J.M.; et al. Legume abundance along successional and rainfall gradients in Neotropical forests. *Nat. Ecol. Evol.* **2018**, *2*, 1104–1111.

41. Hartshorn, G.S.; Hammel, B.; McDade, L.A.; Bawa, K.S.; Hespenheide, H.A.; Hartshorn, G.S. *Vegetation Types and Floristic Patterns. La Selva: Ecology and Natural History of a Neotropical Rain Forest*; The University of Chicago Press: Chicago, IL, USA, 1994.
42. Lojka, B.; Preininger, D.; Lojkova, J.; Banout, J.; Polesny, Z. Biomass growth and farmer knowledge of Inga edulis in Peruvian Amazon. *Agric. Trop. Subtrop.* **2005**, *38*, 44–51.
43. Ghazoul, J.; Sheil, D. *Tropical Rain Forest Ecology, Diversity, and Conservation*; Oxford University Press: New York, NY, USA, 2010; ISBN 0-19-928588-8.
44. Allen, O.; Allen, E. *The Leguminosae*; The University of Wisconsin Press: Madison, WI, USA, 1981.
45. Flores, E.; Vozzo, J. Pentaclethra macroloba. In *Tropical Tree Seed Manual*; Agricultural Handbook 721; USDA Forest Service: Washington, DC, USA, 2002; pp. 601–604.
46. Lorenzi, H. A Guide to the Identification and Cultivation of Brazilian Native Trees. In *Brazilian Trees*, 4th ed.; Instituto Plantarum de Estudos da Flora LTDA: Sao Paulo, Brazil, 2002; Volume I.
47. Baarsma, A. An assessment of afforestation success at REGUA Brazil. *Report: REGUA Reserva Ecológica Guapiaçu Atl. For. Mata Atlântica*, January 2016.
48. Fichtler, E.; Clark, D.A.; Worbes, M. Age and Long-term Growth of Trees in an Old-growth Tropical Rain Forest, Based on Analyses of Tree Rings and 14C1. *Biotropica* **2003**, *35*, 306–313.
49. Kurokawa, H.; Yoshida, T.; Nakamura, T.; Lai, J.; Nakashizuka, T. The age of tropical rain-forest canopy species, Borneo ironwood (*Eusideroxylon zwageri*), determined by ^{14}C dating. *J. Trop. Ecol.* **2003**, *19*, 1–7.
50. Buchmann, N. Biotic and abiotic factors controlling soil respiration rates in Picea abies stands. *Soil Biol. Biochem.* **2000**, *32*, 1625–1635.
51. Whiting, D. *Colorado Master Gardener Garden Notes*; Colorado State University Extension: Fort Collins, CO, USA, 2013.
52. Kuzyakov, Y. Priming effects: Interactions between living and dead organic matter. *Soil Biol. Biochem.* **2010**, *42*, 1363–1371.
53. Plante, A.F.; Conant, R.T.; Carlson, J.; Greenwood, R.; Shulman, J.M.; Haddix, M.L.; Paul, E.A. Decomposition temperature sensitivity of isolated soil organic matter fractions. *Soil Biol. Biochem.* **2010**, *42*, 1991–1996.
54. Alef, K.; Nannipieri, P. *Methods in Applied Soil Microbiology and Biochemistry*; Elsevier, Academic Press: London, UK, 1995.
55. McGee, K.M.; Eaton, W.D.; Shokralla, S.; Hajibabaei, M. Determinants of Soil Bacterial and Fungal Community Composition Toward Carbon-Use Efficiency Across Primary and Secondary Forests in a Costa Rican Conservation Area. *Microb. Ecol.* **2018**, *77*, 148–167.
56. Caporaso, J.G.; Lauber, C.L.; Walters, W.A.; Berg-Lyons, D.; Lozupone, C.A.; Turnbaugh, P.J.; Fierer, N.; Knight, R. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 4516–4522.
57. Wang, Q.; Garrity, G.M.; Tiedje, J.M.; Cole, J.R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **2007**, *73*, 5261–5267.
58. Weiss, S.; Xu, Z.Z.; Peddada, S.; Amir, A.; Bittinger, K.; Gonzalez, A.; Lozupone, C.; Zaneveld, J.R.; Vázquez-Baeza, Y.; Birmingham, A.; et al. Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome* **2017**, *5*, 1–18.
59. Shin, J.; Lee, I. Co-Inheritance Analysis within the Domains of Life Substantially Improves Network Inference by Phylogenetic Profiling. *PLoS ONE* **2015**, *10*, e0139006. [CrossRef]
60. Shim, J.E.; Lee, T.; Lee, I. From sequencing data to gene functions: Co-functional network approaches. *Anim. Cells Syst.* **2017**, *21*, 77–83.
61. Eaton, W.D.; McGee, K.M.; Alderfer, K.; Jimenez, A.R.; Hajibabaei, M. Increase in abundance and decrease in richness of soil microbes following Hurricane Otto in three primary forest types in the Northern Zone of Costa Rica. *PLoS ONE* **2020**, *15*, e0231187. [CrossRef]
62. Anderson, M.J.; Gorley, R.N.; Clarke, R.K. *PERMANOVA+ for Primer: Guide to Software and Statistical Methods*; PRIMER-E Ltd.: Devon, UK, 2008; ISBN 841889821.
63. Clarke, K.R. Non-parametric multivariate analyses of changes in community structure. *Aust. J. Ecol.* **1993**, *18*, 117–143.
64. Anderson, M.J.; Willis, T.J. Canonical analysis of principal coordinates: A useful method of constrained ordination for ecology. *Ecology* **2003**, *84*, 511–525.

65. Tilki, F.; Fisher, R.F. Tropical leguminous species for acid soils: Studies on plant form and growth in Costa Rica. *For. Ecol. Manag.* **1998**, *108*, 175–192.
66. Leblanc, H.A.; McGraw, R.L.; Nygren, P.; Roux, C.L. Neotropical Legume Tree Inga edulis Forms N2-fixing Symbiosis with Fast-growing Bradyrhizobium Strains. *Plant Soil* **2005**, *275*, 123–133.
67. Fisher, R.F. Amelioration of Degraded Rain Forest Soils by Plantations of Native Trees. *Soil Sci. Soc. Am. J.* **1995**, *59*, 544. [CrossRef]
68. Omena-Garcia, R.; Justino, G.; Araujo, V.; Souza, L.; De Camargos, L.; Goncalves, J. Mineral nitrogen associated changes in growth and xylem-N compounds in Amazonian legume tree. *J. Plant Nutr.* **2015**, *4*, 584–595.
69. Justino, G.; Omena-Garcia, R.; dos Santos, A.; de Camargos, L.; Sodek, L.; Gonçalves, J. Nitrogen used strategies of nodulated amazonian legume: Inga edulis. *J. Trop. For. Sci.* **2017**, *29*, 1–9.
70. Eaton, W.D.; Anderson, C.; Saunders, E.F.; Hauge, J.B.; Barry, D. The impact of Pentaclethra macroloba on soil microbial nitrogen fixing communities and nutrients within developing secondary forests in the Northern Zone of Costa Rica. *Trop. Ecol.* **2012**, *53*, 207–214.
71. Louca, S.; Doebeli, M. Transient dynamics of competitive exclusion in microbial communities: Transient dynamics in microbial communities. *Environ. Microbiol.* **2016**, *18*, 1863–1874. [PubMed]
72. Eldridge, D.J.; Delgado-Baquerizo, M.; Travers, S.K.; Val, J.; Oliver, I.; Hamonts, K.; Singh, B.K. Competition drives the response of soil microbial diversity to increased grazing by vertebrate herbivores. *Ecology* **2017**, *98*, 1922–1931. [PubMed]
73. Hardin, G. The Competitive Exclusion Principle. *Science* **1960**, *131*, 1292–1297.
74. Derhé, M.A.; Murphy, H.; Monteith, G.; Menéndez, R. Measuring the success of reforestation for restoring biodiversity and ecosystem functioning. *J. Appl. Ecol.* **2016**, *53*, 1714–1724.
75. Louca, S.; Polz, M.F.; Mazel, F.; Albright, M.B.N.; Huber, J.A.; O'Connor, M.I.; Ackermann, M.; Hahn, A.S.; Srivastava, D.S.; Crowe, S.A.; et al. Function and functional redundancy in microbial systems. *Nat. Ecol. Evol.* **2018**, *2*, 936–943. [PubMed]
76. Wohl, D.; Arora, S.; Gladstone, J. Functional redundancy supports biodiversity and ecosystem function in a closed and constant environment. *Ecology* **2004**, *85*, 1534–1540.
77. Allison, S.D.; Martiny, J.B.H. Colloquium paper: Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11512–11519.
78. Nemergut, D.R.; Schmidt, S.K.; Fukami, T.; O'Neill, S.P.; Bilinski, T.M.; Stanish, L.F.; Knelman, J.E.; Darcy, J.L.; Lynch, R.C.; Wickey, P.; et al. Patterns and Processes of Microbial Community Assembly. *Microbiol. Mol. Biol. Rev.* **2013**, *77*, 342–356.
79. Evans, S.E.; Wallenstein, M.D. Climate change alters ecological strategies of soil bacteria. *Ecol. Lett.* **2014**, *17*, 155–164.
80. Morrissey, E.M.; Mau, R.L.; Schwartz, E.; Caporaso, J.G.; Dijkstra, P.; van Gestel, N.; Koch, B.J.; Liu, C.M.; Hayer, M.; McHugh, T.A.; et al. Phylogenetic organization of bacterial activity. *ISME J.* **2016**, *10*, 2336–2340.
81. Placella, S.A.; Brodie, E.L.; Firestone, M.K. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 10931–10936.