Symbiosis
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RESEARCH

Changes in microbiome assembly of the pioneer Andean tree *Alnus acuminata* under land degradation

Nubia Guzmán[1,2] · Lara A. Quaglini[2,3] · Raffaella Ansaloni[1] · Mayra Jiménez[1] · Rodolfo Gentili[2] · Sandra Citterio[2]

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Abstract

Land degradation in the Andes threatens ecosystems and biodiversity. The recovery of these areas often depends on pioneer species such as *Alnus acuminata*, which relies on symbiotic microorganisms for nutrient acquisition and stress tolerance. Understanding how degradation affects its microbiome is crucial for effective restoration. This study investigated how a land-use trajectory involving deforestation and abandonment impacts the diversity and structure of bacterial communities associated with *Alnus acuminata*. Next-generation sequencing of 16 S rRNA gene amplicons was used to compare bacterial communities in bulk soil, roots, and root nodules between a native forest and degraded forest in the Ecuadorian Andes. Land degradation significantly altered bulk soil bacterial diversity and community structure, with pH and carbon content identified as key environmental drivers. Degraded soils were dominated by Actinomycetota, whereas native forest soils harbored more diverse communities, including Acidobacteriota and Pseudomonadota. While bacterial diversity in root and nodule endophytes remained stable across both sites, their community structures differed significantly between native and degraded forests. This shift reveals that the endophytic compartments harbour distinct bacterial consortia in the degraded environment, suggesting a potential role for host-related factors in shaping the microbiome under environmental stress. In the degraded forest, the endophytic microbiome was characterized by an enrichment of stress-resilient, pathogen-defending, and plant growth-promoting genera such as *Micromonospora*, *Rahnella*, *Rhodanobacter*, *Mycobacterium*, and *Deinococcus*. These findings suggest that the stability and composition of the *Alnus acuminata* microbiome may be linked to its ability to establish in nutrient-poor, degraded environments, highlighting the importance of plant-microbe associations in ecosystem recovery and providing a basis for further mechanistic and ecological studies on restoration outcomes.

Keywords Land degradation · Soil restoration · Andean alder · Bacterial communities · Endophytes · NGS of 16S rRNA

1 Introduction

Land degradation, generally defined as the long-term reduction or loss of biological productivity, ecological integrity, or human value (Olsson et al. 2022), is a pressing global challenge. In Ecuador, especially in the Andean mountains, forest ecosystems are under constant threat from human activities, primarily due to extensive livestock grazing, agriculture, mining, and logging. Additionally, large areas have been converted into plantations of exotic tree species such as *Eucalyptus* spp. and *Pinus* spp. (Hofstede et al. 2002; Günter et al. 2009; Tapia-Armijos et al. 2015). This land-use change has resulted in irreversible biodiversity loss and severely disrupted ecosystem functioning, affecting a significant proportion of native vegetation in an area of

✉ Sandra Citterio
sandra.citterio@unimib.it

[1] Herbario Azuay, University of Azuay, Av. 24 de Mayo 7-77 y Hernán Malo, Cuenca, Ecuador

[2] Department of Earth and Environmental Sciences, University of Milano-Bicocca, Piazza della Scienza 1, Milan 20126, Italy

[3] Sezione di Botanica, Museo di Storia Naturale di Milano, Corso Venezia 55, Milan 20121, Italy

exceptionally high biological diversity. Indeed, nearly half (46%) of the original forest cover in southern Ecuador has been replaced by anthropogenic land cover types (Tapia-Armijos et al. 2015).

These large-scale transformations not only threaten plant and animal biodiversity but can also have profound implications for the microbial communities that drive essential ecosystem functions. Recent research has highlighted the fundamental role of microorganisms in supporting almost all ecosystem processes, from regulating methane emissions in tree bark (Jeffrey et al. 2021) to serving as key indicators of ecosystem multifunctionality (Romero et al. 2025). These communities, primarily bacteria and fungi, regulate the transformation of organic matter, carbon storage, and nutrient cycling (Rawat et al. 2023). Furthermore, plant-associated microorganisms are critical for host health, productivity, and establishment, providing resilience to environmental stress and aiding adaptation to degraded conditions (Fitzpatrick et al. 2018; Muhammad et al. 2024).

The assembly of these microbial communities is primarily driven by soil properties and plant community composition. Land degradation alters key factors such as soil pH and organic carbon, which exert strong selective pressures on specific bacterial taxa and modulate microbial metabolic activity (Zhou et al. 2018, 2024; Li et al. 2024). Simultaneously, shifts in plant communities following land-use change modify the soil microbiome mainly through varied root architectures and rhizodeposition patterns (Oppenheimer-Shaanan et al. 2022; Domeignoz-Horta et al. 2024). These changes in bulk soil and plant-associated niches represent a fundamental challenge for understanding ecosystem responses to degradation (Zhou et al. 2018; Rawat et al. 2023).

Given these consequences, ecological restoration through the re-establishment of pioneer vegetation is a priority. Successful restoration often relies on species capable of colonizing nutrient-poor or compacted soils and initiating positive feedback loops by improving soil structure and fostering beneficial microbial networks (Wang et al. 2024; Xiuyu et al. 2024). Among these, *Alnus* (alder) species are exceptional pioneers due to their tripartite symbiosis with mycorrhizal fungi and nitrogen-fixing bacteria of the genus *Frankia* (Becerra et al. 2005; Chen et al. 2020). Moreover, emerging researchhas identified diverse bacterial taxa beyond *Frankia* associated with alder root nodules, revealing unexpected complexity in these symbiotic relationships (Carro et al. 2013; Aslani et al. 2020; Garneau et al. 2023a). Root nodules represent a highly specialized and biologically filtered niche. While the bulk soil and rhizosphere are directly exposed to environmental fluctuations, the root endosphere and nodules are considered more environmentally buffered. Nevertheless, the assembly of their microbial communities remains mainly dependent on the microbial pool available in the surrounding soil (Carro et al. 2013; Garneau et al. 2023a). Understanding the extent to which land degradation modulates these protected symbiotic compartments is essential for assessing the role of *Alnus*-microbe interactions in plant resilience. Beyond plant–microbe interactions, microbe–microbe dynamics are also critical. For example, *Pseudomonas* has been shown to promote nodulation in *Alnus rubra* Bong. (Knowlton and Dawson 1982), while other bacteria genera such as *Microvirga* and *Streptomyces* can stimulate *Frankia* growth (Garneau et al. 2023a).

While microbial communities of various *Alnus* species have been explored globally (e.g. Aslani et al. 2020; Garneau et al. 2023a; Thiem et al. 2023; Dove et al. 2024), little information is available on *Alnus acuminata* Kunth, the only alder species native to the mountains of South America (Lægaard and Balslev 2014). In Ecuador, where extensive deforestation and land-use conversion threaten the integrity of Andean forest ecosystems, *A. acuminata* has been used for reforestation, yet its belowground microbial associations remain largely unexplored, especially concerning non-*Frankia* bacteria and the broader structure of its root nodules and root associated bacterial communities.

To address this gap, this study investigated the diversity and structure of bacterial communities associated with the bulk soil, roots, and nodules of *A. acuminata* using 16 S rRNA gene amplicon sequencing, comparing individuals and soils from a native and a degraded forest in the southern Ecuadorian Andes. The ultimate goal is to contribute to providing the fundamental knowledge required to exploit pioneer plant-microbe interactions to improve ecosystem recovery in degraded Andean landscapes.

2 Materials and methods

2.1 Study area and sample collection

The study was conducted in the Andean mountains of southern Ecuador in the province of Azuay, across two distinct land-use types: (1) native intact forest (NF; 2°55’ S, 78°50’ W), a patch of high montane evergreen forest located within the Aguarongo Protected Vegetation Area; and (2) degraded forest (DF; 2°57’ S, 78°53’ W), corresponding to a high montane evergreen forest that has been completely deforested, which is located in close proximity to the Aguarongo Reserve (~6 km away) (Fig. 1).

The native forest represents a well-preserved remnant of natural vegetation, showing no evidence of fire, logging, or other anthropogenic disturbances. The list of dominant species characterizing the NF is shown in Table S1 of the

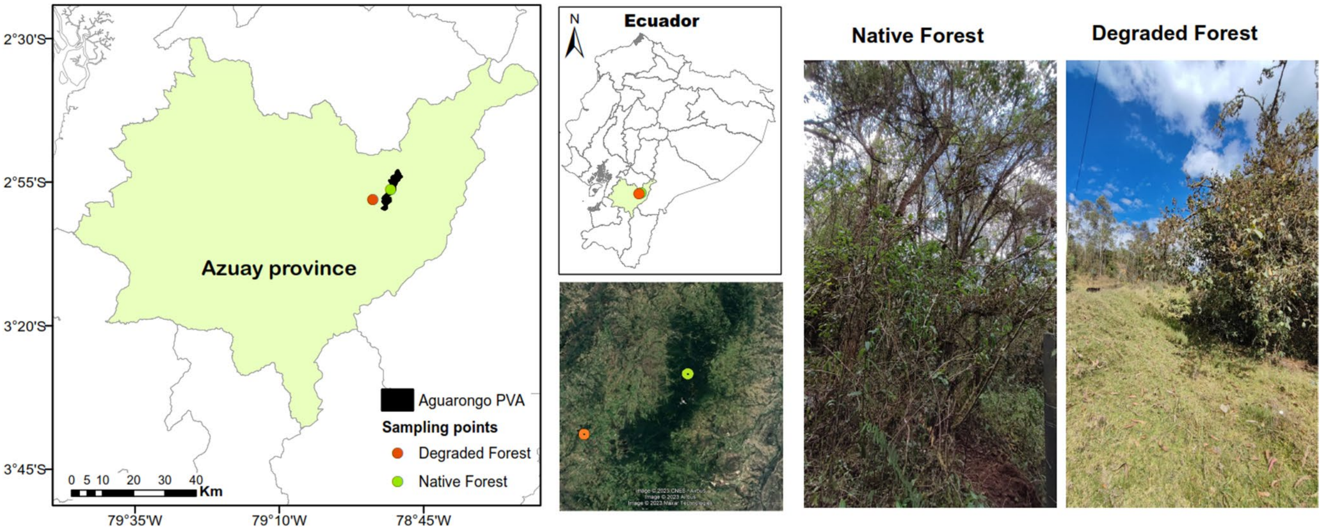


Fig. 1 Map showing the sampling locations in the province of Azuay across two distinct land-use types: native forest and degraded forest

supplementary information. The degraded site, on the other hand, has undergone a complete land-use change. It was deforested over 50 years ago, mainly for livestock grazing and road construction, and has since been abandoned for about 10 years. It is now dominated by the grass *Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone, commonly used as a forage crop, and by other non-native species such as the tree *Eucalyptus globulus* Labill., along with a few native pioneer species, including *Alnus acuminata*, the focal species of this study. Both sites are located at elevations ranging from 2,800 to 3,140 m a.s.l. and share similar climatic conditions. The climate is cold temperate, with annual temperatures ranging from 9 to 12 °C and an annual precipitation of 820 mm. Rainfall varies throughout the year, with a dry season from May to September, during which monthly precipitation ranges from 25 to 60 mm. From October to April, rainfall is more intense, reaching 75 to 110 mm per month (Minga 2002).

Bulk soil, root, and nodule samples were collected from different individuals of *A. acuminata* growing in both the native and degraded forest sites.

At each site, four trees were selected, with an inter-tree distance of approximately 150 m. Selected trees were 4.0–7.5 m tall, with diameters at breast height (DBH) of 6–15 cm. Around each focal tree, a 5 × 5 m plot was established centered on the trunk. Within each plot, all vascular plant species were identified according to the Angiosperm Phylogeny Group classification system (APG IV 2016). From three of the four selected trees, soil was sampled at a minimum of three locations around the trunk, 10–20 cm from the trunk and 20–40 cm deep; subsamples were pooled to form a composite, sieved to 2 mm, homogenized, and a 100 g aliquot was retained for analysis, kept cold in the field, transported to the laboratory the same day. Approximately 50 g of each soil sample was used for the analysis of soil chemical properties, while the remaining portion was retained for DNA extraction of soil bacterial communities.

Composite samples of several pooled roots or pooled nodules were collected from all four trees, including the three used for soil sampling and one additional tree.

In total, six bulk soil samples (three per land-use type) and eight root–nodule samples (four per land-use type) were obtained. All samples were stored at −20 °C until analysis.

To compare root nodulation among *A. acuminata* individuals growing in native and degraded forests, the morphological characteristics of *Frankia* nodules were assessed (see Supplementary Materials for further details).

2.2 Soil analysis

Prior to analysis, soil samples were sieved through a 2 mm mesh and stored at 4 °C. Soil pH was measured in water, while total nitrogen (%) and total carbon (%) contents were determined using a CN elemental analyzer (Flash EA 1112 NC Soil, Thermo Fisher Scientific, Pittsburgh, USA).

2.3 Sample processing

Bacterial community diversity associated with bulk soil, *A. acuminata* roots and nodules (endophytes) in native and degraded forests was defined using 16 S rRNA gene amplicons. DNA from all samples were extracted using the FastDNA® Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) following the manufacturer's protocol. Prior to DNA extraction, roots and nodules of *A. acuminata* were first washed with tap water and then surface-sterilized by immersion in 70% ethanol for 1 min, followed by 3% sodium hypochlorite for 3 min, a second immersion in 70% ethanol for 30 s, and four rinses with sterile distilled water. To verify the effectiveness of sterilization, 100 µL from the final rinse was plated on trypticase soy agar and incubated to check for microbial contamination (Cheng et al. 2019). Sterilized roots and nodules were then ground in liquid nitrogen.

Because root and nodule samples also contain plant plastid-derived 16 S rRNA sequences, they required a different processing workflow compared to bulk soil samples. Specifically, an initial PCR amplification targeting the full-length 16 S rRNA gene was performed only for root and nodule DNA using primers 27 F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). PCR conditions were as follows: initial denaturation at 95 °C for 4 min; 29 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 2 min; followed by a final extension at 72 °C for 5 min. PCR products were then purified using the Wizard® SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI, USA), according to the manufacturer's instructions.

A second PCR amplification targeting the V5–V6 hypervariable regions of the bacterial 16 S rRNA gene was subsequently performed on all samples (bulk soil, roots, and nodules). For root and nodule samples, the purified products obtained from the first PCR were used as template DNA, whereas for bulk soil samples the extracted DNA was used directly. Two different primer sets were used depending on the sample type: primers 783 F and 1027R were used for bulk soil samples (Gandolfi et al. 2024), while primers 799 F and 1107R, specifically designed for endophytic communities, were used for root and nodule samples (Chen et al. 2022). All primers were tagged with custom 6 bp oligonucleotide barcodes (sequences listed in Table S3). PCR conditions were identical for both primer sets and included an initial denaturation at 94 °C for 4 min, followed by 28 cycles of denaturation at 94 °C for 50 s, annealing at 47 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. PCR products were purified, and DNA concentrations were quantified using a Qubit® 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA).

Amplicon libraries were prepared in batches of nine samples, each distinguished by the unique barcode pair (Table S2). Library preparation included the addition of standard Nextera indexes (Illumina, Inc., San Diego, CA, USA), and sequencing was carried out on an Illumina MiSeq platform using a 2 × 300 bp paired-end protocol.

2.4 Bioinformatics

After sequencing, reads were demultiplexed according to their barcodes (Table S2). This operation and the subsequent bioinformatic analyses were performed separately for bulk soil and endophyte (roots and nodules) samples. Amplicon Sequence Variants (ASVs) were separately inferred using the DADA2 pipeline (Callahan et al. 2016). Forward and reverse reads were trimmed after 180 and 150 bases, respectively, for the former, and after 220 and 200 bases, respectively, for the latter. In both cases, quality filtering was set to allow no ambiguous bases and a maximum number of expected errors equal to 0.5 per read. The two reads were then merged and chimeras were removed. Reads were taxonomically assigned with SILVA v132 databases, as previously described (Gandolfi et al. 2024). All non-bacterial ASVs, i.e., those belonging to Archaea or Eukarya domains, those that were not classified at domain level, and those classified as chloroplasts or mitochondria, were discarded. The remaining ASVs were classified again with the latest version of the RDP classifier (ver. 2.14, August 2023).

2.5 Data analysis

Due to the use of different primer sets for bulk soil and endophytes (roots and nodules) samples, these compartments were analyzed separately. All statistical analyses were performed within the R environment v. 4.3.2 (R Core Team 2023).

First, different α-diversity metrics on the rarified bacterial data were calculated using the *vegan* package. Specifically, bacterial richness was estimated as the number of ASV using the specnumber() function. Bacterial evenness was assessed using the Berger-Parker index, which measures the dominance of the most abundant taxa in a community (Berger and Parker 1970). Additionally, the Shannon diversity index was calculated using the diversity() function. Next, differences were analyzed for bacterial α-diversity between land-use types (native and degraded forests), compartments (bulk soil, roots, and nodules), and their interaction. ANOVA was used for bacterial evenness and Shannon diversity index, while generalized linear models (GLMs) with a quasi-Poisson distribution were applied to ASV richness.

To visualize differences in bacterial community structure between land-use types, Non-metric Multidimensional Scaling (NMDS) was conducted based on Bray-Curtis dissimilarities using the metaMDS() function from the *vegan* package. Additionally, significant differences were tested with PERMANOVA using the adonis2() function. To evaluate the influence of soil variables and plant richness on bacterial community composition, a Redundancy Analysis (RDA) was conducted on bulk soil and endophytes (roots and nodules) ASVs, constrained by environmental variables including soil pH, total nitrogen, total carbon, and plant richness. However, because total carbon and soil pH were highly correlated, total carbon was excluded from the analysis, and soil pH was retained. Before these multivariate analyses, the bacterial ASV abundance matrix was transformed using the Hellinger method to downweigh the influence of highly abundant ASVs, emphasize their presence or absence, and address the double-zero problem commonly encountered when comparing community compositions across samples (Borcard et al. 2018).

The 10 most abundant phyla were identified across all land-use types and compartments and compared to their relative abundances between native and degraded forests with one-way ANOVA.

Next, the core endophytic bacterial communities present in the roots and nodules of *A. acuminata* were identified by selecting ASVs detected in at least 80% of samples (i.e., frequency refers to the proportion of samples in which a given ASV was detected), regardless of land-use type (Cheng et al. 2019; Neu et al. 2021). The core microbiome refers to microbial taxa that are consistently associated with a specific host or environment, typically identified by their presence across multiple microbial communities (Neu et al. 2021). These taxa are thought to play key ecological and functional roles within their host or environment under prevailing conditions (Risely 2020).

3 Results

3.1 Differences in soil properties and plant composition between native and degraded forests

Soil properties were altered in the degraded forest (DF) compared to the native forest (NF). The DF exhibited a markedly higher soil pH (5.4 ± 0.04 vs. 4.3 ± 0.05) and a lower total carbon content (4.22 ± 0.06% vs. 5.20 ± 0.02%). However, total nitrogen content did not substantially differ between the two sites (0.35 ± 0.20% in the DF and 0.34 ± 0.06% in the NF).

A total of 19 vascular plant species, belonging to 14 families were recorded in the plots surrounding *A. acuminata* individuals. In the degraded site, the vegetation was almost completely dominated by two introduced herbaceous species: *Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone and *Trifolium repens* L, along with a few native shrubs such as *Baccharis latifolia* (Ruiz & Pav.) Pers., *Cestrum tomentosum* L.f., and *Rubus floribundus* Kunth. In contrast, the native forest was characterized by a dominance of native tree species typical of high mountain forest ecosystems, including *Axinaea macrophylla* Triana, *Lomatia hirsuta* (Lam.) Diels, *Myrica parvifolia* Benth., *Polylepis lanuginosa* Kunth, and *Vallea stipularis* L.f. The understory featured species such as *Valeriana hirtella* Kunth, *Verbesina latisquama* S.F. Blake, and the fern *Lophosoria quadripinnata* (J.F. Gmel.) C. Chr.

3.2 Effect of land degradation on bacterial alpha-diversity

When analysing the differences of bacterial α-diversity between DF and NF, no effect of degradation was found on ASV richness of either bulk soil (GLMs: $p = 0.519$; Table S3), or endophytes (root and nodule samples) of *A. acuminata* (GLMs: $p = 0.918$; Fig. 2a, Table S4). Conversely, bacterial evenness of bulk soil samples was significantly lower in the degraded forest (ANOVA: $F = 32.93$, $p = 0.00457$; Fig. 2b); a similar trend was observed for Shannon index, although the difference was marginally significant (ANOVA: $F = 7.164$, $p = 0.0554$; Fig. 2c, Table S3). Bacterial diversity in endophytic samples did not differ significantly between root and nodule compartments, between land-use types, or due to their interaction (Table S4). Root samples showed a pattern similar to that of bulk soil, with lower evenness and Shannon indices in DF compared to NF; however, these differences were not statistically significant, likely due to high variability in index values. Degradation also appeared not to affect the bacterial diversity of *A. acuminata* nodules (Fig. 2a, b, c).

3.3 Effect of land degradation on the structure and composition of bacterial communities

The results indicate that bacterial community structure across different compartments (bulk soil, roots, and nodules of *A. acuminata*) was influenced by land-use type, as revealed by NMDS analysis (Fig. 3). In bulk soil, PERMANOVA showed that land use explained a substantial portion (86.4%) of the variation in community composition, although the effect was not statistically significant, likely due to the small sample size ($F = 25.38$, $R^2 = 0.864$, $p = 0.1$; Table S5). In contrast, for *A. acuminata* endophytes (roots and nodules), the effect of land use was statistically significant, accounting for 10.6% of the variation ($F = 1.761$, $R^2 = 0.106$, $p = 0.037$; Table S6). These findings suggest that land degradation can alter bacterial community structure, with a marked effect in the bulk soil compared to the endosphere. Additionally, differences between root and nodule compartments explained 8.9% of the variation ($F = 1.472$, $R^2 = 0.089$, $p = 0.088$; Table S6), indicating that compartmentalization within the plant also contributes to shaping the endophytic bacterial community, albeit to a lesser extent than land use.

Among the environmental variables examined, soil pH and plant richness were the main drivers of bacterial community composition, as revealed by the RDA analysis (Fig. 4). Together, these factors explained 91.3% of the variation in bulk soil bacterial communities (RDA: $p < 0.05$), but only 19.5% of the variation in endophytic communities,

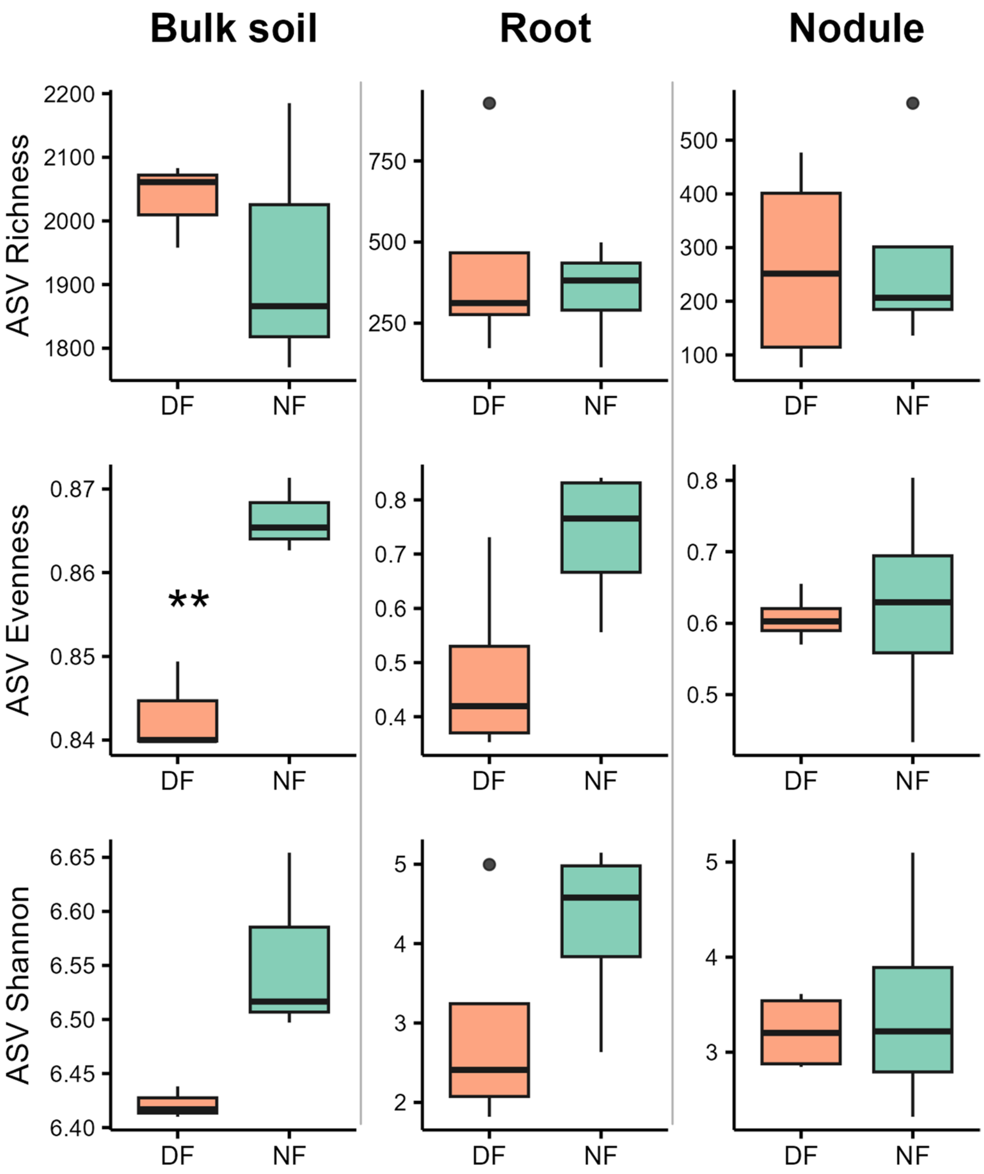


Fig. 2 Comparison of ASV richness, evenness, and Shannon diversity in bulk soil, root, and nodule samples of *Alnus acuminata* from degraded (DF, orange boxes) and native (NF, light green boxes) forest sites

which was not statistically significant (RDA: $p > 0.05$). Soil pH emerged as a key determinant of bacterial community structure in bulk soil, especially under land degradation. In contrast, total carbon, excluded from the plot due to multicollinearity with pH, and plant richness were more strongly associated with bacterial communities in the native forest (Fig. 4).

The relative abundance of the dominant bacterial phyla shifted in response to land degradation, with more pronounced effects in bulk soil bacterial communities compared to the endophytic communities of *A. acuminata* (Fig. 5, Table S7). Within the bulk soil compartment, degradation significantly decreased the abundance of Pseudomonadota, Acidobacteriota, Bacteroidota, Chloroflexota, and Verrucomicrobiota, while increasing the abundance of Actinomycetota compared to native forest (ANOVA: $p < 0.05$; Fig. 5, Table S8).

In the endosphere, bacterial communities associated with the roots and nodules of *A. acuminata* were largely dominated by Pseudomonadota in both DF and NF. Root endophytes in DF showed a reduced relative abundance of the phyla Bacillota, Bacteroidota, and Acidobacteriota, while Bacteroidota, Chloroflexota, and Cyanobacteriota were less abundant in nodules. Conversely, Planctomycetota, Actinomycetota, and Deinococcota showed higher relative abundances in nodule endophytes from DF compared with NF; however, these differences were not statistically significant (Fig. 5, Tables S9, S10).

At lower taxonomic ranks, the dominance of specific bacterial taxa became more apparent within each land-use (Fig. 6). For example, in bulk soil from degraded forest, the

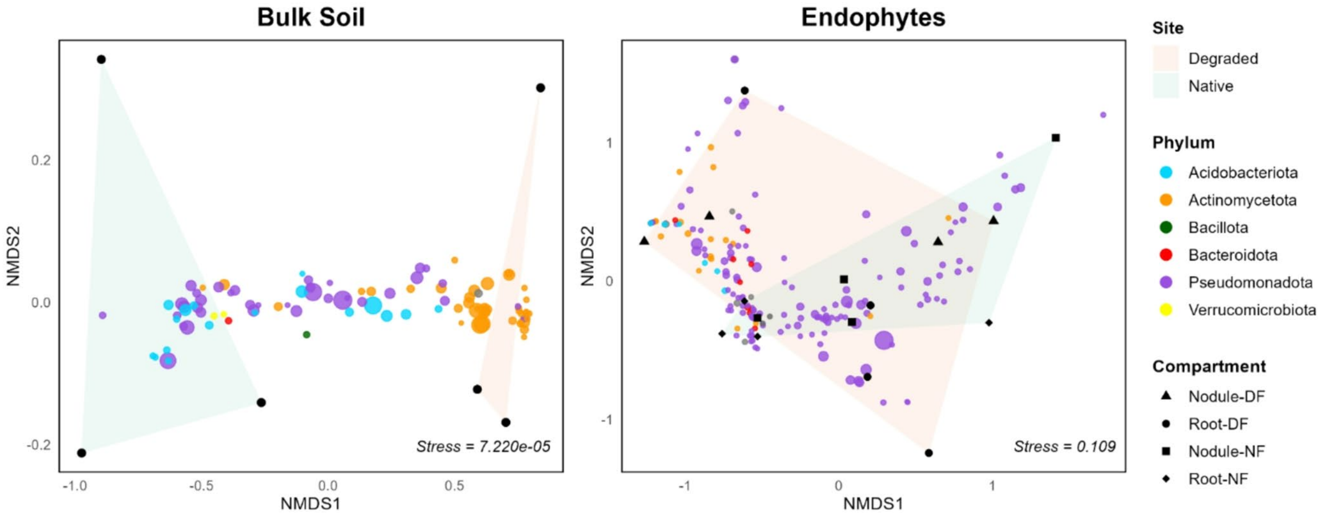
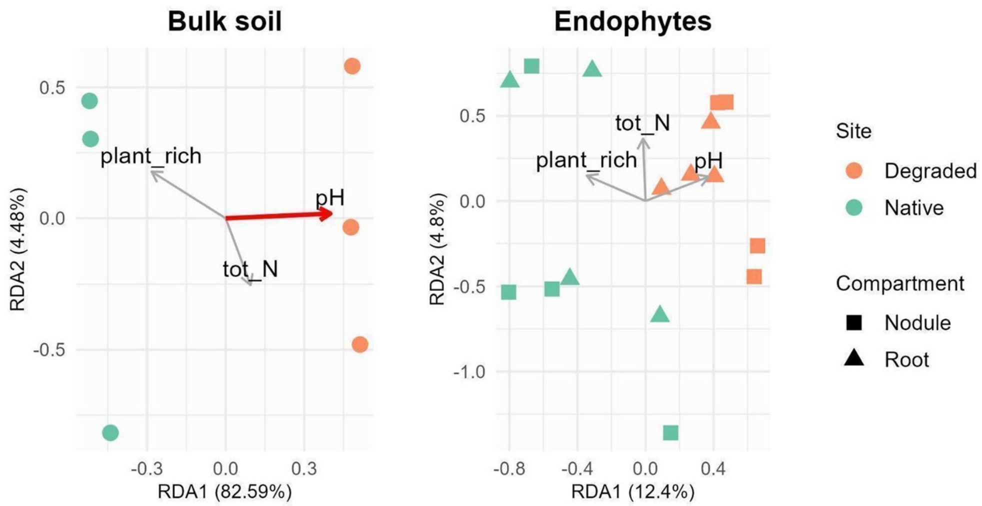


Fig. 3 Non-metric multidimensional scaling (NMDS) of bacterial community associated with *Alnus acuminata* in two land-use types: degraded forest (orange polygons) and native forest (light green polygons). The left plot shows bulk soil; the right plot shows endophytic samples (roots and nodules). The diagram also displays the main bacterial phyla associated with each land-use type and compartment

Fig. 4 Redundancy analysis (RDA) showing the influence of soil pH, total nitrogen, and plant richness on bacterial communities in bulk soil (left) and endophytes (right) of *Alnus acuminata* from degraded forest (orange) and native forest (light green). Significant variables are indicated with red arrows



most abundant genera were *Streptomyces* (relative abundance of 4.7%), *Reyranella* (2.6%), and *Mycobacterium* (2.2%). In contrast, in the native forest, *Paraburkholderia* (5.2%), *GP2* (5%), *Subdivision3_genera_incertae_sedis* (2%), and *Caballeronia* (1.8%) were the most abundant (Fig. 6).

Regarding the endophytic samples, *Buttiauxella*, *Rahnella*, *Variovorax*, and *Rhizobium* dominated the root endosphere of *A. acuminata* in the degraded site, with relative abundances of 8.1%, 5%, 4.4%, and 3.5%, respectively. Conversely, in native forest, the most abundant genera were *Paraburkholderia* (8.3%), *Bradyrhizobium* (7.4%), *Pseudomonas* (2.8%), and *Caballeronia* (1.7%) (Fig. 6). Notably, genera classified within the order *Enterobacterales* (belonging to the phylum Pseudomonadota) were by far the most abundant under both land-use types, accounting for 56% and 30% of the total root endophytic community in DF and NF, respectively. With regard to nodule endophytes, a remarkably higher abundance of *Paraburkholderia* was found in NF compared to DF (42.4% vs. 1.1%, respectively). In DF, *Rhodanobacter* (14.1%), *Rahnella* (12%), and *Caballeronia* (5.7%) were the most abundant genera (Fig. 6).

Interestingly, among nodule endophytes, *Pseudomonas* and *Bradyrhizobium* appeared to be unaffected by land degradation. Both genera showed similar abundances in DF and NF (*Pseudomonas* 17,6% in NF and 21% in DF; *Bradyrhizobium* 1.1% in NF and 2.2% in DF).

3.4 Analysis of the core endophytic bacterial communities of roots and nodules of Alnus acuminata

Among the 3,225 ASVs detected in the root and nodule endosphere of *A. acuminata*, 10 ASVs were identified

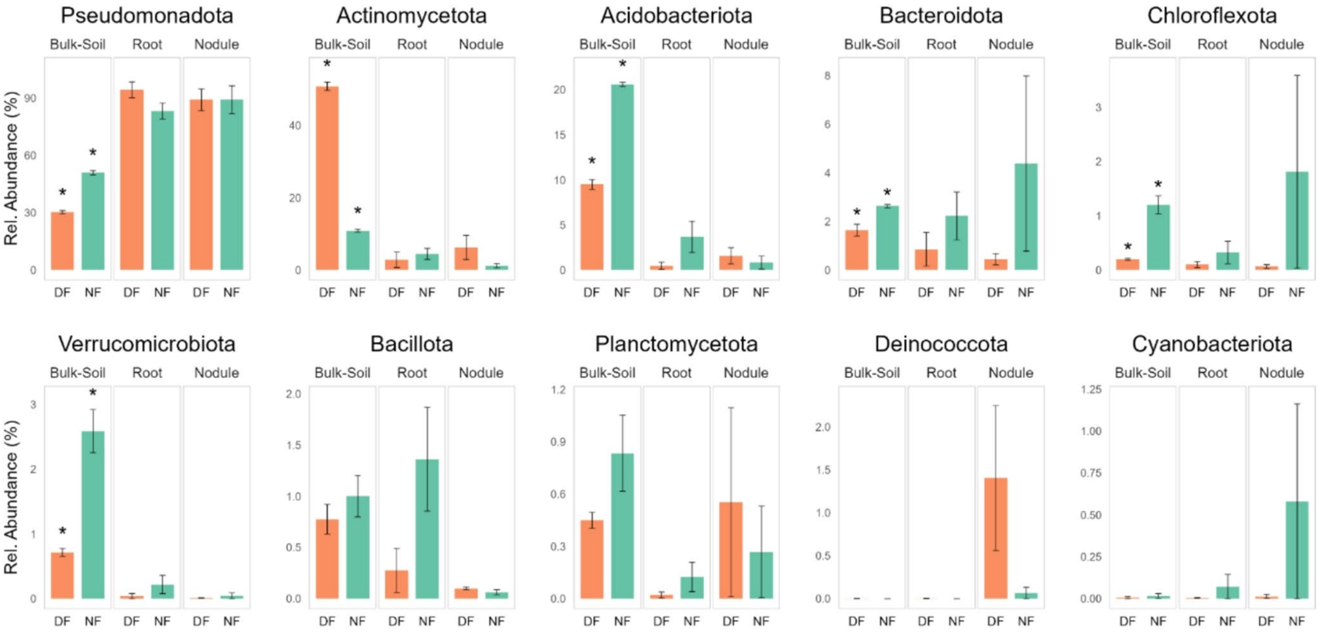


Fig. 5 Comparison of relative abundance (%) of main bacterial phyla across compartments (bulk soil, roots, nodules) in degraded forest (orange bars) and native forest (light green bars). Significant differences between land-use types are indicated by asterisks

Fig. 6 Distribution of relative abundances (%) of bacterial genera across compartments (bulk soil, roots and nodules) in degraded forest (DF) and native forest (NF)

Table 1 Core endophytic bacterial community of *A. acuminata* samples. The table displays taxonomic classification, relative abundance (Ab), and frequency (Frq) of bacterial Amplicon Sequence Variants (ASVs) in root and nodule samples

Phylum	Class	Order	Family	Genus	ASV	Roots		Nodules	
						Ab%	Frq%	Ab%	Frq %
Pseudomonadota	Alphaproteobacteria	*Hyphomicrobiales*	*Bradyrhizobiaceae*	*Bradyrhizobium*	702	0.89	87.5	/	/
	Betaproteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Paraburkholderia*	146	0.38	87.5	0.98	100
					797	1.82	87.5	/	/
				Caballeronia	207	0.61	87.5	3.2	87.5
	Gammaproteobacteria	*Enterobacterales*	*Yersiniaceae*	*Rahnella*	261	0.63	87.5	1.13	100
			/	/	231	3.88	87.5	/	/
			/	/	298	5.53	100	0.94	87.5
			/	/	826	26.85	100	4.5	87.5
		Pseudomonadales	*Pseudomonadaceae*	*Pseudomonas*	775	0.3	87.5	5.92	87.5
		Xanthomonadales	*Rhodanobacteraceae*	*Rhodanobacter*	353	0.14	87.5	1.07	100

consistently present in root samples at a frequency greater than 80%, and 7 ASVs met the same criterion in nodules, independentely of land-use type (Table 1). Notably, all core ASVs identified in nodules were also part of the root core community. These shared ASVs (i.e. ASV146, 207, 261, 298, 353, 775, and 826) were taxonomically assigned to *Paraburkholderia*, *Caballeronia*, *Rahnella*, *Pseudomonas*, and two unclassified *Enterobacterales*. Among root core endophytes, the most abundant ASV was ASV826, assigned to the order *Enterobacterales*, which accounted for 26.85% of the total relative abundance. In nodules, the most abundant core ASV was ASV775 (5.92%), assigned to the genus *Pseudomonas*.

4 Discussion

By comparing bacterial communities between a native forest and a degraded forest in Ecuador, this study investigated how a land-use trajectory (deforestation for pasture followed by abandonment) affects their diversity and structure in the soil, roots, and root nodules of the pioneer tree *Alnus acuminata*. Pioneer trees are key engineer species in the restoration of degraded landscapes. As a nitrogen-fixing pioneer species, *A. acuminata* is critical for facilitating soil recovery and ecological succession in these abandoned pastures. Therefore, understanding the bacterial communities that support its establishment and growth is pivotal. This knowledge can inform effective, long-term recovery strategies that enhance ecosystem resilience.

In our study we found that, compared with native forest, degraded forest shows a marked alteration of soil physicochemical properties, plant community composition and the structure of bacterial communities associated with both bulk soil and the endosphere of *A. acuminata*.

In this regard, this research should be considered a pilot study. While the small number of replicates may limit the statistical power of some comparisons, the strong and consistent trends observed across all compartments provide biologically meaningful insights into how land degradation reshapes the *Alnus*-associated microbiome. Specifically, soil pH increased and total carbon decreased in degraded forest soils compared to native forest, while total nitrogen content remained relatively unchanged. These findings agree with previous research showing that forest disturbance often leads to soil alkalization (Pedrinho et al. 2020; Pereira et al. 2022) and carbon loss due to reduced organic matter inputs and altered nutrient cycling (Zhou et al. 2018; Peng et al. 2022).

The shift in vegetation composition surrounding *A. acuminata* individuals from native tree-dominated communities in native forest to dominance by introduced herbaceous species and early successional shrubs in the degraded site reflects a common successional trajectory following disturbance. Such changes in plant community structure are known to influence soil microbial communities through modifications of litter quality, root exudates, and microhabitat heterogeneity (Oppenheimer-Shaanan et al. 2022; Domeignoz-Horta et al. 2024).

In our study, bacterial richness was not significantly affected by land degradation in either bulk soil or root and nodule endophytes of *A. acuminata*. However, bacterial evenness and Shannon diversity indices were significantly lower in degraded bulk soils but not in both roots and nodules, indicating that degradation affected principally bulk soil community structure by reducing the evenness of bacterial taxa rather than richness. This pattern agrees with findings from other studies where disturbance often leads to dominance by a few opportunistic taxa at the expense of a more balanced community (Sigler and Zeyer 2004). For example, similar reductions in soil bacterial diversity have been reported in degraded semiarid tropical regions (Pereira et al. 2022; Silva et al. 2024). In contrast, other studies have documented increases (Rodrigues et al. 2013; Zhou et al. 2018; Pedrinho et al. 2020) or no significant changes (Lee-Cruz et al. 2013; Lozano et al. 2014; Li et al. 2024) following land degradation, highlighting the context dependency of microbial responses. Such differences likely reflect variation in spatial scale, disturbance type and intensity, soil physicochemical properties and ecosystem characteristics (Zhou et al. 2018), as well as the potential effect of the limited sample size in our study. In contrast to the significant shifts observed in bulk soil evenness, the lack of significant differences in endophytic bacterial diversity between land-use types, suggests a general host filtering effect. This selective process likely allows *A. acuminata* to maintain a stable internal microenvironment in terms of microbial complexity (alpha diversity), buffering its associated microbiome against the environmental degradation that affects the surrounding soil. However, this stability in diversity levels is accompanied by significant compositional shifts, indicating that the host-filtering mechanism is also plastic. This allows the plant to selectively recruit specific bacterial consortia from the available soil pool to better function under degraded conditions. While the use of different primer sets for soil and endophytic compartments (see Methods) precludes a direct quantitative comparison of their respective diversity indices, the contrasting response patterns (stability in the endosphere versus sensitivity in the soil) support the hypothesis of an active plant-driven selection mechanism (Lundberg et al. 2012; Hardoim et al. 2015). Plants usually select beneficial microorganisms to face unfavorable environmental conditions, but the outcome of this selection can vary; for instance, a reduced root-associated bacterial diversity was reported for *Alnus glutinosa* under high-salinity stress (Thiem et al. 2018, 2023), whereas in our study, it appears to have resulted in the maintenance of alpha diversity levels.

The stability of these diversity levels is further contextualized by multivariate analyses. In agreement with the observed changes in evenness, these analyses indicated that land-use type accounted for a large portion of the variance in bulk soil bacterial communities (PERMANOVA: $R^2 = 0.864$), although this effect was not statistically significant. The high variance explained in the bulk soil, despite the lack of statistical significance, likely reflects the limited sample size of our study. Nonetheless, the observed shifts in soil microbial assemblages are consistent with previous findings regarding the impact of land-use change in similar ecosystems (Rodrigues et al. 2013; Lozano et al. 2014; Zhou et al. 2018). In contrast, land degradation exerted a much more constrained, yet statistically significant, influence on root and nodule endophytes ($R^2 = 0.106$). This lower R^2 value, coupled with its statistical significance, reinforces the hypothesis of host-mediated buffering: while the soil community would undergo a substantial restructuring, the host plant would effectively filter the environmental signal, permitting only a targeted shift in its internal microbiome composition. Additionally, the observed compartment effect within *A. acuminata* (roots vs. nodules) also highlights the role of plant microhabitats in shaping endophytic communities, though this effect was less pronounced than land use.

Soil pH along with total carbon emerged as the dominant environmental drivers shaping bulk soil bacterial communities, consistent with numerous studies identifying them as principal determinants of soil microbial diversity and composition worldwide. These factors act as critical environmental filters, determining which bacterial groups can thrive under specific soil conditions (Zhou et al. 2018, 2024; Lopes et al. 2021; Mészárošová et al. 2024).

In the present study, degraded soils were dominated by stress-tolerant Actinomycetota, whereas native forest soils supported more diverse communities rich in Acidobacteriota and Pseudomonadota, typical of resource-rich, acidic environments. Actinomycetota, known for their adaptability, tolerate stressors such as pH fluctuations, salinity, and nutrient scarcity, enabling them to dominate in degraded soils (Mohammadipanah and Wink 2016; Lopes et al. 2021). Their abundance often decreases as ecosystems recover, suggesting a preference for degraded conditions (Li et al. 2024). In contrast, Acidobacteriota, which thrive in acidic soils and typically decline as soil pH increases (Bai et al. 2023; Silva et al. 2024; Jiang et al. 2025), was dominant in NF along with Pseudomonadota, which prosper in high-resource environments with labile carbon inputs (Peng et al. 2022; Bai et al. 2023; Li et al. 2024). Both these taxa declined with degradation. Verrucomicrobiota, another group sensitive to soil fertility changes, also declined with degradation, consistent with previous findings (Navarrete et al. 2015; Lopes et al. 2021).

Similar but less pronounced changes were detected in endophytic communities; notably, Pseudomonadota remained the most abundant phylum across both roots and nodules regardless of land use. This persistence highlights that members of this phylum are the dominant components of the core endophytic microbiome maintained by the host plant (Knowlton and Dawson 1982; Dove et al. 2024). It is important to underline that although Actinomycetota, particularly *Frankia* spp., was typically dominant in *Alnus* nodules (Diagne et al. 2013; Chen et al. 2020), a low relative abundance of *Frankia* was detected. This is likely attributable to the specific physio-morphological state of these endophytes, which may have hindered efficient DNA extraction relative to other genera (Akkermans et al. 1991). Additionally, the universal primers used for endophytic community detection, while designed to capture a broad spectrum, are known to introduce amplification biases, which likely resulted in the underrepresentation of these groups (Thiem et al. 2018). Nevertheless, anatomical observations confirmed its presence in both land-use types (Fig. S1).

At lower taxonomic rank, *Paraburkholderia*, *Caballeronia*, *Rahnella*, *Pseudomonas*, and two unclassified Enterobacterales genera were identified as core members of the root and nodule microbiomes, consistent with their known roles as nitrogen-fixing and plant growth-promoting bacteria in symbiotic associations (Garneau et al. 2023a; Dove et al. 2024; Thompson et al. 2025). Although endosphere communities are primarily shaped by host plant identity (Brown et al. 2020; Mészárošová et al. 2024) significant differences in the endospheric bacterial communities from *A. acuminata* plants grown in degraded and native forests was found. Specifically, changes in the abundance of these core symbionts between degraded and native forests and their association with other bacterial genera suggest that plants can form specific symbiotic bacteria consortia to function better in their specific environments. The marked increase of *Rahnella* in *A. acuminata* roots grown in degraded soil accompanied by other genera, such as *Micromonospora*, suggests the capacity of the plant to select and enter into symbiosis with the most useful endophytes for those environmental conditions. Both *Rahnella* and *Micromonospora* are plant endophytes. *Rahnella* is a plant growth-promoting bacterium (PGPB) which enhances nutrient acquisition, such as phosphate solubilization, and *Micromonospora* has been shown to contribute to plant health and pathogen defense, through the synthesis of many metabolites (Carro et al. 2013; Garneau et al. 2023a, b).

Similarly, the marked decline of Paraburkholderia in nodules from degraded forests, accompanied by an increased abundance of other species such as Rhodanobacter, primarily recognized for its role in biogeochemical cycling, Mycobacterium and Deinococcus, recognized as plant associated resilience enhancers, suggests that land degradation may alter symbiotic partnerships, to potentially maintain or even improve nitrogen fixation efficiency in an environment where the C/N ratio is significantly lower. However, the functional implications of all these shifts remain to be directly tested.

Despite these compositional changes, nodule morphology did not differ between land-use types, indicating that nodulation capacity is maintained under degraded conditions (see Supplementary Materials Fig. S2, Tab. S11). This observation is consistent with potential functional redundancy or physiological plasticity within the nodule microbiome, as previously suggested (Gyaneshwar et al. 2011; Tong et al. 2025), although direct measurements of symbiotic performance would be needed to further support this interpretation.

Overall, the results reported in this study highlight the complex interactions between land degradation and microbial assemblages in soil and in the root of the pioneer plant *A. acuminata*. The strong influence of soil pH and total carbon on soil bacterial communities underscores the need to consider abiotic and biotic factors in restoration strategies. Furthermore, within plant-associated compartments, the microbiome of *A. acuminata* appears stable in terms of alpha diversity yet flexible in composition, suggesting a balance between host control and environmental influence. This apparent adaptive capacity may contribute to the ecological success of this species in degraded environments and highlights the potential importance of plant–microbe interactions in ecosystem recovery.

Future work integrating bacteria selection, functional analyses and experimental inoculations will be needed to confirm these results and determine the appropriate bacteria consortia useful to accelerate targeted restoration.

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Declarations

Competing interests The authors declare no competing interests.

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