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METABOLIC DIVERSITY OF THE MICROBIAL COMMUNITY AND ENDOPHYTIC NITROGEN-FIXING BACTERIA IN THE RHIZOSPHERE OF *Xanthosoma daguense* (Araceae) AND *Oplismenus burmannii* (Poaceae) IN THE COLOMBIAN COFFEE REGION

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ABSTRACT

The rhizosphere is one of the most dynamic areas of biodegradation in soil because of the interaction among microbiota, plant roots and ecosystem productivity processes. In this study, heterotrophic microbial communities and endophytic nitrogen-fixing bacteria associated with the rhizosphere of the plant species *Xanthosoma daguense* and *Oplismenus burmannii* in subandine forest soils at the Colombian coffee-growing region were characterized with respect to their ability to degrade carbon substrates. In this preliminary study we analyzed the rhizosphere of two plant species variation in microbial community functional diversity using sole–carbon–source utilization profiles. The metabolic capacity of heterotrophic microbial communities was evaluated for 31 carbon substrates on Biolog EcoPlates™ grouped as carbohydrates, amino acids, carboxylic acids, amines, carbon phosphates and carbon complex. For the isolation of endophytic diazotrophs, the semisolid media LGI, LGI-P, NFb and JNFb were used and the ability to degrade 47 types of substrates was evaluated using Vitek 2 Compact®. The heterotrophic microbial community associated with the rhizosphere of *X. daguense* and *O. burmannii* exhibited a functional diversity of 90% and 68% respectively. Cluster analysis for the nitrogen-fixing bacterial isolates allowed separation of colonies based on the use of substrates, confirming metabolic diversity. These data demonstrate the differential metabolic capacity of the rhizosphere of *X. daguense* and *O. burmannii* to favor growing microbes in the rizhosphere

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which is important in the potential contribution of microbial communities to the matter decomposition in this type of ecosystem.

Keywords: Rhizosphere, bacterial diazotrophs, semisolid media, heterotrophic microbial community, ecosystem.

1. INTRODUCTION

Soils of the central Colombian coffee-growing region, belonging to the formation process of the Andean mountains during the Pliocene, are relatively young and influenced by volcanic materials. These soils, in which the clay mineral allophane predominates, produce biomass with low lignin contents and litter which readily forms organo-mineral complexes. Under natural conditions, these tropical soils are characterized by high organic matter mineralization that increases acidity. When soils at altitudes up to 2000 meters above sea level are exposed to farming or livestock activities, acidification is accelerated, especially when ammonia fertilizer is applied in excess of crop needs; this can induce the formation of poorly soluble compounds or cause nutrient leaching. Further degradation of soil occurs when agricultural practices are carried out on steep slopes, favoring soil erosion (Van der Hammen, 1992; Mantilla, 1998). About 170 plant species have been reported in the coffee region, of which 15% are classified as beneficial coverage for soil protection (Gómez, 1990). One of these native species with ecological importance is *Xanthosoma daguense* (Araceae), which exhibits pollination interactions with three beetle species in the Colombian cloud forest (Garcia-Robledo et al., 2004). Another relevant species in high mountain ecosystems is *Oplismenus burmannii* (Poaceae), which is widely distributed in both tropical hemispheres and has been reported as a protective cover against soil erosion (Rivera, 2001).

The researches have showed that the microbial communities are highly diverse, highly redundant and ubiquitous distribution and there has been a large increase of knowledge regarding the characterization of microorganism activity in soils but there a misconception for assessment and understanding of the functioning of microbial communities and the pertinence of microbial diversity in ecosystem functioning (Bodelier, 2011). In the soil, the rhizosphere is the ecologically more complex zone due to transfer processes of matter and energy, which are hosted by microbial communities interacting with plant roots and exudates generated from photosynthetic activity (Anderson & Habiger, 2012). The rhizosphere microbial communities are part of the trophic links that contribute to sustained productivity in natural environments by taking part in processes such as organic matter decomposition. The dynamics underlying these processes are driven by catabolism of different carbon substrates released by the root (Bansal & Mukerji, 1996), as well as mineralization and nutrient release (Dakora & Phillips, 2002; Begon et al., 2006). In the scenario of ecosystem functions, it is important to address research on soil

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microorganisms that live the rhizosphere and degrade a variety of organic substrates that return carbon and N to the environment, indicating metabolic diversity with key roles in the ecosystem (Warenborag & Estelrich, 2000). The lack of knowledge about traits of microorganisms involved in specific biologic processes in the soil restrict understanding of services that it supply with supporting and regulating of ecosystem (Bodelier, 2011). With this preliminary study we aimed to compare the metabolic activity of heterotrophic microbial communities and diazotrophs from the rhizosphere of non-leguminous plants species *X. daguense* and *O. burmannii* which are of ecological and sustainable importance in preserved soils.

2. MATERIALS AND METHODS

2.1. Study area and sample collection:

Rhizosphere samples from the two tropical plant species were collected in El Aguila forest located at 6 Km on the road from Manizales to Neira in Caldas State, Colombia. According to Cuatrecasas (1958), this area corresponds to the Subandine forest plant formation, and is located on the western slope of the Central mountain range. The location coordinates are 05° 06' 48" N and 75° 30' 57" W, at an altitude between 1800-2200 meters above sea level.

Three individuals for each species, *X. daguense* (Araceae) and *O. burmannii* (Poaceae), were collected and their identification was confirmed in the Caldas University Herbarium. For each individual, both the soil adhering to the roots and the whole plant were removed using a disinfected shovel. The rhizosphere of each individual was introduced into plastic bags and transported in styrofoam coolers at 4°C. The three rhizosphere samples for each species were mixed and 500 g of sample were separated and processed. Sampling was carried out in October 2014, corresponding to a rainy period; 290 mm of precipitation were registered for that month in the closest meteorological station.

Three adjacent soil samples were taken from the surface 70 cm to measure chemical properties as well as texture in the soil chemistry and fertility laboratory at Caldas University, Manizales. Soils in this coffee-growing area are highly variable due to different cover patterns of volcanic ash and its location in the relief which ranges from flat or slightly undulated to abrupt, with slopes greater than 75%. These soils have been reported as slightly acidic, with a medium to high N content and low P content (Arcila, 2007). Physicochemical analyses for this study showed that the sampled soil is a sandy loam, with pH between 5.7 and 6.2, high N, Ca, Fe, Mn, B concentrations and medium levels of P, Na and S (Table 1), classified as a Entisols (Soil Survey Staff, 2014).

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Table 1. Soil analysis for samples collected in El Aguila forest

Property	Units	Sample 1	Sample 2	Sample 3	Reference Values*		
					Low	Medium	High
pH		5.7	5.4	6.2			
Nitrogen	%	0.61	0.61	0.48	<0.24	0.24 - 0.48	>0.48
Phosphorus	mg/kg	10	11	6	<20.0	20.0-40.0	>40.0
Potassium	cmolc/kg	0.32	0.16	0.27	<0.2	0.2- 0.4	>0.4
Calcium	cmolc/kg	9.47	10.29	11.8	<3	3.0 -6.0	>6.0
Magnesium	cmolc/kg	2.21	2.73	4.72	<1.5	1.5 -2.5	>2.5
Sodium	cmolc/kg	0.261	0.238	0.233	<0.5	0.5-1.0	>1.0
Iron	mg/kg	303	166	75	<25.0	25.0- 50.0	>50.0
Manganese	mg/Kg	62.1	59.04	35.01	<5.0	5.0 -10.0	>10.0
Zinc	mg/Kg	30.67	10.04	6.69	<1.5	1.5 -3.0	> 3.0
Copper	mg/Kg	1.76	1.46	1.66	<1.5	1.5 - 3.0	>3.0
Sulfur	mg/Kg	5.8	13.07	10.96	<10.0	10.0 - 20.0	>20.0
Boron	mg/Kg	0.6	0.7	0.9	<0.2	0.2 -0.4	>0.4
Texture		Sandy Loam	Sandy Loam	Sandy Loam			

* Based on a table given for forest soils (Soil chemistry and fertility lab, Caldas University, 2014)

2.2. Biochemical profile of the microbial community associated with the rhizosphere of *X. daguense* and *O. burmannii* :

From the soil attached to the roots, 2 grams were separated and suspended in 18 ml of PBS, 10^{-1} dilution (8.5 g NaCl, 0.3 g KH_2PO_4, 1.12 g $Na_2HPO_4.7H_2O$, diluted to 1L of distilled water and adjusted to pH 6.8); then, 3 ml of this dilution were added to 27 ml of PBS and subsequent dilutions were made up to 0.5 in the McFarland scale. From each dilution of rhizosphere, 100 ul of the suspension were taken to be placed in the Biolog EcoPlates™ cells composed of 31 carbon substrates with three replications and control cells (water). The substrates were divided into six categories: carbohydrates, carboxylic acids, amines, amino acids, complex carbon and carbon phosphate.

After introducing the soil suspensions, each plate was incubated in a plastic bag at room temperature with wet paper towels to prevent evaporation and maintain a constant humidity. Color changes from tetrazolium (no color) to formazan (purple) were registered in the cells after 5 days, when coloration was stable. Thereafter, the percentage of functional diversity was calculated for the microbial community in the rhizosphere of each plant species based on

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Mulcahy and Edenborn (2007). A comparative analysis of these microbial communities in the rhizosphere was performed by substrate use.

2.3. Biochemical profile of the diazotroph bacteria isolates in the rhizosphere of *X. daguense* and *O. burmannii* :

Roots of 3 individuals from each plant species were mixed and 10 g were weighed to be washed 5 times with sterile distilled water, submerged in 5% sodium hypochlorite solution for 5 minutes and washed with sterile distilled water another 3 times. Subsequently, roots were submerged in 0.5 M phosphate buffer (K_2HPO_4 1M, KH_2PO_4 0.4M) at pH 7 for 3 minutes and washed 5 times with sterile distilled water as indicated by Sampaio et al. (2007).

Then, samples were macerated for 5 minutes using saline solution (containing 1 mL of K_2HPO_4 10%, 0.5 mL of $MgSO_4$ 10%, 0.2 mL of NaCl 10%, 0.5 mL of $CaCl_2.2H_2O$ 10%, 1 mL of FeEDTA 1.64%, 0.5 mL of micronutrient solution, in a total volume of 90 ml at pH 6.5) and kept for one hour as indicated by Döbereiner et al. (1995). Then serial dilutions of 1 ml up to 10^{-8} with three replicates were made. From all dilutions and replicates an aliquot of 0.1 mL was transferred into vials containing 5 mL of the following N-free semisolid isolation media or treatments: LGI, LGI-P, NFb and JNFb (Döbereiner et al., 1995). A total of 96 treatments were evaluated, including serial dilutions by type of inoculated media, after incubation for 7 days at 30°C.

As a positive control, indicating N fixation by halo formation, a strain of *Gluconacetobacter diazotrophycus* (GIBI_000022_AG_B) was used in the semisolid media. For those treatments that formed a halo or a subsurface film, other inoculation with 20 ul in 5 mL of semisolid N-free media LGI, LGI-P, NFb and JNFb was made for 7 days at 30°C. From those semisolid media with halo formation, 10 ul were inoculated into the solid media LGI, LGI-P, NFb y JNFb and incubated at 30°C. These resulting colonies were cultivated in new semisolid media: LGI, LGI-P, NFb and JNFb, and incubated at 30° for 48-72 hours. From these latter semisolid media with halo formation, 20 ul were inoculated into PDA media and incubated for 48-72 hours at 30°C and after that, preserved in 10% glycerol at -20°C.

The isolates obtained in the semisolid media LGI, LGI-P, NFb and JNFb were characterized by Gram stain and by biochemical tests for carbon and N sources using the Vitek 2 Compact® system. Then, N fixation features of the isolates in the semisolid media and the respective metabolic profiles by substrate use for both plant species were compared.

Surface halo formation in semisolid media, as a feature of N-fixing bacteria, was evaluated with a multiple correspondence analysis for each isolate obtained from both plant species, considering

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the following variables: type (consistent or weak), position (inside, medium or surface) and quantity of halo. In addition, color and turbidity of the semisolid media, isolation type (LGI, LGI-P, NFb and JNFb) and plant species were considered. All analyses were performed using IBM SPSS sofware Version 2.2. The Jaccard similarity index was used for comparing by substrate use for all isolates from the rhizosphere for both species, and a cluster analysis with Average linkage was performed using the program INFOSTAT V. 2008.

3. RESULTS

3.1 Biochemical profile of the microbial community associated with the rhizosphere of *X. daguense* and *O. burmannii*:

The ability of the microbial community to metabolize carbon sources showed a functional diversity value of 90% for *X. daguense* and 68% for *O. burmannii*. The similarity index after using carbon substrates by microbial communities for both species showed a 0.67 ratio. As shown in Figure 1, carbohydrates were the most consumed carbon source by the two communities, showing values of 90.5 and 87.5% for *X. daguense* and *O. burmannii* respectively. To a lesser extent, the microbial community of *X. daguense* also consumed amino acids followed by carbon complexes (tween, cyclodextrin, and glycogen) carboxylic acids, amines and carbon phosphate. Carbon phosphate sources were 50% consumed by the microbial community from *X. daguense* but not consumed by microbes associated with *O. burmannii*.

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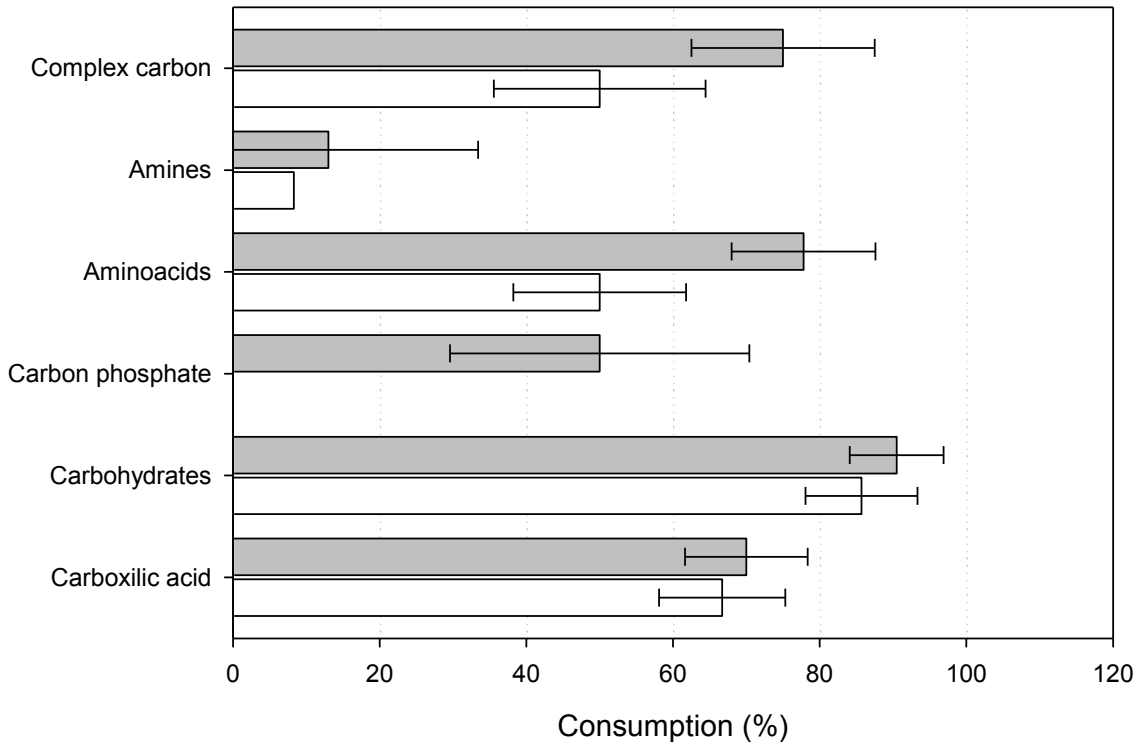

Fig. 1. Consumption percentage of carbon sources by heterotrophic microbial communities associated with the rhizosphere of *X. daguense* and *O. burmannii*

3.2 Biochemical characterization of the endophytic N-fixing bacteria isolates in the rhizosphere of *X. daguense* and *O. burmannii*:

In the N-free semisolid culture media, N-fixing bacteria move towards the central region where the respiration rate is at equilibrium with the oxygen diffusion rate, and there they form a film beneath the surface. Such offset within the media is due to N fixation and N-dependent growth (Döbereiner 1992). From the 96 treatments used for N-fixing bacteria isolates in semisolid media (JNFb, LGI, LGI-P and NFb) evaluated in *X. daguense*, 37.5% formed a white film, 30% showed a consistent film, 28.1% exhibited yellow media, 71.9% developed a blue-green color and 53.1% were murky.

Of the *O. burmannii* treatments, 46.9% were positive for the formation of a white film, 37.5% showed a consistent film, 53.1% were yellow, 46.9% exhibited a blue-green color and 53.1% of media were murky. An evaluation of the halo formation in semisolid media by associating the variables formation and position of white halo, color shift and turbidity with reduced dimensions

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showed Cronbach alphas of 0.896 for the first dimension and 0.701 for the second dimension. At the same time, 89% of variance was explained by only two dimensions (Table 2).

Table 2. Reliability of the variables related to the halo formation in semisolid media

Dimension	Cronbach Alpha	Variance accounted for		
		Total (auto value)	Inertia	% of variance
1	0.896	4.633	0.579	57.912
2	0.701	2.584	0.323	32.300
Total		7.217	0.902	
Media	0.826[a]	3.608	0.451	45.106

Note: [a] Cronbach alpha media values between 0.8 and 0.9 indicate GOOD internal consistency

After evaluating halo formation in semisolid media, the most reliable variable for this condition was plant species, which indicates that rhizosphere isolates from both species share a similarity in their ability to fix N in semisolid media (Figure 2).

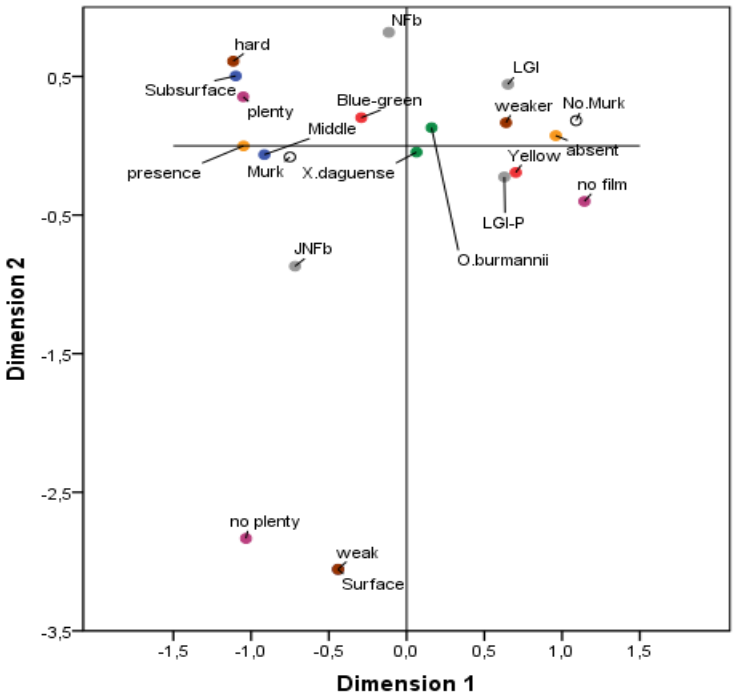

Fig. 2. Point diagram of the variables related to halo formation of N-fixing bacteria isolates in the semisolid media LGI, LGI-P, NFb and JNFb. Circle colors indicate differences in variables as follows: Red= film color (blue-green, yellow); Grey= isolation media (LGI, LGI-P, NFb, JNFb);

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Green= plant species (*X. daguense, O. burmannii*); Blue= film position (surface, subsurface, middle, bottom); Orange= film presence (present, absent); Purple= film quantity (plenty, no plenty, no film); White= Turbidity (murky, no murky); Brown= film type (hard, weak, weaker)

The variables quantity, position, and presence of halo in semisolid media were discriminative measurements in the first dimension in comparison to the second dimension, whereas halo position showed relatively larger values in both dimensions. The position and type of halo showed high values in the second dimension. The variables isolate type and color showed lower discriminative measurements. These results confirm the similarity in N fixation behavior among isolates in semisolid media as indicated by consistent halo formation (Table 3).

Table 3. Discriminative measurements of the variables related to halo formation in semisolid media for endophytic N-fixing bacteria.

	Dimension		average
	1	2	
Presence	.938	.006	.472
Type	.673	.913	.793
Position	.948	.878	.913
Quantity	.938	.503	.721
Color	.117	.004	.060
Turbidity	.756	.000	.378
Isolate media	.256	.280	.268
Plant	.008	.000	.004
Active Total	4.633	2.584	3.608
% of variance	57.912	32.300	45.106

The five isolates obtained from *X. daguense* were all identified as Gram negative bacilli; 2 isolates came from NFb, 2 from JNFb and 1 from LGI-P media. Similarly, for *O. burmannii*, 5 Gram negative bacilli isolates were obtained with 1 from NFb, 2 from JNFb, 1 from LGI and 1 from LGI-P. According to the media selectivity and based on Sampaio (2007), for both plant species the presumptive genera of endophytic N-fixing bacteria grown in JNFb are *Herbaspirillum spp*. and *Sphinghomonas spp.*, the organism from NFb is *Azospirillum spp.* and the LGI-P isolate is *Gluconaceatobacter spp*. In *O. burmannii* those obtained in LGI met the criteria to be classified as *Burkholderia spp.* or *Azospirullum spp.*

The isolated bacteria from *X. daguense* and *O. burmannii* have in common the ability to catabolize sucrose, D-maltose, D-glucose, D-mannose, D-cellobiose, as well as to alkalinize L-

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lactate. These isolates exhibited differences at using substrates which indicates specificity related to enzymatic activity, use of carbon sources and/or resistance to the O/129 (2,4-diamino-6,7-diisopropylpteridine) agent (Table 4).

Table 4. Summary table indicating the substrate specific use for the rhizosphere bacteria isolates in the Vitek 2 Compact® system

Isolate	Plant specie	Substrate	Indicated enzymatic activity or Carbon source utilization
JNFbC2X NFbC2X	*X. daguense*	Glycosides	beta-glucosidase (hydrolysis of non-reducing ends of glycosides)
		Amino acids	L-proline-arylamidase (hydrolysis)
		2,4-diamino-6,7-diisopropylpteridine	Resistance
		Ornithine decarboxylase	Carbon source utilization
		D-mannitol	Carbon source utilization
		Palatinose	Carbon source utilization
		D-threalose	Carbon source utilization
		Succinate	Carbon source utilization and alkalinization
		L-malate	Carbon source utilization
		Tyrosine	Tyrosine arylamidase
		Sodium citrate	Carbon source utilization
		Oligosaccharides	beta-N-acetyl-galactosaminidase
		Amino acids	gamma-glutamyl transferase (synthesis)
		Malonate	(use of salts as carbon source)
		Glycolipids and glycoproteins	alpha-galactosidase
		Glycoproteins	beta-galactosidase (hydrolysis)

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		Glucose fermentation	Carbon source utilization
		D-sorbitol	Carbon source utilization
		5-keto-D-gluconate	Carbon source utilization
		Phosphate groups	phosphatase
LGI-PC1X	*X. daguense*	Amino acids	beta-alanine-arylamidase pNA (hydrolysis)
JNFbC1O JNFbC2O NFbC1O	*O. burmannii*	Glycosides	beta-glucosidase,(hydrolysis)
		2,4-diamino-6,7-diisopropylpteridine	Resistant
		Oligosaccharides	beta-N-acetyl-glucosamidase
		Ornithine	ornithine decarboxylase
		D-mannitol,	Carbon source utilization
		Palatinose,	Carbon source utilization
		D-threalose	Carbon source utilization
		Succinate	Carbon source utilization (alkalinization)
		Tyrosine	Tyrosine arylamidase
		Citrate (sodium)	Carbon source utilization
		Glutathione	Gamma-glutamyl-transferase
		Malonate	Carbon source utilization
		Glycoproteins	alpha-galactosidase (hydrolysis)
		Glycoproteins	beta-galactosidase (hydrolysis)
		Glucose fermentation,	Carbon source utilization
		Phosphate groups	Phosphatase
		5-keto-D-gluconate	Carbon source utilization
JNFbC1O	*O. burmannii*	L-lactate	Carbon source utilization

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JNFbC2O	*O. burmannii*	L-malate	Carbon source utilization
LGI-PC1O	*O. burmannii*	Amino acids	beta-alanine arylamidasa-pNA (hydrolysis)
		Urea	Urease

The similarity in the ability for degrading substrates between isolates coming from both species using the Jaccard measurement showed a Cophenetic correlation index of 0.997. The differences in the use of substrates among colonies from the rhizosphere of both plant species contribute to the total metabolic diversity in this soil zone and provide information to better understand the dynamics of microorganism-plant interactions (Figure 3).

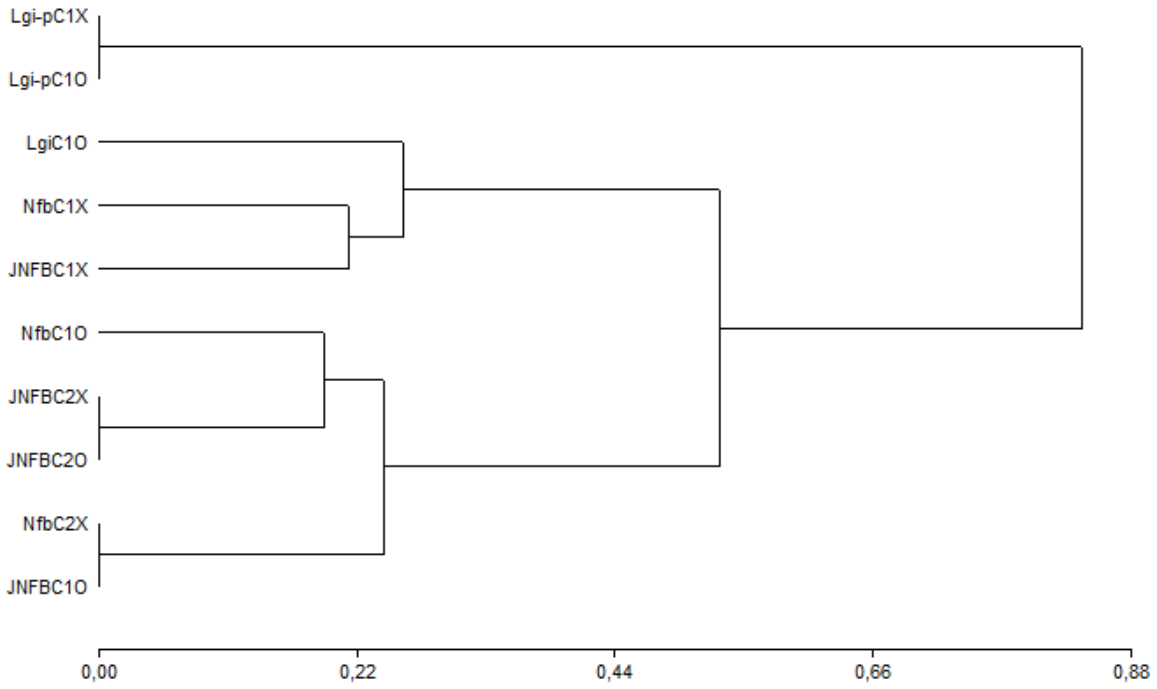

Figure 3. Similarity analysis of catabolic metabolism among the endophytic N-fixing bacteria functional groups in the rhizosphere of the two plant species; C: Colony; X: *X.daguense*; O: *O. burmannii*

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4. DISCUSSION

The heterotrophic microbial communities in the rhizosphere are an integral component of soils that can offer environmental services related to assessing the ecological role in the ecosystem. These rhizosphere microbial communities exposed to the environmental variations generate metabolic responses that affect ecosystem functioning, which is reflected in the supply of regulation and support services (Bodelier, 2011). The microbiota of the rhizosphere have been identified as a key small-scale component of the ecosystem influencing the global carbon cycle by several mechanisms, such as mediating plant-root interactions and plant nutrient acquisition, which are influenced in part by the microbial metabolic dynamics coupled to biogeochemical reactions and promotion of nutrient cycling (Cheng et al., 2011). These microbial communities have specific degradation patterns of carbon and N substrates in natural soils and have shown correlations with environmental supply at the level of variables such as soil management and texture. This information has expanded the components of profile metabolic, functional diversity and ecosystem functioning with practical applications when monitoring the soil quality with biological indicators (Rutgers, 2016).

Analyzing the rhizosphere microbial communities of *X. daguense* and *O. burmannii*, there were differences in the metabolic diversity, which to our knowledge have not been reported previously in these pioneer plants in the Subandine forest of the coffee region. The comparative analysis of the biochemical profile among rhizospheric microbial communities colonizing the two species showed that they share catabolism of 29 substrates comprising carbon complexes, amines, amino acids, carbohydrates and carboxylic acids; however, there was a differentiation in the proportions used.

Greater functional diversity found in *X. daguense* compared to *O. burmannii* is supported by the use of carbon complex sources as tween 80 and cyclodextrin and the use of the amino acids such as L-phenylalanine, L-threonine, and glycyl-L-glutamic acid. The specific use of the carbon phosphate glucose-1-phosphate is a discriminating factor that contributes to the functional diversity of *X.daguense,* since this substrate was not used by the microbial community of *O.burmannii*. Among the analogue substrates to plant root exudates present in the Biolog TM plates are D-xylose, D-malic acid, gamma-hydroxybutyric acid, L-arginine, L-asparagine, L-phenylalanine, L-serine, L-threonine, 4-hydroxybenzoic acid and 2-hydroxybenzoic acid (Campbell et al., 1997).

The communities for the two plant species showed similarity in the degradation of 4-hydroxybenzoic acid, D-xylose, D-malic acid, L-arginine, L-serine and L-asparagine. However, L-phenylalanine and L-threonine were degraded only by the rhizosphere community of *X.*

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daguense wheras 2-hydroxybenzoic acid was degraded by microorganisms from *O. burmannii*. Conversely, gamma-hydroxybutyric acid was not degraded by any community. Winding et al. (2005) stated that the reproducibility in both the use of substrates and the analyzed soil samples reflect the ecological properties of the heterotrophic bacterial community as a soil attribute. Data obtained in the present study are part of characterizing the natural state of conserved soils like those in the studied region.

Part of the rhizosphere microbial community consists of diazotroph bacteria that, as a functional group, contribute to the N dynamics and nutrient balance in the system. These bacteria are stimulated by exudates and therefore colonize the interior of the plant root; furthermore, they are established in niches to fix N and transfer it effectively to the plant (Compant et al., 2010, Santi et al., 2013), and it has been determined that nitrogenase activity depends on carbon supply (Kuhla & Oelse, 1988). Advances in the ecological significance of diazotroph bacteria due to its N contribution to productivity and its effect on the ecosystem functioning is one of the current restraints to fully understanding these organisms. Some studies show that N fixation is stimulated by labile carbon (Keeling et al., 1998, Burgmann et al., 2005) and N sources (Poly et al., 2001).

In this study, the metabolic pattern of carbon and N substrates by diazotroph bacteria isolates of *X. daguense* and *O. burmannii* was determined and presumptive genera were identified. Isolates of endophytic diazotroph bacteria for *X. daguense* have not been reported. Moreover, isolates of endophytic N-fixing bacteria having as a host plant species of the Poaceae family have been already reported in crops of agronomic interest (Reis et al., 2006). Comparing the use of substrates at this functional group level coming from rhizosphere isolates of two plant species, it was found that of 47 carbon sources, six substrates have common catabolism. The remaining 41 carbon sources were used differentially by the bacteria isolates, which highlights differences in the ability to use a set of substrates by the isolates belonging to the rhizosphere of each species. The diazotrophs ability to use various substrates is an advantage to compensate the insufficient amino acids releasing by plants as N sources for microbial community growth in the rhizosphere (Simons et al. 1997). On the other hand, the plant itself might be considered as an environment with high amounts of carbon sources derived from photosynthates (Reinhold-Hurek and Hurek, 1998, 2011; James 1998, 2000). This factor could be key for the success of bacteria by determining the host's distribution and colonization of plant roots and internal tissues. *Gluconacetobacter diazotrophicus* isolated from sugar cane was the endophytic N-fixing bacteria used as a reference, but this species has also been isolated from other plants of agronomic interest such as cereals, spices and fruit trees (Cavalcante & Döbereiner, 1988; Madhaiyan, 2004). It is pertinent to study and evaluate the metabolic capacity of N-fixing bacteria other than *G. diazotrophicus* in preserved soil, since there is frequent agricultural interest to better understand N dynamics in tropical soils.

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5. CONCLUSIONS

This study confirmed the differential responses in catabolic activity for microbial communities of the rhizosphere, including specific activity of N-fixing bacteria, in two non-legume species grown in preserved forest soils. The microbial community associated with *X. daguense* showed a greater functional diversity with more preference for using substrates such as carbon complexes, amino acids and carbon phosphates, whereas the community of *O. burmanii* did not utilize carbon phosphate substrate. For both species, the diazotroph bacteria also showed differences in utilization of carbon sources in four isolates from semisolid media: LgiC1O and NfbC1O for *O. burmannii* as well as NfbC1X and JNFBC1X for *X. daguense*.

It is necessary to continue obtaining information about the degradation processes of carbon compounds by microbial communities of tropical soils, based on plant diversity patterns as well as on their ecological and environmental importance for soil protection against erosion. Moreover, this type of studies will contribute to better describing how environmental services are supported and how microbial communities are regulated in the ecosystems.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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