Effect of land use on the density of nitrifying and denitrifying bacteria in the Colombian Coffee Region

Efecto del uso de suelo sobre la densidad de bacterias nitrificantes y desnitrificantes en la Ecorregión Cafetera Colombiana

Victoria E. Vallejo[1], María M. Gómez[1], Ana M. Cubillos[1], and Fabio Roldán[1, 2]

ABSTRACT

Soil microbial communities involved in the cycling of nitrogen (N) are essential to maintaining and improving soil fertility, productivity and functionality of natural and agricultural ecosystems. However, some compounds generated during the metabolic processes performed by nitrifying (NB) and denitrifying (DB) bacteria are associated with the production of greenhouse gases, groundwater pollution and acidification. Therefore, the study of these bacteria is essential for economic and environmental sustainability. This study evaluated the effect of different land uses in two river basins (La Vieja and Otún) on NB and DB densities. Two sampling events (SE) were conducted by selecting the most representative land uses. Physicochemical (T °, pH, moisture and nitrate) and microbiological properties (NB and DB densities) were evaluated. In both SEs, significantly higher densities of NB and DB were observed in the land uses: pasture, guadua (DB only) and unshaded coffee (La Vieja) and onion (Otún). These land uses, excluding guadua, are dependent on nitrogen fertilizers, which together with the activities of grazing livestock on pastures may lead to greater availability of substrates for the NB. The use of agricultural machinery and overgrazing in pasture and onion uses generate compacted soil and other physical disturbances, encouraging the growth of DB. Forests had the lowest densities of NB and DB possibly due to a reduced availability of N and the releasing of allelopathic compounds from certain plants. Finally, the densities of ammonium-oxidizing bacteria had the greatest differences between the land uses evaluated, demonstrating its high sensitivity to agricultural management practices and livestock. We suggest that changes in the abundance of this community could serve as a relevant and cost-effective bioindicator for soil monitoring.

Key words: nitrifying bacteria, denitrifying bacteria, land use, Colombian coffee region, ammonia oxidizing bacteria.

RESUMEN

Las comunidades microbianas edáficas involucradas en el ciclaje de nitrógeno (N) son fundamentales para el mantenimiento y mejoramiento de la fertilidad, productividad y funcionalidad de los ecosistemas naturales y agrícolas. Sin embargo, algunos compuestos generados durante los procesos realizados por bacterias nitrificantes (BN) y desnitrificantes (BD), se asocian con la producción de gases efecto invernadero, contaminación de aguas subterráneas y acidificación. Por lo tanto, el estudio de estas bacterias resulta esencial para la sostenibilidad económica y ambiental. El presente estudio evaluó el efecto de diferentes usos de suelo en dos cuencas hidrográficas (la Vieja y Otún) sobre la densidad de BN y BD. Se realizaron dos eventos de muestreo (EM) seleccionando los usos de suelos más representativos. Se evaluaron propiedades fisicoquímicas (T°, pH, porcentaje de humedad y nitratos) y microbiológicas (densidad de BN y BD-Número más probable). En los dos EM se observaron densidades significativamente mayores de BN y BD en los usos de pastizal, guadual (solo BD) y cafetal sin sombrío (la Vieja), así como en cebolla (Otún). Estos usos de suelo, excluyendo guadual, son dependientes de fertilizantes nitrogenados, lo cual en conjunto con las actividades de pastoreo del ganado en los pastizales, podrían generar una mayor disponibilidad de sustratos para las BN. El uso de maquinaria agrícola y sobrepastoreo en cebolla y pastizal generan disturbios físicos en el suelo y lo compactan favoreciendo el crecimiento de BD. Los bosques presentaron las densidades más bajas de BN y BD posiblemente por una menor disponibilidad de N y la liberación de compuestos alelopáticos. Finalmente, las densidades de bacterias oxidadoras de amonio fueron quienes presentaron mayores diferencias entre los diferentes usos de suelo evaluados, demostrando su alta sensibilidad frente a prácticas de manejo agrícola y pecuario.

Palabras clave: bacterias nitrificantes, bacterias desnitrificantes, usos de suelo, ecorregión cafetera colombiana, bacterias oxidadoras de amonio.

Introduction

In recent years, there have been increasing concerns about the consequences of land cover change and its effects on the earth's ecosystems. For example, conventional farming systems share many characteristics, such as: large capital investments with rapid technological innovation, large-scale farms with monoculture agricultural practices, and

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[1] Sanitation Unit and Environmental Biotechnology (USBA), Department of Biology, Faculty of Science, Pontificia Universidad Javeriana. Bogota (Colombia).
[2] Corresponding author: fabio.roldan@javeriana.edu.co

extensive use of external chemical inputs (e.g., pesticides and fertilizers) to increase agricultural productivity (Matson *et al*., 1997). However, high demand and dependence of chemical inputs has caused economic and environmental problems (Hurni *et al*., 2008), which have reduce fertility and productive capacity in soil as a result of physical (erosion and compaction), chemical (acidification, nutrient loss and pollution) and biological degradation (biodiversity loss) (Hunter, 1996; Girvan *et al*., 2003).

Agricultural management practices (e.g., tillage, organic amendment addition, chemical fertilization, overgrazing, cropping rotation, and irrigation) can strongly influence the size, composition, and activity of the microbial community in soils. Moreover, a modification of the soil microbiota may in turn affect soil processes, providing a positive or negative feedback on plant productivity (Feng *et al*., 2003; Parfitt *et al*., 2010). Previous studies have established that changes in soil microbial communities are caused by alterations in the physicochemical characteristics of the soil (e.g., organic C, nutrients, bulk density, pH and moisture), and in the quantity, quality and distribution of crop residues as a result of implemented agricultural and livestock management practices (Hayden *et al*., 2010; Kong *et al*., 2010).

To assess the impact of land use on soil microorganisms, two approaches can be used. In the first approach, total microbial community is studied, but diversity changes on this community may not generate effects on ecosystem processes due to a high functional redundancy (Allison and Martiny, 2008; Nyberg *et al*., 2006). The second approach evaluate microbial functional groups which are involved in N cycling, where functional redundancy is lower, and therefore, any diversity change would have a significant impact on soil processes (e.g., nitrification, denitrification and N fixation) (Kowalchuk and Stephen, 2001; Malchair *et al*., 2010).

Historically, the coffee region in Colombia has undergone a transformation and fragmentation of natural ecosystems for the establishment of different crops such as bananas, onions, and citrus, as well as pastures for intensive livestock grazing (IGAC, 2007; Yanine, 2010). These farming practices have increased N inputs in soil through the application of organic and inorganic nitrogen fertilizer (e.g., manure, urea, NPK), generating significant environmental pollution and loss of soil fertility, through the alteration of processes such as nitrification and denitrification (Hurni *et al*., 2008). The first stage of nitrification (conversion of ammonium to nitrite) is carried out by ammonium-oxidizing archaea (AOA) and bacteria (AOB) (Avrahami and Bohannan, 2007). Subsequently, nitrite is oxidized to nitrate by nitrite-oxidizing bacteria (NOB), which can leach and contaminate ground and surface water (Chu *et al*., 2007; Junier *et al*., 2009; Kong *et al*., 2010). AOB can use nitrite as an electron acceptor in environments with a low availability of oxygen to generate NO, via reduction of NO_2^- and produced N_2O (Bock and Wagner, 2006), another problem caused by nitrification is the acidification of the soils, which generates negative effects on the health and productivity of agricultural ecosystems.

Denitrification is a part of the global nitrogen cycle in which fixed nitrogen in the biosphere is returned to the atmosphere, and is mediated by diverse communities of microorganisms. This process is conducted by bacteria that use NO_3^- as an electron acceptor (Paul, 2007). Additionally, the production of N_2O (by AOB or DB) is a major source of air pollution, contributing to the greenhouse effect (Bock and Wagner, 2006), which together with nitrification produces N losses, lower efficiency of fertilizers on crops, and affects the economy of farming (Pacheco *et al*., 2002).

Previous studies have shown that AOB and nitrogen fixing bacteria can be used as indicators of soil quality and health, due to their high sensitivity and low physiological tolerance to natural and anthropogenic disturbances (Nielsen and Winding, 2002; Horz *et al*., 2004; Nyberg *et al*., 2006; Roldán *et al*., 2008). Microorganisms possess the ability to give an integrated measure of soil quality, an aspect that cannot be obtained with physicochemical measures and analyses of diversity of higher organisms. Microorganisms respond quickly to changes so they rapidly adapt to environmental conditions. This adaptation potentially allows microbial analyses to be used in soil quality assessment, and changes in functional groups and activities may therefore function as an excellent indicator of change in soil quality (Nielsen and Winding, 2002).

Despite the biotechnological and environmental interests of N bacteria, few studies have been conducted in Colombia on the effect of different agricultural management practices and land uses in the density of functional groups associated with N cycling has been evaluated (Torres and Lizarazo, 2006; Roldán *et al*., 2008; Cardona *et al*., 2009). This can be related with the difficulties encountered during traditional laboratory culture techniques caused by factors such as slow growth, inadequate selection of the culture medium, ease of contamination by heterotrophic and possible activity loss after cultivation and preservation (Avrahami and Bohannan, 2007; Chu *et al*., 2007; Gómez, 2008).

The main goal of this study was to evaluate the effect of different land uses on the density of NB and DB. For this, it was necessary to standardize the optimal conditions for cultivation and enumeration of AOB, NOB and DB through the technique of most probable number (MPN). Finally, the isolated bacteria were taxonomically identified by amplification and sequencing of the 16S rRNA gene.

Materials and methods

Study area

The study area was located in the basins of the La Vieja and Otún rivers (Colombia Coffee Region), in the departments of Quindio, Valle del Cauca and Risaralda. These sites were selected based on criteria of spatial heterogeneity, altitudinal gradient and landscape structures by means of a canonical correspondence analysis of biotic and abiotic characteristics as well as the presence and representation of different land uses (coverage) of the region (Yanine, 2010). For each basin, the most representative land uses were selected: unshaded coffee (USCF), guadua and cattle pastures in the basin of La Vieja and onion monocultures, guadua and pine plantations (*Pino patula*) for Otún (Camargo, 2006). In each basin, systems with low human disturbance were selected (secondary forests) which were used as a reference site. For each land use, two different farms were selected and sampled (n=3).

Soil sampling

In each farm, three quadrants (2.5 x 2.5 m) were randomly established and samples were taken at the vertices and center. NB samples were taken using an auger (20 x 5 cm) which were mixed and homogenized to form a composite sample. For DB, a core sampler with cylinders (15 cm) was used to ensure low oxygen conditions. The samples were refrigerated (4-6°C) until processing in the laboratory. There were two sampling events (SE) in June and October, 2006.

Physicochemical analysis

We determined the soil *in situ* temperature, pH by the 9045C method (EPA, 1995), nitrate percentage by method No. 366 (HACH, 1994) and humidity (IGAC, 1994)

Microbiological analyzes

For optimal growth of AOB, NOB and DB, different carbon and energy sources reported in the literature were evaluated with the MPN technique (Stienstra *et al*., 1993; Aakra *et al*., 1999; Bigelow *et al*., 2002). NB were evaluated for the following carbon sources at different concentrations (g L^{-1}): $NaHCO_3$ (0.125) and $CaCO_3$ (5.0 and 1.0). As an energy source (g L^{-1}): $(NH_4)_2SO_4$ (0.5 and 0.13) for AOB and $NaNO_2$ (2.0 and 0.035) for NOB. For DB, KNO_3 (2.0) was used as a terminal electron acceptor and as carbon sources: ethanol (1.0), glutamic acid (1.7) and sodium acetate (1.0) were evaluated.

Determining of NB density and isolation

We used the MPN technique in 96-well plates (Rowe *et al*., 1977) for determining the density of NB, using previously standardized mineral salts media. In each well, 100 µL of culture media for AOB and NOB and 50 mL of each dilution of the samples (10^{-2}-10^{-7}) were added with 5 replicates per dilution. The plates were incubated at 24±2°C for four weeks in darkness. AOB and NOB growth were verified by the disappearance of nitrite production, using diphenylamine and *Griess*-llosvay *reagents*, respectively (Rowe *et al*., 1977; Phillips *et al*., 2000). The combination of positive and negative wells was analyzed in the Most Probable Number program (version 4.04) and the results were expressed as MPN of NB/g dry weight (g_{dw}). For the isolation of these bacteria, the dilution to extinction technique was used (Aakra *et al*., 1999).

Determining of DB density and isolation

The MPN technique using the Hungate methodology was employed for DB (Pachón and Posada, 2003), with incubation for 15 d at 28±2°C. Growth and isolation was performed following the protocol described by Gómez (2008) and Pachón and Posada (2003).

Extraction of DNA from pure cultures of bacteria

Two methods were used for extraction of DNA: the first by boiling at 95°C by suspending a colony in 50 µL of TE buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, pH 8.0). The suspension was subjected to an initial heating (100°C for 15 min), followed by cooling on ice (5 min) and subsequent centrifugation at 13,000 rpm for 2 min. In the second method, lysozyme (10 µg ml^{-1}) was used. The two methods followed the protocol described by Sambrook and Russell (2001).

PCR amplification of the gene 16S rRNA

To amplify the V3-V5 regions of the 16S ribosomal subunit, the universal bacterial primers 341F and 907R were used (Casamayor *et al*., 1999). All reactions were performed in a final volume of 25 µL containing 0.2 µM of each primer, 0.25 mM deoxynucleotide triphosphates mixture, 1X buffer, 2.5 mM $MgCl_2$ and 0.025 U μL^{-1} of Taq polymerase (Taq Polymerase Kit, Promega) and 2 µL as DNA template. The program used consisted of 35 cycles with an initial denaturation phase at 94°C for 1 min, an annealing step at 54°C for 1 min and a final extension phase of 72°C for

10 min (Muyzer *et al*., 1993). The PCR product was verified by electrophoresis in agarose 1% (w/v) (120 V for 40 min) (Sambrook and Russell, 2001).

Sequence analysis and determination of the taxonomic affiliation

Sequences were analyzed using the BLAST program StandAlone version, using the Smith-Waterman algorithm (V. 2.2.9) from the National Center of Biological Information (NCBI) page and alignments were compared against the RDP database (Ribosomal Database Project) (v. 10).

Statistical analysis

The data that did not meet the requirements of normality and homogeneity of variances were transformed using logarithm base 10. To evaluate the effect of different land uses on the density of NB and DB, analysis of variance and Tukey-Kramer test ($P \leq 0.05$), using the JMP statistical program (V.9.0), were used. To determine the relationship between soil physicochemical factors on the density of NB and DB, Pearson correlation analysis was performed.

Results and discussion

Physicochemical analysis

In general, soils of the study were characterized by a low pH (4.9-6.3) (Tables 1 and 2). This could be related mainly to the type of fertilizer used and the low buffering capacity of soil (Donahue *et al*., 1981). During the oxidation process of NH_4^+ by microorganisms, soil acidification occurs as a result of the continuous release of H^+ (Kemmitt *et al*., 2006). Additionally, the metabolism of microorganisms and roots generates CO_2 and soluble organic acids, decreasing the pH and behave as free acids of the soil (Inselsbacher *et al*., 2010). Our data showed a significant effect of land use on the pH, where forests had significantly lower pH values in the two river basins ($P \leq 0.05$) (Tables 1 and 2). These land uses had higher organic matter content, which when is decomposed, release active carboxylic and phenolic groups, behaving as weak acids, acidifying the soil (Ritchie and Dolling, 1985).

Forest and guadua values were significantly higher for humidity and lower for temperature ($P \leq 0.05$) (Tabs. 1 and 2). This may be associated with the presence of a large tree cover in the forests which generates more leaf loss input, less solar radiation and decreased wind speed, factors that contribute to lower temperatures and evaporation processes (Sylvia *et al*., 2005). On the other hand, higher temperatures and lower humidity was observed in pasture and onion uses (Tables 1 and 2), possibly caused by the absence of tree cover and the use of conventional agricultural management practices (tillage and overgrazing), which cause soil compaction affecting water infiltration processes (Murgueitio, 2003; Yanine, 2010). Finally, in forests and some guadua sites, the observed lowest values of nitrates could be due to the slow processes of mineralization that could limit the growth of DB (Azam *et al*., 1995).

TABLE 1. Comparison of physicochemical parameters in La Vieja river basin during study.

La Vieja	Land use	pH	Humidity (%)	T°	$N\text{-}NO_3^-$ (mg kg^{-1})
June, 2006	Forest	4.9±1.06	36.6±10.3	19.7±2.19	1.42±0.45
	Guadua	5.5±0.24	39.9±6.8	22.2±0.14	1.97±0.12
	USCF	5.3±0.29	34.8±6.3	22.9±0-64	1.69±0.19
	Pasture	5.2±0.84	32.7±5.0	24.6±2.5	1.70±0.44
October, 2006	Forest	5.0±0.41	32.1±12.0	18.2±1.84	1.40±0.06
	Guadua	6.3± 0.11	23.6±8.24	22.8±0.30	1.49± 0.03
	USCF	6.0±0.43	21.6±6.39	22.4±0.71	1.50±0.34
	Pasture	5.6±0.80	21.2±6.85	26.3±2.83	1.62±0.05

TABLE 2. Comparison of physicochemical parameters in Otún river basin during study.

Otún	Land use	pH	Humidity (%)	T°	$N\text{-}NO_3^-$ (mg Kg^{-1})
June, 2006	Forest	4.9±0.58	41.3±8.07	18.3±0.10	1.31±0.08
	Guadua	5.4±0.24	39.2 ±2.48	18.0 ±0.49	1.74±0.20
	Onion	6.1±0.25	30.3±3.99	23.0±0.97	1.77±0.58
	Pine plantation	5.5±0.51	37.9±7.27	20.7±0.96	1.54±0.65
October, 2006	Forest	5.4±0.18	26.9±12.0	13.9±2.88	1.33±008
	Guadua	5.8± 0.17	27.1±8-24	16.5±8.57	1.42± 0.04
	Onion	5.8±0.17	25.3±6.39	21.1±2.10	1.83±0.17
	Pine plantation	5.7±0.31	31.5±6.85	18.43±1.41	1.55±0.07

Optimal culture conditions for the growth of NB and DB

For all the land uses evaluated, there were significantly higher densities of AOB and NOB using $NaHCO_3$ (0.125 g L^{-1}) as a carbon source ($P \leq 0.05$) (data not shown). Additionally, the use of this compound facilitated the reading of the positive wells, reducing turbidity and minimizing evaporation of the culture medium. We selected the concentration of 0.5 g L^{-1} $(NH_4)_2SO_4$ for AOB, which has been previously reported (Aakra *et al*., 1999; Bruns *et al*., 1999) and 0.035 g L^{-1} of $NaNO_2$ was selected for NOB as an energy source (Stienstra *et al*., 1993). For DB a mixture of ethanol and sodium acetate as a carbon source were selected (Gómez, 2008).

NB and DB densities in the different land uses

AOB densities for the first SE did not differ significantly between the different land uses ($P \leq 0.05$), although pastures and USCF had higher densities (Fig. 1). For the second SE, there was a significant decrease in AOB counts in USCF (La Vieja) and forest (Otún) ($P \leq 0.05$) (Fig. 1). Additionally, onion and forest (Otún) had AOB densities significantly higher and lower, respectively ($P \leq 0.05$), which may be associated with pH values observed in these land uses. It is well known that in agricultural soils, the density and activity of AOB decreases when the pH is below 5.5 or above 9.0, the optimum pH for nitrification is between 7.6-8.0 (Atlas and Bartha, 2002; Nugroho *et al*., 2007), therefore, significantly lower values of pH present in the forest and higher in onion (Table 2) may have affected AOB density.

For all land uses assessed, AOB showed positive correlations ($P \leq 0.05$) with pH, nitrates and temperature (R^2 = 0.95, 0.85, 0.70, respectively) and negatively with moisture (R^2 = -0.84). This could be related to increased moisture in the soil that could reduce the availability and transfer of oxygen, this being the final electron acceptor needed for the oxidation of ammonia.

NOB densities in La Vieja were significantly lower ($P \leq 0.05$) for the second SE (Fig. 2). Unlike AOB, NOB did not differ significantly between land uses for any SE, but the forest and onion counts were lower and higher, respectively, for Otún ($P \leq 0.05$) (Fig. 2). In general, higher counts of NB and mainly AOB in the land uses pasture, USCF and onion can be associated with implemented agricultural management practices, which involve the addition of nitrogen fertilizers (NPK-15: 15: 15, urea and organic fertilizers: cattle and poultry manure) that produce an increased availability of ammonium for NB (Hayden *et al*., 2010; Inselsbacher *et al*., 2010). The results are in agreement with previous studies which shown that agricultural practices, such as mineral N

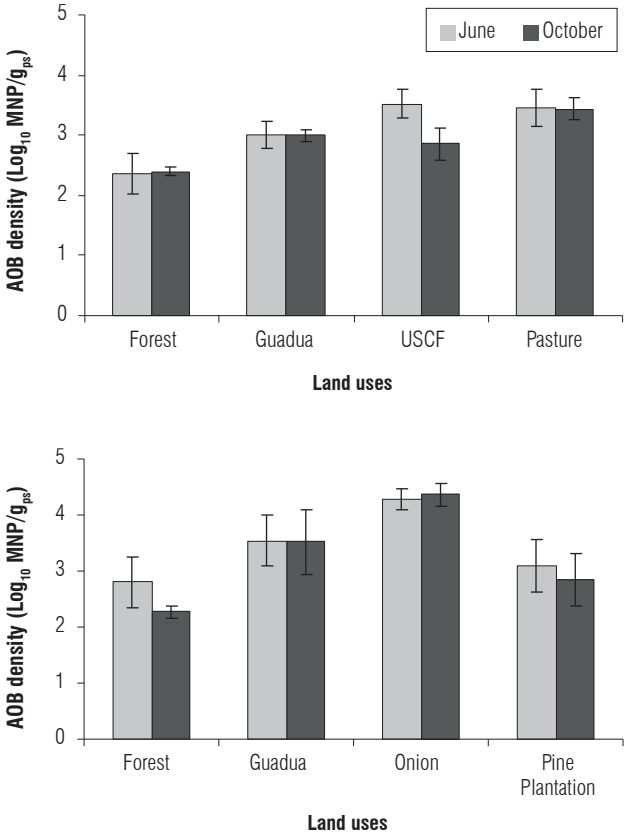

FIGURE 1. AOB density in La Vieja (above figure) and Otún (lower figure). With an average (n=6) ± 1 standard deviation.

fertilizer application, significantly alter AOB populations, which subsequently impact nitrification and production rates of nitrous oxide (N_2O), a potent greenhouse gas, in crop production systems (Kowalchuk and Stephen, 2001). Additionally, the grazing and tillage activities cause changes in physicochemical properties of soil (e.g., porosity, bulk density, penetration resistance, availability of oxygen and nutrients) affecting the density of NB (McNaughton *et al*., 1997; Busso *et al*., 2001; Patra *et al*., 2005; Siavosh *et al*., 2000; Murgueitio, 2003). On the other hand, pastures showed a positive correlation ($P \leq 0.05$) between the density of AOB and NOB and nitrates (R^2 = 0.942 and R^2 = 0.907, respectively), which is the end product of nitrification, reflecting the active role of these two microbial groups in N cycling.

The low densities of NB in forests (Figs. 1 and 2) could be associated with low pH values, but also with low N availability and quality of organic matter. In forests, trees are the predominant vegetation, which produce woody plant residues with high C: N ratios and high concentrations of polyphenolic compounds (Kowalchuk and Stephen, 2001), which have proven to be toxic and inhibitory to

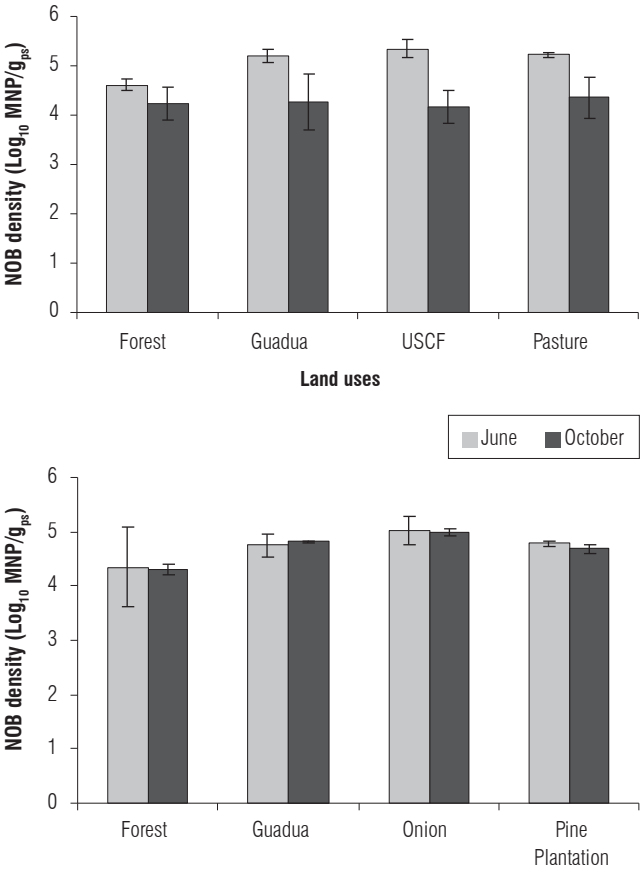

FIGURE 2. NOB density in La Vieja (above figure) and Otún (lower figure). With an average (n = 6) ± 1 standard deviation.

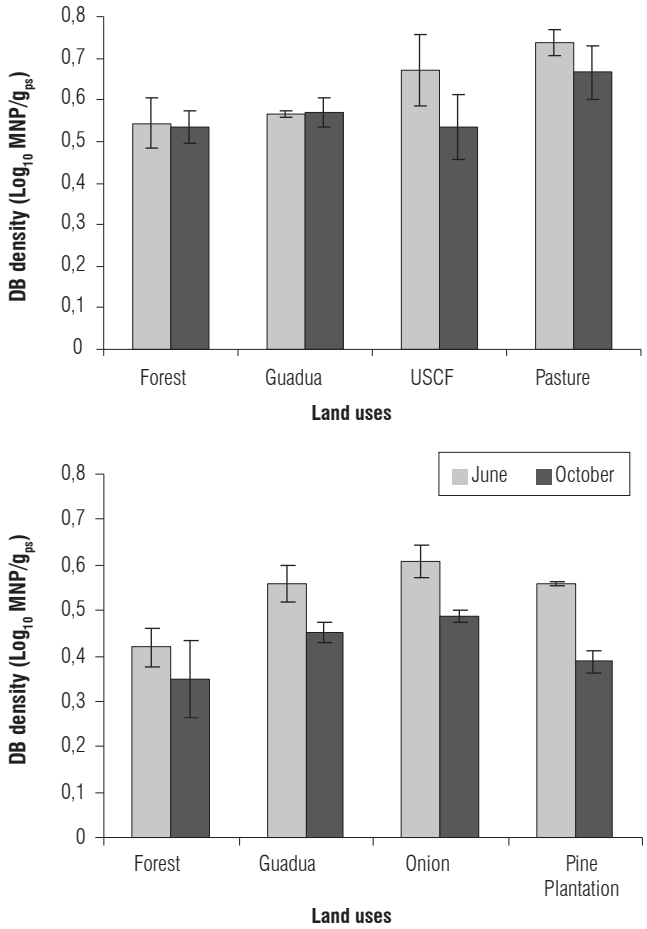

FIGURE 3. DB density in land uses in La Vieja and Otún river basins. With an average (n = 6) ± 1 standard deviation.

AOB, mainly Nitrosomonas (Blanco, 2007; Kowalchuk and Stephen, 2001). The residues that have a high C:N ratio produce strong competition between NB and heterotrophs for available N in the soil, with heterotrophs leading the competition for available NH_4^+, decreasing its density (Verhagen *et al*., 1995).

Finally, DB was significantly higher in USCF and pasture (La Vieja) for the first SE, and for the second SE was significantly higher only in pasture ($P \leq 0.05$). Additionally, there was a significant decrease ($P \leq 0.05$) in counts in the second SE in USCF and pasture (Fig. 3). Furthermore, onion (Otún) presented the highest densities of DB for both SEs (Fig. 3), with the forest and pine plantation land uses having significantly lower densities ($P \leq 0.05$).

DB are favored by the products generated during nitrification by NB, which also are stimulated by organic and inorganic fertilizers (Ellis and Pennington, 1989; Hill and Cardaci, 2004). Thus, in pasture and onion, positive correlations were found between the densities of AOB and DB ($R^2 = 0.90$ and $R^2 = 0.92$, respectively for pasture) and NB and DB ($R^2 = 0.84$ and $R^2 = 0.98$, respectively for onion). On the other hand, pastures showed a positive correlation between DB density and nitrates ($R^2 = 0.981$, $P \leq 0.05$), indicating that the high densities seen in this land use were favored by nitrates. These results agree with those reported by Kong *et al*. (2010), who showed a greater abundance of *nosZ* genes as a result of a higher input of organic C from fertilizations, which contain easily degradable C compounds, which stimulate denitrification. Conversely, the low DB densities in forest are possibly due to the low NB densities, which is consistent with that reported by Rich *et al*. (2003).

Taxonomic identification by the 16S ribosomal subunit of AOB, NOB and DB

Only 13 AOB were isolated due to the difficulty in obtaining pure cultures because the heterotrophic bacterial contamination. Of the strains isolated, 11 showed high similarity (98-99%) with the genus *Hyphomicrobium*. While this genus has been widely recognized as

denitrifying, it has been demonstrated as being capable of using ammonium as an energy source (Brook *et al*., 1987). The 2 others showed similarity with the genera *Mesorhizobium* (95%) and *Bradyrhizobium* (98%) (Supplementary Table S1), which have not been reported as AOB, but as N-fixing (Teske *et al*., 1994; Compton *et al*., 2004).

For NOB, 7 isolates were found; 6 of them had high similarity (97-100%) with the species *Oligotropha carboxidovorans* and the remaining one presented similarity (100%) with *Rhodopseudomonas palustris* (Table supplementary S2), which are phylogenetically related with each other and to genera reported as nitrite oxidizers (Meyer, 2005; Aamand *et al*., 1996).

As for DB, 30 of the 39 isolates were identified, and 9 of them showed similarity (92-99%) with partial 16S rRNA sequences for non-cultivable bacteria. The genera found in La Vieja corresponded to *Pseudomonas*, *Klebsiella*, *Citrobacter*, and *Microvirgula*, while in Otún the genera found corresponded to *Klebsiella*, *Bacillus*, *Enterobacter*, *Microvirgula*, and *Pantoea.,* These genera correspond to the β-proteobacteria, γ-proteobacteria and Bacillales subdivisions (Supplementary Table S3), that have been previously reported by other studies (Cofman and Levine, 1986; Patureau *et al*., 2000; Rösch *et al*., 2002; Braker and Tiedje, 2003; Dandie *et al*., 2007; Morozkina and Zvyagilskaya, 2007). On the other hand, the genus *Bacillus* was only found in USCF and onions, and has been reported as one of the most representative microorganisms in altered soils (Dandie *et al*., 2007).

Conclusions

Land use had a significant effect on NB and DB densities. In addition, the higher densities of these microorganisms were observed in pastures, USCF and onion. Land use and land management practices (e.g., use of nitrogen fertilizers, tillage and grazing) had an effect on the densities of these bacteria associated with the N cycle. On the other hand, AOB densities were significantly different in the evaluated land uses, suggesting its use as a potential indicator of soil quality. On the other hand, forests had the lowest AOB densities, possibly due to reduced availability of N and release of allelopathic compounds. Moreover, although not taxonomically identified, NB bacterial genera and species were found involved in N cycling and closely related to the genera *Nitrosomonas* and *Nitrosospira*, which are reported as abundant in agricultural soils. As for DB, all sequences related to genera and species previously reported in agricultural soils were found.

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