Agronomía Colombiana
ISSN: 0120-9965
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Universidad Nacional de Colombia
Colombia

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Agronomía Colombiana, vol. 34, núm. 1, 2016, pp. 82-91
Universidad Nacional de Colombia
Bogotá, Colombia

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Cambios temporales de las comunidades reductoras de nitrito en un campo de suelo arrocero en Ibagué (Colombia)

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ABSTRACT

Denitrification and nitrification are microbial processes that regulate the cycle of nitrogen and nitrous oxide, which is considered an important greenhouse gas. Rice field soils have been known to have strong denitrifying activities; however, the microorganism structure that is responsible for denitrification and the temporal variation of these communities in the agricultural soils of Ibague (Colombia) is not well known. In this study, the denitrifying community composition was compared between a rice field soil and an uncultivated soil at three different times during the year using a terminal restriction fragment length polymorphism analysis of the *nirS* functional gene, which codes the enzyme that reduces nitrite, one of the key steps in the denitrification process. The results showed changes in the richness, relative abundance and diversity of the operational taxonomic units between the soils and sampling times. The canonical correspondence analysis indicated that the moisture and the pH were the environmental factors that explained the observed changes in the *nirS*-type denitrifiers' community composition in the studied soils.

Key words: denitrification, nitrogen cycle, soil microorganisms, *nirS* gene, diversity.

RESUMEN

La desnitrificación y la nitrificación son procesos microbianos que regulan el ciclo del nitrógeno y del óxido nitroso, el cual es considerado como un importante gas invernadero. Los suelos cultivados con arroz presentan una fuerte actividad desnitrificante, sin embargo, la estructura de los microorganismos responsables de la desnitrificación y la variación temporal de estas comunidades en suelos agrícolas de Ibagué (Colombia) no es muy conocida. En este estudio se comparó la composición de la comunidad desnitrificante entre un suelo cultivado con arroz y uno no cultivado, en tres épocas del año, usando el análisis del polimorfismo de los fragmentos de restricción terminal del gen funcional *nirS* que codifica la enzima que reduce el nitrito, uno de los pasos claves del proceso de desnitrificación. Los resultados mostraron cambios en la riqueza, abundancia relativa y diversidad de unidades taxonómicas operacionales entre los suelos y épocas de muestreo. El análisis de correspondencia canónica indicó que la humedad y el pH fueron los factores ambientales que explicaron los cambios observados en la composición de las comunidades desnitrificantes tipo *nirS* en los suelos estudiados.

Palabras clave: desnitrificación, ciclo del nitrógeno, microorganismos del suelo, gen *nirS*, diversidad.

Introduction

Denitrification is a microbial process that occurs predominantly in flooded soils with 60-90% pore-water (Bateman and Baggs, 2005). During this process, a sequential reduction of nitrate, nitrite, nitric oxide, and nitrous oxide to dinitrogen takes place under low oxygen conditions through different taxonomic groups, including bacteria, archaea and fungi (Laughlin and Stevens, 2002; Zumft and Kroneck, 2006). Some studies suggest that biocides and pesticides additions, changes in soil use and irrigation type could influence the diversity (Avrahami *et al.,* 2002; Zhou *et al.,* 2002, 2011), composition (Nüsslein and Tiedje, 1999), microbial stability (Girvan *et al.,* 2005) and quantity of N_2O emissions from soils (Senbayram *et al.,* 2009).

Nitrogen plays an important role in agricultural production and different microbial communities regulate its transformation in the soil through the expression of functional genes within the resident microbial community. For denitrification, the genes that code for nitrite reductase (*nirS* and *nirK*) and nitrous oxide reductase (*nosZ*) have been used for accessing the denitrifying community diversity (Horn *et al.,* 2006). The *nirS* gene has been widely used to detect the denitrifiers responsible for N_2O production through nitrifier-denitrification and heterotrophic nitrification (Braker and Conrad, 2011) and also to determine that the *nirS* abundance is more related to the nitrate and organic carbon levels and soil water content than to N_2O emissions (Dandie *et al.,* 2011). Other researchers have reported the existence of a relationship between the *nirS*

Received for publication: 10 September, 2015. Accepted for publication: 28 March, 2016. Doi: 10.15446/agron.colomb.v34n1.52993

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gene copy number and the $N_2O/(N_2O+N_2)$ molar ratio (Cuhel *et al.,* 2010) and that the *nirS,* but not the *nirK*-type denitrifiers, is related to denitrifying activity, suggesting that the assembly of communities responds to distinct environmental gradients (Enwall *et al.,* 2010). Moreover, it has been reported that *nirS*-type denitrifiers have the attributes for generating its own niches, which is not possible with the *nosZ* gene (Hallin *et al.,* 2009), and that, with this functional gene, can be detected the nitrite reducers microorganisms laking of nitrite reductase, and consequently generating N_2O as a denitrification final product (Jones *et al.,* 2008). In general, studies of the denitrifier community diversity in soils have been developed with different molecular approaches, including the terminal restriction fragment length polymorphism (TRFLP) (Rösch and Bothe, 2005), RFLP (Priemé *et al.,* 2002; Stress *et al.,* 2004), cloning (Rösch *et al.,* 2002), qPCR and RT-PCR (Kandeler *et al.,* 2006; Philippot *et al.,* 2009; Cuhel *et al.,* 2010), functional microarrays (Berthrong *et al.,* 2009) pyrosequencing (Palmer *et al.,* 2012) and, more recently, stable isotopes probing (Ishii *et al.,* 2011). But, despite advances in studies on the denitrifying diversity in soils, knowledge on how the structure of the community is related to its function is still scarce (Braker and Conrad, 2011).

In Colombia and specifically in the department of Tolima, a large part of the land is used for growing rice, sorghum and corn. These soils are classified as "Entichaplustolls", characterized by moderate levels of organic matter, low phosphorous, nitrogen and pH; and high levels of fertilization (IGAC, 2004). The soils in this area are influenced by strong temperature fluctuations (between 25 and 35°C) and by effects from the "Niño-Niña" event that alter the rain-dry seasons. The studies developed in Colombia involving microbial communities in agricultural areas have focused on the isolation of contaminant-degrading bacteria from soils (Arbeli and Fuentes, 2010), on the composition and enzymatic activity of soil communities (Vallejo *et al.,* 2012), on characterization of microbial communities in relationship with agricultural practices (Avellaneda-Torres, 2014), on the study of microbial communities in the rhizosphere (Vanegas *et al.,* 2013) and on activity of some nitrogen cycling microorganisms in soils with different uses (Cañon-Cortázar *et al.,* 2012; Avellaneda-Torres, 2014). However, at present, there are no studies on the structure and temporal variation of denitrifiers despite their crucial role in the N_2O emissions and nutrient recycling into the soil. Given the lack of studies in this area, the main objective of this research was to analyze the composition of nitrite reducing denitrifying communities in rice field soils at different times during the year, in comparison with uncultivated soils, and determine which environmental factors could drive its structure.

Materials and methods

Collection and manipulation of samples

Soils samples were taken at Ibague (Tolima) from an area cultivated with rice (4°25'30" N - 75°09'27.5" W), which was irrigated regularly and was in the growing phase during the different samplings. The other soil samples were taken from one non-agricultural area or control (4°25'29.3" N - 75°11'59" W), which was near the rice field with similar geomorphological conditions. The sampling was done during the rainy season (October 2008), samples denominated AL08 (agricultural area) and CL08 (control area); in the rain-dry transition (March 2009), samples denominated AT09 and CT09; and during the dry season (July 2009), samples denominated AS09 and CS09. For the soil sample collection, a 10 x 10 m quadrant was established in each area, from which six soil cores (2.5 cm diameter) were taken at random at a depth of 0-20 cm during each sampling time in the same quadrant. Subsequently, the samples were mixed, homogenized, sieved (for 4 mm diameter) and subdivided for the physical-chemical and molecular analyses (0.25 g sample) in sterile whirlpack bags that were frozen at -20°C until their use.

Analysis of physical and chemical variables

The following parameters were determined according to the protocols described by IGAC (2006) for the soil analysis. The soil moisture was determined by drying samples at 100°C for 48 h. The content of soil organic matter was reported as the difference between the dry weight (60°C/24 h) and weight of the residue after combustion at 450°C. The content of total nitrogen was determined by the Kjeldahl method. The content of soil organic carbon was determined by combustion. Nitrate quantification was done with the spectrophotometric-selective electrode method and pH determination was done by the potentiometric method.

DNA extraction and amplification of the nitrite reductase gene

For the DNA extraction, triplicate samples of 0.25 g were homogenized for a total of 3 g of soil, from which DNA was extracted using a MoBio kit following the manufacturer's instructions. Subsequently, 10 ng μL^{-1} of DNA were used to perform the *nirS*-gene amplification using the primers Cd3aF-FAM and R3cd (Michotey *et al.,* 2000; Throbäck *et al.,* 2004). The PCR protocol was standardized under the following conditions: 94°C-5 min, 10 cycles at 94°C-30 s, alignment beginning at 58°C-30 s with subsequent

declining of 0.5°C in each cycle, and extension at 72°C-40 s; the remaining 30 cycles were done at 94°C-30 s, 55°C-30 s and extension at 72°C-40 s. The mixture of PCR reagents (25 µL) included: 200 µM of dNTPs, 10 pmol of each primer, 1.25 U Red AccuTaq polymerase and 1 µL (10 ng μL^{-1}) of DNA. The products of the three replicate PCRs were combined. The PCR-products were purified with the Quiaquick PCR purification kit (Qiagen, Chatsworth, CA) and quantified by spectrophotometry at 230, 260 and 280 nm.

Amplicon digestion

Purified *nirS*-amplicons (100 ng), marked with fluorescence, were digested with enzymes *Msp*I and *Hae*III at 37°C-1 h following the manufacturer's instructions. The digested products were purified with columns AutoSeq G-50 (Amersham Biosciences, Piscataway, NJ). From 2 µL of the digested product were mixed with 12 µL of deionized formamide (Applera, Darmstadt, Germany) and 0.2 µL of a DNA standard (50-1,000 pb marker X-Rhodamine, BioVentures, Murfreesboro, TN). TRFLP were separated by electrophoresis in a DNA 310 sequencer (Applied Biosystems, Foster City, CA). This analysis was performed in duplicate to verify reproducibility of the TRFLP on each assay and sample.

Analysis of TRFLP

From the electropherograms, the size (in base pairs) of each TRF marked with fluorescence was determined by comparison with the DNA internal standard using GeneScan 3.71 (Applied Biosystems). In this analysis, each TRF was equivalent to a specific operational taxonomic unit (OTU) within each sample and the fluorescence detected for each TRF corresponded to the abundance of each OTU within each sample. For the subsequent data analysis, only the TRF, with a fluorescence of > 70 units over the background fluorescence and > 69 bp over the size of primer dimers, was taken into account. The results obtained for all samples were normalized to the total units of fluorescence using the iterative standardization procedure described by Dunbar *et al.* (2001). The relative abundance of each TRF (%) was determined by calculating the ratio between the height of each TRF and the total height of the normalized TRFs from each sample.

Analysis of TRF in relation with the environmental parameters

Since the TRF patterns corresponded to different OTUs in each community, the richness, relative abundance and diversity of the OTUs were assessed within them. The diversity of each community was calculated using the Shannon diversity index. The similarity between the communities was calculated using the Bray Curtis index with Biodiversity Pro 4.0 (The Scottish Association for Marine Science, UK). The relationship between the *nirS*-gene composition and the soil environmental parameters was carried out through a correspondence canonical analysis (CCA) using PC-ORD version 4.01 (MjM Software, Gleneden Beach, OR).

Results

The *nirS* gene amplicons (490 bp) were successfully amplified for all of the DNA extracts obtained from the soil samples. The minor resolution for the structure of the *nirS*-communities in the soils was obtained with the *Hae*III enzyme (16 OTUs, Fig. 1) in comparison with the enzyme *Msp*I (21 OTUs, Fig. 2). The results showed that, although many *nirS*-OTUs were shared between the cultivated and uncultivated soils (75, 113, 116, 132, 136, 224 and 257), there were also unique OTUs in each sample and/or sampling time. For example, the OTU 210 was only observed in the transition time (AT09) and the OTUs 363 and 275 were only observed during the rainy season (AL08) in the cultivated soil. In the rice field soil, the OTU 116 presented a higher relative abundance (~ 70%) during the dry season, whereas the transitional period was dominated by OTU 190 (~ 35%). In the uncultivated soil, 50% of the *nirS*-community corresponded to OTUs 116 and 132, which were observed during the rainy season; while, in the transition time and dry season, the relative abundance of OTUs was mostly equally distributed among them, with the exception of a slight abundance (~ 25%) of OTU 257.

On the other hand, the wide distribution of some OTUs was also confirmed with the similarity analysis, which showed 86% relationship between the *nirS*-denitrifying communities between the rice field soil during the transition time (AT09) and the uncultivated soil during the rainy season (CL08). However, between both communities, the OTU's relative abundance variation and a higher richness of OTUs in the cultivated soil (10 OTU) than in the control soil (8 OTU) was observed. The communities inhabiting the cultivated area during the dry season (AS09) presented the lowest similarity (40%) and OTU richness (4) and diversity (H' = 0.69) (Tab. 1).

The digestion of the amplicons with the *Msp*I enzyme (Fig. 2) generated a higher richness of the OTUs (21) distributed in the studied soils. The *nirS*-type denitrifier community profile obtained with this enzyme showed that both soils also share OTUs, had specific OTUs, and presented changes

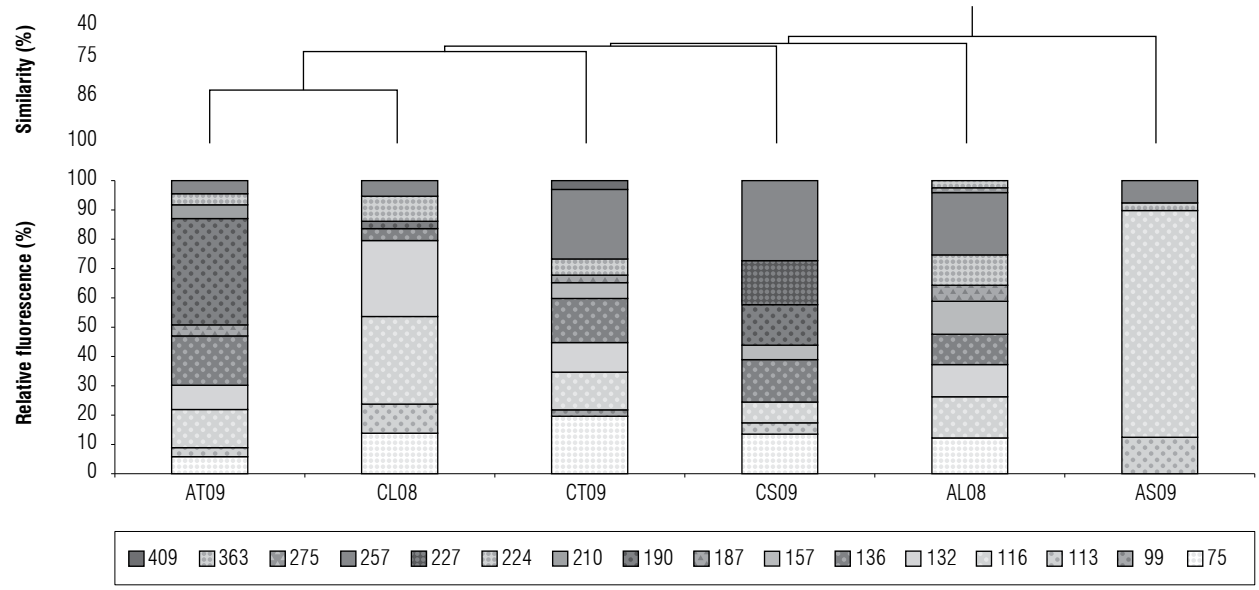

FIGURE 1. Similarity dendrogram and T-RFLP profile showing the relative abundance of each TRF obtained by *nirS*-amplicon digestion with the *Hae*III enzyme in rice soils from Ibague-Colombia. Soil samples of the agricultural (A) and control (C) areas in the rainy (AL08 and CL08), transition (AT09 and CT09) and dry seasons (AS09 and CS09). The size (bp) of each TRF is presented in the legend.

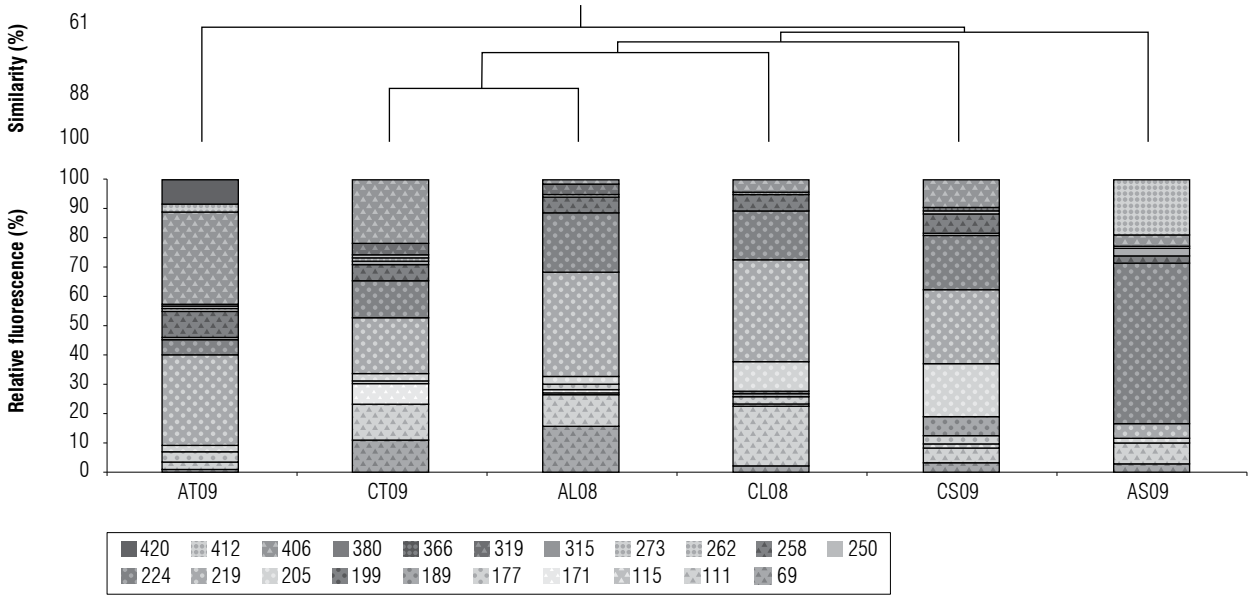

FIGURE 2. Similarity dendrogram and T-RFLP profile showing the relative abundance of each TRF obtained by *nirS*-amplicon digestion with *Msp*I enzyme in rice soils from Ibague-Colombia. Soil samples of the agricultural (A) and control (C) areas in the rainy (AL08 and CL08), transition (AT09 and CT09) and dry seasons (AS09 and CS09). The size (bp) of each TRF is presented in the legend.

TABLE 1. Estimation of *nirS*-OTUs diversity from the rice soil samples (Ibague-Colombia) digested with the enzymes *Hae*III and *Msp*I, based on the Shannon index (H') log base 10.

Enzyme	Samples					
	AT09	CT09	AL08	CL08	AS09	CS09
*Hae*III	1.08	1.08	1.00	0.95	0.69	1.00
*Msp*I	1.15	1.11	1.08	1.11	1.04	1.11

Soil samples of the agricultural (A) and control (C) areas in the rainy (AL08 and CL08), transition (AT09 and CT09) and dry seasons (AS09 and CS09).

in the relative abundance (RA) of TRFs, like the ones observed using the *Hae*III enzyme. From these OTUs, it was observed that nine (69, 111, 177, 205, 219, 224, 258, 315 and 406) of them were distributed in both soils (cultivated and control). In the rice field soil, the OTUs 420 (< 8% RA) and 380 (< 1% RA) were found only in the transition season; and OTU 412 was found in the dry season (~ 15% RA) and also in the transition time (< 2% RA). For the uncultivated soil, OTU 366 (< 1% RA) was typical of the dry season,

OTU 189 was found during the rainy (1% RA) and dry seasons (5% RA); and OTU 199 (1% RA) appeared only in the rainy season.

The similarity analysis of the TRFs obtained from the *nirS*-type denitrifier community with the *Msp*I enzyme showed a different association than the ones observed using enzyme *Hae*III. In this case, for example, a greater similarity (88%) of communities was detected between samples CT09 and AL08, given that they shared 10 of 13 OTUs. This analysis confirmed that the *nirS*-type denitrifier communities inhabiting the agricultural soil during the dry season (AS09) were different from the other communities (63% similarity) given its lowest richness of OTUs (10) (Tab. 1) and the dominance of OTU 224 (60% RA), which confirmed the variability in the structure of this community between the different seasons analyzed.

The results of the chemical and physical parameters measured in each soil showed that the soil moisture values were between 4.5 and 17.7% for the rice field soil and 3.1 to 18.6% for the control soil; the highest value of the soil moisture was recorded during the transition season (18.6% in control soil). The quantity of organic matter ranged from 1.4 to 9.0% with the highest and lowest values recorded in the AS09 and AT09 samples, respectively (Tab. 2).

TABLE 2. Chemical factors quantified in the rice soils (Ibague-Colombia) for each sampling time.

Sample	Moisture (%)	Organic matter (%)	Organic carbon (%)	Organic nitrogen (%)	pH	Nitrate (mg L^{-1})
AL08	10.0	8.6	4.9	0.4	6.4	nd
CL08	8.7	2.2	1.2	0.1	5.7	nd
AT09	17.7	1.4	0.8	0.09	6.5	0.38
CT09	18.6	2.6	1.5	0.1	5.7	0.43
AS09	4.5	9	5.2	0.4	5.2	0.3
CS09	3.1	4.6	2.6	0.2	6.4	0.5

Soil samples of the agricultural (A) and control (C) areas in the rainy (AL08 and CL08), transition (AT09 and CT09) and dry seasons (AS09 and CS09).

The soil organic carbon was also lower in the control soil (1.2 - 2.6%) when compared to the agricultural soil, where this parameter ranged between 0.8% (AT09 sample) and 5.2% (AS09 sample). This result was similar to the one observed for the soil organic nitrogen, which was higher in the cultivated soil during the dry and rainy seasons

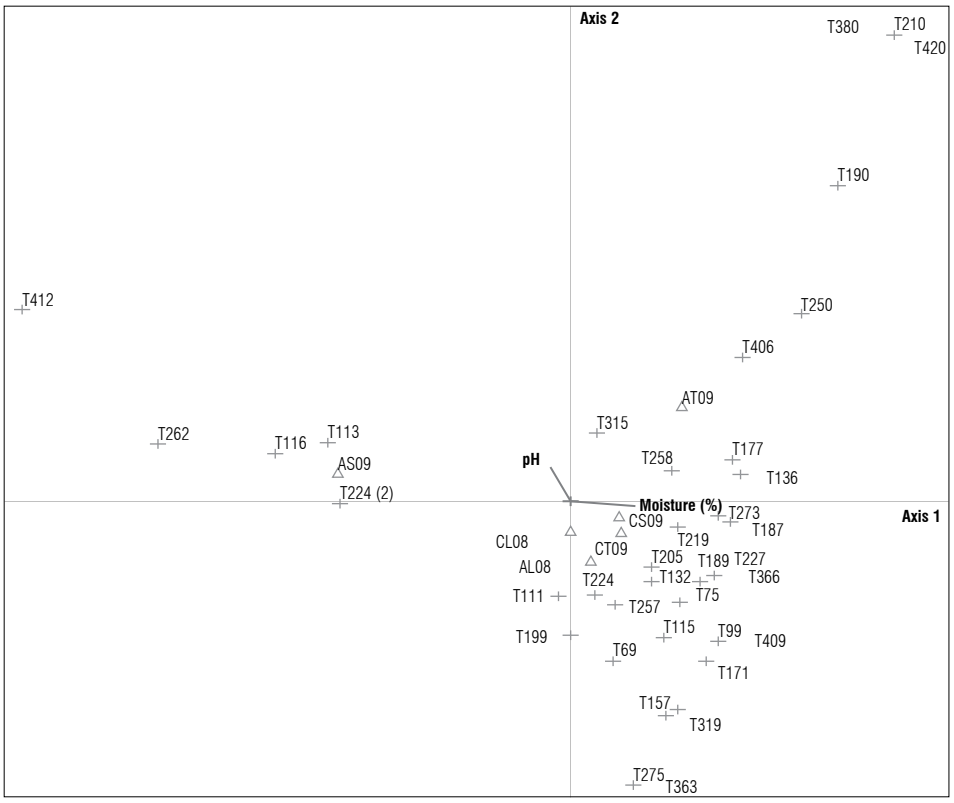

FIGURE 3. Canonical correspondence analysis between the *nirS*-OTUs (crosses) and environmental parameters determined in the different soil types and sampling times for rice soils from Ibague-Colombia. Soil samples (triangles) of the agricultural (A) and control (C) areas in the rainy (AL08 and CL08), transition (AT09 and CT09) and dry seasons (AS09 and CS09).

(0.4%). The pH varied between 5.2 (dry season) and 6.5 (transition time) in the agricultural soil; and between 5.7 (rainy season) and 6.4 (dry season) in the control soil. The nitrate concentration showed a slight decrease between the transition time until the dry season in the rice field soil, contrarily to the one observed for control soil, where a slight increase was detected.

In general, the results showed that the agricultural soil during the dry season (AS09) was characterized by the lowest percentage humidity, a low pH, and a high percentage of soil organic matter, carbon and organic nitrogen, while, during the transition time, the same area (AT09) was quantified by high values of moisture and pH, and low values of organic matter and organic nitrogen and carbon.

The results of the CCA showed a significant effect (P=0.010 by Monte Carlo permutation test) from the examined environmental variables on the *nirS*-type denitrifier community structure in both soils (Fig. 3). The data showed that the pH (correlation of 0.66) and soil moisture content (correlation of 0.58) explained 82.6% of the *nirS*-type denitrifier community structure found in each soil sample. The CCA grouped and separated the unique OTUs (380, 420 and 210) found in the agricultural soils in the AT09 sample, indicating that the *nirS*-type communities were characteristic of this area during this time; also, the CCA separated the OTU 412, which was detected only in the cultivated soil and was dominant in the AS09 sample. The OTUs 113, 116 and 224 were positioned around the AS09 sample, suggesting the importance of these OTUs in the structure of this community. The other OTUs were homogeneously distributed, suggesting that these denitrifiers were part of the permanent community which could be adapted to environmental changes during the year.

Discussion

The results showed that the *nirS*-type denitrifier community was diverse and occupied stable niches in both soils. The richness of the *nirS*-OTUs was similar to that reported for agricultural soils in China (Zhou *et al.,* 2011; Yuan *et al.,* 2012) and, in comparison with the other functional genes of denitrification, the richness was lower than previously reported for *nirK* communities in soils (Bremer *et al.,* 2007) and similar to that reported for *nosZ* communities in forest soils (Rich *et al.,* 2003). Although some studies have reported similarity in the composition of denitrifying communities between agricultural and natural areas (Dandie *et al.,* 2011) observed in the present research, other studies have found greater diversity in cultivated areas (Enwall *et al.,* 2005, 2010). This observation corroborates results found in this study in the rice field soil during the rainy and transition seasons, where a higher OTU richness was detected in both seasons when the rice was in the growing phase. Also, it has been suggested that soil cultivation may create new environmental niches that support a greater diversity (Zhou *et al.,* 2002; Stress *et al.,* 2004) despite the use of pesticides and herbicides (Griffiths *et al.,* 2000), contributing to a major N_2O emission by microbial communities inhabiting the so-called hotspots (Kong *et al.,* 2010). One has to bear in mind that, in soils, it is usual the heterogeneity and the presence of many microhabitat and that in this case, our data are only a snapshot of the *nirS*-type community composition in specific points and times, for which is difficult to generalize the results. Likewise, although functional genes have helped in the understanding of the composition of this polyphyletic community, limitations exisit in its use that are related to the amplification from the environmental DNA extracts, which could include DNA from dead microorganisms, DNA adsorbed by clays, variation in the number of copies of genes, presence of microorganisms in very low abundance that can´t be detected, preferential amplification of specific DNA depending on the selected primer and formation of pseudo-terminal TRF (Egert and Friedrich, 2003). Currently, many of the technical limitations (DNA extraction, PCR amplification and T-RFLP analysis) have been minimized and diversity studies have focused on analyzing all functional genes (*narG*, *nirS*, *nirK*, *nosZ*, *norB*) in each denitrifying community; however, the biggest challenge has been to find a better way to relate the structure and function of these microbial communities in the environment.

In general, the results obtained with both tetrameric restriction endonucleases showed that the *Msp*I enzyme produced a higher richness of OTUs than the *Hae*III enzyme probably because in the environmental *nirS*-sequences predominate the appropriate restriction place for the first one (5´...C↓CGG…3´), which has also been observed in other studies (Avrahami *et al.,* 2002; Castro-González, 2014). The data indicated that the dry season had a stronger impact on the *nirS*-type denitrifier community structure inhabiting the soil cultivated with rice, which decreased the richness and diversity of the OTUs and favored the dominance of particular OTUs (224 and 116) that were probably better adapted to the conditions of water stress. The results also indicated that the *nirS*-type denitrifier community structure (richness and abundance) was mainly dependent on two environmental parameters, the soil moisture and pH. These data supported previous studies where the structure of the *nirK* and *nirS* denitrifier communities was strongly

associated with the pH and soil water content in temperate agricultural soils (Enwall *et al.,* 2010; Dandie *et al.,* 2011), tropical soils (Carney and Matson, 2006); and even, on a continental scale (Fierer and Jackson, 2006; Lauber *et al.,* 2009). Changes in pH has been associated with decreases in *nosZ* community diversity in native soils (Stress *et al.,* 2004), with changes in the *nirK* and *nosZ* community compositions in soils exposed to intensive monoculture (Dell *et al.,* 2010), with the niche separation of the *nirS*-type denitrifier communities (Stress *et al.,* 2004; Enwall *et al.,* 2010; Dell *et al.,* 2010), and with the N_2O/N_2 ratio that is generated during the denitrification process (Philippot *et al.,* 2009). However, pH and soil moisture change the richness, diversity and abundance of denitrifying communities in agricultural soils. In this respect, it has been reported that rice soils fertilized for decades (Chen *et al.,* 2010) and hundreds of years (Bannert *et al.,* 2011) presented less variation in the composition and diversity of the *nirS*-type than *nirK*-type denitrifier community; that the quality of irrigation water has impacted the composition of *nirS* and *nirK* communities (Zhou *et al.,* 2011); that organic and chemical fertilization determine the abundance of *nirS* and *nirK* genes in tropical soils (Hai *et al.,* 2009), and that the soil organic carbon (Chen *et al.,* 2012) and organic nitrogen (Seo *et al.,* 2014) have controlled the composition of *nirK*, *nirS* and *nosZ* genes in rice field soils. In the same sense, and although the CCA did not show a significant contribution of organic matter content to the composition of community, this is a key factor for the denitrification, which is an anaerobic organoheterotrophic process. We speculate that perhaps the high levels of organic matter, organic carbon and organic nitrogen recorded in the agricultural soil during the dry season could favor the increase of the OTUs, and consequently slightly diminished the nitrate levels in the same area with respect to the other soil samples; however, this hypothesis awaits further detailed investigation because the results in general did not reveal a significant effect of nitrate and organic matter on the structure of the community. Our results are in accordance with some studies that demonstrated that even with nitrate additions, the changes in the structure, abundance and copy number of genes on the *nirS*-community were observed only when the anaerobic condition was fully developed in the anoxic rice soil after 20 d of incubation (Yuan *et al.,* 2012); that the abundance and diversity of the *nirS* community presented less variation than the *nirK* community due to the effect of long-term additions of balanced mineral fertilizers (Chen *et al.,* 2010) and that the activity of the *nirS* community and the composition of this community were correlated with the nitrate levels in a global study developed by Enwall *et al.* (2010). These reports, along with our data, suggest that the nitrate levels are more related to the activity and expression of functional genes than with the composition of these denitrifying communities.

The dominance of some OTUs in the agricultural soil during the dry season (AS09) could be explained by the presence of some denitrifying microorganisms with type r survival strategies, which are characterized by rapid responses to the availability of substrate with a high growth rate and therefore competes with carbon or nitrogen sources, as has been suggested by Stress *et al.* (2004) and Yoshida *et al.* (2009). On other hand, although Seo *et al.* (2014) reported that temporality does not affect the abundance of denitrifying genes of areas cultivated with rice, Castro *et al.* (2010) indicated that the precipitation changes if have a very strong effect on the composition of the microbial communities in soils favoring the abundance of some *Acidobacteria* during the dry season, which supports our observations. Likewise, Yoshida *et al.* (2009) and Yuan *et al.* (2012) also reported changes in the composition, diversity, and abundance of *nirS* and *nirK* genes in relation to the sampling time of rice field soils.

Conclusion

In general, the results of this study suggest that *nirS*-type denitrifier communities that inhabited a rice field soil and uncultivated soil in Ibague (Colombia) responded with changes in the relative abundance to soil chemical variations produced during each of the seasons, without affecting the diversity, except in the dry season. Our data suggested that the *nirS*-type denitrifier community from rice field soils could be severely affected if the area is subjected to a prolonged dry season because it could drastically diminish the diversity of this community.

Future studies should focus on the analysis of the impact of global warming on the diversity of microbial communities and how this might translate into changes in the function of the community, specifically in the N_2O production and/or denitrification.

Acknowledgements

Thanks to the office of research and scientific development of the University of Tolima for funding this research, to L. Londoño for her help in the DNA extraction and to Dr. G. Braker for supporting the TRFLP analysis.

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