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Direct seeding mulch-based cropping increases both the activity and the abundance of denitrifier communities in a tropical soil

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ABSTRACT

This study evaluated the impact of direct seeding mulch-based cropping (DMC), as an alternative to conventional tilling (CT), on a functional community involved in N cycling and emission of greenhouse gas nitrous oxide (N_2O). The study was carried out for annual soybean/rice crop rotation in the Highlands of Madagascar. The differences between the two soil management strategies (direct seeding with mulched crop residues versus tillage without incorporation of crop residues) were studied along a fertilization gradient (no fertilizer, organic fertilizer, organic plus mineral fertilizers). The activity and size of the denitrifier community were determined by denitrification enzyme activity assays and by real-time PCR quantification of the denitrification genes. Denitrification activity and total C and N content in the soil were significantly increased by DMC both years, whereas the fertilization regime and sampling year (crop and mulch types, climatic conditions) had very little effect. Similar results were also observed for denitrification gene densities. Denitrification enzyme activity was more closely correlated with C content than with N content in the soil and denitrification gene densities. Principal component analysis confirmed that soil management had the strongest impact on the soil denitrifier community and total C and N content for both years and further indicated that changes in microbial and chemical soil parameters induced by the use of fertilizer were favored in DMC plots. Overall, the alternative DMC system had a significant positive effect on denitrifier densities and potential activities, which was not altered by crop rotation and the level of fertilization. These data also suggest that in these clayey soils, the DMC system simultaneously increased the size of the soil N pool and accelerated the N cycle, by stimulating the denitrifier community. Complementary investigations should further determine in greater detail the influence of DMC on *in situ* N-fluxes caused by denitrification.

1. Introduction

Direct seeding mulch-based cropping (DMC) is a conservation agriculture technique devoted to establishing agricultural sustainability to ensure the perennial productivity of the soil through a reduction in soil erosion and mineral fertilizer inputs and an increase in soil nutrients (Smart and Bradford, 1999). DMC is being increasingly adopted worldwide (about 90 million hectares, Derpsch, 2003), especially in tropical and semi-arid tropical agroecosystems, to cope with soil degradation induced by combinations of arbitrary agricultural practices (e.g. no intercropping, non-optimal rotation, systematic intensive cultivation) and adverse climatic conditions. A large body of literature has reported that alternative practices can favor cascades of beneficial changes to chemical, structural and biological soil properties. Soil structure is an important regulator in soil functioning that can be improved in DMC managed fields through increased aggregation (Paustian et al., 2000) often associated with increased organic matter content (Doran, 1980) and soil moisture (Steiner, 1989). DMC management has been reported as increasing the diversity and abundance of faunal communities (Brévault et al., 2007; Blanchart et al., 2007), as well as several other microbial characteristics (Govaerts et al., 2007;

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Cookson et al., 2008). For instance, increased microbial biomass and various enzyme activities (e.g. β-glucosidase, phosphatase, urease) have often been reported as being enhanced in no-till soils, especially in the upper surface soil layer and in fine textured soils (Doran, 1980; Bergstrom et al., 1998; Rabary et al., 2008).

While conservation agricultural practices are globally beneficial to soil quality, reduced tillage and residue conservation may trigger negative environmental side effects, such as increased N_2O emissions (Baggs et al., 2003; Rochette, 2008). Reduced availability of oxygen and air space in DMC soils, together with the decomposition of mulched crop residues in the superficial soil layers, are likely to favor anaerobic processes such as denitrification (Baggs et al., 2003; Sarkodie-Addo et al., 2003). Increased denitrification rates are also likely to be mediated by an increased denitrifier biomass as suggested by early studies documenting a significant increase in denitrifier counts in no-till fields (Doran, 1980; Broder et al., 1984). Against this background, the denitrification rates in such systems are worth attention since this microbial process can represent a significant source of N_2O (Mosier et al., 1998) contributing to global warming and destruction of the ozone layer (Tabazadeh et al., 2000).

Although a large body of literature describes the significant effects of tillage intensity, fertilizer types or loading rates on potential denitrification activity (review in Philippot et al., 2007) or *in situ* losses through N_2O or N_2 releases (Baggs et al., 2006; Liu et al., 2007), few studies have analyzed the effects of agricultural practices on both denitrification activity and size of the denitrifier community. However, measuring the size and activity of denitrifier community and analyzing the relationship between them are of great interest as the regulation of biogeochemical cycles by the size of the microbial community is still in dispute (Coleman and Whitman, 2005; Philippot and Hallin, 2005; Röling, 2007).

This study was based on the hypothesis that the size and activity of the denitrifier community would be increased under DMC management and that combined mineral and organic fertilizers would strengthen this effect. This hypothesis was studied in the Highlands of Madagascar where DMC systems were initially implemented to deal with extensive soil erosion (Rabary et al., 2008). The study was carried out in an agronomic field trial set up eight years ago to study the long-term effects of DMC management on soil functioning. The impact of DMC was evaluated by comparison with tilled plots in two consecutive years, for soybean/rice crop rotation, along a fertilization gradient including mineral and manure inputs. The activity and size of the denitrifier community were determined by monitoring denitrification enzyme activity and by real-time PCR quantification of the denitrification genes, respectively.

2. Materials and methods

2.1. Field experimental design

The experimental station was located near Antsirabe, in Bemasoandro (19°46′S, 47°06′E), Madagascar. This area has a tropical altitude climate, with around 10–20 days of frost annually (Oldeman, 1990) and a mean annual temperature of 17 °C. The site was 1600 m above sea level with an average rainfall of 1665 and 1203 mm during the 2004/2005 and 2005/2006 seasons, respectively. Rainfall during the rainy season was particularly low in 2006 (223 mm through January and February compared with an average of 556 mm for the same period in the previous 5 years). This soil is andic Dystrustept (Soil Survey Staff, 2003). In 2003 the main characteristics of the 0–25 cm soil layer in a soybean/rice rotation under DMC systems were: pH(water) 5.1, clay 79%, fine silt 10%, coarse silt 2%, fine sand 4%, coarse sand 5%, carbon 2.1%, nitrogen 0.16%, and CEC 17 cmol kg^{-1} (Razafimbelo, 2005). This field experiment was set up in 1997 and consisted of two soil management strategies (conventional tillage (CT) without crop residue conservation and direct seeding (DMC) with mulched crop residue conservation) combined with three fertilization regimes: F0-no fertilizer, F1-organic fertilizer (5 t zebu manure ha^{-1} y^{-1}), F2-organic and mineral fertilizers (5 t zebu manure ha^{-1} y^{-1}, 70 kg N, 30 kg P and 40 kg K ha^{-1}), which resulted in a total of six different treatments. Manure was applied at the beginning of December while seeding, and mineral fertilizers were usually spread a couple of weeks later. Plots (13.5 m^2) were completely randomized with three plots for each combination of treatment. This study focused on a soybean (*Glycine maxima* L.)/rice (*Oryza sativa* L.) annual rotation using rainwater only.

The soil was sampled in two consecutive years during the rainy season on January 24 for the soybean crop (2005) and February 13 for the rice crop (2006) to characterize the denitrifier communities during the period most favorable to denitrification (i.e. high soil moisture, recent fertilizer inputs, plant growth) and to take account of possible seasonal and crop type effects (e.g. residue quality, quantity and quality of root exudates). Soil cores were taken along three parallel lines located between rows (40 cm wide for soybean and 30 cm wide for rice). No samples were taken from the soil between the first two rows of crops on either side of each plot to avoid possible edge effects. For each sampling line, five elementary soil cores (5 cm depth, 5 cm diameter) were collected at three separate locations along the line and mixed to give a total of 3 composite samples per plot (54 composite soil samples each year). Soil samples were immediately air-dried, sieved at 2 mm and stored at room temperature.

2.2. Chemical analyses

The total soil C and N contents were determined by dry combustion using a CHN analyzer (Thermo-Finnigan EA 1112NC Soil Analyzer). Measurements of nitrate-ammonium soil contents were performed by ISO 9001 LAMA Laboratory (Dakar, US Imago, IRD), but only on 2005 samples.

2.3. Activity measurements

Denitrification enzyme activity (DEA) was measured according to the method described by Smith and Tiedje (1979). 20 g (dry weight) sub-sets of soil samples were made anoxic by flushing the flask headspace with helium. 2 mg C g^{-1} dry soil (added as a 50/50 w/w glucose and glutamic acid) and 0.2 mg N g^{-1} soil (added as KNO_3) were added to each sample. The flask contents were incubated with 10% (v/v) acetylene to allow the accumulation of denitrified nitrogen as N_2O. DEA was calculated as the rate of N accumulated as N_2O in the headspace in the presence of acetylene between 2 and 6 h in the dark at 100% water holding capacity and at 25 °C, and analyzed using a gas chromatograph (Varian Star 3900, Varian, Walnut Creek, CA, USA). The same protocol was used to quantify potential N_2O emissions but without acetylene to determine the proportion of N denitrified as N_2O during the assay.

2.4. DNA extraction

DNA was extracted from 0.25 to 1 g of composite soil samples with the Ultra Clean Soil DNA kit according to the manufacturer's instructions (Ozyme, Mo Bio, France). DNA extracts were quantified by spectrophotometry at 260 nm using a BioPhotometer (Eppendorf, Hamburg, Germany). For each plot, three independent soil DNA extractions were performed, corresponding to the three sampling lines per plot, giving a total of 54 DNA extracts, used as PCR templates, for each year.

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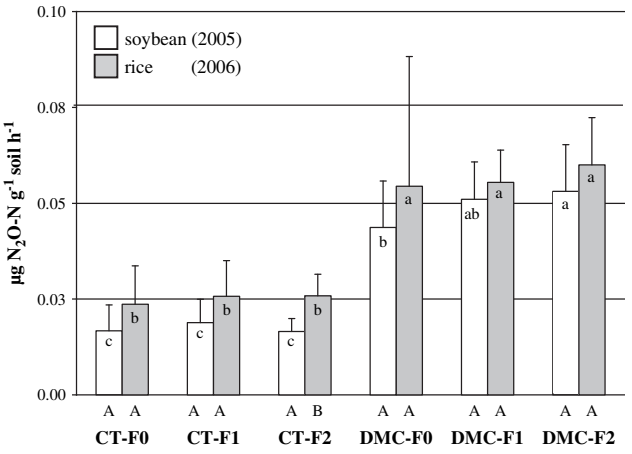

Fig. 1. Denitrification enzyme activity (DEA) (mean and standard deviation, $n = 9$) measured in the presence of acetylene with field samples collected in two consecutive cropping seasons (soybean in 2005 and rice in 2006, respectively). CT: conventional tillage, DMC: direct seeding mulch-based cropping system, F0: no fertilizer, F1: manure, F2: manure + mineral fertilizer. Different capital letters below columns indicate significant differences between sampling years ($P < 0.05$). Different lowercase letters on top of columns indicate significant differences between fertilization/cultivation technique modalities for a given year.

2.5. Quantification of the denitrifier community size

The size of the denitrifier community was estimated by quantitative PCR (qPCR) of the genes encoding the catalytic subunit of the key enzymes of the denitrification pathway. Fragments of the *nirK*, *nirS* and *nosZ* genes encoding the copper and cd_1 nitrite reductases and the nitrous oxide reductase, respectively, were amplified in a 20 µl reaction volume containing SYBR Green PCR Master Mix (Absolute QPCR SYBR Green Rox ABgene, France), 1 µM of each primer, 100 ng of T4 gene 32 (QBiogene, France) and 6.5 ng of template DNA. The primers and PCR conditions are described in López-Gutiérrez et al. (2004) for 16S rDNA, Henry et al. (2004) for *nirK*, Kandeler et al. (2006) for *nirS* and Henry et al. (2006) for *nosZ*. Thermal cycling, fluorescence measurements and data analysis were carried out using an ABI Prism® 7900HT sequence detection system according to the manufacturer's instructions. Two or three no-template controls were run for each quantitative PCR assay. All assays were run using genomic DNA from either *Bradyrhizobium japonicum* USDA110, *Pseudomonas aeruginosa* PAO1, *Agrobacterium tumefaciens* C58 or *Sinorhizobium meliloti* 1021, containing known copy numbers of targeted genes as external standards (Henry et al., 2006). The potential presence of PCR inhibitors in soil DNA extracts was tested by running a real-time PCR assay on serial dilution of soil DNA extracts. No inhibition was detected in any case. To eliminate bias related to the DNA extraction efficiency, gene copy numbers were calculated both by nanogram of DNA and gram of dry soil.

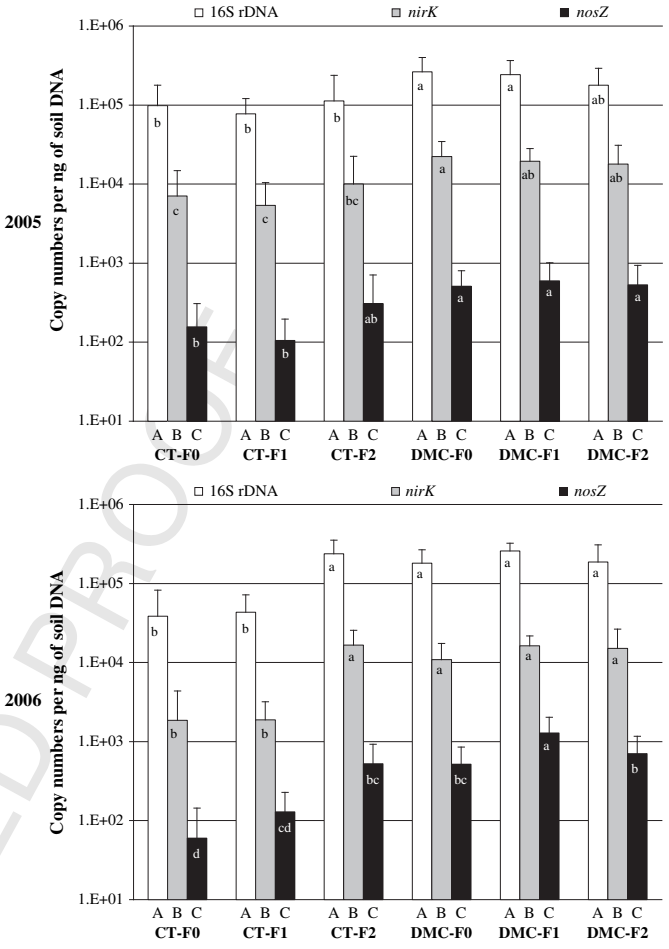

Fig. 2. 16S rRNA, *nirK* and *nosZ* gene densities (mean and standard deviation, $n = 9$) measured with field soil samples collected in two consecutive years (2005: soybean crop and 2006: rice crop). CT: conventional tillage, DMC: direct seeding mulch-based cropping system, F0: no fertilizer, F1: manure, F2: manure + mineral fertilizer. Different capital letters below columns indicate significant differences between the three types of gene densities ($P < 0.05$). Different lowercase letters on top of columns indicate significant differences between fertilization/cultivation technique modalities for a given gene.

2.6. Statistics

The results were analyzed using Fisher's LSD test with XLSTAT software (2007.8.03 version, Addinsoft, Paris, France) on raw data

Table 1
Influence of cultivation technique and fertilization on total C and N, NH_4^+ and NO_3^- content in the soil.

		Conventional tillage			Direct seeding mulch-based system		
		F0[a]	F1[a]	F2[a]	F0	F1	F2
Carbon (%)	soybean (2005)	2.3 (0.3) a	2.4 (0.2) ab	2.6 (0.3) b	3.5 (0.3) c	3.7 (0.4) cd	3.9 (0.5) d
	rice (2006)	2.5 (0.3) a	2.7 (0.2) a	2.9 (0.2) b	3.5 (0.3) c	3.9 (0.3) d	4.5 (0.5) e
Nitrogen (%)	soybean (2005)	0.16 (0.02) a	0.17 (0.02) ab	0.19 (0.03) b	0.25 (0.03) c	0.25 (0.03) c	0.25 (0.05) c
	rice (2006)	0.18 (0.03) a	0.20 (0.02) a	0.23 (0.02) b	0.28 (0.03) c	0.31 (0.03) c	0.37 (0.05) d
Ammonium[b]	soybean (2005)	6.2 (2.0) a	8.5 (2.4) a	15.2 (2.7) b	14.2 (2.5) b	13.1 (2.6) b	15.3 (3.4)b
Nitrate[b]	soybean (2005)	6.8 (1.0) b	4.2 (1.0) a	4.0 (1.4) a	9.9 (1.9) c	6.3 (2.5) b	5.2 (3.0) ab

Numbers represent means ($n = 9$) followed by their standard deviations in parenthesis. Different letters within a row indicate significant differences ($P < 0.05$) between all six treatments for a given crop.

[a] Fertilization levels: F0 no fertilizer, F1 manure, F2 manure + mineral fertilizer.

[b] Expressed in µg NH_4^+/NO_3^- – N g^{-1} soil.

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or log-transformed data for total soil C and N content. Principal component analyses (PCA) were done by using normalized data with ADE4 software (Thioulouse et al., 1997).

3. Results and discussion

This study set out to characterize the impact of zero tillage and residue conservation on the size and activity of the denitrifier community. DMC management significantly stimulated denitrification enzyme activity with respect to conventional tillage (CT) (Fig. 1). Average DEA values for soil collected from tilled plots ranged from 17 to 26 ng N_2O–N g^{-1} dry soil h^{-1} for both years and across the whole fertilization range, while significantly higher rates of 44–60 ng N_2O–N g^{-1} dry soil h^{-1} were observed for DMC (Fig. 1). Tillage has been reported either to stimulate (Calderón et al., 2001) or decrease denitrification (Liu et al., 2006, 2007). The absence of a clear effect of tillage on denitrification can be attributed to the association of tillage with other agricultural practices that can also potentially affect denitrifying communities. Moreover, the effect of tillage depends on the timing, frequency and depth which determine the extent of aggregate disruption, organic matter protection and dissolved organic matter availability, which in turn affect soil microbial functioning (Cookson et al., 2008). Total C and N, together with NH_4^+ and NO_3^- concentrations, also increased with the DMC (Table 1). Previous studies have reported that, in no-till systems, part of the soil organic matter can be physically protected by inclusion in stable aggregates (Six et al., 2000; Oorts et al., 2007). However, this study showed that DEA was significantly correlated with soil C and N contents in both years (R^2 from 0.53 to 0.83 for C and from 0.53 to 0.59 for N, $P < 0.001$). As already reported (Burford and Bremner, 1975), soil C content appears to be a good predictor of DEA across very different soil types.

Denitrifying enzyme activity did not depend on the sampling year (i.e. mulch and crop types, climatic conditions) or fertilization rate (Fig. 1). Organic matter and/or mineral N fertilizers inputs have usually been reported to stimulate denitrification (Mulvaney et al., 1997; review in Philippot et al., 2007), especially under direct seeding (Baggs et al., 2003; Liu et al., 2006, 2007) and it is possible that there may have been a transient fertilizer effect before the samples were taken. No significant potential reduction of N_2O into N_2 was noticed in the absence of acetylene for any of the combinations of treatment (data not shown), suggesting that N_2O is the end product of denitrification in the soil studied. Incomplete denitrification processes have been reported in agricultural soils elsewhere (Hénault et al., 2001). However since our results are based on only two sampling dates, a thorough investigation of *in situ* N_2O emissions is necessary before concluding that DMC also increased emissions of N_2O from the field. A recent study has shown low N_2O fluxes for the whole growing season in no-till plots in the same area (Chapuis-Lardy et al., 2009).

For both sampling dates, the number of 16S rRNA copies per nanogram of soil DNA ranged from 2.32×10^2 to 4.76×10^5 (Fig. 2), which corresponded to 8.12×10^5 and 6.75×10^9 gene copies per gram of dry soil (data not shown). Lower values were observed for *nirK* and *nosZ* gene abundances, with values ranging from 1.16×10^2 to 3.77×10^4 copies and from 1.31×10^3 to 2.86×10^3 copies per ng DNA, respectively (from 3.44×10^5 to 4.79×10^8 and from 2.47×10^3 to 5.16×10^7 copies per gram dry soil, respectively). In this study, the *nirS* gene density was below the detection limit as for the study by Dandie et al. (2008). The density of *nirK* and *nosZ* denitrification genes estimated in this study varied within the ranges previously reported: *nirK* and *nosZ* densities of 2×10^6 to 2×10^8 and 3×10^6 to 8×10^7 copies per gram of soil were reported for various arable soils (Henry et al., 2006; Dandie et al., 2008; Hallin et al., 2009). The effect of DMC on denitrification gene abundances (Fig. 2) was similar to that on denitrification enzyme activity (Fig. 1) with significantly higher denitrification gene copy numbers in DMC than in conventional tillage plots, with the exception of the plots receiving the highest fertilizer inputs. Both *nirK* and *nosZ* gene densities were significantly correlated with N content (data not shown) and more strongly with C content (Fig. 3A). This confirmed the heterotrophic nature of the denitrifying community (Tiedje, 1988), which was stimulated by the increased soil organic matter content (DMC) and by manure inputs. In the field studied, the

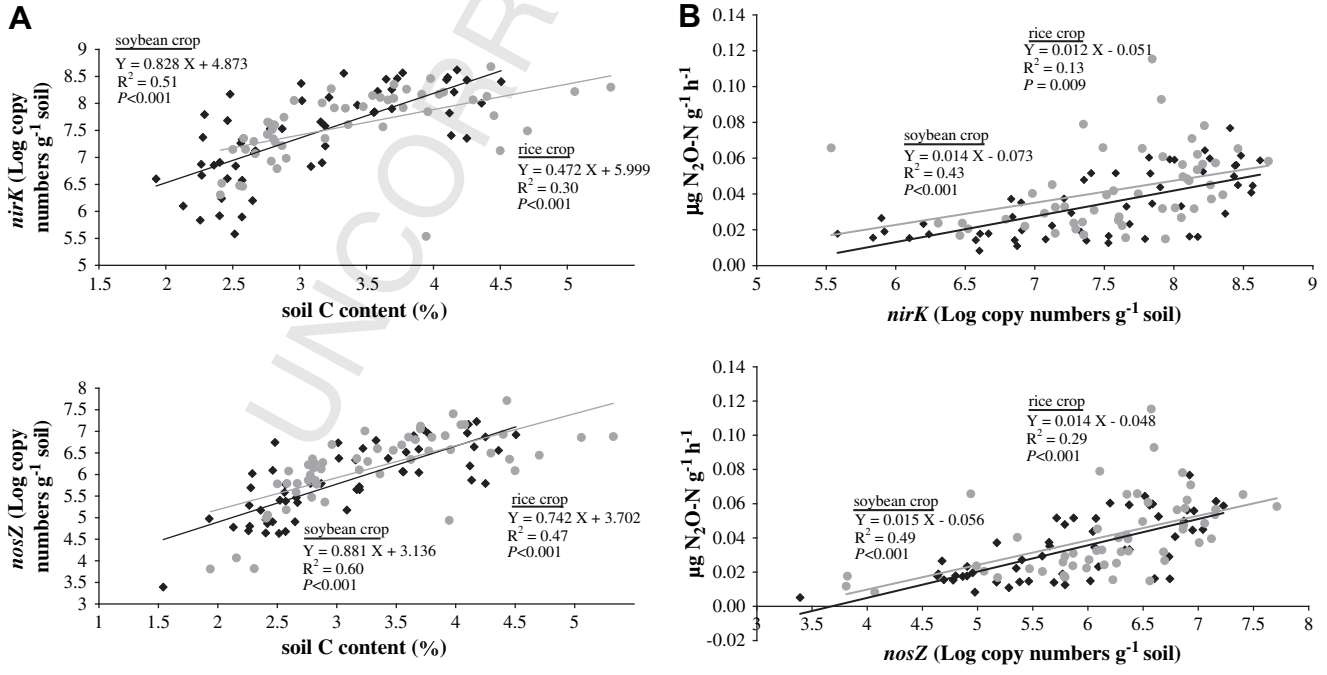

Fig. 3. Linear regressions established between (A) *nirK* or *nosZ* gene densities and soil C contents of samples, (B) denitrification enzyme activity (DEA) and *nirK* or *nosZ* gene densities, collected under soybean (black diamonds) and rice crops (grey circles) on the base of the whole data set ($n = 54$, all treatments).

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Table 2
Influence of cultivation technique and fertilization on the ratios of *nirK* and *nosZ* genes to 16S rDNA gene (*nirK*/16S, *nosZ*/16S) and ratio of *nosZ* gene to *nirK* gene (*nosZ*/*nirK*).

		Conventional tillage			Direct seeding mulch-based system		
		F0[a]	F1[a]	F2[a]	F0	F1	F2
nirK/16S	soybean (2005)	5.23 (3.47) a	6.10 (2.72) ab	8.67 (3.65) bc	9.50 (3.44) c	8.83 (2.69) bc	9.36 (2.06) c
	rice (2006)	3.23 (2.25) a	4.06 (2.32) ab	6.99 (1.09) c	5.79 (1.64) bc	6.38 (1.35) c	7.48 (3.41) c
nosZ/16S	soybean (2005)	0.18 (0.08) a	0.14 (0.06) a	0.39 (0.34) b	0.28 (0.29) ab	0.28 (0.20) ab	0.27 (0.08) ab
	rice (2006)	0.21 (0.18) a	0.42 (0.40) bc	0.20 (0.06) a	0.27 (0.06) ab	0.48 (0.18) c	0.42 (0.16) bc
nosZ/*nirK*	soybean (2005)	3.10 (2.22) ab	2.70 (1.63) ab	4.35 (2.98) b	2.61 (1.19) a	2.95 (1.41) ab	2.85 (0.49) ab
	rice (2006)	2.35 (1.91) a	5.96 (2.93) bc	2.99 (0.97) ab	4.77 (0.93) abc	7.54 (2.37) c	7.83 (6.93) c

Numbers represent means ($n = 9$) followed by their standard deviations in parenthesis. Different letters within a row indicate significant differences ($P < 0.05$) between all six treatments for a given crop.
[a] Fertilization levels: F0 no fertilizer, F1 manure, F2 manure + mineral fertilizer.

proportion of *nirK* and *nosZ* genes to 16S rRNA gene ranged from 3.2 to 9.5% and from 0.14 to 0.48% respectively (Table 2). These results are in agreement with culture-based studies that have found that the proportion of denitrifiers to total bacteria is less than 5% (Tiedje, 1988; Chèneby et al., 2000, 2004). In this study, *nosZ* gene abundance was less than 5% of that of the *nirK* gene (Table 2). The higher abundance of *nirK* genes compared to *nosZ* genes has already been observed in a temperate agricultural soil (Henry et al., 2006), and suggests that, in the fields studied, many of the denitrifiers may lack the *nosZ* gene and, therefore, are genetically unable to reduce N_2O into N_2. However, the *nosZ* primers used may not be as universal as the *nirK* primers, which could account for the high *nirK* to *nosZ* gene ratio reported here. On the other hand, the low proportion of *nosZ* denitrifiers could explain the absence of a detectable potential reduction of N_2O in the DEA assay.

PCA of the chemical and microbial data, all expressed per gram of soil, confirmed that soil management had a greater influence than the fertilization regime. There was a significant segregation of the soil management strategies along the first axis, explaining 76 and 71% of the variance for soybean and rice crops, respectively (only shown for the soybean crop in Fig. 4). PCA ordination of the variables indicated that they were all associated with the first axis, showing that the soil C and N content, gene densities and DEA were all stimulated by DMC (Fig. 4). A higher variability in PCA scores was observed for DMC plots which could reflect the higher structural heterogeneity of the soil after several years of zero tillage. PCA also showed that there was a weak effect between the F1 and F2 fertilization treatments but only with DMC soil management, as indicated by a significant segregation of points along the second axis explaining 17.4% of the total variance. Linear regressions between DEA and denitrification gene densities (Fig. 3B) were significant in all cases suggesting that potential denitrification activity is partly regulated by the size of the denitrifier community (average $R^2 = 0.33$, $P < 0.001$). However, regression coefficients and slopes were rather low indicating a limited predictive value of such regressions. For instance, some soil samples having up to two log differences in denitrifier density exhibited similar denitrification levels. Such weak correlation between denitrifier biomass and denitrification activity has already been reported (Martin et al., 1988; Dandie et al., 2008). Several hypotheses can explain the low correlation between size and activity observed in this study. Firstly, the number of denitrification genes was quantified to estimate the size of the community genetically capable of denitrifying but this did not provide information on the size of the active fraction of this community. Moreover, although *nirS* gene densities were too low to be reliably estimated, it is possible that cytochrome cd_1 nitrite reductase activity significantly influenced the measured potential denitrification activity. Another hypothesis is that agronomic treatments may have modified the composition of the denitrifier community by selecting denitrifier populations with different specific activities or having denitrification enzymes that were susceptible to abiotic factors (Cavigelli and Robertson, 2000). Chèneby et al. (2009) observed, at the same experimental site, significant differences in the nitrate reducer community structure between treatments. Such compositional shifts are likely to loosen the link between denitrification activity and the size of the denitrifier community.

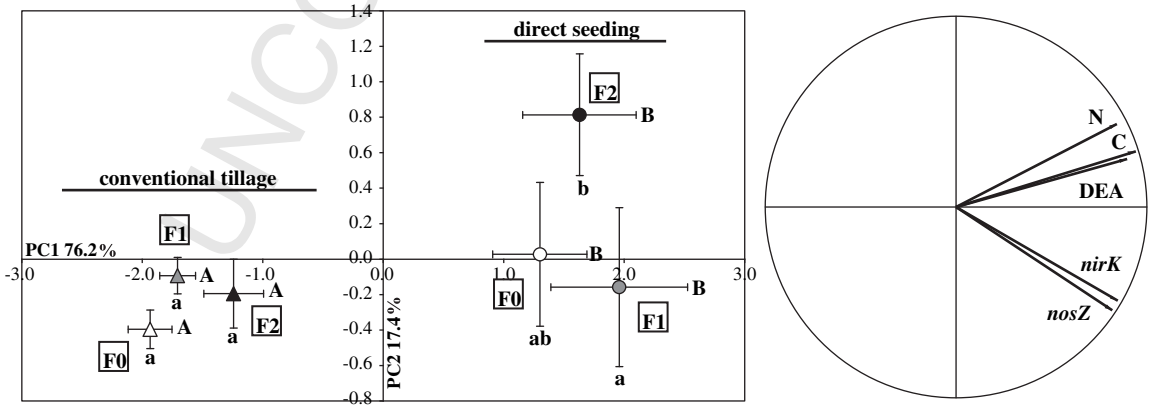

Fig. 4. Principal component analysis and associated correlation circle carried out on the whole data set (total soil C and N contents, *nirK*–*nosZ* gene densities, denitrification enzyme activity (DEA), $n = 54$) of samples collected under the soybean crop. Symbols represent mean coordinates of triplicate samples scores, with associated standard errors. Different capital and lowercase letters indicate significant differences ($P < 0.05$) between mean abscises and ordinates, respectively. Triangle and full circle symbols refer to conventional tillage and direct seeding mulch-based cropping systems, respectively. White, grey and black colors of symbols refer to the fertilization gradient (F0: no fertilizer, F1: manure, F2: manure + mineral fertilizer, respectively).

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4. Conclusions

This study showed that DMC systems may favor soil denitrification potential with the size and potential activity of the denitrifier community increased by direct seeding and crop residue retention compared to conventional tillage. However, different levels of fertilization had no significant impact on the denitrifier community in the tropical clayey soil studied. These results suggest that the tillage system (determining the soil physical status and residue retention) modifies several soil properties, making it the dominant driver of the denitrifier community over fertilization practices in these arable soils. It also seems that even on such clayey soils, the DMC system can simultaneously favor soil N content and N-cycling microbial communities. However, data on *in situ* N-fluxes caused by denitrification are needed to confirm the impact of DMC on nitrogen loss. In addition, the lack of a clear-cut correlation between denitrification gene abundances and potential denitrifying activity highlights the complexity of the mechanisms determining N-fluxes by denitrification. Nevertheless, focusing on the active fraction of the denitrifier community that contributes to the N_2O and N_2 emissions would help clarifying the role of denitrifier community size and diversity in regulating N-fluxes by denitrification.

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