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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.