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SHORT COMMUNICATION

Impact of irrigation water quality on soil nitrifying and total bacterial communities

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Abstract Disturbance induced by two contrasting irrigation regimes (groundwater versus urban wastewater) was evaluated on a sandy agricultural soil through chemical and microbial analyses. Contrary to wastewater, groundwater displayed very high nitrate contents but small amounts of ammonium and organic matter. Despite these strong compositional shifts, soil organic carbon and nitrogen, nitrate and ammonium contents were not significantly different in both types of irrigated plot. Moreover, neither microbial biomass nor its activity, determined as fluorescein diacetate hydrolysis activity, was influenced by irrigation regimes. Bacterial community structure, assessed by denaturing gradient gel electrophoresis (DGGE) of 16S ribosomal DNA fragments, was also weakly impacted as molecular fingerprints shared an overall similarity of 85%. Ammonia-oxidizing bacterial community (AOB) was monitored by DGGE of the functional molecular marker *amoA* gene (alpha subunit of the ammonia monooxygenase). Surprisingly, no *amoA* signals were obtained from plots irrigated with groundwater, whereas signal intensities were high in all plots under wastewater. Among the last, compositional shifts of the AOB community were weak. Overall, impact of irrigation water quality on soil chemistry could not be evidenced, whereas effects were low on the total bacterial compartment but marked on the AOB community.

Keywords Irrigation · Wastewater · Nitrogen · Ammonia-oxydizing bacteria (AOB) · Tropical sandy soil

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Introduction

In Senegal, urban agriculture is estimated to ensure 40% of the national production (Mbaye 1999). In Dakar, it is performed on smallholder farming systems (84% of the producers valorise less than 0.5 ha) with an intensive management (several growth cycles per year, mineral and organic fertilisation, pesticides application; Ba-Diao 2004). Arable soils are sandy, and irrigation, classically performed with groundwater, is essential to farming. Nevertheless, over the past decade, decrease of the aquifer level due to recurrent droughts led farmers to supply groundwater with urban effluents, as irrigation cost with treated municipal water was too expensive (Faruqui et al. 2002). Wastewater is known to introduce large amounts of various nutrients, particulate and dissolved organic matter, detergents, parasites and microorganisms into soil (Cho and Kim 2000; Carr et al. 2004). Fields included in our study have been daily subjected to repeated short-lived additions of wastewater for approximately more than 15 years (Gaye and Niang 2002), and soil microbiota have been faced to considerable fluctuation of their physico-chemical and biological environment. Given the functional significance

of microbial diversity (Cavigelli and Robertson 2000, 2001), its potential shifts in these irrigated soils could have severely alter key soil processes linked to soil fertility such as organic matter mineralisation and biogeochemical cycles. To date, consequences of this agricultural practice on soil microbial community diversity, resistance, resilience or functions are unknown.

The aim of the present research was first to characterise the impact of these long-term irrigation regimes with contrasted qualities of water (groundwater or urban wastewater) on some key chemical soil properties and secondly to examine the size, activity and structure of the total soil bacterial community through measurements of microbial biomass, fluorescein diacetate (FDA) hydrolysis activity and denaturing gradient gel electrophoresis (DGGE) of amplified 16S ribosomal DNA (rDNA) fragments. With regard to the previously evidenced nitrogen pollution on this site (Gaye and Niang 2002), bacterial communities specifically engaged in the nitrogen cycle are of critical interest. As (1) nitritation plays a fundamental and rate-limiting step in the nitrogen cycle, (2) the congruent physiological and phylogenetic characteristics of the β-subgroup ammonia-oxydizers constitute a reliable indicator for environmental disturbance (Stephen et al. 1999), we also chose to target the ammonia-oxidizing bacterial (AOB) community by means of DGGE of the *amoA* gene (alpha subunit of the ammonia monooxygenase).

Materials and methods

Experimental site and sampling

The soil is classified as an Eutric Arenosol (FAO 1998). Average soil texture characteristics in the 0- to 20-cm layer were: clay 3.9%, silt 3.1% and sand 94%. Other soil parameters were largely influenced by the irrigation regime (Table 1). No soil characteristic was available at the time fields were solely irrigated with groundwater. Irrigation was manually performed three to four times a day from ponds dug at the bottom of some dune slopes where water table nears the surface. Urban wastewater was collected from a single sewer pipe and directly introduced in some collecting ponds. In this study, ponds containing mixed groundwater and wastewater were discarded. Each cultivated plot was associated with its own pond, receiving groundwater or wastewater. Soil and water samples were collected in late February 2004 (dry season). Three cultivated plots (growing lettuce) were sampled per type of irrigation water together with their associated ponds. Due to natural site constraints, three slope points (top, medium and bottom) were considered for soil sampling in each plot. Three soil cores (0–20 cm) per slope point were collected between rows and mixed for a total of three independent composite samples per plot. Humid soil was sieved to 2 mm to discard any residue or root debris, and aliquots were immediately processed for NO_3^-, NH_4^+ contents, microbial biomass and enzymatic activity measurements. Remaining soil samples were air-dried to determine their moisture contents and stored at −20°C for subsequent molecular analysis. Air drying of sieved soil samples was completed in less than 24 h owing to ambient temperature (≈27°C) and soil texture. Water was sampled by immersing a 1-l plastic bottle in the middle of the pond (groundwater or wastewater) and immediately processed for chemical analysis.

Chemical analyses

Total soil organic carbon and nitrogen contents were determined by dry combustion using a CHN autoanalyser (ThermoFinnigan Flash EA 1112 series). Mineral N (NH_4^+-N, NO_3^--N) contents in soil were colorimetrically determined from 2 M KCl extracts according to Bremner (1965) using a Technicon Autoanalyzer (Evolution II, Alliance-instruments, Mery-sur-Oise, France). Filtered irrigation water samples (Whatman GF/C) were analysed using

Table 1 Properties of waters (mean±SD, n=3) and irrigated soils (mean±SD, n=9)

	pH (water)	Nitrate[a]	Ammonium[a]	COD (mg l^{-1})	Organic C (mg g^{-1})	Organic N (mg g^{-1})	Microbial biomass (μg C g^{-1})	FDA (μg g^{-1} min^{-1})
Groundwater	7±0.3	350.1±188.6 a	1.5±0.7 a	26.3±7.4 a	ND	ND	ND	ND
Wastewater	7.2±0.3	1.9±0.5 b	108.2±37.5 b	213±23.6 b	ND	ND	ND	ND
Soil under groundwater	7.23±0.15 a	68.32±32.70	2.00±1.21	ND	10.12±4.69	1.02±0.57	95.96±68.15	26.80±4.52
Soil under wastewater	5.68±0.19 b	106.94±28.91	7.90±3.63	ND	7.84±2.38	0.90±0.30	109.85±55.89	33.94±10.06

Data within column followed by different letters indicate significant differences (P<0.05).
COD chemical oxygen demand, *ND* not determined
[a] Expressed as mg N l^{-1} for water and μg N g^{-1} for soil

Technicon for NH_4^+ contents and capillary ion electrophoresis (CIA, Waters) for NO_3^- contents. Chemical oxygen demand (COD) was measured using reagent vials for photometric analysis (Hach) at 420 nm (Hach DR/2000) in accordance with the manufacturer's instructions.

Microbiological analyses

Microbial biomass C was determined by the chloroform fumigation-extraction method (Amato and Ladd 1988). FDA was determined according to Adam and Duncan (2001) with a few modifications. Assay was performed on 1 g fresh soil sample. Incubation steps were set at 30°C for 1 h, and enzymatic reactions were stopped by bringing acetone into the reaction mixture in a 1:1 ratio.

Molecular fingerprints

Total soil DNA was extracted using the method described by Porteous et al. (1997) and modified by Assigbetse et al. (2005). Triplicate DNA extracts (3×500 mg dry soil) were pooled for each composite soil sample corresponding to one out of the three slope points of a given plot. Crude extracts were then purified with Wizard® DNA Clean-Up (Promega, Charbonnières, France) and quantified as described by Ranjard et al. (2003). For both genes, polymerase chain reaction (PCR) amplifications were performed in 25 µl mixtures using puReTaq™ Ready-To-Go™ PCR beads (Amersham-Biosciences, Orsay, France) with 5 ng of template DNA and a GeneAmp PCR System 9700 (Applied Biosystems, Courtaboeuf, France). To ensure the DGGE segregation of amplicons, a 33-bp GC-clamp was added to the 5′ end of each forward primer (Muyzer et al. 1993). Partial bacterial 16S rDNA sequences were amplified with the eubacterial primers 338f-clamp/518r, according to Assigbetse et al. (2005). Partial *amoA* gene sequences were amplified with *amoA*-1F-clamp/*amoA*-2R primers developed by Rotthauwe et al. (1997), but final primers and $MgCl_2$ concentrations were 1 µM (each) and 2 mM, respectively. Thermal profile consisted of an initial denaturation of 5 min at 95°C followed by 35 cycles as follows: 1 min at 95°C, 1 min at 57°C, 45 s at 72°C and a final elongation step of 5 min at 72°C. Specificity of amplification products was checked by agarose (2%) electrophoresis.

Both types of amplicon were resolved by DGGE using 8% acrylamide gels (acrylamide–bisacrylamide 40%, 37.5:1; Sigma-Aldrich, St. Quentin Fallavier, France) and a gradient of 45–70% denaturant (Muyzer et al. 1993) in 1× TAE buffer with the Ingeny phorU system (Ingeny International, Goes, The Netherlands) at 60°C and 50 mA–100 V for 17 h. Staining and scanning of the gels are described elsewhere (Assigbetse et al. 2005).

Statistics

Quantitative data related to chemical analysis of water/soil samples, microbial biomass and enzyme activity were compared by one-factor analysis of variance using Statview software (version 4.55, Abacus Concepts Inc., Berkeley, CA, USA). DGGE profiles similarity was calculated by determining Dice's coefficient from the total number of bands independently of their intensity, and dendrograms were constructed using the unweighted pair group method with arithmetic averages (Assigbetse et al. 2005).

Results

NH_4^+ loads in urban effluents were significantly higher (more than 70 times) than in groundwater which alternatively contained far more NO_3^- (more than 180 times) than wastewater (Table 1). Organic content of wastewater (chemical oxygen demand) was approximately eight times higher than in groundwater. Both irrigation waters displayed similar neutral pH.

The only soil parameter to be statistically influenced by irrigation was pH with an acidification of soil receiving urban effluents (Table 1). Intriguingly, soil irrigated with wastewater did not contain higher amounts of organic C and N (around 1 and 0.1%, respectively). Despite low NO_3^- and high NH_4^+ contents in wastewater, soil balance of these nutrients was inverted. Site heterogeneity (plot location and slope) had no effect on all these parameters with any treatment (not shown).

Similar values for soil microbial biomass and enzymatic activity (FDA) were obtained with both types of irrigation (Table 1). DGGE migration patterns of 16S rDNA amplicons are displayed in Fig. 1A. Bacterial communities inhabiting soil irrigated with groundwater were characterised by a significant higher number of DGGE bands (43±3, $P<0.05$) than those harboured in soil under urban wastewater regime (36±2). Cluster analysis (Fig. 1B) revealed an overall similarity of 85% in the genetic structures. No clustering related to slope point or plot origin of the soil samples was observed. Two distinct groups (P1 and P2) were distinguished at 87% homology. P1 encompassed six samples out of nine related to groundwater regime and one sample out of nine related to wastewater irrigation. Samples gathered in P2 were mostly linked to wastewater treatment (all samples but one).

No *amoA* amplification product was obtained with template DNA extracted from soil sampled in plots irrigated with groundwater (Fig. 2A). Modification of some PCR parameters (hybridation temperature, template DNA and primers concentrations) failed to generate *amoA* amplicons for these nine samples (not shown). Thus, only amplifica-

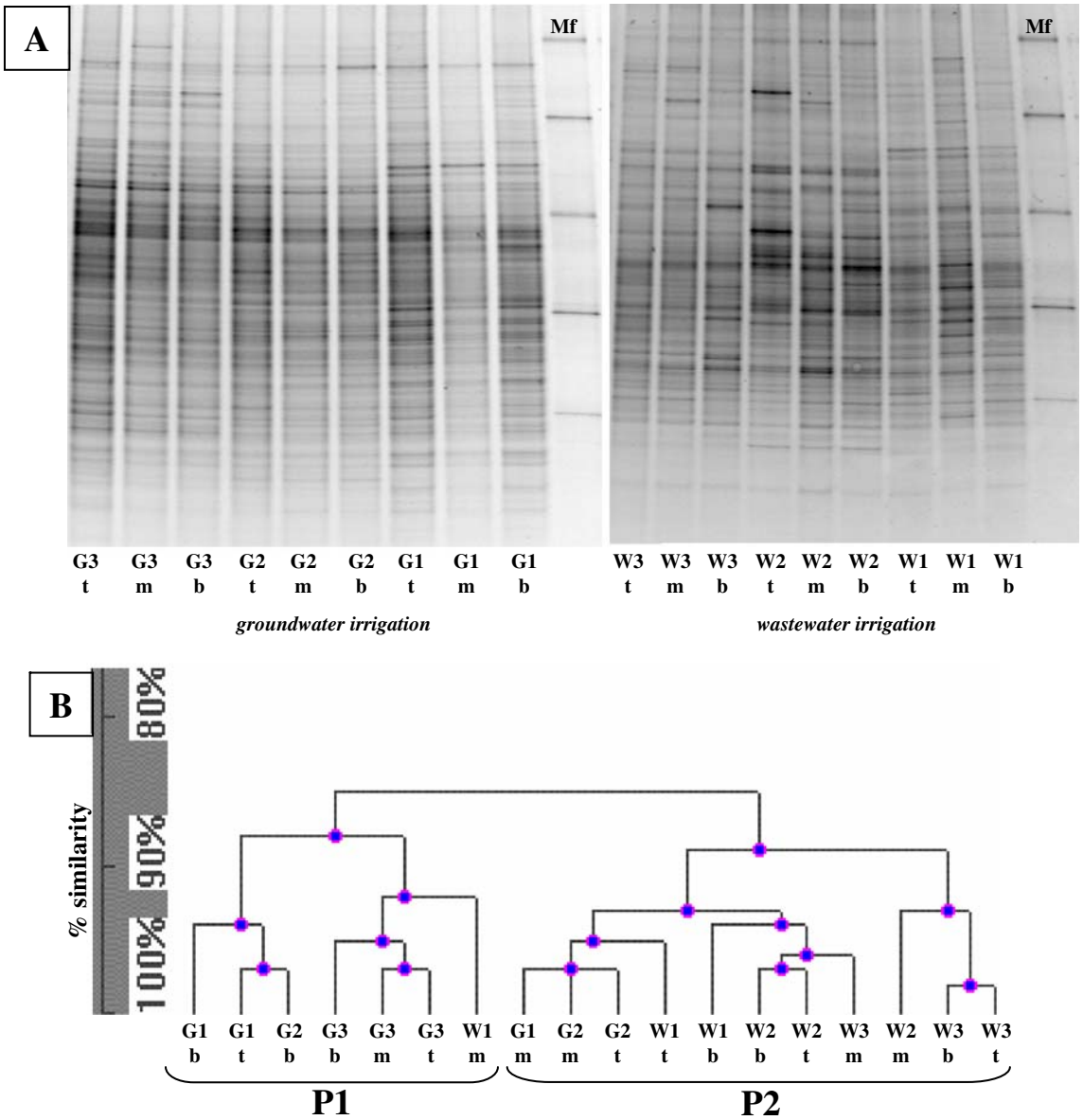

Fig. 1 **A** DGGE fingerprints of 16S rDNA fragments amplified from soil DNA of plots irrigated with groundwater (encoded *G*) and wastewater (encoded *W*). Numbers *1*, *2* and *3* refer to plot numbers; *t*, *m* and *b* letters refer to top, medium and bottom slope points, respectively. *Mf* molecular marker of migration front. **B** UGPMA dendrogram assessing the similarity of DGGE patterns illustrated in **A**. P1 and P2 refer to sample groupings at 87% similarity

tion products obtained for plots under wastewater were analysed by DGGE (Fig. 2B). Cluster analysis indicated that genetic structures shared more than 80% of similarity (Fig. 2C). Again, no clustering specific of a slope point or a plot origin was evidenced.

Discussion

This study aimed at characterizing chemical and microbiological changes that occurred in a sandy agricultural soil irrigated for more than 15 years by groundwater or urban wastewater. Urban effluents contained far more organic material than groundwater. Nevertheless, this discrepancy was not mirrored by soil contents in organic C and N that were unexpectedly similar between irrigation modalities. A similar result was noticed in an agricultural clay soil for pH, phosphorus and nitrogen contents (Heidarpour et al. 2007). The contrast in organic loads between irrigation regimes should have been strong enough to trigger distinct soil organic C/N contents all the more this irrigation type lasted for years. But soil organic matter is a characteristic that requires several years to significantly evolve even in the case of changes linked to human disturbance (Sikora and

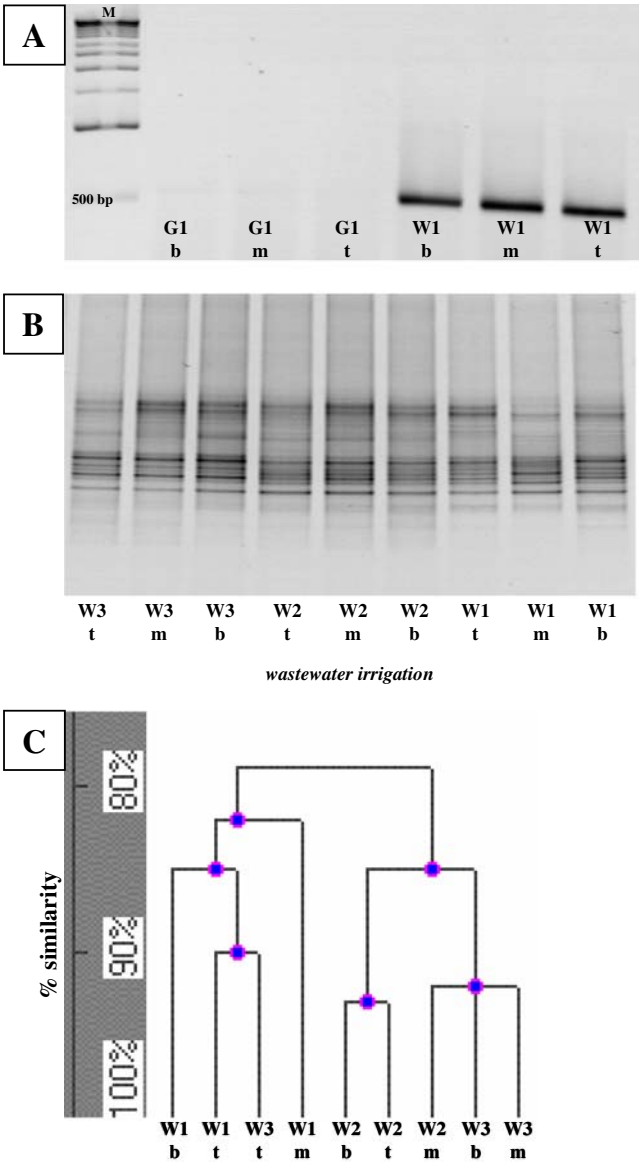

Fig. 2 **A** Agarose (2%) migration patterns showing absence and presence of *amoA* fragments amplified from soil DNA of plot no. 1 irrigated with groundwater (encoded *G1*) and of plot no. 1 irrigated with wastewater (encoded *W1*), respectively. *t*, *m* and *b* letters refer to top, medium and bottom slope points, respectively. *M* size DNA marker, *bp* bases pair. **B** DGGE fingerprints of *amoA* fragments amplified from soil DNA of plots irrigated with wastewater (encoded *W*). Numbers *1*, *2* and *3* refer to plot numbers; *t*, *m* and *b* letters refer to top, medium and bottom slope points, respectively. **C** UGPMA dendrogram assessing the similarity of *amoA*-DGGE patterns obtained from soil DNA of plots irrigated with wastewater (*W*). Numbers *1*, *2* and *3* refer to plot numbers

Stott 1996). For instance, only a 1.4-fold increase in soil organic C was measured over nearly 90 years of wastewater application (Ramirez-Fuentes et al. 2002). Besides, owing to the sandy soil texture of our site, organic particles and soluble organic compounds brought in daily by wastewater may have had a short residence time in the sampled top soil. This leaching hypothesis could also be held back to account for similar soil contents in NO_3^- and NH_4^+. Nevertheless, this physical explanation is not entirely convincing, as plots irrigated by groundwater (high NO_3^- load) still contain substantial amounts of this nutrient known to be easily leached. Thus, leaching alone could not account for the observed similarities in soil chemistry. Besides, distinct levels of biological activities such as mineralisation or nitrification could help fill the gap. For instance, the significant acidification of plots irrigated by wastewater of neutral pH could partly result from allochthonous organic matter and microbial oxidation of its NH_4^+ load (Princic et al. 1998). Mineralisation of this organic matter could then partly explain the absence of differences in organic contents between both types of plots. Influence of root and crop residues derived C can also play a consistent role in this leveling, as both types of plots are continuously cultivated all year long. Nitrification in these plots would also explain the size of their NO_3^- pools that cannot be fuelled by wastewater amendments.

Neither microbial biomass nor microbial activity (FDA) was impacted by irrigation regimes. This finding was unexpected, as readily available nutrients and organic matter contained in wastewater were ideal compounds to sustain increased growth and activity of soil microbiota. Moreover, wastewater contains its own microorganisms that should have at least increased soil microbial biomass. In a similar irrigation assay, CO_2 emission and dehydrogenase activity were found, among others, to be lowered by wastewater supply, even though stimulation of microbial activity and biomass seems to be the rule (Meli et al. 2002; Brzezinska et al. 2006). In particular, recurrent wastewater flooding was shown to increase several parameters, among which microbial biomass and substrate-induced respiration, concurrently to a marked compositional shift in bacterial community structure (Gelsomino et al. 2006). In our case, absence of difference in microbial biomass or enzyme activity probably depended on the resistance of total bacterial community structure that supported a global functional resistance (i.e. FDA activity). High bacterial diversity was recently shown to favour community resistance to chemical perturbation of soil (Girvan et al. 2005). Minor shifts between our 16S rDNA fingerprints could also suggest a high bacterial diversity at this site. This lack of a salient effect of irrigation regimes on the structural diversity of bacterial communities is unlikely to stem from soil storage conditions. Although soil air drying is recognised to lower active microbial biomass and enzyme activities (Rao et al. 2003; Whiteley et al. 2003), these storage conditions were preferred over handling of moist samples for technical convenience and assuming that long-term storage in presence of water could be more detrimental to DNA conservation. This assumption is apparently strengthened by a recent study indicating that air-dried soils can protect microbial DNA for

more than 150 years (Clark and Hirsch 2008). Moreover, repeated air-drying/wetting cycles appear to be more efficient in altering genomic DNA functionality than a single drying (Pietramellara et al. 1997), and our desiccation step was probably too short to allow an extensive DNA degradation.

On the contrary, water quality had a salient impact at the scale of the functional AOB community. The inability to recover an *amoA* signal from plots under groundwater cannot be attributable to PCR inhibitors in template DNA, as the same samples gave 16S rDNA amplicons. Thus, a reduced size of the AOB community can reasonably account for our inability to amplify *amoA* targets whose density in soil template DNA might have been below our threshold detection. Ammonium availability has often been demonstrated to exert a selective control over the AOB community activity and development (Princic et al. 1998; Prosser and Embley 2002; Avrahami et al. 2003; Okano et al. 2004; Geets et al. 2006), especially in a sandy soil irrigated with tertiary-treated wastewater (Cantera et al. 2006). Consequently, poor NH_4^+ concentration of groundwater should be regarded as a major limiting factor of AOB development in these plots. In this background, competition for NH_4^+ with heterotrophic microorganisms and plants was very likely to have magnified the scarcity of available substrate (Verhagen et al. 1992). This postulate could be reinforced by preliminary results of *amoA* gene detection in irrigation waters. Amplicons were obtained for both types of water, especially in wastewater (not shown). Yet, amplicon length was unspecific in all cases despite modifications of the PCR parameters (combined decreased concentration of template DNA and primers together with increased hybridation temperature up to 62°C). Further protocol development is needed to confirm that AOB are present in groundwater but not in the corresponding irrigated plots, owing to a possible enhanced competition for soil NH_4^+. Alternatively, the strong *amoA* PCR bands obtained for plots under wastewater may be indicative of a consistent AOB biomass, and thus of a significant in situ nitrification activity, comforting our view on soil NO_3^- origin in these plots. In the present study, shifts of *amoA* fingerprints between plots were rather weak, which could be indicative of the unique origin of wastewater and of its own AOB community whose members may have been included in the *amoA* fingerprints. Wastewater was recently shown to reduce the diversity of the AOB community (Gelsomino et al. 2006). Here, with regards to the high NH_4^+ and organic matter loads, the recovered *amoA* diversity could refer to AOB members not inhibited by NH_4^+ and organic matter excess, a property often encountered among representatives of *Nitrosomonas* spp. and *Nitrosospira* clusters 1 and 3 (Hastings et al. 1997; Rotthauwe et al. 1997; Princic et al. 1998; Oved et al. 2001; Jordan et al. 2005; Chu et al. 2007).

In conclusion, this study highlighted a double nitrogen pollution in the suburban agriculture area of Dakar. Our results suggest that NO_3^- contamination of belowground water could be partly linked to nitrification of NH_4^+ contained in wastewater. Whereas soil chemistry and total bacterial community did not appear to be affected by the contrasted irrigation regimes, the AOB community was deeply impacted by groundwater supply. Overall, this functional community appeared to be a relevant biological indicator of disturbance induced by waste management and NO_3^- pollution. Further investigation is needed to clarify the extent of nitrification disruption in plots irrigated by groundwater.

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