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ORIGINAL ARTICLE

Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment

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The relative importance of size and composition of microbial communities in ecosystem functioning is poorly understood. Here, we investigated how community composition and size of selected functional guilds in the nitrogen cycle correlated with agroecosystem functioning, which was defined as microbial process rates, total crop yield and nitrogen content in the crop. Soil was sampled from a 50-year fertilizer trial and the treatments comprised unfertilized bare fallow, unfertilized with crop, and plots with crop fertilized with calcium nitrate, ammonium sulfate, solid cattle manure or sewage sludge. The size of the functional guilds and the total bacterial community were greatly affected by the fertilization regimes, especially by the sewage sludge and ammonium sulfate treatments. The community size results were combined with previously published data on the composition of the corresponding communities, potential ammonia oxidation, denitrification, basal and substrate-induced respiration rates, in addition to crop yield for an integrated analysis. It was found that differences in size, rather than composition, correlated with differences in process rates for the denitrifier and ammonia-oxidizing archaeal and total bacterial communities, whereas neither differences in size nor composition was correlated with differences in process rates for the ammonia-oxidizing bacterial community. In contrast, the composition of nitrate-reducing, denitrifying and total bacterial communities co-varied with primary production and both were strongly linked to soil properties.
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Introduction

Ecosystem functioning is a broad term that encompasses, for example, process rates, primary or secondary production and cycling of nutrients (Hooper *et al.*, 2005). As most of these processes are driven by microbial communities, microorganisms are key players for ecosystem functioning. Although microbial species composition may be important for ecosystem functioning, microbiologists struggle with the challenges of distinguishing species and their traits in addition to a poorly defined species concept. Closely related species or strains can have different physiological or metabolic features, whereas distantly related ones can share the same functional trait, making species affiliation a poor predictor of ecosystem functions. Instead, information on functional communities, which are defined as assemblages of populations sharing certain features or carrying out the same process, may forecast specific ecosystem responses or aid in the proper management of agroecosystems. Thus, a trait-centered perspective along environmental gradients, as suggested by McGill *et al.* (2006), would be a tractable way for environmental microbiology to bring general patterns into community ecology. For this, functional communities involved in the nitrogen cycle, such as ammonia oxidizers and denitrifiers, have been suggested as models for ecology studies aiming at elucidating the significance of microbial communities in agroecosystem functioning (Kowalchuk and Stephen, 2001; Philippot and Hallin, 2005). The ammonia-oxidizing prokaryotes

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perform the first step in nitrification, oxidizing ammonia into nitrite. This step is followed by the oxidation to nitrate by nitrite oxidizers; nitrate can then be reduced into nitrite or stepwise further to dinitrogen gas by nitrate reducers and denitrifiers, respectively. In contrast to nitrate reduction and denitrification, which are traits found in many different bacteria, archaea and eukaryotes, oxidation of ammonia is performed only by a few phylogenetically constrained ammonia-oxidizing bacteria (AOB) and the ammonia-oxidizing archaea (AOA).

Research on soil microbial communities involved in nitrogen cycling has concerned more often the analysis of process rates in relation to the composition than to the community size of the corresponding functional communities. Therefore, the relative importance of community size versus composition in ecosystem functioning is still an open question. Taken that the different steps within the nitrogen cycle are performed by communities that may have different levels of diversity, the meaning of community composition would not be the same for different steps. However, one could argue that cell numbers of a given guild would be so strongly correlated with process rates that these become independent of community composition and are purely based on community size. This leads to the question: what matters most for ecosystem functioning—composition or size of functional communities?

We tested how community composition and size correlated with agroecosystem functioning, which we defined as microbial process rates, total crop yield and nitrogen content in the crop. Focus was on the nitrate-reducing and denitrifying bacteria as well as the AOB and AOA as model functional guilds, in addition to the total bacterial community in soil sampled in a long-term fertilizer field experiment established in 1956 in Sweden. The site provides a unique model system to relate microbial communities to ecosystem functioning, as the long-term effects related to fertilization have changed microbial activity and community composition (Enwall *et al*., 2005, 2007), in addition to differentiated soil properties and crop yield. We determined the size of the functional guilds and the total bacterial community by quantifying genes encoding some of the enzymes catalyzing reactions in the nitrogen cycle and 16S rRNA genes, respectively. To explore the effect of correlation structure of community size and composition on ecosystem functions, the density data were subjected to an integrated statistical analysis using environmental factors and crop yield, together with the results on community composition and potential process rates previously reported from the same sampling occasion (Enwall *et al*., 2005, 2007).

Materials and methods

Experimental site, soil properties, crop yield and process rates

The field site was established in 1956 in Ultuna (Sweden) (Kirchmann *et al*., 1994). The soil at the site is a clay loam classified as a Eutric Cambisol according to the system of the Food and Agriculture Organization. The setup is a block design with three independent replicate blocks, in which each block comprises different treatments randomized in plots of 2×2 m separated by frames. This study includes six treatments: unfertilized bare fallow, unfertilized, fertilized with calcium nitrate, ammonium sulfate, solid cattle manure and sewage sludge (Table 1). The organic fertilizers were applied during fall every second year in amounts corresponding to 8000 kg ash-free organic matter per ha, and mineral fertilizers have been applied yearly in spring at a rate of 80 kg N per ha. All treatments have been fertilized yearly with 22 kg phosphorus and 35–38 kg potassium per ha. During the years 1956–1999, spring-sown cereals dominated in the crop rotation and fodder rape and fodder beet were occasionally cultivated (Kirchmann *et al*., 1994), but since 2000, all plots except the bare fallows have been cropped with maize (*Zea mays*). To obtain a good estimate of the primary production in the different treatments, the average crop yield during 2000–2006 for each

Table 1 Soil properties and crop yield for different fertilizer treatments (mean ± s.d., $n = 3$)

Treatment	*Fertilizer regime*	*pH*[a]	*Soil Tot-C*[a] *(% of dw)*	*Soil Tot-N*[a] *(% of dw)*	*C/N*	*Crop yield*[b] *(kg dw)*	*N yield*[c] *(%)*
A	Unfertilized (bare fallow)	5.47 ± 0.16^{d}	1.01 ± 0.03^{d}	0.09 ± 0.00^{d}	11.2^{d}	NA	NA
B	Unfertilized	5.63 ± 0.05^{d}	1.22 ± 0.10^{e}	0.11 ± 0.01^{e}	11.1^{d}	4079 ± 1883^{d}	1.09 ± 0.285^{d}
C	Calcium nitrate $Ca(NO_3)_2$	6.26 ± 0.04^{e}	1.43 ± 0.03^{f}	0.13 ± 0.00^{f}	11.0^{d}	7629 ± 3271^{e}	1.43 ± 0.305^{e}
D	Ammonium sulfate $(NH_4)_2SO_4$	3.97 ± 0.14^{f}	$1.29 \pm 0.01^{e,f}$	0.13 ± 0.01^{f}	9.9^{b}	4258 ± 2313^{d}	1.60 ± 0.263^{e}
J	Solid cattle manure	6.02 ± 0.09^{e}	2.20 ± 0.05^{d}	0.20 ± 0.01^{d}	11.0^{d}	$9081 \pm 4640^{e,f}$	1.16 ± 0.258^{d}
O	Sewage sludge	4.68 ± 0.03^{d}	2.76 ± 0.08^{e}	0.27 ± 0.01^{e}	10.2^{b}	10392 ± 4098^{f}	1.57 ± 0.202^{e}

Abbreviations: dw, dry weight; NA, not applicable; Tot-C, total soil carbon; Tot-N, total soil nitrogen.
All plots, except the bare fallows, were planted with maize. Values followed by the same letter (d–f) within columns are not significantly different ($P > 0.05$).
[a]When the experimental site was established in 1956, the soil pH was 6.5 and the Tot-C and Tot-N were 1.5 and 0.17% of the dry solids, respectively.
[b]Total green biomass.
[c]Nitrogen content in total green biomass.

plot was determined both as dry weight of harvested green biomass and the nitrogen content in the green biomass, measured as Kjeldahl nitrogen (ISO 10694; Table 1). Soils were sampled between plant rows directly after harvest in September 2002 from each plot of the six treatments ($n = 18$). Ten soil cores (2 cm diameter, 20 cm depth) were taken from each plot, mixed into one composite sample, sieved (4-mm mesh) and stored at −20 °C. The 18 soil samples were analyzed separately throughout this and earlier studies (Enwall *et al.*, 2005, 2007).

Total soil nitrogen was measured as Kjeldahl nitrogen (ISO 10694), total content of organic carbon was determined through dry combustion (ISO 13878) and the pH was measured in slurry of 0.01 M calcium chloride (ISO 10390) at the time of sampling (Table 1). Potential ammonia oxidation and denitrification rates were determined in earlier studies using the chlorate and acetylene inhibition techniques, respectively (Enwall *et al.*, 2005, 2007). In the same studies, basal soil respiration and substrate-induced respiration were determined using a respirometer analyzing the accumulating carbon dioxide by automatic conductivity readings during soil incubation.

DNA extraction

For each of the 18 samples, three independent DNA extractions were performed using the FastDNA Kit for Soil (MP Biomedicals, Solon, OH, USA). The three extracts from the same replicate plot were pooled before further analysis. DNA concentration and quality was measured using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Quantification of functional communities

Quantitative PCR (qPCR) of genes encoding the key enzymes of ammonia oxidation (*amoA* encoding the ammonia monooxygenase) in both AOB and AOA, nitrate reduction (*narG* encoding the membrane-bound nitrate reductase) and denitrification (*nirK*, *nirS* and *nosZ* encoding the cd_1 and copper nitrite reductases (NirS and NirK) and the nitrous oxide reductase (Nos), respectively) was used to estimate the density of functional communities involved in the nitrogen cycle. In addition, 16S rRNA genes of the total bacterial community were quantified. The quantification was based on the fluorescence intensity of the SYBR Green dye, which binds to double-stranded DNA. For the denitrification genes and the 16S rRNA genes, the 25 µl PCR mixture contained 12.5 µl of SYBR Green PCR Master Mix (Qiagen, Valencia, CA, USA), 1 µM of each primer, 100 ng of T4 gp32 (Qbiogene) and 12.5 ng of DNA. For the *amoA* genes in the AOB or AOA, the 25 µl PCR mixture contained 12.5 µl of SYBR Green PCR Master Mix (Qiagen), 3% bovine serum albumin, 20 ng of DNA and 0.3 or 0.2 µM of each primer, respectively. Thermal cycling conditions and primers used for each reaction are described in Table 1, Supplementary Information. Reactions were carried out with an ABI prism 7900 (Applied Biosystem, Foster City, CA, USA). Standard curves were obtained using serial dilutions of linearized plasmids containing cloned *amoA* (AOB), *amoA* (AOA), *narG*, *nirK*, *nirS*, *nosZ* and 16S rRNA genes. Two independent qPCRs were performed for all samples, and an average value was calculated. No template controls gave null or negligible values. The presence of PCR inhibitors in DNA extracted from soil was examined by (i) diluting soil DNA extract and (ii) mixing a known amount of standard DNA to soil DNA extract before qPCR. In none of the cases inhibition was detected.

PCR amplification of amoA *genes in AOA for terminal restriction fragment length polymorphism analysis*

Fragments of the *amoA* gene for terminal restriction fragment length polymorphism (T-RFLP) analysis of AOA communities were amplified with the primer pair CrenamoA23f:CrenamoA616r (Toura *et al.*, 2008). The forward primer was hexachlorofluorescein labeled. Amplification was performed in triplicate in a total volume of 25 µl with 2.5 µl PCR buffer, 200 µM of each deoxyribonucleoside triphosphate, 1.25 U Taq Polymerase (GE Healthcare, Amersham, UK), 0.4 µM of each primer, 800 ng µl^{-1} bovine serum albumin and 20 ng of DNA. No *amoA* amplicons were obtained from treatments D and O, and the subsequent T-RFLP analyses were therefore discontinued.

Statistical analysis

Analysis of variance and Tukey's honest significant differences tests of soil properties, microbial process rates (Enwall *et al.*, 2005, 2007), crop yield and the data from qPCR analysis of the communities were performed using the MASS package (Ihaka and Gentleman, 1996) in the R statistical programming environment.

Community composition of AOB, nitrate reducers, denitrifiers and total bacteria, which consisted of AOB 16S rRNA DGGE, *narG* RFLP, *nosZ* DGGE and 16S rRNA T-RFLP data also determined from the same sampling occasion (Enwall *et al.*, 2005, 2007), was analyzed using non-metric multidimensional scaling (NMS) implemented in the vegan (Oksanen *et al.*, 2007) and LabDSV (Roberts, 2006) packages for R. For all NMS ordinations, data matrices of community fingerprints were binary transformed (presence/absence of bands/peaks), and the Sørenson's distance measure was used to generate dissimilarity matrices (Sørensen, 1948). Community size, soil properties, microbial process rates and crop yield were incorporated into the analysis through the use of bi-plot ordinations, in which variables were combined into a secondary matrix and plotted as vector fits against community composition ordinations. Before analyses, the qPCR data were $\log_{10}$ transformed and then the secondary

matrix was normalized by dividing values within each variable with column totals. Optimal NMS configurations were determined using 1000 random starts with 1000 iterations each, and ordinations with the lowest stress values were used. Vector and surface fitting of variables within ordinations were performed using the *envfit* and *ordisurf* functions, respectively, in the vegan package. Permutation tests ($n = 1000$) were used to determine the significance of vector fits with ordination axes, and significant ($P < 0.05$) variables were included in the resulting bi-plots. Confidence ellipses at the 0.95 level for sample sites were included in ordinations to examine the variability of sample positions within each ordination.

Mantel's test (Mantel, 1967) was used to evaluate the correspondence between dissimilarity matrices. For this purpose, the values for the soil properties, crop yield, microbial process rates and size of the communities were transformed to dissimilarity matrices using Euclidian distance measure with the *vegdist* function in the vegan package for R, whereas the community composition dissimilarity matrices were obtained by Sørenson's distance measure. The tests were performed using Pearson's product-moment correlation coefficient and 1000 permutations for each test. All data sets were examined for normality using quantile–quantile plots before creation of distance matrices.

Results and discussion

Fertilization effects on the size of microbial communities

The long-term Ultuna experimental site established in 1956 comprises several fertilization regimes, and the treatments used in this study included unfertilized bare fallow, unfertilized plots with crop, and plots with crop fertilized with calcium nitrate, ammonium sulfate, solid cattle manure or sewage sludge (Table 1). Our results demonstrate that these long-term fertilization regimes have influenced the size of microbial guilds responsible for ammonia oxidation, nitrate reduction and denitrification, which was estimated by qPCR of the genes encoding the main catalytic enzymes from the corresponding processes (Figure 1). These effects were significant when gene copy numbers were expressed both as copy numbers per gram of soil (Figure 1) and as copy numbers per ng of DNA extracted (data not shown). When comparing the two organically fertilized soils, the number of nitrate reducers (*narG*) and denitrifiers (*nirS*, *nosZ*) were significantly lower in the plots treated with sewage sludge than in the ones with manure. The lower pH of 4.7 in the sludge plots compared with that of 6.0 in the manure-treated plot, in combination with the higher heavy metal content measured in the sludge plots (Bergkvist *et al*., 2003) could counteract the positive effects expected by high organic inputs. Overall, the size of these functional guilds was one to two orders of magnitude lower in the ammonium sulfate treatment compared with the other treatments. Again this might be explained by the lower pH of 4.0 in these plots. Similarly, both AOB and AOA were present in lower numbers in the plots treated with ammonium sulfate (Figure 1). Interestingly, the sewage sludge treatment had a negative impact on the size of the archaeal ammonia oxidizers, but not the bacterial counterpart. Thus, our results confirm that the fertilization regimes can affect the AOB/AOA ratio (He *et al*., 2007). Most often it has been

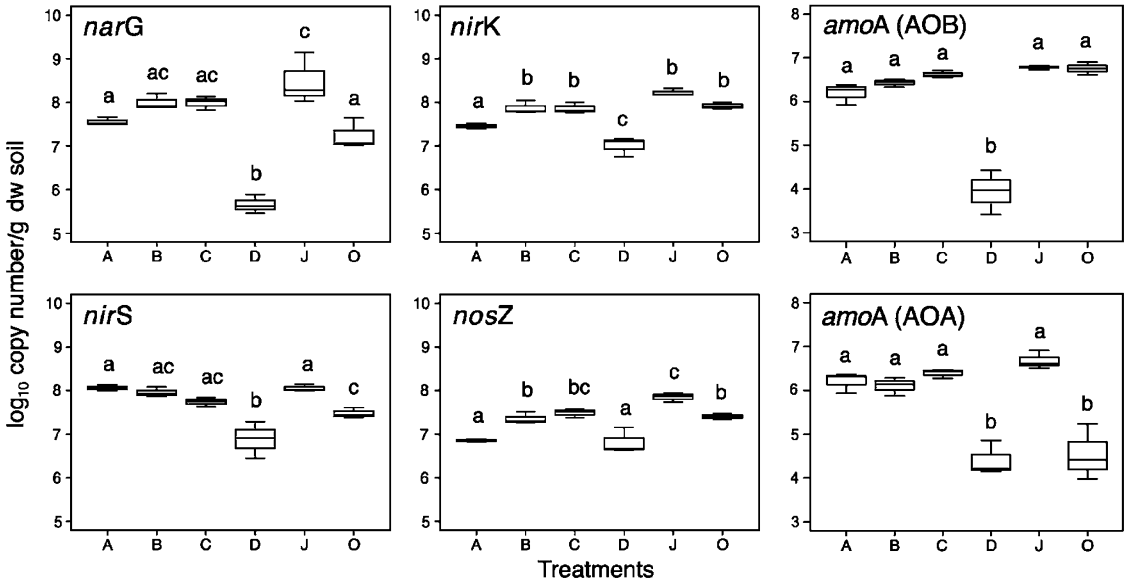

Figure 1 Gene copy numbers per g of dry soil of functional guilds involved in nitrogen cycling (*narG*, *nirK*, *amoA* (AOB), *nirS*, *nosZ* and *amoA* (AOA)) in six different fertilizer treatments (A, unfertilized bare fallow; B, unfertilized; C, fertilized with calcium nitrate; D, ammonium sulfate; J, solid cattle manure and O, sewage sludge). Error bars show data range ($n = 3$). Significant differences between treatments at the $\alpha = 0.05$ level are indicated by different letters for each gene.

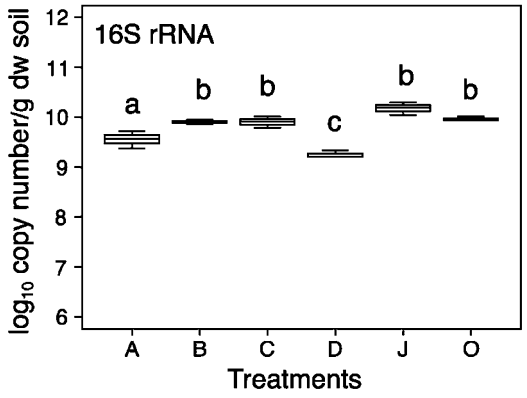

Figure 2 Gene copy numbers per g of dry soil of the total bacterial community (16S rRNA) in six different fertilizer treatments (A, unfertilized bare fallow; B, unfertilized; C, fertilized with calcium nitrate; D, ammonium sulfate; J, solid cattle manure and O, sewage sludge). Error bars show data range ($n = 3$). Significant differences between treatments at the $P = 0.05$ level are indicated by different letters.

shown that the AOA abundance either exceeds or is the same as the AOB abundance in soil (Leininger *et al.*, 2006, Nicol *et al.*, 2008), but as in the sewage sludge treatment, the AOB sometimes dominates (Boyle-Yarwood *et al.*, 2008; Santoro *et al.*, 2008). However, it is not yet clear which conditions favor the numerical dominance of one or the other of the two types of ammonia oxidizers. The size of the total bacterial community followed the same trend as the functional communities (Figure 2), but a direct comparison of 16S rRNA gene copies between treatments should be interpreted with caution. In contrast to the *narG*, *nirK*, *nirS*, *nosZ* and *amoA* gene copy number per cell, which differ only slightly between prokaryotes (1–3), the 16S rRNA gene copy number per cell can vary between 1 and 15. Nonetheless, this study clearly demonstrated that abundance of functional guilds, and probably also total bacteria, is greatly affected by fertilization regimes.

The density of nitrate reducers (*narG*), denitrifiers (*nirS, nirK* and *nosZ*) and ammonia oxidizers (AOB and AOA *amoA*) per biomass unit (μg CO_2-C per g dw soil per h), estimated by substrate-induced respiration (Martens, 1995), was significantly lower in the ammonium sulfate treatment (data not shown). As also the 16S rRNA gene copy number per biomass unit was lower in the ammonium sulfate plots, it is likely that the microbial biomass was dominated by fungi in this treatment. This was shown earlier by Marstorp *et al.* (2000), who found that fungal biomass estimated from ergosterol content per biomass C was higher in the ammonium sulfate plots compared with the other plots at the Ultuna site. In the other treatments, the density per biomass unit was relatively constant for all targeted genes, except for the AOA *amoA* in the sewage sludge treatment. This confirmed the negative impact of sewage sludge on AOA community size compared with the other microbial communities.

Quantification of the *nirS* and *nirK* genes revealed a significantly higher *nirS*/*nirK* ratio in the unfertilized bare fallow soils, being three to ten times higher compared with the treatments with crops (data not shown). Taking into account that the reduction of nitrite by denitrifiers can be performed either by a copper nitrite reductase (NirK) or by a cytochrome cd_1 nitrite reductase (NirS) and that denitrifying bacteria possess only one type of Nir, these findings indicate a selection for an Nir type by the habitat created by the presence or absence of plants. We postulate that NirS and NirK, which are functionally equivalent, are not ecologically redundant. It is therefore tempting to explain the maintenance of two types of nitrite reductases over bacterial evolution by niche differentiation, which can provide insurance against competitive exclusion.

Exploring ecological patterns

Explorative analyses of ecological patterns were performed to find correlations between community composition, community size, soil properties and ecosystem functioning (Figure 3). For the latter, basal respiration, substrate-induced respiration, potential ammonia oxidation and denitrification rates were used as process rates, and the mean annual crop yield during 7 years was used as a measure of primary production. To obtain a measure of both quantity and quality, yield was determined as total above-ground biomass and as the mean nitrogen concentration in the plants, respectively. Both factors were significantly different between most treatments (Table 1). For each community, the soil properties, process rates, crop yield and data on abundance for all the functional genes were compared with separation of sampling sites in ordination space, based on the binary-transformed fingerprint data, by vector fitting of variables with sample ordination scores. We were unable to obtain PCR products for AOA in the D and O treatments. The *amoA* gene abundance was low in these samples (Figure 1) and we were likely below the detection limit for amplification using regular PCR. Without results from all treatments, the subsequent community fingerprinting of the AOA was discontinued.

Our analysis resulted in a clear sorting of soil samples according to treatment with 95% confidence levels for the denitrifier community (Figure 3c). When targeting the nitrate-reducing community, all treatments differed from each other except the unfertilized bare fallow, which was similar to the calcium nitrate and unfertilized treatments (Figure 3a), whereas for the AOB community, only the sludge and ammonium sulfate treatments separated from each other and from all other treatments (Figure 3b). In comparison, only

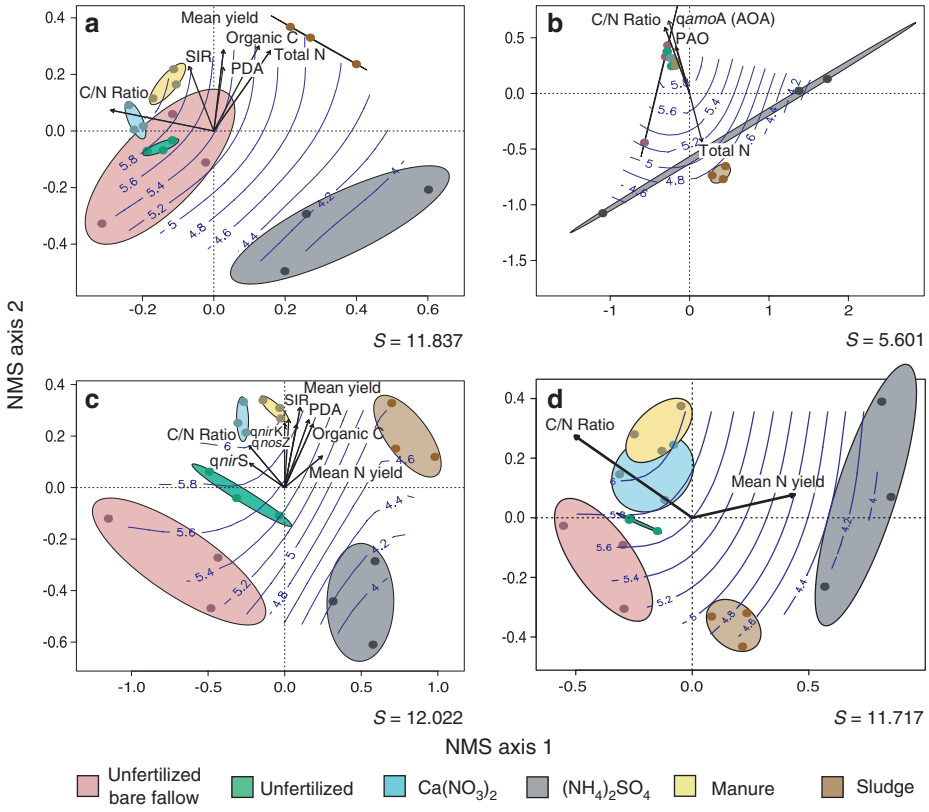

Figure 3 Non-metric multidimensional scaling analysis of fertilization effects on functional guilds involved in nitrogen cycling. (**a**) Nitrate-reducing bacteria fingerprinted by RFLP of *narG*, (**b**) ammonia-oxidizing bacteria (AOB) fingerprinted by DGGE of specific AOB 16S rRNA genes, (**c**) denitrifying bacteria fingerprinted by DGGE of *nosZ* and (**d**) the total bacterial community fingerprinted by T-RFLP of 16S rRNA genes. The size and process rate of the corresponding communities (PAO, potential ammonia oxidation; PDA, potential denitrification activity; SIR, substrate-induced respiration), as well as soil properties (total N, organic C and C/N ratio) and crop yield (mean yield and mean N yield) were incorporated in the analyses using vector fitting with ordination scores. Arrow length is proportional to the strength of correlation in resulting bi-plots. The significance of vector fits was determined using permutation tests ($n = 1000$) at the $P = 0.05$ level. Ellipses indicate 95% confidence interval for replicates. Contours represent the pH data fit to sample ordination scores. Stress values (S) are indicated for each analysis. RFLP, restriction fragment length polymorphism.

the calcium nitrate and manure treatments were similar for the total bacterial community (Figure 3d). As a separation of the fallow treatment plots from the other treatments was seen only for the denitrifier and total bacterial communities, it suggests that the absence of crops during the vegetation period was a less important indicator of community composition patterns compared with other factors. This is in agreement with studies reporting that the extent to which plants affect microbial community composition could depend on the taxonomical (Costa *et al.*, 2006) or functional (Patra *et al.*, 2006) microbial group investigated. For all communities, pH had a strong impact on the differences in community composition between soils treated with ammonium sulfate or sewage sludge and the other treatments (Figure 3). These results are consistent with those of a recent study showing that pH was the best predictor of soil microbial community composition, even at larger scales (Fierer and Jackson, 2006). Secondary effects of fertilization causing changes in pH and affecting functional communities involved in the nitrogen cycle have been widely documented and summarized in the reviews by Philippot *et al.* (2007) and Prosser (2007). Additional variables correlated with the separation of soil samples were the C/N ratio, soil organic C, soil N, process rates and crop yield.

Significance of microbial community composition and size in process rates and crop yield

The significance of relationships between microbial community size, composition and ecosystem functioning explored by NMS was confirmed by comparing dissimilarity matrices of the data (Table 2). It was apparent that β-diversity, defined as the community composition dissimilarities among sites (Whittaker, 1972), was significantly correlated between the different communities. The differences in community size among treatments were also correlated between nearly all of the communities. These results are not surprising as we observed both a similar treatment effect on the size of targeted communities (Figure 1) and a similar separation of some treatments in all the NMS ordinations (Figure 3). Significant correlations were observed between composition and size of all functional

Table 2 Mantel's tests of dissimilarity matrices for soil properties (abiotic), ecosystem functioning (primary production in terms of crop yield; PAO; PDA; SIR; and basal respiration), community size and community composition

	Abiotic	*Yield*	*N yield*	*Basal respiration*	*SIR*	*PAO*	*PDA*	*q16S*	*qAOB*	*qAOA*	*q*nar*G*	*q*nir*K*	*q*nir*S*	*q*nir*(S+K)*	*q*nos*Z*	*16S T-RFLP*	*AOB DGGE*	nar*G RFLP*	nos*Z DGGE*
Abiotic		***	NS	***	*	NS	***	NS	NS	NS	NS	NS	NS	NS	NS	*	*	**	***
Function																			
Yield	0.60		**	***	***	NS	***	*	NS	NS	NS	NS	NS	NS	**	NS	NS	*	***
N yield	0.11	0.58		**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**
Basal respiration	0.81	0.62	0.40		*	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	***
SIR	0.29	0.47	0.11	0.22		***	***	**	NS	NS	NS	*	NS	NS	***	NS	NS	NS	NS
PAO	0.14	0.06	−0.11	0.00	0.76		**	*	NS	*	NS	NS	NS	NS	**	NS	NS	NS	NS
PDA	0.53	0.55	0.16	0.50	0.78	0.53		*	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS
Abundance																			
q16S	0.12	0.21	0.11	0.01	0.39	0.26	0.23		***	*	***	***	**	***	***	***	**	**	*
qAOB	0.06	0.00	−0.01	−0.10	0.02	−0.01	−0.04	0.71		*	***	***	***	**	***	***	***	***	*
qAOA	0.30	−0.01	0.04	0.11	0.01	0.29	−0.06	0.24	0.43		**	*	***	**	NS	***	**	***	***
q*nar*G	0.04	−0.13	−0.03	−0.12	0.08	0.20	−0.07	0.65	0.84	0.60		***	***	***	**	***	***	**	**
q*nir*K	0.12	0.18	0.08	−0.04	0.34	0.23	0.20	0.87	0.81	0.32	0.75		***	***	***	***	**	**	*
q*nir*S	0.07	−0.07	0.15	−0.02	−0.07	0.03	−0.11	0.48	0.79	0.62	0.79	0.63		***	**	***	***	***	**
q*nir*(S+K)	−0.03	−0.15	−0.06	−0.17	0.08	0.13	−0.05	0.67	0.90	0.48	0.87	0.83	0.89		**	***	***	***	NS
q*nos*Z	0.14	0.39	0.24	0.07	0.65	0.46	0.44	0.78	0.46	0.16	0.45	0.78	0.35	0.49		*	*	*	*
Structure																			
16S T-RFLP	0.27	0.09	0.20	0.16	−0.02	0.12	−0.04	0.43	0.71	0.65	0.75	0.57	0.71	0.66	0.28		***	***	***
AOB DGGE	0.37	0.08	0.11	0.20	−0.07	0.05	−0.04	0.45	0.73	0.71	0.70	0.53	0.74	0.66	0.26	0.82		***	***
*nar*G RFLP	0.50	0.20	0.11	0.31	0.01	0.08	0.05	0.38	0.65	0.65	0.60	0.48	0.64	0.56	0.25	0.79	0.87		***
*nos*Z DGGE	0.51	0.41	0.46	0.50	0.06	0.10	0.10	0.29	0.37	0.58	0.37	0.31	0.45	0.28	0.24	0.64	0.69	0.68	

Abbreviations: NS, not significant at $P > 0.05$. PAO, potential ammonia oxidation; PDA, potential denitrification activity; SIR, substrate-induced respiration
$^{***}P \leqslant 0.001$, $^{**}0.001 < P \leqslant 0.01$, $^{*}0.01 < P \leqslant 0.05$;
Abiotic factors, ecosystem functioning and community abundance dissimilarities were calculated using Euclidian distances, whereas community composition dissimilarities were calculated using Sørenson's distances. Data for soil properties were concatenated into a single data matrix.

guilds: nitrate reducers, denitrifiers and ammonia oxidizers in addition to the total bacterial community (Table 2). Thus, differences in community composition appear connected to differences in community size. Whether it is related to the level of diversity, in terms of richness, cannot be concluded from our results, as fingerprinting methods cannot give an estimate of richness.

For process rates, differences in substrate-induced respiration were significantly correlated with differences in total bacterial community size, estimated by the quantification of the 16S rRNA gene copy number (Table 2). When looking at the functional communities, differences in potential denitrification rates were significantly correlated with differences in denitrifier density estimated by the quantification of *nosZ* gene copy numbers (Table 2). These results are consistent with recent studies showing that denitrification enzyme activity was correlated with the size of the denitrifier community estimated by most probable number (Patra *et al.*, 2005) or by the quantification of *nirK* genes (Throbäck *et al.*, 2007), which suggests that the size of the denitrifier community could predict the corresponding process rate. On the other hand, we did not find significant correlations between denitrification rates and the abundance of *nirS* and *nirK* genes. This lack of correlation underlines the fact that the size of a functional gene pool might not reflect the activity of the corresponding enzyme, due to subsequent hierarchical regulation (Röling, 2007). Differences in potential ammonia oxidation rates were significantly correlated with differences in the AOA community size, but not the AOB community size (Table 2). This was again explained by the high abundance of AOB in the sewage sludge treatment while low rates were observed. Our result is in agreement with the study by Schauss *et al.* (2008), showing that AOA could contribute substantially to ammonia oxidation. This is in line with the mounting evidence that archaeal ammonia oxidizers may have greater role than the bacterial counterparts in many environments (see review by Prosser and Nicol, 2008).

No correlations were found between differences in composition of any of the bacterial communities and differences in their corresponding potential activities, which indicate that community composition might play a minor role for process rates compared with density. However, if not only gross rates but also qualitative aspects of process rates are considered, community composition might be of importance. Accordingly, it has been suggested that denitrifier community composition could be crucial for the balance between N_2O and N_2 as denitrification end products (Cavigelli and Robertson, 2000; Holtan-Hartwig *et al.*, 2000). It might also be speculated that under *in situ* conditions, which often are suboptimal for microbial processes (unlike the measurement of potential rates), composition of the corresponding functional microbial communities may play a more important role.

As indicated by the NMS analyses, primary production seemed more related to community composition than size and this was confirmed by the Mantel's tests (Table 2). However, the community composition is likely not directly linked to primary production, but rather to pH, soil nitrogen content and other soil properties that affect plant growth (Table 1). Accordingly, differences in both composition of all bacterial communities and crop yield correlated significantly with the differences in soil properties among the treatments (Table 2). In addition to yield, there was also a qualitative difference in primary production among the treatments, as shown by the higher nitrogen content in the crop harvested in the ammonium sulfate and sewage sludge treatments. The lower C/N ratios observed in these treatments may favor net mineralization that increases the relative amount of plant available nitrogen. In addition, nitrification is likely low as shown by the low potential rates. The main nitrogen source for plants in these treatments would thereby be ammonium, which is favored by maize.

In conclusion, this study demonstrates the potential of agricultural practices to influence size and composition of bacterial communities and the ecosystem processes they mediate. For the denitrifying, AOA and total bacterial communities, differences in size rather than in composition were correlated with differences in potential process rates. The results indicate that community size could be more important to take into account than community composition when developing biogeochemical process models that incorporate bacterial community parameters. We also showed that the composition of some bacterial communities co-varied with primary production, and both were strongly influenced by soil properties. However, neither the size nor the composition of bacterial communities was strongly linked to agroecosystem functioning. Further studies must show if this holds true also for *in situ* process rates and size and composition of gene transcripts in N cycling.

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