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ORIGINAL ARTICLE

Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale

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Little information is available regarding the landscape-scale distribution of microbial communities and its environmental determinants. However, a landscape perspective is needed to understand the relative importance of local and regional factors and land management for the microbial communities and the ecosystem services they provide. In the most comprehensive analysis of spatial patterns of microbial communities to date, we investigated the distribution of functional microbial communities involved in N-cycling and of the total bacterial and crenarchaeal communities over 107 sites in Burgundy, a 31 500 km² region of France, using a 16 × 16 km² sampling grid. At each sampling site, the abundance of total bacteria, crenarchaea, nitrate reducers, denitrifiers- and ammonia oxidizers were estimated by quantitative PCR and 42 soil physico-chemical properties were measured. The relative contributions of land use, spatial distance, climatic conditions, time, and soil physico-chemical properties to the spatial distribution of the different communities were analyzed by canonical variation partitioning. Our results indicate that 43–85% of the spatial variation in community abundances could be explained by the measured environmental parameters, with soil chemical properties (mostly pH) being the main driver. We found spatial autocorrelation up to 739 km and used geostatistical modelling to generate predictive maps of the distribution of microbial communities at the landscape scale. The present study highlights the potential of a spatially explicit approach for microbial ecology to identify the overarching factors driving the spatial heterogeneity of microbial communities even at the landscape scale.
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Introduction

Spatial patterns have long been of concern in ecology and have changed the manner in which studies of plant and animal ecology are designed and analyzed. Characterization of the patterns of species diversity is central for understanding the underlying evolutionary and ecological processes that shape biodiversity across spatial and temporal scales (Levin, 1992). Patterns also have implications for applied ecology, as understanding and predicting spatial patterns are the keys for developing ecosystem management strategies (Levin, 1992).

In contrast to plants and animals, studying spatial patterns is recent for microorganisms (Hughes-Martiny *et al.*, 2006; Ramette and Tiedje, 2007a) and an increasing body of literature supports the idea that microbial communities exhibit spatial pattern at different scales. Thus, in terrestrial ecosystems, several studies reported spatial patterns from the centimetre to the meter scale (Nunan *et al.*, 2002; Franklin and Mills, 2003; Ritz *et al.*, 2004; Philippot *et al.*, 2009a). In contrast, only a few studies have investigated spatial patterns of microbial communities over broad spatial scales even though spatial dependence was also observed at the kilometre scale (Cho and Tiedje, 2000; Dequiedt *et al.*, 2009; Yergeau *et al.*, 2009). However, such investigations at broader spatial scales are of importance as it is well known that patterns can change with the scale of description (Hutchinson, 1953). Indeed a landscape perspective is

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needed to understand the impact of human activities, geomorphology or climate on microbial community distribution. Thus, how microorganisms are spatially distributed at the landscape scale and which the factors, among land management, soil physico-chemical properties and local climate, governing their distribution are therefore central, yet unanswered, questions despite the fact that microbial communities are essential for biogeochemical cycling and ecosystem functioning.

In this study, we investigated microbial distribution at the landscape scale by focusing on the functional communities involved in nitrogen cycling because traits rather than taxa were suggested to be the fundamental units of biodiversity and biogeography (Weiher and Keddy, 1995). The potential of such a functional trait-based approach to microbial biogeography has recently been further emphasized by Green *et al.* (2008). Nitrogen-cycling microbial communities such as the ammonia oxidizers, nitrate reducers and denitrifiers have been described as excellent models of functional communities (Kowalchuk and Stephen, 2001; Philippot and Hallin, 2005), of both agronomic and environmental importance. Thus, microbial transformations within the nitrogen cycle affect the bioavailability of nitrogen, which is one of the nutrients that limit plant growth most often limiting for plant growth. Denitrification and ammonia oxidation are also major contributors to the emission of N_2O, a greenhouse gas with *ca* 300 times the global warming potential of CO_2 (Forster *et al.*, 2007) and the dominant ozone-depleting substance (Ravishankara *et al.*, 2009).

Here, we characterize and explain the spatial variability in the distribution of microbial communities that are involved in nitrogen cycling at the landscape scale. The abundance of the nitrate-reducing, denitrifying and ammonia-oxidizing communities in soil samples, collected using a grid covering 31 500 km^2, was quantified by real-time PCR. Canonical variation partitioning was used to examine the relative contributions of land management, spatial distance, climatic conditions, time and more than 40 soil physico-chemical properties to the distribution of each microbial community. We also used geostatistical modelling to investigate the spatial correlations of the microbial communities and produce maps of their distribution at the landscape scale.

Materials and methods

Experimental site and sampling

Soil sampling was performed using a systematic grid approach. For this purpose, the Burgundy region was divided into 118 cells of about $16 \times 16\,km^2$ and the soil was collected at the center of 107 out of the 118 cells (Supplementary Figure S1). This scale was selected according to the minimum sampling density recommended to monitor soils across Europe (Morvan *et al.*, 2008) and is fully compatible with the unique existing pan-European soil-monitoring network (Lacarce *et al.*, 2009). At each sampling site located in the center of the cell, 25 individual soil cores were collected in the topsoil (0–30 cm), using an unaligned sampling design within a $20 \times 20\,m^2$ area. The 25 core samples were then composited for each site. Samples of known volume were taken for bulk density determination. Soil samples were air-dried and sieved to 2 mm before analysis. Soil sampling was achieved thanks to the French Soil Quality Monitoring Network, which collected soil throughout France over a 10-year period using the same $16 \times 16\,km^2$ sampling grid. In Burgundy the 107 soil samples were collected from October 2002 to October 2008 at all seasons (37 in winter, 39 in spring, 13 in summer and 19 in fall).

Soil, climate and land use data

The following soil characteristics were measured: (i) total organic carbon content and nitrogen measured by dry combustion, (ii) particle-size distribution using five classes (clay (0–2 μm), fine silt (2–20 μm), coarse silt (20–50 μm), fine sand (50–200 μm) and coarse sand (200–2000 μm) using wet sieving and the pipette method (NF X 31–107), (iii) cation exchange capacity and Ca, Mg, K, Na, Al, Mn exchangeable cations (cobaltihexamin method), (iv) total K, Ca, Mg, Na, Fe, Al, Cd, Co, Cr, Cu, Mn, Ni, Pb, Tl, Zn, (v) pH in water (1:5 soil:water ratio), (vi) extractable boron (boiling water method) and (vii) EDTA-extractable Cd, Cr, Cu, Ni, Pb and Zn. Analyses were performed by the Soil Analysis Laboratory of INRA in Arras, France, which is accredited for soil and sludge analysis. Climate data came from the SAFRAN database (Quintana-Segui *et al.*, 2008) and included 1992–2004 averages of monthly and yearly evapotranspiration (ETP), temperature (°C) and rainfall (mm), interpolated on the basis of a $8 \times 8\,km^2$ grid. Land use was classified according to the Corine Land Cover database Classification (Heymann *et al.*, 1994) and grouped in the following broad classes: grasslands, forest, agricultural soil, vineyard and orchards.

DNA extraction

For each of the 107 samples, DNA was extracted from 250 mg to 1 g of soil based on the method developed by Martin-Laurent *et al.* (2001), which is currently under final evaluation by national body members of the ISO before being published as the ISO standard 11063 '*Soil quality—Method to directly extract DNA from soil samples*'. Even though the comparison for the ISO standardization of DNA extraction from air dried, fresh, and frozen soils from different soils did not show any significant effect on 16S rRNA gene copy number per ng of DNA (unpublished results), we cannot exclude the

possibility that our results might have been different for some soils with a different procedure. Briefly, samples were homogenized in 1 ml of extraction buffer for 30 s at 1600 r.p.m. in a minibead beater cell disrupter (Mikro-DismembratorS; B. Braun Biotech International, Melsungen, Germany). Soil and cell debris were eliminated by centrifugation (14 000 *g* for 5 min at 4 °C). After precipitation with ice-cold isopropanol, nucleic acids were purified using both polyvinylpyrrolidone and Sepharose 4B spin columns. Quality and size of soil DNA were checked by electrophoresis on 1% agarose. DNA was quantified using the Quant-iT dsDNA Assay Kit (Invitrogen, Paisley, UK) and a plate reader (Berthold Mithras LB940, Thoiry, France).

Real-time PCR quantification (qPCR)

The total bacterial and crenarcheal communities were quantified using 16S rRNA primer-based qPCR assays described previously (Ochsenrelter *et al.*, 2003). Quantification of the bacterial and crenarchaeal ammonia oxidizers was performed according to Leininger *et al.* (2006) and Tourna *et al.* (2008) whereas quantification of nitrate reducers and denitrifiers was performed according to Bru *et al.* (2007) and Henry *et al.* (2004, 2006), respectively. For this purpose, the genes encoding catalytic enzymes of ammonia oxidation (bacterial and crenarchaeal *amoA*), nitrate reduction (*narG* and *napA*) and denitrification (*nirK*, *nirS* and *nosZ*) were used as molecular markers. Reactions were carried out in an ABI prism 7900 Sequence Detection System (Applied Biosystems, Carlsbad, CA, USA). Quantification was based on the increasing fluorescence intensity of the SYBR Green dye during amplification. The real-time PCR assay was carried out in a 20 μl reaction volume containing the SYBR green PCR Master Mix (Absolute QPCR SYBR Green Rox, ABgene, Courtaboeuf, France), 1 μM of each primer, 100 ng of T4 gene 32 (QBiogene, Illkrich, France) and 0.5 − 2 ng of DNA. Two independent quantitative PCR assays were performed for each gene. Standard curves were obtained using serial dilutions of linearized plasmids containing the studied genes. PCR efficiency for the different assays ranged between 86 and 99%. Two to three no-template controls were run for each quantitative PCR assay and no template controls gave null or negligible values. The presence of PCR inhibitors in DNA extracted from soil was estimated by (i) diluting the soil DNA extract and (ii) mixing a known amount of standard DNA with soil DNA extract prior to qPCR. No inhibition was detected in either case.

As the number of 16S rRNA operons per cell is variable (Klappenbach *et al.*, 2001) the 16S rRNA gene copy data were not converted into cell numbers and the results were expressed as 16S rRNA gene copy numbers per ng of extracted DNA. Calculation of the gene copy number per ng of DNA rather than per gram of soil minimizes the bias related to possible differences in the DNA extraction yield between samples. To obtain an estimate of the relative abundance of the different functional communities within the total bacterial or crenarchaeal communities in the samples, we calculated ratios between the ammonia-oxidation, nitrate reduction and denitrification gene copy numbers and the total bacterial 16S rRNA gene copy number ratios, and between the crenarchaeal ammonia oxidation gene copy numbers and the crenarchaeal 16S rRNA gene copy number ratio.

Statistical analyses

All quantitative (response and explanatory) data were transformed using Box−Cox transformation prior to analyses (the corresponding lamba parameters were estimated by maximum likelihood (Cook and Weisberg, 1999)). Qualitative explanatory variables were transformed into dummy variables. Spatial vectors were constructed by using the Principal Coordinate of a Neighbor Matrix (PCNM) approach (Borcard and Legendre, 2002). This spatial decomposition method was applied to the geographic coordinates of the samples (data were spatially detrended if necessary), which yielded 62 spatial variables that represented all spatial scales present in the sampling scheme. The order of the PCNM variables corresponds to a progression from larger to smaller spatial scales (Borcard *et al.*, 2004). For each response data model, the most significant PCNM variables were chosen by permutational forward model selection and by ensuring that the adjusted R^2 of the reduced models did not exceed the adjusted R^2 of the global models. Explanatory variables were then selected by multiple regression analysis using stepwise selection and by minimizing the Akaike Information Criterion. Statistical significances were assessed by 1000 permutation of the reduced models. The respective effects of each explanatory variable, or combinations thereof, were determined by canonical variation partitioning (Borcard *et al.*, 1992; Ramette and Tiedje, 2007b). *P* values were Bonferroni-corrected to maintain the family-wise error level in multiple testing. All statistical calculations were performed with the R statistical platform using the vegan, PCNM and MASS packages.

Geostatistical interpolation

Kriging or geostatistical interpolation aims to predict the unknown value of a variable Z at a non-observed location x_i using the value z_i at surrounding locations. For this purpose, a stochastic function was used as a model of spatial variation so that the actual but unknown value $z(\mathbf{x}_i)$ and the value at the surrounding location were spatially dependent random variables. A Box–Cox transformation was applied to our data (Box and Cox,

1964) so that **z** was a realization of a Gaussian random function with a covariance matrix **V**.

$$Z^* = \begin{cases} \log(z) & \text{if } t = 0 \\ \frac{z^t - 1}{t} & \text{otherwise,} \end{cases} \quad (1)$$

where t is the parameter of the transformation.

The elements of **V** are expressed as a function of the distance separating two observations (**h**). The elements of **V** are obtained from a parametric function $C(\mathbf{h})$, where **h** is the lag vector separating two observations. In general this parametric function may vary with both the length and direction of **h,** but here we assumed that the function is isotropic and varies only according to the length of **h,** which we denote by h. It is common in the geostatistical literature for the spatialcovariance of a random variable to be expressed in terms of the variogram

$$\gamma(h) = 0.5E(\{(x) - Z(x+h)\}^2) \quad (2)$$

The full details for the calculation of **V** are given in Webster and Oliver (2007). To model the spatial covariance, we used the Matérn function, which has a smoothness parameter ν. When ν is small the spatial process is rough, whereas for large ν it is smooth. We calculated an effective range, which depends both on a and ν, by using the distance at which the Matérn semi-variance equalled 95% of the partial sill variance. The parameters of the Matérn function were obtained by maximum likelihood estimation (Lark, 2000). The validity of the fitted geostatistical models was confirmed by leave-one-out cross-validation. For each sampling site location $i = 1, \ldots, n$, the value of the property at site $\boldsymbol{x}_i$ is predicted by simple kriging upon $\mathbf{z}^*_{(-i)}$, the vector of observations excluding $z^*(\boldsymbol{x}_i)$. The statistic

$$\theta_i = \frac{\{z^*(x_i) - \tilde{Z}^*_{(-i)}\}^2}{\sigma^2_{(-i)}}, \quad (3)$$

where $\tilde{Z}^*_{(-i)}$ and $\sigma^2_{(i)}$ denote the kriging prediction and kriging variance at $\boldsymbol{x}_i$ when $z^*(\boldsymbol{x}_i)$ is omitted from the transformed observation vector. If the fitted model is a valid representation of the spatial variation of the soil property, then $\theta = (\theta_1, \ldots, \theta_n)$ has a χ^2 distribution with mean $\theta = 1.0$ and median $\theta = 0.455$ (Lark, 2002). The mean and median values of θ were also calculated for 1000 simulated realizations of the fitted model to determine the 90% confidence limits. Moreover, the evaluation of the model was also verified by performing a likelihood ratio test. This test was used to compare the fits of the spatial and non-spatial models. The spatial analysis GeoR package was used for the spatial analyses (Ribiero and Diggle, 2001).

Results and Discussion

The largest variations in gene copy numbers across the Burgundy region were observed for the ammonia-oxidizing crenarchaeota (AOA) and total crenarchaeota, with densities ranging between less than 10^2 (detection limit) to 9.8×10^4 and from 4.7×10^1 to 5.9×10^4 gene copies per ng of DNA, respectively (Figure 1, Supplementary Figure S2). In comparison, the abundance of ammonia-oxidizing bacteria (AOB) varied over two orders of magnitude, whereas the abundances of nitrate reducers and denitrifiers mostly varied within one order of magnitude. In accordance with the study of Leininger *et al.* (2006), which showed a good correlation between a membrane lipid biomarker of archaea and the *amoA* gene copy numbers, we found that the abundance of the AOA and the total crenarchaeota were highly correlated ($R^2 = 0.72$, $P < 0.001$; Supplementary Figure S3). In most soils of the Burgundy region, the AOA were largely predominant over the AOB with a ratio of archaeal to bacterial *amoA* copy numbers ranging from 10 to 400 in 77 out of 107 sites, as observed in other studies (Leininger *et al.*, 2006; Nicol *et al.*, 2008; Jia and Conrad, 2009). However, 9 sites without common characteristics had a AOA:AOB ratio ranging between 0.2 and 1.

The relative abundance of coexisting communities is of fundamental interest in ecology (Weiher and Keddy, 1999). Therefore, we also calculated the ratios of the different bacterial N-cycling genes to bacterial 16S rRNA copy numbers and that of the AOA to total crenarchaeal 16S rRNA copy numbers to examine how the proportions of the different N-cycling communities within the prokaryotic community vary across landscapes (Supplementary

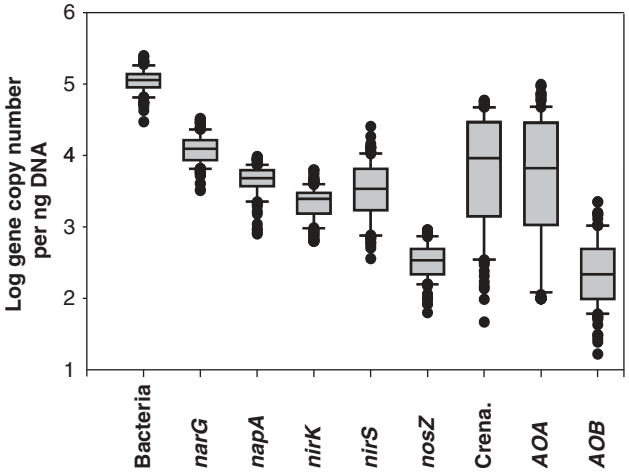

Figure 1 Variation in the abundance of different microbial communities across the Burgundy region. The upper and lower boundaries of each box indicate the 75th and 25th percentile, respectively, and the mid-line marks the median of the distribution of the corresponding qPCR values. Whiskers above and below the box indicate the 90th and 10th percentiles, respectively, while black dots indicate outliers.

Table 1 Partitioning of the biological variation of different microbial communities as a function of contextual parameters

	Overall model[a]			*Respective contribution of contextual variables (% explained variance)*[b]					
	N	*F-ratio*	*Total explained variance (%)*	*Space*	*Land use*	*Climate*	*Time*	*Soil physics*	*Soil chemistry*
Total bacteria	16	14.81***	73.1	7.70***		19.7***		6.5***	20.8***
Total crenarchaea	16	27.01***	85.1	1.3**	1.6*	0.3 NS		1.4*	25.2***
Nitrate reducers									
narG	25	17.33***	55.0	14.6***		1.4*	0.6 NS	2.3**	39.1***
napA	16	10.89***	66.7	6.0**			6.6**	3.9**	49.5***
Denitrifiers									
nirK	12	18.64***	71.1					2.8**	59.3***
nirS	21	16.99***	83.0	2.2**	0.5 NS	1.6**	2.1**	1.2*	35.5***
nosZ	10	17.08***	64.7	3.8**		5.2**		2.3*	41.3***
Nitrifiers									
AOB	8	22.59***	70.8		18.6***			1.3*	16.9***
AOA	15	25.65***	83.5		1.1 NS			0.9*	26.9***

[a]For each quantitative response variable, redundancy analysis models were assessed for significance after selecting the most parsimonious explanatory variables by minimizing the Akaike Information Criterion. *N*, number of retained explanatory variables (details about the individual variables can be found in Table 2). Total explained variance consists of the sum of the respective contributions of each contextual category and of their overall covariation.
[b]The biological variation in each model was partitioned into the respective effects of different categories of explanatory variables by using partial redundancy analyses and by adjusting R^2 values (that is, the percentage of explained variation) to obtain unbiased estimates (Peres-Neto *et al.*, 2006).
Significance was determined by 1000 permutations with ***$P<0.001$; **$P<0.01$; *$P<0.05$; and NS (not significant), $P\geqslant 0.05$.

Figure S4). Interestingly, the AOA to crenarchaeota ratio varied from 0.08 to 2.7, which suggests that (i) not all crenarchaea have the *amoA* gene and are therefore capable of ammonia oxidation and (ii) the proportion of ammonia oxidizers within the crenarchaea is not constant in terrestrial environments and is influenced by environmental changes. However, this might also be partly explained by a variation in the number of *amoA* and 16S rRNA gene copies per cell and/or by the specificity of the primers used. Thus, ratios higher than 1 are likely due to the fact that the crenarchaea primers are not truly universal and are underestimating the total number of crenarchaea. We found that the nitrate reducers, denitrifiers and AOB represented around 5–20%, 1–5% and 0.05–1% of the total bacterial community, respectively, as previously reported (Okano *et al.*, 2004; Henry *et al.*, 2006; Philippot *et al.*, 2009b). It is noteworthy that the percentage of bacteria possessing the *nosZ* gene, which encodes the N_2O reductase, within the denitrifying community (that is, those possessing the *nirK* or *nirS* genes encoding a nitrite reductase) varied within one order of magnitude and was never higher than 56%. This is consistent with the work of Jones *et al.* (2008), which showed that out of approximately 68 complete prokaryotic genomes in the database with either *nirS* or *nirK*, only 43 had the *nosZ* gene. Our findings support the mounting evidence that a significant fraction of the denitrifying community might lack the genetic ability to perform the last step of the reduction pathway, that is, reduction of the potent greenhouse gas N_2O into harmless N_2 (Henry *et al.*, 2006; Richardson *et al.*, 2009; Philippot *et al.*, 2009b).

Using a dataset describing 49 different soil and environmental variables at each sampling site (Supplementary Table S1), we found that between 43 and 85% of the biological variance in the distribution of the studied communities could be explained (Table 1 and Supplementary Table S2). The amounts of explained variation belonged to the upper range of what has been evidenced in other studies in microbial ecology (Yergeau *et al.,* online first; Ramette and Tiedje, 2007b), or in classical community ecology (Cottenie, 2005) using comparable statistical approaches. To better understand the mechanisms driving the spatial distributions observed in this study, all variables were grouped into five categories (spatial effects, land use, climate, soil physics and soil chemistry), and partial regression models were calculated for each dataset (Table 1 and Supplementary Table S2). In almost all cases, soil chemistry was the strongest predictor and explained between 20 and 68% of the total variance (Table 1). When separating the effect of each variable, pH emerged as either an important or the strongest single soil chemistry predictor for most communities (Table 2). Thus, differences in soil pH alone could explain up to 17.8% of the variability in abundance of the total bacterial community, between 15.6 and 21.4% for the denitrifier community, 8.5% for the AOA and 2.9–7.1% for the nitrate-reducing community. The importance of soil pH has

Table 2 Contribution of the first five most important explanatory variables to the variation in microbial community abundances

	% Variance explained by:				
Total bacteria	pH (17.8)	Tp_M (13.1)	Sp. $Dist._y$ (7.1)	Rain (6.0)	Cr_{ext} (6.0)
Total crenarchaea	pH (9.6)	K_{tot} (5.6)	Mg_{tot} (4.5)	Res. water (3.8)	Cd_{ext} (3.3)
Nitrate reducers					
NarG	Carbon (7.6)	pH (7.1)	Sp. $Dist._{V11}$ (5.6)	Sp. $Dist._{V7}$ (4.5)	Mn_{exch} (4.2)
napA	Mn_{exch} (7.4)	Time (6.6)	Pb (6.2)	Sp. $Dist._x$ (6.0)	Cu_{ext} (5.2)
Denitrifiers					
nirK	pH (21.4)	Cu_{tot} (7.3)	Cr (6.0)	Fe_{tot} (5.4)	B (5.1)
nirS	pH (15.6)	Mn_{exch} (6.4)	Mn_{tot} (4.8)	Ca_{tot} (4.5)	Na_{tot} (2.8)
nosZ	pH (15.9)	Mn_{exch} (8.5)	Na_{exch} (5.7)	K_{tot} (5.5)	Tp_M (5.2)
Nitrifiers					
AOB	Land use (18.6)	Carbon (8.4)	Ni (6.6)	Na_{exch} (5.3)	K_{exch} (3.3)
AOA	pH (8.5)	K_{tot} (5.5)	Mg_{tot} (3.6)	Pb_{tot} (3.2)	P_{ass} (2.7)
Relative abundances					
narG/16S	Carbon (23.0)	Sp. $Dist._y$ (15.1)	Na_{tot} (8.9)	Mn_{tot} (6.1)	$Rain_{yr}$ (5.5)
napA/16S	Ca_{exch} (32.0)	Res. water (5.4)	ETP_M (4.3)	—	—
nirK/16S	Ca_{exch} (16.8)	Res. water (10.2)	Tp_M (9.8)	—	—
nirS/16S	Na_{tot} (9.8)	P_{ass} (8.0)	$Rain_{yr}$ (6.8)	Mn_{tot} (5.4)	K_{tot} (5.3)
nosZ/16S	Sp. $Dist._y$ (9.6)	—	—	—	—
AOB/16S	Land (18.0)	Ni_{ext} (9.3)	Ca_{tot} (6.3)	Nitrogen (5.7)	Na_{exch} (5.7)
AOA/16S	pH (23.9)	Res. water (12.6)	Sand (8.3)	Fe_{tot} (7.2)	Mn_{exch} (5.3)
AOA/AOB	pH (12.0)	K_{tot} (9.9)	Nitrogen (7.8)	K_{exch} (6.6)	Mn_{tot} (4.1)

The respective contributions were calculated by taking into account all other significant variables in the model using partial redundancy analyses and adjusting the R^2 values (see Table 1). Only significant fractions based on 1000 data permutations and subsequent multiple-comparison Bonferroni corrections are reported. Subscripts are: M (monthly), Sp. Dist. (spatial distance with the corresponding spatial variables indicated as subscripts), Res. water (residual water), ass (assimilable), tot (total), ext (extractable), exch (exchangeable) and yr (year).

been widely documented for both the total bacterial community and the microbial communities involved in N-cycling (Fierer and Jackson, 2006; Philippot *et al.*, 2007; Hartman *et al.*, 2008; Nicol *et al.*, 2008; Hallin *et al.*, 2009). Despite the fact that soil pH is now recognized as a driver of changes in AOA and AOB communities (Prosser and Nicol, 2008; Erguder *et al.*, 2009), the way in which pH affects AOA communities is still debated and controversial. Thus, decreasing of AOA abundance has been reported both with decreasing soil pH (He *et al.*, 2007; Hallin *et al.*, 2009; Jia and Conrad, 2009) and with increasing soil pH (Nicol *et al.*, 2008). In our study, which included 107 soils with pH ranging from 4.2 to 8.3, we found that the AOA were below the detection limit only in acidic soils and that soil pH was positively correlated with AOA abundance ($R^2 = 0.424$, $P < 0.001$). Soil pH was also the best predictor of the AOB/AOA ratio with 12% of the variance explained ($P < 0.001$). Although soil pH has been shown to influence the abundance of AOB (He *et al.*, 2007; Hallin *et al.*, 2009), it was not a significant factor across the large range of soils examined here. This suggests that pH may be important only in regulating AOB on smaller scales or across specific fertilization regimes (Fierer *et al.*, 2009). Altogether, our results indicate that niche partitioning between AOB and AOA is largely attributable to soil pH, with AOA being more affected by acidic pH than AOB. Interestingly, we also found that the 24% spatial variability of the AOA/crenarchaea ratio could also be explained by changes in pH, which suggests a stronger effect of soil pH on the crenarchaeal ammonia oxidizers than on the rest of the crenarchaeal community. Evidence of AOA and AOB specific niches in terrestrial environments is strengthened by the findings that none of the 42 measured soil properties at the sampling sites could explain the variation in both the abundances of AOA and AOB (Table 2). Among the soil chemical properties other than pH that explained the variance in distribution of the different N-cycling communities, exchangeable manganese availability was a significant predictor of the abundance of the nitrate reducers (4.2–7.4%) and of the denitrifiers (5.0–8.5%) (Table 2). In contrast to soil chemistry, the relative contribution of soil physics was never higher than 6.5% of the variability, although significant for several functional communities (Table 1). Altogether, these findings demonstrate that a very significant proportion of the variation in the distribution of microbial guilds can be predicted across terrestrial ecosystems at the landscape scale.

Three dominant types of ecosystems were distinguished across the 107 Burgundy sites, with forests (with 21 out of 26 being deciduous forests), grasslands and agricultural soils dominating. We found that changes in land use did not strongly influence the abundance of any of the studied communities

other than the AOB, for which 18.6% of the variation could be explained by the pure effects of that factor alone (Table 1). Changes in land use also affected the proportion of AOB within the total bacterial community, further suggesting an inherent sensitivity of this community to land management (Table 2). This coupling between land use managements and abundance of AOB indicates that AOB abundance could be used as a pertinent biological indicator for monitoring soils. Likewise, AOB diversity is affected by land use (Carney *et al.*, 2004) and was recently selected as a top candidate biological indicator of soil quality for national-scale soil monitoring (Ritz *et al.*, 2004).

To examine the relative contribution of climatic variables to the landscape distributions of the functional microbial communities, we used regional patterns of precipitation, net evapotranspiration and temperature data (Supplementary Table S1). Despite important variations, local climate mostly had a significant influence on the distribution pattern of the total bacterial community, with temperature, precipitation and evapotranspiration significantly explaining 13.1, 6.0 and 5.6% of the total variance, respectively (Table 2). We also found that local climate explained around 10–13% of the variability in the relative abundance of *nirS* and *nirK* denitrifiers within the total bacterial community (Supplementary Table S2). When considered separately from the other factors, the sampling period (time) had a weak influence and affected significantly only the *napA* and *nirS* communities (% variance explained of 6.6 and 2.1, respectively), thus indicating that the large time period needed to sample all 107 sites did not strongly affect our results by masking the effects of other environmental variables. Likewise, a recent study reported that temporal variation also had little impact on the distribution of the microbial community composition, despite being sampled in different seasons and different years (Drenovsky *et al.*, 2010). Altogether, the results show that neither local climate nor the sampling time was a major factor influencing the distribution patterns of the studied N-cycling communities over the 31 500 km^2 Burgundy region.

Geographical distance, when separated from the other environmental variables, was a weak but significant predictor of the total bacterial and crenarchaeal communities and of the nitrate-reducing and -denitrifying communities (Table 1). However, the explanatory power of the spatial distance dramatically increased when spatial autocorrelation was explicitly modelled without dissecting the environmental variables and incorporating covariation. Thus, by investigating the spatial correlation of microbial abundance using a geostatistical approach, we found strong spatial patterns in the distribution of some communities, with autocorrelation ranging between 22 and 739 km (practical ranges in supplementary Table S3). Three major types of spatial distributions were found. The predicted map of the distribution of total bacteria was quite smooth with a high density in the north and a low density in the south, while the crenarchaea exhibited a more patchy distribution (Figure 2). Finally, the maps of *nirK* and *nirS* are

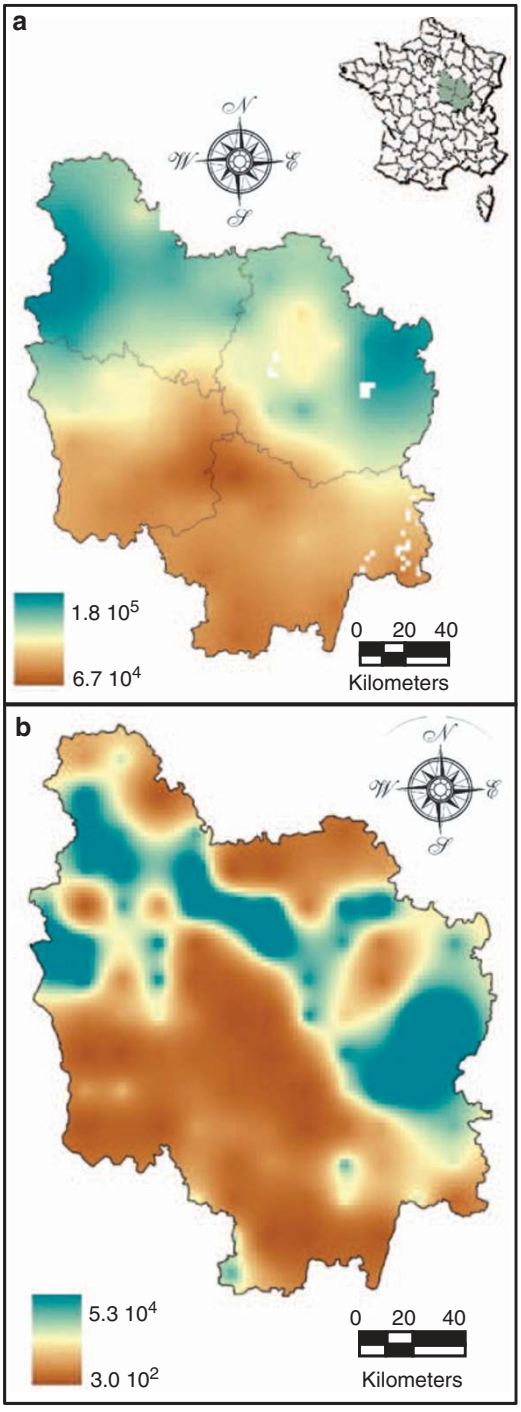

Figure 2 Maps of the abundances of total bacteria and crenarchaea in Burgundy. (**a**) Bacterial 16S rRNA, (**b**) crenarchaeal 16S rRNA. The color scale to the left of each map indicates the extrapolated abundance values (gene copy number per ng of DNA).

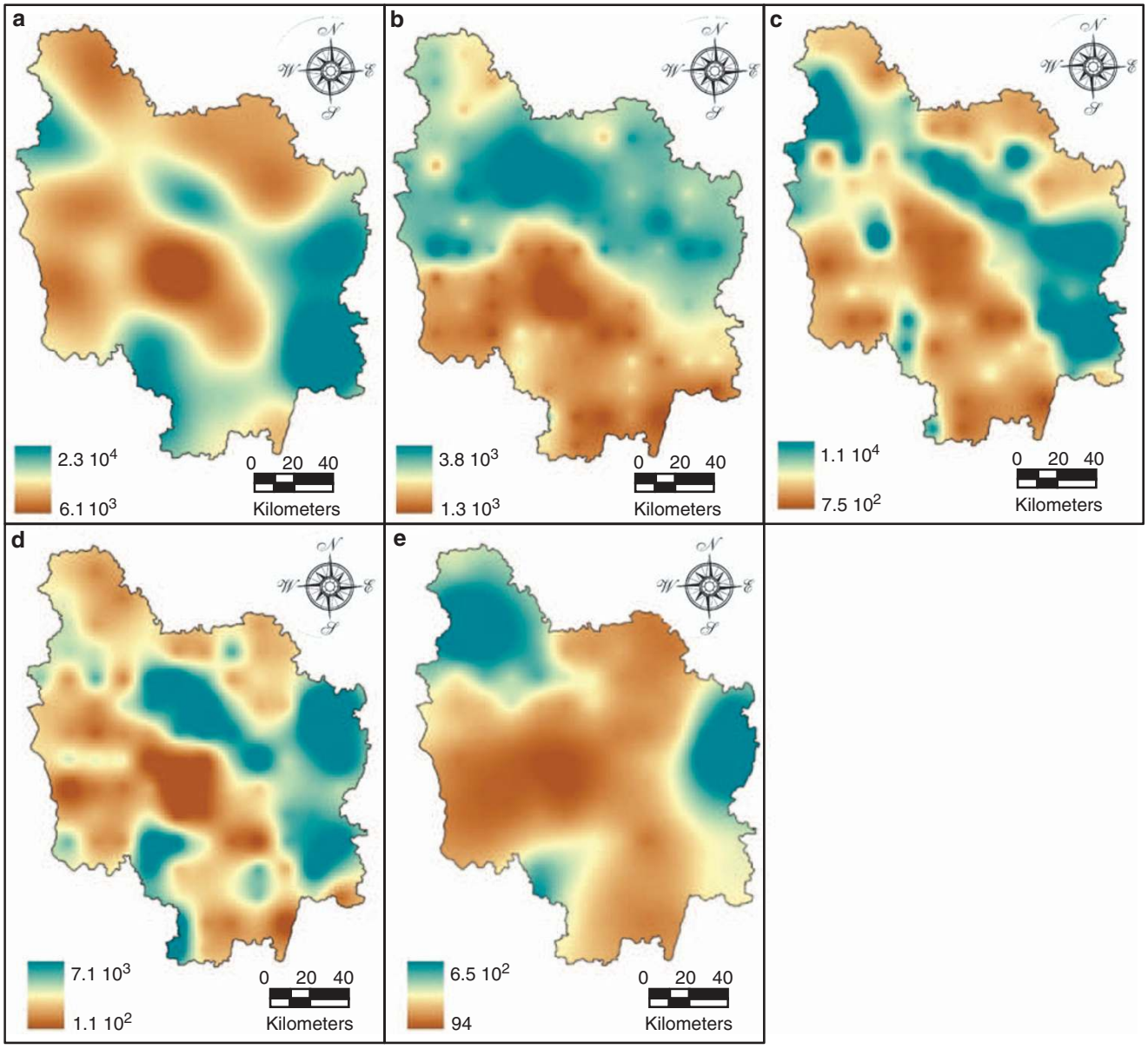

Figure 3 Maps of the abundances of N-cycling genes in Burgundy. (**a**) *narG*, (**b**) *nirK*, (**c**) *nirS*, (**d**) *nosZ*, (**e**) AOB. The color scale to the left of each map indicates the extrapolated abundance values (gene copy number per ng of DNA).

somewhat 'spotty'. These three types of maps are directly related to the roughness of the spatial distributions, which were modelled thanks to the flexibility of Matérn variogram. Interestingly, the significant latitude effect observed both for the distribution of the total bacterial community and also for the relative abundance of the *narG* and *nosZ* genes (Table 2, Supplementary Figure S5) was related to the distribution of soil parental material with limestone plateau in northern Burgundy and crystalline rocks in southern Burgundy. Few differences were observed between the distributions of the *nirS* and *nosZ* denitrifiers, while the distribution of the *nirK* denitrifiers was more related to that of the total bacteria (Figure 3). In contrast, the predicted map of AOB distribution differed considerably from that of all the other studied communities (Figure 3). Those maps supported the results of the canonical variation partitioning analyses, indicating that the AOB was the only community for which soil chemistry was not the main determinant of the spatial distribution (Table 1). Although we know of no other directly comparable studies, a few articles have reported spatial dependence of the distribution of microbial abundance at much lower scales ranging from centimetres to tens of meters (Franklin *et al.*, 2002; Ritz *et al.*, 2004; Philippot *et al.*, 2009b; Enwall *et al.*, 2010). At larger scales, studies using a spatially explicit approach have focused on microbial diversity rather than on

microbial abundance. Thus, spatial dependence of microbial diversity at a kilometer scale was observed by Dequiedt *et al*. (2009) and Cho and Tiedje (2000), while Fierer and Jackson (2006) found that microbial diversity was not related to geographic distance across North and South America. Although spatial variability in the distributions of soil microorganisms is generally regarded as random noise, our results revealed that this variability can be explained even at the landscape scale.

In conclusion, the present study provides an overview of the factors driving the spatial distribution of microbial communities involved in N-cycling and of the total bacterial and crenarchaeal communities across a 31 500 km^2 terrestrial landscape. Our spatially explicit approach showed that no single biogeographical distribution was shared by all the studied microbial communities. However, some common features emerged and soil chemistry—with pH as an overarching controlling factor—was the most important predictor of the distribution of the microbial communities in many cases. Thus, although many environmental variables were significant predictors, only a few accounted for a large amount of the total variance in the distribution of the studied microbial guilds and we could explain between 43 and 85% of this spatial variation in community abundances. Furthermore, our findings illustrate the potential of geostatistic methods, which were successfully used to produce the first maps of the distribution of microbial guilds at a scale of relevance to policy makers and stakeholders for ecosystem management.

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