Biogeographical patterns of soil bacterial communities

Samuel Dequiedt,[1–3] **Jean Thioulouse,**[4]
Claudy Jolivet,[5] **Nicolas P.A. Saby,**[5]
Mélanie Lelievre,[1–3] **Pierre-Alain Maron,**[1–3]
Manuel P. Martin,[5]
Nicolas Chemidlin Prévost-Bouré,[1,2]
Benoit Toutain,[5] **Dominique Arrouays,**[5]
Philippe Lemanceau[1–3] **and Lionel Ranjard**[1–3]*

[1]*INRA-Université de Bourgogne, UMR Microbiologie du Sol et de l'Environnement, CMSE, 17, rue Sully, B.V. 86510, 21065 Dijon, Cedex, France.*
[2]*Université de Bourgogne, UMR 1229, CMSE, 17, rue Sully, B.V. 86510, 21065 Dijon, Cedex, France.*
[3]*Platform GenoSol, INRA-Université de Bourgogne, CMSE, 17, rue Sully, B.V. 86510, 21065 Dijon, Cedex, France.*
[4]*Université de Lyon, F-69000, Lyon; Université Lyon 1; CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, F-69622, Villeurbanne Cedex, France.*
[5]*INRA Orléans – US 1106, Unité INFOSOL, Avenue de la Pomme de Pin – BP 20619 Ardon 45166 Olivet, Cedex, France.*

Summary

This study provides the first maps of variations in bacterial community structure on a broad scale based on genotyping of DNA extracts from 593 soils from four different regions of France (North, Brittany, South-East and Landes). Soils were obtained from the soil library of RMQS ('Réseau de Mesures de la Qualité des Sols' = French soil quality monitoring network). The relevance of a biogeographic approach for studying bacterial communities was demonstrated by the great variability in community structure and specific geographical patterns within and between the four regions. The data indicated that the distribution of bacterial community composition might be more related to local factors such as soil type and land cover than to more global factors such as climatic and geomorphologic characteristics. Furthermore, the regional pools of biodiversity could be ordered: South-East ≥ North > Brittany > Landes, according to the observed regional variability of the bacterial communities, which could be helpful for improving land use in accordance with soil biodiversity management.

Although microorganisms are the most ubiquitous, diverse and abundant living organisms on earth and despite their key role in biogeochemical cycles, in comparison with macroorganisms, few investigations have been carried out on the distribution of soil microbial community diversity on a broader scale than agricultural fields or forest sites. Indeed, most studies have focused on cataloguing the microbial diversity in particular sites and describing how communities have been affected by environmental perturbations (for review see Ranjard *et al.*, 2000) without integrating the spatial scale in microbial community assembly (Papke and Ward, 2004). As a result, the survey of microbial diversification and the distribution patterns of microbial diversity on a large scale are poorly documented and understood (Martiny *et al.*, 2006). Ecologists have long recognized that beta-diversity (how community composition changes across a landscape) can offer valuable insights into the relative influence of dispersal limitations, environmental heterogeneity and environmental and evolutionary changes in shaping the structure of ecological communities (Green *et al.*, 2004).

In this context, the aim of our study was to investigate the geographic distribution patterns of bacterial community structure by considering a broad spatial scale of sampling. For this, we characterized the bacterial communities from soils in the RMQS soil library ('Réseau de Mesures de la Qualité des Sols' = French soil quality monitoring network) (Fig. 1). This library represents 2200 soils sampled with a 16 km × 16 km systematic grid over the entire French territory and representative of the different land uses, soil types and climatic conditions occurring in France (for more details see Arrouays *et al.*, 2002 and Supporting information). We focused on characterizing the bacterial community structure in 593 soils sampled from four different geographical regions (North, Brittany, Landes and South-East, Fig. 1). These regions were chosen for their particular geographic, pedo-climatic and land use characteristics (as described in the JRC Soil Atlas of Europe, http://eusoils.jrc.ec.europa.eu/projects/soil_atlas). The soil bacterial community structure was directly genotyped from soil DNA extracts, using a B-ARISA (Bacterial-Automated Ribosomal Intergenic Spacer Analysis) fingerprinting approach (Ranjard *et al.*, 2003) optimized for medium throughput in the platform GenoSol (http://www.dijon.inra.fr/plateforme_genosol). The ARISA fingerprinting was used as it allows the rapid examination of the genetic structure of complex bacterial
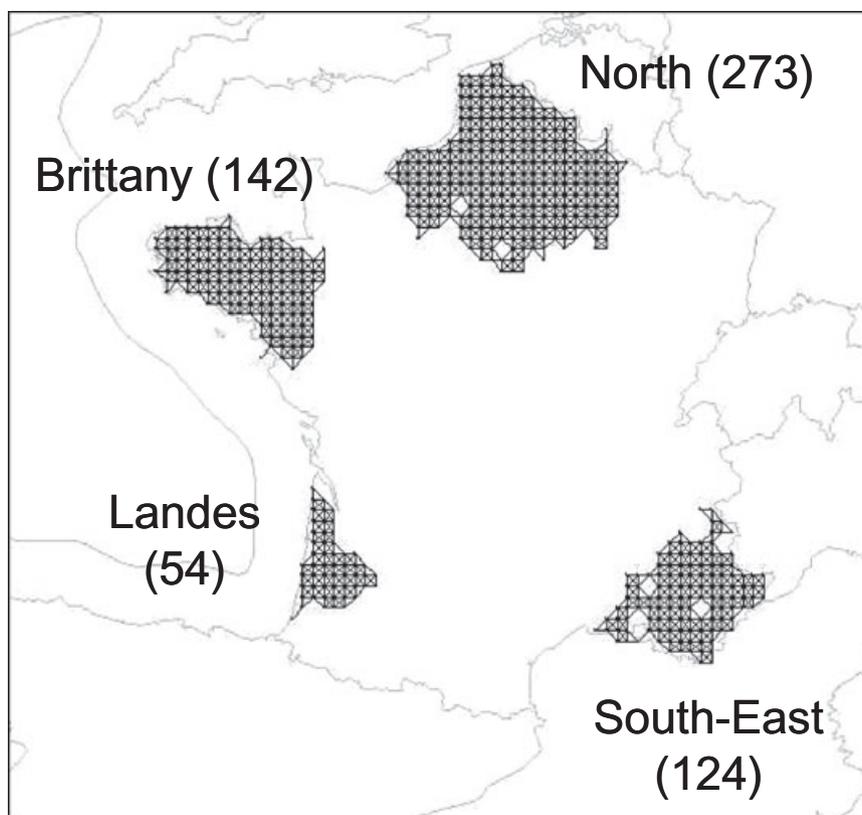
Fig. 1. Location and delimitation of the four studied regions in France. Numbers in parentheses indicate the number of soils samples analysed in each region. The neighbouring relationship between the sampling sites is indicated on the map (this relationship corresponds to a one-square move of the Queen on a chess board: each site can have up to eight neighbours).

communities and has been demonstrated to be sensitive and relevant for evaluating modifications in community composition in space and time (Ranjard *et al.*, 2001; 2003).

Complex B-ARISA profiles were obtained for each soil and compiled into a single matrix (Ranjard *et al.*, 2003), which was analysed using a generalization of multivariate spatial correlation analysis called MULTISPATI (see Dray *et al.*, 2008 and Supporting information for a detailed description of this method). This analysis takes into account the spatial position of sampling sites on the basis of neighbouring relationships between sites (see Fig. 1). One of the biggest advantages of MULTISPATI scores is that they maximize spatial autocorrelation. Consequently, they can be used to draw easily interpretable geographical maps. Computations were conducted using the R software (R Development Core Team, 2008), with the ade4 (Chessel *et al.*, 2004; Dray and Dufour, 2007) and spdep (Bivand *et al.*, 2008) packages (for more details see the 'Materials and Methods' section in the Supporting information file).

The factorial map deduced from the MULTISPATI analysis of all B-ARISA profiles demonstrated the considerable variability of the bacterial community structure within and between the four different regions (for more detailed results see Supporting information for Fig. S1). Significant regional and national variations of soil bacterial communities were apparent, which were consistent to those observed on micro- or field scales (Ranjard and Richaume, 2001; Ranjard *et al.*, 2001) and with other studies on broader spatial scales (Fierer and Jackson, 2006). The site scores of the first three axes of global MULTISPATI analysis were first interpolated using a geostatistical interpolation method and then geographically mapped for each studied region (Fig. 2) to determine the distribution patterns of soil bacterial community variability (for more details see Supporting information). Mapping of the first MULTISPATI score revealed a moderate regional variability within Landes and Brittany and a high similarity in community structure between these two regions (Fig. 2 Axis 1). In contrast, the North and South-East regions exhibited much greater regional variability and distinct community structures compared with Landes and Brittany. Mapping of the second MULTISPATI score (Fig. 2 Axis 2) discriminated between Brittany and Landes as well as between North and South-East whereas mapping of the third MULTISPATI score (Fig. 2 Axis 3) revealed greater regional heterogeneity and the evidence of new spatial
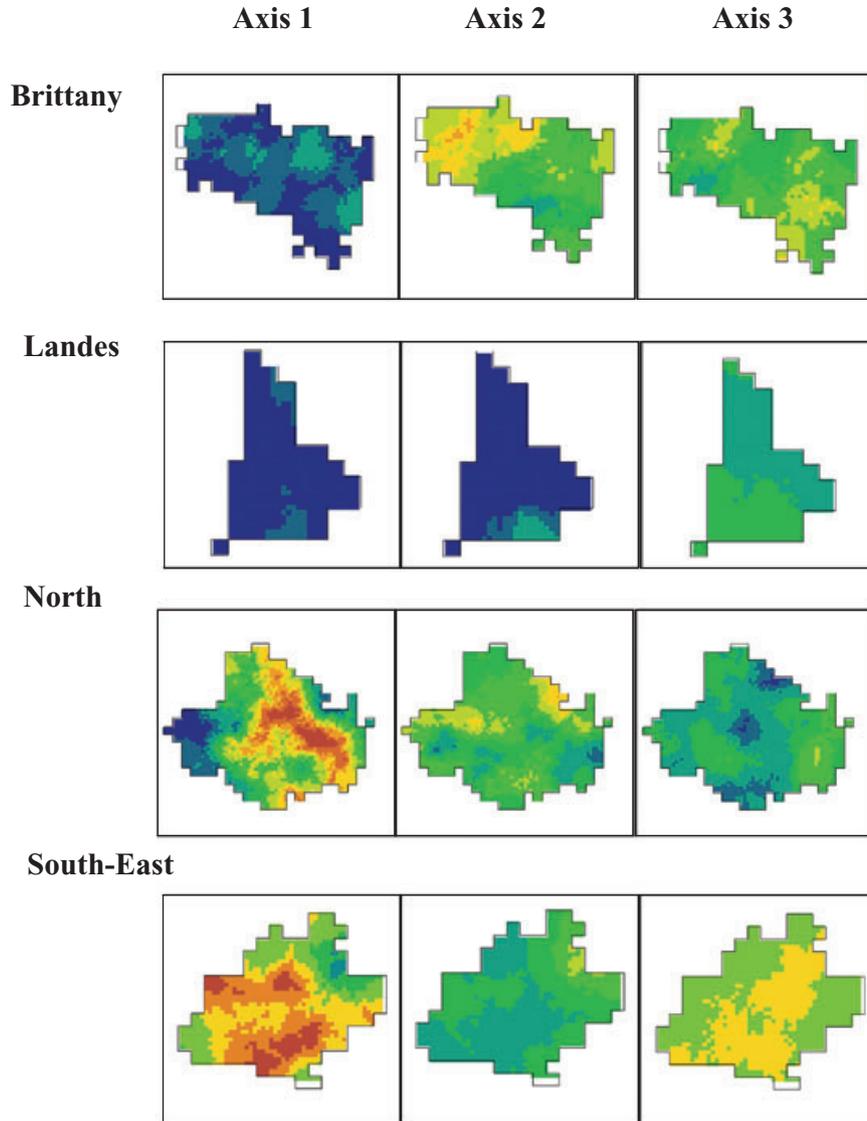
Fig. 2. Maps of interpolated MULTISPATI scores for the first three MULTISPATI axes (columns) and for the four geographical regions (rows). Each map is a spatial synthesis of the B-ARISA genetic structure of indigenous bacterial communities from the corresponding soils sampled in the four regions of France.

Axis 1, Axis 2 and Axis 3 represent the mapping of MULTISPATI scores for the first three axes. Colours on the map are proportional to the score values (see Fig. S1 in Supporting information), and the colour code is given by the following scale:

	−0.174/−0.129			−0.037/−0.011
	−0.128/−0.102			−0.01/0.022
	−0.101/−0.083			0.023/0.054
	−0.082/−0.062			0.055/0.079
	−0.061/−0.038			0.08/0.108

Similar colours and scores on a given axis indicate similar genetic structure of bacterial community.

structures. On the basis of these maps, the null hypothesis of microbial biogeography, corresponding to a random spatial distribution of microorganisms, could be rejected (Martiny *et al.*, 2006). These maps further evidenced that bacterial community can have biogeography with significant and specific spatial assemblages according to the studied region. Interestingly, similar community structures appeared to occur in non-contiguous regions, suggesting that environmental conditions (geomorphologic, pedo-climatic, land cover …), rather than geographic distance, could be of major importance in shaping community structure (Martiny *et al.*, 2006). This observation contrasts with previous suggestions that geographic distance could be a useful predictor of microbial community turnover and assemblage for other types of ecosystems (mountain lakes, Reche, 2005) as well as for other types of microorganisms (fungi in desert ecosystem, Green *et al.*, 2004).

The particular and homogeneous assemblage of bacterial communities in the Landes and Brittany regions could not be explained by a geographic isolation of these regions due to the presence of natural barriers (mountain, sea, desert . . . ; http://eusoils.jrc.ec.europa.eu/projects/soil_atlas, elevation p. 121) or by particular climatic conditions (http://eusoils.jrc.ec.europa.eu/projects/soil_atlas, climate p. 122). However, the mapping of French soil types according to their physico-chemical characteristics (http://gissol.orleans.inra.fr/programme/bdgsf/carte.php) appeared to match well with the bacterial community distribution within and between regions, supporting the hypothesis of a strong influence of edaphic parameters. More precisely, in the Landes region a single sandy acidic soil type (podzol) has been described for all the studied sites (IUSS Working Group WRB report 2006), which might partly explain the low variability of the bacterial community in this region. This hypothesis is supported by previous studies dealing with the description of bacterial diversity on continental or field scales that demonstrated the more important influence of soil characteristics, such as pH (Fierer and Jackson, 2006) and/or soil texture (Johnson *et al.*, 2003), than climatic or land cover characteristics. The higher variability of soil types described in the North and South-East regions (http://gissol.orleans.inra.fr/programme/bdgsf/carte.php) might support also a relationship between soil-type distribution and community assemblage on a broad scale. In parallel, Mantel tests and partial Mantel tests were used to investigate the relationships between geographical distances, physico-chemical characteristics of soil samples (texture, pH, CEC, Corg, N, $CaCO_3$, P, Ca, Mg, K contents) using Euclidean distances, and Sørensen similarity coefficient between B-ARISA profiles. All computations were done with the R software (R Development Core Team, 2008), using the vegan (Oksanen *et al.*, 2009) and labdsv

(Roberts, 2007) packages. A simple Mantel test showed that there is a very highly significant correlation between B-ARISA profiles and soil physico-chemical characteristics ($r = 0.326$, $P < 0.001$). There is also a significant correlation between B-ARISA profiles and geographical distances ($r = 0.225$, $P < 0.001$). But, after controlling for geographical distances, a partial Mantel test showed that there is still a very highly significant correlation between B-ARISA profiles and soil physico-chemical characteristics ($r = 0.277$, $P < 0.001$).

The spatial variation of community structure might also be related to the more or less regional variability of land cover (http://image2000.jrc.it/; http://eusoils.jrc.ec.europa.eu/projects/soil_atlas, land cover p. 123). The Landes region is mainly characterized by a monospecific forest cover with (monoclonal) *Pinus pinaster*, intensively exploited for wood production. Such a homogenous over exploitation of soil in this region might be consistent with the low regional variability observed for community assemblage. In contrast, the higher variability recorded in the three other regions could be related to the greater land use variability, which includes forest, grassland and agricultural crops. Consequently, the Landes region can be defined as a biotic province with very few types of particular habitats in terms of climatic, pedological and land cover characteristics, leading to a homogenous distribution of bacterial community composition.

This study represents the first exploratory step of an extensive biogeographical study to be applied to the whole French territory. Although our results remain descriptive, they implicitly support the second hypothesis of Bass-Becking (1934) deduced from the work of Beijerinck (1913), i.e. '*everything is everywhere*, but, *the environment selects*', implying that different contemporary environments maintain distinctive microbial assemblages. In other respects, our data also evidenced that microbial biogeography differs fundamentally from the biogeography of macro-organisms, which appears to be more influenced by global parameters such as climate or geomorphology (Green and Bohannan, 2006). Finally, regional pools of microbial diversity could be ordered according to the regional variability of the bacterial community structure, i.e. South-East ≥ North > Brittany > Landes, thus demonstrating the need to better understand the biogeographical patterns of microbial communities in order to improve our capacity to manage and protect soil biological diversity on a large scale.

References

Arrouays, D., Jolivet, C., Boulonne, L., Bodineau, G., Saby, N., and Grolleau, E. (2002) Une initiative nouvelle en France: la mise en place d'un Réseau multi-institutionnel de Mesures de la Qualité des Sols (RMQS). *Cpte Rend Ac Agr Fr* **88:** 93–105.

Bass-becking, L.G.M. (1934) *Geobiologie of inleiding tot de milieukunde*. The Hague, the Netherlands: Van Stockum & Zoon.

Beijerinck, M.W. (1913) De infusies en de ontdekking der backterien. In *Jaarboek van de Knoniklijke Akademie van Wetenschappen*. Amsterdam, the Netherlands: Muller.

Bivand, R., Anselin, L., Assunçaõ, R., Berke, O., Bernat, A., Carvalho, M., *et al.* (2008) spdep: Spatial dependence: weighting schemes, statistics and models. R package version 0.4-29. URL http://cran.at.r-project.org/web/packages/spdep.

Chessel, D., Dufour, A.B., and Thioulouse, J. (2004) The ade4 package-I- One-table methods. *R News* **4:** 5–10.

Dray, S., and Dufour, A.B. (2007) The ade4 package: implementing the duality diagram for ecologists. *J Statist Soft* **22:** 1–20. URL http://cran.at.r-project.org/web/packages/ade4.

Dray, S., Said, S., and Debias, F. (2008) Spatial ordination of vegetation data using a generalization of Wartenberg's multivariate spatial correlation. *J Veget Sci* **19:** 45–56.

Fierer, N., and Jackson, R.B. (2006) The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA* **103:** 626-631.

Green, J.L., and Bohannan, B.J.M. (2006) Spatial scaling of microbial diversity. *Trends Ecol Evol* **21:** 501–507.

Green, J.L., Holmes, A.J., Westoby, M., Oliver, I., Briscoe, D., Dangerfield, M., *et al.* (2004) Spatial scaling of microbial eukaryote diversity. *Nature* **432:** 747–750.

Johnson, M.J., Lee, K.Y., and Scow, K.M. (2003) DNA fingerprinting reveals links among agricultural crops, soil properties, and the composition of soil microbial communities. *Geoderma* **114:** 279–303.

Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Furhman, J.A., Green, J.L., *et al.* (2006) Microbial biogeography: putting microorganisms on the map. *Nature* **4:** 102–112.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Solymos, P., *et al.* (2009) Vegan: community ecology package. R package version 1.15-2. URL http://CRAN.R-project.org/package=vegan.

Papke, R.T., and Ward, D.M. (2004) The importance of physical isolation to microbial diversification. *FEMS Microbiol Ecol* **48:** 293–303.

R Development Core Team (2008) R: a language and environment for statistical computing. ISBN 3-900051-07-0. URL http://www.R-project.org.

Ranjard, L., and Richaume, A. (2001) Quantitative and qualitative microscale distribution of bacteria in soil. *Res Microbiol* **152:** 707–716.

Ranjard, L., Poly, F., and Nazaret, S. (2000) Monitoring complex bacterial communities using culture-independent molecular techniques: application to soil environment. *Res Microbiol* **151:** 167–177.

Ranjard, L., Poly, F., Lata, J.C., Mougel, C., Thioulouse, J., and Nazaret, S. (2001) Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. *Appl Environ Microbiol* **67:** 4479–4487.

Ranjard, L., Lejon, D., Mougel, C., Scherer, L., Merdinoglu, D., and Chaussod, R. (2003) Sampling strategy in molecular microbial ecology: influence of soil sample size on DNA fingerprinting analysis of fungal and bacterial communities. *Environ Microbiol* **5:** 1111–1120.

Reche, I. (2005) Does ecosystem size determine aquatic bacterial richness? *Ecology* **86:** 1715–1722.

Roberts, D.W. (2007) Labdsv: ordination and multivariate analysis for ecology. R package version 1. URL http://ecology.msu.montana.edu/labdsv/R.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Factorial map obtained by MULTISPATI analysis of B-ARISA profiles of soils sampled in the four regions studied. (A) Axis1 × Axis 2; (B) Axis 1 × Axis 3.
Appendix S1. Materials and methods.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.