SOILS, SEC 2 · GLOBAL CHANGE, ENVIRON RISK ASSESS, SUSTAINABLE LAND USE · RESEARCH ARTICLE

Spatial distribution of the abundance and activity of the sulfate ester-hydrolyzing microbial community in a rape field

Xavier Goux · Bernard Amiaud · Séverine Piutti · Laurent Philippot · Emile Benizri

Abstract

Purpose Sulfur (S) plays a vital role in plant metabolism, and the detrimental impact of S deficiency in several field crops has increased over the last 30 years. The bio-availability of organic S to plant depends on arylsulfatase (ARS), a key enzyme for S mineralization in soil. In this study, we characterized the spatial variability of ARS activity in an agricultural soil cropped with the rape plant (*Brassica napus*). Because rape requires relatively large amounts of S per yield unit compared to most grain crops, it is very sensitive to S deprivation similarly to the other plants of the Brassicaceae family, with consequences for seed quality and yield.

Materials and methods The spatial variability of (a) ARS activity, (b) the abundance of culturable bacteria possessing the ARS, and (c) soil properties (temperature, soil pH, SO_4^{2-}-S (sulfate-S) content, labile carbon (C) and nitrogen (N), soil microbial biomass carbon SMB-C, and nitrogen SMB-N) was estimated at 40 sites within a rape field, using a 4×5-m sampling grid. Geostatistics were used to model the spatial distribution of the measured variables, and relationships between variables were tested using linear statistical analyses.

Results and discussion The total ARS activity showed a low variability ranging between 69.0 and 153.1 $\mu g p$-nitrophenol g^{-1} dry soil h^{-1} while the abundance of the culturable ARS community ranged within one order of magnitude. The distribution of both the abundance and activity of the ARS community exhibited spatial dependence in 800 m^2 agricultural field.

Conclusions The spatial pattern of ARS activity in the field was correlated with several soil properties, and results suggest that soil pH, labile C and N, and SBM-C/SBM-N ratio were the main parameters linked to the ARS activity rather than the abundance of the culturable ARS bacterial community or the SO_4^{2-}-S concentration.

Keywords Arylsulfatase · Geostatistic · Rape · Soil bacterial community · Spatial distribution · Sulfur

X. Goux · B. Amiaud · S. Piutti · E. Benizri
UMR 1121 INRA Agronomie et Environnement Nancy-Colmar,
Université de Lorraine,
2 avenue de la Forêt de Haye, BP 172, 54505
Vandoeuvre-lès-Nancy, France

L. Philippot
INRA UMR 1347 Agroécologie,
BP 86510, 21065 Dijon Cedex, France

E. Benizri (✉)
UMR 1120 INRA Sols et Environnement, Université de Lorraine,
2 avenue de la Forêt de Haye, BP 172, 54505
Vandoeuvre-lès-Nancy, France
e-mail: emile.benizri@ensaia.inpl-nancy.fr

1 Introduction

The spatial variability of microbial processes in soil has been investigated for several decades (Parkin 1993). Soil microbial processes are controlled by several factors, such as organic matter, soil moisture, texture, and pH, all of which exhibit spatial variability (Shouse et al. 1995; Aşkin and Kizilkaya 2006). A better characterization of the variability of microbial processes in soil would therefore help identifying their main driving factors and understanding of soil functioning. Spatially explicit approaches have become of great interests for studying the distribution of microbial processes and diversity at different scales and have been successfully applied to a few functional communities involved in different biochemical cycles. For example, it has been shown that some N-cycling processes exhibit spatial patterns, which can be mapped using geostatistical analyses (Yanai et al. 2003; Grundmann 1990; Philippot et al. 2009; Enwall et al. 2010).

In contrast to other biogeochemical cycles such as C and N cycles, little attention has been paid to the sulfur (S) cycle. However, S is an essential element for plant development and growth which is now considered as one of the most plant limiting macronutrients (Zhao et al. 1999). S limitation mostly results from the decreased atmospheric depositions since the beginning of the 1980s and the increasing use of S-free fertilizer products. The bio-availability of S to plants requires microbial mineralization and therefore depends on the organic sulfur-transforming processes that occur in the soil. The nature of organic S in soil is still not well defined, but it is believed that sulfate esters are the most prevalent organic form of S in agricultural soils, while sulfates are the main form assimilated by plant roots (Leustek and Saito 1999) and are generally less abundant (<5 %). Therefore, the arylsulfatase sulfohydrolase (ARS), which hydrolyzes sulfate esters to sulfates in the soil, is considered as the key enzyme of soil organic S mineralization (Tabatabai and Bremner 1970; Fitzgerald 1978; Klose and Tabatabai 1999). Large variability in ARS activity in soil has previously been reported (Aşkin and Kizilkaya 2006; García-Ruiz et al. 2008; Cregut et al. 2009). However, our understanding of this spatial variability for identification of the factors driving ARS activity is limited despite being crucial for modeling the S turnover and S management in arable soil.

In this study, we characterized the spatial variability of ARS activity in an agricultural soil cropped with rape (*Brassica napus*) to identify the physicochemical and biological variables influencing this process. For this purpose, temperature, soil pH, SO_4^{2-}-S (sulfate-S) content, labile C and N, MB-C and MB-N together with the size and activity of the microbial community involved in the hydrolysis of sulfate esters to sulfates were characterized and their distribution modeled using geostatistical analyses. A rape field was used as model system because S deficiency is known to affect both yield and quality of the crop harvested products (McGrath and Zhao 1996; Janzen and Bettany 1984; Scherer 2001; Dubousset et al. 2009). Indeed, rape requires relatively large amount of S per yield unit compared to most cereal crops (Bhupinderpal-Singh and Bowden 2006). Zhao et al. (1999) also underlined the fact that deficiency of S has been recognized as a limiting factor for crop production in many regions in the world. In particular, S deficiency has increasingly been reported in *Brassica* crops in western Europe over the last decade, mainly as a consequence of a massive decrease of atmospheric S inputs.

2 Materials and methods

2.1 Site description and sample collection

The study was carried out in an area 50×16 m in a 5-ha agricultural field located in northeastern France (48.44°N,

6.18°E). It is a clayey soil (Vertic Stagnic Cambisol in the WRB scheme 2001) and the landform of the studied area is flat. The field was planted with winter rape (*B. napus* cv. Grizzly), which was sown on September in sequential rotations: wheat, spring barley, and rape. Rape plants received in April 160 kg ha^{-1} ammonium nitrate containing 33.5 % N. No sulfated fertilization was made.

Soil samples were collected in April (rape flower bud formation stage) in the middle of each of the 40 cells of about 4×5 m within the sampling grid (the soil sampling intervals were chosen in order to avoid any putative effect due the tractor wheel tracks, Fig. 1). At each sampling location, 200–300 g soil was collected from the top layer (25 cm depth, 10 cm diameter). The soil temperature was determined on site at each sample location, and then the soil samples were sieved (<5 mm) and stored at 4 °C less than 7 days until soil physicochemical and microbiological analysis.

2.2 Soil physicochemical properties

Soil physicochemical properties were determined by the Soil Analysis Laboratory of INRA (Arras, France). The soil contained 573, 369, and 58 g kg^{-1} soil clay, silt, and sand, respectively. The main soil chemical characteristics were 30.5 g kg^{-1} organic C, 3.73 g kg^{-1} N, and 746 mg kg^{-1} soil total S. For extraction of free and adsorbed SO_4^{2-}-S, an equivalent of 30 g dry soil was weighed into 200-ml polyethylene pots (Singh 1984). The SO_4^{2-}-S contents of the soils were extracted with 0.009 M Ca(H$_2$PO$_4$)$_2$ solution (1/5 w/v) on an end-over-end shaker for 30 min and then filtered through a Whatman no. 42 paper disk. The SO_4^{2-}-S contents were measured by turbidimetry at 460 nm (Lisle et al. 1994). The soil residue was dried at 37 °C, and 12 g dry soil was put into a 200-ml polyethylene pot with 60 ml distilled water and incubated at 70 °C for 18 h in an oven (Sparling et al. 1998). After manual shaking and filtering through a Whatman no. 42 paper disk, labile C and N in the hot water extracts were determined with a TOC analyzer (Shimadzu TOC-VCSH). Soil pH was measured using a pH meter (pH meter, CG840, Schott, Germany) in a 10-g/25-ml soil/water solution mixture.

2.3 Microbial C and N biomass

The soil microbial biomass C and N (SMB-C and SMB-N, respectively) were estimated using the fumigation extraction technique previously described by Vance et al. (1987). Extractable C and N were measured by the automated UV-persulfate oxidation method in a TOC analyzer (Wu et al. 1990). The microbial biomass C was determined using the formula: microbial biomass C=(C$_{fumigated\ extract}$−C$_{unfumigated\ extract}$)/K_c. The calculated K_c factor of 0.45 was used to convert extractable C into microbial biomass C (Jenkinson

Fig. 1 Distribution of the 40 samples points in the studied field (830 m²) located in northeastern France (48.44°N, 6.18°E)
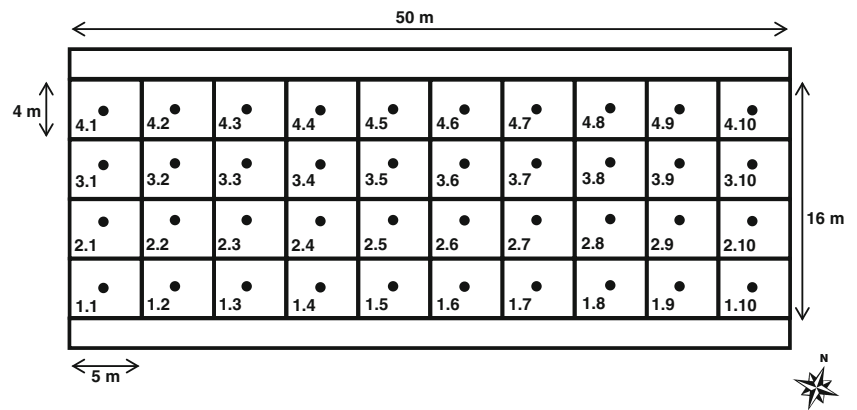
and Ladd 1981). The microbial biomass N was determined using the formula: microbial biomass N=(N$_{fumigated\ extract}$−N$_{unfumigated\ extract}$)/K$_n$. The calculated K$_n$ factor of 0.54 was used to convert extractable N into microbial biomass N (Brookes et al. 1985).

2.4 Quantification of total culturable bacteria and culturable bacteria possessing ARS activity

Three grams of each soil sample was mixed with 30 ml phosphate buffer (8 g NaCl, 1.44 g Na$_2$HPO$_4$, 0.24 g KH$_2$PO$_4$/l, pH 7.2). Soil suspensions were serially diluted. The dilutions (10^{-2} to 10^{-4}) were used for the determination of the number of culturable cells as colony forming units (CFU) by spread-plating serial dilutions onto tryptone soy agar 10 % (Difco). Three replicates for each dilution were prepared. The agar plates were incubated in the dark at 26 °C for 2 days. The number of bacteria was expressed as log CFU g^{-1} of dry soil. The abundance of ARS bacteria was estimated by plate counting on M9-X-sulf medium (Cregut et al. 2009). Soil suspensions were serially diluted, and 100-μl aliquots of appropriate dilutions (from 10^{-2} to 10^{-4}) were plated in triplicate on M9-X-sulf agar plates. X-sulf is a chromogenic substrate that reports the bacterial hydrolysis of this sulfate ester with the appearance of a blue color (Wyss 1989). The blue colonies (expressing ARS activity) were counted after 21 days of incubation at 27 °C.

2.5 Arylsulfatase activities

Chloroform-fumigated soil and its non-fumigated counterpart were simultaneously used to measure arylsulfatase activity according the method described by Klose et al. (1999). In the chloroform-fumigated soil samples, 1 g of soil was incubated with 1 ml of chloroform for 2 h, and then the chloroform-fumigated soil samples were placed for 12 h under a laminar flow unit to remove the chloroform. Soil samples (1 g) were then incubated with 4 ml of 0.5 M acetate buffer (pH 5.8) and 1 ml of 50 mM of potassium p-nitrophenyl sulfate at 37 °C for 1 h. Next, 1 ml of 0.5 M

CaCl$_2$ and 4 ml of 0.5 M NaOH were added. Lastly, the solutions were filtered. The p-nitrophenol released was extracted and determined colorimetrically. Total arylsulfatase activity (ARS$_t$) was then measured in fumigated soil, extracellular arylsulfatase activity (ARS$_e$) was measured in non-fumigated soil, and the intracellular arylsulfatase activity (ARS$_i$) was the resulting difference between the ARS$_t$ and ARS$_e$ (Klose and Tabatabai 1999). Results were expressed as micrograms p-nitrophenol per gram dry soil per hour.

2.6 Geostatistical and statistical analysis

The soil physicochemical and biological parameters were analyzed by calculating a semivariogram for all the parameters using the following formula:

$$\gamma(h) = 1/2N(h)\Sigma[z(x_i + h) - z(x_i)]^2$$

where $\gamma(h)$ is the experimental semivariogram value at distance interval h, $N(h)$ is the number of sample pairs within the distance interval h, and $z(x_i)$ and $z(x_i+h)$ are the sample values at two points separated by the distance interval h. All pairs of points separated by a distance h (lag h) were used to calculate the experimental semivariogram. Different models were fitted to the empirical semivariograms. Semivariogram models, based on the calculation of semivariance, provide the necessary information for kriging, i.e., interpolation of data at non-sampled points. The parameters nugget semivariance, range, and sill were determined. Nugget semivariance is the variance at zero distance, the sill is the constant value of the variogram when the variables do not influence each other, and the range is the distance at which the value of one variable becomes spatially independent of another. One index of spatial dependence was employed in the analysis: the Q value [calculated as (sill/sill+nugget), which indicated the spatial structure at the soil sampling scale (Görres et al. 1998). The Q value varies between 0 and 1. When it is close to 0, no spatial structure is detected at the sampling and support scale used. When the Q value approaches 1, the spatial variation can be explained by the

semivariogram model at the analysis scale used. Maps were then computed using ordinary kriging to evaluate the regional patterns of variation rather than local details. The geostatistical software GeoR version 1.6-17 under R version 2.8.1 for Windows® (Gamma design Software) was used in the analysis (Ribeiro and Diggle 1999). The resulting maps provide visualizations of patterns. Linear statistical analyses between variables and principal components analysis (PCA) were carried using StatBox version 6, Grimmersoft, Paris, France.

3 Results

3.1 Spatial distribution of the ARS activity, abundances of culturable bacteria possessing ARS activity, and microbial biomass

The average ARS_t activity in the rape-planted field varied between 69.0 and 153.1 μg p-nitrophenol g^{-1} dry soil h^{-1} with an average of 108.8 and a coefficient of variation (CV) of 16.8 %. Similarly, the ARS_e activity with an average of 58.4 μg p-nitrophenol g^{-1} dry soil h^{-1} was characterized by a coefficient of variation of 14.6 % (Table 1). Calculation of the intracellular (ARS_i) activity revealed that it represented approximately 46.3 % of total enzyme activity (ARS_t). The number of culturable bacteria-hydrolyzing sulfate esters was about 5.5×10^5 cfu g^{-1} dry soil. In contrast to the ARS activities, a higher CV of 37.5 % was observed (see Table 1). For the ARS activities, Q values, which indicate the spatial structure at the soil sampling scale, ranged between 0.52 and 0.55. A higher value was found for the abundances of the

culturable bacteria-hydrolyzing sulfate esters (0.82). On the other hand, the range of spatial autocorrelation was 2 m for the later while it was up to 40 m for the ARS_i activity. Kriged maps showed similar spatial distributions between the ARS_t and ARS_e activities with both the highest and lowest activities in the west areas of the field (Fig. 2).

3.2 Spatial variability of the soil physicochemical properties

At the field site, soil temperature (average 14 °C) and pH (average 7.0) showed a moderate variability with a coefficient of variation below 10 %. The sulfate concentrations (average 3.1 ppm), labile C (average 0.7 mg C g^{-1} dry soil), and labile N (average 0.06 mg N g^{-1} dry soil) were slightly more variable, with CV ranging from 13.1 to 15.6 % (see Table 1). The spatial variability of the soil physicochemical was evaluated by fitting different variogram models (spherical, exponential, and circular) to the full data set of each parameter to provide a single model of the spatial variance structure in the field (see Table 1). A strong spatial structure was detected at the scale of sampling for all soil properties, but the sulfate concentrations were indicated by the Q value ranging between 0.80 and 1.0 and spatial autocorrelation ranging from 4 to 11 m (see Table 1). Among these parameters, temperature, soil pH, and labile N were characterized by a null nugget variance, which indicates the absence of spatial structure at a finer scale than that investigated in our study. The kriged maps show similar distribution of pH and temperature with an east–west gradient (Fig. 3). In overall, distribution of labile C, labile N, and soil moisture also varied between the east and west areas of the field but with an opposite gradient.

Table 1 Descriptive statistics and geostatistical analysis of physiochemical and microbiological parameters determined from the 40 different soils

Parameters	Min	Max	Mean	SD	CV (%)	Model	Range (m)	Nugget	Sill	Q
TOC	12.0	17.0	14.0	9.3	9.3	Circular	11	0	4	1.00
Moisture	28.9	44.4	37.9	4.3	11.4	Spherical	10	5	27	0.84
pH	6.5	7.5	7.0	0.2	3.8	Spherical	4	0	0.08	1.00
SO_4^{2-}–S	1.7	4.0	3.1	0.5	15.6	Exponential	10	0.15	0.3	0.67
Labile C	0.4	0.9	0.7	0.09	13.1	Spherical	4	0.002	0.008	0.80
Labile N	0.04	0.09	0.06	0.009	15.2	Circular	3	0	0.00009	1.00
ARS_t	69.0	153.1	108.8	18.3	16.8	Exponential	25	280	300	0.52
ARS_e	39.9	75.4	58.4	8.5	14.6	Exponential	10	50	60	0.55
ARS_i	21.3	84.4	50.4	15.7	nd	Exponential	40	225	240	0.52
SMB-C (mg kg^{-1})	150.9	301.5	240.9	34.0	14.1	Exponential	4	500	900	0.64
SMB-N (mg kg^{-1})	14.7	29.4	21.9	3.3	15.1	Exponential	25	9	10	0.53
Total cfu (×10^7 g^{-1})	8.2	28.0	15.6	4.1	26.1	Exponential	2	50×10^6	150×10^6	0.75
Functional ARS cfu (×10^5 g^{-1})	1.5	10.0	5.46	2.0	37.5	Exponential	2	10^5	4.5×10^5	0.82

SD standard deviation, *CV* coefficient of variation, *Q* Q value, *nd* not determined

Fig. 2 Kriged maps of the abundance of the culturable ARS microbial community, ARS activity, and microbial biomass
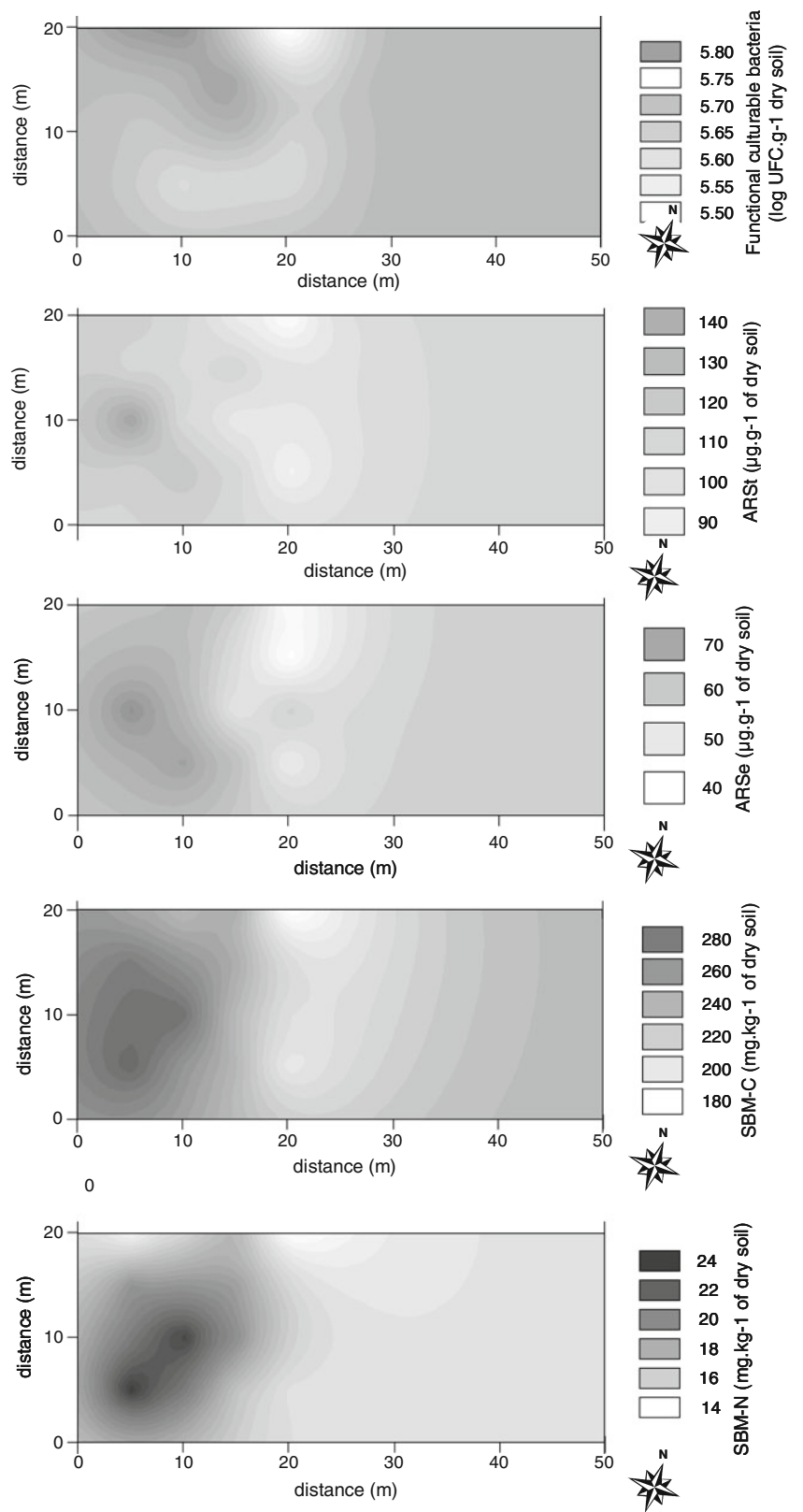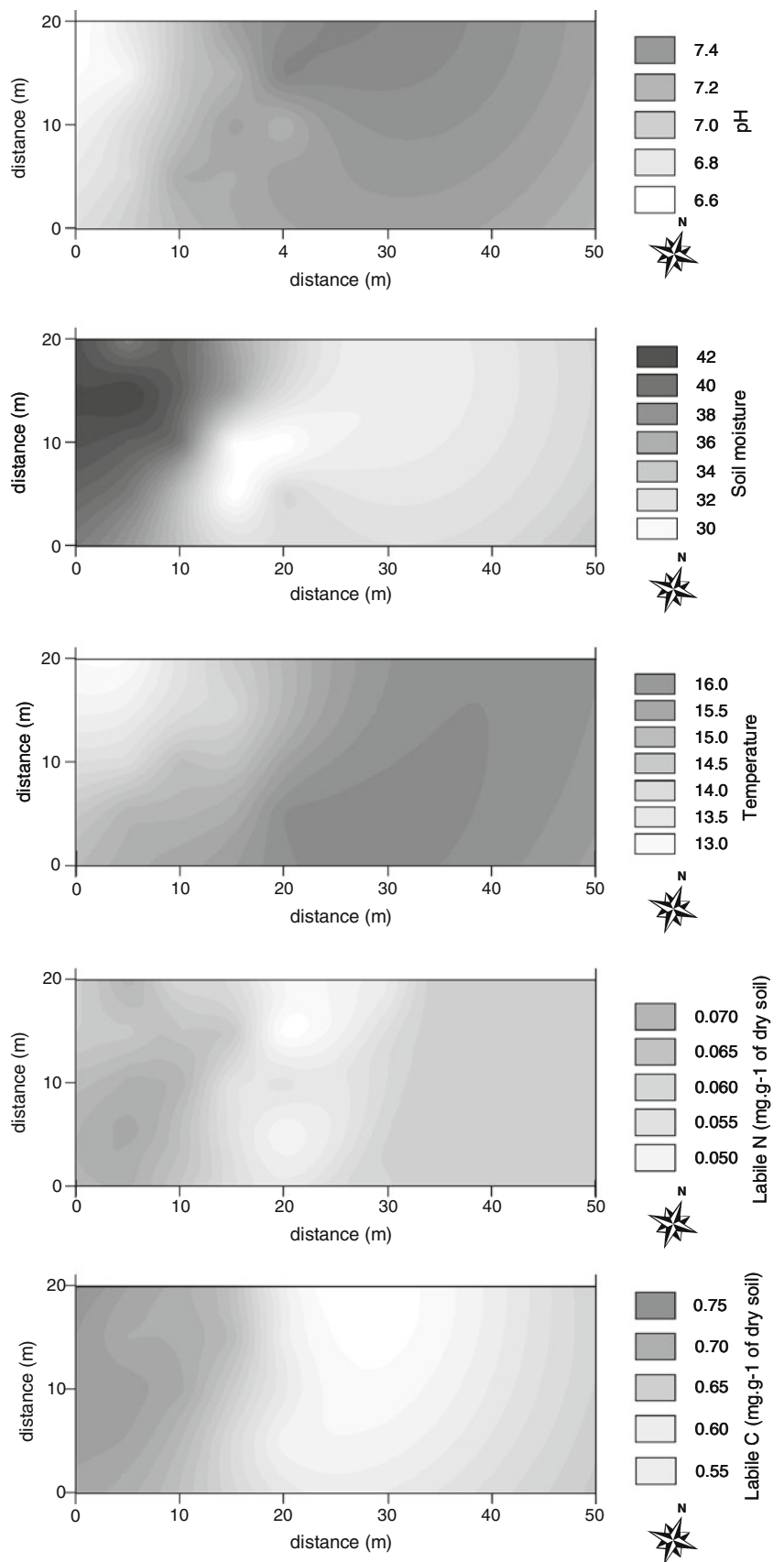
Fig. 3 Kriged maps of soil physicochemical properties

3.3 Determinants of the abundance and activity of bacteria-hydrolyzing sulfate esters

PCA was performed for exploratory analysis to find ecological patterns in the distribution of the abundance and activity of bacteria-hydrolyzing sulfate esters and of the soil physicochemical properties (Fig. 4a, b). Eigenvalues from the PCA analysis indicate that the two principal components (PC) accounted for 54.7 % of the variance of data (PC1 38.6 %, PC2 16.1 %). While most of the sub-site samples where centered on the factorial map, several sub-site samples (4-1, 4-3, 4-4, and 4-5), which were all located on the northwest border of the field, could be distinguished along the second component. The highly weighed variables under PC1 were SMB-C, labile C and N, soil moisture, and pH. These variables appear to be positively correlated with ARS activities (positive coordinates on PC1) while sulfates and pH were negatively correlated with these activities (negative coordinates on PC1). SMB-N was highly weighed variables under PC2 (see Fig. 4a).

Correlation coefficients between variables are presented in Table 2. The total and extracellular arylsulfatase activities showed the strongest correlations with SBM-C/SBM-N ratio (r^2=0.256, P<0.001) and SBM-C (r^2=0.287, P<0.001), respectively. Significant correlations were also observed between ARS_t and labile C, labile N, and pH. The ARS_e and the abundance of culturable bacteria-hydrolyzing sulfate esters were correlated to labile C, labile N, pH, and the SBM-C/SBM-N ratio. The abundance of culturable bacteria-hydrolyzing sulfate esters also correlated to temperature and soil moisture.

4 Discussion

Microorganisms are not distributed uniformly in the environment; rather, their abundance and activity change along environmental gradients (Robertson et al. 1988; Li and Sarah 2003; Franklin and Mills 2003; Philippot et al. 2009). Even within a homogeneous system, biological processes may produce aggregations of organisms at various spatial scales. Soil systems are particularly heterogeneous, and this heterogeneity arises as a result of the interaction of interrelated variables that fluctuate at many different spatial scales (Saerte and Bååth 2000). The factors that affect microbial processes in soils are known to be both biotic and abiotic (e.g., temperature, pH, and substrate availability). Indeed, soil properties do not vary independently; rather, the general perception is that any such variable measured at a certain point in space is the outcome of several physical, chemical, and biological processes, all of which are spatially variable. The driving factors of microbial community spatial patterns are not only related to soil physicochemical properties but also to human activity. Indeed, many studies have shown that soil microbial communities and enzyme activities are sensitive to soil management practices, such as organic fertilization by animal manures or green manures/crop residues, as well as tillage regime and herbicide additions (Inamura et al. 2004; Nayak et al. 2007; García-Ruiz et al. 2008).

Analysis of the 40 soil samples from the rape-planted field showed an average ARS_t activity of approximately 108 μgp-nitrophenol g^{-1} dry soil h^{-1}, which was similar to that reported in other previous studies (Klose et al. 1999; Dedourge et al. 2004; Vong et al. 2004; Cregut et al. 2009). However, a much lower ARS activity of about 2.27 μgp-nitrophenol g^{-1} dry soil h^{-1} was reported by Aşkin and Kizilkaya (2006). These lower values of ARS_t activity could be due to the analysis of bulk soil instead of the rhizosphere soil; in the rhizosphere, organic C released by root exudation is a strong driver of the density and activity of the microbial community (Bowen and Rovira 1999; Lugtenberg and Dekkers 1999). In our study, extracellular enzyme activities represented approximately 53.7 % of total enzyme activity, with a resulting calculated intracellular activity of 46.3 % (see Table 1). Our data are in accordance with Klose and Tabatabai (1999) reporting an average intracellular arylsulfatase activity of 57.7 % of total enzyme activity in the upper layer of an agricultural field. A higher proportion of the intracellular to total arylsulfatase activity was observed by Li and Sarah (2003) in an arid Israeli soil, which was attributed to denaturation of the extracellular arylsulfatase due to high temperatures, salt concentrations, and low moisture contents in the environment studied. We found a relatively low variability of the ARS_t activity ranging between 69.0 and 153.1 μg p-nitrophenol g^{-1} dry soil h^{-1} within the 800-m^2 arable field in agreement with previous studies (Vong et al. 2004; Cregut et al. 2009). Interestingly, the same range of variation was reported by Aşkin and Kizilkaya (2006) despite of the lower ARS activity observed.

The number of sequences for the *atsA* gene encoding the key enzyme catalyzing the hydrolysis of sulfate esters available in GenBank is limited, preventing the development of direct molecular approaches to target the ARS microbial community as previously done for other microbial communities (Smith and Osborn 2009). As a consequence, a cultivation-based approach using plate counting was performed to estimate the abundance of this functional guild. The abundance of the culturable ARS bacterial community displayed high variability with the minimum, maximum, and CV of 1.5 and 10.0×10^5 g^{-1} dry soil and of 37.5 %, respectively (see Table 1). The same range of variation has been found for the abundance of other functional communities at the field scale despite the field being subjected to different agricultural practices (Philippot et al. 2009; Wessén et al. 2011).
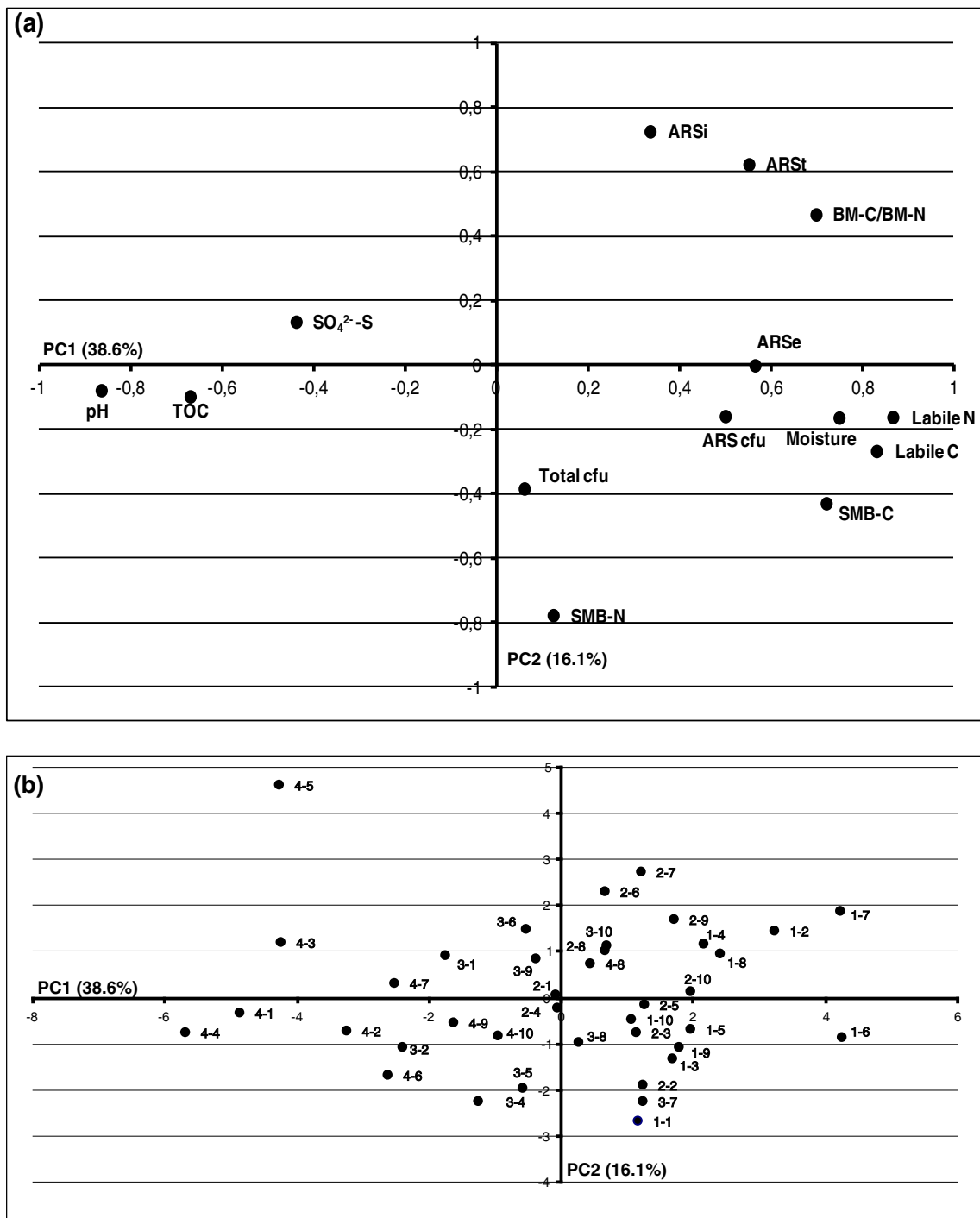
Fig. 4 Principal components analysis of the 40 soil samples based on the analysis of the physicochemical and microbiological parameters. **a** Physicochemical and microbiological parameters involved in the discrimination of the 40 soil samples. **b** PCA ordination of the 40 soil samples

Geostatistical analysis of the spatial distribution of the soil properties together with the activity and abundance of the ARS community revealed differences in the ratio Q, which is used as an indicator of spatial structure at the sampling scale. Indeed, the ratio Q was close to 1 for the abundance of the culturable ARS community and most of the soil physicochemical parameters except for the SO_4^{2-} concentration. Interestingly, a low Q ratio of 0.52 was also observed for the ARS_t activity (see Table 1). These results indicate that the sampling distance used in this study was adequate for most measured variables but the ARS activity for which a smaller sampling grid would have improved its

Table 2 Coefficients of correlation (R^2) for the relationships between physiochemical and microbiological parameters

	ARS_t	ARS_e	ARS_i	Total cfu	ARS cfu	SMB-C	SMB-N	BM-C/ BM-N	Labile C	Labile N	Moisture	pH	SO_4^{2-}-S	TOC
ARS_t														
ARS_e	0.264***													
ARS_i	0.783***	0.003												
Total cfu	0.036	0.000	0.049											
ARS cfu	0.001	0.021	0.001	0.048										
SMB-C	0.090	0.287***	0.003	0.000	0.106*									
SMB-N	0.015	0.026	0.052	0.014	0.000	0.445***								
BM-C/ BM-N	0.256***	0.178**	0.130*	0.014	0.141*	0.120*	0.215**							
Labile C	0.104*	0.131*	0.032	0.010	0.114*	0.438***	0.120*	0.118*						
Labile N	0.163**	0.239*	0.042	0.003	0.109*	0.418***	0.066	0.207**	0.735***					
Moisture	0.021	0.042	0.003	0.014	0.163**	0.230**	0.004	0.244**	0.402***	0.343***				
pH	0.149*	0.160**	0.054	0.019	0.265***	0.178**	0.014	0.410***	0.428***	0.599***	0.501***			
SO_4^{2-}-S	0.007	0.030	0.000	0.027	0.002	0.156*	0.015	0.092***	0.112*	0.114*	0.141*	0.073		
TOC	0.073	0.028	0.050	0.032	0.160**	0.082	0.015	0.242**	0.272***	0.192**	0.296***	0.403***	0.070	

$n=40$

ARS arylsulfatase activity, *SMB* soil microbial biomass, *Cfu* colony-forming unit

*$p=0.05$; **$p=0.01$; ***$p=0.001$

geostatistical modeling. However, our results show that the distribution of both the activity and abundance of the ARS community was not random but exhibited spatial dependence at the field scale. Comparison of the interpolated maps for the ARS communities and the soil properties revealed different or even contrasted patterns, and both exploratory and correlation analyses were performed to identify the soil factors driving the ARS community. Previous studies have shown the influence of soil moisture, soil pH, or temperature on the activities of soil enzyme activities (Li and Sarah 2003; Chaer et al. 2009) such as the phosphatase (Kang and Freeman 1999) and aryl-sulfatase activities (Kang and Freeman 1999; Niemi and Vepsäläinen 2005). In contrast, in our study, we found the ARS activities to be only correlated to soil pH among the soil properties measured. The effect of pH on soil enzyme activity has been described in several studies (Taylor et al. 2002; Aşkin and Kizilkaya 2006; Acosta-Martínez et al. 2007) and has been attributed to the fact that slight deviations in pH can disrupt the interactions between the enzymes and their sub-strates (Acosta-Martínez et al. 2007). Similarly, soil pH was also the soil property most closely correlated with the abundance of culturable bacteria-hydrolyzing sulfate esters. Similarly, several recent studies have shown that soil pH is a strong driver of the abundance of microbial communities (Hallin et al. 2009). Indeed, Bru et al. (2011) reported that soil pH alone explained 17.1 and 21.4 % of the spatial variability in the abundance of the total bacterial community and of a functional microbial community involved in N-cycling, respectively.

It is well-known that edaphic parameters affect enzyme activities by influencing microbial growth and substrate availability (Li and Sarah 2003). However, we did not find any significant correlation between the abundance of the culturable ARS bacterial community and ARS activities. This could result from the fact that we targeted only the culturable fraction of this functional community. In a comprehensive review, Röling (2007) also highlighted that microbial process rates are regulated by the size and properties of the enzyme pool and by the concentrations of substrates and that it is therefore often not sufficient to count the numbers of cells performing a particular step in a biogeochemical process in order to estimate its flux. Interestingly, no significant correlation between ARS activity and the concentration of the corresponding substrate, SO_4^{2-}-S, was observed in this study. An alternative explanation for the lack of relationship between abundance and activity of the ARS community observed in this work is that the ARS activity has not only a bacterial but also a fungal origin. This is supported by the strong correlation between ARS activities and the SBM-C/SBM-N ratio. Indeed, a higher fungal abundance in soil results in a higher ratio of the C/N biomass (Strong et al. 2004; Bhupinderpal-Singh and Bowden 2006; White 2006). In contrast to our results, Baum and Hrynkiewicz (2006) and Cregut et al. (2009) reported significant but weak correlation between the abundance of the ARS community and the ARS activity in soil.

5 Conclusions

To conclude, we found that the distribution of the abundance and activity of a functional microbial community

involved in S cycling in 800 m^2 agricultural field exhibits spatial dependence. The ARS activity showed a relatively low spatial variability while the abundance of the culturable ARS community ranged within one order of magnitude. The spatial pattern of ARS activity in the field was correlated with several soil properties, and results suggest that soil pH, labile C and N, and SBM-C/SBM-N ratio were the main parameters driving the ARS activity. Since microbial processes can vary not only in space but also in time, future work should focus on bridging spatial and temporal scales for a better understanding of S cycling in arable soil.

References

Acosta-Martínez V, Cruz L, Sotomayor-Ramírez D, Pérez-Alegría L (2007) Enzyme activities as affected by soil properties and land use in a tropical watershed. App Soil Ecol 35:35–45

Aşkin T, Kizilkaya R (2006) Assessing spatial variability of soil enzyme activities in pasture topsoils using geostatistics. Eur J Soil Biol 42:230–237

Baum C, Hrynkiewicz K (2006) Clonal and seasonal shifts in communities of saprotrophic microfungi and soil enzyme activities in the mycorrhizosphere of *Salix* spp. J Plant Nutr Soil Sc 169:481–487

Bhupinderpal-Singh RZJW, Bowden JW (2006) Carbon, nitrogen and sulphur cycling following incorporation of canola residue of different sizes into a nutrient-poor sandy soil. Soil Biol Biochem 38:32–42

Bowen GD, Rovira AD (1999) In: Sparks DL (ed) The rhizosphere and its management to improve plant growth. Advances in agronomy. Academic, New York, pp 1–102

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842

Bru D, Ramette A, Saby NPA, Dequiedt S, Ranjard L, Jolivet C, Arrouays D, Philippot L (2011) Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. ISME J 5:532–542

Chaer GM, Myrold DD, Bottomley PJ (2009) A soil quality index based on the equilibrium between soil organic matter and biochemical properties of undisturbed coniferous forest soils of the Pacific Northwest. Soil Biol Biochem 41:822–830

Cregut M, Piutti S, Slezack-Deschaumes S, Vong PC, Crovisier I, Benizri E (2009) Density, structure and diversity of the arylsulfatase bacterial community in the rhizosphere of field-grown rape and barley. Soil Biol Biochem 41:704–710

Dedourge O, Vong PC, Lasserre-Joulin F, Benizri E, Guckert A (2004) Effects of glucose and rhizodeposits (with or without cysteine-S) on immobilized-^{35}S, microbial biomass-^{35}S and arylsulphatase activity in a calcareous and an acid brown soil. Eur J Soil Sci 55:649–656

Dubousset L, Abdallah M, Desfeux AS, Etienne P, Meuriot F, Hawkesford MJ, Gombert J, Ségura R, Bataillé MP, Rezé S, Bonnefoy J, Ameline AF, Ourry A, Le Dily F, Avice JC (2009) Remobilization of leaf S compounds and senescence in response to restricted sulphate supply during the vegetative stage of oilseed rape are affected by mineral N availability. J Exp Bot 60:3239–3253

Enwall K, Throbäck IN, Stenberg M, Söderström M, Hallini S (2010) Soil resources influence spatial patterns of denitrifying communities at scales compatible with land management. Appl Environ Microbiol 76:2243–2250

Fitzgerald JW (1978) Naturally occurring organosulfur compounds in soil. In: Nriagu JO (ed) Sulfur in the environment, part 2. Wiley, New York, pp 391–443

Franklin RB, Mills AL (2003) Multi-scale variation in spatial heterogeneity for microbial community structure in an eastern Virginia agricultural field. FEMS Microbiol Ecol 44:335–346

García-Ruiz R, Ochoa V, Hinojosa B, Carreira JA (2008) Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. Soil Biol Biochem 40:2137–2145

Görres JH, Dichiaro MJ, Lyons JB, Amador JA (1998) Spatial and temporal patterns of soil biological activity in a forest and an old field. Soil Biol Biochem 30:219–230

Grundmann GL (1990) Utilisation des méthodes géostatistiques en microbiologie du sol: revue bibliographique. Rev d'Ecol Biol Sol 27:353–369

Hallin S, Jones CM, Schloter M, Philippot L (2009) Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. ISME J 3:597–605

Inamura T, Goto K, Iida M, Nonami K, Inoue H, Umeda M (2004) Geostatistical analysis of yield, soil properties and crop management practices in paddy rice fields. Plant Prod Sci 7:230–239

Janzen HH, Bettany JR (1984) Sulfur nutrition of rapeseed. I. Influence of fertilizer nitrogen and sulfur rates. Soil Sci Soc Am J 48:100–107

Jenkinson DS, Ladd JN (1981) Microbial biomass in soil measurement and turnover. In: Paul EA, Ladd JN (eds) Soil biochemistry, vol 5. Marcel Decker, London, pp 415–471

Kang H, Freeman C (1999) Phosphatase and arylsulphatase activities in wetland soils: annual variation and controlling factors. Soil Biol Biochem 31:449–454

Klose S, Tabatabai MA (1999) Arylsulfatase activity of microbial biomass in soils as affected by cropping systems. Biol Fert Soils 29:46–54

Klose S, Moore JM, Tabatabai MA (1999) Arylsulfatase activity of microbial biomass in soils as affected by cropping systems. Biol Fert Soils 29:46–54

Leustek T, Saito Z (1999) Sulphate transport and assimilation in plants. Plant Physiol 120:637–643

Li X, Sarah P (2003) Arylsulfatase activity of soil microbial biomass along a Mediterranean-arid transect. Soil Biol Biochem 35:925–934

Lisle L, Lefroy R, Anderson G, Blair G (1994) Methods for measurement of sulphur in plants and soil. Sulphur Agric 14:45–54

Lugtenberg BJJ, Dekkers LC (1999) What make *Pseudomonas* bacteria rhizosphere competent? Environ Microbiol 1:9–13

McGrath SP, Zhao FJ (1996) Sulphur uptake, yield responses and the interactions between nitrogen and sulphur in winter oilseed rape (*Brassica napus*). J Agric Sci 126:53–62

Nayak DR, Babu YJ, Adhya TK (2007) Long-term application of compost influences microbial biomass and enzyme activities in a tropical Aeric Endoaquept planted to rice under flooded condition. Soil Biol Biochem 39:1897–1906

Niemi RM, Vepsäläinen M (2005) Stability of the fluorogenic enzyme substrates and pH optima of enzyme activities in different Finnish soil. J Microbiol Meth 60:195–205

Parkin TB (1993) Spatial variability of the microbial process—a review. J Environ Qual 22:409–417

Philippot L, Čuhel J, Saby NPA, Chèneby D, Chroňáková A, Bru D, Arrouays D, Martin-Laurent F, Šimek M (2009) Mapping field-scale spatial patterns of size and activity of denitrifier community. Environ Microbiol 11:1518–1526

Ribeiro PJ Jr, Diggle PJ (1999) GeoR/geoS: a geostatistical library for r/s plus. Technical report ST-99-09. Dept maths and stats, Lancaster University, Lancaster, UK

Robertson GP, Huston MA, Evans FC (1988) Spatial variability in a successional plant community: patterns of nitrogen availability. Ecology 69:1517–1524

Röling WFM (2007) Do microbial numbers count? Quantifying the regulation of biogeochemical fluxes by population size and cellular activity. FEMS Microbiol Ecol 62:202–210

Saerte P, Bååth E (2000) Spatial variation and patterns of soil microbial community structure in a mixed spruce–birch stand. Soil Biol Biochem 32:909–917

Scherer HW (2001) Sulphur in crop production. Eur J Agron 14:81–111

Shouse PJ, Russel WB, Burden DS, Selim HM, Sisson JB, van Genuchten MT (1995) Spatial variability of soil water retention functions in a silt loam soil. Soil Sci 159:1–12

Singh BR (1984) Sulfate sorption by acid forest soil: 3. Desorption of sulfate from adsorbed surface as a function of time desorbing ion, pH, and amount of adsorption. Soil Sci 138:440–447

Smith CJ, Osborn AM (2009) Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. FEMS Microbio Ecol 67:6–20

Sparling GP, Vojvodic-Vukovic M, Schipper LA (1998) Hot-water soluble C as a simple measure of labile soil organic matter: the relationship with microbial biomass C. Soil Biol Biochem 30:1469–1472

Strong DT, De Wever H, Merckx R, Recous S (2004) Spatial location of carbon decomposition in the soil pore system. Eur J Soil Sci 55:739–750

Tabatabai MA, Bremner JM (1970) Arylsulfatase activity of soils. Soil Sci Soc Am Proc 34:225–229

Taylor JP, Wilson B, Mills MS, Burns RG (2002) Comparison of microbial numbers of enzymatic activities in surface soils and subsoils using various techniques. Soil Biol Biochem 34:387–401

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

Vong PC, Dedourge O, Guckert A (2004) Immobilization and mobilization of labelled sulphur in relation to soil arylsulphatase activity in rhizosphere soil of field-grown rape, barley and fallow. Plant Soil 258:227–239

Wessén E, Söderström M, Stenberg M, Bru D, Hellman M, Welsh A, Thomsen F, Klemedtson L, Philippot L, Hallin S (2011) Spatial distribution of ammonia-oxidizing bacteria and archaea across a 44-hectare farm related to ecosystem functioning. ISME J 5:1213–1225

White RE (2006) Principles and practice of soil science: the soil as a natural resource. Blackwell, Malden, 36 pp

WRB (2001) World reference base for soil resources. In: Driessen P, Deckers J, Spaargaren O, Nachtergaele F (eds) Lecture notes on the major soils of the world. FAO World soil resources reports, vol. 94. Food and Agriculture Organization of the United Nations (FAO), Rome

Wu J, Joergensen RG, Pommering B, Chaussod R, Brookes PC (1990) Measurement of soil microbial biomass C by fumigation-extraction: an automated procedure. Soil Biol Biochem 22:1167–1169

Wyss C (1989) *Campylobacter–Wolinella* group organisms are the only oral bacteria that form arylsulfatase-active colonies on a synthetic indicator medium. Infect Immun 57:1380–1383

Yanai J, Sawamoto T, Oe T, Kusa K, Yamarkawa K, Sakamoto K, Naganawa T, Inubshi K, Hatano R, Kosaki T (2003) Spatial variability of nitrous oxide emissions and their soil-related determining factors in an agricultural field. J Environ Qual 32:1965–1977

Zhao FJ, Hawkesford MJ, McGrath SP (1999) Sulphur assimilation and effects on yield and quality of wheat. J Cereal Sc 30:1–17