This paper was peer-reviewed for scientific content.
Pages 486-493 In: D.E. Stott, R.H. Mohtar and G.C. Steinhardt (eds). 2001. Sustaining the Global Farm. Selected papers from the 10th International Soil Conservation Organization Meeting held May 24-29, 1999 at Purdue University and the USDA-ARS National Soil Erosion Research Laboratory.

Regional-Scale Analysis of Soil Microbial Biomass and Soil Basal CO_2-Respiration in Northeastern Germany

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ABSTRACT

Regional-scale surveys of soil microbiological, physical and chemical properties related to soil quality indication were performed across the northeastern German lowland. It was hypothesized, soil microbiological properties would follow spatial patterns and trends of soil physical and chemical properties across the region, being detectable via a regional transect sampling strategy. At each of 89 cereal crop cultivation sites along a transect (total distance: 151 km; medium lag distance: 1.7 km), five nested soil samples were collected (0-15 cm, lag distance: 2 m) in autumn 1996, 1997, and 1998. Soil microbial biomass (C_{mic}) and soil basal respiration were assayed as integrative, overall properties using an automated infrared gas analysis system. Additionally, organic carbon (C_{org}), hot-water soluble carbon (C_{hwl}), total nitrogen (N_t), potential cation exchange capacity (CEC), pH and texture were analyzed to assess spatial distribution patterns, spatial autocorrelation, and inter-annual variability. As a result, C_{mic} displayed an increasing spatial trend from the southern to the northern end of the transect (overall mean: 484 µg g^{-1} soil, range: 162-967 µg g^{-1} soil). Correspondingly, an increasing but less steep trend was detected for basal CO_2-respiration (overall mean: 0.48 µg CO_2-C g^{-1} h^{-1}, range: 0.13-1.55 µg CO_2-C g^{-1} h^{-1}), as well as for C_{org} (overall mean: 0.91 %, range: 0.47-1.45 %), and N_t (overall mean: 0.09 %, range: 0.04-0.15 %), respectively. Spearman Rank Order correlation analyzes across the transect revealed highly significant correlates between C_{mic} and N_t ($r_s = 0.748$), C_{org} ($r_s = 0.766$), CEC ($r_s = 0.698$), and pH ($r_s = 0.664$). Correlates for basal CO_2-respiration were highly significant, but less strong. No significant inter-annual differences were found for soil microbial biomass along the transect comparing three autumn sampling campaigns, but soil basal respiration activities differed significantly among years. The spatial continuities of C_{mic}, basal CO_2-respiration, and soil properties were analyzed geostatistically via semivariogram modeling. C_{mic} displayed a spatial autocorrelation, i.e., a model range was identified at the regional scale (56 km), whereas soil basal CO_2-respiration displayed no autocorrelation, i.e., a pure nugget effect. Thus, the transect sampling strategy and the semivariogram analyzes proved to be suitable to evaluate spatial patterns of soil properties at the regional scale, including C_{mic} as a reliable, quantitative measure of the soil microflora, closely related to soil physical and chemical properties.

INTRODUCTION

In northeastern Germany, the moraine landscape after the Pommern / Brandenburg Stadium of the Weichsel glacial period is determined by mosaic-type, high spatial heterogeneity of surface topography and soil types (Franz et al., 1970; Schmidt, 1991), as well as soil physical and chemical properties. For the assessment and analyzes of soil organic carbon dynamics at the regional scale such as the northeastern German agricultural landscape, basic information on spatial patterns and continuity of soil microbiological properties is required on the corresponding scale, but concepts and research activities at these scales are still sparse and being developed (Brockman and Murray, 1997; Goovaerts, 1998; Gustafson, 1998). Spatial patterns and variability of soil microbial activities is manifested at different scales. As suggested by Parkin (1993), four scales can be defined: the microscale, the plot or field scale, the landscape scale and the regional scale. Most research has been done at the microscale and the plot or field scale. Such studies include reports of specific microbial activities with respect to soil nutrient cycling and soil quality (Bergstrom et al., 1998; Bonmati et al., 1991; Pankhurst et al., 1995), but few studies report on the spatial distribution of soil microbial properties at the landscape scale (Myrold et al., 1989; Ruess and Seagle, 1994; Staddon et al., 1998; Wirth, 1999), including assays of soil microbial biomass, microbial diversity, or basal CO_2 respiration.

Moreover, surveys of organic carbon transformation processes and fluxes in soils, which are controlled by the activities of microorganisms (Paul and Clark, 1996), are considered essential for evaluating the function of soil resources within a region (Burke, 2000). Thus, the soil microbial biomass (C_{mic}) as an integrative measure of the physiologically active part of the soil microflora and the soil basal CO_2-respiration as a measure of overall soil biochemical activity were studied as a part of a ZALF Research Group, entitled "Biological parameters of soils and plant phyllospheres along a transect across the northeastern German lowland: Studies of the spatial continuum and spatial co-variance structure with special reference to selected site properties". Integrative microbiological parameters were selected as potential indicators of soil quality, or soil health as suggested by Boehm and Anderson (1997), or Pankhurst et al. (1995), and moreover, assayed in context with soil physical and chemical properties (carbon

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and nitrogen contents, pH, texture). It was hypothesized; soil microbiological properties would follow spatial patterns and trends of soil physical and chemical properties across an agricultural region. A regional transect was assumed to be a suitable sampling strategy. The aims of this study were to: (1) explore spatial distribution and variability of soil microbiological, physical and chemical properties across the northeastern German lowland, (2) identify soil physical and chemical properties that correlate with soil microbiological properties at the regional scale, and (3) identify spatial continuity of soil microbiological, physical and chemical properties using geostatistical methods (semi-variography) to analyze the autocorrelation of soil properties.

MATERIALS AND METHODS

A regional, one-dimensional transect (total length: 151 km, medium lag distance: 1.7 km) was established across the northeastern German lowland, which is an upper-Pleistocene, agricultural landscape (Franz et al., 1970), characterized by high spatial variability of surface topography, soil types, as well as soil physical and chemical characteristics. The climate is semi-continental with a mean annual temperature of 8.5ºC and a mean annual precipitation of 450-600 mm. Along the transect, 89 cereal crop production sites (1996: 36 winter wheat, 30 winter rye, 14 triticale, 6 barley, 1 corn, 1 rape, and 1 fallow) were studied, i.e., *Site 1: Neuendorf im Sande* at the southern end of the transect (Gauss-Krueger right value: 5439023, high value: 5807211) up to *Site 89: Wolfshagen* at the northern end of the transect (Gauss-Krueger right value: 5409466, high value: 5924585). The sites are located between 21 m and 151 m above sea level. Soil types along the transect include, according to the German and the FAO soil classification system (Arbeitsgruppe Boden, 1994 / FAO, 1998), Braunerde/ Cambisol (17 sites), Fahlerde/Podzoluvisol (34 sites), Parabraunerde/Luvisol (2 sites), Pseudogley/Gleysol (9 sites), Kolluvisol (10 sites), Pararendzina/calcaric Regosol (9 sites), Tschernosem/Phaeozem (4 sites), Kalk-tschernosem/calcaric Phaeozem (1 site), and Anmoorgley/ Gleysol derived from peat (1 site). Soils were sampled in August-September 1996, 1997, and 1998, immediately after crops were harvested. At each site, five nested samples were taken from the top 0-15 cm of soil with a local lag distance of 2 m. Samples were sieved (2 mm) and stored at 4ºC until analysis. Soil microbial biomass (C_{mic}) was determined with a physiological, substrate-induced respiration method (Anderson and Domsch, 1978) at 20°C using an automated infrared gas analysis system developed by Heinemeyer et al. (1989). C_{mic} was calculated from the maximum initial respiratory response on the addition of glucose (5000 µg g^{-1} soil), i.e., C_{mic} (µg g^{-1} soil) = [(µl CO_2 g^{-1} soil h^{-1})·40.04]+0.37. Soil basal respiration (CO_2^-respiration without the addition of glucose) was measured hourly under continuous aeration of the soil samples, using the automated infrared gas analysis system over a period of at least 8 up to 18 h at 20°C. Soil water content was determined gravimetrically (105°C, 24 h). Soil pH was measured potentiometrically in 0.01 M $CaCl_2$ suspension (1:2.5 w/v). Total soil carbon content and total soil nitrogen content was analyzed after dry combustion using a CNS-2000 analyzer (LECO Corporation, St. Joseph, MI, USA). C_{org} was calculated after deduction of $CaCO_3$. Hot-water soluble carbon (C_{hwl}) as a measure of labile soil organic matter was determined as described by Körschens et al. (1997). Soil texture and potential cation exchange capacity were analyzed according to DIN ISO 19683 and DIN ISO 13536 protocols, respectively. All laboratory analyzes were carried out in triplicate, referred to oven dry-weight basis of soil and finally plotted as mean values. Data-sets were mostly not normally distributed with equal variances. Consequently, the non-parametric Spearman Rank Order Correlation was used at significance levels of $P<0.05$, $P<0.01$ and $P<0.001$ to calculate strength of association by correlation coefficients (r_s). The Kruskal-Wallis One Way ANOVA on Ranks was performed to test for differences among years of sampling. Descriptive statistics, Kolmogorov-Smirnov normality tests, regressions, correlation analyses and ANOVA were performed using the software SigmaStat version 2.0 (Jandel Scientific, San Rafael, CA). Spatial autocorrelations were calculated and modeled using the geostatistical software GS+ version 2.3b (Gamma Design, Plainwell, MI). Isotropic (direction independent) semivariance of data was calculated and plotted versus separation distance between samples (lag distance). Best-fit models were applied to estimate the range, i.e., the maximum separation distance within which samples are spatially autocorrelated, reflecting the spatial continuity of observations.

RESULTS AND DISCUSSION

Immediately after harvest in late summer 1996, 1997, and 1998, respectively, a linear transect was sampled across the northeastern German lowland including 89 cereal crop production sites. Thus, a regional sequence of arable sites was under study, including a range of different soil types, and soil physical and chemical properties. Distinct spatial trends were revealed along the transect with respect to soil texture (sand, Fig. 1A), potential cation exchange capacity (Fig. 1B), and soil pH (Fig. 1C), characterized by high variability in between the sites, i.e., by factors of 1.7-10 (Table 1). Similarly, distinct trends were detected for soil organic carbon (Fig. 2A) and soil nitrogen contents (Fig. 2C), except for hot-water soluble carbon (Fig. 2B) which displayed no clear spatial trend along the transect. Against this background of soil physical and chemical properties across the region (see Table 1 for summarized descriptive statistics), spatial distribution patterns of soil microbial properties were analyzed. The soil microbial biomass was assayed via substrate-induced respiration to provide a measure of the total, physiologically active part of the microflora (Anderson and Domsch, 1978). Furthermore, the soil basal CO_2-respiration was studied as a measure of overall, potential soil microbial activity (Gray, 1990). Both approaches were automated, laboratory-based methods, which are commonly applied to characterize the microbiological status of soils (Stenberg et al., 1998), and are furthermore discussed as potential, integrative bioindicators of soil health or soil quality (Pankhurst et al., 1995; Gregorich et al., 1994). As a result, soil microbial biomass at the central out of five local/nested sampling points displayed an increasing trend from the southern to the

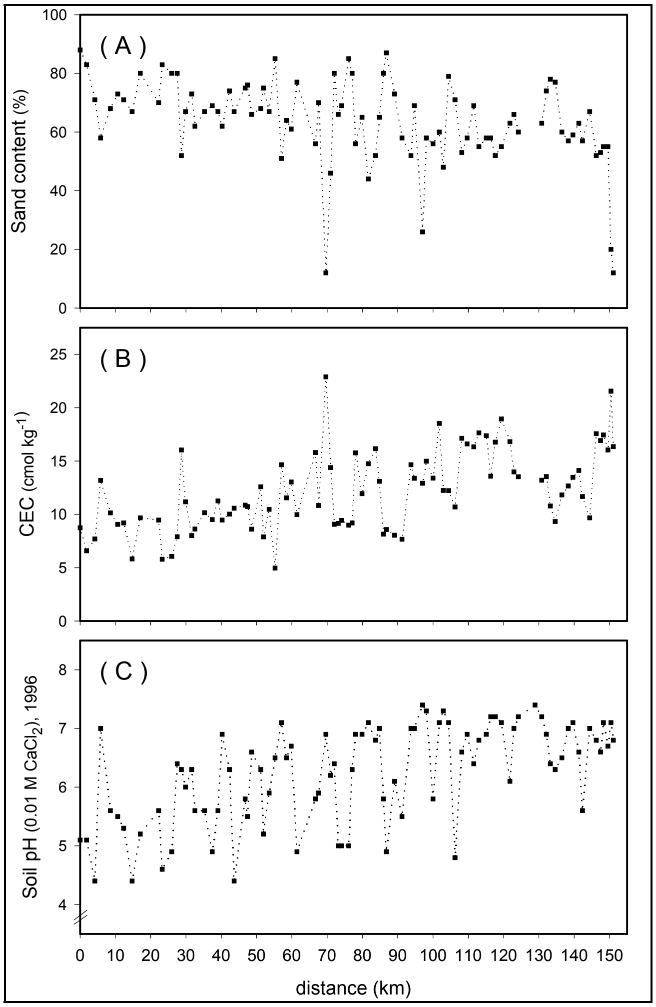

Figure 1: Spatial distribution of selected soil physical and chemical properties along a regional transect across the northeastern German lowland: A) sand fraction, B) potential cation exchange capacity (CEC), and C) soil acidity. Total distance: 151 km. Medium lag distance: 1.7 km. Sampling date: autumn 1996.

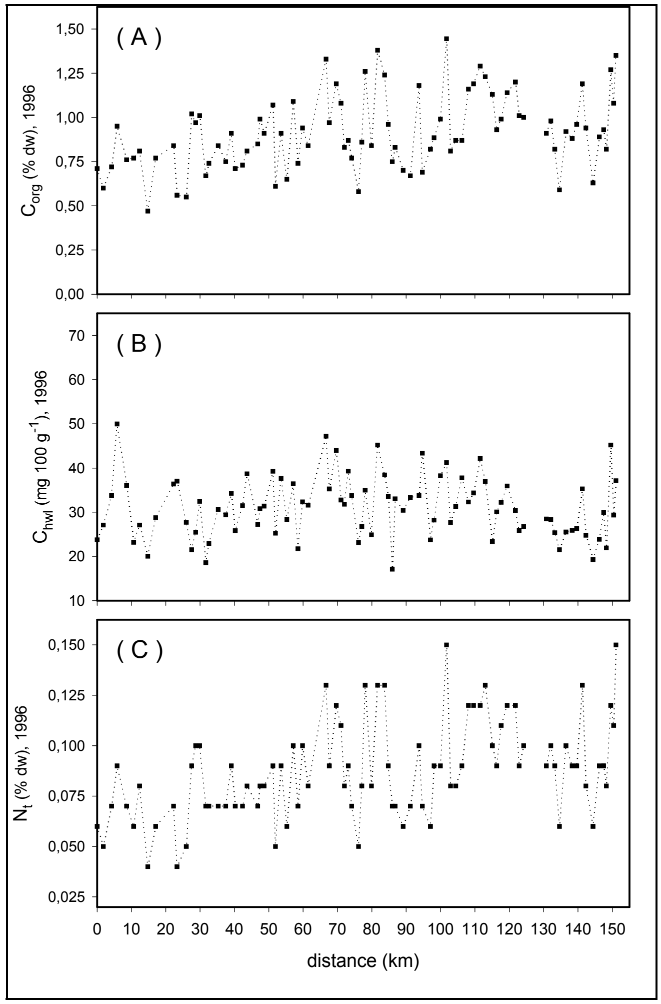

Figure 2: Spatial distribution of selected soil chemical properties along a regional transect across the northeastern German lowland: A) soil organic carbon (C_{org}), B) hot water soluble soil organic carbon (C_{hwl}), and C) total soil nitrogen content (N_t). Transect sampling details appear in Figure 1.

northern end of the transect in between 162 and 966 µg C_{mic} g^{-1} soil (Fig. 3A; $y = 354.33 + 1.627\,x$). Including all of five local/nested samples, i.e., the small scale or site variability by means of standard errors, an almost identical trend was revealed (Fig. 3B; y=341.10+1.667x). Furthermore, values for microbial biomass were in a range commonly reported for arable field soils (Beck et al., 1997; Martens, 1995), or sites from the respective region (Grimm and Wirth, 1998; Wirth, 1999). In contrast, soil basal respiration displayed a less steep, increasing spatial trend from the southern to the northern end of the transect (Fig. 4A; y=0.431+0.00033x), which was hardly detectable after the inclusion of local/nested samples (Fig. 4B; y=0.383+0.00124x). With the exception of distinct maximum values above 1.0 µg CO_2-C g^{-1} h^{-1}, the values found in between 0.13 and 1.55 µg CO_2-C g^{-1} h^{-1} were in a range commonly described for soil basal respiration of arable soils (e.g., Beck et al., 1997; Grimm and Wirth, 1998). Concerning temporal, i.e., inter-annual variability of soil microbial properties along the transect, data sets from three sampling campaigns at harvest 1996, 1997, and 1998 were compared by Kruskal-Wallis One Way ANOVA on Ranks. As a result, no significant differences were found for soil microbial biomass along the transect in between the years of sampling (H=5.216 with 2 degrees of freedom, P=0.074). In contrast, soil basal respiration was statistically significant different among the years (H=35.313 with 2 degrees of freedom, P<0.001). Consequently, soil microbial biomass proved to be a stable and reliable parameter for quantitative regional-scale analyses with respect to a specific soil sampling date, such as the end of the vegetation period, or more conveniently, early spring. Moreover, soil microbial biomass is suitable and commonly used as an potential indicator of soil quality (Dalal, 1998), as an indicator of change and future trends in soil organic matter levels and equilibria (Gregorich et al., 1994), or used in long-term soil monitoring programs (Grimm and Wirth, 1998).

With respect to correlates of soil microbial and soil physical and chemical properties along the transect, the non-parametric Spearman Rank Order Correlation Analysis was applied, since most of the data sub-sets were not normally distributed and failed tests for equal variance even after logarithmic or square root transformation. With respect to the 1996 data set, soil microbial biomass was highly significant correlated with soil organic carbon (r_s=0.766, P<0.001), total nitrogen (r_s=0.748, P<0.001), CEC (r_s=0.698, P<0.001), and soil pH (r_s=0.664, P<0.001), but less clear with hot-water soluble carbon (r_s=0.321, P<0.01). The latter was an unexpected result, but more data on the composition of soil organic matter are required to explain this finding. Soil basal respiration was less clear, but highly significant correlated with the respective soil physical and chemical properties (Table 2). Notably, these correlations are valid across a regional scale including 89 arable sites characterized by highly variable soil types and soil substrates. Similar correlates were found by other authors at the field scale, e.g., Kaiser et al. (1992), stating significant logarithmic or square root transformation. With respect to the 1996 data set, soil microbial biomass was highly significant correlated with soil organic carbon (r_s=0.766, P<0.001), total nitrogen (r_s=0.748, P<0.001), CEC (r_s=0.698, P<0.001), and soil pH (r_s=0.664, P<0.001), but less clear with hot-water soluble carbon (r_s=0.321, P<0.01). The latter was an unexpected result, but more data on the composition of soil organic matter are required to explain this finding. Soil basal respiration was less clear, but highly significant correlated with the respective soil physical and chemical

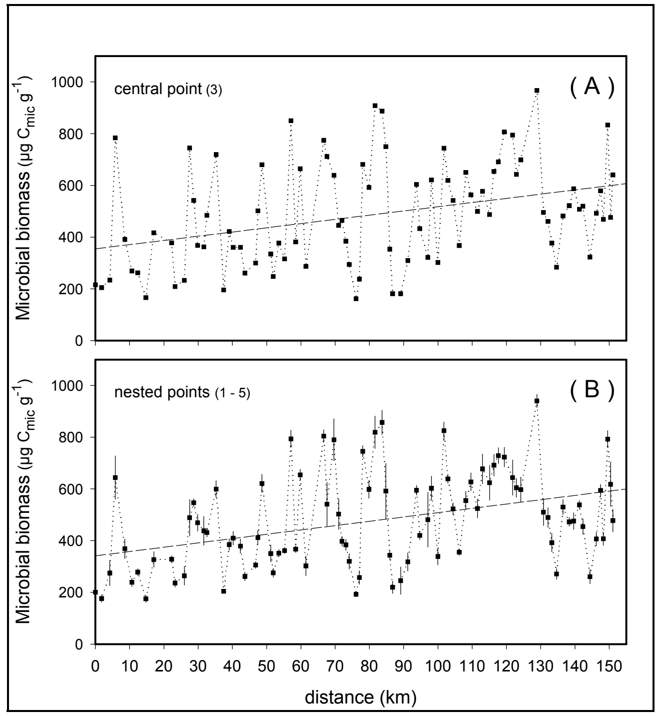

Figure 3: Spatial distribution of soil microbial biomass (C_{mic}) along a regional transect across the northeastern German lowland with respect to A) singular, and B) nested local soil sampling. Transect sampling details appear in Figure 1. Local lag distance: 2 m.

Table 1: Descriptive statistics of soil microbial properties and soil variables along a regional transect (autumn 1996).

Property	Mean	SD	SE	Maximum	Minimum	Median	Skewness	Kurtosis
C_{mic}	484.31	200.07	21.21	966.74	161.95	476.14	0.341	-0.757
CO_2	0.482	0.264	0.028	1.550	0.130	0.380	1.540	2.760
C_{org}	0.913	0.211	0.023	1.445	0.470	0.887	0.391	-0.275
C_{hwl}	31.06	6.875	0.733	49.96	17.11	30.68	0.407	-0.101
N_t	0.087	0.024	0.003	0.150	0.040	0.090	0.465	-0.111
S	63.74	14.41	1.537	88.00	12.00	66.00	-1.340	3.323
U	25.96	10.06	1.072	65.00	8.00	26.00	1.477	4.217
T	10.31	5.66	0.604	40.00	4.00	9.00	2.054	7.752
CEC	12.17	3.70	0.395	22.89	4.90	11.75	0.437	-0.191
pH	6.23	0.837	0.088	7.40	4.40	6.40	-0.577	-0.832

C_{mic}: soil microbial biomass (µg C_{mic} g^{-1} soil), CO_2: soil basal respiration (µg CO_2-C g^{-1} soil h^{-1}), C_{org}: soil organic carbon (% dw), C_{hwl}: hot water soluble soil organic carbon (mg 100 g^{-1} soil), N_t: total soil nitrogen (% dw), S: sand content (%), U: silt content (%), T: clay content (%), CEC: potential cation exchange capacity (cmol kg^{-1} soil), pH: soil pH (0.01 M $CaCl_2$), data: autumn 1996, SD: standard deviation; SE: standard error.

Table 2. Spearman Rank Order Correlation coefficients (r_s) between soil microbial properties and soil variables across the entire regional transect.

Property	CO_2	C_{org}	C_{hwl}	N_t	S	CEC	pH	site type
C_{mic}	0.730***	0.766***	0.321**	0.748***	-0.626***	-0.698***	0.664***	0.523***
CO_2		0.524***	0.347***	0.512***	-0.412***	0.526***	0.472***	0.466***
C_{org}			0.498***	0.944***	-0.614***	0.805***	0.470***	0.531***
C_{hwl}				0.494***	-0.246*	0.326**	-0.001	0.172
N_t					-0.659***	0.828***	0.504***	0.631***
S						-0.774***	-0.602***	-0.533***
CEC							0.672***	0.697***
pH								0.576***

*P < 0.05; ** P < 0.01; *** P < 0.001. Abbreviations and units of properties are explained in Table 1. Data: autumn 1996

Table 3: Semivariogram model parameters for soil microbial properties and soil variables across a regional transect.

Property	Nugget c_0	Sill $c_0 + c$	Nugget/Sill (%)	s^2	Model	Range (km)	r^2
C_{mic}	0.163	0.213	76.53	0.203	linear/sill	56.2	0.167
CO_2	0.069	0.069	100	0.070	linear	> 100	0.117
C_{org}	0.035	0.049	71.43	0.045	linear/sill	70.4	0.229
C_{hwl}	43.30	48.77	88.78	47.27	Gaussian	50.4	0.029
N_t	0.051	0.103	49.51	0.080	linear	> 100	0.533
S	158.0	217.0	72.81	207.79	linear/sill	69.0	0.146
U	81.6	112.0	72.86	101.12	linear	> 100	0.125
T	22.48	31.48	71.41	32.06	linear/sill	15.90	0.046
CEC	7.29	100.0	7.29	13.71	linear	> 100	0.689
pH	0.360	1.01	35.64	0.701	linear	> 100	0.810

After testing for normal distribution, a ln (z+0) transformation was performed except for C_{org}, C_{hwl} and CEC, which were normally distributed. Finally, best-fit models were applied at an active lag distance of 100 km. Abbreviations and units of properties appear in Table 1. Date: autumn 1966.

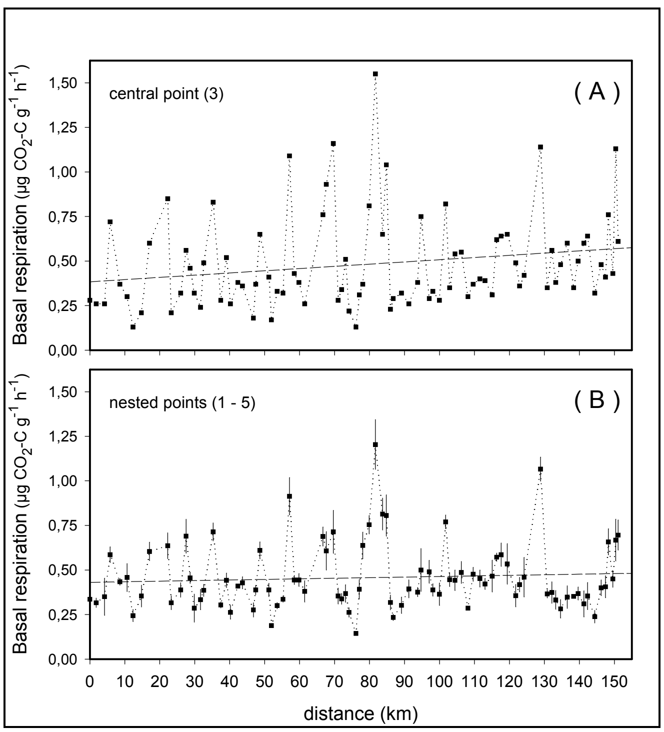

Figure 4. Spatial distribution of soil basal respiration along a regional transect across the northeastern German lowland with respect to A) singular, and B) nested local soil sampling. Transect sampling details appear in Fig. 1. Local lag distance: 2 m.

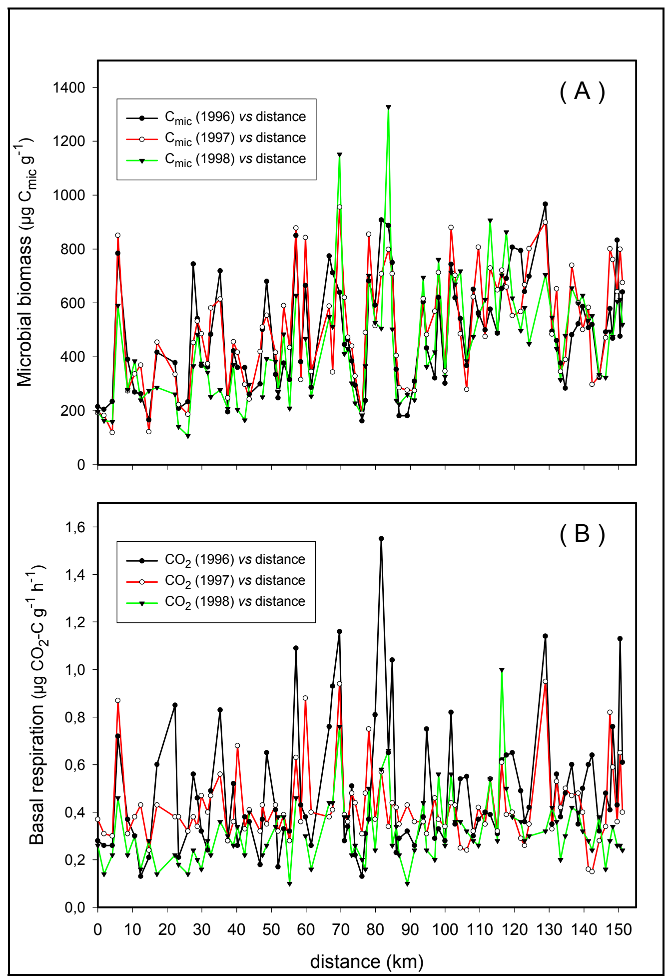

Figure 5. Inter-annual variability of A) soil microbial biomass, and B) soil basal respiration along a regional transect across the northeastern German lowland. Transect sampling details appear in Figure 1. Sampling dates: autumn 1996 (black line), 1997 (red line), and 1998 (green line).

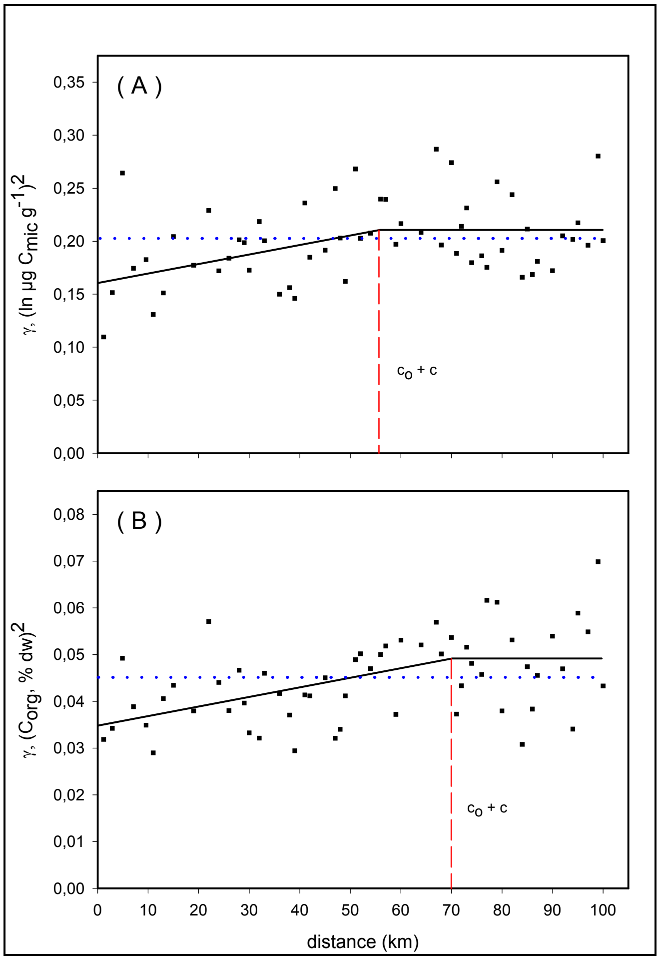

Figure 6. Semivariograms for A) soil microbial biomass (C_{mic}), and B) soil organic carbon (C_{org}) along a regional transect across the northeastern German lowland. Semivariance (γ) is plotted against an active lag distance of 100 km. Sampling date: autumn 1996. Semivariogram model parameters appear in Table 3. Horizontal blue-dotted lines indicate overall sample variance for each variate, vertical red dashes indicate the range.

properties (Table 2). Notably, these correlations are valid across a regional scale including 89 arable sites characterized by highly variable soil types and soil substrates. Similar correlates were found by other authors at the field scale, e.g., Kaiser et al. (1992), stating significant correlation coefficients of r=0.58 and r=0.84 for soil basal respiration and soil organic carbon and total nitrogen, respectively, analyzing 27 arable soils including two peat soils. With respect to correlates of soil microbial biomass, the authors reported coefficients of r=0.25 for soil organic carbon and r=0.58 for total nitrogen, while Robertson et al. (1997) stated r=0.37 for total soil carbon and r=0.18 for soil moisture. Consequently, correlates of soil microbial biomass, soil basal respiration and soil physical and chemical variables are considered to be valid at various scales.

Geostatistical methods have been used to better understand spatial patterns and continuity of soil properties (Goovaerts, 1998; Warrick et al., 1986; Wendroth et al., 1997), which is a prerequisite to predict and ultimately model the distribution of soil microbiological properties across heterogeneous systems (Brockman and Murray, 1997). Most reports focus on spatial analyzes of soil fertility or soil quality criteria (Cahn et al., 1994; Robertson et al., 1997; Smith et al., 1993), but rarely at the regional scale, e.g., such as a study in landscape-scale changes of soil quality indicators based on landform element complexes (Pennock et al., 1994). Other research activities dealing with the spatial distribution of soil microbial activities at the landscape scale concentrate on carbon mineralization (Halvorson et al., 1995; Smith et al., 1994), or soil enzyme activities (Bergstrom et al., 1998). Reports published so far on the regional variability of soil microorganisms at the species level (e.g., Horn and Dorner, 1998) are based on classical statistics or biogeographic patterning of microbial diversity across climatic gradients (Staddon et al., 1998). In this study, semi-variography was applied to explore the spatial continuity of selected soil microbial properties and soil variables along a transect at the regional scale. Isotropic (direction independent) semi-variance of data was calculated and plotted versus separation distance between samples (lag distance), after log transformation of data sets to pass tests for normal distribution. Best-fit models were applied to estimate the range, i.e., the maximum separation distance within which samples are spatially auto-correlated. Beyond the range, the semi-variogram is constant at a value termed the sill (c_0+c, Nugget variance plus structural variance), which is theoretically equal to the total variance of the data set. The Nugget variance (c_0) reflects the residual, non-structural variance of observations, which is extrapolated from the semi-variogram model at lag distance =0. As a result, soil microbial biomass (Fig. 6A) displayed a moderate spatial autocorrelation, revealing a model range of 56 km, whereas for soil organic carbon a model range of 70 km was detected (Fig. 6B). In contrast, total nitrogen, soil pH, and CEC were characterized by strong spatial trends (Table 3). At the field scale, autocorrelations for soil microbial biomass and soil respiration were reported at a range of 21 m and 61.2 m, respectively (e.g., Robertson et al., 1997), or at a range of 28 m and 22 m at the encatchment scale, respectively (Wirth, 1999). Thus, spatially structured variance of soil microbial properties needs to be considered to represent a major proportion of the total variance at the landscape-scale, and moreover may be prevalent at the regional scale. In conclusion, further soil monitoring activities at the regional scale are required to analyze temporal stability of spatial autocorrelations and covariance structures of soil properties, as well as functional interrelationships among geomorphological features, soil physical and chemical properties, and soil microbial activities. Moreover, spatial databases are required for the validation of biogeochemical models, or for scaling-up soil properties to regional scales (Burke, 2000; Pennock et al., 1994). Using a regional transect sampling design in combination with semivariance analyses is considered to provide a promising approach.

ACKNOWLEDGEMENTS

I am grateful to Mrs Martina Wiemer for her excellent technical assistance. Furthermore, I would like to thank Dr Wilfried Hierold, Mr B. Grossmann and Dr. Peter Lentzsch for site selection, GPS measurements, information on site properties, and co-ordination. Soil chemical assays of C_{org}, C_{hwl}, N_t, texture and CEC were performed by ZALF Central Laboratory. This study was supported by Federal Ministry of Consumer Protection, Food and Agriculture (BMVEL) and the Ministry of Agriculture, Environmental Protection and Regional Planning of the Federal State of Brandenburg (MLUR).

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