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Topographic control of soil microbial activity: a case study of denitrifiers

Igor V. Florinsky[a,*], Shawna McMahon[b], David L. Burton[c,d]

[a] Institute of Mathematical Problems of Biology, Russian Academy of Sciences, Pushchino, Moscow Region 142292, Russia
[b] Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331-7306, USA
[c] Climate Change Research Chair, Nova Scotia Agriculture College, 203 Resource Stewardship Building, 20 Tower Road, P.O. Box 550, Truro, NS, Canada B2N 5E3
[d] Department of Soil Science, University of Manitoba, 362 Ellis Building, Winnipeg, MB, Canada R3T 2N2

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Abstract

Topography may affect soil microbial processes, however, the use of topographic data to model and predict the spatial distribution of soil microbial properties has not been widely reported. We studied the effect of topography on the activity of denitrifiers under different hydrologic conditions in a typical agroecosystem of the northern grasslands of North America using digital terrain modelling (DTM). Three data sets were used: (1) digital models of nine topographic attributes, such as elevation, slope gradient and aspect, horizontal, vertical, and mean land surface curvatures, specific catchment area, topographic, and stream power indices; (2) two soil environmental attributes (soil gravimetric moisture and soil bulk density); and (3) six attributes of soil microbial activity (most probable number of denitrifiers, microbial biomass carbon content, denitrifier enzyme activity, nitrous oxide flux, denitrification rate, and microbial respiration rate). Linear multiple correlation, rank correlation, circular–linear correlation, circular rank correlation, and multiple regression were used as statistical analyses. In wetter soil conditions, topographically controlled and gravity-driven supply of nutritive materials to microbiota increased the denitrification rate. Spatial differentiation of the denitrification rate and amount of denitrifying enzyme in the soil was mostly effected by redistribution and accumulation of soil moisture and soil organic matter down the slope according to the relative position of a point in the landscape. The N_2O emission was effected by differentiation and gain of soil moisture and organic matter due to the local geometry of a slope. The microbial biomass, number of denitrifiers, and microbial respiration depended on both the local geometry of a slope and relative position of a point in the landscape. In drier soil conditions, although denitrification persisted, it was reduced and did not depend on the spatial distribution of soil moisture and thus land surface morphology. This may result from a reduction in soil moisture content below a critical level sufficient for transient induction of denitrification but not sufficient to preserve spatial patterns of the denitrification according to relief. Digital terrain models can be used to predict the spatial distribution of the microbial biomass and amount of denitrifying enzyme in the soil. The study demonstrated a feasibility of applying digital terrain modelling to investigate relations of other groups of soil microbiota with topography and the system 'topography–soil microbiota' as a whole.

Keywords: Digital terrain models; Topography; Denitrification; Spatial variation; Microbial activity; N_2O flux

* Corresponding author. 1026-110 Adamar Road, Winnipeg, MB, Canada R3T 3M3. Tel.: +1-204-261-4453.
E-mail address: iflorinsky@yahoo.ca (I.V. Florinsky).

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1. Introduction

Soil moisture, soil temperature, and soil organic matter are acknowledged to be among the most important soil physiochemical properties influencing population dynamics, activity, and ecology of the soil microbiota (Stotzky, 1997). It is common knowledge that topography significantly effects the spatial distribution of soil moisture, temperature, and organic matter (Beven and Kirkby, 1979; Romanova et al., 1983; Burt and Butcher, 1985; Moore et al., 1993; Florinsky and Kuryakova, 2000; Florinsky et al., 2002). In this connection, soil studies often include digital terrain modelling (Moore et al., 1991; Florinsky, 1998; Shary et al., 2002). Thus, it would be logical to suggest that (1) topographic characteristics may affect soil microbial processes, and (2) quantitative topographic data, such as digital terrain models (DTMs), may be used to analyse, model, and predict the spatial distribution of soil microbial properties. However, no mention has been made of these ideas in monographs (e.g., Alexander, 1977; Tate, 1995; Van Elsas et al., 1997) and analytical reviews (Macura, 1974; Insam, 2001) on soil microbiology, although topography is the generally recognised state factor of soil development (Huggett, 1975; Gerrard, 1981).

It would be incorrect to state that relations between topography and soil microbiota have not been studied. These works have been initiated by E.N. Mishustin in the end of 1940s. At a regional scale, some works have been carried out across mesoslopes. In high mountains of the Caucasus and Central Asia, soil microbial diversity as well as population size of spore-forming, nitrifying, and aerobic ammonification bacteria increased downslope (Mishustin and Mirzoeva, 1950; Timofeev, 1966). In the northern grasslands of North America and subtropical forests of Southeast Asia, microbial biomass C and N contents also increased downslope (Tracy and Frank, 1998; Chen and Chiu, 2000). This topographic effect was associated with variation in plant production in different vegetation altitude zones. Dobrovolskaya and Lysak (1986) found a strong influence of the slope aspect (A) on the population of spore-forming and coryneform bacteria in mountain soils of the dry subtropics of Central Asia; the highest number of bacteria was found on northern slopes as the moisture supply was preserved there.

At a landscape scale, some studies were done across catenas. It was found that activities of soil enzymes (i.e., dehydrogenase, urease, phosphatase, arylsulfatase, β-glucosidase, chitinase) depend on slope positions (Bergstrom et al., 1998; Decker et al., 1999). Ulrich and Wirth (1999) observed that the total number of soil bacteria decreased downslope while a population density of cellulolytic bacteria ignored topography. More productive and active microbial communities were found in downslopes marked by the highest levels of soil moisture, plant biomass, N and C contents comparing with up- and midslopes (Broughton and Gross, 2000). Sveshnikova et al. (2001) found that the amount of bacteria and actinomycete mycelia in Sod Podzolic soils on southern slopes was higher than on northern ones. The number of bacteria in Grey Forest soils at up- and downslope positions was higher than at the midslope; the number of fungal mycelia increased downslope. These summer trends disappeared in autumn.

However, these regional and catenary studies included two methodological shortcomings. First, soil data were collected in a limited number of points across mesoslopes and catenas. This approach based on a single transect limits the ability to examine two spatially distributed phenomena, topography and soil. Second, topography was described qualitatively. To obtain impartial results and to develop a quantitative model of the topographic influence on soil microbial properties, one should use (a) spatially distributed soil and topographic data, and (b) a quantitative description of topography, such as DTMs.

This was done, within certain limits, in studies of soil nitrification and denitrification at a landscape scale. Garten et al. (1994) observed greater N mineralisation and urease enzyme activity in valleys comparing with up- and midslopes in Appalachians. For simulated catchments, Beaujouan et al. (2002) found that the highest denitrification rates occurred in concave areas and downslopes. These studies were performed with topographic index (TI) (Table 1) (Beven and Kirkby, 1979; Beven et al., 1995) derived from a digital elevation model (DEM). In the northern grasslands of North America, downslopes and depressions were marked by the highest rates of N_2 and N_2O emission comparing with up- and midslopes (Pennock

Table 1
Definitions and physical interpretations of some topographic variables (Florinsky, 1998)

Variable	Definition and interpretation
Local variables	Describe local geometry of the land surface.
Slope gradient (G), °	Angle between a tangent plane and a horizontal one at a given point on the land surface. A measure of the velocity of substance flows.
Slope aspect (A), °	Angle clockwise from north to a projection of an external normal vector to a horizontal plane at a given point on the land surface. A measure of the direction of substance flows.
Vertical curvature (k_v), m^{-1}	A curvature of a normal section of the land surface by a plane, including gravity acceleration vector at a given point. A measure of the relative deceleration of substance flows.
Horizontal curvature (k_h), m^{-1}	A curvature of a normal section of the land surface. This section is orthogonal to the section of vertical curvature at a given point on the land surface. A measure of the convergence of substance flows.
Mean curvature (H), m^{-1}	A half-sum of the horizontal and vertical curvatures. A representation of the flow convergence and relative deceleration with equal weights.
Nonlocal variables	Describe location of a point in a landscape.
Specific catchment area (CA), $m^2\ m^{-1}$	Ratio of an area of an exclusive figure formed on the one hand by a contour intercept with a given point on the land surface and, on the other, by flow lines coming from the upslope to the ends of this contour intercept, to length of this intercept. A measure of the contributing area.
Combined variables	Describe both local geometry of the land surface and location of a point in a landscape.
Topographic index (TI)	Natural logarithm of a ratio of the catchment area to the tangent of the slope gradient. A measure of the extent of flow accumulation.
Stream power index (SI)	Natural logarithm of a product of the specific catchment area by the tangent of the slope gradient. A measure of the extent of potential flow erosion.

et al., 1992; Van Kessel et al., 1993; Corre et al., 1996). These three works used terrain segmentation based on horizontal (k_h) and vertical (k_v) curvatures and slope gradient (G) (Table 1) derived from DEMs (Pennock et al., 1987). Using an elevation map, McMahon (2001) concluded that N_2O flux and soil nitrate levels did not exhibit patterns relating to relief in the Canadian prairies, while the highest denitrification potential and denitrifier enzyme activity was found in downslope positions.

However, unfounded choice of a single topographic attribute or a single scheme to describe the land surface is unsuited to obtain reasonably sophisticated and complete notion of the topographic control of soil microbial activity. This is because relief is characterised by at least nine attributes, each being a measure or indicator of a particular aspect of the gravity-driven overland and intrasoil lateral transport of water and dissolved substances (Table 1). Therefore, to gain a better insight into the system 'topography–soil microbiota', there is a need to examine a representative set of topographic attributes.

Our study of topographic control of soil microbiota examined denitrifiers, a genetically diverse and metabolically versatile bacterial group (Murray et al., 1989). A unique aspect of these bacteria is their response to changes in aeration status: most denitrifiers are aerobes under aerobic conditions, but they can use nitrate as an alternative terminal electron acceptor oxidising C for respiration when exposed to anaerobic conditions (Murray et al., 1990). The ability to denitrify allows organisms to survive in oxygen-depleted habitats, such as poorly drained soils (Groffman and Tiedje, 1989). Denitrification, a process of biological conversion of NO_3^- into N_2O and N_2 gases (Payne, 1981), is important for understanding N dynamics at regional and global scales (Mishustin and Shilnikova, 1971; Khalil and Rasmussen, 1992), and contribution of N_2O emission into global warming, stratospheric ozone depletion, and photochemical air pollution (Conrad, 1996; Meixner and Eugster, 1999). Denitrification is influenced by soil water content, and the distribution of denitrifying activity is anticipated to respond to hydrological differences in a landscape (Groffman and Hanson, 1997).

The objective of this study was to study the effect of topography on the activity of denitrifiers under different humidity conditions in northern grasslands at a landscape scale using digital terrain modelling with a representative set of topographic variables.

2. Material and methods

2.1. Study site

The study site is located about 260 km west of the city of Winnipeg, Manitoba, Canada (Fig. 1). The site measures 1680 × 820 m, and the maximum elevation difference is 13 m (Fig. 2). It is situated in the Newdale Plain at an elevation of about 580 m above sea level. The site is representative of a broad region of undulating-to-hummocky glacial till landscapes in Western Canada (Clayton et al., 1977).

The site is located within a continental climate zone with warm summers and prolonged, cold winters. Mean annual temperature is 2.5 °C, mean summer temperature is 16 °C, and mean winter temperature is − 11 °C. Mean annual precipitation is 460 mm including 310 mm of rainfall and 150 mm of snowfall.

The parent material consists of loamy textured glacial till deposits (Clayton et al., 1977). For the most part, soils at the site are Black Chernozems (Soil Classification Working Group, 1998). The Newdale Orthic and Cordova Calcareous series were predom-

Fig. 1. Geographical position of the study site.

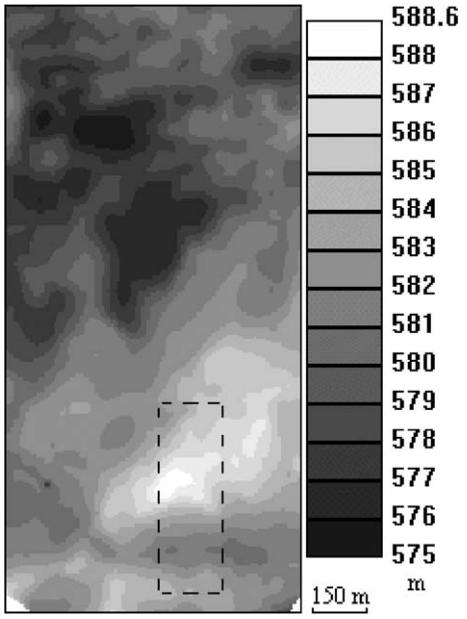


Fig. 2. The study site, elevations. Dashed lines indicate the plot.

inant on well-drained crests and midslopes. The Beresford and Varcoe Gleyed Carbonated Rego series were indicative of imperfectly drained downslopes, often in association with the Angusville Gleyed Eluviated series. The Drokan Gleysols series predominate in poorly drained depressions (Bergstrom et al., 2001b).

The site is located in the aspen parkland of the Canadian prairies, the northern extension of open grasslands in the Great Plains of North America. Native vegetation of willows (*Salix sp.*), aspen (*Poppulus tremuloides*), and sedges (*Carex sp.*) surrounds water-saturated depressions. The site is a conventional tilled field cultivated by a deep-tiller, with one pass in autumn and one or two passes in spring. Historically, crops have included wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), rape (*Brassica napus*), and flax (*Linum usitatissimum* L.) (Bergstrom et al., 2001a). The site was cropped to rape in 2000 and wheat in 2001.

2.2. Field sampling

A plot was previously selected within the study site to include a typical soil catena (Bergstrom et al., 2001b). The plot measures about 500 × 200 m with an elevation difference of about 8 m (Fig. 2). Soil samples were collected along four parallel transects about 500 m long, each about 50 m apart. There were 10 sampling points along transects spaced at intervals of approximately 50 m, for a total of 40 sampling points in the plot (Fig. 1). This design allowed us to measure and describe variations of soil properties within the catena. Each of the 40 points was previously georeferenced with horizontal accuracy of 0.03 m by a global positioning system (GPS receivers Trimble TSC1 Asset Surveyors) (Bergstrom et al., 2001b).

We sampled two sets of soil attributes. First, there were two environmental properties influencing soil microbial activity: gravimetric soil moisture (%) and soil bulk density (g cm^{-3}). Second, there were six indices of soil microbial activity: most probable number of denitrifiers, microbial biomass carbon, denitrifier enzyme activity, denitrification rate, microbial respiration rate, and N_2O flux (Table 2).

Sampling of all soil attributes took place at two times, July 2000 and July 2001, to assay the effect of topography on the activity of denitrifiers in different hydrologic situations. The 2000 sampling date occurred during a period of elevated rainfall, the 2001 sampling occurred following a period of limited rainfall. Monthly precipitation of 133 and 26 mm was observed in July 2000 and July 2001, correspondingly, at the nearest weather station in the city of Brandon located 40 km southward of the site.

Soil samples were collected using aluminium soil cores 5 cm in diameter and 5 cm in height. Depth of sampling was approximately 0.1 m because each core pressed into the ground passed surface litter and

Table 2
Interpretations of soil microbial variables under study

Variable	Interpretation
Most probable number of denitrifiers, $\#_{organisms}\ g_{soil}^{-1}$	A measure of the number of denitrifiers in the soil.
Microbial biomass carbon, $\mu g_C\ g_{soil}^{-1}$	A measure of the microbial biomass expressed as carbon.
Denitrifier enzyme activity, $\mu g_N\ g_{soil}^{-1}\ h^{-1}$	A measure of the amount of denitrifying enzymes in the soil.
Denitrification rate, $\mu g_N\ g_{soil}^{-1}\ h^{-1}$	A measure of the total gas N production from the soil.
Microbial respiration rate, $\mu g_{CO_2}\ g_{soil}^{-1}\ h^{-1}$	A measure of the rate of the total microbial respiration in the soil.
N_2O flux, $ng_{N_2O}\ m^{-2}\ s^{-1}$	A measure of the rate of N_2O emission from the soil.

discontinuities (about 2 cm). To allow direct comparison, analyses were conducted on a single sub-sample taken from each soil core within 1 h of collection. Measurements were made sequentially (microbial respiration, denitrification, then denitrifier enzyme activity) within 48 h of sampling.

To minimise an impact of temporal variability of denitrification and storage of samples, collection of soil core and N_2O flux measurements occurred simultaneously. N_2O flux was estimated using vented static chambers (Hutchinson and Mosier, 1981). Chambers were inserted within 1 m of each sample point. After 1 h of accumulation, a 15-ml gas sample was taken of each chamber by a syringe and injected into 10-ml Vacutainers™ and returned to the laboratory for analysis (Burton et al., 2000).

2.3. Laboratory methods

Soil moisture was determined by drying 10-g soil subsamples at 105 °C for 24 h (Topp, 1993). Bulk density was calculated from the moist weight of soil, water content, and volume of the core (Culley, 1993).

Microbial biomass C was determined using fumigation-direct extraction (Voroney et al., 1993). Two 15-g samples were weighed into square French bottles. One sample was extracted immediately using 30 ml 0.5 M K_2SO_4. The second sample was fumigated for 24 h under chloroform atmosphere, and then extracted. Filtrate was analysed for C using Technicon Auto-analyser (Industrial Method #455-76W/A).

The most probable number of denitrifiers was determined with a modified method of Tiedje (1994). Aliquots (0.5 ml) of serial dilutions from 10^{-3} to 10^{-6} were added to 4.5 ml of sterile nutrient broth in 10-ml Vacutainers™. They were incubated for approximately 7 days at 25 °C. Denitrifier presence was determined by measuring N_2O accumulation in the headspace using a Varian 3800 gas chromatograph.

To measure denitrifying enzyme activity, we applied a modified method of Beauchamp and Bergstrom (1993). Soil (5 g) was incubated at ambient temperature with 4 ml of buffer solution under a helium/acetylene atmosphere in 20-ml headspace vials. The buffer solution consisted of 10 mM glucose, 10 mM KNO_3, and 50 mM K_2HPO_4. N_2O in the headspace was measured at 30-min intervals using a Varian 3800 gas chromatograph.

To measure denitrification rate, we used a modified method of Beauchamp and Bergstrom (1993). Soil (5 g) was incubated in ambient temperature in 20-ml headspace vials containing atmospheric air and 10% acetylene. After 24 h, headspace was analysed for N_2O concentration using a Varian 3800 gas chromatograph. The acetylene blocks the conversion of N_2O to N_2 (the last step of denitrification), so in this case, the N_2O production is a reasonable equivalent to the evolution of N_2O and N_2 without the addition of acetylene. Therefore, the rate of N_2O accumulation can be used as a proxy for total denitrification ($N_2O + N_2$). This is not analogous to the measurements of the N_2O flux in situ (see above) when the conversion of N_2O to N_2 can proceed.

To measure microbial respiration rate, we used a modified method of Zibilske (1994). Soil (5 g) was incubated at ambient temperature in 20-ml headspace vials for 2 h, after which time the headspace was sampled, and CO_2 concentration was determined by a Varian 3800 gas chromatograph.

The N_2O flux samples were analysed using a Varian 3800 gas chromatograph (Burton et al., 2000).

2.4. Digital terrain modelling

An irregular DEM of the study site based on 7193 points was previously constructed (Bergstrom et al., 2001b) with a GPS technique by Cansel (Winnipeg, Canada). Single-frequency Trimble 4600LS Surveyors were mounted on all-terrain vehicles; data were collected cinematically (Parkinson and Spilker, 1996). Vertical and horizontal accuracy of the DEM was 0.05 and 0.03 m, respectively.

The irregular DEM was converted into a regular one (Fig. 2) by the Delaunay triangulation and piecewise smooth interpolation (Watson, 1992). The grid interval of the regular DEM was 20 m, corresponding to typical sizes of topographic elements within the site. Digital models of five local topographic attributes—G, A, k_h, k_v, and mean (H) curvatures (Table 1)—were calculated by the method of Evans (1980). The method of Martz and De Jong (1988) was applied to calculate a digital model of a non-local topographic variable—specific catchment area (CA), and digital models of two combined topographic attributes: TI and stream power index (SI) (Table 1). The grid interval of all derived DTMs was 20 m (Fig. 3). Then

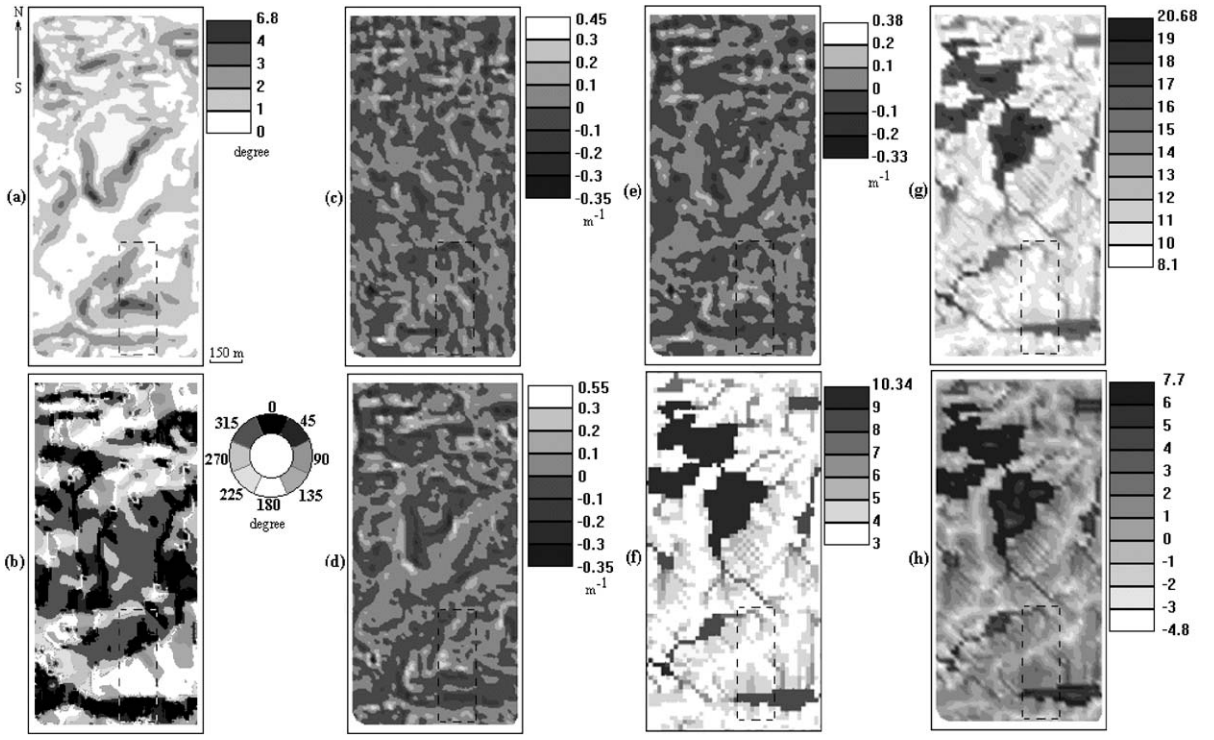


Fig. 3. The study site, topographic variables: (a) gradient, (b) aspect, (c) horizontal curvature, (d) vertical curvature, (e) mean curvature, (f) natural logarithm of specific catchment area, (g) topographic index, (h) stream power index. Dashed lines indicate the plot.

we used the Delaunay triangulation and a piecewise smooth interpolation of these DTMs to determine values of elevation (z), G, A, k_h, k_v, H, CA, TI, and SI at each of the sampling points.

Digital terrain modelling and deriving maps of topographic attributes (Fig. 3) were performed by LandLord software (Florinsky et al., 1995). Mathematical expressions for derivation of the topographic variables can be found elsewhere (Florinsky, 1998).

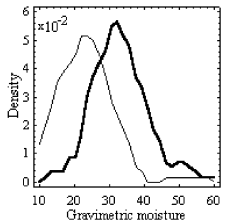
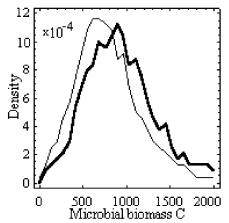
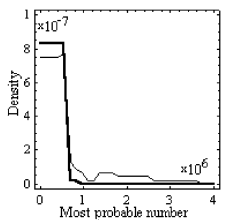
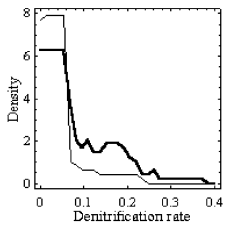
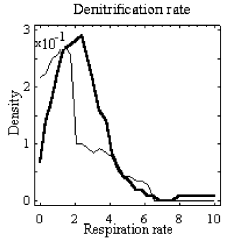
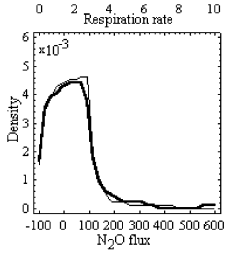
2.5. Statistical analyses

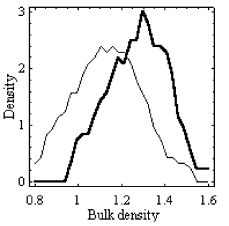
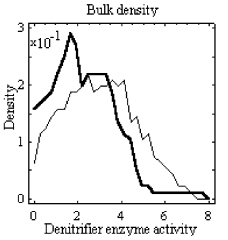
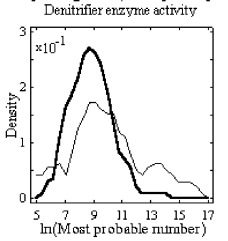
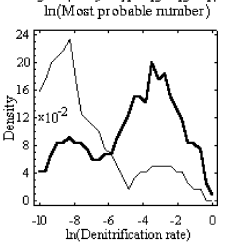
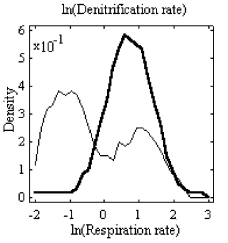
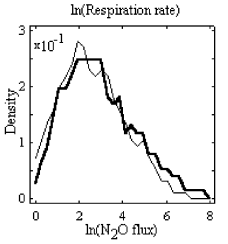
2.5.1. Distributions

To assess distinctions in statistical distributions of the soil samples due to different hydrologic situations in 2000 and 2001, we carried out three procedures. First, we plotted density traces for each soil variable using 40-point samples (Table 3). The density traces were constructed using an unweighted boxcar method with an interval of 30% of an x-axis width. Second, mean ($\bar{x}$), standard deviation (s), and standard error of the mean ($s_{\bar{x}}$) were determined for each soil variable (Table 3). Third, to estimate whether two distributions of each soil property measured in the wetter and drier soil conditions are different, we applied the Kolmogorov–Smirnov two-sample test (Daniel, 1978) to each soil property (Table 3). According to the Kolmogorov–Smirnov test, if the test statistic $D > D_{0.05}$, one can assume that there is a statistically significant difference between the two distributions at the 95% confidence level. $D_{0.05} = 0.300$ for $n = m = 40$ (Daniel, 1978). To estimate distinctions in the spatial distribution of the soil properties within the plot due to different hydrologic conditions, the coefficient of variation (CV) was determined (Table 3) for each soil variable.

To estimate a topographic representativeness of the plot relative to the entire area of the site (Figs. 2 and 3), we plotted density traces for each topographic attribute within the plot and site using 40- and 3193-point samples, correspondingly (Table 4). $\bar{x}$, s, and D were also calculated for each topographic variable,

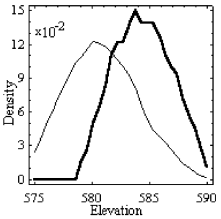
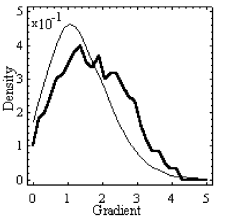
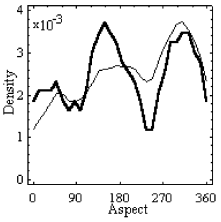
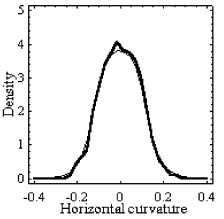
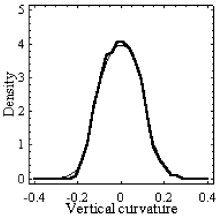
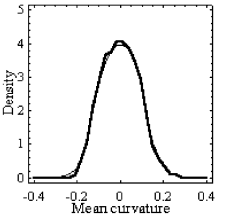
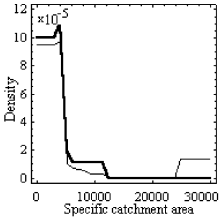
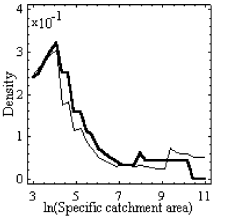
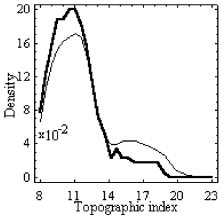
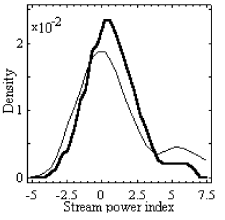
Table 3
Density traces and statistics for soil attributes in the wetter and drier conditions

Density trace		2000	2001
	**Gravimetric moisture,** %		
	$\bar{x}$	33	23
	s	7	8
	$s_{\bar{x}}$	1	1
	CV	21	33
	W	0.930	0.926
	D	0.650	
	**Microbial biomass C,** $\mu g_C g^{-1}_{soil}$		
	$\bar{x}$, $\times 10^2$	9.27	8.04
	s, $\times 10^2$	3.65	3.62
	$s_{\bar{x}}$, $\times 10^2$	0.58	0.57
	CV	39	45
	W	0.954	0.916
	D	0.225	
	**Most probable number,** $\#_{organisms} g^{-1}_{soil}$		
	$\bar{x}$, $\times 10^4$	1.5	23.9
	s, $\times 10^4$	4.6	63.2
	$s_{\bar{x}}$, $\times 10^4$	0.7	1.0
	CV	299	265
	W	0.271	0.440
	D	0.325	
	**Denitrification rate,** $\mu g_N g^{-1}_{soil} h^{-1}$		
	$\bar{x}$	0.06	0.01
	s	0.08	0.04
	$s_{\bar{x}}$	0.01	0.01
	CV	136	276
	W	0.746	0.418
	D	0.550	
	**Respiration rate** $\mu g_{CO_2} g^{-1}_{soil} h^{-1}$		
	$\bar{x}$	2.39	1.40
	s	1.50	1.54
	$s_{\bar{x}}$	0.24	0.24
	CV	63	110
	W	0.788	0.758
	D	0.575	
	**N_2O flux,** $ng_{N_2O} m^{-2} s^{-1}$		
	$\bar{x}$	41.9	31.5
	s	113.4	58.6
	$s_{\bar{x}}$	17.9	9.3
	CV	270	186
	W	0.493	0.530
	D	0.200	

Density trace		2000	2001
	**Bulk density,** gcm^{-3}		
	$\bar{x}$	1.27	1.13
	s	0.12	0.14
	$s_{\bar{x}}$	0.02	0.02
	CV	9	13
	W	0.945	0.965
	D	0.475	
	**Denitrifier enzyme activity,** $\mu g_N g^{-1}_{soil} h^{-1}$		
	$\bar{x}$	2.25	2.92
	s	1.36	1.56
	$s_{\bar{x}}$	0.22	0.25
	CV	60	54
	W	0.898	0.948
	D	0.300	
	**1n(Most probable number)**		
	$\bar{x}$	8.76	9.47
	s	1.04	2.67
	$s_{\bar{x}}$	0.16	0.42
	CV	12	28
	W	0.926	0.925
	**1n(Denitrification rate)**		
	$\bar{x}$	-4.54	-7.41
	s	2.49	2.42
	$s_{\bar{x}}$	0.39	0.38
	CV	55	33
	W	0.895	0.811
	**1n(Respiration rate)**		
	$\bar{x}$	0.72	-0.28
	s	0.58	1.13
	$s_{\bar{x}}$	0.09	0.18
	CV	81	404
	W	0.955	0.872
	**1n(N_2O flux)**		
	$\bar{x}$	2.80	2.46
	s	1.49	1.38
	$s_{\bar{x}}$	0.24	0.22
	CV	53	56
	W	0.956	0.968

Heavy and thin lines describe samples collected in 2000 and 2001, correspondingly.

Table 4
Density traces and statistics of topographic variables within the plot and site

Density trace		Plot	Site	Density trace		Plot	Site
	**Elevation**, m				**Gradient**, °		
	$\bar{x}$	584	581		$\bar{x}$	1.7	1.3
	s	2	3		s	0.9	0.8
	W	0.945			W	0.944	
	D	0.551			D	0.258	
	**Aspect**, °				**Horizontal curvature**, m^{-1}		
	$\bar{\phi}_1$	336	333		$\bar{x}$	0.00	0.00
	s_1	31	36		s	0.06	0.07
	U^2	0.061			W	0.980	
					D	0.103	
	**Vertical curvature**, m^{-1}				**Mean curvature**, m^{-1}		
	$\bar{x}$	0.00	0.00		$\bar{x}$	0.00	0.00
	s	0.08	0.08		s	0.05	0.06
	W	0.963			W	0.967	
	D	0.073			D	0.073	
	**Specific catchment area**, m^2m^{-1}				**ln(Specific catchment area)**		
	$\bar{x}, \times 10^2$	9.2	39.2		$\bar{x}$	4.66	4.94
	$s, \times 10^2$	23.8	93.8		s	1.79	2.45
	W	0.402			W	0.789	
	D	0.334					
	**Topographic index**				**Stream power index**		
	$\bar{x}$	11.1	11.9		$\bar{x}$	0.7	1.0
	s	1.9	2.6		s	1.6	2.5
	W	0.782			W	0.912	
	D	0.172			D	0.179	

Heavy and thin lines describe topographic variables within the plot and site, correspondingly.

except for A, within the plot and site (Table 4). $D_{0.05} = 0.216$ for $n = 40$, $m = 3193$ (Daniel, 1978).

A is a circular variable, so linear statistics is not appropriate to describe its distribution. It is necessary to apply methods of circular statistics based on concepts of the mean angle and angular deviation, equivalents of $\bar{x}$ and s in linear statistics, correspondingly (Batschelet, 1981, pp. 10 and 34). Because of the relief (Figs. 2 and 3b), A samples are bimodal: there were northwestern and southeastern extrema on the density traces of A for both the plot and site (Table 4). Bimodal mean angle ($\bar{\phi}_1$) and angular deviation (s_1) (Batschelet, 1981, pp. 25 and 35) were determined (Table 4). To estimate whether distributions of A in the plot and site are different, we applied the Watson two-sample test (Batschelet, 1981, p. 114) to this topo-

graphic attribute (Table 4). According to the test, if the statistic $U^2 > U^2_{0.05}$, one can suppose that there is a statistically significant difference between two distributions at the 95% confidence level. For $n = 40$ and $m = 3193$, $U^2_{0.05} = 0.187$ (Batschelet, 1981, p. 348).

A test for normality of the samples was carried out. For sample sizes of $3 \le n \le 50$, the Shapiro–Wilk test is recommended (Shapiro and Wilk, 1965). Thus, the Shapiro–Wilk statistics (W) were determined for each soil variable (Table 3) and topographic attribute within the plot, except for A (Table 4). A is a circular variable, so a linear test for normality is of no practical importance. According to the Shapiro–Wilk test, if a sample is marked by $W > W_{0.01}$, one can assume that the sample comes from a normal distribution with 99% confidence. For $n = 40$, $W_{0.01} = 0.919$ (Shapiro and Wilk, 1965).

Based on the Shapiro–Wilk statistics (Tables 3 and 4), hypotheses about normality may not be rejected for the sample distributions of soil moisture in 2000 and 2001, bulk density in 2000 and 2001, microbial biomass C in 2000, denitrifier enzyme activity in 2001, z, G, k_h, k_v, and H. The samples of microbial biomass C in 2001, denitrifier enzyme activity in 2000, and SI were close to normal distributions, and their discrepancies may be ignored (Tables 3 and 4). Hypotheses about normality may be rejected for the samples of CA, TI, most probable number, denitrification rate, microbial respiration rate, and N_2O flux for both years (Tables 3 and 4). These distributions were approximately lognormal that is usual for many soil properties (Webster and Oliver, 1990). For the samples of those soil properties and CA, we did lognormal transforms, plotted density traces, and calculated $\bar{x}$, s, $s_{\bar{x}}$, and W (Table 3). D statistics were not estimated since they are not affected by scale changes (Daniel, 1978).

2.5.2. Correlations

Correct linear correlation analysis implicates processing of normally distributed samples (Webster and Oliver, 1990). Therefore, we can use linear correlation analysis to measure the strength of associations between soil moisture, bulk density, microbial biomass C, denitrifier enzyme activity, z, G, k_h, k_v, and H. There are two options to measure the strength of associations between non-normally distributed variables. First, scales of measurements of non-normal samples may be transformed to achieve normality (Webster and Oliver, 1990), and then linear correlation analysis may be applied. Second, rank correlation analysis may be applied to non-normally distributed samples (Daniel, 1978). We used both of these options. ln(CA) was used in subsequent correlation and regression analyses. Rank correlation analysis was chosen to measure the strength of associations for most probable number, denitrification rate, microbial respiration rate, and N_2O flux versus other normally distributed soil and topographic variables. We did not transform TI, a logarithmic variable by itself (Table 1), as it is just the logarithmic nature allowing one to use TI successfully in soil and hydrological predictions. TI was used in subsequent correlation and regression analyses in its original form.

To measure the strength of association between soil environmental and microbial properties, we estimated suitable pairwise correlation coefficients, that is, either the Pearson linear correlation coefficients (r) or the Spearman rank correlation (r_s) (Table 5) using 40-point samples. To estimate strength of associations between soil and topographic attributes, except for A, we also determined appropriate correlation coefficients: either r or r_s (Table 6).

To analyse relations between A and soil properties, linear statistics would not do as A is a circular variable. To measure strength of associations between

Table 5
Pairwise coefficients of the Pearson linear correlation (r) and the Spearman rank correlation (r_s) between indices of soil microbial activity and soil environmental properties in wetter and drier conditions

Index of soil microbial activity		Soil environmental property			
		Gravimetric moisture		Bulk density	
		2000	2001	2000	2001
Most probable number	r_s	0.38	–	−0.41	–
Microbial biomass C	r	0.71	0.61	−0.64	–
Denitrifier enzyme activity	r	0.66	–	−0.31	–
Denitrification rate	r_s	0.36	–	–	–
Microbial respiration rate	r_s	0.33	–	–	–
N_2O flux	r_s	0.47	–	–	–

$n = 40$; $P \le 0.05$ for statistically significant correlations; dashes are statistically non-significant correlations.

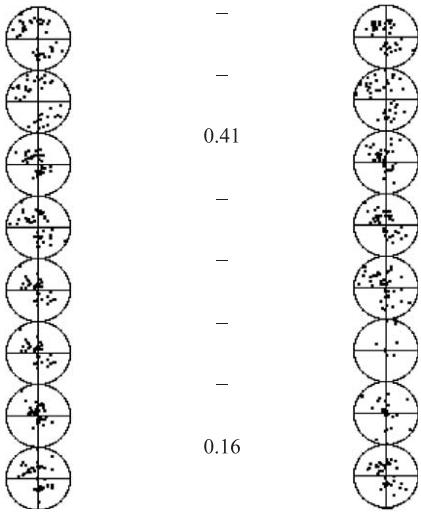
Table 6
Pairwise coefficients of the Pearson linear correlation (r) and the Spearman rank correlation (r_s) between soil properties and topographic variables in the wetter and drier conditions

Soil property		Topographic attribute															
		z		G		k_h		k_v		H		ln(CA)		TI		SI	
		2000	2001	2000	2001	2000	2001	2000	2001	2000	2001	2000	2001	2000	2001	2000	2001
Gravimetric moisture	r	−0.52	−0.47	−0.33	–	–	–	−0.62	−0.42	−0.49	−0.34	0.63	0.54	0.72	0.50	0.64	0.51
Bulk density	r	0.55	–	0.32	–	–	–	0.67	–	0.57	–	−0.64	–	−0.59	–	−0.51	–
Most probable number	r_s	–	–	−0.45	−0.38	–	–	–	–	–	–	–	–	0.46	0.36	–	–
Microbial biomass C	r	−0.51	–	−0.38	–	–	–	−0.34	–	–	–	0.54	0.36	0.58	0.38	0.48	0.46
Denitrifier enzyme activity	r	–	–	–	–	–	–	–	–	–	–	–	−0.32	0.50	–	0.40	–
Denitrification rate	r_s	–	–	–	–	–	–	–	–	–	–	0.31	–	0.40	–	0.42	–
Microbial respiration rate	r_s	–	–	−0.42	–	–	–	–	–	–	–	–	–	0.44	–	–	–
N_2O flux	r_s	–	–	–	–	–	–	−0.34	–	–	–	–	–	–	–	–	–

$n = 40$; $P \leq 0.05$ for statistically significant correlations; dashes are statistically non-significant correlations.

Table 7
Circular scatter diagrams and pairwise coefficients of the Batschelet circular–linear correlation (r_{cl}) and the Mardia circular rank correlation (D_n) between soil properties and A in wetter and drier conditions

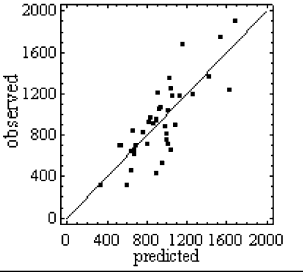
Soil property	r_{cl}/D_n	2000	2001
Gravimetric moisture	r_{cl}	–	–
Bulk density	r_{cl}	–	–
Most probable number	D_n	0.54	0.41
Microbial biomass C	r_{cl}	–	–
Denitrifier enzyme activity	r_{cl}	–	–
Denitrification rate	D_n	–	–
Microbial respiration rate	D_n	0.21	–
N_2O flux	D_n	0.20	0.16

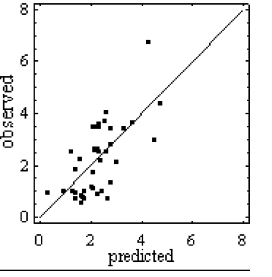


$n = 40$; $P \leq 0.05$ for statistically significant correlations; dashes are statistically non-significant correlations.

Table 8
Parameters and statistics of regression equations for some soil microbial properties in wetter soil conditions

	Dependent variable	Microbial biomass C				Denitrifier enzyme activity			
		Estimate	Standard error	95% confidence interval		Estimate	Standard error	95% confidence interval	
				Lower	Upper			Lower	Upper
	Constant	317×10^2	138×10^2	36×10^2	598×10^2	2.52	0.41	1.68	3.35
Topographic predictors	z	−46.9	23.4	−94.4	0.6				
	G	−499	160	−823	−175	−0.56	0.21	−0.98	−0.13
	k_h	-657×10^4	232×10^4	-1131×10^4	-183×10^4	-293×10^2	104×10^2	-503×10^2	-82×10^2
	k_v	-657×10^4	232×10^4	-1131×10^4	-183×10^4	-293×10^2	104×10^2	-503×10^2	-82×10^2
	H	131×10^5	46.5×10^5	36.7×10^5	226×10^5	586×10^2	207×10^2	164×10^2	1007×10^2
	TI	−258	117	−496	−19				
	SI	394	117	155	632	0.66	0.15	0.35	0.96
R^2 (Adjusted R^2)		0.64 (0.57)				0.45 (0.37)			
F-ratio		8.30				5.53			
P-value		0.00				0.00			
Standard error of the estimate		240				1.08			
Mean absolute error		168				0.82			
Durbin-Watson statistic		2.21				1.90			





normally distributed soil attributes and A, pairwise coefficients of the Batschelet circular–linear correlation (r_{cl}) were determined (Batschelet, 1981, p. 193) (Table 7). To estimate strength of associations between other soil variables and A, we determined pairwise coefficients of the Mardia circular rank correlation (D_n) (Batschelet, 1981, p. 195) (Table 7). It is imperative to use r_{cl} and D_n together with related circular scatter diagrams to correctly interpret associations. This is due to the indistinguishability between positive and negative correlation in the circular–linear case: r_{cl} and D_n indicate association occurrence, other than its directionality. Thus, circular scatter diagrams were plotted (Table 7). Forty-point samples were used.

2.5.3. Regression

To predict soil microbial properties within the entire area of the site using data from the plot, cokriging (Goovaerts, 1999) was abandoned in favour of regression. A reason was that we operated with 40-point soil and topographic samples (Section 2.2). However, a minimum sample of 100 points is required to compute valuable variograms (Webster and Oliver, 1992; Lark, 2000).

One of the assumptions of multiple regression is a normal distribution of a dependent variable for any fixed combination of predictors (Kleinbaum et al., 1988). So, as dependent variables, we analysed microbial biomass C, denitrifier enzyme activity, as well as logarithmical transforms of most probable number, N_2O flux, denitrification, and microbial respiration rates. We did not perform regressions for soil moisture and bulk density since this was outside the scope of the study and has been done before (Florinsky and Kuryakova, 2000; Florinsky et al., 2002).

To find models fitting the data on soil microbial properties measured within the plot, the 'best' combinations of predictors (z, G, k_h, k_v, H, ln(CA), TI, and SI) were chosen by stepwise multiple regression (Seber, 1977) using 40-point samples. As a circular variable, A was not included into the regression analysis. Since some of the independent variables are functions of other predictors (e.g., $TI = \ln(CA/\tan G)$, $SI = \ln(CA \times \tan G)$; Table 1), the regression equations obtained (Table 8) are nonlinear in the predictors. However, the equations can be considered as linear multiple regression models, since in linear regression analysis, an equation must be linear in the parameters, while predictors can include cross products, transformations, and powers of the original variables (Seber, 1977).

Based on the Kolmogorov–Smirnov and Watson statistics (Table 4), hypotheses about statistically significant differences in distributions of topographic variables within the plot and site may be rejected for k_h, k_v, H, TI, SI, and A, while may not be rejected for z, G, and CA. Thus, we can presume that the plot is

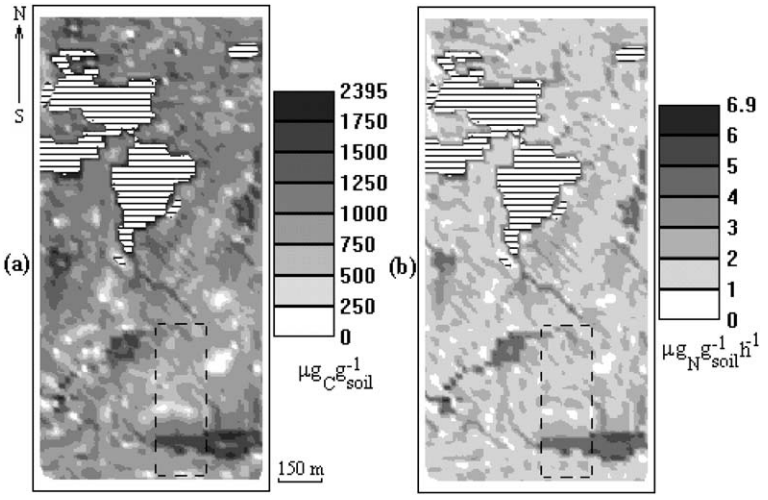


Fig. 4. Prediction of the spatial distribution of (a) microbial biomass C and (b) denitrifier enzyme activity (both for the wetter sampling data, July 2000). Dashed lines indicate the plot. Hatching indicates depressions omitted from the prediction.

generally representative of the site for the most topographic attributes, except for z, G, and CA. Indeed, the plot does not include values of $z < 581$ m, $G > 3.5°$, and CA>8000 $m^2\ m^{-1}$. They are typical for bottoms and some slopes of depressions in the northern part of the site (Figs. 2 and 3a and f). Since CA was not inserted into the regression equations as a predictor (Table 8), z and G give us concern about a possibility to use the equations for prediction of the soil properties within the entire area of the site. It is risky to apply a regression equation to estimate a response if current values of a predictor are beyond its range used in the regression analysis. This is because a relation considered as linear may be in fact a part of a curvilinear one detected after expansion of the predictor range (Seber, 1977; Webster and Oliver, 1990).

Thus, we derived predictive maps of denitrifier enzyme activity and microbial biomass C of the entire area of the site, except for 'critical' depressions (Fig. 4). The maps were obtained using digital models of topographic attributes (Fig. 3) inserted into the corresponding regression equations as predictors (Table 8). Predictive values were initially calculated for 3193 points of the DTM square-spaced grid of the site. Areas of 'critical' depressions were then omitted.

Linear statistical analysis was carried out by Statgraphics Plus 3.0. Circular statistical procedures were developed with Microsoft Excel 97. Maps of predicted soil properties (Fig. 4) were produced by LandLord software (Florinsky et al., 1995).

3. Results and analysis

Different rainfall conditions in July 2000 and July 2001 resulted in different levels of soil water in the landscape: the means of soil moisture content in the plot differed by 10% in July 2000 and July 2001 (Table 3). In the wetter and drier soil conditions, there were strong differences in relationships between soil environmental and microbial properties (Table 5) as well as between soil properties and topographic attributes (Tables 6 and 7).

3.1. Wetter soil conditions

The correlation analysis demonstrated significant dependence of all soil microbial variables, notably microbial biomass C and denitrification enzyme activity, on soil moisture in the wetter soil conditions of July 2000. Microbial biomass C, most probable number, and denitrification enzyme activity also depended on the bulk density (Table 5).

These results are consistent with previous observations of the influence of soil moisture and bulk density on denitrification (Myrold and Tiedje, 1985; Groffman and Tiedje, 1989; Webster and Hopkins, 1996). Indeed, gravimetric moisture content and bulk density are indicative of aeration status in the soil. These parameters also influence the movement of water through soil and thereby the distribution of N and organic C, proximal regulators of denitrification. Either increased water content (percent water-filled pores) and/or an increase in bulk density (decreased total porosity) will result in lower air-filled porosity and therefore a greater number of anaerobic sites in the soil, increasing the suitability of the environment to support denitrification. Bulk density influences the ratio of pore sizes and thus for a given water content, soils of lower bulk density will have a greater number of air-filled pores and therefore greater aerobic microbial activity. These trends were reflected in the positive and negative correlations of general soil microbial activity with soil moisture and bulk density, correspondingly, in the wetter soil conditions (Table 5).

There was a relatively strong influence of topography on the spatial distribution of soil moisture for the wetter soil conditions of July 2000 (Table 6). This was expected and supported by interpretations of topographic variables (Table 1) and previous results (Burt and Butcher, 1985; Florinsky and Kuryakova, 2000; Florinsky et al., 2002). Soil moisture was highest where values of k_v were negative (concave profiles), values of G were low (flat areas), while values of CA were high. Detailed physical interpretation of these usual trends can be found elsewhere (Florinsky et al., 2002).

Relatively strong correlations between bulk density and topographic attributes (Table 6) resulted from its dependence on soil moisture, soil texture, and soil organic matter usually distributed according to land surface morphology. Consequently, upslopes are marked by higher values of bulk density than downslopes and depressions within the plot.

A topographic influence on the spatial distribution of the soil organic C content, a proximal regulator of

denitrification (Myrold and Tiedje, 1985), has been previously demonstrated at this site in the wetter soil conditions using the same sampling grid (Bergstrom et al., 2001b). This was expected and stems from the spatial differentiation of organic matter and moistening according to the land surface morphology (Moore et al., 1993; Arrouays et al., 1998; Florinsky et al., 2002).

All soil microbial properties, in one way or another, depended on topographic variables in the wetter soil conditions (Tables 6 and 7). This was expected as essential factors for denitrification, such as soil moisture, soil organic C, and bulk density, depended on topographic attributes within the plot (see above). Denitrifier enzyme activity, microbial biomass C, and denitrification rate depended basically on nonlocal and combined topographic variables, CA, TI, and SI (Table 6). Microbial biomass C also depended on some local topographic variables, z, G, and k_v; N_2O flux was effected by k_v, while the number of denitrifiers and microbial respiration rate was influenced by G and TI (Table 6). Correlations of soil microbial variables with CA, TI, and SI are positive, while they are negative with z, G, and k_v.

Thus, under wetter soil conditions, spatial variability of the denitrification rate and the amount of denitrifying enzyme was mostly effected by redistribution and accumulation of soil moisture and soil organic matter due to their gain along a slope from top to bottom because of their additional amounts contributed from upslope, that is, according to relative position of a point in the landscape. However, the N_2O emission was affected by the distribution of other attributes of the environment related to the local geometry of the slope. Both topographic factors of spatial redistribution and accumulation of soil moisture and organic matter influenced the microbial biomass, number of denitrifiers, and microbial respiration. Thus, topographically controlled and gravity-driven aspects of the system increased the denitrification rate. These observations are consistent with previous observations that 'hot spots' of denitrification are associated with downslope positions (Pennock et al., 1992; Van Kessel et al., 1993; Corre et al., 1996).

There is an effect of A on the number of denitrifiers, microbial respiration rate, and N_2O flux (Table 7). Based on the circular scatter diagrams of these soil microbial properties, we may suppose that these correlations are connected with anisotropy of scatters associated with northwest slopes of the plot. Although there were no statistically significant correlations of denitrifier enzyme activity, microbial biomass C, and denitrification rate with A, their scatter diagrams are marked by the same anisotropy (Table 7). It is unlikely that this dependence of soil microbial properties on A is associated with insolation and soil temperature. This is because A did not control soil moisture (Table 7) owing to the spatial differentiation of soil moisture may not sufficiently be effected by insolation in the relatively flat landscapes within this climatic zone.

The dependence of soil microbial properties on A may indirectly reflect an influence of erosion, as A is a measure of the direction of substance flows (Table 1). Indeed, points of some circular scatter diagrams are stretched along the northwest–southeast direction (Table 7) while a northeast-striking crest with northwest and southeast slopes crosses the plot (Figs. 2 and 3b). It is clear that eroded soil material is moved by gravity from the crest in two general directions of its slopes. Occurrence of positive correlations between SI, a measure of the extent of potential flow erosion (Table 1), and microbial biomass C, denitrifier enzyme activity, and denitrification rate (Table 6) also testifies that erosion and deposition affected soil microbial properties under study.

3.2. Drier soil conditions

Based on the Kolmogorov–Smirnov statistics (Table 3), hypotheses about significant differences in statistical distributions of soil properties measured in the wetter and drier soil conditions may not be rejected for soil moisture, bulk density, most probable number, denitrification, and microbial respiration rates, while may be rejected for microbial biomass C, denitrification enzyme activity, and N_2O flux. Thus, in the drier soil conditions, there were marked decreases in soil moisture and bulk density, decreases in denitrification and microbial respiration rates, a slight increase of the number of denitrifiers, and no significant changes in microbial biomass C, denitrification enzyme activity, and N_2O flux (Table 3).

Comparisons between the two sampling events demonstrated significant changes in the spatial differentiation of the soil properties. In drier soil conditions,

the CV values showed pronounced increases in variation of soil moisture, denitrification, and microbial respiration rates, and a pronounced decrease in variation of the N_2O flux (Table 3). All correlations between indices of soil microbial activity and selected soil environmental properties became statistically insignificant in the drier soil conditions, except for the dependence of the microbial biomass on soil moisture (Table 5).

We observed a decrease of topographic control of soil moisture, microbial biomass, and number of denitrifiers in the drier July 2001, while associations of other soil properties with topographic attributes became insignificant (Tables 6 and 7). It was not surprising that we found different correlations between soil and topographic attributes in the different years for the same area: this demonstrates a phenomenon of temporal variability in topographic control of dynamic soil properties (Florinsky et al., 2002).

From the observed distribution of denitrification rate and denitrifier enzyme activity, it may be deduced that the denitrifier activity continued to persist under the drier soil conditions, but it was reduced and ceased to depend on the spatial distribution of soil moisture and thus land surface morphology. This likely reflects a transition of some critical level of soil moisture status, and the ability of denitrifiers to be effective aerobic heterotrophs under aerobic conditions. Soil moisture status was still sufficient for the activity of these organisms, but was no longer a dominant force in influencing their spatial patterns.

3.3. Regression-based prediction

Regression equations of most probable number, N_2O flux, denitrification, and microbial respiration rates in either year, as well as denitrifier enzyme activity and microbial biomass C in the drier soil conditions of July 2001 had adjusted R^2 (Seber, 1977) less than 0.35; we did not include them in Table 8. In the wetter soil conditions, regression equations explained 64% of the variability of the microbial biomass, and 45% of the variability of the amount of denitrifying enzyme (Table 8). The equations are obviously site- and DTM grid size-specific: they may be used in the same type of a landscape marked by ranges of predictors close to their ranges within the plot (Table 4), and DTMs with the grid size of 20 m.

Predictive patterns of denitrifier enzyme activity and microbial biomass C (Fig. 4) generally resemble the structure of some morphometric maps, particularly TI and SI (Fig. 3). We were unable to validate these predictive maps as unfortunately there were no data on soil properties outside the plot. Standard errors of the estimate (Table 8) may be used to assess roughly the accuracy of the prediction outside the plot.

4. Discussion and conclusions

It is possible to conclude that for topography to control of the distribution of denitrifiers and their activity, the landscape should contain some sufficient amount of soil moisture. Physically, this idea seems reasonable as the topographically controlled gravity-driven lateral transport of substances generally acts through the medium of gravimetric soil water. Different aspects of this lateral transport affect distinct manifestations of the denitrifier population and its activity. Indeed, in the wetter soil conditions, topographically controlled aspects of the system clearly increased the denitrification rate and denitrifier enzyme activity. Spatial differentiation of the denitrification rate and amount of denitrifying enzyme in the soil occurred according to relative position of a point in the landscape. The N_2O emission was influenced by the local geometry of a slope. The microbial biomass, number of denitrifiers, and microbial respiration were influenced by both topographic factors for spatial redistribution of soil moisture and organic matter. However, in the drier soil conditions, the various measures of denitrifier activity (denitrification rate and denitrifier enzyme activity) were reduced and ceased to depend on the spatial distribution of soil moisture and hence topography, reflecting a transition beyond a critical level of soil moisture status sufficient to allow transient denitrification to occur but not to allow the expression of spatial patterns of the denitrification according to relief.

In the drier soil conditions, the number of denitrifiers continued to depend on topography, while the denitrifier enzyme activity was essentially the same on both sampling dates, ceasing to be effected by relief (Tables 3, 6 and 7). This result reinforces the view that under field conditions, there is seldom a direct relationship between the number of denitrifiers and the

amount of denitrifying enzyme in the soil (Parsons et al., 1991). This is as a result of the dual aerobic/anaerobic nature of the ecology and physiology of denitrifiers. The occurrence of denitrifying bacteria in any given habitat is primarily controlled by their ability to compete as heterotrophs rather than ability to denitrify (Groffman and Tiedje, 1989). The expression of denitrifying enzyme, however, is in response to anaerobic conditions and reflects soil aeration status.

Although the spatial distribution of denitrifying bacteria is effected by topography in both years, other groups of soil microbiota are probably more sensitive to land surface morphology. This is reflected in the higher topographic effect on microbial biomass C compared with the most probable number (Table 6). Microbial biomass C measures the total microbial biomass in the soil including both denitrifiers and other organisms (Table 2). The higher topographic control of the microbial biomass but lower topographic control of the denitrifiers may imply different dependencies of diverse groups of microorganisms on relief.

The decrease in the denitrification rate without a significant change in the N_2O flux in the drier soil condition demonstrates, that in some situations, these parameters are independent even though denitrification is one of the primary processes producing N_2O. This independence reflects the role of denitrification as both a source and sink for N_2O as well as role of physical factors such as diffusion and solubility of N_2O in the soil profile at very high levels of water-filled porosity in determining the relative amount of N_2O lost to the atmosphere during denitrification. So, although total denitrification ($N_2O + N_2$) is lower in drier soils, the potential for N_2O to diffuse from the site of production, thus preventing further reduction to N_2, is greater as a result of higher air-filled porosity. This may increase the N_2O/N_2 ratio of the denitrification process and result in N_2O emissions from drier soils of similar magnitude to those from wetter soils (Webster and Hopkins, 1996). The decrease in denitrification rate without a significant change in the N_2O flux suggests that most of the N released was in the form of N_2O in the drier soil condition. This may reflect a greater rate of N_2O diffusion from the site of denitrification as a result of higher air-filled porosity under the drier condition. This is not always the case however as other workers have observed that N_2O flux is a function of soil moisture content (Van Kessel et al., 1993; Corre et al., 1996).

The lower topographic control of the spatial differentiation of the N_2O emission compared with other soil microbial variables in the wetter soil conditions as well as the disappearance of this control in the drier soil conditions (Tables 6 and 7) may reflect the high temporal and spatial variability of this attribute (Parsons et al., 1991) plus the nature of N_2O production. Since N_2O production results from both autotrophic, aerobic processes (nitrification) and heterotrophic, anaerobic processes (denitrification) and is merely an intermediate in denitrification, it is not surprising that N_2O production and flux are highly variable and do not always reflect environmental prerequisites of either of the microbial groups producing this gas.

The results reported here demonstrate no relationship between N_2O flux and landscape. This is in apparent contrast to observations in the Saskatchewan grasslands: Corre et al. (1996) found seasonally sustained associations between the highest rates of N_2O emission and downslopes. One possible explanation for the discrepancy is that the two systems simply behaved differently with respect to role of landscape. Whether due to pedogenic aspects of the site or site management, the processes responsible for N_2O production may have been different at the two sites. The two hydrologic conditions examined here are at the two extremes of the optimal water content for N_2O production of 40–80% water-filled pore spaces (Davidson, 1991). The hydrologic condition during the 2000 sampling was at the upper end of the optimal region for N_2O production (mean water-filled pore space was 81%). At water-filled pore space ratios of this magnitude, there is a shift from N_2O towards N_2 emission. Under the drier condition of 2001, the average water-filled pore space was 46%. In this situation, the primary source of N_2O is nitrification, rather than denitrification. This might explain why the N_2O production was not very different between the 2 years despite significant differences in water content. This also highlights the challenge in describing unique relationships between N_2O flux and soil environmental parameters.

Another possible explanation relates to the manner in which the landscape attributes were character-

ized. Corre et al. (1996) utilized a terrain segmentation technique while the current study utilizes a technique of terrain description with continuous functions. A comparative study of these approaches (Florinsky et al., 2002) has demonstrated that the strong spatial and temporal variability in soil–topography relationships can be observed if one analyses an association of soil properties with topography described *quantitatively* by continuous functions. However, this spatial and temporal variability decreases if one simplifies a task and analyses an association of soil properties with topography described *qualitatively* by several types of landforms segmented by some rules. This is because DTM-based terrain segmentation methods result in a generalisation of soil and topographic properties (Florinsky et al., 2002). So, relationships between slope position and N_2O emissions observed by Corre et al. (1996) may be the result of the spatial integration inherent in the analysis.

Spatial heterogeneity of some other soil microbial attributes can be predicted using DTM-based regression, as demonstrated by the predictive maps of denitrifier enzyme activity and microbial biomass C (Fig. 4). Notice that geostatistic methods are at times applied to predict spatial distribution of soil microbial properties (Webster and Boag, 1992; Kuperman et al., 1998). Taking into account advantages of regression over cokriging (Lark, 2000), DTM-based regression may be preferable to geostatistics in some situations. Specifically, this can be topical for studies operating on small soil microbial samples, as with this work, inadequate to compute valuable variograms (Webster and Oliver, 1992; Lark, 2000). However, DTM-based regression should be used with caution as different regression equations may be obtained in different seasons for dynamic soil properties (Florinsky et al., 2002).

Predictive maps of the biological properties such as denitrifier activity may be useful for spatially variable N fertiliser application since N requirements and losses depend on slope position (Fiez et al., 1995). Indeed, the highest levels of actual and potential denitrification occurred in low, imperfectly drained areas of the landscape. In wet years, crop productivity in such areas is generally lower than in other parts of a field, so uniformly applied fertiliser nitrate is not utilized efficiently, and can accumulate resulting in higher denitrification. Spatially variable fertiliser application may improve this situation.

The results obtained relate to the microbial activity in the upper soil layer wherein samples were collected and may vary with depth. This is because soil microbial communities and enzyme activity change with depth (Zvyagintsev, 1994; Polyanskaya et al., 1995; Bergstrom et al., 1998), and topographic control of soil properties (e.g., the number of bacteria and soil moisture) decreases with depth (Sveshnikova et al., 2001; Florinsky et al., 2002). Regularities may vary with time due to several factors: temporal variability in topographic control of soil properties (Florinsky et al., 2002), seasonal dynamics of soil microbiota (Golovchenko and Polyanskaya, 1996), soil microbial succession (Polyanskaya and Zvyagintsev, 1995), and spatio-temporal oscillations of soil microbial populations (Semenov et al., 1999).

The study demonstrated the feasibility of applying digital terrain modelling to investigate relations of particular groups of soil microorganisms with topography and the system 'topography–soil microbiota' as a whole. The implementation of a representative set of DTM-based topographic variables offers undeniable advantages. First, DTM-based design allows one to carry out a simple and flexible analysis of spatially distributed activity of soil microorganisms in the landscape. Second, DTM-based design enables one not only to examine relations between soil microbiota and topography, but also to investigate effects of particular aspects of overland and intrasoil flows on soil microbial activity using rigorous physico-mathematical concepts of the topographically controlled gravity-driven lateral transport of substances (Shary et al., 2002) rather than direct field observations of these processes. Third, the design including a representative set of topographic variables insures against failures in detecting topographic control of soil microbial activity. The reason is that a dependence of soil property on topography is often manifested in its dependence on some particular topographic attributes rather than on all of them. There is no way to know a priori these 'effective' topographic variables.

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