A spatial approach to soil-ecological experimentation at landscape scale§

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Abstract

The upscaling of soil-ecological processes to larger landscape units represents a special challenge to soil ecology. Results from micro- or mesocosms cannot easily be transferred to other scales because effects are often scale-dependent. In this context, field experiments which take into account the heterogeneity of the landscape may be promising. Therefore, we carried out an experiment based on a transect study in the agrolandscape of NE Germany on heterogeneous sandy soil in which the feeding activity of the soil-organism community was assessed by means of the bait-lamina test at each of the 101 transect locations. At every 4th position, prior to the measurement the soil biota were stimulated by a treatment consisting of adding easily available C and water to the soil. Our aim was to test whether this kind of spatial approach enables to separate effects induced by treatments from landscape effects. The results showed a highly variable feeding activity along the transect after 4 weeks. Despite this variability, a basic trend could be identified which was related to a landscape factor, *i.e.*, the relief. On upper-slope positions, the feeding activity tended to be less in comparison to positions down-slope. At every 4th position of the transect, the stimulating effect of the substrate and water addition could be clearly detected and quantified with spectral and cross-spectral analysis. It is concluded that effects of treatments in heterogeneous landscapes may be distinguished from site effects when the signal-to-noise ratio is high and soil and treatment effects on the variable of interest are sufficiently different from one another. In a heterogeneous landscape with gradients of site properties, a treatment based on the frequency domain and applied in regular intervals can be distinguished with spectral analysis techniques.

Key words: bait-lamina test / soil organisms / spatial variability / spectral analysis / experimental design / terrain elevation

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1 Introduction

The upscaling of soil-ecological processes to larger landscape units represents a special challenge to soil ecology for which new concepts are needed. For the upscaling of functional traits of soil organisms and their environmental controls, a thorough knowledge of causal relationships in the soil system is necessary, which often times can only be acquired by experimental approaches (*Scheu,* 2002). Results from micro- or mesocosm experiments, however, cannot easily be transferred to the field scale. Also, experiments carried out on small plots often do not sufficiently represent the variability of nature (*Nielsen* and *Wendroth,* 2003). Spatial heterogeneity of landscapes (*Wu* et al., 2000) may affect the response of a dependent variable to treatments and should therefore be considered in the experimental approach.

Landscape heterogeneities may be identified and quantified by means of spatial statistics (*Nielsen* and *Wendroth,* 2003). The spatial approach may also be helpful for designing

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§ Parts of this paper were presented at the Workshop "Upscaling – Soil organisms and soil ecological processes up to the landscape scale", February 23–24, 2006 at the University of Vechta, Germany. The workshop was organized by the Soil Ecology Working Groups of the German Soil Science Society (DBG) and the Ecological Society of Germany, Austria, and Switzerland (GfÖ), convenor Gabriele Broll and co-convenors Volkmar Wolters and Anton Hartmann experiments at the landscape or regional scale. *Nielsen* and *Wendroth* (2003, p. 206) suggested that treatments may be applied at regularly spaced positions to a spatial series and subsequently be analyzed with techniques of spectral and cross-spectral analysis. If the experimental design allows the application of a treatment and the subsequent analyses in a large number of replicates, spatial approaches should equally allow to separate landscape effects from effects induced by treatments in soil-ecological studies. This separation is crucial for the clear identification of treatment effects, since no *ceteris paribus* conditions exist in a heterogeneous landscape and confounding variables cannot be kept constant. It was therefore the objective of this study, to test this assumption by carrying out a field experiment based on a transect study in agricultural sandy soil.

Along the transect, a simple treatment to the soil-organism activity was to be applied whose effects could be distinguished from the natural variability induced by the landscape. For the treatment, we chose the stimulating effect of substrate and water addition on the activity of soil organisms, assessed by a simple screening test. According to *Scheu* (2002), the addition of substrate or resources is a suitable method for soil-ecological perturbation experiments in the field. At 101 sample points, with equal distances of 1 m between points, the feeding activity of the soil-organism community was quantified by means of the bait-lamina test (*von Törne,* 1990). It was hypothesized that the water- and substrate-limited soil biota would react to this treatment with increased feeding activity. For identifying treatment effects in the landscape context, spectral and cospectral analysis was used.

Since landscape heterogeneity may induce different responses to treatments, this experimental approach may yield further insight into landscape functions. Differential responses to treatments might be used for the characterization of functional subunits in the landscape. This additional aspect was also addressed in our study.

2 Material and methods

2.1 Site description

The study was carried out in October 2006 at a 74 ha field belonging to the Komturei Lietzen, Märkisch-Oderland, Brandenburg (NE Germany). The field is located in a ground-moraine area with an undulating relief. Soil texture ranges between sand and loam, with Luvisols as dominating soil type (*Seyfarth* et al., 1999). At the experimental site, reduced tillage by a heavy tine cultivator (18 cm tillage depth) had been carried out since 1996. The current crop was winter wheat.

2.2 Experimental design

In October 2006, a transect oriented in the direction of tillage was set up between rows of the planted winter wheat comprising 101 locations, with a distance of 1 m between observations (Fig. 1).

Figure 1: Transect in tillage direction with 101 locations, 1 m apart, between two winter wheat rows; October 2006 (Photo: Brandt).

2.3 Stimulation experiment

For stimulating the soil organisms, cellulose (Carl Roth, article number 5873.1) and glucose (“Traubenzucker”, Müller’s Mühle GmbH) were used in a two-step treatment. On October 13, at a mean soil temperature of 14.1°C, a cellulose suspension (4.25 g in 1 L water) was carefully poured onto the soil at every 4th transect point ($n = 25$). For applying the solution to the soil, a polypropylene cylinder (∅ 10.5 cm, equivalent to 85.9 cm^2, and height of 12 cm) was pushed into the soil to a depth of 1–2 cm. The cellulose was mixed with water just before applying it to the soil. After approx. 30 min, the solution had infiltrated the soil. Subsequently, the soil surface was softly stirred with a fork to a soil depth of approx. 1 cm in order to incorporate the substrate into the soil. On the 76 control positions which were not treated, the soil surface was also stirred with a fork to a soil depth of approx. 1 cm, in order to impose the same mechanical disturbance to all transect locations. The cellulose amount of 4.25 g corresponded to a concentration of approx. 5000 ppm in 850 g soil. Twelve days later, on October 25th, similarily, a glucose solution (4.25 g in 1 L water), which was prepared beforehand in the lab, was applied to the soil at the same transect locations. The infiltration lasted for approx. 35–65 min.

The concentration of 5000 ppm of C to be added to the soil was chosen because glucose additions between 3000 and 7000 ppm have been found to optimally stimulate microbial activity in soils of the studied region for microbial-biomass determinations after *Anderson* and *Domsch* (1978). The combination of cellulose and glucose was chosen for optimally stimulating the soil-organism community during the experiment (4 weeks, see below). The stimulation was considered to comprise the following phases: (1) activation of all microorganisms by addition of glucose (*Anderson* and *Domsch,* 1978), (2) activation of cellulase-forming organisms by addition of cellulose, (3) after depletion of the cellulose attack of these groups on the bait-lamina which to a large extent consisted of cellulose (see below), (4) activation of soil fauna by flourishing microorganism populations.

2.4 Assessment of feeding activity

For assessing the soil-organismic activity, bait-lamina strips (*von Törne,* 1990) with 16 holes were used. The holes which had a size between between 0.85 and 1.65 mm with a mean hole size of 1.22 mm, were filled with a bait consisting of 70% cellulose, 30% wheat bran, and active charcoal (von Törne, personal communication).

On October 13, 2006, at a gravimetric soil water content of 5.46% (Oct. 12, 2006) and a mean air temperature of 13.1°C, 101 bait-lamina strips were applied to the soil at all locations so that the upper hole was approx. 1 cm below the soil surface. At the treated locations ($n = 25$), the bait-lamina strips were applied after substrate addition. The development of feeding activity which was not interrupted by frost or cold temperatures during the mild winter was checked every day with additional 52 strips positioned near the transect.

After 4 weeks, on November 10, 2006, the experiment was terminated, and the strips were collected. They were carefully extracted and transported horizontally to the lab. On the same day, the strips were visually inspected on a light box to evaluate each bait hole for the extent of feeding activity. Bait holes which showed signs of feeding activities were recorded ("eaten" *vs.* "not eaten"). Holes which were most probably artifacts, judged by the existence of parallel structures to the hole border, were excluded from further analysis.

2.5 Acquisition of elevation data

On November 8, terrain elevation was measured using a surveyor's level (Zeiss Ni 025) and a leveling staff, at all 25 treated locations of the transect, that is every 4 m. Elevation at positions in between these points was linearly interpolated.

2.6 Statistical analysis

2.6.1 Spectral and cross-spectral analysis: theory

With spectral analysis, repetitious features in a data set may be identified, by "partitioning the total variance of a set of observations among different frequencies" (*Nielsen* and *Wendroth,* 2003). The spectrum indicates the frequencies with maximal amplitude.

With cross-spectral analysis, two different spatial series can be compared with respect to synchrony of their cyclic patterns. Coherency indicates the degree of linear dependency between two series at a certain frequency, comparable to the correlation coefficient of a linear regression between two variables (*Nielsen* and *Wendroth,* 2003).

2.6.2 Spectral and cross-spectral analysis: data preparation and application

The distribution of feeding activity along the transect was analyzed with Q–Q plots (*Timmer,* 1998). As these plots showed reasonable consistency with normal distribution, the raw data were used in further analysis. Subsequently, autocorrelation and crosscorrelation functions were calculated with STATISTICA. With the same software, spectral and cross-spectral analysis were carried out. After detrending and subtracting the mean, the spectral estimates were smoothed with a Hamming window of width 5.

2.6.3 Additional statistics

In addition, for comparison of means from stimulated and control positions from three regions along the transect, t-tests for independent samples were carried out (STATISTICA).

3 Results

The feeding activity assessed with the bait-lamina test at 101 locations along the transect is shown in Fig. 2. Feeding activity ranged between 0 and 12 eaten baits per lamina strip corresponding to 0% and 75% of potential feeding activity. The variability of feeding activity was high, yielding a coefficient of variation of 84.5%.

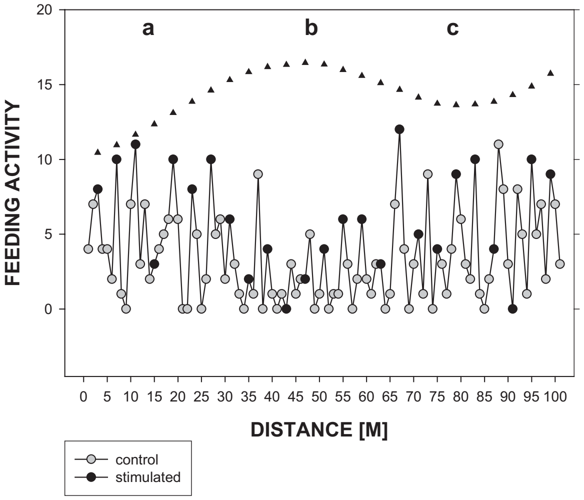

Figure 2: Feeding activity (number of eaten baits per strip, circles) and elevation (triangles) *vs.* distance at 101 positions along the transect; section a comprises position 1–37, section b position 38–65, and section c position 66–101, black symbols: stimulated positions.

Despite this variability, the feeding activity showed a spatial pattern, which was characterized by decreasing and rising trends along the transect. A polynomial fitted to the feeding-activity values along the transect revealed an u-shaped distribution (data not shown). Based on visual inspection of the pattern, three transect sections with local means (only controls considered) of 3.4 (a) corresponding to 21%, 1.3 (b) corresponding to 8%, and 3.9 (c) eaten baits corresponding to 24% of potential feeding activity could be distinguished along the transect (Fig. 2). Within these sections, the feeding activity fluctuated randomly (only controls considered); along the whole transect, autocorrelation of data was found when only control positions were used, with the missing fourth data point being interpolated.

Elevation as surrogate variable for landscape position is shown in Fig. 2 together with the feeding-activity distribution along the transect. Low values of feeding activity between position 38 and 65 corresponding to section b are related to positions on the hill top. Feeding activity after position 90, seem to follow a different pattern. Despite this deviation, a clear spatial relationship between feeding activity (control positions, with the 4th data point being interpolated) and elevation could be ascertained by a significant negative cross-correlation between both variables (data not shown).

In contrast to the larger scale pattern of feeding activity, probably induced by landscape position, the treatment, *i.e.*, the stimulation of soil biota, was applied in a small-scale regular pattern with each 4th position being treated. Therefore, in order to identify possible effects of the stimulation treatment, spectral analysis was carried out. As shown in Fig. 3, the results indicate a periodic structure of the data. At a frequency of 0.25 corresponding to every 4th position, the amplitude is extraordinarily high indicating a significant effect of the treatment.

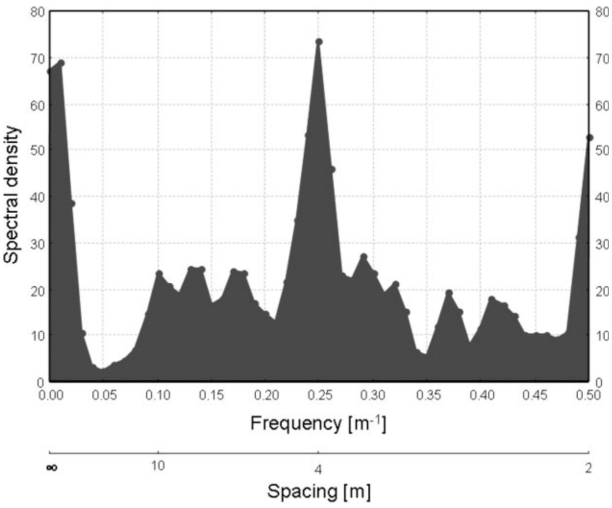

Figure 3: Spectrum of feeding activity along the transect indicating a cyclic variability structure which corresponds to the stimulation treatment at every 4th position.

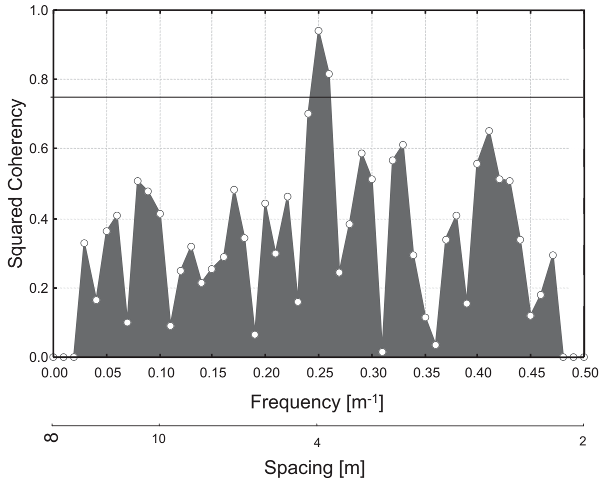

Figure 4: Coherency between the spectra of treatment (stimulation at every 4th position of the transect) and feeding activity, indicating close relationship ($coh^2 = 0.94$) between treatment and increase of feeding activity at a frequency of 0.25. Straight line represents the 5%-significance level for the hypothesis of zero coherency (*Timmer* et al., 1998).

The application of cross-spectral analysis enabled to quantify the extent, to which the feeding activity at every 4th location was related to the stimulation treatment. A squared coherency coefficient between treatment pattern and feeding activity of 0.94 (Fig. 4) indicated that 94% of the variability of feeding activity at the frequency 0.25 could be explained by the treatment.

For the complete data set, if locations are ignored and means are compared, t-test statistics revealed significantly increased feeding activity after stimulation (Fig. 5). The average feeding activity after stimulation was more than twice the average feeding activity along the transect. If we try to account for the spatial process of feeding-activity distribution along the transect, three sections with more or less constant local means can be distinguished (Fig. 2). When means for these transect sections are calculated, it is evident that not only the feeding activity in the control is lower in the middle section, but also the relative treatment effect is reduced (Fig. 6). This middle section b corresponds to the area with the maximum elevation, *i.e.*, the hilltop (Fig. 2). A similar, though more complicated relationship is indicated when single values from 25 stimulated positions along the transect are analyzed and related to elevation (Fig. 7).

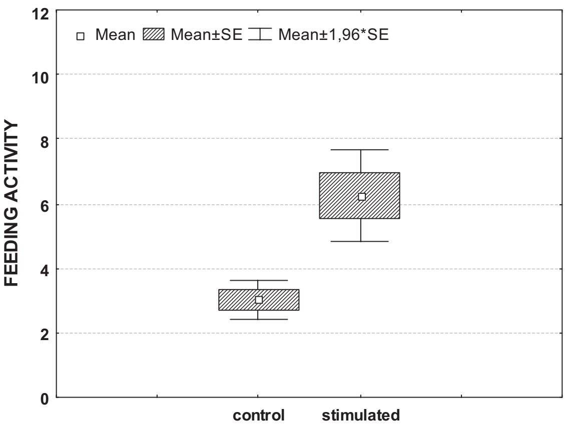

Figure 5: Box plots of feeding activity (number of eaten baits per strip) of all control and stimulated positions.

4 Discussion

It was the objective of this study, to explore the potential for experiments in heterogeneous landscapes which are not confined to small plots but are carried out in the landscape context. For that purpose, a spatial-transect study on agricultural soil was carried out, with the bait-lamina test for assessment of soil-organism activity. At each position, one bait strip was inserted into the soil; the 16 baits in the strip provide an estimate of the soil biological activity at one location.

As hypothesized, the addition of easily available C and water at every 4th transect position stimulated the soil-organism community which reacted to the treatment with increased feeding activity. The effect of treatment could be distinguished from the landscape-induced effects on the feeding activity of soil biota, since both variability patterns were extremely different from each other. The landscape-induced variability was characterized by larger scale trends overriding small-scale point-to-point variability. The treatment, in contrast, induced a regular, small-scale variability pattern of the feeding activity. We can consider the treatment as signal and the basic background feeding activity as noise. The signal-to-noise ratio is high in this case, and therefore both could be separated from each other. The large-scale pattern of feeding activity, despite high variability, exhibited a trend, which could be related to elevation as surrogate for landscape position. The small-scale pattern, consisting in cyclic fluctuations with increased feeding activity at almost every 4th position, could be related to the stimulation treatment.

Thus, it could be shown that spatial approaches are a suitable methodology for carrying out experiments at landscape scale, as it has been suggested by *Nielsen* and *Wendroth* (2003). It is the merit of these authors to stress the importance of considering locations of observations in landscape studies in contrast to approaches with random-sampling designs which do not satisfactorily reflect the nature of gradually changing landscapes.

Prerequisite for this type of experiment and analysis is that the pattern of landscape- and treatment-induced variability is sufficiently different from each other. In a landscape with increasing and decreasing trends of soil properties, a cyclic treatment pattern is obviously suited to be identified in the landscape context, as it can be distinguished from the natural landscape-induced variability of the variable of interest. With spectral and cross-spectral analysis, the treatment effect can be identified. In addition, it is possible to quantify the relationship between treatment and the subsequent increase of feeding activity with the coherency—similar to a regression coefficient (*Nielsen* and *Wendroth,* 2003). This capability inherent in spectral and cross-spectral analysis is of increased importance when different treatments are applied to a spatial series.

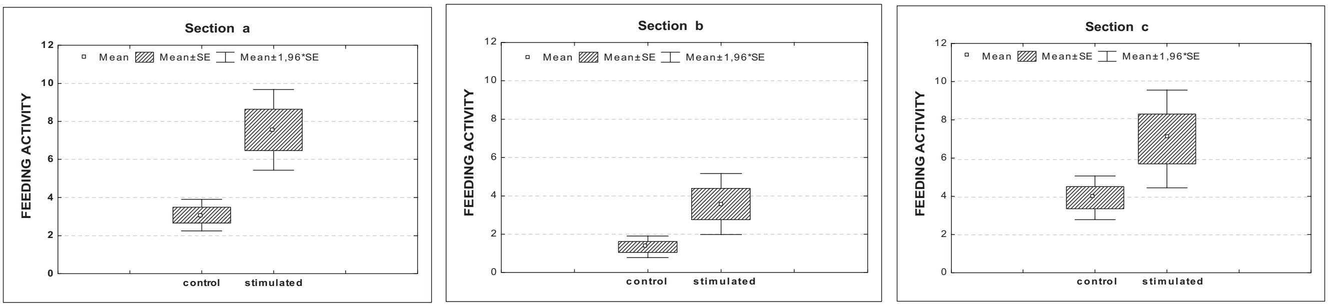

Figure 6: Box plots of feeding activity (number of eaten baits per strip) of control and stimulated positions grouped by landscape position.

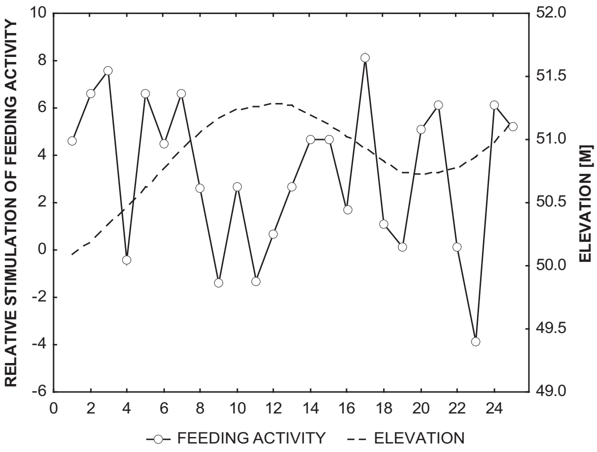

Figure 7: Elevation and relative effect of stimulation (difference between feeding activity at stimulated positions and the local mean of feeding activity) at 25 positions, 4 m apart from each other, along the transect.

Information about the spatial process of feeding activity along the transect enabled us to analyze changes of the basic feeding activity and of treatment effects in course of the transect. In our study, the basic feeding activity and the treatment effect was low in the medium transect section, coinciding with the hillslope positions (Fig. 6 and 7). We may speculate that adverse soil conditions, such as low organic-C content and low soil moisture content support the low feeding activity and the smaller treatment effect at this landscape position. Our results suggest that the degree of stimulation varies according to landscape position for which elevation was used as surrogate variable. While the basic feeding activity of soil biota was already related to landscape properties, *i.e.*, the elevation, the treatment seemed to be even more affected by landscape position. Thus, the treatment helped to identify different parts of the transect and thus the landscape.

While this relationship seems to be clear in Fig. 6, where means are used, the detailed analysis of the spatial process of relative stimulation (Fig. 7) shows that the reality is more complex and deserves increased attention. Potentially relevant factors are quantity of organic matter, moisture availability, temperature-range fluctuations, and differences in soil texture at the different slope positions.

Even though a thorough spatial analysis of treatment effects was not possible with this data set due to the limited number of stimulated positions, the advantage of spatial approaches is obvious. Only the spatial context allowed the distinction of different transect sections characterized by more or less constant local means of soil biological activity. This information will help in developing models for predicting soil-biota distributions across the landscape.

It is envisaged that in future studies, more sophisticated experimental designs could be applied. We used a semiquantitative measure, the feeding activity of the edaphon, assessed by means of a simple screening test, *i.e.*, the bait-lamina test after *von Törne* (1990), as dependent variable. In similar studies, quantitative measurements and a more sophisticated pattern of treatment could be used. As *Nielsen* and *Wendroth* (2003) suggested, a gradually changing treatment pattern would allow studying the effect of different treatment intensities.

Further development of the approach includes the modeling of this kind of spatial processes. As shown by *Sampson* (1996), a time series–based cycle-trend model is suited for spatial distributions with cyclic variability structures.

5 Conclusions

With spatial approaches, experiments may be carried out at the landscape scale. Effects of treatments in heterogeneous landscapes may be distinguished from site effects when the signal-to-noise ratio, *i.e.*, the variability induced by the treatment and by the site, are sufficiently different from one another. In a heterogeneous landscape with gradients of site properties, a treatment based on the frequency domain and applied in regular intervals can be distinguished with spectral-analysis techniques.

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