The spatial structure of soil microbial properties in an upland grassland

Jorge Paz-Ferreiro and Richard D. Bardgett

[A]Department of Biological Sciences, Lancaster University, Lancaster, United Kingdom, Email jpaz@udc.es

Abstract
We characterised the spatial structure of soil microbial properties (microbial biomass-C, microbial biomass-N, dissolved organic carbon (DOC), dissolved organic nitrogen (DON), dissolved inorganic nitrogen (DIN), soil respiration and net nitrogen mineralization, metabolic quotient (qCO_2) and C/N ratio in the microbial biomass) in an unimproved upland grassland in the Yorkshire Dales, United Kingdom. The grassland was selected because it had been ungrazed for thirty years. A 20 m x 20 m plot was marked and 100 soil samples were taken on a regular grid of 2 m x 2 m. The botanical composition of the vegetation associated with each soil sample was also determined. Both, descriptive statistics parameters and semivariogram analysis were considered for assessing data variability. Soil properties showed very high variability (CV from 50% to 84%). Total plant biomass was rather homogeneous (CV=8%), however, individual plant species distribution was highly skewed. Correlations between soil properties were generally strong. Soil respiration, net N-mineralization and DIN did not exhibit spatial dependence at the sampled scale, indicating that data for these variables collected at 2m distance are independent. The spatial dependence of the remaining soil properties was described by a nugget component plus a structure, where the proportion of structural variance was highest for DOC and DON. Also the spatial dependence of plant biomass was either absent or scarce. In cases where spatial dependence was found, ranges extended from approximately 3.2 to 8 m, dispersed throughout both soil properties and vegetation; places further apart than this are spatially independent. Furthermore, mapping of the soil properties and vegetation allowed description and interpretation of interaction between them. Our data demonstrate high spatial variation in microbial properties in upland grasslands, and suggests plant cover may influence the different soil microbial properties.

Key Words
Microbial community, spatial variability, grasslands, geostatistics, microbial biomass, soil respiration

Introduction

Large-scale determinants of soil microbial properties are relatively well known, with climate, soil type, land-use, management practices and topographic feature being among the most influential factors. At smaller scales, however, there is much less agreement about the environmental factors that drive soil microbial communities and the processes that they regulate. On the other hand, although the mechanisms generating plant spatial patterns have been widely studied (Batllori *et al*., 2009), few studies concentrated on the consequences of these patterns on belowground soil properties. Thus, at present, community level associations between vegetation and microbial assemblages are not well understood. There is stronger evidence that there may be characteristic microbial communities associated with particular plant species (Bardgett *et al*., 1998). The mechanisms by which such associations are generated are generally supposed to be related to the quantity and quality of substrates deposited below-ground by plants, in the form of root and shoot litter and root exudates. This means that there would be, at least to a certain extent, spatial coupling between plant and microbial communities (Grayston *et al*., 1998).

In ecosystems where plants tend to be sparse, there is evidence that soil organisms are affected by proximity to individual plants. In grasslands, plant density is very high and there is much less information available about the spatial organisation of microbes relative to plant types. Previous studies on temperate upland grasslands have shown some evidence for community-level coupling (Clegg *et al*., 1999). However, such relationships were obscured by high levels of spatial variation as the composition of communities a few metres apart within a grassland can be as different as those separated by hundreds of kilometres (Clegg *et al*., 1999). One approach to explore drivers between soil microbial community and plant community would be to determine statistically natural variation in soil microbial community and analyse associations with plant community.

In the present study, the spatial properties of soil microbial community structure, and associated vegetation, were measured in an unimproved upland grassland. The grassland had not been grazed for the last 30 years, so plant growth and community structure are mostly reliant upon natural processes of nutrient cycling and

abiotic environmental conditions. Since the underlying soil type and micrometeorology were effectively constant between samples, such factors would not confound the detection of any vegetation-microbe associations that may occur in multiple-site studies. Thus, the aim of this study was to characterize the spatial variability and distribution of soil biological properties and try to relate them with the variability of plant vegetation.

Methods

The study area is located in the south-western corner of the Yorkshire Dales, on top of Ingleborough (United Kingdom) 54°9′ N 2°23′ W and 700 m.a.s.l. The soil is located in an acid bog (45 % average organic carbon, pH=3.5) and the parent material is limestone. A 10x10 m area of ostensibly uniform grassland was randomly selected within the field site, avoiding evident major topological variation (emergent rock, erosion points, slopes, etc.). Soil and plant were sampled every two meters, using a regular grid (thus, the total number of samples was 100). At each sampling point, an intact sample of soil (5 cm diameter, 15 cm deep) was taken using a core auger. Vegetation was sampled (hand removed) in a 30x30 cm area around the soil sampling point. Sampling took place at the end of June 2009. Microbial biomass C and N were measured using the fumigation–extraction techniques of Vance *et al*. (1987). Briefly, soil samples (5 g) were fumigated with $CHCl_3$ for 24 h at 25º C. After the removal of the $CHCl_3$, soluble C was extracted from the fumigated and from unfumigated samples with 0.5M K_2SO_4 for 30 minutes on an orbital shaker. Total organic C (TOC) in filtered extracts (Whatman No. 1) was determined using a Shimadzu 5000A TOC analyser. Microbial C flush (difference between extractable C from fumigated and unfumigated samples) was converted to microbial biomass C using a k_{EC} factor of 0.35 (Sparling *et al*., 1990). Extractable N in the above extracts was determined by oxidation with $K_2S_2O_8$ using the methodology of Ross (1992), and measurement of the resultant NO_3-N and NH_4-N by auto-analyser procedures using a Bran-Luebbe Autoanalyser. The microbial N flush was converted to microbial biomass N using a k_{EN} factor of 0.54 (Brookes *et al*., 1985).
Microbial activity was measured as basal respiration, using the method described by Bardgett et al. (1997). The pots were placed in Kilner jars and incubated for 24 h at 25ºC. One millilitre headspace gas was then removed and CO_2 concentration was measured against a 1% standard gas on an infra-red gas analyser and expressed as ml CO_2 g^{-1} dry soil hr^{-1}. Dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (DIN) were measured by adding 35 ml distilled water to 5 g moist soil samples, which were shaken on an orbital shaker for 10 min prior to being filtered through Whatman No. 1 paper. Total and inorganic C in the extract were determined using a Shimadzu 5000A TOC analyser, and DOC was then calculated by subtracting the amount of inorganic C from the total C in the samples. Total N was determined by oxidation with $K_2S_2O_8$ using the methodology of Ross (1992), and measurement of the resultant NO_3-N and NH_4-N by auto-analyzer procedures using a Bran-Luebbe Autoanalyzer. DON was calculated by subtracting the amount of inorganic N from the total N in the samples.

To determine net N mineralisation, 10 g soil samples were extracted for 30 min with 50 ml of 1 M KCl before and after incubation for 14 days at 25°C at field moisture content. Total inorganic N was determined in the extracts using an autoanalyzer. Net nitrogen mineralisation (mg kg^{-1} day^{-1}) was calculated as the difference between the values obtained before and after incubation. At each sampling points, plant samples were classified according to their species, air dried for 48 hours (70ºC) and weighted. Eight plant species were detected at the study site, belonging to the following genera: *Lycopodium, Racomitrium, Sphagnum, Deschampsia, Agrostis, Eriophorum, Vaccinium* and *Carex* and were present, respectively, in the following amounts in the studied area: 10.82, 18.29, 4.70, 13.62, 37.90, 0.96, 10.92 and 2.97 g m^{-2}.
The patterns of spatial variability of the studied soil properties and plant biomass data sets were assessed by univariate geoestatistics. Semivariograms were computed and models were fitted for each study soil and plant variable. Model adjustment was performed by visual inspection and statistical fitting Maps were obtained by two methods: inverse distance interpolation and, if spatial dependence was evidenced, also by kriging interpolation.

Results

A consistent feature of many of the soil properties measured in this study was a large range in the magnitude of the parameters, exhibiting high levels of soil spatial heterogeneity. As an example, soil respiration showed a 40-fold variation between its maximum and its minimum value varied. This variation was also very high for DIN (23-fold) and for DOC (11-times). Coefficients of variation of soil properties ranged between 50% and 84%. Variation was also very high in the plant cover, with coefficients of variation always higher than 78 % and as high as 300 % for *Eriophorum* (see Table 1). Plant biomass distribution of each individual

species was strongly skewed, because most of them showed areas without some of the plant species. In spite of the variation in the individual plants, the total weight of the plant cover was quite homogeneous (minimum value of 129 g m^{-2} and maximum value of 176 g m^{-2} and CV was 8%). Pearson correlation coefficients among most of the soil properties measured were high (data not shown).

Table 1. Statistical summary for all properties measured.

	Minimum	Maximum	Average	Median	Standard deviation	CV	Skewness	Kurtosis
Biomass C (mg kg^{-1})	420	2762	1292	1115	710	55	0.433	-1.114
Respiration(ml CO_2 g^{-1} hr^{-1})	0.83	33.50	14.13	12.80	8.19	58	0.193	-0.885
DOC (mg kg^{-1})	56	634	266	207	171	64	0.809	-0.469
Biomass N (mg kg^{-1})	128	997	414	293	244	59	0.924	-0.378
DON(mg kg^{-1})	31	179	83	70	44	53	0.712	-0.761
DIN (mg kg^{-1})	1	23	10	11	5	50	-0.005	-0.434
N mineralization (mg kg^{-1} day^{-1})	0.9	6.7	2.6	2.2	1.6	62	0.894	-0.101
qCO_2	0.08	12.32	2.63	2.12	2.20	84	2.333	6.868
C/N biomass	0.56	15.93	3.60	3.03	2.24	62	2.452	9.077
Soil humidity	33	840	343	310	191	56	0.505	-0.265
Sphagnum (g m^{-2})	0	138	17	0	33	194	2.104	3.573
Racomitrium (g m^{-2})	0	131	28	19	34	121	1.266	0.892
Lycopodium (g m^{-2})	0	93	7	0	19	271	2.809	7.415
Deschampsia (g m^{-2})	0	91	20	18	17	85	1.645	3.796
Agrostis (g m^{-2})	0	199	59	50	46	78	0.587	-0.457
Eriophorum (g m^{-2})	0	14	1	0	3	300	2.166	4.095
Vaccinium (g m^{-2})	0	107	17	12	20	118	1.805	4.182
Carex (g m^{-2})	0	130	5	0	14	280	7.017	58.392
Total plant biomass (g m^{-2})	129	176	153	155	13	8	-0.058	-0.9112

Table 2. Model type and parameters (C_0 = nugget value; C_1 = sill) for indicated semivariograms.

Parameter	Model	C_0	C_1	$C_0/(C_0+C_1)$	range (cm)	r^2
Biomass C	Spherical	294440.0	294549.0	0.50	690.3	0.775
Respiration			Pure nugget effect			
DOC	Spherical	6925.1	19195.5	0.27	800.0	0.887
Biomass N	Spherical	49498.9	9738.0	0.84	600.0	0.074
DON	Spherical	601.5	1161.4	0.34	800.0	0.742
DIN			Pure nugget effect			
N mineralization			Pure nugget effect			
qCO_2	Spherical	2.5	5.5	0.31	319.8	0.436
C/N biomass	Spherical	4.2	1.4	0.75	357.4	0.028
Humidity	Spherical	20138.9	15131.3	0.57	600.0	0.449
Sphagnum	Exponential	86.1	1141.5	0.07	600.0	0.794
Racomitrium	Spherical	400.0	694.5	0.37	327.3	0.158
Lycopodium	Spherical	153.5	265.8	0.37	536.9	0.643
Deschampsia	Spherical	59.8	209.0	0.22	282.4	0.040
Agrostis	Spherical	1190.9	771.1	0.61	600.0	0.395
Eriophorum			Pure nugget effect			
Vaccinium	Spherical	184.5	164.9	0.53	404.3	0.258
Carex	Spherical	84.9	100.3	0.46	600.0	0.298
Total plant biomass	Spherical	0	152.3	0	356.1	0.537

The most formal way to analyse spatial variation statistically is by means of semivariance analysis and here it revealed the presence of spatial structure in variance for many of the parameters measured. Generally, the spatial dependence found was quite low (see Table 2). There was no evidence for spatial structure in soil respiration, DIN and N mineralization at the 2 m sampling interval. The remaining soil properties showed very week spatial dependence, with the exception of DON, DOC, biomass-C and *Sphagnum*. In the case of vegetation, Sphagnum showed the most important spatial correlation. Therefore, the pattern of spatial dependence of the studied soil properties and plant biomass was described by a nugget component, i.e. no spatial dependence or by spherical or exponential models plus a nugget component (Table 2). The fit of the

models to experimental semivariograms was rather poor, except for DON, DOC, biomass-C and *Sphagnum*, again. *Sphagnum* abundance showed a statistically significant negative correlation with both, DON and DOC. The ranges of spatial dependence found for DOC, DON, biomass C and *Sphagnum* varied from about 320 to 800 cm meaning that for distances longer than these the data were not spatially correlated. Samples taken 8 m apart will not exhibit errors due to spatial dependence. Selected examples of spatial distribution are mapped in Figure 1. Kriging maps for DOC and for *Agrostis* illustrate a patchy structure, where the average diameter of the patches is represented by the range of spatial dependence. In contrast, the map for respiration shows no detectable pattern of variation as individual high and low values may occur as neighbours which lead to a pure nugget effect model of spatial dependence. Mapping of the soil properties and vegetation is useful in the description and interpretation of interaction between them.

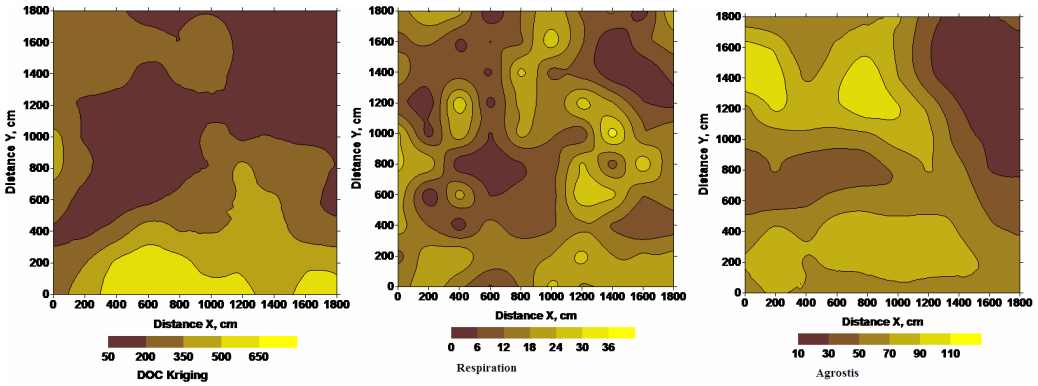

Figure 1. Maps for DOC, respiration and *Agrostis*.

Conclusion

Our study has showed the high levels of spatial complexity in soil properties and vegetation patterns that prevail in unimproved upland grasslands, and suggests that a complex set of interactions influence the spatial patterns of different soil microbial properties. Soil biological properties showed a wide difference in values at the small plot scale and high coefficients of variation. Individual plant species biomass was strongly skewed (asymmetric), whereas total plant biomass was rather homogeneous. In general the variograms of the soil properties and plant biomass were flat or nearly flat, and therefore spatial structure was absent or scarce (exceptions were DON, DOC, C-biomass and *Sphanum*). Our study suggests that a better understanding of spatial patterns of soil microbial properties requires careful study of the vegetation cover around the samples.

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