Taxa-Area Relationship (TAR) of Microbial Functional Genes with Long-Term Fertilization

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ABSTRACTS

Diversity and spatial patterns in plant and animal communities are well documented as a positive-power law of a taxa-area relationship (TAR). At present little is known whether this also applies to soil microbial communities and whether long-term fertilization has an influence on the underlying microbial diversity. To test the effects of long-term fertilization on above-ground botanical diversity and below-ground microbial diversity, a nested sampling approach on Park Grass plots (12d & 11/2c) of Rothamsted Reseach in United Kingdom, both at ~ pH 5 but with plant diversities of between 42 and 13 respectively were used. GeoChip 3.0, covering approximately 57, 000 gene sequences of 292 gene families involved in nitrogen, carbon, sulfur and phosphorus cycling, metal reduction and resistance, and organic contaminant degradation, was used to determine the gene area relationships for both functional and phylogenetic groups and the relationship to plant diversity. Our analysis indicated that the microbial communities were separated by different plant diversity based on DCA. The soil microbial diversity was in accord with plant diversity. Soil microbial community exhibited different z value with different plant diversity, z = 0.0449 with higher plant diversity and z = 0.0583 with lower plant diversity ($P < 0.0001$). These results suggest that the turnover in space of microorganisms may be higher with long-term fertilization.

MATERIALS AND METHODS

Site Description and Sampling Park Grass experiment at Rothamsted Research, which is the oldest experiment on permanent grassland in the world, was initiated in 1856 to investigate the continuing effects of inorganic fertilizers and organic manure on plant production and species diversity.

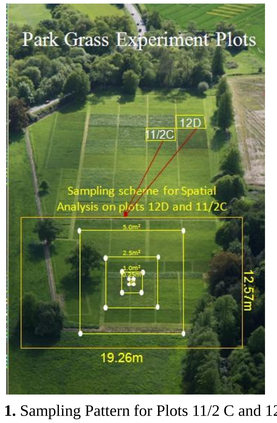

Figure 1. Sampling Pattern for Plots 11/2 C and 12D

Single soil cores were taken at the four corners (as replicates) of the 5 nested squares at increased distances as shown in Figure 1 (10 cm^2, 25 cm^2, 1 m^2, 2.5 m^2, and 5 m^2) at the depth of 0 – 10 cm. One soil core was taken at the center of the area. Survey of spatial distribution of plant species in the same area at the same scale was taken. Plot 12D (nil plot) was of no fertilizer and organic manure and lime was applied since 1856. Plot 11/2C was fertilized with N (144 kg N as ammonium sulphate), P (35 kg P as triple superphosphate), K (225 kg K as potassium sulphate), Na (15 kg Na as sodium sulphate), Mg (10 kg Mg as magnesium sulphate), Si (450 kg of sodium silicate), and limed to keep the pH at 5.

The microbial community genomic DNA was extracted from 5 g of well-mixed soil samples from each individual core (Zhou et al., 1996), labeled and hybridized to GeoChip 3.0 (He, et al., 2010). GeoChip 3.0 is a new generation of functional gene arrays with ~28 000 probes covering approximately 57 000 gene variants from 292 functional gene families involved in carbon, nitrogen, phosphorus and sulfur cycles, energy metabolism, antibiotic resistance, metal resistance and organic contaminant degradation (Table 1).

Table 1. The summary of probe on GeoChip3.0

Gene category	No. of genes	No. of probes	Sequence-specific probes	Group-specific probes	Coved CDS
Carbon cycling	41	5196	1765	3431	10573
Nitrogen cycling	16	3763	2148	1615	7839
Phosphorus utilization	3	599	183	416	1220
Sulfur cycling	4	1504	1083	421	2042
Energy process	2	508	410	98	671
Metal resistance	41	4870	603	4267	10962
Organic contaminant degradation	173	8614	2165	6449	17441
Antibiotic resistance	11	1594	265	1329	3944
Phylogenetic marker (*gyrB*)	1	1164	629	535	2298
Total	292	27812	9251	18561	56990

RESULTS AND DISCUSSIONS

z value of microbial functional and phylogenetic groups

z values were determined by fitting the linear regression equation ($\log S = \log c + z \log A$), where *S* is species richness and *A* is area. The *z* values for individual functional and phylogenetic groups were estimated based on the individual functional gene sequences within each group. *t* and *P* values are from one-sample *t* tests on bootstrapping (9,999 times) for testing significance of *z* values.

Table 2. The slopes of taxa-area relationships for various functional and phylogenetic groups.

	Plot 12D (Nil)					Plot 11/2C (Fertilized)				
microbial group	z value	95% C.I.	n	t	*P*	z value	95% C.I.	n	t	P
all functional genes	0.0449	1.47E-03	4006	-60.21	<0.001	0.0583	2.10E-03	3405	-54.59	<0.001
functional groups										
C degradation	0.0588	1.90E-03	432	-59.87	<0.001	0.063	2.26E-03	414	-53.5	<0.001
C fixation	0.0421	1.36E-03	141	-58.7	<0.001	0.0754	2.52E-03	129	-57.77	<0.001
Energy process	0.0715	2.35E-03	81	-60.92	<0.001	0.0683	2.44E-03	59	-54.26	<0.001
Methane	0.0669	2.66E-03	33	-51.83	<0.001	0.0687	2.68E-03	22	-50.99	<0.001
Metal resistance	0.0561	1.89E-03	773	-58.01	<0.001	0.0496	1.79E-03	630	-53.5	<0.001
Ammonification	0.0514	1.69E-03	43	-59.33	<0.001	0.0565	2.13E-03	36	-51.99	<0.001
Assimilatory N reduction	0.066	2.18E-03	34	-58.73	<0.001	0.0582	2.23E-03	29	-51.71	<0.001
Dissimilatory N reduction	0.0599	1.94E-03	28	-57.59	<0.001	0.0592	2.29E-03	25	-51.08	<0.001
Nitrification	0.0671	2.91E-03	14	-45.42	<0.001	0.0842	2.86E-03	7	-56.99	<0.001
Denitrification	0.0543	1.81E-03	190	-58.93	<0.001	0.0613	2.21E-03	148	-54.73	<0.001
N fixation	0.041	1.38E-03	174	-58.95	<0.001	0.0365	1.36E-03	151	-52.5	<0.001
Organic remediation	0.0351	1.17E-03	1366	-57.63	<0.001	0.0588	2.12E-03	1145	-55.63	<0.001
Phosphorus	0.0234	8.02E-04	76	-55.08	<0.001	0.0609	2.18E-03	56	-57.55	<0.001
Sulfur	0.0367	1.22E-03	193	-58.82	<0.001	0.0724	2.56E-03	176	-54.44	<0.001
Phylogenetic groups										
Archaea	0.0428	1.42E-03	94	-58.68	<0.001	0.0492	1.72E-03	79	-55.57	<0.001
Fungi	0.0604	2.02E-03	182	-58.93	<0.001	0.0704	2.61E-03	153	-54.13	<0.001
Bacteria	0.0444	1.47E-03	3191	-58.03	<0.001	0.0565	2.04E-03	2705	-53.7	<0.001
α-proteobacteria	0.0356	1.20E-03	763	-59.71	<0.001	0.0567	2.02E-03	661	-54.92	<0.001
β-proteobacteria	0.0437	1.45E-03	429	-60.84	<0.001	0.0557	2.07E-03	356	-54.61	<0.001
γ-proteobacteria	0.0496	1.65E-03	522	-58.56	<0.001	0.0528	1.89E-03	399	-53.76	<0.001
δ-proteobacteria	0.0619	2.03E-03	144	-59.35	<0.001	0.0583	2.13E-03	126	-54.88	<0.001

Significant TARs were observed for all functional and phylogentic groups of both fertilized site and control site, for all functional genes, *z* = 0.0449 in control site (12D) and *z* = 0.0583 (11/2C) in fertilized site (Table 2).

Table 3. *z* value comparison between fertilized and control sites.

Functional Group Comparisons (Plot 11/2C vs. Plot 12D)		
Microbial group	t	*P*
all functional genes	10.2243	0.0001
functional groups		
C degradation	2.7885	0.0053
C fixation	22.8042	0.0001
Energy process	1.8486	0.0647
Methane	0.9349	0.3499
Metal resistance	4.9	0.0001
Ammonification	3.6841	0.0002
Assimilatory N reduction	4.9057	0.0001
Dissimilatory N reduction	0.4568	0.6479
Nitrification	8.2137	0.0001
Denitrification	4.8142	0.0001
N fixation	4.5427	0.0001
Organic remediation	19.1801	0.0001
Phosphorus	31.7112	0.0001
Sulfur	24.6464	0.0001
Phylogenetic groups		
Archaea	5.6092	0.0001
Fungi	5.939	0.0001
Bacteria	9.4367	0.0001
α-proteobacteria	17.5887	0.0001
β-proteobacteria	9.3051	0.0001
γ-proteobacteria	2.5051	0.0123
δ-proteobacteria	2.3986	0.0165

Considerable variations in the *z* values were observed among different functional and phylogenetic groups. The mean *z* value was 0.0522 (± 0.0144) for different functional gene groups and 0.0483 (± 0.009) for phylogenetic groups in non-fertilized site (12D). In comparison, the mean *z* value was 0.0624 (± 0.0115) for different functional gene groups and 0.0571 (± 0.007) in fertilized site (11/2C).

To determine whether the estimated *z* values were significantly different between fertilized site and non-fertilized site, *t* test was used to compare the *z* values of all functional genes, functional groups and phylogenetic groups (Table 3). Our results revealed that the estimated *z* values of all functional genes were significantly different between 12D and 11/2C ($P = 0.0001$). For different functional groups, *z* values were significantly different for most nitrogen cycling process, such as ammonification ($P = 0.0002$), assimilatory nitrogen reduction ($P = 0.0001$), nitrification ($P = 0.0001$), denitrification ($P = 0.0001$), nitrogen fixation ($P = 0.0001$), except dissimilatory nitrogen reduction ($P = 0.6479$). In the mean time, it seems fertilization had a significant effects on *z* values of other functional groups such as carbon degradation ($P = 0.0053$), carbon fixation ($P = 0.0001$), metal resistance ($P = 0.0001$), phosphorus cycling ($P = 0.0001$), sulfur cycling ($P = 0.0001$) and organic remediation ($P = 0.0001$). Also, *z* values were significantly different in all phylogenetic groups ($P < 0.05$) between non-fertilized site (12D) and fertilized site (11/2C).

z value comparison of plants and microbial communities in fertilized and control sites

Long-term fertilization significantly decreased the microbial functional diversity ($P = 0.002$), as well as plant diversity ($P < 0.0001$) , which is in accordance with previous study. To compare the effects of long-term fertilization on both microbial and macrobial spatial patterns, the slopes of TARs were estimated by a linear regression with the log transformed gene and plant richness data (Figure 2 (A)(B)). Both microbial functional genes and plant exhibited higher *z* values with long-term fertilization.

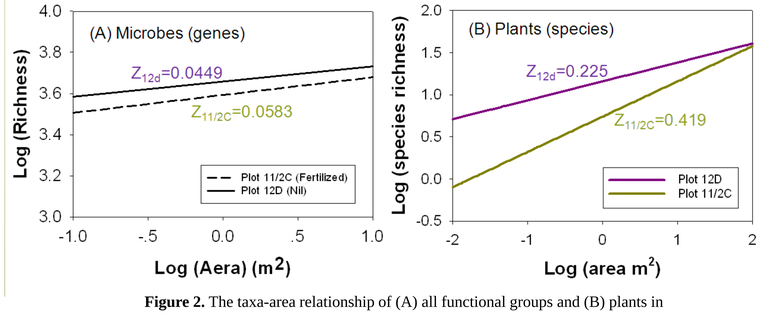

Figure 2. The taxa-area relationship of (A) all functional groups and (B) plants in both fertilized and non-fertilized sites

The *z* value obtained in this study were compared with previously studies to obtain a general insights on the spatial scaling of biodiversity across different organisms (Figure 3). The *z* values for the plants (0.322 ± 0.137) were consistent with previous studies. The turnover rate of microorganisms in space (0.0527 ± 0.007) appears to be much lower than those of other organisms.

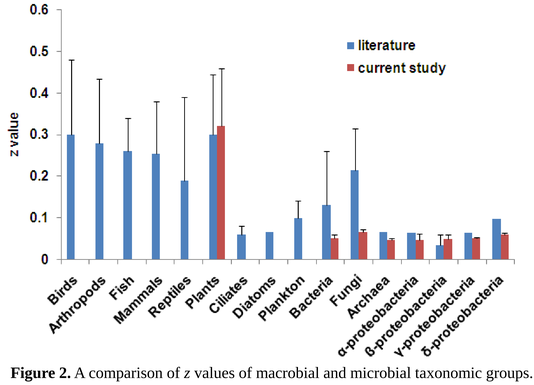

Figure 2. A comparison of *z* values of macrobial and microbial taxonomic groups.

SUMMARY

Significant difference of microbial functional and phylogenetic spatial turnover was observed between long-term fertilized site and control site. *z* values for both plant and microbial communities were higher with long-term fertilization. These results suggest the turnover in space of microorganisms may be affected by human activities such as long-term fertilization.

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