Short communication

Spatial distribution of fungal communities in a coastal grassland soil

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ABSTRACT

In grasslands, saprotrophic fungi, including basidiomycetes, are major decomposers of dead organic matter, although spatial distributions of their mycelial assemblages are little described. The aim of this study was to characterise the scale and distribution of saprotrophic fungal communities in a coastal grassland soil using terminal restriction fragment length polymorphism (T-RFLP).
Soil fungi were sampled at Point Reyes, California, USA, by taking forty-five 26 mm diam. cores in a spatially defined manner. Within each sampled core, complete core sections at 1–2 cm and 14–15 cm depths were removed and sub-sampled for DNA extraction and amplification using the primer pairs ITS1F-FAM/ITS4 (general fungi) or ITS1F-FAM/ITS4B (basidiomycete-specific).
Nonmetric Multidimensional Scaling showed that general fungal communities could be clearly separated by depth, although basidiomycete communities could not. There were no strong patterns of community similarity or dissimilarity for general or basidiomycete fungal communities at horizontal geographical distances from 25 cm to 96 m in the upper horizon. These results show considerable vertical, but little horizontal, variability in fungal community structure in a semi-natural grassland at the spatial scales measured here.

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Little is known about the vertical and horizontal distributions of decomposer fungal communities as mycelia in grassland soils (e.g. Feeney et al., 2006). Even to begin to develop a more general understanding of spatial patterns of fungal community structure, more studies describing vertical and horizontal patterns are necessary. To start to fill this gap, we carried out a study examining vertical (2–15 cm depth) and horizontal (25 cm–96 m) patterns of fungal community composition in a coastal grassland, using T-RFLP. We characterised general fungal communities, and basidiomycete communities more specifically to see whether this group differed in spatial structure.

The study site (38°2′N, 122°53′W) was located on a low ridge (20–30 m a.s.l.) at Point Reyes National Seashore, California, USA. The area is extensive coastal grassland interspersed with coastal scrub dominated by *Baccharis pilularis*. Typically, the A_1 horizon of this Inceptisol is a brown sandy loam about 30 cm in depth (pH 5.6–6.5; 1–6% organic matter). Plant roots are distributed throughout this horizon.

In October 2003, three sets of three 1 m long transects, orientated randomly, were identified for sampling. An indication of the vegetation at each transect is given in Table S1 (Supplementary data). On 22 January 2004, five cores per 1 m transect, at a horizontal distance 25 cm apart, were removed from the grassland soil, giving 45 cores in total. The cores were taken in PVC pipes: each 26 mm internal diam., 4 mm wall and cut diagonally at one end to sharpen, giving 27 cm short length and 35 cm long length. Each PVC tube was driven into the ground to 19.5 cm depth and the core removed inside the PVC pipe. The core and pipe were then immediately sealed inside a large plastic bag, which was placed on ice. The cores were transported back to the laboratory as soon as possible, and stored at 4 °C. Within 7 d of collecting (Izzo et al., 2005), two soil samples were removed from each core. The upper horizon was taken as the complete core section at 1–2 cm depth and the lower horizon was the complete core section at 14–15 cm depth. Each sample was stored separately in sealed plastic bags at 4 °C, and processed within 3 d.

Soil samples were homogenised inside each plastic bag, and obvious roots were removed. Total soil DNA was extracted using the UltraClean™ soil DNA kit (Mo Bio Laboratories Inc., Solana Beach,

CA, USA) from 500 mg (fresh weight) aliquots of freeze-dried soil, one from each of the upper and lower horizons of each core. The nuclear rDNA internal transcribed spacer (ITS) region was amplified with the fungal-specific primer pair ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990), or the basidiomycete-specific ITS1F and ITS4B (Gardes and Bruns, 1993) primer pair. [Although the ITS1F and ITS4B primer pair does not amplify DNA of some basidiomycetes (e.g. *Tulasnella*), it does eliminate ascomycete and plant DNA very well (Gardes and Bruns, 1993)]. For T-RFLP, the ITS1F primer 5′ in each pair was labelled with the fluorescent dye 6-FAM (synthesised by Operon Biotechnologies Inc., Huntsville, AL, USA). Full details of DNA extraction, amplification, digestion and T-RFLP analysis are provided in Supplementary data.

Only the presence or absence of fragments was considered in this study and, throughout the following text, the term frequency refers to the number of soil cores that contained a particular fragment (after Genney et al., 2006). In the current study, because such a large number of relatively closely spaced different fragments resulted and it was difficult to discriminate adjacent peaks (Avis et al., 2006), frequency data for each separate fragment size were used. The mean numbers of fragments per core horizon were analysed between transects ($n = 5$ per transect) and between horizons ($n = 45$ per horizon) by General Linear Models (GLM), followed by Tukey's HSD. Each enzyme/primer combination was analysed separately.
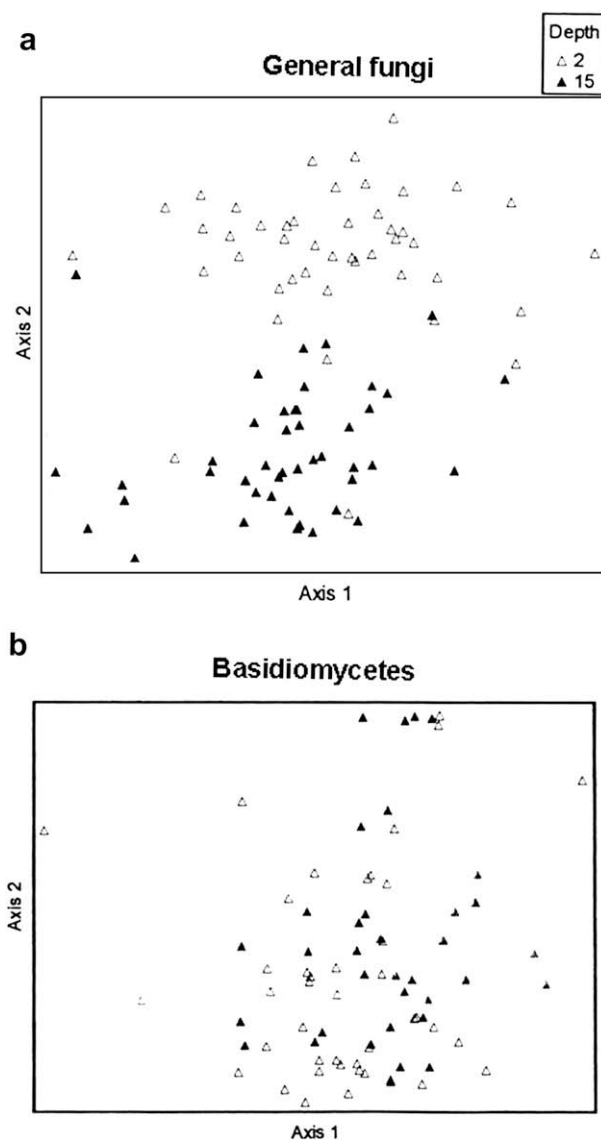
Nonmetric Multidimensional Scaling (NMS) was used to ordinate the fungal or basidiomycete community data by different (i) horizons, (ii) transects and (iii) sets of transects. To determine whether a significant amount of the variation in fungal community composition, as indicated by T-RFLP profiles, could be explained by geographic horizontal distance between samples, Mantel's test was calculated as follows. Similarities were calculated between fungal or basidiomycete communities for the upper horizon: (i) at 25, 50 or 75 cm distances, (ii) across individual transects (1 m distances), (iii) in six pairs of individual transects (distance range 11–38 m) and (v) in six pairs of transects from different sets of transects (distance range 64–96 m). The similarity measure used was the Sørensen (Bray–Curtis) co-efficient. The method for evaluating the Mantel test statistic was the t-distribution with infinite degrees of freedom, using the asymptotic approximation of Mantel (1967) and the standardised Mantel statistic (r) as a measure of effect size (McCune and Grace, 2002). Further details of statistical analyses are given in Supplementary data.

The fungal communities were found to be so heterogeneous in horizontal space that horizontal NMS and Sørensen similarity index/Mantel tests were carried out for the upper horizon only. Distance ranges between transects (11–38 m) or sets of transects (64–96 m) were used for the Sørensen similarity indices/Mantel tests because, in comparison with 25, 50, 75 cm and 1 m distances within transects, this allowed testing of spatial patterns at horizontal distances over two orders of magnitude (i.e. at tens of centimetres to tens of metres). There was so much overlap in the fungal communities within and between sets of transects (NMS data), that for the Sørensen similarity indices/Mantel tests, six randomly chosen pairs of transects were compared within the sets, and six randomly chosen pairs of transects were compared between the sets.

From the 90 soil samples (45 cores), the total number of fragments for general fungi was 223 with *Alu*I ITS1F-FAM/ITS4 and for basidiomycetes was 177 with *Hin*fI ITS1F-FAM/ITS4B. The mean number of fragments per core horizon per transect ($n = 5$ cores for each of 9 transects), ranged from 19 to 37 for general fungi (*Alu*I ITS1F-FAM/ITS4) and 8 to 21 for basidiomycetes (*Hin*fI ITS1F-FAM/ITS4B), and was not significantly different between transects. Results from all 9 transects were combined for analysis of variance (GLM) by core horizon, giving a mean number of fragments per core horizon ($n = 45$), for general fungi, upper horizon 24 ± 1.3, lower horizon 28 ± 1.6, $P = 0.071$; for

basidiomycetes, upper horizon 14 ± 1.6, lower horizon 10 ± 1.3, $P = 0.110$. *Cryptococcus podzolicus* (100% similarity) was identified, by direct sequencing, as the dominant product of non-fluorescent ITS1F and ITS4B PCR products from the original soil DNA extracts. From the T-RFLP results, in the total number of soil samples per horizon (i.e. 45), this species occurred in the upper horizon of 23 cores and the lower horizon of 10 cores. Unfortunately, time constraints prevented further cloning and sequencing.

Grassland soils are stratified vertically (e.g. Bruneau et al., 2005) but, for grasslands, stratification of fungal communities by depth/horizon has received little attention. In the current study, NMS suggested that general fungal communities could be clearly separated by depth (Fig. 1a), even though both depths sampled in each core were within the A_1 soil horizon. Basidiomycete communities, however, were not

Fig. 1. Results of Nonmetric Multidimensional Scaling by depth for communities from cores resulting from (a) restriction with *Alu*I; primers ITS1F-FAM/ITS4 (general fungi), (b) restriction with *Hin*fI; primers ITS1F-FAM/ITS4B (basidiomycetes). Open triangles represent communities from the upper (1–2 cm) soil horizon and solid triangles represent those from the lower (14–15 cm) horizon. In (a), final stress for 3-dimensional solution = 20.564, final instability = 0.00196, Axis 1 alone accounted for 23% of the variance, Axis 2 alone for 31%: all 3 axes together accounted for 73% of the variance, in (b), final stress for 3-dimensional solution = 14.123, final instability = 0.00007, Axis 1 alone accounted for 11% of the variance, Axis 2 alone for 32%: all 3 axes together accounted for 76% of the variance.

separated by depth (Fig. 1b). It is likely that the determinants of fungal community structure in vertical (and horizontal) space are extremely complex, involving a combination of soil physical and chemical factors and vegetation type (Ritz et al., 2004; Kasel et al., 2008). Interspecific fungal interactions may also be important; for example, the numbers of ascomycete species were reduced where basidiomycete mycelium was present (Warcup, 1951).

Here at Point Reyes, there were no strong patterns of community similarity or dissimilarity at horizontal distances from 25 cm to 96 m for general or basidiomycete fungal communities (Fig. 2a and b; NMS results within and between sets of transects). In the current Point Reyes study, we may have missed the appropriate scale of sampling if the patterns in fungal communities and soil properties are scale-dependent. For example, the scale of patchiness in root length was 3–4 cm in prairie (Partel and Wilson, 2002). Replication in time would be likely to detect additional diversity and any further community structure.

Only one species was identified in our work presented here because of time constraints. Ideally, future studies would combine T-RFLP analysis with clone libraries of selected samples (Lindahl et al., 2007) in order to enable processing of a large set of samples with identification of key taxa.

Estimates of diversity based on peak counts from T-RFLP can be exaggerated (Avis et al., 2006), because it is possible that multiple fragments (i.e. extra peaks) occur caused by restriction enzyme inefficiency and intracollection ribosomal DNA ITS variation (i.e. multiple T-RFLP profiles within a single species; Avis et al., 2006; Dickie and FitzJohn, 2007). In addition, there are two mechanisms by which the numbers of unique DNA fragments may be underestimated: (1) restriction enzyme digestion in conserved as opposed to variable gene regions and (2) shared profiles by different species in the same fungal assemblage (Avis et al., 2006; Dickie and FitzJohn, 2007). The profiles obtained are also partly dependent on the amount of soil DNA used and how soil cores are pooled (Schwarzenbach et al., 2007). Despite these potential problems concerning T-RFLP and the numbers of unique DNA fragments obtained, the Mantel test and NMS results in the current study are particularly valid because they are based on contrasting samples processed with methods which are internally consistent within sets of soil samples with a specific primer/enzyme combination.

This study has demonstrated that: (1) general fungal communities could be clearly separated by depth, although basidiomycete communities could not, (2) there were no strong patterns of community similarity or dissimilarity for general or basidiomycete fungal communities at horizontal geographical distances from 25 cm to 96 m in the upper horizon.

Acknowledgements

Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.soilbio.2008.10.021.

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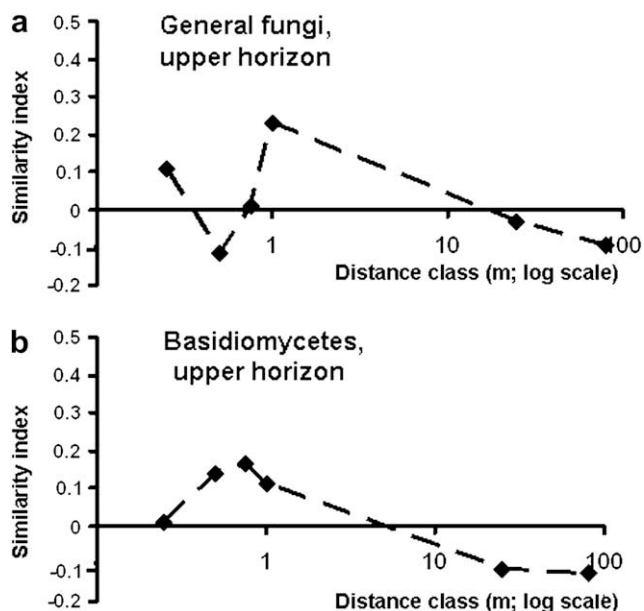
Fig. 2. Similarity values, using the Sørensen (Bray–Curtis) index and the Standardised Mantel statistic (r), between communities at increasing horizontal geographical distance classes in the upper soil horizon, resulting from either (a) AluI ITS1F-FAM/ITS4 (general fungi) or (b) HinfI ITS1F-FAM/ITS4B (basidiomycetes). The Sørensen (Bray–Curtis) index was used as the similarity measure for the first and second matrices; if $r < 0$, then a negative association (dissimilarity) was indicated, if $r > 0$, then a positive association (similarity) was indicated. None of the r values was statistically significant. For each of the distance ranges 11–38 m and 64–96 m, the similarity index is plotted against the mid-point of the distance range.