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NOTE AND SHORT COMMUNICATIONS

Phyllosphere Fungal Communities Differentiate More Thoroughly than Bacterial Communities Along an Elevation Gradient

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The distance-decay pattern of similarity in ecological communities has been recognized by ecologists for decades [1]. Such a pattern has been shown for diverse macroorganisms and microorganisms [2–5], including phyllosphere bacterial communities [6]. It results from four eco-evolutionary processes: selection, drift, dispersal and mutation [2, 7]. Selection and drift generate the decay in compositional similarity with geographical distance, while dispersal counteracts it [2]. Mutation decreases the similarity among locations, regardless of the distance between them [2]. The shape of a distance-decay curve is therefore expected to differ between taxonomic or functional groups of species [8]. For instance, groups of smaller species such as bacteria are expected to have a slower distance-decay because of their higher dispersal abilities. They are also expected to have a lower compositional similarity among samples of a given location, because of their higher rates of mutation [9]. Here, we compared the distance-decay curves of fungal and bacterial communities along an elevation gradient. Since bacterial cells (with a diameter of a couple of micrometers) are smaller than the smallest fungal individuals (unicellular yeasts, measuring tens of micrometers across) [9], we hypothesized that (i) like fungi, bacterial communities differentiate along the elevation gradient, but (ii) they differentiate less thoroughly (i.e., they have a slower distance-decay), and (iii) they display lower levels of compositional similarity among samples of each elevation.

To test these hypotheses, we compared the distance-decay curves of fungal and bacterial communities inhabiting the European beech (*Fagus sylvatica L.*) phyllosphere along an elevation gradient extending from 488 to 1533 m over a distance of 23 km. The sampling design was composed of 12 samples, corresponding to 4 elevation sites with 3 forest plots per site [10]. Previous analyses showed considerable variation of the fungal community composition along the elevation gradient, suggesting that selection by climatic conditions (temperature, in particular) drives variations in the composition of phyllosphere fungal communities [10]. Here, we characterized the bacterial communities along the same elevation gradient, by 454 pyrosequencing the bacterial 16S rDNA in the same samples that had been used previously for sequencing the fungal internal transcribed spacer region [10]. Bioinformatic analyses are detailed in Table S1. To avoid biases due to differences in sequence variability between both barcode regions, sequence clustering into operational taxonomic units (OTUs) was performed using four identity cutoff thresholds (90, 95, 97, and 99 %).

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Table 1 Statistics characterizing distance-decay relationships for phyllosphere bacteria and fungi as a function of the identity threshold used for OTU clustering

Taxa	Identity threshold (%)	Effect of elevation site	Correlation with elevation difference	Correlation with geographical distance	Initial similarity (%)	Slope	Halving distance (km)
Bacteria	99	0.49**	0.70***	0.56***	64 (63, 65)	−0.004 (−0.005, −0.002)	182 (133, 289)
	97	0.5***	0.66***	0.51**	65 (64, 67)	−0.004 (−0.005, −0.002)	191 (134, 331)
	95	0.52***	0.65***	0.48**	70 (69, 71)	−0.003 (−0.004, −0.002)	243 (168, 441)
	90	0.57***	0.65**	0.38**	76 (75, 78)	−0.002 (−0.004, −0.001)	307 (191, 778)
Fungi	99	0.67***	0.79***	0.68***	50 (47, 53)	−0.016 (−0.021, −0.012)	42 (33, 58)
	97	0.74***	0.89***	0.79***	53 (51, 56)	−0.018 (−0.022, −0.014)	39 (32, 48)
	95	0.75***	0.89***	0.8**	55 (52, 57)	−0.018 (−0.021, −0.014)	39 (33, 48)
	90	0.75***	0.9***	0.83***	57 (55, 59)	−0.017 (−0.02, −0.014)	41 (35, 50)

The effect of site on community composition was assessed with permutational analyses of variance. Percentages of variance explained by site (R^2) and significance of the site effect (***$p<0.001$; **$0.01<p<0.001$) are reported. The correlation between community dissimilarity and elevation difference or geographical distance was tested using Mantel tests. Pearson correlation coefficients and significance of the correlations are reported. Initial similarities, slopes of the relationships, and halving distances were estimated from the linear regressions of Fig. 1. 95 % Confidence intervals are reported into parenthesis. The overlap of confidence intervals was used to test for significant differences between bacteria and fungi

Compositional similarity between samples was then assessed with the Sørensen index based on OTU tables rarefied to even sampling depth (784 sequences per sample). The shape of the distance-decay curves was characterized by three parameters: the slope (s), which reflects the degree of community differentiation along the geographical gradient; the initial similarity (IS), which reflects the similarity of communities over very small distances; and the halving distance (HD), which corresponds to the distance over which the initial similarity is halved [1]. IS was defined as the similarity of the communities located 50 m apart, because this was the shortest distance between two plots in our experimental design. Linear regression analyses of the relationship between community similarity (log-transformed) and geographic distance between plots were performed to estimate the three parameters. IS and HD were calculated as $\mathrm{IS} = \exp(0.05s + i)$ and $\mathrm{HD} = 0.05\text{-}\log 2/s$, where s and i are the slope and the intercept of the linear regression line, respectively.

Consistent with our first hypothesis, the composition of bacterial communities differed significantly between elevation sites (Table 1 and Fig. S1), and compositional dissimilarities were significantly correlated with both the elevation difference and geographic distance between plots (Table 1). Consistent with our second hypothesis, bacteria showed a lower degree of differentiation along the elevation gradient than fungi (Figs. 1 and S2). The slope of the distance-decay curve was significantly lower for bacteria than for fungi, while the halving distance was significantly greater (Table 1). In contrast to our third hypothesis, the initial similarity was significantly higher for bacteria than for fungi (Table 1).

In conclusion, this study demonstrates that phyllosphere fungal communities differentiate more thoroughly than bacterial communities over small distances (c.a. 50 m), and also over longer distances (c.a. 20 km) along an elevation gradient. Higher dispersal ability of phyllosphere bacteria, and higher sensitivity of phyllosphere fungi to temperature variations, may account for these findings. Further experiments are required to assess the relative importance of dispersal and selection by climatic conditions on the spatial structure of phyllosphere fungal and bacterial communities.

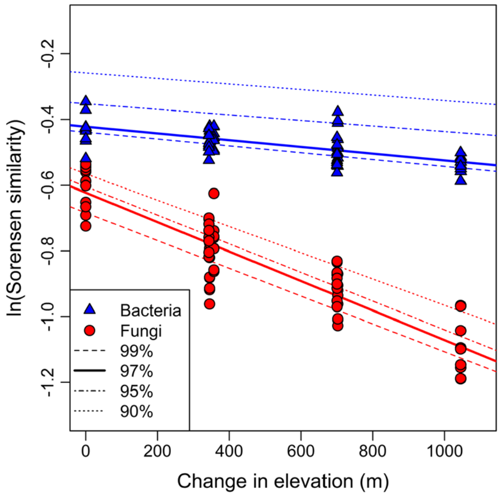

Fig. 1 Decay of similarity in phyllosphere fungal and bacterial communities (in *red* and *blue*, respectively) along an elevation gradient, as a function of the elevation difference between sampling plots and the identity threshold used for OTU clustering. *Circles* and *triangles* represent compositional similarities between samples at the 97 % identity threshold. *Lines* represent linear regressions for the various thresholds

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