

**Method or madness: does OTU delineation bias our perceptions of fungal ecology?**

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37 *al.*, 2012) , resulting in clades with a range of intraclade sequence similarities. This method is  
38 analogous to the virtual taxon approach developed by Öpik et al. (2008). Lekberg et al. find that  
39 the method employing a universal cutoff results in substantially greater numbers of OTUs  
40 (alpha diversity) than the method using the evolutionary metric, with the greatest disparity at  
41 the local scale (Figure 1A). However, both methods produced OTUs that exhibited consistent  
42 ecological sorting among different spatial scales and environmental gradients in their datasets  
43 (beta diversity). The range of spatial extents over which this pattern is observed is substantial,  
44 from a scale of metres to hundreds of kilometres, and while there may be variation in the  
45 differences between alpha and beta diversity estimates at these different scales (Figure 1A and  
46 B, with the largest differences in alpha and beta diversity being observed for the 'local' and  
47 'regional' datasets, respectively), the statistical significance of different ecological variables did  
48 not appear to be affected by the OTU delineation method.

49 These results largely agree with a previous evolutionary model-based approach to OTU  
50 delineation (Powell *et al.*, 2011), and together they suggest that ecologically significant OTU  
51 delineation may occur using a level of sequence similarity that is lower than commonly used  
52 universal cutoffs. These findings support AM fungal community assembly as a product of  
53 phylogenetic niche conservatism of functional traits that affect fitness along certain  
54 environmental gradients. Previous work has focused on deeply rooted clades of AM fungi that  
55 mainly differ in their capacity to produce extensive networks of intraradical or extramatrical  
56 hyphae, which can influence the outcome of interspecific interactions among closely related  
57 species (Maherali & Klironomos, 2007). Although we lack a full understanding of the autecology  
58 of AM fungi, species distribution modeling suggests some coherence in species responses  
59 within families to long gradients in edaphic properties (Veresoglou *et al.*, 2013). Although  
60 encompassing multiple spatial scales, each of the datasets analysed by Lekberg et al. and  
61 Powell et al. encompass significant shifts in land management and vegetation types, as well as  
62 severe disturbance, so these conclusions are primarily being drawn when considering long  
63 gradients in the environment. In this case, and for taxa exhibiting this degree of phylogenetic  
64 niche conservatism, a clustering approach that results in fewer, monophyletic OTUs may be  
65 preferred to improve the power to detect correlations among environmental variables and the  
66 distribution of OTUs across samples. Although not statistically significant, this benefit is likely  
67 indicated by the improved ability of 'MCA' OTUs to explain environmental variation at the  
68 'regional' spatial scale compared to the universal cutoff OTUs (Figure 1B). An additional benefit  
69 is that there is less opportunity for sequence variation (whether real variation within  
70 populations or occurring due to errors during sequencing) to generate noise in OTU-sample  
71 tables.

72 But could small trait differences among closely related taxa be functionally more important  
73 along short environmental gradients? While phylogenetic niche conservatism might be the

74 norm when comparing quite divergent environments, genetic variation within species of AM  
75 fungi might result in differential fitness of populations across small shifts in the environment.  
76 Delineating OTUs that detected these AM fungal ecotypes may require higher resolution than  
77 monophyletic taxa or the universal cutoff currently used. The supervised learning approach  
78 employed by Lekberg et al. (Figure S5 of their letter) may actually provide some indirect  
79 support for this; when assigning AM fungal assemblage to plant communities, Lekberg et al.  
80 observed that the 90% and 99% universal cutoffs produced similar error rates. However, the  
81 lowest error rate was observed using a universal cutoff of 98 %, suggesting even better  
82 correspondence between delineated OTUs and this axis in the environment. While substantial  
83 intraspecific functional variation in some isolates of AM fungi has been observed (e.g.,  
84 Munkvold *et al.*, 2004), our understanding of the incidence of ecotypes in the natural  
85 environment lags behind that of other groups of fungi (e.g., Taylor *et al.*, 2014).

86 Other community assembly mechanisms, including dispersal and local extinction may also  
87 overwhelm environmental filtering and biotic interactions within short environmental  
88 gradients. For example, Caruso et al. (2012) also used the 'sites' data analysed here by Lekberg  
89 et al. and observed differing patterns of community assembly under the different treatments  
90 included in the study. Colonisation of *Plantago lanceolata* roots, in the presence or absence of  
91 disturbance, resulted in highly divergent assemblages of AM fungi within roots, indicative of  
92 environmental filtering along an unidentified gradient or strong biotic interactions among  
93 colonisers. However, AM fungal assemblages associated with roots sampled from the  
94 surrounding plant species converged upon a common composition, indicating a deterministic  
95 assembly process, even though the identities of the host species likely changed among samples.  
96 These patterns were observed following the MCA approach for OTU delineation, so perhaps  
97 alternative approaches to OTU delineation could result in different, and possibly more intuitive,  
98 patterns. While researchers are often constrained to demonstrating statistically significant  
99 shifts in communities as a prerequisite to publish their work, there is a need to focus on AM  
100 fungal community assembly along small environmental gradients to determine at what level of  
101 environmental variation scientists need to concern themselves with comparing delineation  
102 methods (Figure 2). Alternatively, empirical demonstrations that ecological inferences are  
103 robust to OTU delineation approaches along small environmental gradients would help to  
104 confirm the claims by Lekberg et al. that the method chosen is relatively unimportant.

105 The final point made by Lekberg et al. (2014) is an important one: we need to keep in mind that  
106 the end goal of efforts to mass sequence DNA from the environment is the advancement of our  
107 understanding of AM fungal ecology and biogeography, and those of microorganisms in  
108 general. While there are very real issues to be dealt with regarding, for example, error  
109 correction and OTU clustering approaches, too much focus on what is the correct way to do this  
110 may impede progress in achieving this goal and evaluating the sensitivity of our inferences to

111 employing multiple, imperfect approaches (including approaches that are not based on high-  
112 throughput sequencing) may provide even more insight into these processes.

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158 **Figure Legends:**

159 Figure 1: Differences in alpha and beta diversity at different spatial scales (site, local and  
160 regional) based on data in Lekberg et al. 2014. Panel 1A shows the percentage increase in OTU  
161 numbers based on delineation using the 97% cutoff method relative to the MCA evolutionary  
162 method. Panel 1B shows the differences in variance explained at the different scales in both the  
163 ANOSIM and the BEST model.

164  
165 Figure 2: Hypothesized relationships between OTU delineation approach and environmental  
166 variation (low, medium and high) on estimates of alpha and beta diversity. The box to the right  
167 of the figure indicates the environmental gradient over which Lekberg et al. (2014) and Powell  
168 et al. (2011) have likely evaluated this relationship. The y-axis (not-scaled) is similar to figure 1  
169 indicating the predicted differences in the metrics based on OTU delineation method alone.

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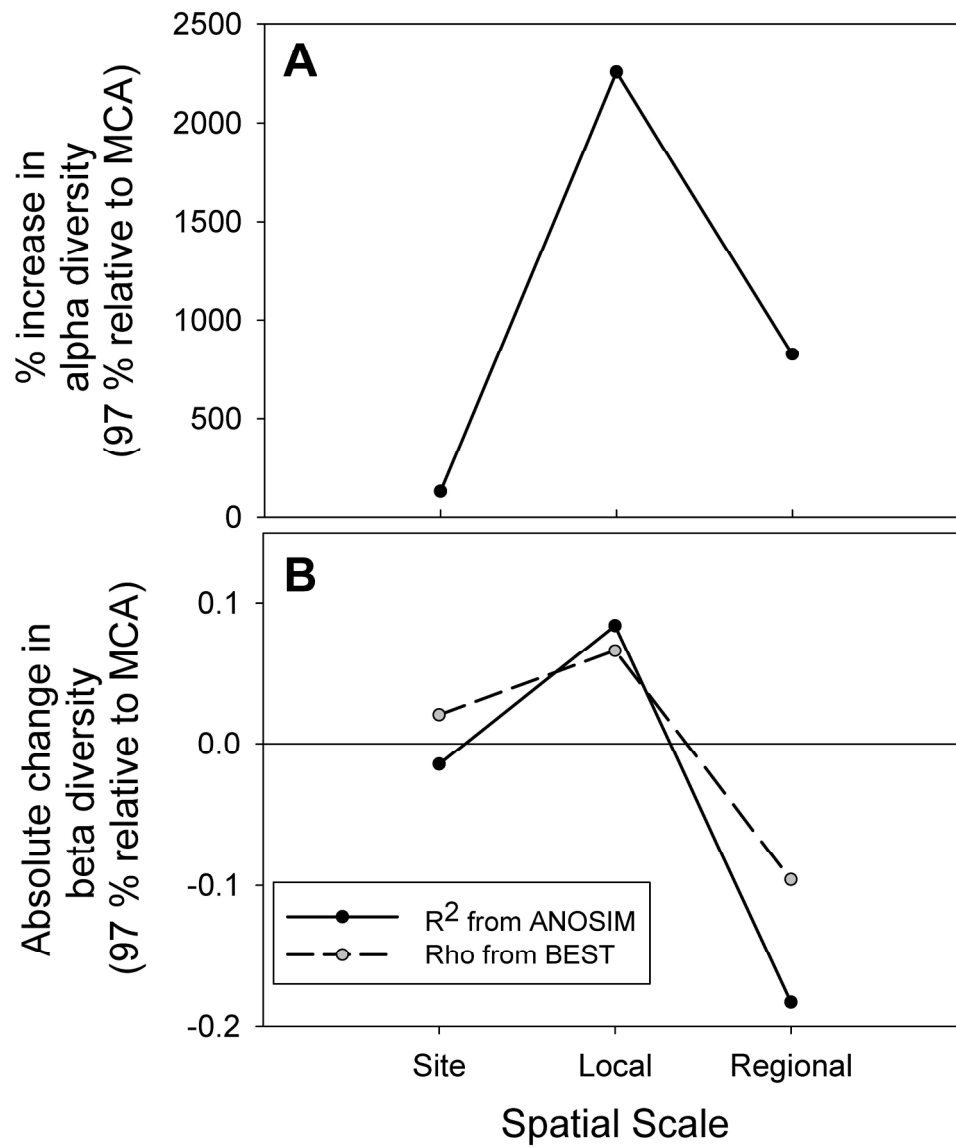


Fig. 1  
216x259mm (300 x 300 DPI)

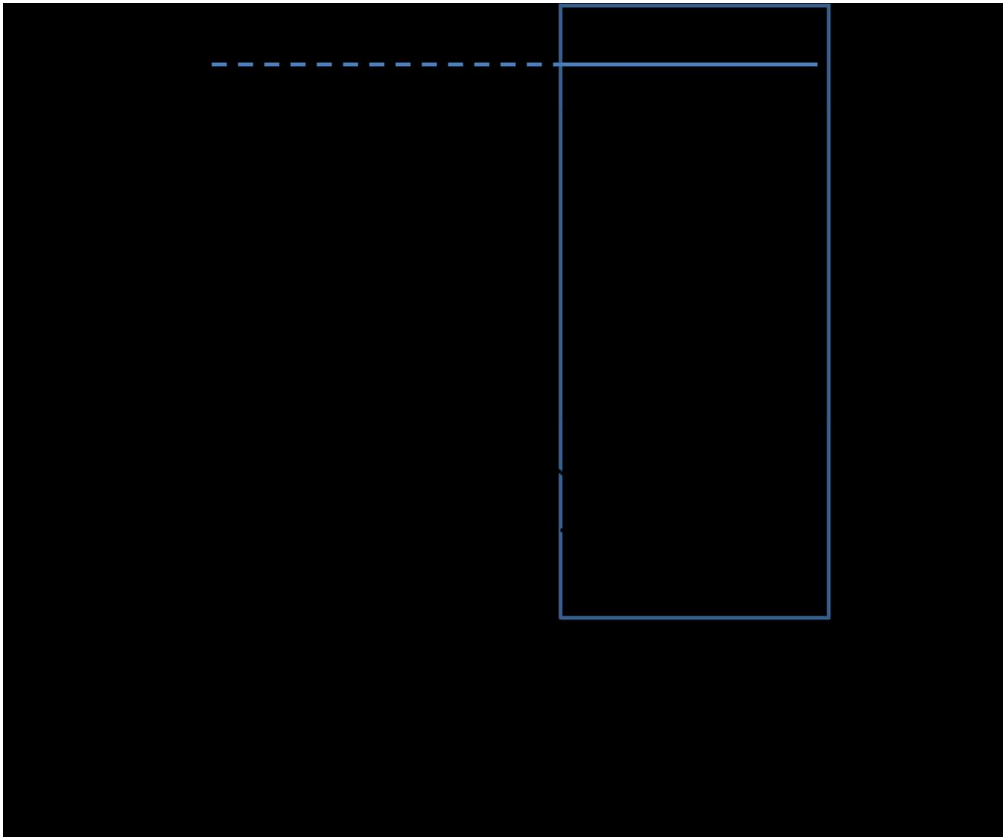


Fig. 2  
180x151mm (150 x 150 DPI)