Phylogenetic congruence of lichenised fungi and algae is affected by spatial scale and phylogenetic diversity

The role of species' interactions in structuring biological communities remains unclear. Mutualistic symbioses, involving close positive interactions between two distinct organismal lineages, provide an excellent means to explore the roles of both evolutionary and ecological processes in determining how positive interactions affect community structure. In this study, we investigate patterns of co-diversification between fungi and algae for a range of New Zealand lichens at the community, genus, and species levels and explore explanations for possible patterns related to spatial scale and pattern, phylogenetic diversity of the taxa considered, and the levelsampling replication. We assembled six, independent datasets to compare patterns in phylogenetic congruence with varied spatial extent of sampling, phylogenetic diversity and level of specimen replication. For each dataset, we used the DNA sequences from the ITS regions of both the fungal and algal genomes from lichen specimens to produce genetic distance matrices. Phylogenetic congruence between fungi and algae was quantified using distance-based redundancy analysis and we used geographic distance matrices in Moran's eigenvector mapping and variance partitioning to evaluate the effects of spatial variation on the quantification of phylogenetic congruence. Phylogenetic congruence was highly significant for all datasets and a large proportion of variance in both algal and fungal genetic distances was explained by partner genetic variation. Spatial variables, primarily at large and intermediate scales, were also important for explaining genetic diversity patterns in all but one very small-scale dataset. Interestingly, spatial structuring was stronger for fungal than algal genetic variation. As the spatial extent of the samples increased,so too did the proportion of explained variation that was shared between the spatialvariables and the partners' genetic variation. Different lichen taxa showed some variation in their phylogenetic congruence and spatial genetic patterns and where greater sample replication was used, the amount of variation explained by partner genetic variation increased. Our results suggest that the phylogenetic congruencepattern, at least at small spatial scales, is

likely due to reciprocal co-adaptationor co-dispersal. However, the detection of these patterns varies among different lichen taxa, across spatial scales and with different levels of sample replication. This work provides insight into the complexities faced in determining how evolutionary and ecological processes may interact to generate diversity in symbiotic association patterns at the population and community levels. Further, it highlights the critical importance of considering sample replication, taxonomic diversity and spatial scale in designing studies of co-diversification.

1 **Phylogenetic congruence of lichenised fungi and algae is affected by spatial scale and phylogenetic**

2 **diversity**

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7 INTRODUCTION

8 Ecologists still do not have a full understanding of how species' interactions affect the structure of

9 biological communities. We have a long history of theoretical and empirical work on the roles of

10 competition and predation (Chase & Leibold 2003; Tilman 1982), but a much poorer understanding of

11 how positive interactions, such as facilitation and mutualisms, drive community phenomena, such as

12 species' diversity (Gross 2008; Stachowicz 2001). Over the last few decades, there has been an increase

13 in interest in the role of positive interactions, with many empirical studies showing that it is the balance

14 between positive and negative interactions that is important in structuring communities (e.g., Bertness &

15 Callaway 1994; Elias et al. (2008; LaJeunesse 2002; Thrall et al. 2007; Waterman et al. 2011).

16　　　　Because mutualistic symbioses involve very close positive interactions between two distinct

17 organismal lineages, they provide an excellent opportunity to specifically explore how positive

18 interactions influence community structure and to evaluate the relative importance of evolutionary and

19 ecological processes in the way that positive interactions affect community structure. Tightly interacting

20 taxa, such as host-parasite systems and certain plants and their insect pollinators, typically show high

21 degrees of phylogenetic congruence between hosts and associates, where the phylogeny of one taxon

22 closely tracks the phylogeny of the partner taxon (Cuthill & Charleston ; Light & Hafner 2008; Quek et al.

23 2004; Subbotin et al. 2004), largely due to co-evolutionary processes. What is not as clear is whether

24 most obligate species-level symbiotic relationships, such as ~~lichens~~ and corals, also have measureable　seen in

25 levels of codivergence. Typically, these diffuse symbiotic and mutualistic interactions are complicated by

26 variation in the specificity of the partners or where more than one symbiotic partner is involved, such as

27 vascular plants and their root-inhabiting mutualists (Hollants et al. 2013; Lanterbecq et al. 2010; Walker et

28 al. 2014). Further, it is much less clear that the causal processes involved in these coevolving symbiotic

29 relationships will produce a pattern of codivergence given the increased opportunity for host switching.

30　　　　Lichens are a classic example of a mutualistic symbiosis. Lichen thalli are the result of an

31 association between a fungus (the mycobiont) and a photobiont, which is usually a green alga, but may

32 also be a cyanobacterium (Nash 2008). Around 3-4% of lichens are tripartite involving a symbiosis of

33   both a green alga and a cyanobacterium (Henskens et al. 2012). In all lichens, the photobiont provides

34   photosynthate to the mycobiont, which in turn provides habitat, water and nutrients to the photobiont

35   (Honegger 1991; Nash 2008). The symbionts of lichens are relatively poorly known because they are very

36   often difficult to culture and identify, particularly many of the photobiont partners (Ahmadjian 1993; del

37   Campo et al. 2013; Grube & Muggia 2010; Honegger 1991). However, in the last two decades a great

38   deal of molecular work has been done to address this showing variable patterns in partner identity and

39   other patterns of association (DePriest 2004). This variable, and seemingly diffuse, mutualism provides a

40   complex model system for addressing questions regarding the role of evolutionary processes in forming

41   and driving ecological patterns in species interactions and how this affects community structure.

42   The first step in understanding how a mutualistic symbiosis might affect community structure is to

43   determine whether or not there is specificity in the symbiosis. In the case of lichens, we know that

44   although there is no evidence for very tight co-evolutionary relationships at the species level, phylogenetic

45   patterns have been observed where particular fungal taxa preferentially partner with particular algal taxa

46   (Fernández-Mendoza et al. 2011; Yahr et al. 2004) resulting in a correlation in the respective genetic

47   distances of each partner. For example, Widmer et al. (2012) found similar genetic structures for the

48   lichen symbiosis between the mycobiont, *Lobaria pulmonaria*, and its photobiont, *Dictyochoropsis*

49   *reticulata* within Europe. Conversely, it seems that some lichenised algal taxa are capable of partnering

50   with a range of fungal taxa (Beck 1999); thus, the specificity (*sensu* Piercey-Normore & DePriest 2001)

51   of the symbiosis appears to be driven by fungal selectivity (*sensu* Piercey-Normore & DePriest 2001).

52   For example, Nelsen & Gargas (2008) observed a lack of phylogenetic congruence between the algae and

53   fungi in specimens from the lichen genus *Lepraria*. Thus, it appears that there is variability in association

54   patterns among different lichen taxa (Fahselt 2008).

55   Several mechanisms are proposed to underpin the patterns in phylogenetic congruence observed

56   for lichens. First, co-evolutionary processes, whereby one partner adapts to take better advantage of the

57   symbiosis, may lead to a reciprocal adaptive evolutionary change in the other partner, although there is

58   little evidence for this in the literature (Yahr et al. 2006). Second, many lichens asexually reproduce,

59   either by fragmentation or specialised structures (Walser 2004), so that the resulting offspring lichens

60   contain clones of their parents, otherwise known as 'vertical transmission' (Dal Grande et al. 2012; Werth

61   & Scheidegger 2011). Sexual reproduction in green algal photobionts (apart from those in the

62   Trentepholiales) is thought to be extremely rare within lichen thalli (Friedl & Büdel 2008; Sanders 2005),

63   despite evidence of recombination within these taxa (Kroken & Taylor 2000). If symbiont co-dispersal is

64   coupled with genetic drift, a pattern of co-diversification is likely to emerge. However, 'horizontal

65   transmission' of photobionts into newly forming thalli is thought to occur, such as in the form of escaped

66   zoospores (Beck et al. 1998), and population genetics studies show evidence of algal switching (Dal

67   Grande et al. 2012; Kroken & Taylor 2000; Nelsen & Gargas 2008; Piercey-Normore & DePriest 2001).

68 Further, most green algal photobionts commonly occur in a free-living state, although for some taxa, such

69 as *Trebouxia*, much about their life cycles and availability in the environment is unknown (Sanders 2005).

70 A free-living state is likely to decrease the importance of vertical transmission and decrease the

71 congruence of phylogenetic patterns. Third, spatial structure in fungal and algal distributions could drive

72 patterns in phylogenetic congruence. Spatial structure in either fungi or algae could arise through

73 dispersal limitation, habitat fragmentation, or niche differentiation, such as variation in habitat

74 preferences. For example, (Werth et al. 2007) showed that the mycobiont of the cyanobacterial lichen,

75 *Lobaria pulmonaria*, varied genetically over spatial scales of less than a few kilometres in a pasture-

76 woodland landscape and suggested that this could be caused by dispersal limitation among habitat patches.

77 Peksa & Škaloud (2011) showed that the spatial distribution patterns of ~~the~~ *Asterochloris* in Europe and

78 North America, the green algal photobiont for two different lichen genera, were driven by substrate type

79 and relative exposure to rain and sun. If spatial structure in fungal and algal distributions resulted in

80 limited availability of one or both partners relative to the other, this would lead to a congruent

81 phylogenetic pattern. example, Marini et al. (2011) found that communities of the 822 species of

82 epiphytic lichens within Italy with different photobiont types (Chlorococcoid green algae, Cyanobacteria

83 or *Trentepohlia*) showed different biogeographic patterns across climatically different areas. Thus, if

84 spatial structure in genetic variation results from differential distributions of algal or fungal ecotypes, this

85 could result in phylogenetic congruence for specimens compared across environmental gradients. For

86 example, the patterns in variable algal selectivity that Vargas Castillo & Beck (2012) observed within the

87 genus *Caloplaca* in the Atacama Desert in Northern Chile were related to changing habitat conditions

88 along an altitudinal gradient.

89   Although much recent research has shown that lichenised fungi specialise on particular algae

90 regardless of the availability of other species, most work has been conducted at the within-species and

91 within-genus levels, and much less often at higher levels of phylogenetic diversity. Such patterns and

92 their explanations, like most ecological phenomena, are likely to be scale-dependent and related to both

93 small scale processes, such as the dispersal of lichen propagules, as well as larger scale biogeographic

94 processes and climatic variation. In addition, these patterns are likely to depend on the amount of

95 phylogenetic diversity contained within the dataset considered. We expect that if co-evolutionary

96 processes play a role, then phylogenetic congruence should be stronger when considering higher levels of

97 phylogenetic diversity because they are the accumulation of a longer period of evolutionary change. To

98 examine these patterns and effects, we tested patterns of association for a range of New Zealand lichens at

99 the community, genus, and species levels. We assembled six independent datasets that varied in the

100 spatial extent of sampling, phylogenetic diversity and the level of specimen replication so that we could

101 compare patterns in phylogenetic congruence across these variables. We have taken a novel approach to

102 the analysis of phylogenetic congruence that uses Moran's eigenvector mapping, distance-based

103   redundancy analysis and variance partitioning, which allows us to evaluate the effects of sampling on the

104   quantification of phylogenetic congruence. We interpret the patterns in the light of the relative importance

105   of the mechanisms driving variation in co-diversification patterns.

106   METHODS

107   *Lichen specimen collection*

108   Lichen thallus samples were collected from many mapped locations around both the North and South

109   Islands of New Zealand (under New Zealand Department of Conservation low-impact research and

110   collection permit, CA-31641-FLO). Samples were taken either from the ground, or from trees and

111   structures like fence posts and stored in paper envelopes. Each sample was identified to the lowest

112   taxonomic level possible and assigned a specific sample code. Six, non-overlapping sample sets were

113   collected (Table 1): (1) Multiple lichen species collected on mountain beech (*Nothofagus solandri* var.

114   *cliffortioides*) trees within less than 1 km^2 of Flock Hill Station (Buckley 2011), (2) *Usnea* spp. specimens

115   from Flock Hill Station, (3) *Usnea* spp. specimens from Craigieburn Forest Park, (4) *Usnea* spp.

116   specimens from sites around New Zealand, and (5) *Ramalina* spp. specimens from around New Zealand

117   (Figure 1).

118   *Molecular analysis*

119   Total DNA was extracted from surface sterilised lichens using Plant DNA Mini Kit (Bioline,

120   London, UK) following the manufacturer's instructions. Photobiont and mycobiont ITS rRNA were

121   amplified using specific algal (nr-SSU-1780-5' Algal and ITS4) and fungal (nr-SSU-1780-5' Fungal and

122   ITS) primers described by (Piercey-Normore & DePriest 2001). PCR amplification was performed in a 25

123   µl reaction volume. Each 25 µl reaction contained 1× GoTaq® Green Master Mix, 5 pmol of each primer,

124   10 µg purified bovine serum albumin (BSA; New England BioLabs, Ipswich, MA, USA) and 1 µl of the

125   extracted DNA (25-30 µg/µl). The thermal cycle for the algal reaction was as follows: initial denaturation

126   at 94°C for 2 min, then cycles of denaturation at 94°C for 30 s, annealing at 50°C for 45 s, extension at

127   72°C for 2 min, then a final extension at 72°C for 7 min. The thermal cycle for fungal rRNA amplification

128   was the same as the algal one except the annealing time was increased to 54°C.

129   *Data analysis*

130   For each of the six datasets, algal and fungal DNA sequences were aligned separately using 'Prankster'

131   (Löytynoja & Goldman 2005) with the default parameters. These alignments were used to calculate

132   genetic distance matrices from the raw distances using uncorrected p-distances (Paradis 2006)

133   implemented by the 'dist.dna' function in the 'ape' package in R (R Core Team 2013). We repeated the

134   analyses using a genetic distance matrix calculated using the TN93 substitution model (Tamura & Nei

135 1993). The results (not shown) were congruent with those obtained using p-distances, so we present the p-

136 distance results only.

137   To relate fungal and algal genetic distances to each other and to describe their variation in space,

138 distance matrices were used in a combined analysis using Moran's eigenvector mapping and variance

139 partitioning, following (Borcard et al. 2011, pp. 258). This analysis was used to describe and partition the

140 variation in algal genetic distances between (a) the fungal genetic distance matrix and (b) a matrix of

141 spatial variables. The spatial variables were derived using the Moran's eigenvector maps (MEMs)

142 procedure (Borcard et al. 2011) implemented using the 'pcnm' function in the R package 'vegan', which

143 uses a principal coordinates analysis to represent different scales of spatial variation for the given set of

144 sample locations (Borcard et al. 2011). MEM analysis produces one fewer spatial variables than there are

145 sample points, describing all possible spatial variation in the data from broad scale variation to very fine

146 scale variation. Only the most important subset of these spatial variables (MEMs) was included in a

147 distance-based redundancy analysis (db-RDA) analysis, which relates multivariate data (algal genetic

148 distance matrix) to explanatory matrices (fungal genetic distance matrix and spatial variables). The

149 selected MEMs were those that were significantly related to the algal distance matrix in a distance-based

150 RDA and forward selection procedure using the 'capscale' and 'ordistep' functions in the 'vegan' package

151 in R (Oksanen et al. 2013). Variance partitioning calculations were conducted following procedures

152 outlined in (Borcard et al. 2011). The 'capscale' function performs a redundancy analysis that seeks the

153 series of linear combinations of the explanatory factors that best describe variation in the response matrix,

154 constrained by the two explanatory matrices (Borcard et al. 2011). The variance partitioning procedure

155 computes R^2 canonical values analogous to the adjusted R^2 values produced in multiple regression (Peres-

156 Neto et al. 2006). The analysis indicates how much total variation in the response matrix (e.g., algal

157 genetic distance) is explained by each of the explanatory matrices alone, as well as the component of

158 shared variation, e.g., spatially structured variation in fungal genetic distances. This analysis was also

159 performed using the fungal genetic distance matrix as the dependent matrix and the algal genetic distance

160 matrix as the explanatory matrix to allow comparison of the degree of spatial correlation in each of the

161 matrices.

162   To test the significance of the phylogenetic congruence between fungi and algae for each of the

163 six datasets, we used the Procrustes approach to co-phylogeny (PACo, Balbuena et al. 2012). This

164 procedure performs a principal coordinates analysis on the algal genetic distance matrix followed by a

165 Procrustes rotation of the fungal genetic distance matrix, while retaining the information that algae and

166 fungi are paired in particular lichen specimens (Balbuena et al. 2012). A sum of squares is calculated from

167 the individual residuals for each specimen that represents the lack of fit of the fungal genetic distance

168 matrix onto the principal coordinate analysis result for the algal genetic distance matrix (Balbuena et al.

169 2012). The algal-fungal pairing matrix, i.e., which alga is paired with which fungus, is then randomised

170 10,000 times and the sums of square values recalculated. The observed sum of squares value is then

171 compared to the distribution of values from the randomisations to determine the probability of obtaining

172 the observed result under random expectation (Balbuena et al. 2012). The magnitude of the residual for

173 each lichen specimen shows its relative lack of fit to a co-diversification pattern. Therefore, for three

174 datasets for which we had additional information on specimen traits, we compared individual residuals

175 among specimens to determine which ones contributed most to the observed association pattern. These

176 three datasets (and trait information) were the Flock Hill community (growth form), Flock Hill *Usnea* and

177 New Zealand *Usnea* datasets (apothecia present or absent). Raw genetic and geographic distance matrices

178 are provided as supplemental files.

179 RESULTS

180 The six datasets captured a wide range of geographic extent and, unsurprisingly, the datasets with greatest

181 numbers of different lichen morphotypes contained the greatest fungal genetic diversities (Table 1). Algal

182 genetic diversity had only a weak, positive correlation with fungal genetic diversity (Pearson's r = 0.39; n

183 = 6).

184 The db-RDA showed that spatial variables were important for explaining genetic diversity patterns in

185 all but the Flock Hill community dataset (Table 2, Significant MEMs). Large-scale variables, e.g. low

186 numbered MEMs, were important for all spatially-structured datasets. Genetic variation in *Ramalina*

187 algae and fungi was significantly related to several large-scale MEMs, showing that spatial pattern in

188 relatedness varied at the larger scales within this spatial extent, such as between the North and South

189 Islands (Table 2). Some intermediate scale MEMs were also important, illustrating additional, more

190 complex, spatial patterns. Similarly, for the *Usnea* datasets, large-scale MEMs were of greatest

191 importance, along with some intermediate-scale, but no very fine-scale, MEMs.

192 Variance partitioning showed that a large proportion of variance in both the algal and fungal genetic

193 variation was explained by genetic variation in the partner (Figure 2). In general, the proportion of

194 explained variation was high, at 75% or more (Table 2, Figure 2). Interestingly, across all datasets for

195 which spatial variation was important, the fungal genetic variation was better explained by spatial

196 variables than was the algal genetic variation for the same lichens (Figure 2). As the spatial extent of the

197 samples increased, so too did the proportion of explained variation that was shared between the spatial

198 variables and the partners' genetic variation (Figure 2).

199 PACo analysis showed that the co-diversification for all datasets, regardless of whether the dependent

200 matrix was the algae or the fungi, was significant at the alpha = 0.05 level (Table 1), indicating that it is

201 very unlikely that the correlations in the fungal and algal genetic distances have arisen by chance. When

202 comparing the individual residuals from the PACo analysis, for the Flock Hill community dataset, the

203   *Usnea* and *Ramalina* specimens, which made up all but one of the foliose specimens, had relatively good

204   fit to the co-diversification pattern, compared to most other taxa (Figure 3). For the Flock Hill *Usnea* and

205   New Zealand *Usnea* datasets, the presence of apothecia did not appear to be related to the relative

206   specimen contribution to the lack of phylogenetic congruence (Figure 3).

207   DISCUSSION

208   We show that there is a strong and significant pattern of phylogenetic congruence for the algae and fungi

209   found in New Zealand lichens, both across multiple taxa within a community and within two widespread

210   genera sampled at spatial extents from less than 1 km^2, to the whole country (*c*. 260,000 km^2). For

211   instance, at the small spatial scales of the Flock Hill study site, there was surprisingly high phylogenetic

212   congruence, as demonstrated by the high variance explained by partner genetic variation. Such

213   congruence suggests that fungi and algae are not randomly distributed across the lichen symbiosis for the

214   taxa considered. Our results show that this non-random pattern is affected by (1) spatial scale, (2) taxon

215   considered, (3) phylogenetic diversity and (4) level of sample replication.

216   The amount of genetic variation explained by spatially-structured partner genetic variation

217   increased with increasing spatial extent (Figure 2) suggesting that a large amount of phylogenetic

218   congruence is likely to be due to the distributions of fungi and algae at larger, rather than smaller, spatial

219   scales. In addition, the spatial structuring of *Usnea* and *Ramalina* fungal genetic distances was more

220   prominent than for algae, suggesting that drivers of fungal distributions were more important in

221   determining these congruence patterns than drivers of algal distributions. It appears that the algae are

222   more dependent on the distribution of the fungi than the fungi are on the algae. This is in contrast to the

223   findings of (Marini et al. 2011), who suggested that the richness of lichens within Italy was dependent on

224   the distribution of different algal types. However, this study encompassed much larger phylogenetic

225   diversity, i.e., kingdom-level diversity in photobionts, than is considered here. Thus, the strong spatial

226   signal in our results show that at large spatial scales, at least some of the co-diversification pattern appears

227   likely to be due to processes other than co-evolution or vertical transmission. If factors such as variation

228   in habitat preferences or photobiont availability were important, we would expect to see an increase in the

229   proportions of genetic variance being explained by spatially-structured variation in the genetic distances

230   of the partners. This is indeed the result we observe across the four *Usnea* datasets. One other paper that

231   mentions the effects of spatial scale on phylogenetic congruence patterns is a study of *Cladonia* lichens

232   across six discrete rosemary scrub sites in three regions in Florida (Yahr et al. 2004). Their findings

233   illustrated that, across Florida, photobiont genetic variation was not significantly spatially structured,

234   despite different fungi occurring at different sites. These contrasting findings suggest that different

235   patterns of phylogenetic congruence may occur in different lichen taxa.

236   The variation in the patterns from the PACo for individual lichen specimens also suggest that the
237   taxon considered may affect the observed phylogentic congruence pattern. The fruticose taxa had lower
238   residual values in the PACo analysis showing that they contributed relatively more to the phylogentic
239   congruence pattern than did crustose and foliose taxa. However, these lichen specimens were primarily
240   *Usnea* and *Ramalina* suggesting that this variation may have a phylogenetic basis, rather than being due to
241   the life form. There was little pattern observed when we related the presence of apothecia to their
242   contribution to the co-diversification pattern for the *Usnea*-only datasets (Figure 3) suggesting that, at
243   least at this scale and level of replication, ability for the fungal component to sexually reproduce had little
244   to do with the observed pattern. Residual values are not comparable among datasets, so it is not possible
245   to evaluate these differences among spatial scales. Overall, these results are consistent with the variable
246   patterns in lichen co-diversification in the literature (Ruprecht et al. 2012; Vargas Castillo & Beck 2012).

247   Our study encompassed a range of phylogenetic diversities for both fungi and algae. Specifically,
248   when fungal diversity was very high, algal diversity was also relatively high, despite the very small spatial
249   scale (Table 1, Flock Hill community dataset). For *Usnea*, when fungal genetic diversity was low, algal
250   diversity was low or high depending on whether the spatial scale was small or large, respectively (Table
251   1). However, if we consider only the two datasets at the smallest spatial scale, despite the variation in
252   genetic diversity, the pattern of phylogenetic congruence did not vary; the Flock Hill datasets both show
253   high levels of phylogenetic congruence despite having the largest difference in genetic diversity (Figure
254   2). This is consistent with arguments suggesting that coevolution is not an important driver of the lichen
255   symbiosis (Yahr et al. 2006), because if coevolution was important, then we would expect to see an
256   increase in the degree of phylogenetic congruence with increasing genetic diversity.

257   By contrasting the result from the NZ *Usnea* dataset and the NZ *Usnea* replicated dataset, we can
258   consider the effects of within-site replication on the phylogenetic congruence signal. This result shows
259   that where greater replication was used, the amount of variation explained by partner genetic variation
260   increased. This highlights the importance of considering sampling design in studies of phylogenetic
261   congruence.

262   This work leads us to more questions regarding variation in phylogenetic congruence patterns and
263   its causes, which are likely to be scale-dependent. We need better understanding of the factors influencing
264   association patterns including algal availability and niche differentiation, dispersal (metapopulation
265   dynamics), and reproductive traits. In particular, we need to understand the effects of lichen reproductive
266   modes better as many lichens reproduce both sexually and asexually. When the fungal component
267   sexually reproduces, the symbiosis must re-form which gives the fungus an opportunity to change
268   symbiotic partners. With asexual reproduction, the fungus and alga disperse together, and so, a
269   predominance of asexual reproduction may be one of the reasons we see such a strong co-diversification

270 signal in these taxa. However, the results of this study suggest that the spatial distributions of the fungi

271 and algae may also be important in determining the nature of the symbiosis, particularly at larger spatial

272 scales, as has been observed in some parasite lineages (e.g., du Toit et al. 2013). Thus, we need to do

273 more work on algal and fungal availability to determine if habitat preferences and/or dispersal limitation

274 led to some of the spatial patterns that we see. In addition, we need better understanding of the

275 availability of free living algae to lichens and what their microhabitat preferences are.

276   This study is consistent with previous research showing that, despite the diffuse nature of the

277 lichen mutualistic symbiosis, there is strong selectivity within the association. We show that despite

278 spatial structuring in algal, and particularly fungal, distributions at large spatial scales, the phylogenetic

279 congruence pattern, at least at small spatial scales, is due to reciprocal co-adaptation or, more likely, to co-

280 dispersal. However, the influence of these processes is likely to differ among different lichen taxa. This

281 work gives us insight into some of the complexities we face in determining how evolutionary and

282 ecological processes may interact to generate diversity in symbiotic association patterns at the population

283 and community levels.

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Figure 1 (on next page)

Map

Figure 1: Maps showing sample collection locations for (A) the three New Zealand datasets, (B) the Craigieburn *Usnea* dataset and (C) the Flock Hill community and *Usnea* dataset. Note that the Craigieburn samples were collected along a road running along an elevation gradient.
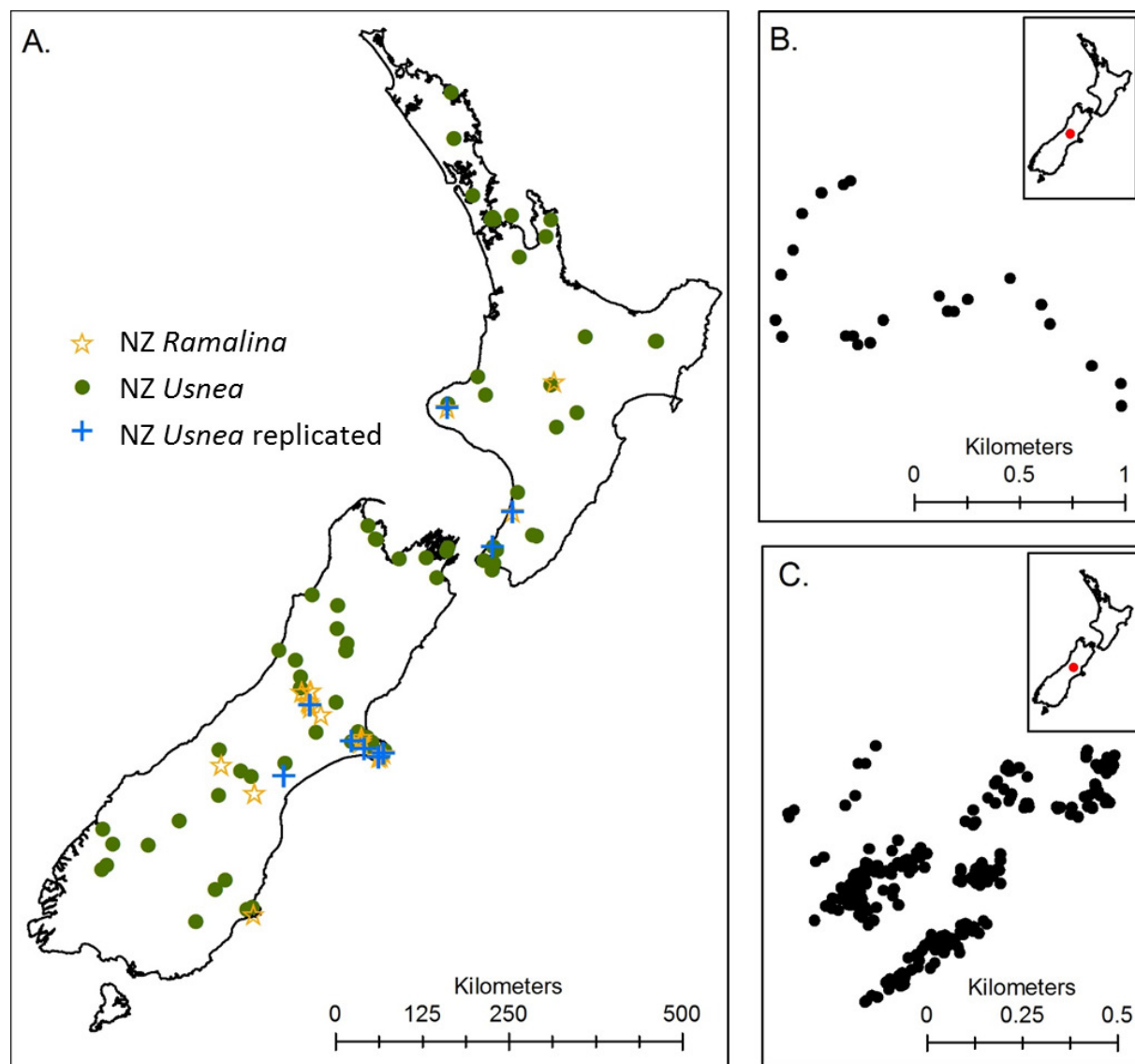
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Figure 2(on next page)

Variance partitioning graph

Figure 2: Bar charts showing variance partitioning for six independent datasets modelled as algal genetic variance as a function of fungal genetic variance and spatial variation (left) and fungal genetic variance as a function of algal genetic variance and spatial variation (right). The total variation in genetic distance is explained by partner genetic distance (red), independent spatial variation (blue), and spatially-structured variation in partner genetic distances (purple). Unexplained variation is shown in grey. The number of specimens sampled (n) is given for each dataset.
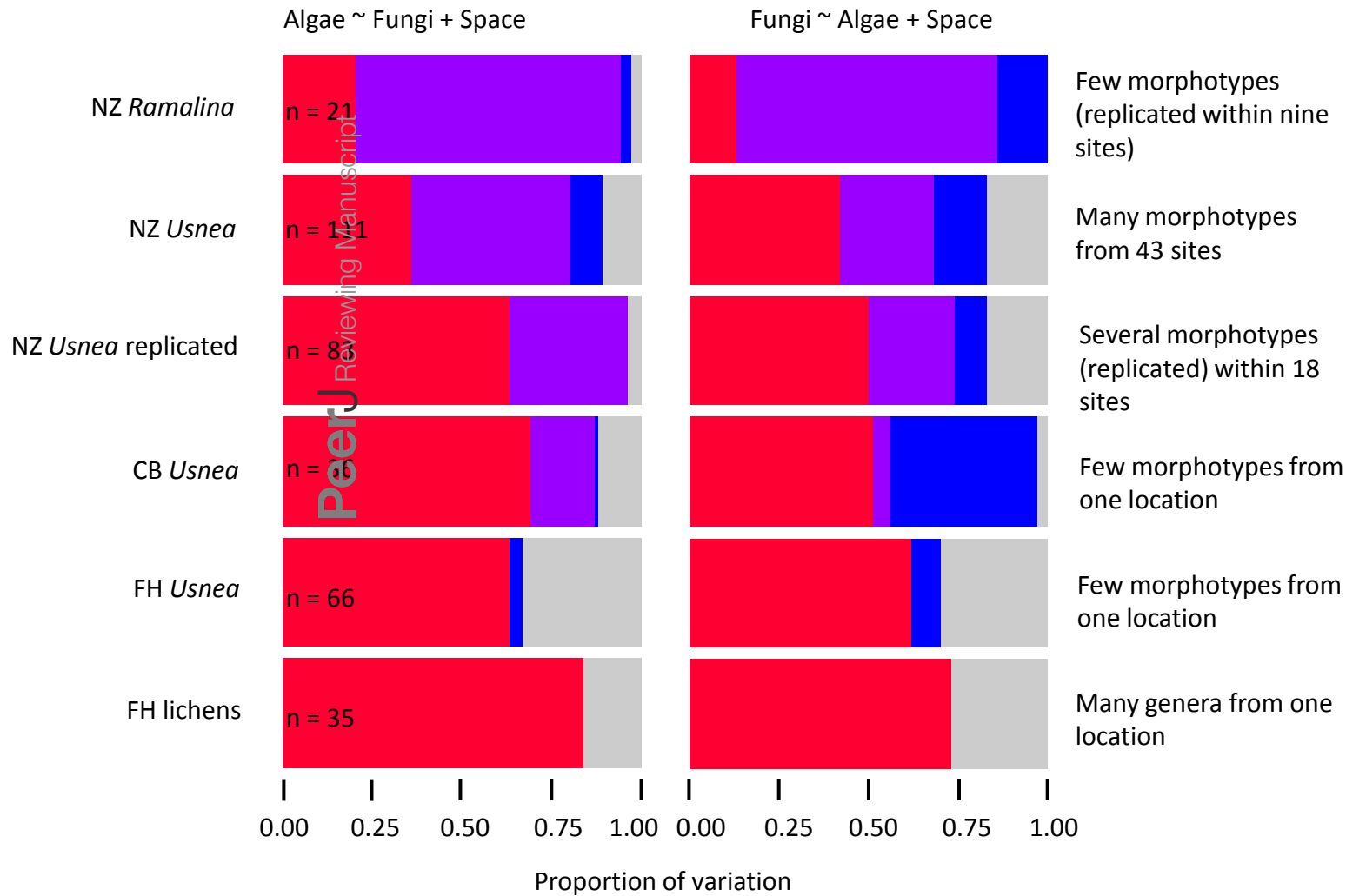
Algae ~ Fungi + Space   Fungi ~ Algae + Space

NZ *Ramalina*   n = 21   Few morphotypes (replicated within nine sites)

NZ *Usnea*   n = 111   Many morphotypes from 43 sites

NZ *Usnea* replicated   n = 83   Several morphotypes (replicated) within 18 sites

CB *Usnea*   n = 0   Few morphotypes from one location

FH *Usnea*   n = 66   Few morphotypes from one location

FH lichens   n = 35   Many genera from one location

0.00 0.25 0.50 0.75 1.00   0.00 0.25 0.50 0.75 1.00

Proportion of variation

Figure 3(on next page)

Species' bar charts

Figure 3: Bar charts showing the individual lichen specimen contribution to the Procrustes sums of squares for (A) the New Zealand *Usnea* dataset (n = 111), (B) the Craigieburn *Usnea* dataset (n = 36) and (C) the Flock Hill all specimen dataset (n = 67). Dashed line indicates the median sums of squares value. Bars for (C) are coloured by growth form: crustose (black), foliose (red) and fruticose (green) and for the other two datasets black bars indicate specimens that had apothecia and white bars are those that were asexual. Note that in (C) all but the first from the left of the fruticose specimens were specimens of *Usnea* or *Ramalina*.
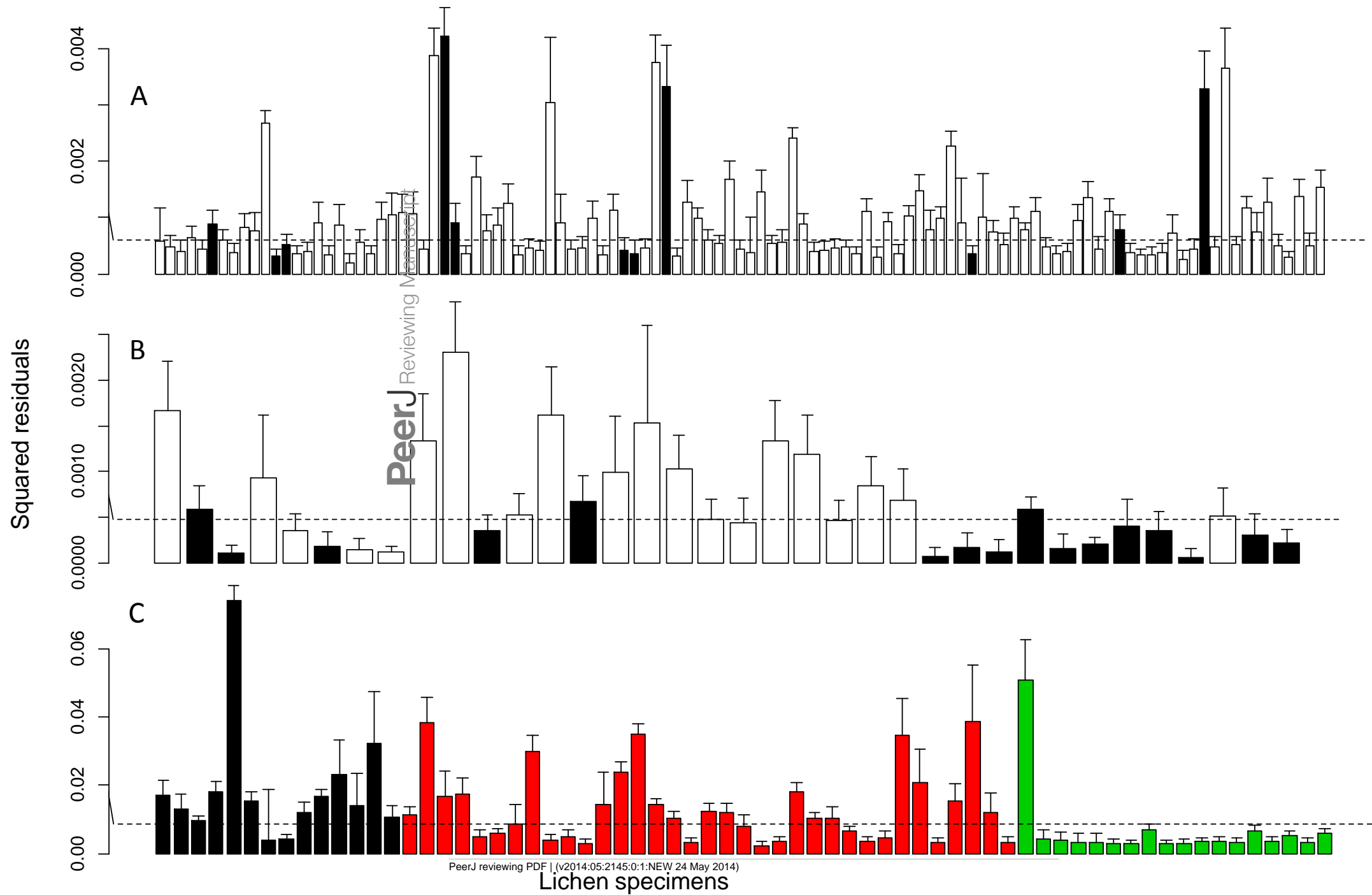
Table 1 (on next page)

List of datasets

Table 1: List of the datasets analysed showing the number of specimens sampled, the approximate number of lichen morphotypes, and the number of sites samples. Also given are the maximum distance between two sample points (Spatial extent) and the mean (standard deviation) genetic distance for each matrix. Note that only 35 of the 67 Flock Hill multiple species dataset specimens were mapped and were therefore analysed separately.

Table 1: List of the datasets analysed showing the number of specimens sampled, the approximate number of lichen morphotypes, and the number of sites samples. Also given are the maximum distance between two sample points (Spatial extent) and the mean (standard deviation) genetic distance for each matrix. Note that only 35 of the 67 Flock Hill multiple species dataset specimens were mapped and were therefore analysed separately.

Dataset	Number of specimens	Taxonomic variation	Number of sites	Spatial extent (m)	Algal genetic diversity		Fungal genetic diversity	
					Mean (S.D.)	Range	Mean (S.D.)	Range
NZ *Ramalina*	21	Few morphotypes (3)	9	581,576	0.08	0.0 – 0.16	0.06	0.0 – 0.11
NZ *Usnea*	111	Many morphotypes (17)	43	1,251,276	0.09	0.0 – 0.18	0.05	0.0 – 0.10
NZ *Usnea* replicated	83	Several morphotypes (9)	18	882,910	0.10	0.0 – 0.17	0.05	0.0 – 0.09
Craigieburn *Usnea*	36	Several morphotypes (6)	1	1,775	0.04	0.0 – 0.15	0.03	0.0 – 0.06
Flock Hill *Usnea*	66	Few morphotypes (3)	1	1,095	0.03	0.0 – 0.14	0.03	0.0 – 0.08
Flock Hill community mapped	35	Many lichen genera	1	796	0.06	0.0 – 0.22	0.13	0.0 – 0.33
Flock Hill community	67	Many lichen genera	1	1,141	0.08	0.0 – 0.49	0.15	0.0 – 0.38

Table 2(on next page)

Results table

Table 2: Results from db-RDA with variance partitioning and Procrustes approach to co-phylogeny giving the *P*- alue from a test randomising the association matrix for fungi and algae for the six independent datasets and thefull Flock Hill community dataset. Note that only 35 of the 67 Flock Hill multiple species dataset specimens were mapped and were therefore analysed separately from the full dataset. Variance partitioning divides the total variance up into portions explained by the partner genetic distance matrix (Partner), the purely spatial portion (Space), the spatially structured variation in the partner matrix (Shared) and unexplained variation (Unexpl.). The significant MEMs are given in order of their significance. For each dataset, there are n-1 MEMs in the total set and smaller MEM numbers represent larger-scale spatial pattern.

Table 2: Results from db-RDA with variance partitioning and Procrustes approach to co-phylogeny giving the *P*-value from a test randomising the association matrix for fungi and algae for the six independent datasets and the full Flock Hill community dataset. Note that only 35 of the 67 Flock Hill multiple species dataset specimens were mapped and were therefore analysed separately from the full dataset. Variance partitioning divides the total variance up into portions explained by the partner genetic distance matrix (Partner), the purely spatial portion (Space), the spatially structured variation in the partner matrix (Shared) and unexplained variation (Unexpl.). The significant MEMs are given in order of their significance. For each dataset, there are n-1 MEMs in the total set and smaller MEM numbers represent larger-scale spatial pattern.

Dataset	Significant MEMs	Partner	Shared	Space	Unexpl.	*P*
Dependent matrix: Algae						
NZ *Ramalina*	1, 5, 3, 2, 7	0.21	0.65	0.09	0.06	0.016
NZ *Usnea*	1, 4, 6, 12, 17, 7, 3, 9, 2, 5, 45, 8, 63, 38, 41, 28, 54	0.39	0.41	0.08	0.12	< 0.001
NZ *Usnea* replicated	4, 5, 1, 3	0.89	0.07	0.00	0.04	< 0.001
Craigieburn *Usnea*	2, 9	0.87	0.00	0.01	0.12	0.021
Flock Hill *Usnea*	16	0.62	0.01	0.01	0.35	0.030
Flock Hill community mapped	none	0.84	0.03	0.02	0.12	< 0.001
Flock Hill community	-	0.71	-	-	0.29	< 0.001
Dependent matrix: Fungi						

1

NZ *Ramalina*	1, 3, 5, 7, 9, 10, 2,	0.32	0.62	0.02	0.03	0.025
NZ *Usnea*	1, 4, 12, 3, 8, 6, 9, 45, 17, 54, 13, 38, 41, 16, 5, 56, 7	0.45	0.23	0.12	0.2	< 0.001
NZ *Usnea* replicated	4, 3, 31, 1, 17, 5, 9	0.72	0.01	0.02	0.25	< 0.001
Craigieburn *Usnea*	7, 12, 13, 15, 16, 3	0.55	0.02	0.3	0.14	0.024
Flock Hill *Usnea*	1	0.62	0.00	0.08	0.30	0.022
Flock Hill community mapped	none	0.73	0.02	0.03	0.22	< 0.001
Flock Hill community	-	0.6	-	-	0.40	< 0.001

2