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Soil foraging animals alter the composition and co-occurrence of microbial communities in a desert shrubland

Eldridge, D.J.; Woodhouse, J.; Curlevski, N.; Hayward, M.W.; Brown, M.; Neilan, B.

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1 **Title page**

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3 **Soil foraging animals alter the composition and co-occurrence of microbial communities in**
4 **a desert shrubland**

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7 David J Eldridge¹, Jason N Woodhouse², Nathalie JA Curlevski^{2,3}, Matthew Hayward^{4,5}, Mark V
8 Brown² and Brett A Neilan²

9

10

11 1. Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences,
12 University of NSW, Sydney, 2052, Australia

13

14 2. School of Biotechnology and Biomolecular Sciences, University of NSW, Sydney, NSW,
15 2052, Australia

16

17 3. Faculty of Science, Aquatic Processes Group, University of Technology, Ultimo, NSW,
18 2007, Australia

19

20 4. Australian Wildlife Conservancy, P.O. Box 432, Nichol's Point, Victoria, 3501, Australia

21

22 5. School of Environment, Natural Resources and Geography; and School of Biological
23 Science, Bangor University, Bangor LL57 2UW United Kingdom.

24

25 Correspondence: D J Eldridge, Centre for Ecosystem Science, School of Biological, Earth and
26 Environmental Sciences, University of NSW, Sydney, 2052, Australia. E-mail:

27 d.eldridge@unsw.edu.au

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29 *Running title:* Animal foraging alters microbial community

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Abstract

Animals that modify their physical environment by foraging in the soil can have dramatic effects on ecosystem functions and processes. We compared bacterial and fungal communities in the foraging pits created by bilbies and burrowing bettongs with undisturbed surface soils dominated by biocrusts. Bacterial communities were characterized by *Actinobacteria* and *Alphaproteobacteria*, and fungal communities by Lecanoromycetes and Archaeosporomycetes. The composition of bacterial or fungal communities was not observed to vary between loamy or sandy soils. There were no differences in richness of either bacterial or fungal Operational Taxonomic Units (OTUs) in the soil of young or old foraging pits, or undisturbed soils. Although the bacterial assemblage did not vary among the three microsites, the composition of fungi in undisturbed soils was significantly different from that in old or young foraging pits. Network analysis indicated that a greater number of correlations between bacterial OTUs occurred in undisturbed soils and old pits, while a greater number of correlations between fungal OTUs occurred in undisturbed soils. Our study suggests that digging by soil disturbing animals is likely to create successional shifts in soil microbial and fungal communities, leading to functional shifts associated with the decomposition of organic matter and the fixation of nitrogen. Given the primacy of organic matter decomposition in arid and semi-arid environments, the loss of native soil-foraging animals is likely to impair the ability of these systems to maintain key ecosystem processes such as the mineralization of nitrogen and the breakdown of organic matter, and to recover from disturbance.

Keywords: animal foraging/ microbial connectivity/ decomposition/ cyanobacteria/ arid/ soil disturbance

Subject category: Microbial ecology and functional diversity of natural habitats

Introduction

63
64 Australia has suffered one of the highest rates of global mammal extinctions over the past 200
65 years since European settlement (Woinarski *et al.*, 2012). Losses have been most pronounced in
66 the critical weight range (35-5500 g) mammals, which were once common over large areas of
67 continental Australia (Johnson, 2006). The loss of these animals, or the contraction of their
68 ranges, has been attributed to multiple causes associated with European settlement and pastoral
69 practices such as altered fire regimes, overgrazing by livestock, competition with exotic pests
70 including the European rabbit (*Oryctolagus cuniculus*), and predation by introduced species such
71 as the domestic cat (*Felis catus*) and the red fox (*Vulpes vulpes*) (Johnson, 2006). Two species
72 that have suffered substantial range restrictions are the greater bilby (*Macrotis lagotis*) and the
73 burrowing bettong (*Bettongia lesueur*). Recent attempts have been made to reintroduce these
74 animals into predator-proof enclosures within their former range in an effort to re-establish viable
75 populations (James and Eldridge 2007).

76
77 Many of Australia's locally extinct animals forage extensively in the soil for seeds, bulbs,
78 invertebrates and fungi (Robley *et al.* 2001; James *et al.*, 2011; Eldridge *et al.*, 2012). Foraging
79 disturbs the soil surface and breaks up the surface crust (biocrust), altering rates of water
80 infiltration, and creating small pits and depressions that trap water, soil, organic matter and seed
81 (James *et al.*, 2009). These pits develop into patches of higher nutrients, with greater
82 concentrations of plant-available nitrogen and carbon than the surrounding soil matrix (James,
83 2010) and often a different vegetation community (Lavelle *et al.*, 2006). Studies worldwide have
84 shown that modification of the abiotic environment by these animals, a process referred to as
85 ecosystem engineering (*sensu* Jones *et al.*, 1994), alters energy flows and resource availability,
86 increases species richness, diversity and productivity, through niche construction, ultimately
87 controlling the availability and distribution of resources to other organisms (e.g. Whitford and
88 Kay, 1999; Jones *et al.*, 2010).

89
90 An important process moderated by soil disturbing animals in arid environments is the
91 decomposition of organic matter. Litter and organic matter in these systems is spatially and
92 temporally variable, and is often concentrated within the foraging pits of animals (James and
93 Eldridge, 2007). Litter is a source of carbon, nitrogen and other trace elements, and provides

94 habitat for a range of micro- and macro-invertebrates involved in the decomposition of organic
95 matter (Haslem *et al.*, 2011). Litter falling into pits comes into close contact with soil, where it is
96 held *in situ* more effectively than if it remained on the soil surface where it is subject to removal
97 by wind and water (Whitford, 2002). Together with reduced evaporation resulting from lower
98 temperatures in the pits than the undisturbed surface (Eldridge and Mensinga, 2007), this
99 increases the time period over which soil moisture is optimum for microbial decomposition and
100 nutrient mineralization (Steinberger and Whitford, 1983; Jacobson and Jacobson, 1998;
101 Whitford, 2002). Litter remaining on the surface, however, is subject to photodegradation (Austin
102 and Vivanco, 2006), potentially reducing the return of carbon to the soil organic pool.

103
104 Soil disturbing animals therefore play an important role in bringing surface resident organic
105 matter into contact with soil microorganisms. The accumulation of litter in the pits is also likely
106 to exert a strong selective pressure on microorganisms essential for the decomposition process.
107 Given the marked differences in the biotic (litter cover and composition) and abiotic (e.g. surface
108 temperature, soil moisture) environments between pits and undisturbed soils; i.e. those soils
109 undisturbed by animal activity (e.g. Vossbrinck *et al.*, 1979; Wallwork *et al.*, 1985; Eldridge and
110 Mensinga, 2007), we expected that the pits would differ in the composition of soil
111 microorganisms. For example, studies of foraging disturbances constructed by the short-beaked
112 echidna (*Tachyglossus aculeatus*) indicate a greater diversity and abundance of micro-arthropods
113 and higher rates of microbial respiration in the pits than undisturbed soil (Eldridge and Mensinga,
114 2007), suggesting that there are differences in the abundance or structure of microbial
115 communities. Over time, pits collect organic matter, and research has shown that pits over about
116 12 months old have high levels of organic carbon. This compares with young pits (< 3 months
117 old), which have relatively low levels of litter and organic matter (D J Eldridge, unpublished
118 data). We would expect pit age to influence microbial community structure, as these old pits (~
119 12 months) would have more time to establish seedlings and accumulate litter and
120 microorganisms that are present on adjacent, undisturbed surfaces. Furthermore, older pits could
121 provide a greater range of different environments, with differences in depth, shape and
122 orientation, and therefore different soil chemistry and organic matter at varying stages of
123 decomposition.

124

125 We compared the community structure of soil microbial communities in old and young pits with
126 the undisturbed surface soil on two soil types in an area where bilbies and bettongs have been
127 reintroduced into their former range. Both bilbies and bettongs construct pits while foraging for
128 buried seed, invertebrates and plant roots. The pits of these two species are indistinguishable, and
129 range from cylindrical-shaped excavations about 15 cm wide and up to 20 cm deep to shallow
130 basin-like structures (Eldridge *et al.*, 2012). Pits are constructed only once, and unlike cache pits
131 of heteromyid rodents (Geluso, 2005), are rarely reworked. Because pits vary in depth and shape,
132 and are constructed in soils of different texture, they provide a range of different physical
133 environments that influences the trapping and retention of litter and the breakdown of organic
134 matter.

135
136 We hypothesized that the microbial community in pit soils would support more microorganisms
137 commonly associated with decomposing litter. Conversely, we expected that the microbial
138 community composition in undisturbed soils would support a community dominated by
139 cyanobacteria, given the extensive cover of biocrusts on the soil surface. We used microbial
140 network analysis to examine the structure of microbial communities, particularly in relation to
141 resilience and reactivity (Ruiz-Moreno *et al.*, 2006; Bissett *et al.*, 2013). Examination of
142 microbial networks improves our understanding of why undisturbed soils might be resistant to
143 nutrient amendment, how microbial community structure is altered following pit construction,
144 and how digging promotes nutrient enrichment within these microsites (James *et al.*, 2009).

145

146 **Methods**

147

148 *The study area*

149

150 Our study was undertaken within the Australian Wildlife Conservancy's Scotia Sanctuary in
151 south-western, New South Wales, Australia (33°43'S, 143°02'E) where locally extinct bilbies
152 and bettongs have been released into predator-proof exclosures. Soil samples were collected from
153 two systems; (1) mallee (*Eucalyptus* spp.) west-east-trending dunes of Quaternary alluvium
154 characterized by calcareous and siliceous sands (Rudosols) and (2) the inter-dunal swales and
155 plains extending to these dunes, which are up to 500 m wide, comprised mainly of loamy,

156 calcareous soils (Calcarosols). The vegetation on the dunes is moderately dense mallee
157 (*Eucalyptus socialis*, *E. dumosa*) and the plains are dominated by open mallee woodland with
158 scattered belah (*Casuarina pauper*) and sugarwood (*Myoporum platycarpum*), and a variable
159 cover of shrubs such as punty bush (*Senna artemisioides*), hopbush (*Dodonaea viscosa*),
160 turpentine (*Eremophila sturtii*), pinbush wattle (*Acacia burkittii*) and assorted bluebushes
161 (*Maireana* spp.), depending on whether trees had been removed. Shrubs covered about 50% of
162 the area of the plains. The climate in the area is semi-arid, with cool winters (mean $\leq 17^{\circ}\text{C}$) and
163 hot summers (mean 30°C). Rainfall is highly spatially and temporally variable and averages 243
164 mm yr⁻¹. Rainfall is evenly distributed between the six warmer months and the six cooler months.

165

166 *Field sampling*

167

168 The location, size, depth and age of all foraging pits constructed by bilbies and bettongs have
169 been monitored at 36 large sites at the Scotia Sanctuary since 2007. Because sites were visited
170 every three months, we were able to calculate the relative age of particular pits. In October 2009
171 we collected soil samples from six sites: three on sandy dunes and three on loamy plains. At each
172 of the six sites we sampled three microsites: (1) young foraging pits, i.e. pits constructed since
173 the previous measurements (<3 months old), (2) old foraging pits, i.e. pits older than 12 months,
174 and (3) undisturbed non-pit surface soils at least 3 m from any pit. At each of the six sites we
175 sampled each microsite at 10 locations. For the young pits, soil was removed from the uppermost
176 10 mm layer of the soil surface or from the base of the pits after removing any existing organic
177 material. Biocrust was not removed from the soil prior to sampling. Approximately 5 g of soil
178 was collected with a sterilized spatula. The material from the 10 locations was then bulked and
179 stored on ice before being transported back to the laboratory. The same procedure was used to
180 collect samples from old pits and undisturbed surfaces. This resulted in a database of 18 bulked
181 samples (3 replicate sites of 2 soil types x 3 microsites).

182

183 *Molecular analysis*

184

185 Environmental DNA was isolated from 500 mg of soil using the FASTDNA™ Spin Kit for Soil
186 (MP Bio) according to the manufacturer's instructions and stored at -80°C until use. DNA was

187 quantified using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific) and the
188 quality checked by PCR amplification of the 16S rRNA gene using the primer pair 27f/519r
189 (Weisburg, 1991). Bacterial and fungal specific tag-encoded FLX amplicon pyrosequencing
190 (TEFAP) of each sample was carried out, using the primers 27f/519r and funSSUF/funSSUR
191 respectively (Lucero, 2011) on a Roche GS-FLX Titanium at the Research and Testing
192 Laboratory (Lubbock, TX). Sequence reads were analyzed using MOTHUR v1.22
193 (www.mothur.org) software package (Schloss *et al.*, 2009). Initial quality processing of 454
194 sequence reads was performed using the mothur implementation of PyroNoise (Quince *et al.*,
195 2011) using default settings. Sequences containing < 200 bp, containing ambiguous bases and
196 homopolymers longer than 8 bp in length were removed. The remaining sequences were aligned
197 to either the bacterial or fungal alignments of the SILVA release 102 reference alignment.
198 Chimeric sequences were identified and removed using the mothur implementation of uchime
199 (Edgar *et al.*, 2011). The taxonomic identity of each unique sequence was determined by
200 comparison against the SILVA release 102 reference database. Taxonomic assignment was made
201 at each level, given a bootstrap value greater than 80, using the RDP classifier (Wang *et al.*,
202 2007). Sequences that failed to be classified at the phylum level or were classified as either
203 Mitochondria, Archaea, or Eukaryota/Prokaryota in the respective datasets, were removed. Sub-
204 sampling was performed at a level of 400 sequences per sample for the bacterial dataset and 1300
205 sequences per sample for the fungal dataset. Implementation of this process resulted in the
206 exclusion of a bacterial young loam soil sample and bacterial young sand soil sample, as these
207 samples contained fewer than the 400 sequences required. To ensure a balanced design across the
208 bacterial dataset, the corresponding samples were subsequently excluded from the bacterial old
209 pit soil and bacterial surface pit soil sets (2 replicate sites of 2 soil types x 3 microsites.
210 Uncorrected pairwise distances were calculated between sequence reads with the final clustering
211 of OTUs performed at a 0.03 distance threshold using the average neighbor algorithm (Schloss,
212 2011). The identity of each OTU defined at 0.03 a distance threshold was obtained from the
213 consensus of each sequence within that OTU at a confidence threshold of 80. From these data,
214 two individual data matrices were generated, one for bacteria and one for fungi, each matrix
215 containing every OTU and the number of reads assigned to it from each sample. In this instance
216 the relative proportion of each OTU was used as a proxy for abundance, as absolute abundance
217 measures were not obtained.

218

219 *Statistical analysis*

220

221 We used permutation multivariate analysis of variance (PERMANOVA; Anderson *et al.*, 2008)
222 to examine differences in the composition of a data matrix of 2500 bacterial OTUs, defined at
223 0.03 distance threshold, and a data matrix of 5895 fungal OTUs, defined at 0.03 distance
224 threshold, in relation to microsite (undisturbed soils, young pits, old pits) and soil type (loam,
225 sand). Relative abundance data were, used after resampling, in order to ensure an equivalent
226 number of sequences. The first stratum of this analysis considered soil type and the second
227 stratum microsite and its interaction with soil type. Pair-wise *a posteriori* comparisons were
228 made, where necessary, using a multivariate analogue of the *t* statistic, the probability levels
229 being obtained by permutation. We tested for differences in richness and diversity of taxa with a
230 mixed-model ANOVA with the same structure as the PERMANOVA analysis. Richness and
231 diversity data were checked for homogeneity of variance (Levene's test) and normality using
232 diagnostic tests but no transformations were needed. For all analyses, significant differences
233 between means were examined using Fisher's Protected Least Significant Difference (LSD) test.
234 The procedure was repeated for the fungal data.

235

236 The degree of association of OTUs with respect to microsite was measured with Indicator
237 Species Analysis in R (De Caceres, 2013) using a data matrix consisting of 2500 bacterial OTUs
238 and 5895 fungal OTUs. Indicator values combine information on relative abundance and
239 frequency of species, and the indicator value is maximal (IV=100%) when all individuals of a
240 given species are restricted to a particular microsite (e.g. old pit), and all samples from the
241 particular microsite contain an occurrence of that species. Data (at the OTU level) were
242 randomized among the treatments and a Monte Carlo randomization procedure performed with
243 1000 iterations in order to determine the statistical significance of the indicator values.

244

245 The degree of association of OTUs with respect to one another within each microsite was
246 measured using the Pearson's correlation coefficient (*r*). Bacterial and fungal OTU tables,
247 defined at 0.03 distance threshold, were separated on the basis of microsite then reduced by
248 removing any OTUs that did not occur across at least 75% of available samples. A Pearson's *r*

249 score and P -value were calculated pairwise for each bacterial OTU using the `rcor.test` algorithm,
250 available from the `ltm` package (available from
251 <http://rwiki.sciviews.org/doku.php?id=packages:cran:ltm>) as implemented in R version 3.0.2. For
252 each correlation, P -values were generated and the false discovery rate was maintained below 5 %
253 using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995). Visualization of
254 these interactions, incorporating taxonomic, abundance and microsite occurrence information,
255 was made with the freely available Cytoscape package version 2.8.3 (available at:
256 www.cytoscape.org). For each network, topological metrics of connectivity and density were
257 calculated using the network analysis plug-in (Assenov *et al.*, 2008). Networks pre-embedded
258 with sample and OTU specific information are provided in the Supplementary Material.

259

260 **Results**

261

262 *Richness of bacterial and fungal taxa*

263

264 Most bacterial and fungal OTUs occurred at very low abundances, with a substantial number of
265 abundances equal to one. Of the original 2500 bacterial OTUs after resampling, 320 (14%)
266 contributed 50% of total OTU abundances. For fungal OTUs, 525 (9%) of the 5895 OTUs
267 contributed 50% of total fungal abundances. There were no differences in bacterial OTU richness
268 (i.e. different number of OTUs) among the different soils ($P = 0.24$; range 238-332 OTUs) or
269 among the three microsites ($P = 0.47$). Similarly, fungal richness did not vary with soil texture (P
270 $= 0.81$; range 397 – 873) or among the three microsites ($P = 0.17$).

271

272 *Community composition of bacterial and fungal taxa*

273

274 Bacterial communities were observed to contain a high proportion of Actinobacteria, and to a
275 lesser extent, Alphaproteobacteria and Acidobacteria (Figure 1A). Cyanobacteria appeared to
276 constitute a large proportion of the bacterial community, particularly in undisturbed soils. Fungal
277 communities were observed to contain a high proportion of Lecanoromycetes, and to a lesser
278 extent, Archaeosporomycetes (Figure 1B).

279

280 There was no significant difference in the composition of either bacterial or fungal OTUs
281 assemblages between loamy and clay soils ($P > 0.30$). The composition of the bacterial
282 assemblage did not vary among the three microsites ($P = 0.21$; Figure 2A), but there was a
283 significant effect for fungi (Pseudo $F_{2,8} = 3.08$, $P(\text{Perm}) = 0.003$). The composition of fungi in
284 undisturbed soils was significantly different from that in old (pairwise $t = 2.14$, $P = 0.029$) or
285 young ($t = 2.02$, $P = 0.02$) pits, but there was no significant differences between old and young
286 pits ($P = 0.47$; Figure 2B).

287

288 *Microsite indicators*

289

290 Six cyanobacterial OTUs (Gp I [3 OTUs], Gp X, Gp VII and an unclassified OTU) were
291 indicators of undisturbed pits, as were the single *Asanoa* OTU (Actinobacteria), a *Segetibacter*
292 OTU (Sphingobacteria) and an unclassified alphaproteobacterial OTU. A single *Hylangium* OTU
293 (Myxobacteria, Deltaproteobacteria), *Microvirga* OTU (Rhizobiales, Alphaproteobacteria) and a
294 Gp IV actinobacterial OTU were indicators of old pits. A single *Rubrobacter* OTU
295 (Actinobacteria), *Ammoniphilus* OTU (Bacilli, Firmicutes) and *Actinaurispora* OTU
296 (Actinobacteria) were indicators of young pits (Table 1). Overall, fungal taxa were better
297 discriminators of the three microsites, with 20 Orders containing 170 OTUs, with indicator
298 values > 0.70 , and almost exclusively from sub-phylum Pezizomycotina. These included orders
299 *Dothideales* (genera *Columnosphaeria*, *Delphinella*), *Chaetothyriales* (genus *Glyphium*),
300 Lecanorales (genera *Sphaerophoraceae*, *Cladoniaceae*), *Myxotrichaceae* (genus *Geomyces*),
301 *Mycocaliciales* (*Sphinctina*) and *Pleosporales* (genera *Leptosphaeria*, *Trematosphaeria*,
302 *Phaeosphaeria*). Ten fungal genera (particularly *Eupenicillium*, *Hamigera*, *Bionectriaceae* and
303 an unclassified taxon from the family Bulgaria) were highly indicative ($IV > 0.81$) of young pits.
304 Old pits contained a wide range of different OTUs, with the orders *Chaetothyriales*, *Dothideales*,
305 *Hypocreales*, *Lecanorales*, *Mycocaliciales* and *Pleosporales* having a large number of OTUs that
306 were strongly indicative ($IV > 69\%$) of older pits (Table 2).

307

308 *Network analysis*

309

310 Within the bacterial networks, the mean number of correlations between OTUs was greater in old
311 pit soils (3.45) than either undisturbed (2.516) or young pit (1.294) soils, consistent with a larger
312 number of OTUs co-occurring across the samples (Table 3). The majority of the associations
313 present in young pit soils were between a small number of alphaproteobacterial and
314 actinobacterial OTUs. Young pit soils returned the lowest values for network metrics of
315 clustering (0), density (0.081) and centralization (0.050). Undisturbed soils and old pit soils were
316 similar in relation to clustering (undisturbed = 0.566, old pits = 0.547), density (undisturbed =
317 0.084, old pits = 0.088) and centralization (undisturbed = 0.089, old pits = 0.096) (Table 3).

318
319 Within the fungal networks, the highest mean number of correlations between OTUs (20.497)
320 was observed in undisturbed soils, where many more OTUs (1814 OTUs) were present across
321 multiple samples than in young (321) or old (485) pit soils. Similar to the bacterial networks,
322 young pit soils returned the lowest values for density (0.009), but were also the most centralised
323 (0.116). Old pit soils were the least clustered (0.472) and the least centralised (0.067).
324 Undisturbed soils were similar to young pit soils in terms of clustering (undisturbed =0.652,
325 young pits = 0.647), whereas fungal young and old pit soils were only similar in relation to the
326 mean number of correlations between OTUs.

327

328 **Discussion**

329

330 Soil foraging by semi-fossorial animals in arid areas disrupts surface crusts, alters rates of water
331 infiltration, and creates small pits and depressions that trap water, soil, organic matter and seed
332 (James and Eldridge, 2007). We expected to detect substantial differences in the soil microbial
333 community between intact undisturbed soils and recently-excavated or older, more established
334 pits in response to differences in plant and litter cover, organic matter decomposition and soil
335 nutrient concentrations. Although we detected some significant differences in the fungal
336 community composition between the soil surface and the pits (described below), there were no
337 discernible differences in the bacterial community and in the fungal community between young
338 and old pits, largely because of the high variability among microsites (Figure 1). Consequently,
339 we undertook an analysis that would test whether the physical variability that is observed in pit
340 soils, in regard to moisture and nutrient trapping, influenced the occurrence of individual species

341 or the manner in which individual species exhibited correlations to one another. Indicator species
342 analysis was implemented to identify specific OTUs that were more strongly associated with a
343 particular microsite type. Critically, indicator species analysis has been previously shown to be
344 suitable for identifying variable taxa where there was no prior assessment, or no significant
345 variation, in the larger community composition (De Caceres & Legendre, 2009, De Caceres et al.,
346 2009, De Caceres, 2013). That it was possible to identify species that were statistically indicative
347 of particular microsites when the multivariate (PERMANOVA) analysis was insignificant
348 highlights the fact that there is substantial heterogeneity within microsites, and suggests a level of
349 functional redundancy within microbial taxa that prevents large-scale perturbation of the
350 community despite the loss of species. Based on the indicator species (De Caceres & Legendre,
351 2009, De Caceres et al., 2009, De Caceres, 2013) and microbial network (Chaffron, 2010;
352 Bissett, 2013) analyses, there is sufficient evidence to suggest that pits may be associated with a
353 reduction in autotrophic groups (Figure 1, Tables 1 and 2) that are compensated for by an
354 emergence of taxa capable of decomposing organic material (Tables 1 and 2) and reduced
355 resilience in the microbial communities (Table 3).

356

357 *Compositional differences between pit and undisturbed soils*

358

359 Consistent with information from arid soils worldwide, the bacterial community contained high
360 proportions of Actinobacteria and Alphaproteobacteria (Figure 1A) (Yeager *et al.*, 2004; Kuske,
361 2012). At the community level, we detected no significant differences in bacterial community
362 composition between pits and undisturbed soils (Figure 2). However, consistent with our first
363 hypothesis, filamentous diazotrophic (Cyanobacteria GpI), baeocystous (Cyanobacteria GpVIII),
364 and unicellular (Cyanobacteria GpX) cyanobacteria were found to be indicators of undisturbed
365 soils (Table 1) with a reduction in the observed abundance of cyanobacterial sequence reads
366 when soils were disturbed (Figure 1, Table 1). Cyanobacteria were present in undisturbed soils,
367 however the presence of these taxa as indicators was reflective of both a decrease in the
368 abundance of cyanobacterial groups and a shift within the morphological and physiological
369 nature of cyanobacteria between undisturbed and pit soils. Among the heterotrophic population,
370 actinobacterial members of the *Rubrobacteridae* that are pioneers in biological crust formation
371 (Yeager *et al.*, 2004) dominated both undisturbed and pit soils, with a single *Rubrobacter* OTU

372 an indicator of young pit soils. In addition to cyanobacterial groups, the Sphingobacterial genus
373 *Segetibacter* has been previously affiliated with the decomposition of cyanobacteria- and plant-
374 derived phytodetritus (Li *et al.*, 2011).

375
376 Fungal communities in undisturbed and pit soils comprised a wide range of saprotrophs, with
377 *Lecanoromycetes*, the largest class of lichenized fungi, and to a lesser extent,
378 *Archaeosporomycetes*, comprising about 80% of sequences across the three microsites (Figure
379 1B). Along with *Pezizomycotina*, these fungal taxa perform a diverse array of ecological
380 functions including wood and litter decomposition, mycorrhizal associations and lichen
381 symbioses, animal and plant pathogens (Spatafora *et al.*, 2006). Evidence for active recession, or
382 at least competitive inhibition, of microbial groups from the old pits was found, with the insect
383 and plant pathogenic fungi, *Delphinella*, *Leptosphaeria*, *Trematosphaeria* and *Columnosphaeria*,
384 found almost exclusively in undisturbed and young pit soils. *Glomeromycetes*, which comprise
385 arbuscular mycorrhizal species, represented about 3% of sequences in young pits and 2% of
386 sequences in old pit and undisturbed soils.

387
388 *Community development with pit age*

389
390 *Rubrobacter*, *Ammoniphilus* and *Actinaurispora* were the only bacterial indicators of young pits
391 and likely represent remnants of the sub-surface community. *Rubrobacter* is a cosmopolitan and
392 abundant taxon in arid zone soils (Yeager *et al.*, 2004). The presence of *Ammoniphilus* and
393 *Actinaurispora* in young pit soils is likely due to the deposition of plant material. *Amminophilus*
394 has been reported as a strictly aerobic oxalotroph utilizing plant and algae derived oxalic acid as a
395 sole carbon. *Actinaurispora* are known plant endophytes, inhabiting *Camptotheca acuminata*
396 species (Zhu *et al.*, 2012). The family Micromonosporaceae, to which *Actinaurispora* belongs,
397 however has been tentatively correlated with increasing moisture content in arid and semi-arid
398 soils (Bachar *et al.*, 2010), which may contribute to the presence of this species as an indicator of
399 young soils. *Trichocomaceae* species were the primary fungal indicators of young pits. A single
400 *Trichocomaceae* species was a key fungal indicator of undisturbed soils, suggesting that fungal
401 communities of young pits contain residual surface taxa prior to the colonization and
402 diversification of fungal communities observed in older pits. *Trichocomaceae* species are

403 predominantly saprotrophic, have aggressive colonisation strategies, and a high tolerance to
404 extreme environmental conditions such as soil drying, high temperature and metal toxicity
405 (Houbraken and Samson, 2011). Their presence in young pits could indicate opportunistic
406 colonisation of recently disturbed soil.

407
408 Based on the criteria used to select the microsites, progression of the microbial community from
409 young to old pits occurs over a period of 9-12 months. Over this time, while little change
410 occurred within the microbial community composition between pit stages, a discernable
411 difference was observed between the undisturbed and pit bacterial and fungal communities,
412 irrespective of their age. Microbial richness among microsites, however, remained unchanged.
413 Spore propagule density and arbuscular mycorrhizal fungi (AMF) diversity are known to decline
414 with increasing tillage associated with agriculture (Brito *et al.*, 2010; Schalamuk *et al.*, 2013).
415 However, this was not reflected in our fungal species richness, which remained unchanged over
416 time. The progressive accumulation of fungal species attached to organic matter and seed in the
417 pits is consistent with the presence of several lichenized lecanoralean genera including
418 Parmeliaceae and *Myrangium* (Smith, 1948) and the epiphytic melanized taxon *Sarcinomyces*
419 (Wollenzien *et al.*, 1997). The Lecanorales are predominantly lichen-forming fungi that are
420 mycobionts of the genera *Xanthoparmelia*, *Parmotrema* and *Xanthoria*, which are common
421 corticolous lichens of *Callitris glaucophylla* trees that occur in the study area (Filson and Rogers,
422 1979). These taxa are typically found in the soil surface or in the pits on detached plant material.
423 At some sites we also recorded the vagant lichen *Chondropsis semiviridis* from within the pits.
424 This lichen, which has no attachment to the soil, moves freely along the surface by wind action
425 (Eldridge and Leys, 1999). Similarly, *Cladonia* spp., another common soil lichen genus, was
426 found on undisturbed surfaces. Along with the lichen genera *Endocarpon* and *Placidium*, it is one
427 of the most common lichens forming biocrusts on stable soils in arid and semi-arid areas
428 (Eldridge and Koen, 1998).

429
430 Despite our inability to discriminate between the bacterial community of old and young pits, we
431 recorded three indicator species, *Hyalangium* and *Microvirga*, and a Gp IV Acidobacteria. The
432 two proteobacterial species were indicative of the presence of established vascular plants.
433 *Hyalangium*, belongs to the group of Myxobacteria that uses plant lignin and produces small

434 bioactive molecules. *Microvirga* has been implicated in nodule formation, facilitating nitrogen-
435 fixing processes within the rhizosphere (Ardley *et al.*, 2012). The occurrence of these groups in
436 old pit soils is likely to enhance nitrogen fixation, presumably to levels greater than those in the
437 undisturbed and young pit soils, and support the growth of vascular plants occurring in these
438 microsites.

439

440 *Microbial co-occurrence in pit and undisturbed soils*

441

442 Our analyses thus far indicate that initial disturbance reduces the abundance of key
443 photoautotrophic groups, and that over extended periods of time, capture of organic matter leads
444 to changes in the abundance of some taxa, with increases in those taxa likely reflecting an
445 increased capacity for the assimilation of organic carbon and nitrogen matter.

446

447 Resilience is the ability of a system to recover from large disturbances, typically over short time
448 frames. Reactivity, however, is the capacity of a system to respond to small perturbations over
449 extended periods. Under such circumstances, the apparent equilibrium may appear stable, despite
450 moving to a new steady state over long time periods (Neubert *et al.*, 2009). Modularity, defined
451 by the number and size of groups of highly interconnected nodes within a network, is positively
452 correlated with reactivity, and negatively correlated with resilience (Ruiz-Moreno *et al.*, 2006).
453 Analysis of both bacterial and fungal microbial networks revealed stark differences in
454 modularity, reflected in the values of clustering, density and centralization, of microbial co-
455 occurrence networks between undisturbed soils and pit soils at different developmental stages
456 (Table 3). Clustering coefficients and density (network connectivity) scores tending towards a
457 value of 1 indicate a highly modular system while those tending towards zero represent the
458 opposite (Bissett *et al.*, 2013). Low values of clustering and density associated with microbial
459 communities from contaminated and reference estuarine sediments, indicate historical
460 community “stress” contributing to functional redundancy and reduced correlations among
461 species (Sun *et al.*, 2013). This was reinforced by marginally lower values, for each of these
462 metrics, in contaminated sediments, with the suggestion that this anthropogenic perturbation has
463 contributed an additional stress.

464

465 In the present study, bacterial species-species correlations within the young pit soils were almost
466 non-existent. A clustering coefficient of zero and a slightly lower density value were consistent
467 with reduced modularity, and an increase in functional redundancy associated with a recent
468 external stress (Sun *et al.*, 2013). In contrast, undisturbed and old pit soils were more consistent
469 with increased modularity, suggesting a lack of functional redundancy, with greater species-
470 species correlations, and increased clustering and density. This suggests to us that the bacterial
471 community present in undisturbed soils and old pit soils are more reactive and less resilient than
472 young pit soils. Within the fungal communities, the number of correlations among species,
473 clustering coefficients and density, and hence modularity, were highest in surface soils and
474 lowest in the old pit soils, suggesting that fungal communities within old pits are less reactive and
475 more resilient. In contrast, the young pit soils exhibit reduced modularity, and increased
476 resilience, suggesting that they are likely to respond to nutrient amendments over the short-term,
477 thereby driving large and dramatic structural changes. This is largely because of the high degree
478 of physical disturbance created when foraging pits are established. Within the old pit soils, the
479 bacterial community has largely regained the modularity observed within the undisturbed soils.
480 The fungal community, however, is apparently more resistant at this stage than in the undisturbed
481 soils, suggesting it is able to continue to drive structural changes in response to events such as
482 litter deposition.

483
484 A high level of centralization, as a consequence of the high frequency of centralized nodes, was
485 observed amongst the fungal community in young pit soils (Bulgariaceae, Myxotrichaeae,
486 Trichomaceae, Tubeufiaceae) and among the bacterial community in undisturbed
487 (Rubrobacteriaceae, Geodematophiliaceae, Bradyrhizobiaceae) and old pit (Rhodobacteriaceae,
488 Bradyrhizobiaceae, Geodermatophiliaceae, Beijerinckiaceae, Comamondaceae,
489 Methylobacteriaceae) soil (Supplementary Information). Centralised nodes have been proposed
490 to represent keystone species, exhibiting a large influence of the “information” transfer
491 throughout the community (Bissett *et al.*, 2013). It has been speculated that these nodes
492 represent critical control points in the cycling of nutrients within the system (Ruiz-Moreno *et al.*,
493 2006; Bissett *et al.*, 2013). Thus it is realistic to suggest that these centralized taxa act to stabilize
494 the microbial community. It should be highlighted that these observations were made in the
495 context of a small number of samples defining each microsite, as well as few sequence reads

496 being available to identify species-species correlations. Our observations between the bacterial
497 and fungal datasets suggest that these metrics are susceptible to sequence depth, and pre-
498 treatment of the data by retaining only semi-ubiquitous (occurring across at least 75% of
499 samples) OTUs, suggests that these values may also be influenced by the level of heterogeneity
500 within microsites. Despite this, our analyses of network metrics from the bacterial communities
501 suggested that the community structure of old pit soils reflect that of undisturbed soils. Over the
502 long term this would tend towards decreased responses to nutrient inputs into these soils. This,
503 however, may be partially offset by frequent deposition of plant matter due to the establishment
504 and growth of vascular plants within old pit soils, and subsequent assimilation of this matter by
505 saprotrophic fungi.

506

507 **Conclusions**

508

509 Our study suggests that digging by soil disturbing animals is likely to create successional shifts in
510 soil microbial and fungal communities, which could account for increases in organic matter of
511 nitrogen in old foraging pits (James *et al.* 2009). The observed richness of fungal and bacterial
512 OTUs, in undisturbed soils, and young and old pits did not differ, though fewer correlations, and
513 hence an increased resilience, were observed between bacterial OTUs in young pits, and fungal
514 OTUs in young and old pits. This suggests that these communities are more likely to respond
515 over the short term to nutrient amendment, thus promoting nutrient enrichment and contributing
516 to a form of patchiness that is critical for the functioning of arid systems. The action of soil
517 disturbing animals therefore leads to the development of a mosaic of different patches with a
518 varying complement of microorganisms. Given the wide variety in pit size, depth, substrate and
519 spatial configuration, this differential microbial activity will likely lead to the creation of a
520 mosaic of patches of differing resource availability, analogous to larger surface-resident biotic
521 patches such as hummocks and debris mounds. Our work suggests that microbial community
522 composition and co-occurrence change with physical disturbance during the formation of
523 foraging pits. Given the primacy of organic matter decomposition in arid and semi-arid
524 environments, the loss of native soil-foraging animals from these systems may well impair the
525 ability of these systems to maintain key ecosystem processes and to recover from disturbance.

526

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533
534 **Supplementary Information**

535
536 Supplementary information is available at the ISME Journal's website

537
538 **Conflict of interest**

539
540 The authors declare no conflict of interest

541
542 **References**

- 543
544 Ardley JK, Parker MA, De Meyer SE, Trengove RD, O'Hara GW, Reeve WG, *et al.* (2012).
545 *Microvirga lupini* sp. nov., *Microvirga lotononidis* sp. nov. and *Microvirga zambiensis* sp. nov.
546 are alphaproteobacterial root-nodule bacteria that specifically nodulate and fix nitrogen with
547 geographically and taxonomically separate legume hosts. *Int J System Evol Microb* **62**: 2579-
548 2588
- 549
550 Austin AT, Vivanco L. (2006). Plant litter decomposition in a semi-arid ecosystem controlled by
551 photodegradation. *Nature* **442**: 555-558.
- 552
553 Bachar A, Al-Ashhab A, Soares MIM, Sklarz MY, Angel R, Ungar ED, Gillor O (2010). Soil
554 microbial abundance and diversity along a low precipitation gradient. *Microb Ecol* **60**: 453-461.
- 555
556 Bader GD, Hogue CW. (2003). An automated method for finding molecular complexes in large
557 protein interaction networks. *BMC Bioinformatics* **4**: 2.

558
559 Bissett A, Brown MV, Siciliano SD, Thrall PH. (2013). Microbial community responses to
560 anthropogenically induced environmental change: towards a systems approach. *Ecol Lett* **16**
561 **Suppl 1**:128-139.
562
563 Brito I, Goss MJ, de Carvalho M, Chatagnier O, van Tuinen D. (2010). Impact of tillage system
564 on arbuscular mycorrhiza fungal communities in the soil under Mediterranean conditions. *Soil*
565 *Till Res***121**: 63-67.
566
567 Chaffron S, Rehrauer H, Pernthaler J, von Mering C. (2010). A global network of coexisting
568 microbes from environmental and whole-genome sequence data. *Genome Res* **20**: 947-959.
569
570 Chee Sanford JC, Sanford RA, Löffler FE, Thomas SH, Sims GK. (2006). Investigating
571 anaerobic microbial processes in agricultural soils using *Anaeromyxobacter dehalogenans* as a
572 cosmopolitan model. *Intern Soc Micro Ecol* **11**: 2025.
573
574 De Cáceres M, Legendre P. (2009). Associations between species and groups of sites: indices and
575 statistical inference. *Ecology*, **90**: 3566-3574.
576
577 De Cáceres M, Legendre P, Moretti M. (2010). Improving indicator species analysis by
578 combining groups of sites. *Oikos*: **119**: 1674-1684.
579
580 De Cáceres M. (2013). How to use the indicpecies package (ver.1.7.1)
581
582 Dufrene M, Legendre P. (1997). Species assemblages and indicator species: The need for a
583 flexible asymmetrical approach. *Ecol Monog* **67**: 345-366.
584
585 Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. (2011). UCHIME improves sensitivity
586 and speed of chimera detection. *Bioinformatics* **27**: 2194–2200.
587

588 Eldridge DJ, Mensinga A. (2007). Foraging pits of the Short-Beaked Echidna (*Tachyglossus*
589 *aculeatus*) as small-scale patches in a semi-arid Australian box woodland. *Soil Biol Biochem* **39**:
590 1055-1065.

591

592 Eldridge DJ, Leys JF (1999). Wind dispersal of the vagant lichen *Chondropsis semiviridis* in
593 semi-arid eastern Australia. *Aust J Bot* **47**: 157-164.

594

595 Eldridge DJ, Koen TB (1998). Cover and floristics of microphytic soil crusts in relation to
596 indices of landscape health. *Plant Ecol* **137**: 101-114.

597

598 Eldridge DJ, Koen TB, Huang N, Killgore A, Whitford WG. (2012). Animal foraging as a
599 mechanism for sediment movement and soil nutrient development: evidence from the semi-arid
600 Australian woodlands and the Chihuahuan Desert. *Geomorph* **157/158**: 131-141.

601

602 Geluso K. (2005). Benefits of small-sized caches for scatter-hoarding rodents: influence of cache
603 size, depth, and soil moisture. *J Mammal* **86**: 1186-1192.

604

605 Haslem A, Kelly LT, Nimmo DG, Watson SJ, Kenny SA, Taylor RS *et al.* (2011). Habitat or
606 fuel? Implications of long-term, post-fire dynamics for the development of key resources for
607 fauna and fire. *J Appl Ecol* **48**:247-256.

608

609 Hill R, Nagarkar S, Jayawardena A. (2002). Cyanobacterial crust and soil particle detachment: a
610 rain-chamber experiment. *Hydrol Process* **16**: 2989-2994.

611

612 Houbraken J, Samson RA. (2011). Phylogeny of *Penicillium* and the segregation of
613 *Trichocomaceae* into three families. *Studies Mycol* **70**: 1-51.

614

615 Jacobsen KM, Jacobsen PJ. (1998). Rainfall regulates decomposition of buried cellulose in the
616 Namib Desert. *J Arid Environ* **38**: 571-583.

617

618 James AI, Eldridge DJ (2007). Reintroduction of fossorial native mammals and potential impacts
619 on ecosystem processes in an Australian desert landscape. *Biol Conserv* **138**: 351-359.
620

621 James AI, Eldridge DJ, Hill BM. (2009). Foraging animals create fertile patches in an Australian
622 desert shrubland. *Ecography* **32**: 723-732.
623

624 James AI, Eldridge DJ, Moseby KE. (2010). Foraging pits, litter, and plant germination in an arid
625 shrubland. *J Arid Environ* **74**: 516-520.
626

627 James AI, Eldridge DJ, Koen TB, Moseby KE. (2011) Can the invasive European rabbit
628 (*Oryctolagus cuniculus*) assume the soil engineering role of locally-extinct natives? *Biol Invas* **13**:
629 3027-3038.
630

631 Johnson C (2006) Australia's mammal extinctions: a 50000 year history. Cambridge University
632 Press, Melbourne.
633

634 Jones CG, Lawton JH, Shachak M. (1994). Organisms as ecosystem engineers. *Oikos* **69**: 373-
635 386.
636

637 Jones CG, Gutierrez JL, Byers JE, Crooks JA, Lambrinos JG, Talley TS. (2010). A framework
638 for understanding physical ecosystem engineering by organisms. *Oikos* **119**: 1862-1869.
639

640 Kuske CR, Yeager CM, Johnson S, Ticknor LO, Belnap J. (2012). Response and resilience of
641 soil biocrust bacterial communities to chronic physical disturbance in arid shrublands. *ISME* **6**:
642 886-897.
643

644 Lamont BB, Ralph CS, Christensen PS. (1985). Mycophagous marsupials as dispersal agents for
645 ectomycorrhizal fungi on *Eucalyptus calophylla* and *Gastrolobium bilobum*. *New Phytol* **101**:
646 651-656.
647

648 Lavelle P, Daecans T, Aubert M, Barot S, Blouin M, Bureau F. *et al.* (2006). Soil invertebrates
649 and ecosystem services. *Eur J Soil Biol* **42**: S3-S15.
650

651 Li H, Xing P, Chen M, Bian Y, Wu QL. (2011). Short-term bacterial community composition
652 dynamics in response to accumulation and breakdown of *Microcystis* blooms. *Water Res*
653 **45**:1702-1710.
654

655 Lucero ME, Unc A, Cooke P, Dowd S, Sun S. (2011). Endophyte microbiome diversity in
656 micropropagated *Atriplex canescens* and *Atriplex torreyi* var *griffithsii*. *PLoS One* **6**:e17693.
657

658 Neubert MG, Caswell H, Solow AR. (2009) Detecting reactivity. *Ecology* **90**: 2683-2688.
659

660 Quince C, Lanzen A, Davenport RJ, Turnbaugh PJ. (2011). Removing noise from pyrosequenced
661 amplicons. *BMC Bioinf*, **12**: 38.
662

663 Rivas R, Velázquez E, Willems A, Vizcaíno N, Subba-Rao NS, Mateos PF, *et al.* (2002). A new
664 species of *Devosia* that forms a unique nitrogen-fixing root-nodule symbiosis with the aquatic
665 legume *Neptunia natans* (L.f.) Druce. *Appl Envir Micro* **68**: 5217-5222.
666

667 Robley, A.J., Short,J. and Bradley, S. (2001). Dietary overlap between the burrowing bettong and
668 the European rabbit in semi-arid coastal Western Australia. *Wildl Res* **28**: 341-349.
669

670 Ruiz-Moreno D, Pascual M, Riolo R. (2006). Exploring network space with genetic algorithms:
671 modularity, resilience and reactivity. In: Pascual M, Dunne JA, (eds). *Ecological networks:*
672 *linking structure to dynamics in food webs*. Oxford University Press: UK, pp 187-208.
673

674 Schalamuk S, Velázquez S, Cabello M. (2013). Dynamics of arbuscular mycorrhizal fungi spore
675 populations and their variability under contrasting tillage systems in wheat at different
676 phenological stages. *Biol Agric Hortic* **29**: 38-45.
677

678 Schloss PD, Westcott SL, Ryanbin T, Hall JR, Hartmann M, Hollister EB *et al.* (2009).
679 Introducing mothur: open-source, platform-independent, community-supported software for
680 describing and comparing microbial communities. *Appl Environ Micro* **75**: 7537-7541.
681

682 Schloss PD, Westcott SL. (2011). Assessing and improving methods used in operational
683 taxonomic unit-based approaches for 16S rRNA gene sequence analysis. *Appl Environ Micro* **77**:
684 3219-3226.
685

686 Singh AJ, Bhadouriya R. (2013). Microbes in green technology and carbon sequestration in mine
687 degraded land. In: Jamaluddin, Singh AK (eds) *Microbes and sustainable plant productivity*,
688 Scientific Publishers: Jodhpur, India, pp 170-178.
689

690 Smith, H.D. (1948). Biological control of citrus insects. In: Webber HJ, Batchelor LD (eds) *The*
691 *citrus industry. The production of the crop* **Vol. II**, California Press, USA, pp 597-626.
692

693 Spatafora JW, Sung GH, Johnson D, Hesse C, O'Rourke B, Serdani M *et al.* (2006). A five-gene
694 phylogeny of Pezizomycotina. *Mycologia* **98**: 1018-1028.
695

696 Steinberger Y, Whitford WG. (1983). The contribution of rodents to decomposition processes in
697 a desert ecosystem. *J Arid Environ* **6**:177-182.
698

699 Vossbrinck CR, Coleman DC, Woolley TA. (1979). Abiotic and biotic factors in litter
700 decomposition in a semiarid grassland. *Ecology* **60**: 265-271.
701

702 Wallwork JA, Kamill BM, Whitford WG. (1985). Distribution and diversity patterns of soil mites
703 and other microarthropods in a Chihuahuan desert site. *J Arid Environ* **9**: 215-231.
704

705 Wang Q, Garrity GM, Tiedje JM, Cole JR. (2007). Naive Bayesian classifier for rapid
706 assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Micro* **73**: 5261-
707 5267.
708

709 Weisburg WG, Barns SM, Pelletier Da, Lane DJ. (1991). 16S ribosomal DNA amplification for
710 phylogenetic study. *J Bacteriol* **173**: 697-703.
711

712 Whitford WG (2002). *Ecology of Desert Systems*. Elsevier Science, London.
713

714 Whitford WG, Kay FR. (1999). Biopedturbation by desert mammals: a review. *J. Arid Environ.*
715 **41**: 203-230.
716

717 Woinarski JCZ, Burbidge AA, Harrison PL (2012). *The Action Plan for Australian Mammals*
718 *2012*. CSIRO Publishing, Melbourne, Australia.
719

720 Wollenzien U, Hoog GS, Krumbein WE, Uijthf MJJ. (1997) . *Sarcinomycespetricola*, a new
721 microcolonial fungus from marble in the Mediteranean Basin. *Antone van Leeuwenhoek* **71**: 281-
722 288.
723

724 Yeager CM, Kornosky JL, Housman DC, Grote EE, Belnap J, Kuske CR. (2004). Diazotrophic
725 community structure and function in two successional stages of biological soil crusts from the
726 Colorado Plateau and Chihuahuan Desert. *Appl Environ Micro* **70**: 973-983.
727

728 Zhu WY, Zhao LX, Zhao, GZ, Duan XW, Qin S, Li J, *et al.* (2012). *Plantactinospora*
729 *endophytica* sp. nov., an actinomycete isolated from *Camptotheca acuminata* Decne.,
730 reclassification of *Actinaurispora siamensis* as *Plantactinospora siamensis* comb. nov. and
731 emended descriptions of the genus *Plantactinospora* and *Plantactinospora mayteni*. *Int J Syst*
732 *Evol Micro* **62**: 2435–2442.

Captions for figures

Figure 1. Relative abundance of major (A) bacterial and (B) fungal taxa within each microsite. Larger circles indicate greater abundance.

Figure 2. Multi-dimensional scaling biplot of the first two dimensions of an ordination of a reduced matrix of (A) 280 bacterial OTUs and (B) 135 fungal OTUs. Note the clustering of undisturbed samples for both bacteria and fungi.

1 Table 1. Bacterial taxa, to the level of genus, that are significantly associated with different microsites using Indicator Species
 2 Analysis.

3

Order	Family	Genus	Microsite	IV	P-value	No of OTUs
Cyanobacteria	Family I	Group I	Undisturbed	0.866	0.047	3
Cyanobacteria	Family X	Group X	Undisturbed	0.866	0.046	1
Cyanobacteria	Unclassified	Unclassified	Undisturbed	0.866	0.049	1
Actinomycetales	Micromonosporaceae	Asanoa	Undisturbed	0.866	0.046	1
Cyanobacteria	Family VIII	Group VIII	Undisturbed	0.866	0.047	1
Sphingobacteriales	Chitinophagaceae	Segetibacter	Undisturbed	0.866	0.049	1
Alphaproteobacteria	Alphaproteobacteria	Alphaproteobacteria	Undisturbed	0.866	0.047	1
Myxococcales	Cystobacteraceae	Hyalangium	Old	1.000	0.008	1
Acidobacteria	Acidobacteria	Group IV	Old	0.913	0.030	1
Rhizobiales	Methylobacteriaceae	Microvirga	Old	0.812	0.028	1
Rubrobacterales	Rubrobacteraceae	Rubrobacter	Young	0.905	0.012	1
Bacillales	Paenibacillaceae	Ammoniphilus	Young	0.866	0.040	1
Actinomycetales	Micromonosporaceae	Actinaurispora	Young	0.866	0.040	1

4

1 Table 2. Fungal taxa, to the level of genus, that are significantly associated with different microsites using Indicator Species Analysis.

2 Only taxa with an indicator value (IV) > 0.75 are shown.

3

Subclass	Order	Family	Genus	Microsite	IV	P	No of OTUs
Eurotiomycetidae	Eurotiales	Trichocomaceae	Unclassified	Undisturbed	0.94	0.002	1
Pleosporomycetidae	Pleosporales	Leptosphaeriaceae	Leptosphaeria	Undisturbed	0.91	0.006	8
Pleosporomycetidae	Pleosporales	Melanommataceae	Trematosphaeria	Undisturbed	0.91	0.006	4
Skeletonemataceae	Skeletonema	Unclassified	Unclassified	Undisturbed	0.91	0.002	1
Strombidiidae	Strombidium	Unclassified	Unclassified	Undisturbed	0.91	0.007	1
Dothideomycetidae	Dothideales	Dothioraceae	Delphinella	Undisturbed	0.90	0.003	3
Naviculaceae	Navicula	Unclassified	Unclassified	Undisturbed	0.90	0.003	4
Pleosporomycetidae	Pleosporales	Phaeosphaeriaceae	Phaeosphaeria	Undisturbed	0.88	0.013	6
Pleosporomycetidae	Pleosporales	Pleosporaceae	Pleospora	Undisturbed	0.88	0.011	4
Dothideomycetidae	Dothideales	Dothioraceae	Columnosphaeria	Undisturbed	0.86	0.012	17
Chaetothyriomycetidae	Chaetothyriales	Chaetothyriales	Glyphium	Undisturbed	0.86	0.012	19
Pleosporomycetidae	Pleosporales	Phaeotrichaceae	Phaeotrichum	Undisturbed	0.86	0.011	2
Chaetothyriomycetidae	Chaetothyriales	Chaetothyriales	Sarcinomyces	Undisturbed	0.86	0.011	2
Mycocaliciomycetidae	Mycocaliciales	Sphinctrinaceae	Sphinctrina	Undisturbed	0.86	0.012	11
Dothideomycetes	Tubeufiaceae	Letendraea	Unclassified	Undisturbed	0.86	0.012	10
Leotiomycetes	Myxotrichaceae	Myxotrichaceae	Geomyces	Undisturbed	0.85	0.012	6
Sporadotrichida	Halteriidae	Halteria	Unclassified	Undisturbed	0.85	0.014	7

Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	Botryosphaeria	Undisturbed	0.82	0.016	1
Lecanoromycetidae	Lecanorales	Lecanorineae	Cladoniaceae	Undisturbed	0.82	0.016	1
Dothideomycetidae	Dothideales	Dothideales	Hortaea	Undisturbed	0.82	0.015	2
Xylariomycetidae	Xylariales	Xylariaceae	Hypoxylon	Undisturbed	0.82	0.023	1
Xylariomycetidae	Xylariales	Amphisphaeriaceae	Pestalosphaeria	Undisturbed	0.82	0.012	1
Helotiales	Bulgariaceae	Bulgaria	Unclassified	Undisturbed	0.82	0.012	1
Dothideomycetes	Kirschsteiniothelia	Unclassified	Unclassified	Undisturbed	0.82	0.016	2
Pezizales	Pezizaceae	Peziza	Unclassified	Undisturbed	0.82	0.016	1
Sordariomycetidae	Magnaporthales	Magnaporthaceae	Pseudohalonectria	Undisturbed	0.81	0.021	3
Lecanoromycetidae	Lecanorales	Lecanorineae	Sphaerophoraceae	Undisturbed	0.81	0.018	5
Agaricomycetidae	Agaricales	Lycoperdaceae	Lycoperdon	Undisturbed	0.76	0.039	1
Dothideomycetidae	Dothideales	Dothioraceae	Delphinella	Old	0.91	0.004	1
Eurotiomycetidae	Eurotiales	Trichocomaceae	Chromocleista	Old	0.82	0.015	1
Chaetothyriomycetidae	Chaetothyriales	Chaetothyriales	Glyphium	Old	0.82	0.025	6
Dothideomycetes	Tubeufiaceae	Letendraea	Unclassified	Old	0.82	0.025	1
Chaetothyriomycetidae	Chaetothyriales	Chaetothyriales	Sarcinomyces	Old	0.80	0.025	1
Mycocaliciomycetidae	Mycocaliciales	Sphinctrinaceae	Sphinctrina	Old	0.80	0.027	3
Sporadotrichida	Halteriidae	Halteria	Unclassified	Old	0.80	0.024	2
Dothideomycetidae	Dothideales	Dothioraceae	Columnsphaeria	Old	0.79	0.025	5
Pleosporomycetidae	Pleosporales	Leptosphaeriaceae	Leptosphaeria	Old	0.79	0.030	4
Hypocreomycetidae	Hypocreales	Hypocreaceae	Hypocrea	Old	0.75	0.034	2
Xylariomycetidae	Xylariales	Amphisphaeriaceae	Pestalosphaeria	Old	0.74	0.045	1

Eurotiomycetidae	Eurotiales	Trichocomaceae	Hamigera	Young	0.87	0.011	4
Hypocreomycetidae	Hypocreales	Bionectriaceae	Bionectriaceae	Young	0.86	0.013	2
Dothideomycetidae	Dothideales	Dothioraceae	Columnosphaeria	Young	0.86	0.013	1
Eurotiomycetidae	Eurotiales	Trichocomaceae	Eupenicillium	Young	0.84	0.016	5
Leotiomycetes	Myxotrichaceae	Myxotrichaceae	Geomyces	Young	0.82	0.021	1
Mycocaliciomycetidae	Mycocaliciales	Sphinctrinaceae	Sphinctrina	Young	0.82	0.012	1
Pleosporomycetidae	Pleosporales	Leptosphaeriaceae	Leptosphaeria	Young	0.79	0.041	2
Dothideomycetes	Tubeufiaceae	Letendraea	Unclassified	Young	0.79	0.026	2

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Table 3. Metrics obtained from analysis of scale-free microbial networks of bacterial and fungal microbial communities. Edges represent the number of significant positive and negative Pearson correlation coefficients identified following implementation of the Benjamini-Hochberg procedure at a minimum false discovery rate of 5%.

Taxon and microsite	OTUs	Edges	Mean number of neighbours	Clustering Coefficient	Density	Centralization
Bacteria						
Old	40	70	3.450	0.547	0.088	0.096
Undisturbed	31	39	2.516	0.566	0.084	0.089
Young	17	11	1.294	0	0.081	0.050
Fungi						
Old	135	485	7.185	0.472	0.054	0.067
Undisturbed	177	1814	20.497	0.652	0.166	0.095
Young	81	321	7.926	0.647	0.009	0.116

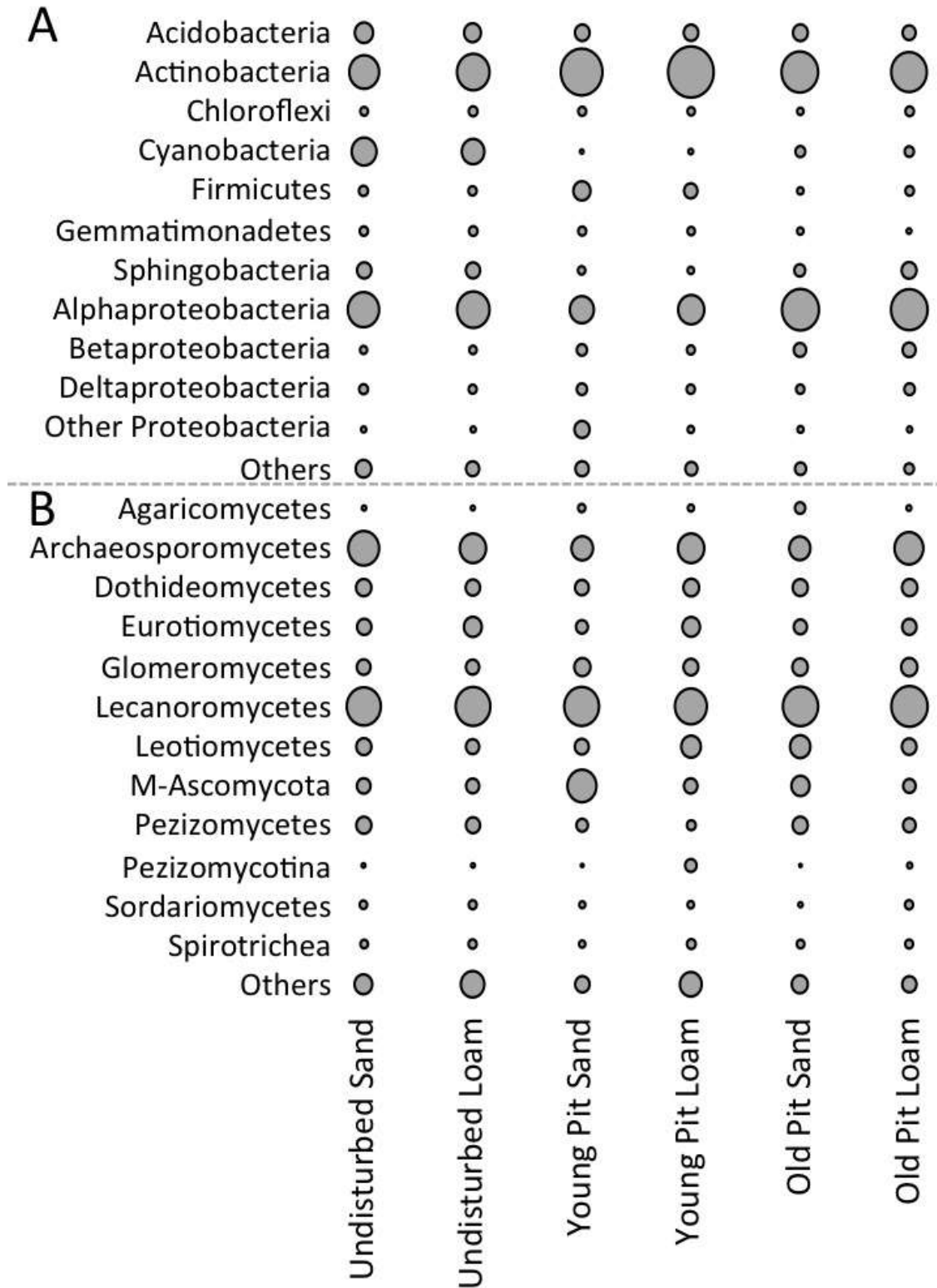


Figure 1. Relative abundance of major (A) bacterial and (B) fungal taxa within each microsite. Larger circles indicate greater abundance.

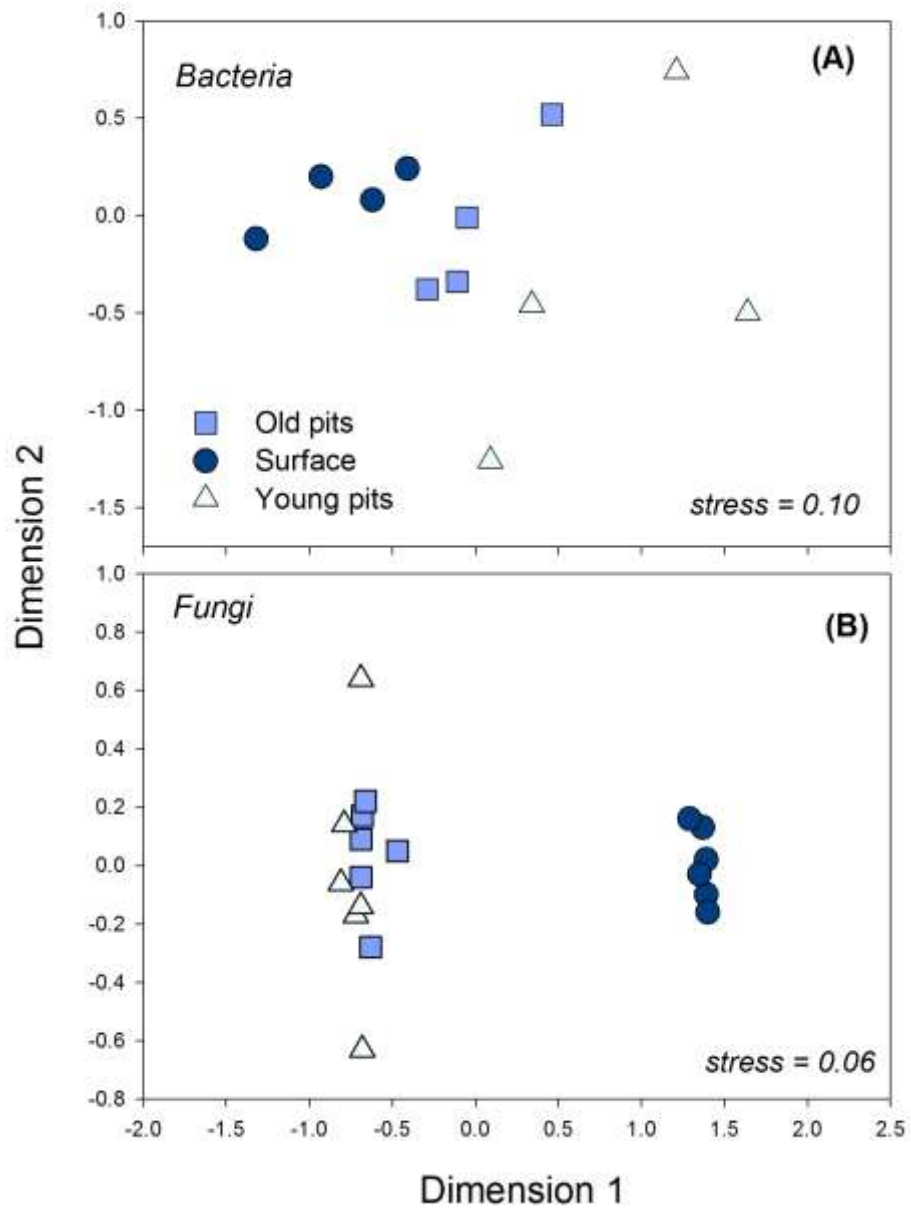


Figure 2. Multi-dimensional scaling biplot of the first two dimensions of an ordination of a reduced matrix of (A) 280 bacterial OTUs and (B) 135 fungal OTUs. Note the clustering of undisturbed samples for both bacteria and fungi.