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### *Niche partitioning of bacterial communities in biological crusts and soils under grasses, shrubs and trees in the Kalahari*

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*Published in:*

Biodiversity and Conservation

*DOI:*

[10.1007/s10531-014-0684-8](https://doi.org/10.1007/s10531-014-0684-8)

*Publication date:*

2014

*Citation for published version (APA):*

Elliott, D. R., Thomas, A. D., Hoon, S. R., & Sen, R. (2014). Niche partitioning of bacterial communities in biological crusts and soils under grasses, shrubs and trees in the Kalahari. *Biodiversity and Conservation*, 23(7), 1709-1733. <https://doi.org/10.1007/s10531-014-0684-8>

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2 **Niche partitioning of bacterial communities in biological**  
3 **crusts and soils under grasses, shrubs and trees**  
4 **in the Kalahari**

5 **David R. Elliott · Andrew D. Thomas · Stephen R. Hoon ·**  
6 **Robin Sen**

7 Received: 22 November 2013 / Revised: 21 March 2014 / Accepted: 24 March 2014  
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9 **Abstract** The Kalahari of southern Africa is characterised by sparse vegetation inter-  
10 spersed with microbe-dominated biological soil crusts (BSC) which deliver a range of  
11 ecosystem services including soil stabilisation and carbon fixation. We characterised the  
12 bacterial communities of BSCs (0–1 cm depth) and the subsurface soil (1–2 cm depth) in  
13 an area typical of lightly grazed Kalahari rangelands, composed of grasses, shrubs, and  
14 trees. Our data add substantially to the limited amount of existing knowledge concerning  
15 BSC microbial community structure, by providing the first bacterial community analyses  
16 of both BSCs and subsurface soils of the Kalahari region based on a high throughput 16S  
17 ribosomal RNA gene sequencing approach. BSC bacterial communities were distinct with  
18 respect to vegetation type and soil depth, and varied in relation to soil carbon, nitrogen, and  
19 surface temperature. *Cyanobacteria* were predominant in the grass interspaces at the soil  
20 surface (0–1 cm) but rare in subsurface soils (1–2 cm depth) and under the shrubs and  
21 trees. *Bacteroidetes* were significantly more abundant in surface soils of all areas even in  
22 the absence of a consolidated crust, whilst subsurface soils yielded more sequences affil-  
23 iated to *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, and *Firmicutes*. The common detec-  
24 tion of vertical stratification, even in disturbed sites, suggests a strong potential for BSC  
25 recovery after physical disruption, however severe depletion of *Cyanobacteria* near trees  
26 and shrubs may limit the potential for natural BSC regeneration in heavily shrub-  
27 encroached areas.

A1 Communicated by Guest Editors of S.I. : Biocrust.

A2 **Electronic supplementary material** The online version of this article (doi:10.1007/s10531-014-0684-8)  
A3 contains supplementary material, which is available to authorized users.

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28 **Keywords** Biological soil crust · 454 Pyrosequencing · Bacterial community ·  
29 Kalahari sand · Carbon · Vegetation

30

## 31 Introduction

32 Soils are vital for agricultural productivity, biodiversity and carbon storage (Stringer 2008),  
33 properties that are dependent upon the presence and activities of soil microbial commu-  
34 nities (Brussard 2012). Most studies on soil microbes have focused on productive agri-  
35 cultural systems and typically target bulk soil or the plant-associated rhizosphere (e.g.  
36 Rousk et al. 2010; Pereira et al. 2012; Fierer et al. 2012a; Phosri et al. 2012). Because  
37 drylands typically support a patchy vegetation cover, light reaches the soil surface in plant  
38 interspaces and facilitates the formation of a complex autotrophic and heterotrophic  
39 microbial community, which binds soil into a crust. These biological soil crusts (BSC) are  
40 a major component of dryland biodiversity (Büdel et al. 2009) and of global carbon and  
41 nitrogen cycles (Elbert et al. 2012). However, we have very little information on the  
42 microbial content of dryland soils and BSCs, and even less on how the soil microbes affect  
43 dryland ecosystem function. A key factor is the lack of a detailed characterisation of the  
44 microbial composition of BSCs, with the exception of a few important groups such as the  
45 *Cyanobacteria* (e.g. Dojani et al. 2013). Addressing this research gap is essential if future  
46 management and conservation of drylands is to be effective in the face of increasing  
47 climatic and anthropogenic pressures (Stringer et al. 2012). This is a major area of concern  
48 because drylands cover approximately 40 % of the global land surface and support 38 % of  
49 the human population (Reynolds et al. 2007).

50 Many of the ecosystem functions of BSCs are attributed to *Cyanobacteria*, which  
51 comprise a large fraction of BSC biomass (Gundlapally and Garcia-Pichel 2006). *Cya-*  
52 *nobacteria* can sequester carbon through photosynthesis and fix nitrogen (Elbert et al.  
53 2012), enabling BSCs to perform similar ecosystem functions to plants (Bowker et al.  
54 2010). BSCs also facilitate numerous ecosystem services of importance to land manage-  
55 ment, conservation and productivity (Thomas 2012). These include soil stabilisation  
56 (Thomas and Dougill 2007), nitrogen fixation (Aranibar et al. 2003), moisture retention  
57 (Menon et al. 2011), and modulation of surface runoff (Eldridge and Greene 1994; Belnap  
58 2006).

59 Despite the recognition of a significant non-phototrophic component of BSC commu-  
60 nities (Garcia-Pichel et al. 2003; Bowker et al. 2010) there have been relatively few studies  
61 characterising functionally important bacterial heterotrophs in BSCs. Most studies of  
62 dryland BSC communities have used a combination of cultivation and molecular 16S  
63 rRNA gene fingerprinting techniques followed by identification of isolates and molecular  
64 types by sequencing. These methods have revealed much about BSC community structure,  
65 including relationship to ecosystem functioning (Castillo-Monroy et al. 2011), demon-  
66 stration of vertical stratification (Garcia-Pichel et al. 2003), similarity of diversity  
67 regardless of nearby plants (Nagy et al. 2005), and identification of numerically dominant  
68 phyla (e.g. Gundlapally and Garcia-Pichel 2006). However, the methodology employed in  
69 these studies severely limits microbial identification to no more than a few hundred species  
70 or operational taxonomic units (OTUs). This is insufficient to make rigorous community  
71 comparisons, particularly in a well-replicated study where more samples must be identi-  
72 fied, thus yielding a lower number of sequences per sample. Current high-throughput



73 sequencing technologies such as 454 pyrosequencing overcome this practical limitation  
74 and have recently been used by Steven et al. (2013) to reveal differences in BSC and soil  
75 bacterial community structure in relation to parent material in Colorado, USA. In a broader  
76 study, Fierer et al. (2012b) went a step further by performing a cross-biome metagenomic  
77 survey of soil (0–5 cm depth) microbial communities in tandem with a high-throughput  
78 phylogenetic survey, enabling a comparison of functional gene frequencies as well as  
79 microbial taxa. This study showed that hot and cold desert communities are quite distinct  
80 from forest, grassland and tundra on both a taxonomic and functional level.

81 In this paper we present the first description of bacterial community structure within  
82 BSCs and soils of the Kalahari in the south west of Botswana, and assess niche partitioning  
83 with respect to depth and nearby vegetation based on high-throughput sequencing of the  
84 bacterial 16S rRNA gene. Weakly developed BSCs in the Kalahari Sand soils are found in  
85 large areas that are subjected to disturbance by livestock and wildlife activity, which  
86 prevents succession into more developed stages (Thomas and Dougill 2006). Grazing also  
87 selectively removes palatable grasses and eventually leads to woody shrub encroachment  
88 which renders the land useless for continued grazing (Thomas 2012). Thus, both vegetation  
89 and co-occurring BSC cover in Kalahari rangelands are strongly affected by human  
90 activity and can be influenced by land management decisions relating to factors such as  
91 animal stocking density, fencing, and water access.

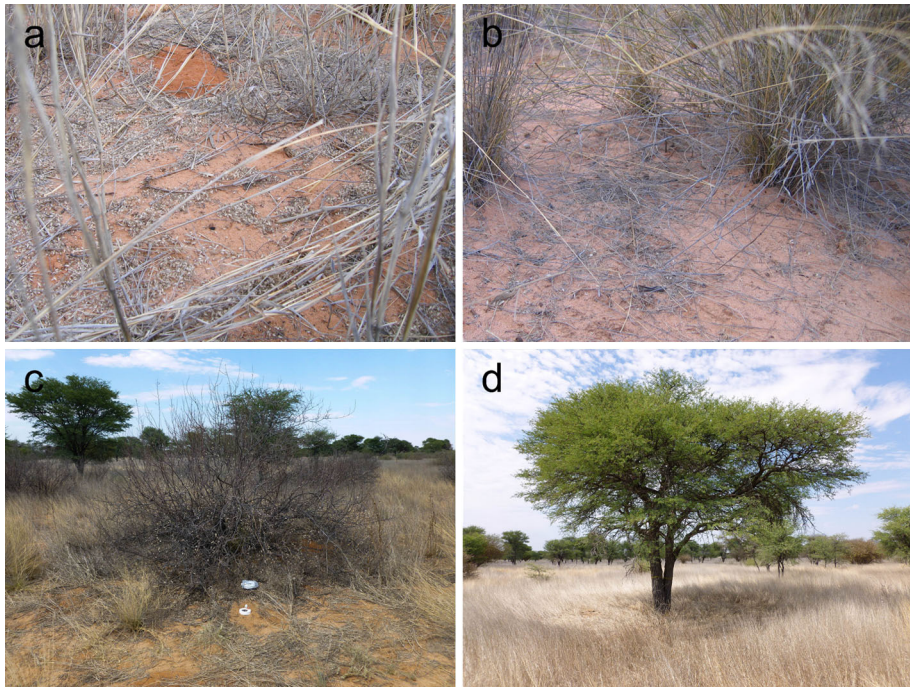
92 Our hypothesis is that landscape impacts related to grazing pressure such as disturbance  
93 and shrub encroachment drives functionally significant soil surface bacterial community  
94 changes and thus should be included in land management decision-making. Our specific  
95 objectives were to determine whether there are significant differences in bacterial popu-  
96 lations associated with: (i) soils under tree, shrub and grasses, (ii) subsurface soils and  
97 BSCs.

## 98 **Materials and methods**

### 99 **Study site**

100 Samples were collected from a long-term research site near Tsabong in south west Bots-  
101 wana (25°56'51"S, 22°25'40"E) at the end of the dry season in November 2011 and during  
102 the wet season in March 2012. Soils are formed on Kalahari Sands and are weakly acidic,  
103 fine sand-sized Arenosols (FAO 1990), with little or no horizon development. In lightly  
104 grazed areas, around 80 % of the surface is covered in a 3–4 mm deep BSC, which has  
105 been described in detail elsewhere (Thomas and Dougill 2007; 2012). BSC cover is  
106 inversely related to grazing intensity and in frequently grazed areas, cover is typically  
107 <10 % of the surface. The organic matter, carbon and nitrogen content of the Kalahari  
108 Sand soils is low, reflecting the limited biological productivity and highly oxidising nature  
109 of the soils (Thomas et al. 2012). BSCs, however, are enriched in ammonium, total N and  
110 organic C compared to the mineral soil (Thomas and Dougill 2007; 2012).

111 Vegetation cover is typical of an open-canopy, fine-leaf savannah, with a mix of  
112 perennial (*Eragrostis lehmanniana*) and annual (*Schmidtia kalahariensis*) grasses, woody  
113 shrubs (*Grewia flava* and *Acacia mellifera*) and trees, predominantly *Acacia erioloba*  
114 (Fig. 1). Mean annual precipitation is 331 mm (1996–2013), with a low of 114 mm in  
115 2006–07 and a high of 532 mm in 2001–02. Seasonal variations in air temperature are  
116 extreme, with summer maxima frequently in excess of 40 °C and winter below 0 °C.



**Fig. 1** Photographs showing examples of the four sampling zones. Letters in square brackets indicate abbreviations used throughout the manuscript. **a** Annual grass [AG] *Schmidtia kalahariensis* interspace. **b** Perennial grass [PG] *Eragrostis lehmanniana* interspace. **c** Shrub [S] *Grewia flava*. **d** Tree [T] *Acacia erioloba*

## 117 Sample collection and preparation

118 Soils and BSCs were collected from within a fenced 800 × 500 m paddock where grazing  
119 animals had been excluded since the previous year. Sites were selected according to the  
120 overlying vegetation type, with three replicate sites under trees (*A. erioloba*), woody shrubs  
121 (*G. flava*), perennial (*Eragrostis lehmanniana*) and annual (*Schmidtia kalahariensis*)  
122 grasses (Fig. 1).

123 Soils at all sites, except those under trees, were covered in a BSC, previously classified  
124 as type 1 (weakly consolidated with no surface discolouration) and 2 (more consolidated  
125 with black or brown speckled surface) by Thomas and Dougill (2006, 2007). Soils  
126 underneath trees were not crusted, but unconsolidated and slightly darkened by fragmented  
127 litter. There was evidence of severe animal disturbance under all tree canopies, a legacy of  
128 cattle seeking shade in the year prior to sampling. Soils under *G. flava* canopies were  
129 covered in a dry layer of leaf litter but were also well crusted.

130 Samples were collected using aseptic techniques from 0 to 1 cm (incorporating the  
131 BSC) and 1–2 cm (the soil immediately below the BSC). The same sites were sampled in  
132 November 2011 and March 2012, giving a total of 96 samples (48 in each season). Soils  
133 were dry at the time of sampling.



134 Physico-chemical analyses

135 Total carbon and nitrogen content of BSC and subsurface soils were determined using a  
136 CN element analyser (Leco TruSpec). Soil surface temperature was measured at approx-  
137 imately 2-h intervals during the day at each sampling site for the duration of each field  
138 campaign, using an infrared thermometer.

139 Molecular analyses of bacterial community composition

140 DNA was extracted from soil samples within 18 h of sampling using a Powersoil DNA  
141 extraction kit (MoBio Inc.). Prior to extraction, samples of approximately 20 g were  
142 homogenised by shaking followed by cutting with a scalpel to disaggregate. Extractions  
143 were performed according to the manufacturer's instructions except that the soil mass was  
144 increased slightly from 0.25 to 0.4 g based on laboratory extraction tests and consultation  
145 with the manufacturer. DNA was eluted into 50 µl of buffer (10 mM Tris). Phylogeneti-  
146 cally informative DNA sequences were obtained from each sample by tag-encoded FLX  
147 amplicon pyrosequencing targeting the bacterial 16S rRNA gene (Dowd et al. 2008). This  
148 analysis was performed by Research and Testing Laboratory (Lubbock, TX, USA), using a  
149 Roche 454 FLX instrument with Titanium reagents.

150 DNA was amplified for pyrosequencing using forward and reverse fusion primers. The  
151 forward primer was constructed with the Roche A linker (CCATCTCATCCCTGCGT  
152 GTCTCCGACTCAG), an 8–10 bp barcode (see Online Resource 1), and the 341F primer  
153 (CCTACGGGAGGCAGCAG) (Muyzer et al. 1993). The reverse fusion primer was  
154 constructed with a biotin molecule, the Roche B linker (CCTATCCCCTGTGTGCCTT  
155 GGCAGTCTCAG), and the 907R primer (CCGTCAATTCMTTGTAGTTT) (Muyzer  
156 et al. 1998). Amplifications were performed in 25 µl reactions with Qiagen HotStar Taq  
157 master mix (Qiagen Inc, Valencia, California), 1 µl of each 5 µM primer, and 1 µl of  
158 template. Reactions were performed on ABI Veriti thermocyclers (Applied Biosystems,  
159 Carlsbad, California) under the following thermal profile: 95 °C for 5 min, then 35 cycles  
160 of 94 °C for 30 s, 54 °C for 40 s, 72 °C for 1 min, followed by one cycle of 72 °C for  
161 10 min and 4 °C hold. PCR products were visualized with eGels (Life Technologies,  
162 Grand Island, New York), pooled equimolar, and size selected before sequencing following  
163 manufacturer protocols.

164 Bioinformatics and statistical analyses

165 Sequence data were processed through the QIIME pipeline (Caporaso et al. 2010) and  
166 further analyses were performed using R (R Core Team 2012). Denoising, quality filtering,  
167 OTU assignment, OTU table generation, and phylogenetic determinations were all per-  
168 formed in QIIME. Sequences shorter than 200 bp or having an average quality score <25  
169 within a 50 base pair window were discarded. A 97 % sequence similarity was used to  
170 define OTUs (approximately species level; Stackebrandt and Goebel 1994) which were  
171 assigned using UCLUST (Edgar 2010), and chimeras were removed using chimeraSlayer  
172 (Haas et al. 2011). After quality control there were on average 1,004 sequences per sample  
173 and a total of 2,705 OTUs (further details are provided in Online Resource 1). OTUs were  
174 identified through the RDP classifier (Wang et al. 2007) using the Greengenes database  
175 release of October 2012 (DeSantis et al. 2006). Identified OTUs were assembled into an  
176 OTU table summarising the frequency of observation in each sample. These results formed  
177 the basis for the determination and comparison of community structure.



178 Thousands of different taxa were detected, so constrained correspondence analysis  
179 (CCA) was used to discern community features which specifically relate to vegetation zone  
180 or depth. Rare species comprising  $<0.01\%$  of the sequences detected in the study were  
181 excluded from correspondence analysis because rare species can obscure community  
182 patterns and may be differentially detected depending on sample sequencing depth. Cor-  
183 respondence analysis was based on the Bray-Curtis distance measure and performed using  
184 the Phyloseq (McMurdie and Holmes 2013) wrapper to the Vegan package (Oksanen et al.  
185 [2013](#)) for R. Unconstrained correspondence analysis (CA) was performed to visualise the  
186 overall community structure (shown in supplementary data Online Resource 2 only) whilst  
187 CCA was employed to discern community features which specifically relate to vegetation  
188 zone or depth. Permutation tests ( $n = 1,000$ ) were used to test the significance of measured  
189 environmental variables to the ordination.

190 Differences in community structure were assessed using ADONIS, a permutational  
191 multivariate analysis of variance test in the R package Vegan (Oksanen et al. 2013), to  
192 determine whether communities differ with respect to vegetation zone, depth, or sampling  
193 month. We used the Bray-Curtis distance measure and performed the test at all taxonomic  
194 levels from phylum to species. OTU richness and community diversity were estimated  
195 using the Chao1 and Shannon methods respectively, implemented in the Phyloseq package.  
196 Richness and diversity calculations used the full data set.

197 Kruskal–Wallis tests were used to determine whether each OTU relative abundance  
198 differed between vegetation zone, depth, or sampling month. The significance of OTU  
199 abundance correlations with continuous variables (e.g. carbon or richness) was assessed  
200 using Spearman's test. P-values were corrected to account for multiple comparisons using  
201 the false discovery rate method (Benjamini and Hochberg 1995). Significant findings were  
202 tested further using post hoc tests to identify the changes responsible. We regarded results  
203 with corrected  $p < 0.05$  as being significant.

204 Availability of sequence data

205 Sequence data and metadata are available on the MG-RAST metagenomics analysis server  
206 (Meyer et al. 2008) at <http://metagenomics.anl.gov/linkin.cgi?project=6691>.

## 207 Results

208 Soil chemistry

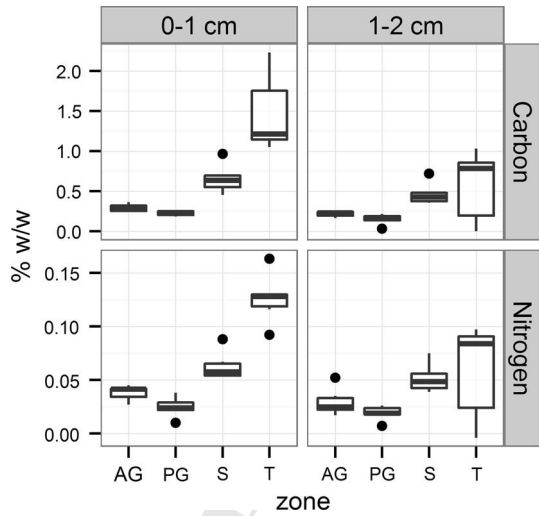
209 Soil carbon was significantly higher in the soil surface ( $0.7\% \pm 0.1$  SE) compared to the  
210 subsurface soil ( $0.4\% \pm 0.1$  SE), and also differed significantly between vegetation  
211 classifications (Fig. 2) but not by month. Total soil carbon and nitrogen were closely  
212 correlated and the mean C:N ratio was 9:1. The C:N ratio was significantly higher in BSCs,  
213 (ANOVA  $F = 8.91$ ,  $df = 1$ ,  $p = 0.0047$ ), but did not vary significantly with respect to  
214 vegetation zones.

215 Bacterial diversity

216 The Chao1 richness estimate and shannon diversity index (Fig. 3) provide an indication of  
217 the total number of species and the microbial diversity (taking account of number of



**Fig. 2** Total carbon and nitrogen in BSC (0–1 cm depth) and soil (1–2 cm depth) samples at each site (n = 6). Boxes represent the interquartile range (IQR), and error bars extend to the most extreme values within 1.5 \* IQR of the box. Median values are shown as a line within the box and outliers are shown as black spots. Sample coding: AG annual grass, PG perennial grass, S shrub, T tree. Note that y axes differ



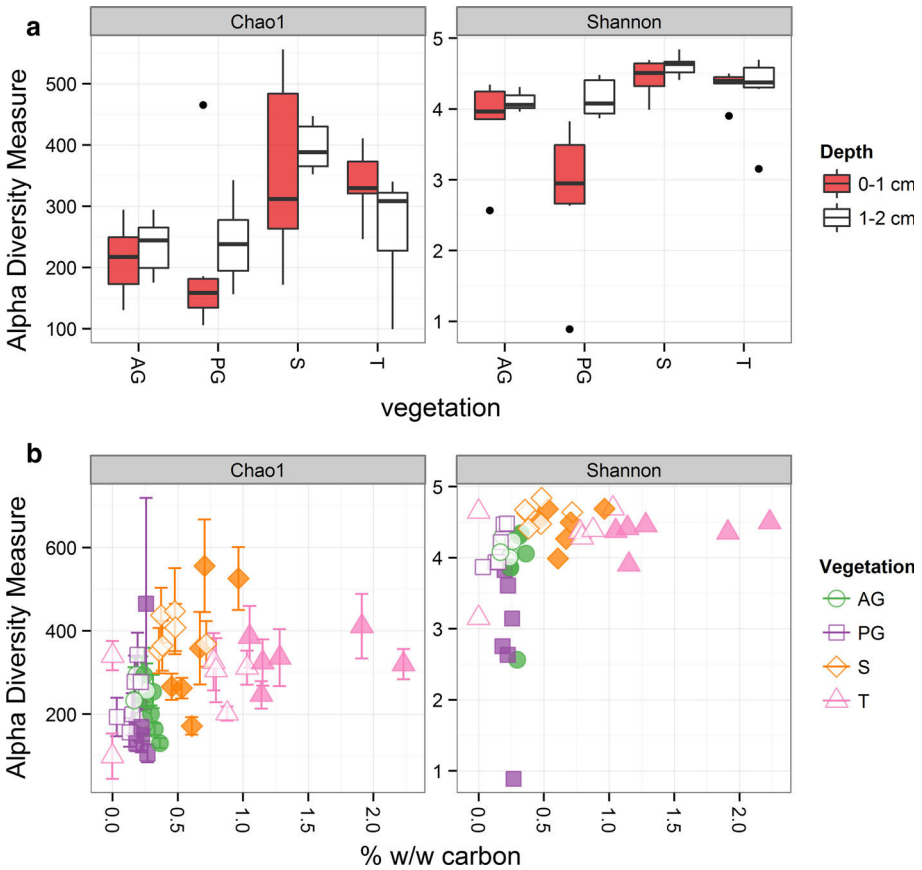
218 species and evenness) in each niche. Richness and diversity differed significantly with  
 219 respect to vegetation zone (Kruskal–Wallis Chi squared = 22.91, df = 3, p  
 220 value =  $4.22 \times 10^{-5}$ ) but not depth, although diversity was close to our significance  
 221 threshold for depth (Kruskal–Wallis Chi squared = 2.93, df = 1, p-value = 0.087). Both  
 222 diversity (Spearman’s rank correlation rho = 0.5, p value =  $3 \times 10^{-4}$ ) and richness  
 223 (rho = 0.5, p value =  $3 \times 10^{-4}$ ) were positively correlated with carbon content of the  
 224 soil. Diversity measures for each sample are provided in Online Resource 1.

## 225 Bacterial community structure

226 ADONIS showed that the bacterial communities differed by depth and by vegetation zone  
 227 ( $p < 0.05$ , see Online Resource 3 for test statistics) at all taxonomic ranks from phylum to  
 228 species, but did not differ in relation to sampling month except for at the rank of Family.  
 229 Interactions were detected ( $p < 0.05$ ) between vegetation zone and depth at all taxonomic  
 230 ranks except for Genus ( $p = 0.051$ ), and also between month and vegetation zones at  
 231 family level and higher taxa. A total of 28 bacterial phyla were detected, and the top 9  
 232 shown in Fig. 4 account for 99 % of sequences. Sequences from *Actinobacteria* and  
 233 *Proteobacteria* numerically dominated the samples, together representing 63 % of  
 234 sequences. Most phyla detection frequencies differed significantly ( $p < 0.05$ , see Online  
 235 Resource 4 for test statistics) with respect to depth or vegetation zone, or both (Table 1).  
 236 *Cyanobacteria* and *Chloroflexi* were more abundant in grass areas, especially the *Cya-*  
 237 *nobacteria* which were only rarely detected in tree and shrub areas. *Cyanobacteria* were  
 238 also very rare in all subsurface soils (1–2 cm depth), only being found in large number in  
 239 the BSCs of grass interspaces. *Bacteroidetes* and *Cyanobacteria* were significantly asso-  
 240 ciated with BSCs (0–1 cm depth), whilst *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, and  
 241 *Firmicutes* were significantly associated with subsurface soil (1–2 cm depth). Phylum  
 242 composition did not vary significantly in relation to the sampling season for any of the top  
 243 9 phyla. Data for rarer phyla is also provided in Online Resource 4.

244 Constrained CA was used to generate a visual representation of the microbial com-  
 245 munity structure differences between depths and vegetation zones. Sequences accounting



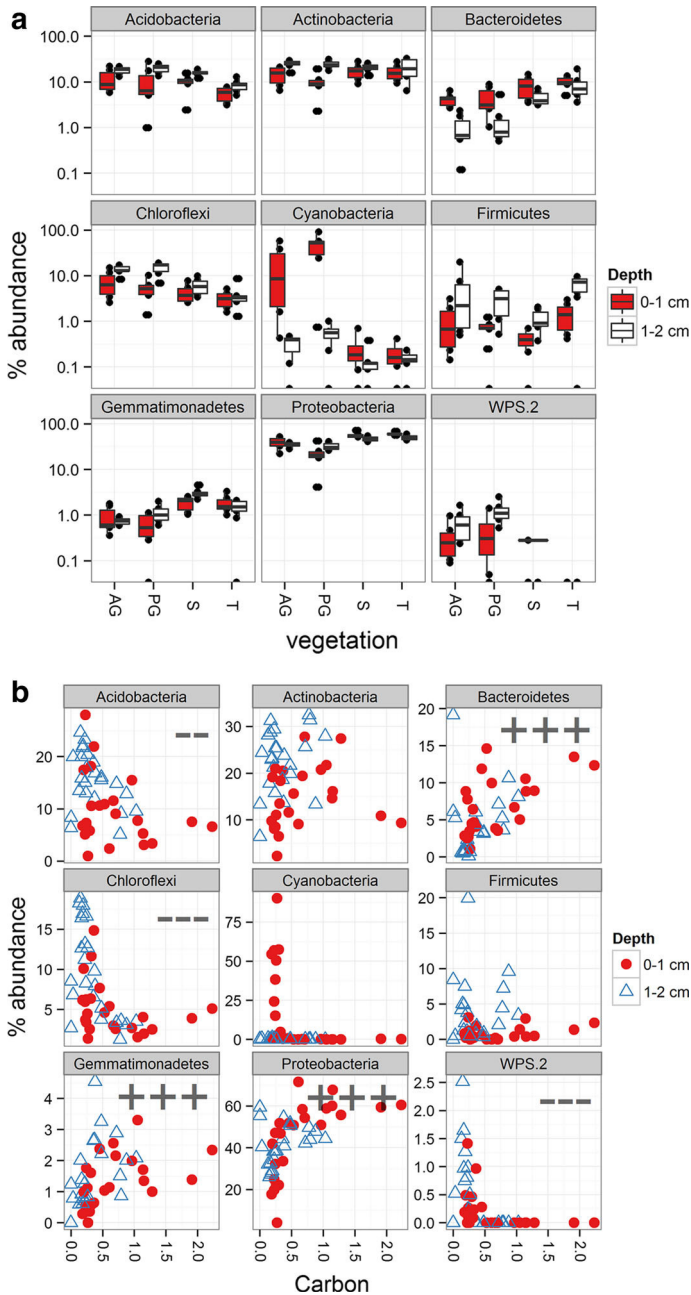


**Fig. 3** OTU richness estimation (Chao1) and diversity index (Shannon). Results are plotted with respect to **a** sampling site; and **b** sample carbon content. Boxes represent the interquartile range (IQR), and error bars extend to the most extreme values within  $1.5 * IQR$  of the box ( $n = 5$  or  $6$ ). Median values are shown as a line within the box and outliers are shown as black spots. Error bars in **b** indicate the standard error of the individual Chao1 estimations. Sample coding: AG annual grass, PG perennial grass, S shrub, T tree. Filled symbols = 0–1 cm depth, hollow symbols = 1–2 cm depth

246 for  $<0.01\%$  of the library were excluded from this analysis, leaving 934 OTUs. The  
247 microbial communities were separated by vegetation type (tree/shrub or grasses) on axis 1,  
248 and by depth on axis 2 (Fig. 5). Unconstrained CA yielded similar but less well defined  
249 patterns (Online Resource 2). Soil carbon (and nitrogen) content and soil temperature were  
250 significant to the ordination as determined by permutation tests ( $n = 1,000$ ). Vectors show  
251 that soil carbon and nitrogen increase with axis 1 (direction of tree and shrub samples),  
252 whilst soil surface temperature increases in the opposite direction (i.e. in the direction of  
253 grass interspace samples).

254 Abundant taxa (OTU level)

255 The detection frequencies of the most abundant 9 OTUs are shown in Fig. 6. Together  
256 these 9 OTUs accounted for 27 % of sequences and all of them had different detection



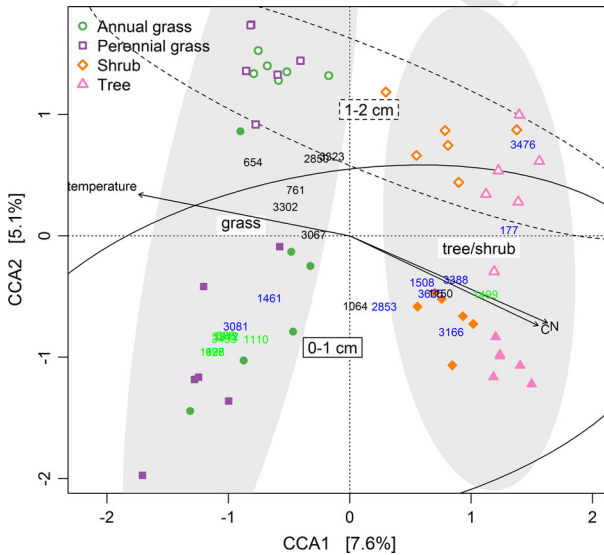
**Fig. 4** Phylum abundance by **a** site and **b** soil carbon content. *Boxes* represent the interquartile range (IQR), and *error bars* extend to the most extreme values within  $1.5 * IQR$  of the *box* ( $n = 6$ ). Median values are shown as a *line* within the *box* and *outliers* are shown as *black spots*. Sample coding: AG annual grass, PG perennial grass, S shrub, T tree. Significance and direction of correlation between phylum abundance and soil carbon is indicated by + or - (determined by Spearman test). Significance codes for positive correlation: +++ < 0.001; ++ < 0.01; + < 0.05. Similar plots for less abundant phyla are included in Online Resource 4



**Table 1** Significance of factors depth and vegetation on phylum abundance, as determined by Kruskal–Wallis test

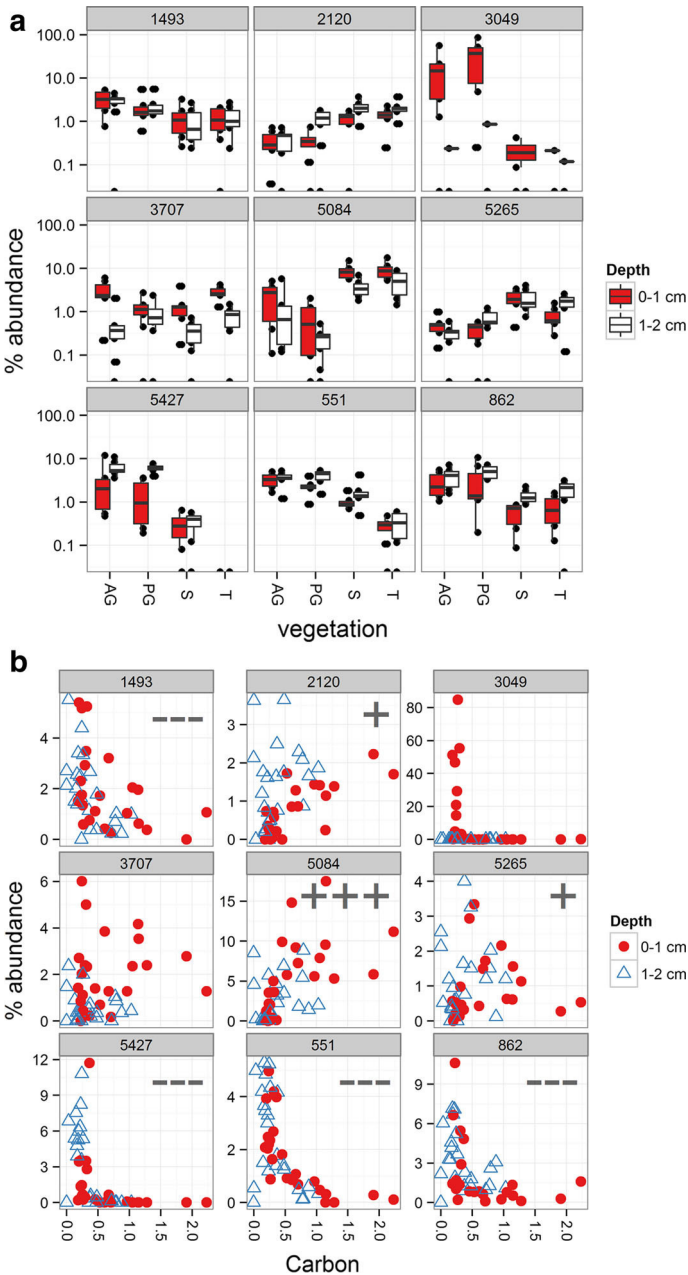
Phylum	Depth	Veg.
<i>Chloroflexi</i>	*	***
<i>Proteobacteria</i>		***
<i>Bacteroidetes</i>	**	***
<i>Acidobacteria</i>	**	**
<i>Firmicutes</i>	**	
<i>Actinobacteria</i>	**	
<i>Gemmatimonadetes</i>		***
<i>Cyanobacteria</i>	**	**
WPS-2		***

Significant effects were further tested by post hoc analyses which are provided in Online Resource 4. Significance codes: \*\*\* < 0.001; \*\* < 0.01; \* < 0.05; < 0.1



**Fig. 5** Constrained correspondence analysis of the microbial community. Coloured markers indicate individual samples, and dispersion ellipses show the 95 % standard deviation confidence interval for different depths and tree/shrub vs grass interspace classifications. Filled symbols = 0–1 cm depth, hollow symbols = 1–2 cm depth. OTU identification numbers are shown in different colours for the 9 most abundant OTUs belonging to the following groups: full dataset (black), phylum *Cyanobacteria* (green), and phylum *Bacteroidetes* (blue). Environmental variables with significance  $p < 0.05$ , are shown as biplotted vectors (based on permutation tests;  $n = 1,000$ )

257 frequencies ( $p < 0.05$ , see Online Resource 4 for test statistics) with respect to depth or  
 258 vegetation zone (Table 2). Similar plots and tables for the top 9 *Bacteroidetes* and *Cya*-  
 259 *nobacteria* are shown in Fig. 7 and Table 3, which account for 10 % of sequences. The  
 260 OTUs of these two phyla were selected because they were detected more often in BSC  
 261 samples compared to subsurface soil. OTU composition did not vary significantly in



**Fig. 6** Relative abundance of the top 9 OTUs detected in the study, by **a** site and **b** soil carbon content. Boxes represent the interquartile range (IQR), and error bars extend to the most extreme values within  $1.5 * IQR$  of the box ( $n = 6$ ). Median values are shown as a line within the box and outliers are shown as black spots. Significance and direction of correlation between OTU abundance and soil carbon is indicated by + or - (determined by Spearman test). Significance codes for positive correlation: +++ < 0.001; ++ < 0.01; + < 0.05. Sample coding: AG annual grass, PG perennial grass, S shrub, T tree



262 relation to the sampling month for any of the top 9 OTUs (Online Resource 4). In addition  
263 to the overall top OTUs shown in Fig. 6, we present in Online Resource 4 similar plots and  
264 tables for the most abundant OTUs belonging to each phylum. These are included to permit  
265 readers interested in particular taxa to easily investigate these in our data, however space  
266 and time do not permit detailed presentation of more than a few OTUs here.

267 The OTUs shown in Figs. 6 and 7 are additionally plotted on the ordination in Fig. 5,  
268 illustrating their contributions to the community structure of samples in the ordination.  
269 Cyanobacterial OTUs are all clustered near the grass interspace BSC samples (0–1 cm  
270 depth) whilst *Bacteroidetes* OTUs are more spread out but tending towards the tree and  
271 shrub soil surface samples (0–1 cm depth). The overall most abundant OTUs are spread  
272 out on the ordination but with more near the grass area samples.

## 273 Discussion

### 274 Phylum level community structure

275 The distribution of the top 9 bacterial phyla accounting for 99 % of sequences (Fig. 4;  
276 Table 1) indicate that *Bacteroidetes* and *Cyanobacteria* are significantly associated with  
277 BSCs (Fig. 7; Table 3). The crucial role of *Cyanobacteria* in BSC carbon and nitrogen  
278 cycling is already widely recognised, whereas *Bacteroidetes*, although ubiquitous in soil,  
279 are not commonly regarded as key BSC community members in the current literature.

280 Using similar methods to this study, Steven et al. (2013) aimed to determine BSC  
281 microbial community differences with respect to soil type (sandstone, shale, and gypsum).  
282 Their samples were numerically dominated by the same top 6 phyla found in this study:  
283 *Proteobacteria*, *Cyanobacteria*, *Chloroflexi*, *Bacteroidetes*, *Actinobacteria*, and *Acido-*  
284 *bacteria*. They found *Cyanobacteria* and *Proteobacteria* to be associated with BSCs whilst  
285 we found *Cyanobacteria* and *Bacteroidetes* to be associated with BSCs. The difference  
286 may be because *Bacteroidetes* are particularly dominant in the soil surface under trees and  
287 shrubs (e.g. Figure 5) which were not a factor in the work of Steven et al. (2013). In the  
288 underlying soil they found enrichment of *Chloroflexi*, which we also found in addition to  
289 *Acidobacteria*, *Actinobacteria*, and *Firmicutes*. Steven et al. (2013) suggested that the  
290 *Chloroflexi* might be involved in anaerobic processes including photoheterotrophy and  
291 chemoheterotrophy, which would increase the productivity of BSCs and enable them to  
292 continue functioning under a wider range of environmental conditions.

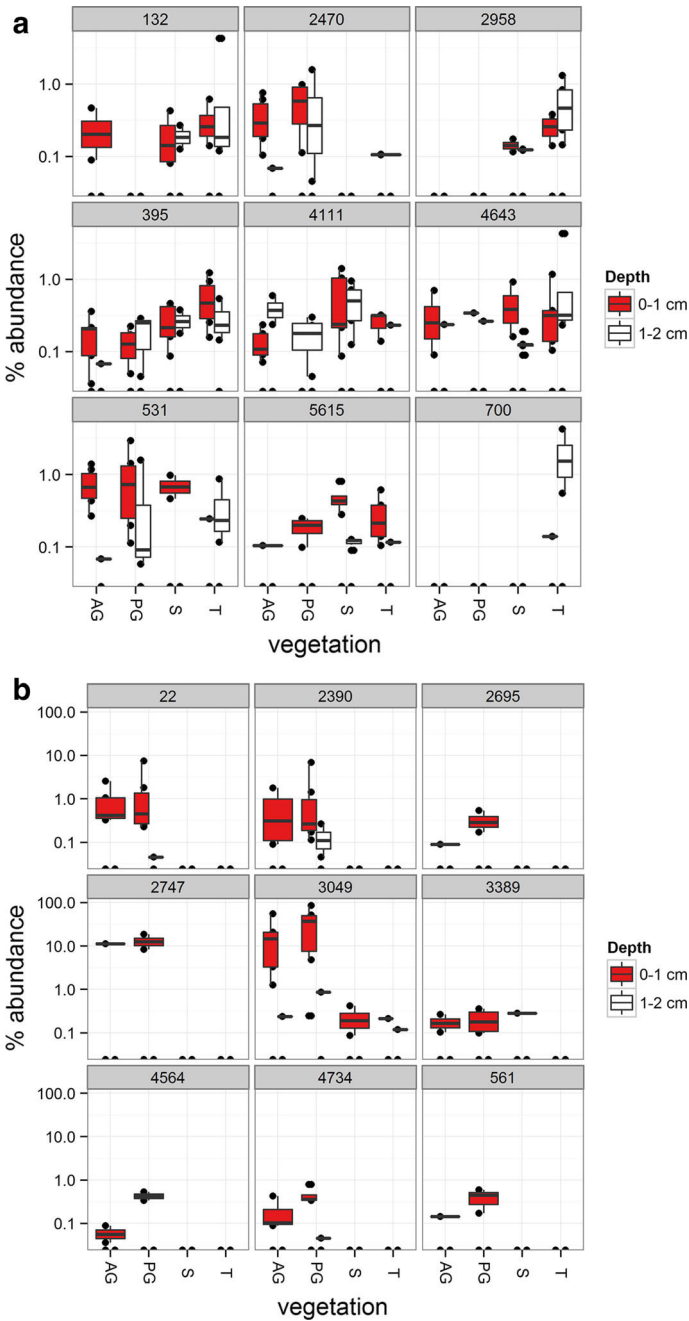
293 *Cyanobacteria* comprised 8.1 % of our sequences as 38 different OTUs. They were  
294 found predominantly in BSCs of the grass areas and are dominated by a single *Phormidium*  
295 species (OTU 1912). Other typical cyanobacterial genera found in BSCs include *Mi-*  
296 *crocoleus*, *Leptolyngbya*, *Nostoc*, and *Scytonema* species (Büdel et al. 2009), but these  
297 were not identified in our samples. The majority of cyanobacterial sequences obtained  
298 could not be classified to genus level however and it is known that molecular identification  
299 of *Cyanobacteria* is problematic (Dojani et al. 2013), so it is possible that these genera  
300 were present but not detected or identified. A higher frequency of *Cyanobacteria* was  
301 detected in the perennial grass compared to the annual grass, probably reflecting the more  
302 stabilised interspaces of perennial grass being slightly more developed. Due to the dom-  
303 inance of *Cyanobacteria* in grass interspace BSCs, it is likely that some of the phyla or  
304 OTUs found to be more abundant in subsurface soil are not specifically adapted for the soil  
305 niche, but may be excluded from the surface by competition.



**Table 2** Taxonomic classification of the most abundant 9 OTUs found in the study

Phylum	Class	Order	Family	Genus	Depth	Veg.
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	Rhodospirillales	Acetobacteraceae			***
<i>Acidobacteria</i>	<i>Solibacteres</i>	Solibacterales	Solibacteraceae	<i>Candidatus Solibacter</i>		***
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	Rhizobiales	Methylobacteriaceae	<i>Methylobacterium</i>	***	
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	Rhizobiales	Bradyrhizobiaceae	<i>Balneimonas</i>	*	***
<i>Cyanobacteria</i>	<i>Oscillatoriothycideae</i>	Oscillatoriales	Phormidiaceae	<i>Phormidium</i>	***	*
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	Rhodospirillales	Rhodospirillaceae		***	**
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	Rhodospirillales	Acetobacteraceae			*
<i>Actinobacteria</i>	<i>Actinobacteria</i>	Actinomycetales	Kineosporiaceae			***
<i>Actinobacteria</i>	<i>Actinobacteria</i>	Actinomycetales	Pseudonocardiaceae		***	

Significance of depth and vegetation on OTU abundance is indicated by \*\*\* < 0.001; \*\* < 0.01; \* < 0.05; < 0.1., as determined by Kruskal–Wallis test. Significant effects were further tested by post hoc analyses which are provided in Online Resource 4



**Fig. 7** Relative abundance of the top 9 OTUs from the phyla **a** Bacteroidetes; **b** Cyanobacteria. Boxes represent the interquartile range (IQR), and error bars extend to the most extreme values within 1.5 \* IQR of the box (n = 6). Median values are shown as a line within the box and outliers are shown as black spots. Sample coding: AG annual grass, PG perennial grass, S shrub, T tree. Similar plots for other phyla are included in Online Resource 4



**Table 3** Taxonomic classification of the most abundant 9 *Bacteroidetes* and *Cyanobacteria* OTUs found in the study

Phylum	Class	Order	Family	Genus	Depth	Veg.
<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	Sphingobacteriales	Chitinophagaceae			**
<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	Sphingobacteriales	Chitinophagaceae	<i>Flavisolibacter</i>		
<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	Sphingobacteriales	Chitinophagaceae	<i>Flavisolibacter</i>		
<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	Sphingobacteriales	Chitinophagaceae			*
<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	Sphingobacteriales	Flexibacteraceae	<i>Segetibacter</i>	**	
<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	Sphingobacteriales	Chitinophagaceae	<i>Segetibacter</i>		*
<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	Sphingobacteriales	Chitinophagaceae			
<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	Sphingobacteriales	Chitinophagaceae			
<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	Sphingobacteriales	Chitinophagaceae		*	
<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	Sphingobacteriales	Chitinophagaceae		*	
<i>Cyanobacteria</i>	<i>Oscillatoriothyrificideae</i>					
<i>Cyanobacteria</i>	<i>Oscillatoriothyrificideae</i>					
<i>Cyanobacteria</i>	<i>Oscillatoriothyrificideae</i>					
<i>Cyanobacteria</i>	4C0d-2	MLE1-12				
<i>Cyanobacteria</i>	<i>Oscillatoriothyrificideae</i>					
<i>Cyanobacteria</i>	<i>Oscillatoriothyrificideae</i>	Oscillatoriales	Phormidiaceae	<i>Phormidium</i>	**	*
<i>Cyanobacteria</i>	<i>Oscillatoriothyrificideae</i>					*
<i>Cyanobacteria</i>	<i>Oscillatoriothyrificideae</i>	Oscillatoriales	Phormidiaceae			*

Significance of depth and vegetation on OTU abundance is indicated by \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; \*  $p < 0.05$ . < 0.1., as determined by Kruskal–Wallis test. Significant effects were further tested by post hoc analyses which are provided in Online Resource 4





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306 Like the *Cyanobacteria*, *Bacteroidetes* were also significantly more abundant in the soil  
307 surface compared to the subsurface soil, however in this case the relationship held also for  
308 soils under tree and shrub canopies. Furthermore, whereas the cyanobacterial abundance  
309 was mostly achieved through a single OTU being very abundant in grass area BSCs, the  
310 *Bacteroidetes* phylum abundance is due to the collective abundance of many OTUs across  
311 all of the vegetation zones (Fig. 7). Overall the *Bacteroidetes* were slightly less abundant  
312 than the *Cyanobacteria*, comprising 5.1 % of sequences, but there were many more OTUs  
313 (297).

314 *Bacteroidetes* are ubiquitous in the soil and have a vast catabolic repertoire, particularly  
315 in the breakdown of complex carbohydrates. Thomas et al. (2011) point out that many  
316 carbohydrates are niche specific, and this seems a very likely scenario for BSCs because  
317 their constituent organisms are subject to unique survival challenges which will select for  
318 production of specialist biomolecules. *Bacteroidetes* may therefore play a vital role in the  
319 recycling of niche specific carbohydrates and associated molecules. It is clear that the soil  
320 surface in grass and tree/shrub areas will be fundamentally different in terms of carbo-  
321 hydrate composition because only the grass interspace areas receive significant primary  
322 production from *Cyanobacteria* and the stress of midday sun, therefore different *Bacter-*  
323 *oidetes* OTUs may be providing specialist degradation roles in these distinct niches. A key  
324 role for BSC *Bacteroidetes* in carbon cycling is further supported by the work of Bailey  
325 et al. (2013), which demonstrated a link between *Chitinophagaceae* family *Bacteroidetes*  
326 abundance with  $\beta$ -glucosidase activity in soil. Eight of the top 9 *Bacteroidetes* in our  
327 samples belonged to the *Chitinophagaceae* family.

328 In an attempt to ecologically classify soil bacteria, Fierer et al. (2007) have shown in a  
329 meta-analysis that phylum abundances in soil are correlated with carbon availability. This  
330 enabled them to broadly classify *Bacteroidetes* and  $\beta$ -*Proteobacteria* as copiotrophs, and  
331 *Acidobacteria* as oligotrophs based on whether phylum abundance is positively or nega-  
332 tively correlated with carbon availability respectively. Previously Smit et al. (2001) had  
333 made a similar suggestion: that the ratio of  $\alpha$ - and  $\gamma$ -*Proteobacteria* to *Acidobacteria* could  
334 provide an indication as to the nutritional status of the soil. Copiotrophic and oligotrophic  
335 groupings are similar to the r- and K-strategists recognised in macroscopic ecology  
336 (MacArthur and Wilson 1967). Copiotrophs therefore can be expected to maximise their  
337 growth rates when resources are plentiful whereas oligotrophs are adapted for maximal  
338 efficiency in the use of rare resources.

339 Based on correlation with total soil carbon (Fig. 4b, Online Resource 4) our data  
340 identify *Bacteroidetes*, *Gemmatimonadetes* and all proteobacterial classes ( $\alpha$ ,  $\beta$ ,  $\delta$ ,  $\gamma$ ) as  
341 potential copiotrophs, and *Acidobacteria* and candidate division WPS-2 as potential oli-  
342 gotrophs in Kalahari soils. *Chloroflexi* abundances were also negatively correlated with  
343 carbon, however due to their carbon fixing abilities soil carbon data are not suitable to  
344 attempt classification. In general, oligotrophs may be expected to function as primary  
345 colonisers in the development of BSCs on nutrient-poor soils such as Kalahari Sand, and  
346 copiotrophs may become more dominant as the crust develops and becomes more pro-  
347 ductive. The mean carbon content in our samples was 0.5 %  $\pm$  0.1 SE with a maximum of  
348 2.2 %, which is low compared to typical mesic soils (Lal 2004) so an abundance of  
349 oligotrophs might be expected, especially in the grass interspaces where there was least  
350 carbon. However, the oligotroph/copiotroph classification is a continuum and may relate  
351 not only to soil total carbon, but more specifically to available carbon and carbon turnover  
352 rate. From a microbial growth perspective the available carbon fraction is determined by  
353 the physiological capabilities of the community and this also defines the amount of soil  
354 carbon which can be regarded as recalcitrant (Schmidt et al. 2011). High carbon turnover



355 rate has previously been calculated for cryptogamic crusts (Elbert et al. 2012). This could  
356 help copiotrophs to maintain high catabolic rates even when soil carbon is low, so long as  
357 there is primary production or other nutrient input available.

### 358 Bacterial community diversity

359 Previous studies have found that dryland BSC and soil bacterial richness and diversity do  
360 not vary with respect to depth or presence of plants, although the community composition  
361 does vary (Garcia-Pichel et al. 2003; Saul-Tcherkas and Steinberger 2011; Steven et al.  
362 2013). Furthermore Nagy et al. (2005) found no difference in community composition  
363 between plant canopies and interspaces, interpreting this to indicate probable independence  
364 of BSC communities for vascular plant resources. Conversely, our results show that  
365 samples from different depths and near different plants have distinct microbial commu-  
366 nities (Fig. 4) which also differ in richness and diversity (Fig. 3). This is not necessarily a  
367 contradiction, however, due to differences in methodology, edaphic factors and disturbance  
368 regime in the previous and current studies. In our samples it seems most likely that the tree  
369 and shrub bacterial communities are at least partly, if not significantly, dependent on  
370 vascular plant and animal-derived resources because they lack phototrophs. Animal distur-  
371 bance could be a key factor in differentiating soil microbial communities because severe  
372 animal disturbance would lead to burial of phototrophs, selectively disadvantaging these  
373 organisms but not significantly disadvantaging heterotrophs which can function at any  
374 depth. In the case of our grass interspaces, animal disturbance is less concentrated and  
375 plant and animal derived inputs are expected to be less, suggesting that the phototrophic  
376 component of BSCs will be more resilient compared to those near trees and shrubs.

### 377 Niche partitioning

378 Our results clearly demonstrate niche partitioning of the microbial community between  
379 BSCs and the subsurface soil, and between soils under different vegetation types. This is  
380 evident from the phylum level breakdown (Fig. 4), the most abundant OTUs overall  
381 (Fig. 6), the OTUs within each phylum (Fig. 7, Online Resource 4), and the overall  
382 community structure (Fig. 5). Although in principle different microbial communities can  
383 be functionally identical, we expect that the differences observed between sites and depths  
384 are at least partly driven by different environmental conditions requiring a different  
385 functional response.

386 From our field observations and soil chemistry results (Fig. 2), we would expect the  
387 grass area BSCs to be functionally similar on the macroscopic scale because the conditions  
388 are similar. One difference is that the BSC patches in perennial grass interspaces are likely  
389 to be older than the patches in annual grass interspaces, because the interspaces have  
390 become stabilised near perennial grasses.

391 A remarkable result was the detection of a clear difference between the soil bacterial  
392 community in the surface and below the surface under trees. At the time of sampling, it  
393 appeared that the homogenisation of the soil resulting from animal disturbances, had  
394 removed all possibility of retaining any depth dependent structure in the biological com-  
395 munity. In fact it can be seen clearly in Fig. 5 and other figures that the surface (0–1 cm  
396 depth) and subsurface (1–2 cm depth) communities from under the trees were quite dif-  
397 ferent, and also that they cluster closely with the same respective communities under the  
398 shrub which were not subject to disturbance by cattle. The separation of BSC and sub-  
399 surface soil communities for both tree/shrub and grass locations on axis 2 of the ordination



400 in Fig. 5 suggests that there are community structure patterns characterising surface and  
401 sub-surface microbial communities, which apply regardless of the presence of BSC or  
402 nearby vegetation.

403 The clear identification of niche partitioning leads to questions about the functional  
404 significance of different communities, and the ecosystem services delivered by BSCs  
405 compared to the subsurface soil. The experimental design of this study means that we  
406 cannot thoroughly address these questions but it does highlight the need for future targeted  
407 studies to do so. We can use the taxonomic identification of sequences to infer possible  
408 functions based on existing knowledge about microbial function in the environment, but  
409 proof of function needs to be addressed separately.

410 Although we have taken microbial community niche partitioning as an indicator of  
411 functional differences between soil depths and vegetation zones, this does not imply that  
412 BSC community structure is shaped only by deterministic factors. There is a growing  
413 recognition of the applicability of neutral models in determining microbial community  
414 structure. These models confer key roles to the stochastic processes of birth, death and  
415 immigration (Sloan et al. 2006) whilst ignoring deterministic factors such as a species  
416 adaptation to drought. Caruso et al. (2011) have suggested that both stochastic and  
417 deterministic processes interact in the assembly of dryland microbial communities and that  
418 the role of niche partitioning at fine scales in dryland ecosystems has been previously  
419 underestimated. They suggest it is incorrect to assume that extreme conditions are the main  
420 determinant of species distribution, because this would lead to the conclusion that com-  
421 munities in extreme environments should converge towards stable low diversity commu-  
422 nities. The most extreme micro-environments in our study were the BSCs in grassed areas,  
423 but they should not necessarily be regarded extreme for adapted microbes. These areas did  
424 have the lowest diversity in our study, although the community structure varied greatly  
425 between individual samples. Whilst this variability in BSC community structure may be  
426 explained in the context of classical ecology as resulting from unobserved environmental  
427 heterogeneity and community succession, it is also quite plausible that stochastic processes  
428 may be playing a major role in community assembly. As BSCs have been recognised as  
429 ecosystem engineers (Bowker 2007), the prospect of stochastic community assembly is  
430 very important because it may directly drive diversity of BSC function and soil properties,  
431 thus diversifying the landscape itself.

#### 432 Possible ecological significance of the most abundant OTUs

433 Three of the top 9 OTUs (Fig. 6; Table 2) were significantly associated with BSCs  
434 (0–1 cm depth) based on frequency of sequence detection. These were a cyanobacterium of  
435 the genus *Phormidium* (OTU 1912), a *Methylobacterium* species (OTU 1064) belonging to  
436 the phylum *Proteobacteria*, and a *Balneimonas* species (OTU 1150) of the phylum *Pro-*  
437 *teobacteria*. In addition, sequences of proteobacterial OTU 2850 and actinobacterial OTU  
438 3323 were found to be significantly more abundant in subsurface soil compared to BSC. It  
439 can be seen in Fig. 6 that in several cases differential abundance between BSC and sub-  
440 surface soil appears to be affected by the vegetation classification, particularly grasses vs.  
441 tree or shrub (e.g. OTU 1912). This is supported by the ADONIS results (Online Resource  
442 3) which suggest that there is a significant interaction between depth and vegetation zone  
443 driving microbial community structure.

444 *Phormidium* species have previously been described as typical *Cyanobacteria* of early  
445 successional stage BSCs by Büdel et al. (2009), consistent with our present observation in  
446 weakly developed type 1 and type 2 BSCs (Thomas and Dougill 2006; 2007). They are



447 widespread filamentous *Cyanobacteria* which are found commonly in hot and cold arid  
448 soils but are also found in aquatic habitats including ultra-oligotrophic Antarctic seasonal  
449 lakes (Keskkitalo et al. 2013), demonstrating a great plasticity for the contrasting envi-  
450 ronmental conditions of relevance to the Kalahari Sand soil surface.

451 Chen et al. (2012) found *P. tenue* to be potentially useful for the stabilisation of sand  
452 dunes by inoculation due to the desiccation tolerance afforded by its extracellular poly-  
453 saccharide (EPS). The EPS of *P. tenue* has also been shown to promote the germination of  
454 seeds and the fitness of seedlings (Xu et al. 2013), which may have been related to  
455 numerous mechanisms including water retention, provision of nutrients, and protection  
456 from oxidative stress. Furthermore, Boopathi et al. (2013) showed that a *Phormidium*  
457 species associated with mangroves produces indole-3-acetic acid (IAA) which is an  
458 important plant hormone associated with diverse responses including enhanced germina-  
459 tion and root growth.

460 BSCs have been associated with both enhancement and inhibition of plant growth  
461 through a variety of mechanisms and with some controversy (e.g. Beyschlag et al. 2008;  
462 Prasse and Bornkamm 2000). We tentatively suggest that microbial secretion of plant  
463 hormones may be an as-yet unrecognised process of relevance to BSC-plant interactions,  
464 and that *Phormidium* species could influence plant cover in the Kalahari if they are  
465 secreting IAA similarly to the species studied by Boopathi et al. (2013). The recruitment of  
466 plants is potentially at odds with the maintenance of a photosynthetic BSC due to com-  
467 petition, so one might expect the exclusion of plants rather than their promotion to be a  
468 more successful ecological strategy for BSC communities. Another effect of IAA on plant  
469 roots is to reduce cell wall integrity, causing the release of nutrients such as sugars which  
470 can promote microbial populations near the root. Thus it is possible that in addition to  
471 being a significant primary producer in BSCs, *Phormidium* species may also be able to  
472 derive carbon from plants, reducing reliance on photosynthesis and potentially promoting  
473 further plant-microbe interactions. Again, this supposition is dependent upon the pro-  
474 duction of plant hormones by the *Phormidium* species in the BSC which has not been  
475 tested. The dominance of sequences assigned to this *Phormidium* OTU in our samples  
476 strongly suggests a numerical dominance of the BSC bacterial community, and leads to the  
477 expectation of a large amount of *Phormidium*-derived EPS in the BSCs and soils examined  
478 in this study. The phyla *Bacteroidetes* and *Cyanobacteria* were both more abundant in BSC  
479 compared to subsurface soil, however unlike the *Bacteroidetes* which were represented by  
480 many OTUs, the *Cyanobacteria* were dominated by the single *Phormidium* OTU 1912.  
481 This may be a reflection of the different life strategies of the phyla. The *Bacteroidetes*  
482 likely being specialist degraders in BSCs as discussed earlier, are strongly dependent on  
483 the soil makeup as determined by the life history and current activity in the soil they  
484 inhabit, so a large genetic (functional) diversity is called for. On the other hand *Cyano-*  
485 *bacteria* being principally photosynthetic are probably less sensitive to soil life history, but  
486 their distribution is likely to be controlled more by abiotic factors such as weather and  
487 hydrology. Since the Kalahari Sand substratum is so uniform over very large areas this  
488 suggests a possibility for one or a few *Phormidium* species to be strongly influencing the  
489 Kalahari soil surface on a very large scale far exceeding our study site.

490 The *Balneimonas* (OTU 1150) abundance was positively correlated to soil carbon,  
491 suggesting it could be functioning as a copiotroph. Six of the other abundant OTUs which  
492 were not significantly associated with BSCs were negatively correlated with soil carbon, so  
493 although potential oligotrophs are not significantly increased in crusts, they do appear to  
494 form a large fraction of the BSC and subsurface soil communities.



495 *Balneomonas* was recognised as a new genus in 2004 (Takeda et al. 2004), however it  
496 has since been proposed for reclassification as *Microvirga* by Weon et al. (2010). In any  
497 case the original description of the type species is of a thermophilic (40–45 °C optimum)  
498 cellulose producing species isolated from a bath fed by a hot spring in Japan and related to  
499 *Methylobacterium* which is the genus of our other OTU of interest here. Cellulose pro-  
500 duction at high temperature was identified as a remarkable property of the type species *B.*  
501 *flocculans*, and noted for causing flocculation of the cells via adhesive cellulose fibrils  
502 similar to those thought to be involved in nonspecific binding of other rhizobial bacteria to  
503 plant host cells. The production of EPS is recognised as an important factor in the  
504 development and survival of BSCs, and is normally attributed to cyanobacteria (Mager and  
505 Thomas 2011). This result suggests that BSC stabilisation by EPS may also be facilitated  
506 by heterotrophic bacteria, and a role for *Balneomonas* species as BSC pioneers in advance  
507 of cyanobacterial establishment seems quite plausible.

508 Our *Methylobacterium* OTU was significantly associated with BSCs but not with any  
509 particular vegetation zone, suggesting a role in the soil surface which is not related to the  
510 presence of a particular vegetation type. The type species *M. organophilum* (Patt et al.  
511 1976) and others are claimed to be facultative methylotrophs—capable of growth on  
512 methane or other C1 carbon sources, however although facultative methylotrophy has now  
513 been proved for some organisms there has been some controversy over this (Theisen and  
514 Murrell 2005). It is thought that 50–90 % of methane released in soils is oxidised by  
515 methylotrophs before reaching the atmosphere (Nazaries et al. 2013), therefore it is an  
516 important ecosystem function with relevance to climate change. None of the known 21  
517 obligate methane oxidising genera (Nazaries et al. 2013) were detected in our samples.

518 The most widely recognised role for *Methylobacterium* species is as epiphytes which  
519 consume methanol emitted from stomata and secrete plant growth promoting hormones  
520 (Lidstrom and Chistoserdova 2002). Several *Methylobacterium* isolates have been con-  
521 firmed as aerobic anoxygenic phototrophs in BSCs (Csotonyi et al. 2010), meaning that  
522 their photosynthetic pathway does not produce oxygen, and that they are obligate aerobes  
523 (in contrast to most other anoxygenic phototrophs). This is an important finding because it  
524 increases the potential light harvesting efficiency of BSCs. Csotonyi et al. (2010) found  
525 that anoxygenic phototrophs represented up to 5.9 % of the cultivable BSC bacterial  
526 community, and we found *Methylobacterium* OTU 1064 alone represented 3 % of  
527 sequences in annual grass zone BSCs (Online Resource 4).

## 528 Methodological limitations

529 The determination of bacterial community structure through analysis of ribosomal RNA  
530 genes as carried out in this study has some limitations which should be kept in mind when  
531 interpreting results and planning future studies. Methodological aspects including DNA  
532 extraction efficiency differences, PCR bias, and primer specificity can affect observed  
533 sequence frequencies. Furthermore, natural rRNA gene copy number variation will affect  
534 results. For instance, it is known that oligotrophs in general carry fewer rRNA gene copies  
535 than copiotrophs (Klappenbach et al. 2000), which could lead to under-representation of  
536 oligotrophs in our study.

## 537 Implications for land management

538 The management of BSCs through protection, restoration, or engineering has the potential  
539 to deliver environmental benefits from local to global scales, and is relevant to numerous



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540 land management challenges. Landscape changes associated with moderate grazing in the  
541 Kalahari include a shift from perennial grass to annual grass species and the promotion of  
542 thorny shrubs (Skarpe 1990; Ward 2009). We characterised the microbial communities  
543 from soils near these plants and found that in all cases a surface specific community can be  
544 detected, even beneath the *Acacia* canopy where there was no consolidated BSC. This  
545 indicates a strong tendency of the soil community to become vertically stratified and  
546 suggests that once grazing pressure is removed a rapid recovery of the BSC is likely. Soil  
547 communities beneath tree/shrub canopies were distinct from those in grass interspaces as  
548 has been shown previously (Saul-Tcherkas and Steinberger 2011), showing that typical  
549 grazing induced vegetation change can be associated with a change in soil microbiota,  
550 notably a loss of *Cyanobacteria*. The extreme reduction in cyanobacterial inoculum may  
551 retard BSC establishment in heavily shrub-encroached areas, for instance after fire or  
552 attempts to reclaim the land by removal of shrubs.

553 The deliberate rehabilitation of BSCs to restore ecosystem function has been discussed  
554 by Bowker (2007) and recently confirmed by cyanobacterial inoculation of shifting sand  
555 dunes to establish BSCs which facilitated vascular plant succession from early nitrogen  
556 fixing legumes to latter successional grasses (Lan et al. 2014). A problem with the inoc-  
557 ulation approach is that it usually relies upon a sacrifice zone, and may not work if there are  
558 nutrient or stability limitations. These problems can potentially be overcome by the  
559 industrial preparation of a designed mixed inoculum. Candidates for a BSC inoculum in the  
560 Kalahari rangelands could be identified for testing based upon our data, which is ideal for  
561 this purpose because early stage BSCs are likely to still contain in large number the pioneer  
562 species which helped them to become established. The *Phormidium* genus of *Cyanobac-*  
563 *teria* (*P. tenue* specifically) has been shown by Chen et al. (2012) to possess excellent  
564 qualities for the stabilisation of sand, and its abundance in our study suggests that delib-  
565 erate establishment on degraded land in the Kalahari could be feasible. A suitable inoc-  
566 ulum to help the establishment of *Cyanobacteria* in general might include oligotrophic  
567 organisms such as *Balneomonas* species which are able to utilise recalcitrant carbon and  
568 quickly stabilise the soil by release of EPS, including beneath the photic zone. The idea  
569 that the early stages of BSC recovery or development could be helped by oligotrophic non-  
570 photosynthetic bacteria has been confirmed by Wu et al. (2010) in laboratory and field  
571 experiments, however the identity of organisms involved was not known.

## 572 Conclusions

573 We found that the bacterial OTU (approximate species level) diversity was greater in  
574 subsurface soil (1–2 cm depth) compared to the BSC at the surface (0–1 cm depth), and  
575 community composition exhibited clear spatial patterns. Crusted grass interspaces were  
576 dominated by a single cyanobacterial OTU from the genus *Phormidium*, but *Cyanobac-*  
577 *teria* were very rare in tree and shrub areas. The BSC community structure was not defined  
578 by the presence of *Cyanobacteria* alone nor by other phototrophs as is often presumed. In  
579 all areas a characteristic but variable BSC bacterial community was present, and this was  
580 defined in part by non-cyanobacterial OTUs which were associated with the soil surface,  
581 and especially the *Bacteroidetes* phylum. The functions of these BSC specific bacteria are  
582 unknown, but we speculated possible roles for the most abundant ones in soil stabilisation,  
583 carbohydrate catabolism, photosynthesis, and plant interactions based on similar species  
584 reported in the literature. Our data suggest that shrub encroachment in Kalahari rangelands  
585 can almost eliminate *Cyanobacteria* from soil surfaces in some circumstances, depriving



586 soils of the ecosystem services delivered by well-developed phototrophic crusts and lim-  
587 iting the potential for natural BSC regeneration.

588 **Acknowledgments** Financial support was provided by the Leverhulme Trust (F/00 426/H) and Man-  
589 chester Metropolitan University. Research in Botswana was conducted with the Republic of Botswana  
590 Research Permit No. EWT8/36/4 VIII(4). We would like to thank Jill Thomas of Berrybush farm for her  
591 considerable support and access to her land. We also thank Peter Harding for assistance with computer  
592 resources and configuration. The constructive feedback of an anonymous reviewer and Matthew Bowker is  
593 greatly appreciated.

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