Fire impacts bacterial composition in *Protea repens* **(**Proteaceae) infructescences

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Abstract

The diverse bacterial communities in and around plants provide important benefits, such as protection against pathogens and cycling of essential minerals through decomposition of moribund plant biomass. Biodiverse fynbos landscapes generally have limited dead-wood habitats due to the absence of large trees and frequent fire. In this study, we determined the effect of a fire disturbance on the bacterial communities in a fynbos landscape dominated by the shrub *Protea repens* using 16S rRNA amplicon sequencing. The bacterial community composition in newly formed fruiting structures (infructescences) and soil at a recently burnt site was different to that in an unburnt site. Bacteria inhabiting *P. repens* infructescences were similar to well-known taxa from decomposing wood and litter. This suggests a putative role for these above-ground plant structures as reservoirs for post fire decomposer bacteria. The results imply that inordinately frequent fires, which are commonplace in the Anthropocene, are a significant disturbance to bacterial communities and could affect the diversity of potentially important microbes from these landscapes.

Introduction

Plants are colonized by a diversity of bacteria, many of which are beneficial to their hosts (Andrews & Harris, 2000, Turner *et al.*, 2013). These benefits vary from protection against pathogens to supplying growth-promoting substances or essential nutrients (Andrews & Harris, 2000, Turner *et al.*, 2013). The bacterial community composition varies widely between different plant species, but also between plant organs and tissues, depending on the abiotic environments and substrate. For example, rhizosphere and phyllosphere bacteria, which have been extensively studied in many plant species, differ widely (Andrews & Harris, 2000, Lindow & Brandl, 2003, Vorholt, 2012, Turner *et al.*, 2013). In contrast, the microbiomes of other plant organs, such as flowers, seeds and fruits, have not received much

attention (Hardoim *et al.*, 2015). While knowledge of the bacterial composition on more unusual plant organs could reveal novel and interesting biodiversity, it would also be important in understanding how the loss of these structures and their associated bacteria affect ecosystems.

The fruiting structures of some serotinous plants growing in the fire-prone Fynbos biome of the Cape Floristic Region (CFR) biodiversity hotspot (Mittermeier *et al.*, 2011) in South African are unique, fire resistant, seed-bearing structures, and they occur widely in the genus *Protea*. These infructescences are formed from mature inflorescences when the leafy bracts surrounding the florets close to form a cone around the seeds (Pretorius, 1985). Senescent (old) infructescences resemble a dead-plant substrate similar to litter, but remain intact on *Protea* plants for several years, with only occasional damage caused by insect borers (Bond, 1985). The infructescences of *Protea* spp. are inhabited by many different insects (Coetzee & Giliomee, 1987, Roets *et al.*, 2006), mites (Roets *et al.*, 2009, Roets *et al.*, 2011, Theron *et al.*, 2012), fungi (Lee *et al.*, 2005, Roets *et al.*, 2013), and actinomycete bacteria (Human *et al.*, 2016, Human *et al.*, 2018). While a substantial base of knowledge has been accumulated on organisms associated with *Protea* infructescences, very little is known regarding their bacterial communities or the interactions of these bacteria with other organisms.

At a microbial level, it is believed that the CFR is a habitat to a considerable fungal diversity (Crous *et al.*, 2006), although most studies have focused on fungal species in the genera *Sporothrix* and *Knoxdaviesia* (Marais & Wingfield, 1994, Wingfield *et al.*, 1999, Roets *et al.*, 2013; Marais *et al.*, 1998, Roets *et al.*, 2013, Aylward *et al.*, 2015). Studies on plant-associated bacteria in the Fynbos have focused on nitrogen-fixing symbionts in the roots of various native legumes (Beukes *et al.*, 2013, Lemaire *et al.*, 2016, Conradie & Jacobs, 2020) and the microbiomes of fynbos plants used for commercial purposes (Postma *et al.*, 2016; Brink *et al.*, 2020). Few studies have considered fynbos plant- and soil-associated

microbiomes, which are strongly structured in space and time and influenced by the native host plant community (Miyambo *et al.*, 2016; Keet *et al.*, 2019) as well as by plant invasions (Slabbert *et al.*, 2014; Jacobs *et al.*, 2020). Likewise, very little is known about the microbial diversity associated with the majority of Fynbos plants, even though it is likely that bacteria are integral in the survival of most plants in this nutrient scarce biome.

Post-fire succession in Fynbos ecosystems follows a distinct pattern, where plants with different fire survival strategies are dominant at different times (Kruger, 1977, Kruger & Bigalke, 1984). The fire frequency, or fire return interval (FRI) in fynbos landscapes is important in maintaining the diversity and survival of many plant species. The optimal recruitment success of most *Proteaceae* has been shown to occur with fire frequencies between 10 and 20 years (Kraaij and Van Wilgen, 2014). Although very long FRIs may also be detrimental to plant diversity, shorter FRIs are of more concern. Juvenile periods of re-seeding fynbos plants typically vary from four to eight years, and FRIs shorter than the juveline period may result in the elimination of these reseeding plants from a landscape (Kruger and Bigalke, 1984; Kraaij and Van Wilgen, 2014). Proteaceae are known as keystone members of mountain fynbos, and it is expected that many organisms obligately associated with these plants and their reproductive structures (e.g. older *P. repens* infructescences and litter) may be lost from landscape due to inordinately frequent fires.

It was previously shown that unburnt *P. repens* populations adjacent to post-fire populations are an important source for the recolonization of *P. repens* infructescences by important fungal symbionts, such as *Knoxdaviesia proteae*, after fire (Aylward *et al.*, 2015). Even so, this process can take a considerable period of time as most *P. repens* infructescences are not rapidly recolonized by actinomycetes (Human *et al.*, 2018) after fire (Human *et al.*, 2016). These studies clearly show that fire has important, yet substantially understudied effects on

the microbial colonization of *P. repens* infructescences, but this may extend to the surrounding litter and soil.

In this study, we sought to determine the differences in microbial diversity in *P. repens* infructescences and the surrounding litter and soil in a fire-affected site and a neighbouring unaffected site. This was in order to predict the effect of fire on bacterial diversity in fire-prone fynbos and the implications of inordinately high fire-frequencies on the maintenance of this unique ecosystem. We determined whether bacteria that inhabit *P. repens* infructescences are similar to taxa known to decay wood or litter substrates, which are particularly rare in recently burned areas (Smith *et al.*, 2008). The shared bacterial communities between infructescences and the surrounding litter and soil were compared to assess whether litter and soil are potential sources of inoculum to colonize newly formed infructescences or vice-versa.

Materials and Methods

Study area and sample collection

Two adjacent patches of mountain fynbos near Franschhoek in the Western Cape Province of South Africa were selected. One of these sites (S 33.926.21 E 19.158.40) was burnt in a wildfire four years prior to sampling (2009) and the other (S 33.925.32 E 19.159.71) was previously burnt c. 15 years before. The closest two sampling points between the burnt and unburnt samples were c. 30 m apart, and the furthest two points were c. 80 m apart. Sampling was conducted in 2013, when vegetation in the burnt site was at the end of the first regeneration phase (4 years old) and *P. repens* plants were ca. 1m tall and had produced a single newly formed infructescence. Here, *P. repens* individuals reached reproductive maturity for the first time after fire and thus no older infructescences (from the previous flowering season) were present in this population. The vegetation canopy remained dominated by resprouting shrubs (Kruger, 1977) and the sparse leaf litter comprised leaves and twigs

98 from ericoid species. In the unburnt site, the vegetation was in its mature phase and was
99 dominated by 3-m-tall *P. repens* individuals that were at least 15 years old (Kruger, 1977). In
100 addition to bearing-infructescences from the current flowering season (newly formed
101 infructescences), individuals also carried numerous infructescences from previous flowering
102 seasons (mature infructescences).

103 In the burnt site, five newly formed (young) infructescences from five different *P. repens*
104 individuals were collected, while mature infructescences had not yet formed and thus were
105 not sampled. In the unburnt site, five newly formed infructescences but also five mature
106 infructescences from the previous flowering seasons from five different *P. repens* trees (i.e.,
107 two structures from each tree) were sampled. Four litter samples were collected from the same
108 trees where infructescences were collected. In addition, five 60-mm cores were collected from
109 the soil beneath each *P. repens* tree that was sampled for infructescences in both sites, and
110 these were bulked for each tree. Collectively, five bulk soil samples were collected from each
111 site. In total 10 newly formed infructescences (5 burnt and 5 unburnt), 5 mature
112 infructescences from the unburnt site, 10 soil samples (5 burnt and 5 unburnt) and 8 litter
113 samples (4 burnt and 4 unburnt) were used in this study.

114 Infructescences were opened with sterilized pruning scissors and separated into florets,
115 involucral bracts and seeds using sterile forceps and dissecting scissors. Three randomly
116 chosen floret pieces (30 mm long), three randomly chosen bract pieces (10-mm^2), and three
117 randomly chosen seeds were selected from each infructescence and used for DNA isolation.
118 For DNA extractions from litter and soil samples, 5 g were as input for the respective DNA
119 extraction kits. Collected soil samples were first sieved to remove fine roots. DNA was
120 extracted from infructescence material and plant litter with a ZymoResearch Bacterial/Fungal
121 DNA extraction kit following the instructions provided. DNA extracted from the three
122 infructescence organs was pooled in equimolar concentrations. DNA extractions from soil

samples were conducted using a MoBio PowerMax® Soil DNA Isolation Kit. DNA concentrations and quality were checked using a Nanodrop ND1000 spectrophotometer.

Sequencing of the 16S ribosomal RNA (rRNA)

Detailed methods used for amplification and library preparation are provided in the supplementary materials. Briefly, pooled DNA extracted from infructescences was submitted to Molecular Research LP (Shallowater, TX, USA) where the V4 region of the 16S rRNA gene was amplified using the universal primers 515f and 816r (Caporaso *et al.*, 2010) and sequenced with a barcode on the forward primer. DNA from soil and litter samples was sent to the sequencing center at the University of Michigan and the V4 region of the 16S rRNA gene region was amplified and sequenced following the methods described by Kozich *et al.* (2013).

Raw sequence reads were processed using the DADA2 package (version 1.14.0; Callahan *et al.*, 2016) in R 3.6.1 (R Development Core Team, 2017). Forward reads were trimmed to 240 base pairs and reverse reads trimmed to 180 base pairs. All reads containing ambiguous bases were removed. Sequences were dereplicated and the sequence variants inferred using the parametric error model in DADA2 (Callahan *et al.*, 2016), removing indels and substitutions in sequences. Error-corrected forward and reverse reads were merged, and forward and reverse reads that did not have an identical overlap were discarded. Chimeric sequences among merged sequences were removed using DADA2 (Callahan *et al.*, 2016). These analyses were performed following the guidelines on the DADA2 Github webpage (http://benjjneb.github.io/dada2/tutorial.html) and in Callahan *et al.* (2016).

Exact amplicon sequence variants (ASVs) rather than operational taxonomic units (OTUs) at classified 97% similarity were used because this provides more precise and reproducible results in amplicon sequencing studies (Callahan *et al.*, 2017, Edgar, 2018). Taxonomic

assignment of inferred sequences was done using the RDP Bayesian classifier (Wang *et al.*, 2007) against the SILVA SSU database release 138 (Quast *et al.*, 2012) in DADA2 (Callahan *et al.*, 2016). This version of the SILVA SSU database no longer contains the phylum Betaproteobacteria, which has been introduced as the Burkholderiales in the Gammaproteobacteria (Parks *et al.*, 2018). Before proceeding with further analyses, all mitochondrial and chloroplast sequences were removed from the data (Table S1).

Diversity and Statistical Analyses

For diversity analyses, the number of sequences were rarefied to 8000 sequences per sample. Species richness (observed ASVs) and diversity (Shannon index) were calculated using the Phyloseq package (version 1.30.0; McMurdie & Holmes, 2013) in R version 3.6.1 (R Development Core Team, 2017). Species diversity estimators were not normally distributed. Therefore, we compared the differences in species richness with a Kruskal–Wallis rank-sum test.

Differences in species assemblage composition between the burnt and unburnt sites for soil, litter and newly formed infructescences and between newly formed and mature infructescences in the unburnt site were tested using permutational multivariate analysis of variance (PERMANOVA (Anderson, 2008) using the vegan package (version 2.5-6; Oksanen *et al.*, 2017) in R (R Development Core Team, 2017). Homogeneity of dispersions of communities was tested by performing a permutational analysis of multivariate dispersions (PERMDISP; Anderson, 2004) on Bray–Curtis dissimilarity values, using the Vegan package in R. The species assemblage structure was examined by ordination of Bray-Curtis distances using non-metric multidimensional scaling (NMDS) of the same Hellinger transformed, sub-sampled dataset. This was performed in the Phyloseq (McMurdie & Holmes, 2013) and *vegan* packages in R (R Development Core Team, 2017).

Sequence variants occurring at least once per sample group (total microbiome/ASVs), and those occurring in four out of five samples in a group (core microbiome/ASVs) were determined using the Microbiome R package (version 1.8.0; Lahti *et al.*, 2012). In litter samples, we considered ASVs occurring in three out of four samples as part of the core microbiome. Subsets of the total- and core microbiomes were assembled and subsequently used to determine the number of these taxa shared by different sample groups.

Results

Bioinformatics results

A total of 2,581,808 raw read pairs were generated from infructescence, soil and litter samples to produce a total of 2,038,910 merged, non-chimeric sequences of which 1,853,151 sequences remained after non-bacterial amplicons were removed (Supplementary Table 1). From these, the DADA2 parametric error estimation model (Callahan *et al.*., 2016) produced 6448 bacterial amplicon sequence variants (ASVs; Callahan *et al*., 2017). The number of ASVs ranged from 162 to 708 after rarefaction. In litter samples, the number of ASVs ranged from 192-379 in the burnt site and 380 – 400 in in the unburnt site. In soil, the number of ASVs ranged between 461 and 567 ASVs in the burnt site and 525 to 601 in the unburnt site. In newly formed infructescences there were 333 to 398 ASVs in the burnt site and 377 to 480 in the unburnt site. The number of ASVs in mature infructescences ranged from 340 to 708. Raw sequence data are available from the NCBI short-read archive (SRA) under the accession PRJNA494799

Overview of bacterial communities

In litter samples from the burnt site, the majority of sequences were from the Class Alphaproteobactera (relative abundance, 47-57%), followed by Acidobacteria (11-22%), Veruccomicrobia (3-11%) and Planctomycetes (6-8%; Fig. 1a). In litter from the unburnt site,

195 the majority of sequences were from the Class Alphaproteobactera (31 – 35%), followed by
196 Acidobacteria (17–33%) and Veruccomicrobia (6-9%), Planctomycetes (4-8%) (Fig. 1a).
197 Changes between mean relative abundance of these groups between samples from the burnt
198 and unburnt sites were detected in the Alphaproteobacteria (burnt = 48%, unburnt =31%;
199 K-W, $\chi^2 = 5.33$, p = 0.02) and Gammaproteobacteria (burnt = 4% , unburnt = 7%, Kruskal-
200 Wallis Rank Sum test (K-W), $\chi^2=4.74$, p=0.03).

201 Most sequences from soil samples from the burnt site were from the Alphaproteobacteria (21-
202 24%) and Acidobacteria (17-25%), followed by Planctomycetes (11-18%), Verrucomicrobia
203 (6–12%) and Actinobacteria (6-12%). In soil samples from the unburnt site, the majority of
204 sequences were from the Alphaproteobacteria (20-24%) and Acidobacteria (20-22%),
205 followed by Verrucomicrobia (12–19%), Planctomycetes (7-18%) and Gammaproteobacteria
206 (7–11%). The group means of the relative abundance of the most abundant taxa at phylum
207 level were similar in the burnt and unburnt sites, except for Verrucomicrobia (burnt=9%,
208 unburnt=15% K-W, $\chi^2 = 6.36$, p=0.01).

209 In newly formed infructescences from the unburnt site, most sequences were from Alpha-
210 (45–49%) and Gammaproteobacteria (12–36%), Acidobacteria (3–15%), Actinobacteria (6–
211 15%) and Bacteroidetes (3–13%). In newly formed infructescences from the burnt site, most
212 sequences were from Alpha- (31–46%), Gammaproteobacteria (22–32%), Acidobacteria (3–
213 8%), Actinobacteria (5–7%) and Bacteroidetes (11–17%). The only phylum with statistically
214 significant differences in the mean relative abundances in newly formed infructescences from
215 the burnt and unburnt sites was Bacteroidetes (burnt=13, unburnt=8%; K-W, $\chi^2=3.96$,
216 p=0.04).

217 In mature infructescences, most sequences were from Alphaproteobacteria (28-46%),
218 Actinobacteria (15%), Gammaproteobacteria (11-19%) and Acidobacteria (8-12%).Compared

to newly formed infructescences, there was an increase in Actinobacteria (newly-formed = 8, mature =15% K-W, χ^2=7.77, p=0.005).

A *Rhizobium, Luteibacter, Pseudomonas, Tardiphaga*, *Mycobacterium*, *Duganella* and a Magnetospirrilaceae were amongst the most abundant ASVs in newly formed infructescences (Fig 1b). The most abundant ASVs in mature infructescences were of *Bradyrhizobium*, *Paraburkholderia,* Solirubrobacterales, Acidibacter and *Luteibacter*. In litter, members of *Sphingomonas, Bryocella*, *Stenotrophomonas*, *Terriglobus*, *Granulicella* and a Beijerinckiaceae were most common and in soil, *Bradyrhizobium*, *Paraburkholderia* were among the most abundant ASVs detected. Among highly abundant taxa shared between litter and infructescences were *Sphingomonas*, *Amnibacteria, Granulicella*, *Bryocella* and *Pseudomonas*. Several different ASVs identified as *Bradyrhizobium*, *Paraburkholderia* were among the most abundant taxa in soil and infructescences.

Species diversity

Species richness and diversity in samples from the burnt and unburnt site were compared using the observed species and Shannon index metrics. In newly formed infructescences, observed ASVs were significantly lower in the burnt site than in the unburnt site (Kruskal-Wallis rank sum test (K-W), χ^2=5.77, p=0.02; Fig. 2). However, no significant differences were detected in the soil samples (K-W, χ^2=0.88, p=0.34; Fig. 2). Mature infructescences had a significantly higher number ASVs than newly formed infructescences (K-W, χ^2=4.33, p=0.03). Shannon's diversity index not was significantly different between soil samples from the burnt and unburnt site (K-W, χ^2=2.45, p=0.12; Fig. 2). However, the Shannon index values were significantly higher in newly formed infructescence samples from the unburnt site (K-W, χ^2=3.94, p=0.04). In mature infructescences, the Shannon index was significantly higher than in newly formed infructescences (K-W, χ^2=9.38, p=0.002). As a separate analysis, the bacterial diversity in litter samples from the burnt and unburnt sites as a consequence of

differences in plant diversity were compared. Observed ASVs was higher in the burnt site (Kruskal-Wallis rank sum test, $\chi^2 = 4.08$, $p = 0.04$) and no difference was present in Shannon index values (K-W, $\chi^2=3$, $p=0.88$). As expected, the bacterial diversity was significantly different in samples from the burnt and unburnt sites.

Differences in community assemblage

To determine the similarity of bacterial community assemblages between samples from the burnt and unburnt sites, bacterial communities were compared using PERMANOVA, and the homogeneity of dispersions tested using PERMDISP. In litter, where the difference in plant communities determines litter bacterial composition, the bacterial diversity was significantly different between samples from the burnt and unburnt sites. According to PERMDISP analysis, the in-group dispersions in litter samples were significantly different ($F=7.71$, $p<0.001$). The the burn effect comparison, bacterial communities in *P. repens* infructescences and soil samples, communities in the burnt and unburnt sites were significantly different in both soil and newly formed infructescences (Table 1). The bacterial community assemblages in mature infructescences and newly formed infructescences from the burnt and unburnt sites were also significantly different. Group dispersions in newly formed infructescences ($F = 0.33$, $p = 0.6$), all infructescences ($F = 2.1$, $p = 0.2$) and soil ($F = 1.74$, $p = 0.2$) were not significantly different. Samples from infructescences, litter and soil were distinctly separated in the NMDS (Dimensions=2; Stress=0.07. Fig. 3), while communities from the burnt and unburnt sites within each sample group clustered separately. These results were expected because of the different stages of vegetation succession and the removal of soil and litter-associated bacteria by fire.

Shared microbiomes

The possible distribution of ASVs between infructescences, litter and soil in the core and total microbiomes were determined by calculating the number of ASVs shared between different sample types from the burnt and unburnt sites. Newly formed and mature infructescences shared a large number of the ASVs present in their total microbiomes (n=409; Fig. 4), although all three these sample groups had some unique ASVs, with the largest number in mature infructescences (n=652) (Table 2). Newly formed infructescences from the unburnt site shared a number of ASVs with mature infructescences that were unique to these groups and not found in the burnt site (n=252). The same was true for core ASVs (n=37). When comparing the number of shared and total microbiomes ASVs shared between infructescences, litter and soil, a much higher proportion of ASVs were shared between these sample groups in the unburnt site, many of which were present in the core microbiomes of litter, infructescences and soil (Fig. S1). These results confirm that soil, litter and newly formed infructescences from the unburnt shared a higher proportion of ASVs compared to the burnt site. These results confirm that soil, litter, and infructescences share inoculum, possibility accumulating over several flowering seasons, but that mature infructescences remain the largest possible source of infructescence-associated bacteria.

Discussion

The results of this study describe the bacterial diversity in the newly-formed and mature infructescences of *P. repens*, a unique plant organ where only fungi and actinomycetes have previously been studied (Human *et al.*, 2016; Human *et al.*, 2017), as well as in the associated soil environment. Bacterial communities in newly formed infructescences and soil were different in a recently burnt *P. repens* population when compared to an adjacent unburnt population. Many of the bacterial taxa were detected only in infructescences, suggesting that this is a unique, specialized bacterial habitat, as is true for its fungal component (Lee *et al.,*

291 2005*)*. The data also suggest that the bacterial communities in mountain fynbos landscapes
292 may be negatively affected by fire.

293 Bacterial diversity in newly formed infructescences differed significantly between the
294 recently burnt and the unburnt site, demonstrating the microbial associates of fire-affected
295 plants may require long periods to reach their pre-fire composition. Infructescences from the
296 burnt site also had unique bacterial ASVs, demonstrating that fire may be a driver of plant-
297 associated bacterial diversity. The effects of fire on the bacterial communities in fruits or
298 infructescences have not, to the best of our knowledge been studied previously, making this ,
299 the first such report. Aboveground plant microbiomes, such as those in the phyllosphere, are
300 altered in the post-fire environment (Huang *et al*., 2016; Dove *et al*, 2021). The likely drivers
301 of these changes are not well understood but believed to be due to fire-driven changes in soil,
302 a major potential source of microbial inoculum for aboveground plant organs (Grady *et al.*,
303 2019). The results presented suggest that fire alters bacterial communities, even if only at
304 regional scales.

305 Few of the dominant groups of microbiota in the studied infructescences such as
306 *Pseudomonas,* members of Rhizobiaceae (excluding *Rhizobium* spp.), Enterobacteriaceae and
307 Sphingomonadaceae in infructescences are common flower and fruit microbiota (Aleklett *et*
308 *al*., 2014; Gaube *et al*., 2021). Instead, the majority of common taxa detected in the
309 infructescences such as species of Group 1 Acidobacteria, *Luteibacter*, *Granulicella* and
310 *Rhizobium* are known from decaying wood (Kielak *et al.*, 2016, Tláskal *et al.*, 2017),
311 decomposing fungal mycelia (Brabcová *et al.*, 2016) and litter (Gołębiewski *et al.*, 2019), and
312 the roots and rhizosphere of many different plants (Compant *et al.*, 2010, Noll *et al.*, 2010,
313 Hardoim *et al.*, 2015). Infructescences, which remain on aerial branches for several years,
314 consequently represent a unique bacterial habitat for important bacterial. Because seed-
315 associated microorganisms are often the first to establish in the rhizosphere microbiome after

316 seeds are propagated (Truyens *et al.*, 2014; Mitter *et al.*, 2017), we hypothesize that various
317 taxa associated with seeds in *P. repens* infructescences may be founders in the rhizosphere of
318 post-fire fynbos populations.

319 Even though the newly formed and mature infructescences originated from the same trees,
320 their bacterial diversity and community assemblages were significantly different. In mature
321 infructescences, taxa known from late stages of wood and litter decomposition, soil and roots,
322 such as members of the Actinobacteria, Rhizobiales and the Acidobacteria, became highly
323 abundant. The source of many bacterial taxa inhabiting mature infructescences, mostly
324 associated with below ground habitats, remains unclear. Older infructescences represent a
325 common habitat for mites and insects that move from desiccating mature infructescences to
326 newly formed infructescences (Coetzee & Giliomee, 1987, Roets *et al.*, 2009, Theron *et al.*,
327 2012). Other insects that have been found in infructescences belong to groups commonly
328 associated with litter (Coetzee & Giliomee, 1987, Roets *et al.*, 2006), and known for their
329 ability to disperse litter and wood decomposing bacteria and fungi. They are likely a source of
330 many decomposer-type bacteria in older *P. repens* infructescences.

331 Members of the Rhizobiales and Burkholderiales, known for their ability to fix N_2, were
332 abundant in *P. repens* infructescences, similar to many other nutrient-scarce environments,
333 such as dead wood (Hoppe *et al.,* 2014; Hoppe *et al.,* 2015). During the late stages of wood
334 and litter decomposition, available nitrogen is depleted, which restricts fungal decomposition.
335 Proteoid litter is known to have a very low nutrient quality and consists predominantly of
336 lignin, smaller amounts of cellulose and very little nitrogen and phosphorus (Mitchell &
337 Coley, 1987). It seems probable that N_2-fixing bacteria may alleviate N limitations on the
338 surrounding microbial community, especially fungal decomposers.

339 The species richness in soil samples from the burnt and unburnt sites did not differ. However,
340 the community composition, which considers both the number and abundance of species, was

significantly different in samples from the different sites. This was possibly because soils are often not completely sterilized by fires (Pietikäinen and Fritze, 1995; Bárcenas-Moreno *et al.*, 2011), as is also the case in mountain fynbos ecosystems (Kruger and Bigalke, 1984). Therefore, the differences between the burnt and unburnt sites in these communities in the post-fire environment was small. The low nutrient levels, especially low nitrogen and phosphorus of soils that characterize fynbos vegetation (Specht and Moll, 1983; Witkowski and Mitchell, 1987), most likely also shape the soil bacterial community composition, along with aboveground plant diversity.

Species richness and composition was significantly different in the litter samples from the burnt and unburnt sites. This can be explained by the differences in plant species composition (Kruger, 1977; Kruger and Bigalke, 1984), which was the main contributor to litter in the two adjacent plots. It is known that the plant communities in burnt and unburnt sites of fynbos are very different in the first years after fire, with burnt sites rich in annual plants and older sites normally dominated by larger Proteaceae (Kruger, 1977; Perez *et al.*, 2003; Ferrenberg *et al.,* 2013). The absence of *Protea*- derived litter in the burnt site is also the most likely reason why few ASVs were shared between litter and infructescence samples.

The results of this study have shown that infructescences and surrounding soil and litter associated with *P. repens* plants at different stages of development are inhabited by different bacterial assemblages. This suggests that landscapes with a mosaic of plants at different developmental stages would support increased overall bacterial diversity, as is known for birds (Chalmandrier *et al.*, 2013) and insects (Usher & Jefferson, 1991). Unfortunately, anthropogenically induced increases in fire frequency and increases in the extent of fires are responsible for a homogenization of these landscapes, which would also result in disturbance to the microbial communities. Clearly, further studies on the effect of fire in fynbos ecosystems should strongly consider plant-associated microorganisms, especially because

366 they could provide important ecosystem services, such as the provision of nitrogen and
367 phosphorus to the environment.

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373 **Conflict of interest**

374 The authors declare that there is no conflict of interest

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586 **Tables and Figures**

587 **Table 1.** Results of PERMANOVA using the Bray-Curtis distances calculated from bacterial
588 assemblages from litter, soil and newly-formed infructescences; from burnt vs unburnt
589 patches and newly-formed and mature infructescences from the unburnt patch

Sample	R^2	p-value
Burnt against Unburnt		
Newly formed infructescences	0.18	0.014
Soil	0.22	0.012
Litter (as response to plant communities)	0.42	0.024
Newly formed against mature infructescences		
Burnt Newly formed- against Mature Infructescences	0.55	0.007
Unburnt Newly formed- against Mature Infructescences	0.2	0.038

590

591 **Table 2.** A summary of core and total bacterial microbiome ASVs for each of the sample
592 groups separated into burnt and unburnt patches, including the number of unique ASVs in
593 each core microbiome.

Samples	Site	Core ASVs	%Core ASVs	Core ASVs Unique	% Core ASVs Unique	Total ASVs in sample	Unique ASVs in total microbiome	% Unique ASVs	Total ASVs in sample group
Newly formed infructescences	Unburnt	198	19%	38	20%	1040	197	19%	1318
Newly formed infructescences	Burnt	172	21%	42	23%	836	215	26%	
Mature infructescences	Unburnt	200	13%	71	30%	1484	652	44%	1484
Litter	Unburnt	234	30%	143	61%	768	357	46%	1317
Litter	Burnt	97	13%	39	39%	774	451	58%	
Soil	Unburnt	127	6%	74	58%	1714	1121	65%	3206
Soil	Burnt	97	5%	49	48%	2001	1382	66%	

594

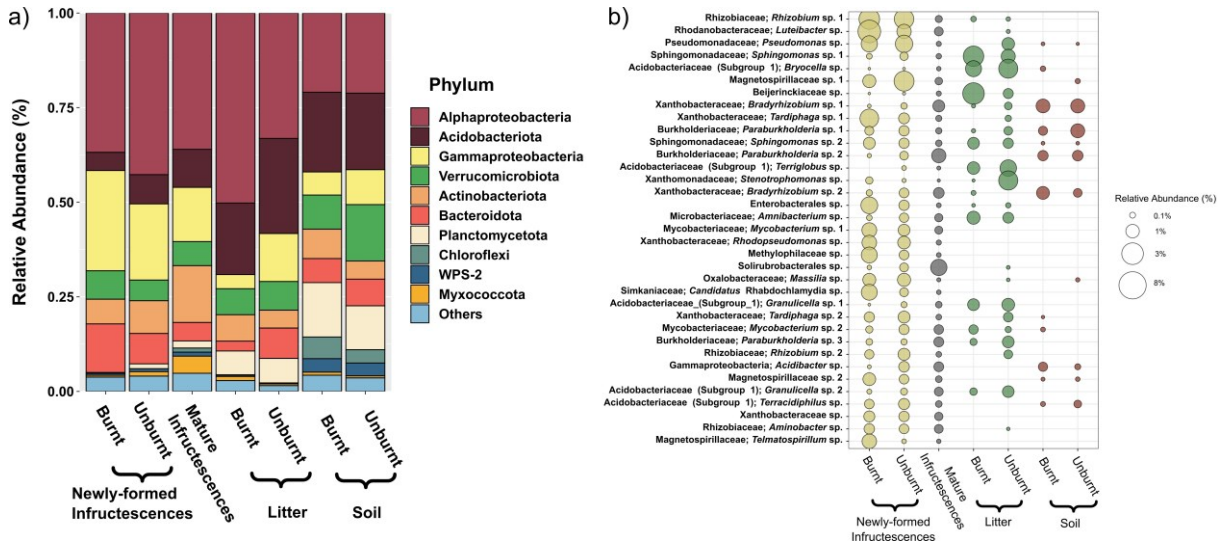

Fig. 1a. Stacked barplot showing the relative abundance of bacterial phyla and classes within Proteobacteria in different sample groups and b) bubble plot of the ASVs with the highest relative abundance in each sample group and often shared between sample groups in the burnt and unburnt site.

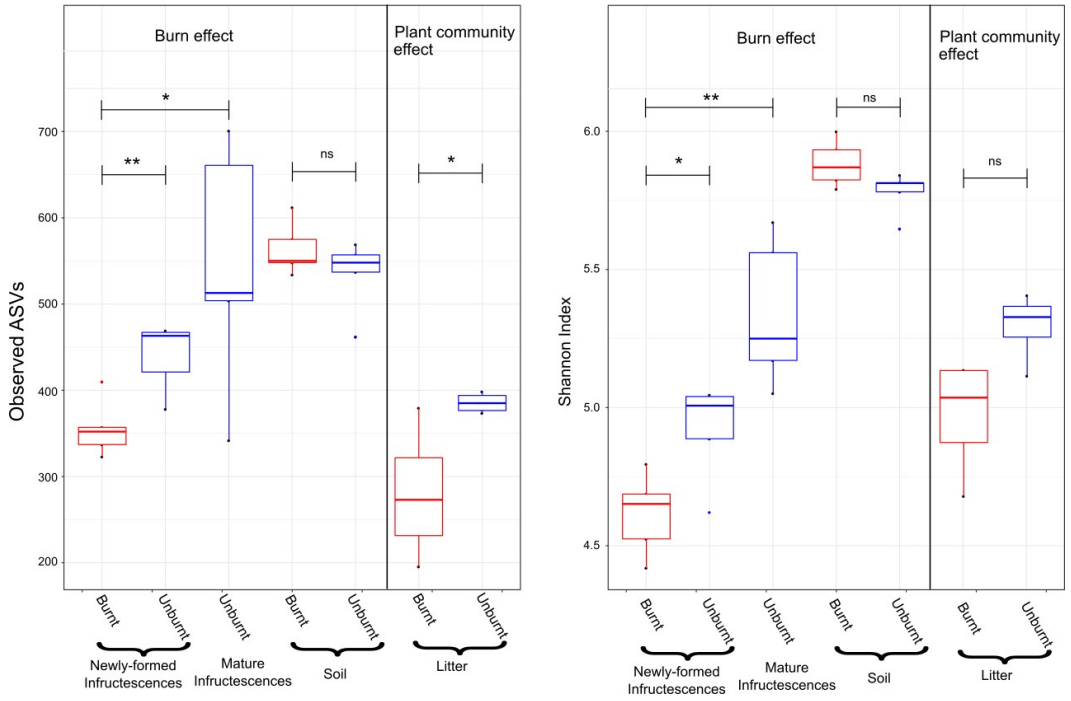

Fig 2. Boxplots of observed ASVs (species richness) and Shannon-index as metrics for α-diversity in different sample groups in the burnt and unburnt site. Statistical significance is indicated as * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

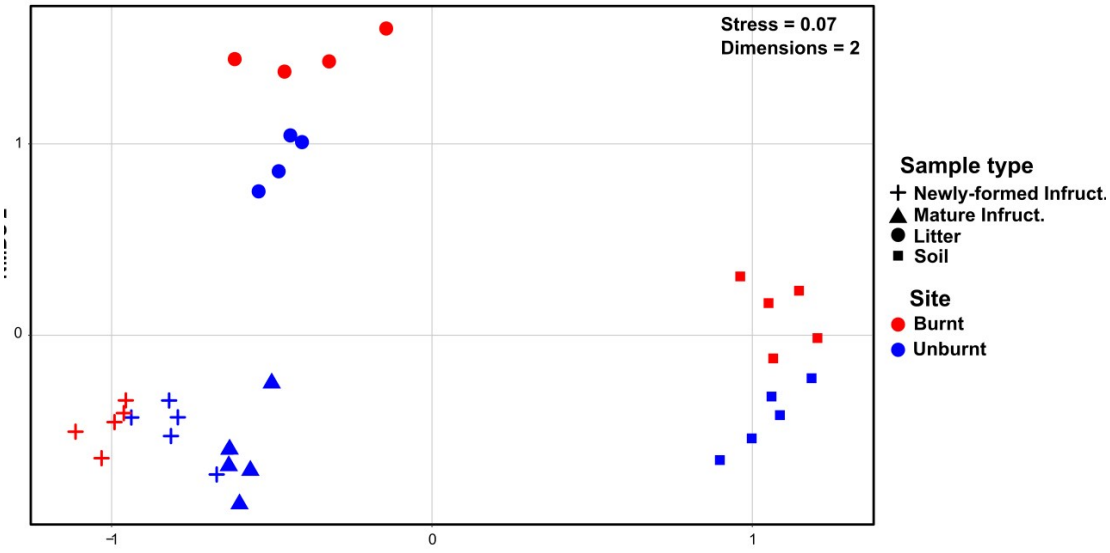

Fig 3. Non-metric multidimensional scaling (NMDS) plot of Bray-Curtis distances of bacterial assemblages in different sample groups in the burnt and unburnt sites. The optimal number of dimensions were 2 and stress equal to 0.07.

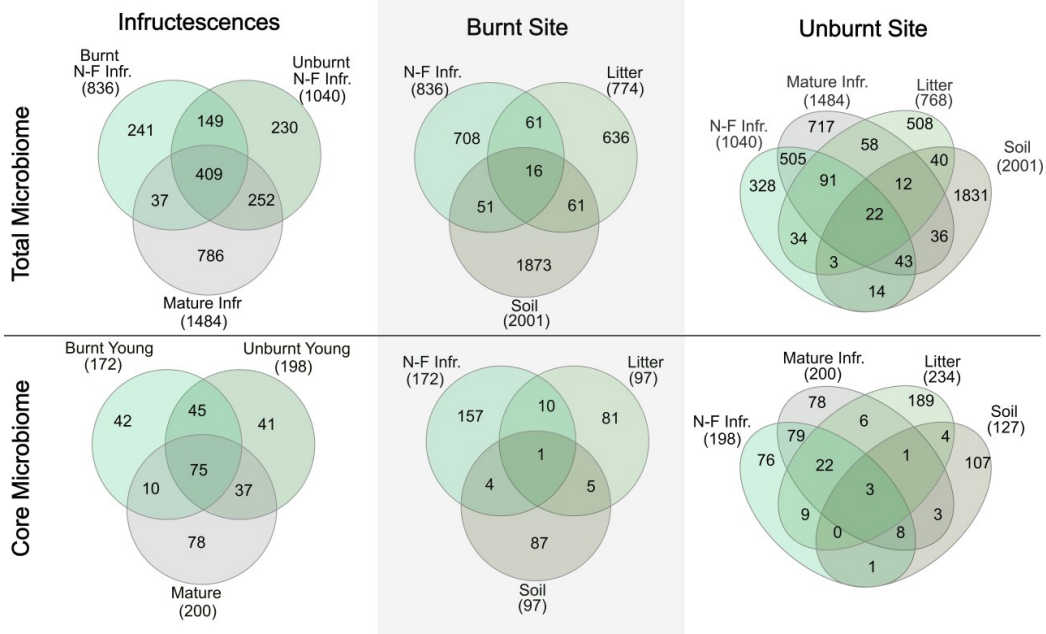

Fig 4. Venn diagrams of the number of core- and total bacterial microbiome ASVs shared between infructescences and sample groups from the burnt and unburnt sites. The number of total and core ASVs in each sample group is indicated in brackets. Newly-formed and mature infructescences are abbreviated as N-F Infr. and Mature Infr. respectively.