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Microbial diversity and community structure in Fynbos soil

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Abstract

The Fynbos biome in South Africa is renowned for its high plant diversity and the conservation of this area is particularly important for the region. This is especially true in the case of endangered vegetation types on the lowlands such as Sand Fynbos, of which only small fragments remain. The question is thus whether the diversity of the above-ground flora is mirrored in the below-ground microbial communities. In order to determine the relationship of the above- and below-ground communities, the soil community composition of both fungal and bacterial groups in Sand Fynbos was characterized over space and time. A molecular approach was used based on the isolation of total soil genomic DNA and automated ribosomal intergenic spacer analysis of bacterial and fungal communities. Soil from four different sites was compared to resolve the microbial diversity of eubacterial and fungal groups on a local (alpha diversity) scale as well as a landscape scale (beta diversity). The community structures from different sites were compared and found to exhibit strong spatial patterns which remained stable over time. The plant community data were compared with the fungal and the bacterial communities. We concluded that the microbial communities in the Sand Fynbos are highly diverse and closely linked to the above-ground floral communities.

Keywords: 16S rDNA, 18S rDNA, bacteria diversity, bacterial communities, fungal communities, fungal diversity, fynbos soil

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Introduction

The Cape Floristic Region falls within an area recognized as one of the world's biodiversity hotspots (Cowling *et al.* 2003). Within the region, Sand Fynbos is among the most poorly conserved of all vegetation types in South Africa and has been classified as endangered (Cowling 1992; Rouget *et al.* 2006). Approximately 95% of this vegetation type has been transformed into agricultural land, invaded by alien species or developed (Low & Rebelo 1996; Rebelo *et al.* 2006). The result is the fragmentation of the vegetation which now only occurs in isolated pockets (Rebelo 1992; Heijnis *et al.* 1999). Plant studies have shown that in Fynbos areas, fragments have significantly fewer plant species than areas of the same size located inside a more extensive pristine area (Bond *et al.* 1984; Kemper *et al.* 1999; Kongor 2009). Smaller land fragments are also more adversely affected by outside factors compared to larger areas. The effect and significance of fragmentation on microbial populations, particularly the beta diversity, has thus far not been considered (Lozupone & Knight 2007).

The soil environment harbours a large proportion of the earth's undiscovered biodiversity. The diversity of soil micro-organisms is not as well studied and it is believed that only about 1% of the organisms that occurs in soil have been cultured, identified, and characterized (Torsvik *et al.* 1990; Hawksworth 2001). Studying the diversity in different ecosystems contributed to the understanding of the extent of microbial diversity (Jessup *et al.* 2004; Bertin *et al.* 2008; Zhou *et al.* 2008). The exact factors that are responsible for diversity and

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community patterns are, however, not well understood (Zhou *et al.* 2002).

The general factors that influence microbial populations are the soil properties, which may be physical or chemical. Physical properties include soil moisture, aeration, texture, structure and temperature (Gaur & Misra 1978; Fomsgaard & Kristensen 1999; Chen *et al.* 2007). Chemical properties include nutrient characteristics and pH (Gaur & Misra 1978; Jonasson *et al.* 1999; Lipson *et al.* 1999; Turrión *et al.* 2002). The above-ground vegetation structure and makeup also have an influence on the microbial population within the soil (Nüsslein & Tiedje 1999; Hamilton & Frank 2001; Tangjang *et al.* 2009). The relationship between microbes and the above-ground vegetation, specifically in Fynbos, has been focused on in one previous study (Stafford *et al.* 2005).

The diversity and variation of soil micro-organisms have been studied in a number of different ecosystems (Saetre & Baath 2000; Bhatnagar & Bhatnagar 2005; Bezemer *et al.* 2006). This includes studies which looked at soil microbial diversity at a landscape scale and meso-scale (Green *et al.* 2004). Many of these studies were, however, only focused on the rhizosphere communities or diversity of mycorrhizal fungi (Allsopp & Stock 1995; Caravaca *et al.* 2002; Bergero *et al.* 2003; Spriggs *et al.* 2003). The beta diversity of plants in the Fynbos biome is characteristically high (Cowling 1992). This high species turnover results in a significant variation in the plant community diversity and composition between sites. Thus, we believe that this species turnover may also drive variations in the microbial communities between different locations. In this study above-ground plant diversity, microbial community structure, and relative diversity of fungal and eubacterial groups were investigated and compared in four Atlantis Sand Fynbos fragments.

Materials and methods

Study area

The sites at Pella and Riverlands are part of a conservation area administered by CapeNature. The site at Kalbaskraal is in the process of being rehabilitated from Port Jackson (*Acacia saligna*) infestation after its introduction to stabilize the sandy soil. The site at Camphill Village is surrounded by farmland with no planned or natural fires having occurred for 40 years and we thus consider this to be a neglected site. The soil type observed in Atlantis Sand Fynbos is acidic tertiary grey regic sand (Goldblatt & Manning 2000) derived originally from Cambrian Cape Granite (Rebelo *et al.* 2006) and with Aeolian origin. The soils of the study sites are all on average 2 m in depth (Deacon et al. 1983; Rebelo 1996) with a pH that ranges from 3.6 to 4.7 (Low 1983). The soil typically has an organic matter content of between 1–3% and available carbon content of less than 1% (Low 1983; Mitchell *et al.* 1984). The soil of the study areas are low in nutrients and are especially poor in phosphorus (Kruger 1979; Richards *et al.* 1997). This is due to the low amounts of P_2O_5 found in the parental palaeozoic rocks, sandstones, shales, schists and granites, which are typically below 1% (Marchant & Moore 1978; Low & Bristow 1983).

Sampling

Soil samples were taken at four different sites in the Atlantis Sand Fynbos vegetation type (Western Cape, South Africa) (Mucina & Rutherford 2006). The study sites were located between 160 and 220 m above sea level and sloped slightly between 0% and 4% (Witkowski & Mitchell 1987). The sites were located at Riverlands (1100 ha), Pella (600 ha), Kalbaskraal (37 ha) and Camphill Village (16 ha) (Fig. 1, Table 1). On every site, three random plots of 10 m × 10 m were identified. The plots were selected and divided in four quadrants. Ten soil samples were taken randomly in each quadrant within the first 10 cm of the surface away from plant to avoid roots. The 10 samples of 50 g each were homogenized and sieved using a 2-mm mesh sieve to remove plant debris and produce a composite sample for each quadrant (Coutinho *et al.* 1999). Sampling was conducted in February (Summer), March (Autumn), July (Winter) and September (Spring) 2007 to acquire representative samples for each season.

The above-ground plant species inventory was carried out between September and December of 2004 and 2005. During the period between 2005 and 2007, the vegetation was unlikely to change, since no fire events occurred during this period. The inventory was conducted using a 50 m × 20 m modified Whittaker plot design (Stohlgren *et al.* 1995). The 50 m × 20 m plots had 0.1 m^2, ten 1 m^2, three 5 m^2 and three 100 m^2 subplots. In each sub-plot, presence–absence data of plant taxa were recorded. The data of the 10 m × 10 m plots were used for comparison with below-ground communities.

DNA extraction and PCR amplification

DNA was extracted from 0.35 g of soil within 12 h of sampling using the ZR Soil Microbe DNA kit (Zymo Research) and the presence of genomic DNA was checked on a 1% agarose gel, stained with ethidium bromide. PCR reactions were performed on the genomic DNA using fungal and eubacterial primers sets to evaluate its application in automated ribosomal internal transcribed spacer (ITS) analysis (ARISA). Eubacterial

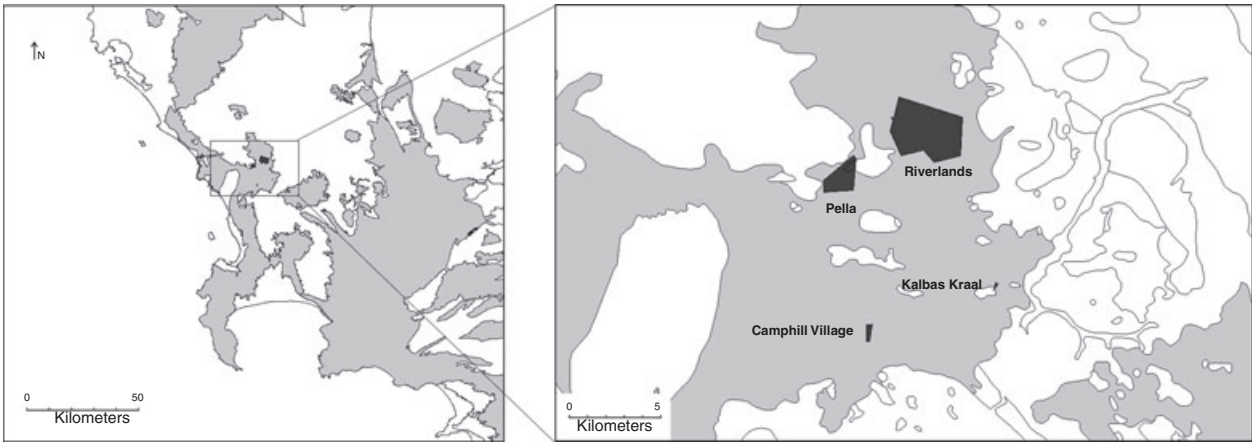

Fig. 1 Location of the study sites within the Sand Fynbos.

Table 1 Location of the study plots

Site	Location of site (GPS)
Pella	
Plot 1	S 33°,51022 E 18°,55236
Plot 2	S 33°,50960 E 18°,54925
Plot 3	S 33°,52011 E 18°,54766
Riverlands	
Plot 1	S 33°,49795 E 18°,58931
Plot 2	S 33°,49788 E 18°,58608
Plot 3	S 33°,49608 E 18°,58388
Camphill Village	
Plot 1	S 33°,59701 E 18°,56554
Plot 2	S 33°,59787 E 18°,56433
Plot 3	S 33°,59774 E 18°,56327
Kalbas Kraal	
Plot 1	S 33°,57061 E 18°,62861
Plot 2	S 33°,57133 E 18°,62773

specific primers, ITSReub and FAM (carboxy-fluorescein) labelled ITSF, were used to determined bacterial diversity using ARISA (Cardinale *et al.* 2004). The fungal diversity was determined by using fungal primers ITS4 and FAM labelled ITS5 (White *et al.* 1990) in the PCR reactions. PCR reactions were done using a GeneAmp PCR System 2400 (Applied Biosystems). The reaction mixture contained 0.5 μL of the purified genomic DNA extracted from soil, 500 nM of each primer and 5 μL of 2× KapaTaq Readymix (KapaBiosystems, South Africa) in a total volume of 10 μL. The PCR conditions consisted of an initial denaturing step of 3 min at 95 °C followed by 40 cycles of 95 °C, for 30 s, 51 °C for 30 s and 72 °C for 30 s. The reaction was completed with a final extension at 72 °C for 5 min and then cooled and held at 4 °C. PCR for each sample was performed in triplicate and pooled to eliminate background noise and reduce the PCR variability occurring. PCR samples were separated on a 1% agarose gel, stained with ethidium bromide and visualized using ultraviolet light.

Automated ribosomal intergenic spaceranalysis

The PCR products were run on an ABI 3010xl Genetic analyser to obtain an electropherogram of the different fragment lengths and fluorescent intensities. F-ARISA PCR samples were run along with LIZZ 600 size standard which contained sizes from 60 to 660 bp in length. B-ARISA samples were run with ROX 1.1 size standard which varied from 20 to 900 bp (Slabbert 2008). ARISA data was analysed using Genemapper 4.1 software. The software converted fluorescence data to an electropherogram and the peaks which represented fragments of different sizes are termed operational taxonomic units (OTU). Only fragment sizes larger than 0.5% of the total fluorescence ranging from 100 to 1000 base pairs in length was considered for analysis. A bin size of 3 bp for fragments below 700 bp and 5 bp for fragments above 700 bp was employed to minimize the inaccuracies in the ARISA profiles (Brown *et al.* 2005).

Data analysis

Species accumulation curves were plotted for fungi and bacteria for each of the four sampling events during the year. The fungal and bacterial data sets used were randomized by re-sampling the data (10 times) using Poptools 2.7 software and transformed to 100% of the maximum value. Twelve species-accumulation models commonly used were evaluated against the species accumulation curves for both bacteria and fungi using Statistica 8 (Statsoft 2008). The estimation methods used included Chao (based on presence and absence data),

1st order Jack-knife, Bootstrap and Michaelis–Menten models. The various estimators appear to perform differently depending on the system (Colwell & Coddington 1995). The suitability of the richness estimators was evaluated according to Bartels & Nelson (2006).

The peak heights were used to calculate the diversity indices for each ARISA profile using Microsoft Excel™ software. The Shannon (H') index was calculated for each sample and each plot to determine the disorder in the species distribution of the community. The increase of beta diversity was determined over the different samples, plots and sites. The Whittaker (βw) index for beta diversity was determined over all scales and comparisons made between samplings (Whittaker 1972; Mena & Vázquez-Domínguez 2005).

The Whittaker similarity index was calculated for bacterial and fungal profile data between all plots. The distance relationship between the samples was illustrated by performing a complete linkage cluster analyses using the Whittaker (Sw) similarity indices (Hewson *et al.* 2006). After initial grouping with cluster analysis, the significance of the groupings were tested by performing a non-parametric analysis of similarity analysis (ANOSIM) with 10 000 permutations (Clarke 1993).

The vegetation data were expressed as a data matrix based on the presence or absence of a specific plant species in the plot. Fynbos communities at the different sites were compared by calculating the Jaccard's similarity index (Jaccard 1912). The relationship between the bacterial and fungal community similarity data and the plant community similarity was tested by using a non-parametric partial Mantle test (Sokal & Rohlf 1981). The partial mantel test was performed using ZT-software with a confidence level of 95% (Bonnet & Van der Peer 2002).

Results

DNA extraction and ARISA–PCR

Genomic DNA extractions from soil from different sites resulted in consistent yields of more than 100 ng/μL (Fig. S1). PCR reactions resulted in smears ranging from 100 to 900 bp with bacterial primers and 150 to 800 bp with fungal primers (Figs S2 and S3). Bacterial ARISA electropherogram data produced peaks from 100 to 900 bp as extrapolated by Genemapper from the ROX 1.1 size standard. The fungal ARISA electropherogram results show peaks ranging from 150 to 800 bp as extrapolated using the LIZZ 600 size standard. Genemapper detected approximately 150 unique peaks for F-ARISA and 250 for B-ARISA in all the samples.

Frequency of bacterial and fungal OTUs

Bacterial ARISA demonstrate a dominance of fragments between 150 and 500 bp in length for the bacterial population (Fig. 2). This size category constitutes 90.6% of the total operational taxonomic units observed. The size data based on the frequency of fungal OTUs indicate the dominance of peak heights between 550 bp and about 650 bp (Fig. 3). This region of the histogram constitutes 45.6% of the total number of peaks observed.

Below-ground richness and diversity

The number of bacterial OTUs remained significantly higher than fungal OTUs across all plots, and this observation was consistent from February to September (Fig. S4). The number of bacterial OTUs in each sample in February ranged from 42 to 68, in April from 40 to 56, in June from 40 to 51 and in September from 43 to 49. The number of unique fungal OTUs in each sample was consistently less than the number of bacterial OTUs, and varied between 12 and 34 in February, from 21 to 37 in April, from 21 to 35 in June and from 27 to 36 in September.

Due to the relatively high species number, the bacterial diversity is expected to be higher than the fungal diversity. The Shannon index for the bacterial communities remained very high (H' = 3.60–4.4) throughout the year (Fig. S5). The lowest fungal diversity was detected at Kalbaskraal (H' = 2.9), and the highest (H' = 4.3) at Riverlands during the same sampling time

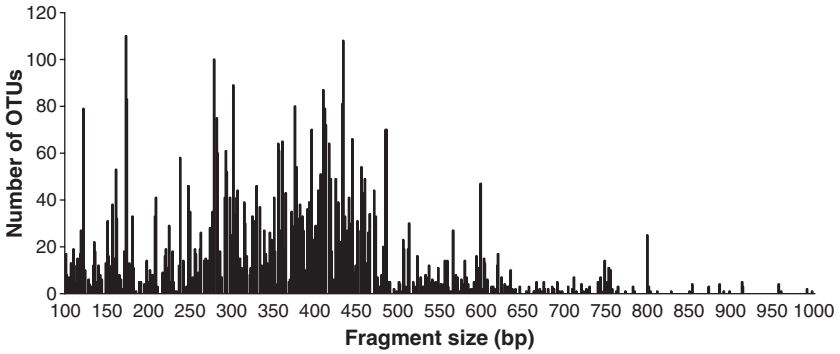

Fig. 2 Summary of the number of bacterial operational taxonomic units detected of each size category.

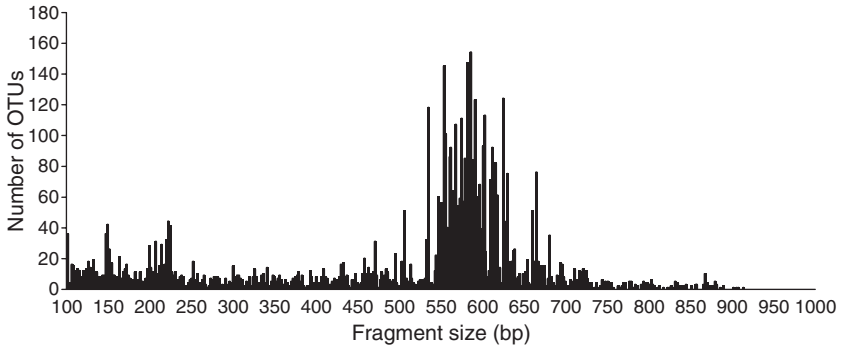

Fig. 3 Summary of the number of fungal operational taxonomic units detected for each size category.

in February 2007 (Fig. S5). Although diversity indices varied spatially and over time, no significant differences between sites were observed.

Below-ground species accumulation and estimation

The parametric Beta-P models showed a consistent good fit for bacterial accumulation curves over all four sampling dates (Fig. S6). In addition to the Beta-P, the three parametric Weibull models also showed significant fit with fungal accumulation data. It is apparent from the fungal species accumulation curves that the number of fungal species is still increasing relatively fast compared to the bacterial equivalents (Fig. S7). The Bootstrap method performed better than the other species estimation models tested. The results predicted a detection level of around 90–92.6% for bacterial species (Table 3) and around 87.5% for fungi (Table 4) using ARISA.

Below-ground community structure

The cluster analysis performed illustrated the relationship between the intra-fungal and bacterial communities according to occurrence and abundance of operational taxonomic units. The tree diagrams for bacteria and fungal communities display high similarities between plots from the same sites (Figs. 4 and 5).

Below-ground beta diversity

The beta diversity of bacterial and fungal samples is very high, with values above 4.0 observed except for the bacterial samples in the month of September when it only reached 3.6 (Table 2). The beta diversity of both the fungal and bacterial communities decreased with an increase in scale due to the increase likelihood of species-overlap occurring. The comparative beta diversity for fungi is significantly higher at larger scales during each sampling event. Locally occurring microbial species also represent relatively unique communities.

Table 2 Summary of the Whittaker beta diversity index over the three area sizes considered

	Whittaker (Bw)		
	Sample	Plot	Site
Bacteria			
February	5.0965	1.7154	0.67683
April	4.0091	1.3848	0.43928
June	4.8353	1.6719	0.68521
September	3.6056	1.543	0.67315
Fungi			
February	5.647	2.1989	1.1232
April	5.0414	2.2043	1.0892
June	4.7284	1.7455	0.85542
September	4.8423	1.9685	0.87013

Relationship between plant and microbial communities

The partial Mantel test showed a strong, to very strong correlation between the bacterial and the plant community absence and presence data. This indicates a link between the occurrences of some bacterial species with the presence of certain plant species. The high beta diversity of above-ground plant species can thus be expected to directly influence the diversity of soil bacteria (Table 5). The fungal community showed a strong relationship between plant presence and absence data when performing the Mantel test. When controlling for the influence of space, this relationship becomes insignificant.

Where above-ground plant species diversity varied greatly, large variations in the microbial soil communities was also shown. The above-ground community composition changed very little during the entire year of soil sampling. Bacterial communities correlated better with the plant communities and fungal community structure appeared to be linked to spatial regression. The species accumulation characteristics categorize these fungal communities as having a high risk of local extinction.

Table 3 The performances of the Bootstrap model based on half the number individuals sampled, the estimated number of species and the percentage of the estimate observed with ARISA

	Bacteria					
	Number of samples used	Model	Number observed	Estimation using half the individuals	Estimate number of species	Percentage observed with ARISA
February	21	Bootstrap	276	275.03	306	90.19608
April	25	Bootstrap	240	242	259	92.66409
June	23	Bootstrap	265	259	290	91.37931
September	23	Bootstrap	215	214	239	89.95816

Table 4 The performances of the Bootstrap model based on half the number individuals sampled, the estimated number of species and the percentage of the estimate observed with ARISA

	Fungi					
	Number of samples used	Model	Number observed	Estimation using half the individuals	Estimate number of species	Percentage observed with ARISA
February	18	Bootstrap	179	168	205	87.31707
April	24	Bootstrap	164	163.75	188	87.23404
June	19	Bootstrap	154	145	176	87.5
September	23	Bootstrap	180	177	205	87.80488

Discussion

In this study we successfully used ARISA to characterize the microbial communities in the soil of the Sand Fynbos. The data obtained could effectively be used to analyse and compare ecological information. Previous studies have shown ARISA to be a reproducible and extremely sensitive technique for assessing microbial community structure (Fisher & Triplett 1999; Green *et al.* 2004; Torzilli *et al.* 2006). The supporting data from above-ground ecological studies provide considerable insight into soil microbial diversity in the Fynbos ecosystem which was previously unexplored.

PCR reactions with universal eubacterial primers resulted in an abundance of shorter fragments that most likely represent gram-positive bacteria. As most gram-positive bacteria have no tRNA in the spacer region which would results in shorter fragment lengths (Gürtler & Stanisich 1996). This would be expected due to the well-recognized dominance of gram-positive bacterial species in soil (Torsvik *et al.* 1990; Dunbar *et al.* 2002; Smalla *et al.* 2001). Similarly, the dominance of fragments between 550 and 650 bp observed with F-ARISA are expected and previous studies also report similar fragments in soil (Cardinale *et al.* 2004).

ARISA has been shown to be relatively sensitive in the detection of low frequency templates in mixed samples (Danovaro *et al.* 2006). This sensitivity is evident in the accumulation curves, which shows that the numbers of unique fungal OTUs are still increasing relatively rapidly with the addition of new samples compared to the bacterial equivalents. The relatively steep increase towards the tail of the curve is an indication of the presence of high numbers of low frequency fungal OTUs (Ugland *et al.* 2003). High beta diversity and subsequent heterogeneity in the fungal communities is demonstrated by the accumulation curve that is less steep compared to the bacterial accumulation curve. This would imply that bacteria are more evenly distributed throughout the soil than fungi. The species accumulation characteristics categorize these fungal communities as having a high risk of local extinction compared to bacteria. To determine the estimated number of taxa in the area, non-parametric methods were shown to be more accurate (Chazdon *et al.* 1998). Different methods have been shown to be effective for specific applications and specific niches (Bartels & Nelson 2006), but the Bootstrap method proved most useful in estimating total soil microbial OTUs in this study (Tables 3 and 4).

The diversity indices varied spatially and over time, but there were no significant differences between sites. Although the diversity between the plots in most cases did not differ considerably, the peak composition of the ARISA profiles differed significantly as indicated by the Whittaker similarity indices (Figs. 4 and 5). The higher

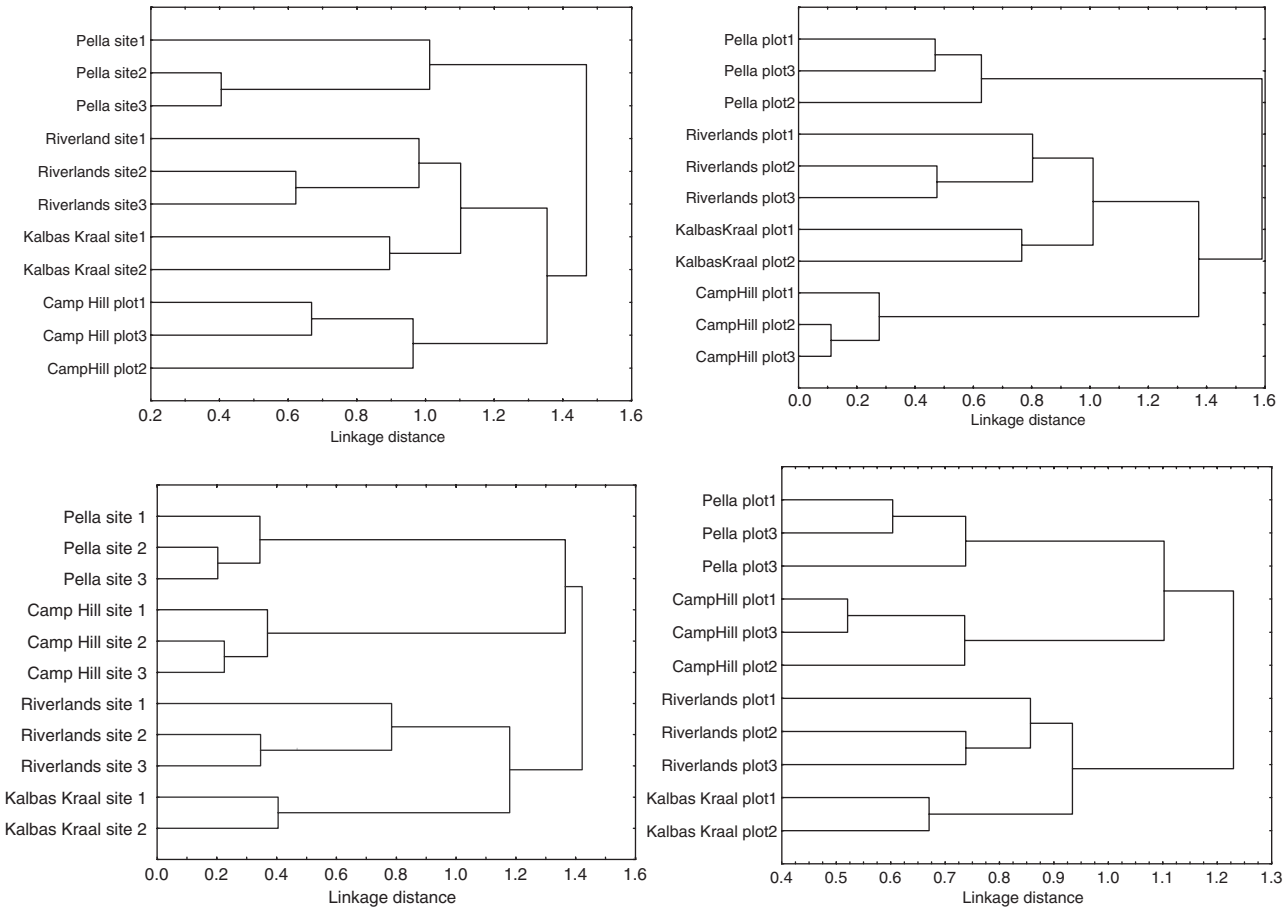

Fig. 4 Tree diagrams of the Cluster analysis of the Whitaker similarity analysis for the bacterial OTUs during February 2007.

similarity between bacteria and fungal communities from the same sites is expected when considering that a decline in similarity over distance is known to occur between communities (Green *et al.* 2004; Ramette & Tiedje 2006; Peay *et al.* 2007). The similarities between plots remained constant for both bacterial and fungal OTUs over all four sample events. The distribution of microbial communities was shown not to be random and strong spatial patterns exist with relatively stable fungal and bacterial communities (Figs. 4 and 5). Over short time scales, Fynbos plant community composition changes very little from season to season, unless impacted by fire (Cowling *et al.* 1997). As there was not a fire event during the study period, the above-ground community composition changed very little during the entire year. This stability in community composition is mimicked by the microbial communities, suggesting a relationship between the above and below-ground communities.

It is well recognized that the primary factor influencing Fynbos diversity is not its alpha diversity at a specific site but the beta diversity (Cowling 1992). The beta diversity of both the fungal and bacterial communities decreased with an increase in scale due to the increase likelihood of species-overlap occurring (Table 2). The spatial separation between sites and the scale at which beta diversity is observed proved to be important factors when looking at the beta diversity of bacteria and fungi in the Sand Fynbos. The exact extent of microbial diversity in a system is not only a measure of local diversity, but also beta diversity is an important factor when considering the overall diversity of an ecosystem. The particular species turnover demonstrated by the ARISA profiles is an important primary factor contributing to the diversity of fungal and bacterial species of the Sand Fynbos. The large species turnover seen in Fynbos is of vital importance when considering the conservation of Fynbos (Cowling 1992; Rouget *et al.* 2003). Locally existing microbial species also represent unique communities. The fungal community has significantly higher beta diversity, even though dispersal by aerial spores poses less of a limitation to dispersal than is the case of bacteria (Unterseher & Tal 2006). The high beta diversity, which is the main contributing factor to the high biodiversity seen in Fynbos (Cowling *et al.* 2003; Kongor 2009), appears to play an important role in the

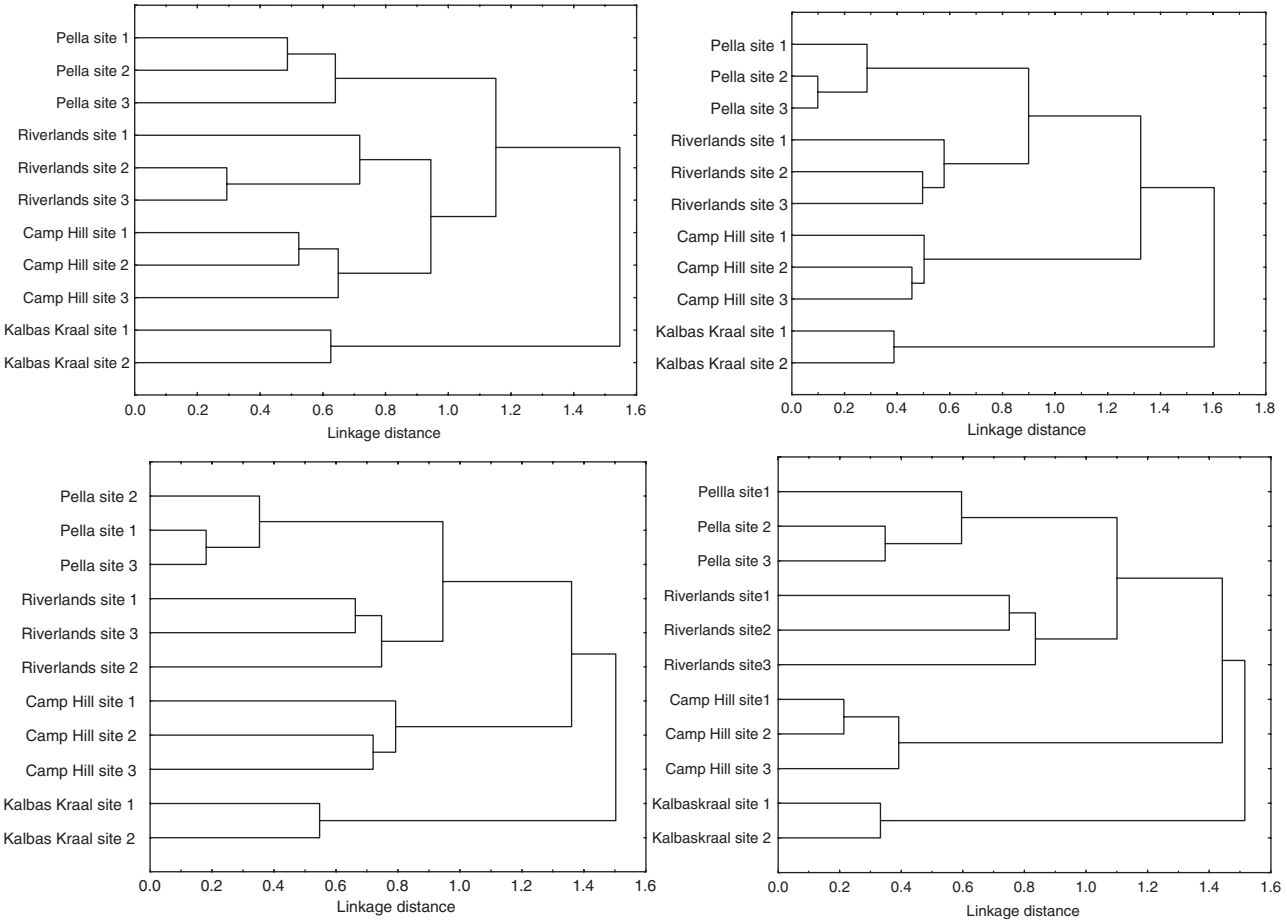

Fig. 5 Tree diagrams of the Cluster analysis of the Whitaker similarity analysis for the fungal OTUs during February 2007.

Table 5 Results for the Mantel test and partial Mantel test

	Fungi		Bacteria	
	Mantel test	Partial Mantel test	Mantel test	Partial Mantel test
February 2007	0.706	0.212	0.59	0.6
April 2007	0.442	0.112	0.37	0.62
June 2007	0.398	0.091	0.24	0.57
September 2007	0.659	0.124	0.11	0.49

biodiversity of bacterial and especially the fungal communities.

The results from the Partial Mantel tests indicate that bacterial community structure is closely linked to plant communities, although it follows a similar clustering pattern to the fungal communities during all four sampling dates. The higher similarities between sites on the larger fragments, Pella and Riverlands, are indicative of a community structure which lends itself to spatial patterning (Peay *et al.* 2007). A link between the occurrence of some bacterial OTUs with the presence of certain plant species was suggested. Thus, the high beta diversity of above-ground plant species is expected to influence the diversity of soil bacteria (Table 5).

From the data it is apparent that a correlation between plant and microbial communities exists although the exact causes of the relationship remains unclear. A few possibilities may explain the link between plants and micro-organisms. First, it is possible that both the plant and the microbial populations have an effect on the abiotic environment and on each other (Reynolds *et al.* 2003; Ehrenfeld *et al.* 2005; Hättenschwiler *et al.* 2005). A second possibility is that the same prevailing soil and environmental conditions that affect the above-ground species composition affect the microbial population structure and the two do not affect each other to a very high degree (Cornelissen *et al.* 2003; Ramette & Tiedje 2006). Finally, it is possible that a one-way interaction between the plant communities and the soil micro-organisms exists, where the plant litter is the main contributing factor and driver of the microbial community structure (Xiao & Zheng 2001; Liu *et al.* 2005). The effect of above-ground spatial distribution on

microbial communities can, therefore, not be discounted and seems to play a bigger role in the fungal communities than in the bacterial communities.

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Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Agarose gel electrophoreses total extracted DNA from Riverlands on Feb 2007. Lanes 1 and 14: Hyper Ladder I, Lanes 2–5: Riverlands plot1A–D, Lanes 6–9: Riverlands plot A–D, Lane 10–13: Riverlands plot A–D PCR amplification.

Figs S2 and S3 Agarose gel electrophoreses of PCR amplification of total extracted DNA from Riverlands on Feb 2007 with fungal specific primer set ITS5 (FAM) and ITS4 and bacterial specific primer set ITSF (FAM) and ITSR. Lanes 1 and 14: Hyper Ladder I, Lanes 2–5: Riverlands plot1A–D, Lanes 6–9: Riverlands plot A–D, Lanes 10–13: Riverlands plot A–D PCR amplification.

Fig. S4 The number of bacterial and fungal OTU's detected in each plot during February, April, June and September 2007.

Fig. S5 Comparative Shannon–Weaver diversity between fungi and bacteria for February, April, June and September 2007.

Fig. S6 The accumulation curves of bacterial operational taxonomic units for June 2007.

Fig. S7 The accumulation curves of fungal operational taxonomic units.

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