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Moisture and substrate stability determine VA-mycorrhizal fungal community distribution and structure in an arid grassland

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The arid central dune field of the Namib Desert is characterized by a pronounced rainfall gradient across its west–east, 160 km breadth, and a correlated increase in sand stability and grass community complexity. In addition to these macro-gradients, micro-gradients of sand stability and available moisture across each dune slope result in stratified grass communities on the dunes. The effects of abiotic factors and plant associations on the community structure of VA-mycorrhizal fungi in a naturally arid and unstable grassland could thus be investigated. Mycorrhizal fungal communities associated with five grass species were sampled at sites located across the gradients. Diversity and abundance of spores, as well as percent mycorrhizal colonization of plant roots, were used to characterize the fungal communities and their plant specificity. Five *Glomus* species (Glomales) were associated with grasses at all sites, but no plant specificity was observed. Rather, the fungal communities varied in diversity and abundance both within a dune site and across the dune field. Regression analyses showed that spore abundance and colonization levels were significantly affected by abiotic factors. Sand stability affected spore abundance and thus determined the limits of distribution of the fungal community in the dune grassland. In contrast, colonization levels were primarily affected by moisture availability, and fungal growth and spore production following an isolated rain event were closely associated with moisture availability. A rapid and opportunistic growth response to moisture, production of resilient spores in response to declining moisture, and lack of plant symbiont specificity are characteristics which allow mycorrhizal fungal communities to function under hyperarid conditions.

Keywords: dune ecology; fungal diversity; moisture; mutualism; Namib Desert; Namibia; pulse-reserve paradigm; mycorrhiza

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Introduction

VA-mycorrhizal fungi are common mutualistic symbionts of plant roots in grasslands and deserts (Trappe, 1981; Miller, 1987; Allen, 1991; Brundrett, 1991). Mycorrhizal associations play an important role in enhancing primary production (e.g. Lopez-Sanchez *et al.*, 1992; Brejda *et al.*, 1993), and nutrient status of plants, particularly in nutrient poor soils such as those found in deserts (Brundrett, 1991). Numerous studies have documented significant increases in phosphorus uptake in mycorrhizal plants under such conditions (Koide, 1991; Cui & Nobel, 1992; Lopez-Sanchez *et al.*, 1992; Trent *et al.*, 1993).

Mycorrhizae can also increase water uptake by plants, which is of particular importance in arid and semi-arid ecosystems (Allen, 1982; Cui & Nobel, 1992). Increased root hydraulic conductivity (Safir & Nelsen, 1985) in mycorrhizal plants has been attributed to hyphae within roots providing a low resistance pathway for radial water flow across the cortex (Safir & Nelsen, 1985) as well as possible changes in membrane permeability to water, resulting from improved phosphorus nutrition (Radin & Eidenbock, 1984).

Despite the importance and ubiquity of VA-mycorrhizal fungi in natural ecosystems, relatively few studies have examined how abiotic and biotic factors affect mycorrhizal community structure (Miller, 1987). Early studies showed that VA-mycorrhizal fungi were not host specific and until recently VA-mycorrhizal fungal community composition was primarily attributed to abiotic factors such as soil pH (Abbott & Robson, 1977), soil moisture (Anderson *et al.*, 1986), temperature (Koske, 1987) and total soil C and N (Johnson *et al.*, 1991). Physical soil disturbance has negative effects on VA-mycorrhizal fungal abundance resulting from a reduction or loss of propagules, depending on the type of disturbance and its duration (Reeves *et al.*, 1979; Allen & Allen, 1980; Gould & Liberta, 1981; Doerr *et al.*, 1984; Waaland & Allen, 1987; Aziz & Habte, 1989; Jasper *et al.*, 1991; Veenendaal *et al.*, 1992; Brejda *et al.*, 1993).

Recent studies have, however, also demonstrated that in some grassland ecosystems, biotic factors such as plant community structure may be as important as soil parameters in regulating the species composition of VA-mycorrhizal fungal communities (Johnson *et al.*, 1992). In addition, Dhillion (1992) showed that indigenous VA-mycorrhizal fungal isolates may indeed show a preference for particular plant symbionts.

The relative importance of plant symbiont *vs.* moisture or other abiotic factors in structuring VA-mycorrhizal communities in arid ecosystems has never been examined. The pulse-reserve paradigm provides a useful framework for examining community structure and function of many desert animals and plants (Noy-Meir, 1973). Because moisture is the primary limiting factor in desert ecosystems, organism adaptation and functioning is primarily attributed to surviving the long dry periods and responding rapidly and effectively to moisture inputs. The principle aspects of applying the paradigm to a specific organism are determining the types of 'reserve' that it uses to survive extended dry periods, and how it regulates the transfer of energy from the reserve to the active pulse in response to moisture inputs.

Can the activity and structure of mycorrhizal fungal communities in arid ecosystems be effectively described as a response to the limiting moisture regime? Given that VA-mycorrhizal fungi are obligate mutualists, is mycorrhizal community structure in deserts a complex interaction of plant symbiont, moisture and other abiotic factors? An understanding of how these factors naturally affect fungal community structure and function would provide an important baseline for subsequent studies of how mycorrhizal communities are affected by natural climatic variation and various land use strategies.

In the Namib Desert, a steep rainfall gradient across the width of the central dune field is positively correlated with a sand stability gradient (Yeaton, 1988). The grasses

which occur in the region have different levels of tolerance for moisture and substrate stability stress, resulting in stratified grass communities on the dune slopes (Yeaton, 1988; Boyer, 1989). The linear dunes, with their uniformly structured plant communities and relatively simple abiotic gradients, provide an ideal natural system for determining abiotic (moisture and stability) *vs.* plant symbiont effects on fungal community structure. The purpose of this study was to investigate the limits of VA-mycorrhizal fungal distribution and the factors affecting community structure in a naturally unstable and arid grassland.

Site descriptions and methods

Site descriptions

Primary transect

A gradient, from less than 20 mm in the west to about 100 mm annual rainfall in the east, extends across the central dune field of the Namib Desert (Lancaster *et al.*, 1984). Four sampling sites (A, B, C, D) were established on the western (windward) slopes of the linear dunes that lie perpendicular to this moisture gradient (Fig. 1). Annual precipitation, sand stability, grass community complexity and plant density increase from west to east across the dune field (from site A to site D) (Yeaton, 1988; Boyer, 1989). Soil nutrient levels are low throughout the region with available phosphorus ranging from undetectable to 1·53 $\mu g \cdot g^{-1}$, and organic matter varying from 0·013–0·062% (Jacobson, 1992). Soil pH levels are fairly constant throughout the dune field (6·61–7·62) (Jacobson, 1992)

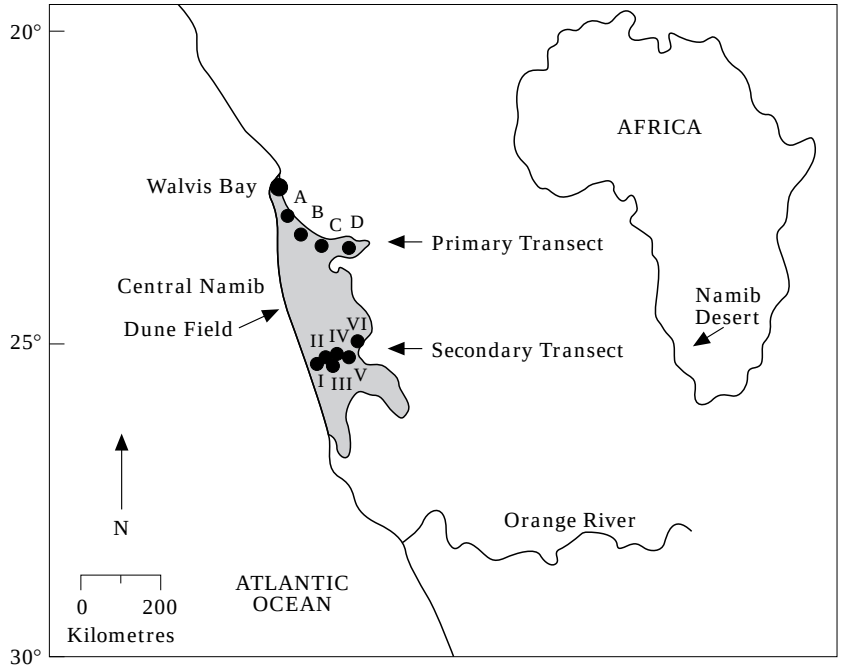

Figure 1. Location of primary and secondary transect study sites in the Central Namib Dune Field. Site A = Nara Valley; Site B = Homeb West; Site C = Bushman's Circle; Site D = Far East Dune. (Yeaton, 1988; Boyer, 1989; Jacobson, 1992).

Table 1. *Micro-gradients characterizing the primary dune Sites C and D. Compiled from Yeaton (1988), Boyer (1989) and Jacobson (1992). (See text for descriptions of the parameters)*

Parameters	Plains	Dune level		
		Base	Plinth	Top
Sand depth	Shallow	Deeper	Deep	Deep
Moisture availability	Low	Medium	High	Medium
Sand movement	Lowest	Low	High	Highest
Number of grass species	1	1–2	2–4	1–2
Annual or perennial grass	A	A or P	P	P
Grass cover density	Medium	Highest	High to low	Low

In addition to macro-gradients across the dune field, 'micro-gradients' of available soil moisture (Jacobson, 1992), and sand movement (Yeaton, 1988; Boyer, 1989), exist across the dune slopes at sites C and D (Table 1). Highly stratified grass communities exist on the dune slopes (Fig. 2), resulting from different tolerance levels of moisture and substrate stress among the grasses (Yeaton, 1988; Boyer, 1989). *Stipagrostis sabulicola* (Pilg.) De Winter has the greatest tolerance for instability and is present at the most westerly sites A and B, and the highly unstable upper regions of the eastern sites, C and D. *Stipagrostis seelyae* De Winter and *Cladoraphis spinosa* (L.f.) S.M. Phillips, have fairly high tolerance of instability, and are found in the upper regions of the eastern dune slopes. *Stipagrostis lutescens* (Nees) De Winter var. *marlothi* (Hack.) De Winter, with an intermediate tolerance of instability, is prevalent throughout the plinth regions at sites B, C and D. All of the above grasses are perennial

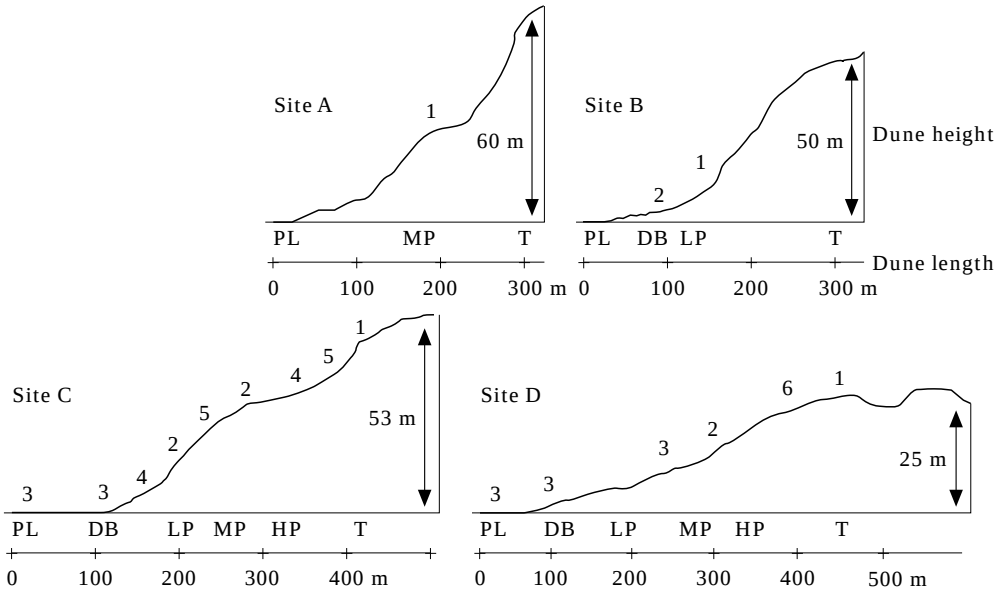

Figure 2. Grass species distribution on the dune slopes of the primary sites. 1 = *Stipragostis sabulicola*; 2 = *Stipagrostis lutescens*; 3 = *Stipagrostis ciliata*; 4 = *Centrapodia glauca*; 5 = *Stipagrostis seelyae*; 6 = *Cladoraphis spinosa*. PL = plains; DB = dune base; LP = low plinth; MP = mid plinth; HP = high plinth; T = top. For example, *Stipagrostis lutescens* was present at sites B, C and D, and at site C it ranged from the lower to mid-plinth.

and access moisture reserves stored deep in the dune for year-round growth (Yeaton, 1988).

Two other species, *Stipagrostis ciliata* (Desf.) De Winter and *Centropodia glauca* (Nees) Cope, may be perennial or annual (facultatively annual), depending on moisture availability. With only minimal rainfall (single event of more than 12 mm), these grasses will develop and set seed as annuals (Jacobson, 1992). If sufficient moisture is available, they develop perennial rooting structures which re-sprout during subsequent rainy seasons. *Stipagrostis ciliata* is limited to the most stable regions of the dune profile: the plains and base of the two eastern sites. At site D, substrate instability in the plinth region is not as great as that at site C, and the range of *S. ciliata* extends up the dune profile into the plinth region. The range of *Centrapodia glauca* is limited to the middle of the dune field (site C), but here it grows over much of the dune profile. Annual plants are abundant at the base of the dune, and perennial plants extend into the more unstable upper regions of the dune slope.

Stipagrostis seelyae, *S. sabulicola* and *S. lutescens* var. *marlothi* are endemic to the Namib dune field, whereas *Centrapodia glauca*, *Cladoraphis spinosa* and *S. ciliata* are more widely distributed in arid to semi-arid regions of southern Africa (Gibbs Russell *et al.*, 1991).

Secondary transect

A second dune transect was established in the southern part of the dune field (Fig. 1) to determine whether trends observed across the primary transect occurred elsewhere in the dune field. Six sampling sites (I–VI, from west to east) were chosen based on grass community complexity. Abiotic data were not recorded at these sites, but rainfall patterns are similar across this transect (0–150 mm). Site I was less than 5 km from the coast, and site VI was on the eastern edge of the dune field, approximately 80 km inland. Dunes in this region are short and reticulate, rather than tall and linear like those of the primary dune transect. Site VI was the only location in the secondary transect that exhibited a linear dune profile and corresponding grass community stratification. Grass species distributions across the secondary transect were similar to those of the primary transect: those species with the greatest tolerance for instability were at the western sites and at the top of the dune at site VI (*S. sabulicola, S. seelyae, Cladoraphis spinosa*); those requiring more stability were only at site VI (*S. ciliata*).

As both transects lie within the Namib-Naukluft Park, grazing is limited to occasional small, nomadic herds of oryx (*Oryx gazella gazella* L.) and springbok (*Antidorcas marsupialis* Zimmermann).

Description and sampling of abiotic parameters

Depth to wetting front (a measure of moisture availability)

For the purposes of this study, available moisture was quantified as the depth of the visibly moist layer of sand, or ‘wetting front’ (Noy-Meir, 1973). This sand has a moisture content of more than 2·0%, whereas visibly dry sand has a moisture content of less than 0·4% (Jacobson, 1992). Depth to wetting front was measured (N = 3) at 7–10 day intervals following rain events over a 3-year period at the various levels of the dune sites (unpublished data). As an example, moisture availability at the various levels of site D, 23 days following a single 12 mm rain, are illustrated in Fig. 3. For the purposes of this study it should be noted that moisture availability over time is greatest at the mid to lower plinth regions of the dune, and intermediate at the top of the dune. The plains have the lowest amount of available moisture following rains because the sand layer covering the underlying calcrete is only 15–30 cm deep.

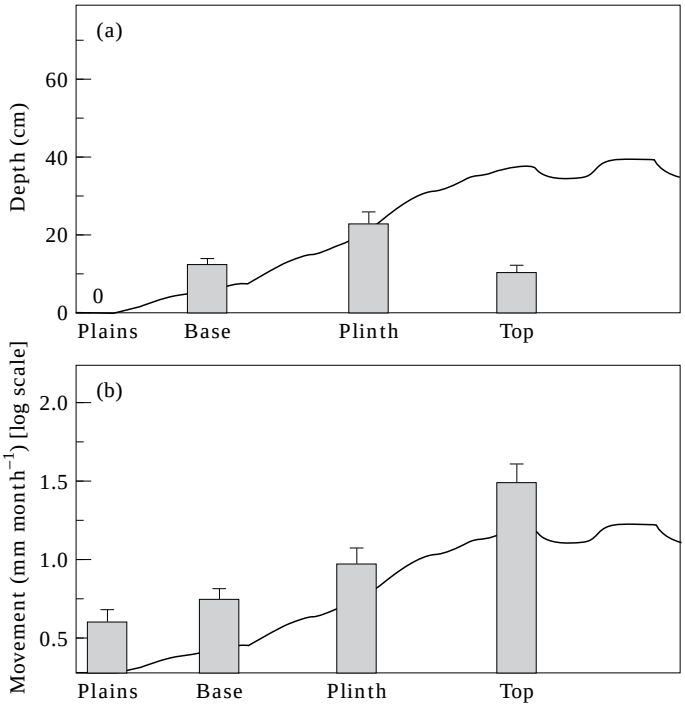

Figure 3. (a) Moisture availability as measured by the depth to wetting front 23 days after a 12 mm rainfall, and (b) sand movement (adapted from Yeaton, 1988).

Sand stability

Yeaton (1988) measured sand movement at sites A, C and D and showed that sand movement decreases from west to east across the dune field, as well as down individual dune slopes. Figure 3 illustrates the general trend of decreasing sand movement down the dune slope at Site D. This trend is even more extreme at the more westerly sites where the dune slope is steeper and there is less grass cover (Table 1, Fig. 2). For the purposes of this study, sand stability is defined as the inverse of sand movement and thus shows an increasing trend from west to east across the dune field as well as down individual dune slopes.

Sampling fungal communities

Five replicates of each grass species from various levels on the dunes at the four sites of the primary transect were taken in November 1990 and May 1991. Seasonal differences in spore diversity, abundance and mycorrhizal colonization were thus examined at the end of the dry and wet seasons, respectively. The latter sampling occurred 1 month after a single rain event at Sites C and D, recorded as 25 and 12 mm, respectively. All grass species at the five sites of the secondary transect were sampled only once, in May 1991.

Sampled plants were chosen randomly within the range of the plant and at specific levels of the dune slope. Root and sand samples were taken from a standard depth of 15–25 cm, within 20 cm of the plant, in order to minimize differences due to micro-site heterogeneity. Roots were stored in 50% ethanol and dry sand was stored in sealed plastic bags.

Spore abundance and diversity determinations across the gradients

The five sand replicates from the rhizosphere of each grass species were combined into one sample from which a single 150 cc quantity was removed to determine spore abundance and species diversity. Trends in species distribution and spore abundance across the primary and secondary transects were examined in this manner. Spores were separated from the sand by centrifugation in sucrose gradients (Daniels & Skipper, 1982). Whole and squashed spore mounts were characterized by size, colour, wall layer thickness and composition, and hyphal attachment. Permanent slides and a photographic record aided comparisons of spores from different samples. Species identifications were attempted according to Schenk & Perez (1990).

Counts were made of total spore abundance, number of species, and relative abundance of each species. Because the samples were 'collections' rather than random samples, Brillouin's Index was used to calculate species diversity for each sample (Krebs, 1989).

Similarities between fungal communities within the primary transect were examined via cluster analysis using the multivariate statistical package, NTSYS (Rohlf, 1988). Relative abundances of each spore type were used as characters. Similarity matrices, generated using the product-moment correlation coefficient, were used to construct phenograms for the 1990 and 1991 sampling, using the unweighted pair group method analysis (UPGMA).

Pearson's correlation coefficient was used to determine whether there was a relationship between spore abundance and mycorrhizal colonization levels, and multiple regression analysis was used to determine the effects of sand stability and moisture availability on spore abundance. All statistical tests were performed with NCSS Software Version 5·03 (Hintze, 1992).

Percent mycorrhizal colonization

Roots were stained using the trypan blue method of Koske & Gemma (1989). Percent mycorrhizal colonization of each replicate was determined by averaging 40 randomly chosen 1 cm root segments (systematic slide method discussed by Kormanik & McGraw (1982) and Giovannetti & Mosse (1980)). The five replicate means were then averaged to obtain a colonization level for a particular grass sample. Percentage data were arc-sine transformed prior to statistical analysis (Zar, 1984). Seasonal differences in mycorrhizal colonization levels were examined using individual *t*-tests for each set of samples from a particular grass. One-way ANOVAs were used to determine whether there were significant differences between the colonization levels of the different grasses within both the primary and secondary transects. Duncan's Multiple Range Test was subsequently used to determine which samples were significantly different. Regression analysis was used to determine the effects of moisture availability and sand stability on mycorrhizal colonization levels.

Fungal growth and phenology

The response of fungal growth and phenology to variable moisture availability was subsequently examined by serial sampling of *S. ciliata* seedling roots following rain events previously described at sites C and D. Percent mycorrhizal colonization of germinating seedlings at the various dune levels were determined at 7–10-day intervals until the plants set seed or wilted. Spore germination and sporulation in the rhizosphere was also noted in each sample.

Results

Species diversity and abundance

Twelve VA-mycorrhizal fungal species were distinguished in the primary dune transect, and nine of these were also found in the secondary transect. Across both transects, five *Glomus* species accounted for 95–97% of the spore abundance. All *Glomus* spores were small, ranging in size from 20–110 μm. While the *Glomus* species were easily distinguished from one another, identification from the literature at hand was not successful. Species determinations will therefore be reported in a subsequent publication following taxonomic work-up.

Primary transect

There was little variation in total spore abundance between the two sampling periods, despite some differences in fungal species richness (Table 2). The greatest variation in both parameters was seen at the upper dune levels at Sites C and D, where spores were less abundant than at the lower dune levels. At the higher levels, seasonal differences in diversity, as determined by Brillouin's Index, therefore resulted from differences in species richness and abundance. In contrast, seasonal differences in fungal community diversity at the lower levels (i.e. Site C: *Stipagrostis ciliata* from the plains, *Centrapodia glauca* from the base) were due to differences in species richness, but not spore abundance.

Fungal community diversity and spore abundance were lower at Sites A and B than at Sites C and D (Table 2). At Sites C and D, spore abundance and species diversity varied within the sites. Community diversity was greatest in the rhizospheres where spores were most abundant, namely at the lower to mid-dune levels of Sites C and D.

Cluster analyses for the 1990 and 1991 samplings (Fig. 4), based on relative species abundance, did not reveal any distinctive patterns of association between fungal community diversity and grass species, dune level or site. While fungal communities associated with *Stipagrostis sabulicola* were highly similar, this was a reflection of much lower spore abundance and diversity than was associated with the other grasses (Table 2). The correlation coefficients for the clusters suggest a good fit of the phenogram to the data: 0·81 and 0·84 for the 1990 and 1991 samplings, respectively.

While fungal diversity varied throughout the dune field, total spore abundance showed an increasing trend from Site A to D (Table 2). Within Sites C and D, spore abundance was greatest at the lower dune levels, and decreased up the dune slope. There was no significant correlation between mycorrhizal colonization levels and spore abundance (r^2 = 0·4455, p = 0·0639). Rather, regression analysis showed that the model which best explained the variation in spore abundance used both moisture availability and sand stability as independent variables (Table 3). A model using sand stability alone also provided a highly significant model for predicting spore abundance, with only a slightly smaller r^2 (0·7447 *vs.* 0·7763). Sand stability is thus clearly the primary determinant of spore abundance in the dune field, with only a minor contribution from moisture availability.

Secondary transect

VA-mycorrhizal fungal community diversity increased across the secondary dune transect from west to east, as a result of increasing species richness and abundance (Table 4). Diversity was lowest in communities at the western sites and also at the top of the eastern site VI. The highest level of species diversity and abundance were observed at the lowest, most stable dune level at Site VI. These results corroborate the

Table 2. *Percent mycorrhizal colonization of roots; diversity (Brillouin's Index); abundance; and species richness of VA-mycorrhizal fungal spores associated with grasses from different dune levels in the primary transect (N=5; numbers in parentheses are standard errors of the mean)*

	Percent colonization		Diversity		Abundance		Species richness	
	1990	1991	1990	1991	1990	1991	1990	1991
Site A								
S. sabulicola	1 (1)	1 (1)	0·05	0·05	2	2	2	2
Site B								
S. lutescens	–	1 (1)	–	1·77	–	32	–	4
S. sabulicola	–	3 (2)	–	0·00	–	2	–	1
Site C								
Plains								
*S. ciliata**	24 (6)	25 (2)	2·25	1·54	194	174	5	3
Base								
*S. ciliata**	23 (3)	21 (9)	2·92	2·53	331	361	8	6
Base								
*C. glauca**	35 (7)	30 (3)	1·54	2·49	181	191	3	6
Lower plinth								
S. lutescens	55 (3)	69 (7)	2·51	1·95	266	254	6	4
Mid plinth								
S. lutescens	27 (7)	33 (9)	2·19	2·19	94	96	5	5
Mid plinth								
S. seelyae	13 (6)	10 (4)	1·20	1·44	10	40	3	3
Top								
C. glauca	3 (3)	1 (1)	1·94	0·65	23	4	5	2
Top								
S. seelyae	4 (4)	4 (3)	1·54	0·93	8	44	5	2
Top								
S. sabulicola	6 (6)	5 (3)	0·00	0·72	3	6	1	2
Site D								
Plains								
*S. ciliata***	24 (4)	29 (3)	2·53	2·75	336	404	6	7
Base								
S. ciliata	74 (3)	81 (3)	2·76	2·76	535	524	7	7
Mid plinth								
S. ciliata	63 (4)	73 (5)	2·23	2·47	144	140	5	6
Mid plinth								
S. lutescens	48 (7)	52 (12)	2·24	2·70	167	198	5	7
Top								
C. spinosa	3 (3)	5 (4)	0·00	1·32	3	17	1	3
Top								
S. sabulicola	2 (1)	4 (4)	0·00	0·00	1	3	1	1

*Annual grasses sampled. All other grasses were perennial.

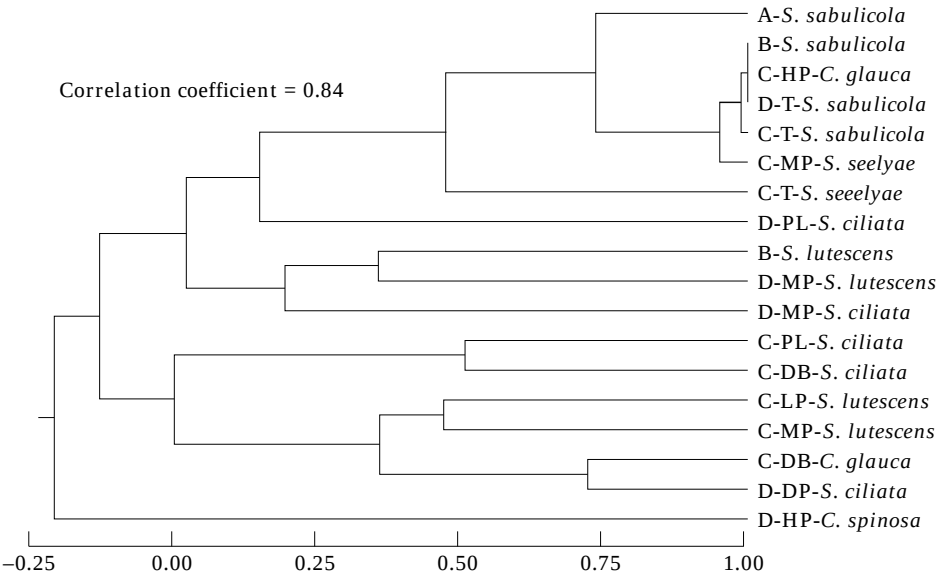

Figure 4. Phenogram illustrating similarities between VA-mycorrhizal communities of the primary transect, based on relative species abundance in the May 1991 samples.

trends observed in the primary transect of increasing spore abundance and diversity with increasing sand stability.

Percent mycorrhizal colonization of roots

Preliminary observations of the grass roots showed dematiacious and un-pigmented non-VA mycorrhizal hyphae, in addition to VA-mycorrhizal hyphae. VA-mycorrhizal hyphae ranged from 2–15 μm in width. Non-VA mycorrhizal hyphae ranged from 2–5 μm in width and were not easily differentiated from narrow VA-mycorrhizal hyphae at 80 × magnification. Closer examination showed that the non-VA mycorrhizal hyphae were regularly septate and produced thick-walled chlamydospores in roots, distinct from VA-mycorrhizal structures. To accurately differentiate mycorrhizal from non-mycorrhizal colonization, the systematic slide method was preferred to the gridline-intersect method (Giovannetti & Mosse, 1980).

All grass species were colonized by VA-mycorrhizal fungi. Hyphae and vesicles were seen most frequently. Arbuscules were seen only in *Stipagrostis lutescens* at Sites C and D during the November sampling but were seen in all plants during the May sampling.

Table 3. *Multiple regression analysis of the effects of sand stability and moisture availability on mycorrhizal spore abundance in the rhizosphere of grasses in the primary dune transect (*N=*18)*

Model (v = spore abundance)	F	r^2	p
v=408 (stability) –213·76	46·68	0·7447	0·001
v=19·36 (moisture availability) +85	0·27	0·0165	0·611
v=26·80 (moisture availability) +413·58 (stability) –287·82	26·02	0·7763	0·001

Table 4. *Percent mycorrhizal colonization of roots; diversity (Brillouin's Index); abundance; and species richness of VA-mycorrhizal fungal spores associated with grasses from the secondary transect. (*N=*5, numbers in parentheses are standard errors of the mean)*

	Percent colonization	Diversity	Abundance	Species richness
Site I				
Stipagrostis sabulicola	0 (0·2)	0	2	1
Site II				
Stipagrostis sabulicola	0	0	2	1
Cladoraphis spinosa	0 (0·4)	0	5	1
Site III				
Stipagrostis sabulicola	0 (0·4)	0·66	5	2
Cladoraphis spinosa	0 (0·2)	0	0	0
Stipagrostis seelyae	9 (5·8)	0·88	20	2
Site IV				
Cladoraphis spinosa	9 (6·0)	1·72	25	4
Stipagrostis seelyae	6 (3·5)	1·38	24	3
Stipagrostis lutescens	11 (5·3)	1·36	21	3
Centrapodia glauca	18 (7·6)	2·16	77	5
Site V				
Stipagrostis lutescens	23 (9·1)	2·42	89	6
Site VI				
Top *Stipagrostis sabulicola*	2 (1·4)	0·83	13	2
Top *Cladoraphis spinosa*	8 (4·6)	1·44	38	3
Upper plinth *Centrapodia glauca*	12 (3·2)	2·21	117	5
Upper plinth *Stipagrostis ciliata*	21 (6·1)	2·49	194	6
Lower plinth *Stipagrostis ciliata*	55 (6·0)	2·94	461	8

Non-VA mycorrhizal hyphae were also found in all grass species throughout the dune field.

Primary transect

t-tests showed that percent colonization of the grass roots sampled in November 1990 and May 1991 from the primary transect differed significantly only for grasses with high levels of colonization (60–80%) from the lower plinth and base levels of Sites C and D: *Stipagrostis lutescens* and *Stipagrostis ciliata*, respectively. In each case, percent colonization was significantly higher in May 1991, following the rains, than in November 1990, during the dry period. None of the other grasses showed significant seasonal differences in percent colonization.

During each of the sampling periods, there was significant inter- and intra-site variation in mycorrhizal colonization levels. Duncan's Multiple Range Test showed that there were three significantly different mycorrhizal classes: 0–15%, 15–60% and 60–80% (Table 2). These classes were not plant species specific. Rather, where the plants were sampled at each of the sites and within the dune field determined the mycorrhizal colonization levels. Perennial grasses of the most unstable regions had uniformly low or no mycorrhizal colonization (*Stipagrostis sabulicola* and *Cladoraphis spinosa*). All of the other grasses were consistently mycorrhizal but had varying levels of colonization depending on their positions on the dune. Perennial grasses which occupied the lower to upper dune at Sites C and D had highest colonization at the lower dune levels, and lower colonization higher up the dune slope (e.g. *Stipagrostis lutescens, Centrapodia glauca, Stipagrostis seelyae*).

Mycorrhizal colonization levels of *Stipagrostis ciliata* differed significantly between perennials and annuals from different levels of the dune slope. Colonization values were intermediate (20–30%) for plains and base annuals, and high for perennial plants from the mid plinth and base levels of Site D (74–83%) (Table 2).

Multiple regression analysis showed that using moisture availability as the sole independent variable resulted in a highly significant model for explaining the variation in mycorrhizal colonization levels (r^2 = 0·4678, p = 0·002) (Table 5). Sand stability alone did not significantly affect mycorrhizal colonization (r^2 = 0·1366, p = 0·131). Rather, the model with the highest r^2 was that which included both moisture and stability as independent variables (r^2 = 0·6349, p = 0·001). The differences in mycorrhizal colonization of grass roots at different dune levels can thus be significantly explained by moisture availability, but accounting for the effects of sand stability does improve the accuracy of the model. Because sand stability is the primary determinant of spore abundance (see above), the effects of sand stability on colonization levels can be explained as the effect on inoculum levels available for active colonization under appropriate moisture conditions.

Table 5. *Multiple regression analysis of the effects of sand stability and moisture availability on percent mycorrhizal colonization levels of grasses in the primary dune transect (*N=*18)*

Model (v=% mycorrhizal colonization)	F	r^2	p
v=25·85 (stability)+1·08	2·53	0·1366	0·131
v=15·21 (moisture availability)–16·57	14·06	0·4678	0·002
v=15·73 (moisture availability) +28·65 (stability)–42·39	13·04	0·6349	0·001

Secondary dune transect

There was significant inter- and intra-site variation in colonization for grasses from the secondary dune transect (Table 4). Duncan's Multiple Range Test showed that the percent colonization of *Stipagrostis ciliata* from the lower plinth at site VI was significantly different from those of all of the other samples taken. While these other samples were not significantly different from one another, a trend of increasing VA-mycorrhizal colonization across the rainfall gradient corroborates that of the primary dune transect.

VA-mycorrhizal fungal and seedling phenology

Seedling response to rainfall

Four days following rain, germinating *Stipagrostis ciliata* seedlings were visible at the sand surface of the plains, base and plinth levels of D, and at plains and base levels of Site C (Table 6). At Site D, 12 mm was insufficient for grass life cycle completion on the plains, as all seedlings wilted by day 23 without setting seed. However, because of greater moisture availability on the dune, 12 mm was sufficient for seed production at the base and plinth levels of Site D. At the dune base, the wetting front was barely detectable by 23 days when seed production was observed, and similarly at the plinth level, seed set at 31 days was associated with low moisture availability. At Site C, higher rainfall was associated with longer periods of vegetative growth prior to seed set, than at Site D. Plants set seed and were senescing by 31 days on the plains and 45 days at the dune base. *Stipagrostis ciliata* thus showed an opportunistic growth pattern; germinating rapidly in response to variable but low rainfall amounts, and continuing to grow vegetatively as long as stored moisture was available. Seed set was associated with declining moisture availability at all levels except the plains at Site D where 12 mm was insufficient for seed production.

VA-mycorrhizal fungal response to rainfall

Newly germinated seedlings were not mycorrhizal at 4 days, but showed uniformly low levels of colonization at all levels by 7 days (ANOVA, $p = 0{\cdot}1$) (Table 6). Mycorrhizal colonization levels continued to increase as long as the wetting front was detectable.

Table 6. *VA-mycorrhizal fungal and* Stipagrostis ciliata *phenology in response to rain at Sites D and C. Root samples and plant observations were taken on days 4,7,15,23,31 and 45 following the rain event (*N = *5; numbers in parentheses are standard errors of the mean)*

	1	2	3	4	5	6	7	8
Site D (12 mm)								
Plains	4	None	23	7	7(3)	15	12(3)	None
Base	4	23	31	7	5(1)	23	28(2)	None
Plinth	4	31	45	7	11(3)	31	49(2)	31
Site C (24 mm)								
Plains	4	31	45	7	6(5)	31	36(7)	31
Base	4	45	>45	7	6(2)	31	38(4)	45

(1) First day after rains when germinating seedlings were visible; (2) First day when seed set was observed; (3) First day when the wetting front was no longer detectable; (4) First day when colonization of roots was observed; (5) Initial percent colonization observed at 7 days; (6) First day that maximum colonization was observed; (7) Maximum percent mycorrhizal colonization levels achieved; (8) First day that sporulation was observed.

The highest colonization levels were observed when moisture was still barely detectable. The mycorrhizal colonization levels achieved depended on the persistence of the wetting front: plains and base level seedlings at Site D had significantly lower colonization than the other three sites where moisture persisted in the sand for at least 31 days (p = 0·002). The first observations of spore production corresponded with seed production by the plants. Twelve millimeters and 24 mm was sufficient for spore production at all levels where moisture persisted for at least 31 days. Spores were not produced on the plains and base at Site D where moisture was not detectable at 23 and 31 days, respectively, and seedlings were wilting. As with the seedlings, the mycorrhizal fungi showed an opportunistic growth pattern in response to low and variable rainfall amounts. The fungi continued to grow vegetatively as long as the moist layer persisted, and spore production was associated with declining moisture availability.

Discussion

The VA-mycorrhizal fungal communities of the arid Namib grasslands were composed of 12 species, of which five small spored *Glomus* species predominated. No plant symbiont, site, or dune level specificity was observed, but in both transects there was a general trend of increasing fungal diversity with increasing rainfall and stability. Other studies conducted by the author suggest that the five predominant fungal species of the central Namib dune field may be cosmopolitan with *Stipagrostis, Cladoraphis, Eragrostis, Enneapogon*, *Rhynchelytrum* and *Anthephora* grasses in other arid and semi-arid regions of southern Africa: Namaqualand, the Kalahari Desert, and the northern Namib dune field (Jacobson *et al.*, 1993; unpublished data). Four common species found in the Namib grasslands were also associated with *Welwitschia mirabilis* Hook. F. (Gnetales) on the central Namib gravel plains and in northern Namibia in the mopane-savanna (Jacobson *et al.*, 1993).

Substrate stability and moisture availability prescribed the limits of tolerance for VA-mycorrhizal fungal communities in this arid grassland. Mycorrhizal fungi are obligately dependent on the plant symbiont for carbohydrates, and therefore the western distribution of fungal communities was limited by the tolerance of plants to the diminishing rainfall regimes across the east–west transect.

Fungal communities were not found with all plants, however. Low sand stability restricted the establishment of mycorrhizal associations with grass species at the western sites in both transects, as well as the upper dune slopes at the eastern sites. Plant roots in this region are constantly exposed and buried during the dry season, resulting in low retention of any spores produced in the rhizosphere. Spore abundance at these sites was very low, but occasionally plants with mycorrhizal colonization were found, suggesting that establishment of the association was random, and limited by the availability of inoculum in the presence of actively growing roots.

Seely (1991) proposed that in dune deserts the primary factors affecting biotic activity and ecosystem function are abiotic parameters. She argued that while biotic interactions such as competition, predation and mutualism do occur, ‘they are relatively unimportant in establishing community patterns’. This study suggests that even the community composition and functioning of obligate mutualists, such as VA-mycorrhizal fungi, may be primarily determined by abiotic conditions. In this arid grassland of the Namib, substrate stability and moisture availability not only determined the absolute limits of tolerance of the mycorrhizal flora, but were also the primary factors which significantly explained the distribution and functioning of the established fungal communities.

Stepwise regression analysis showed that spore abundance associated with plant roots was significantly affected by substrate stability. Spore abundance increased down the dune slope with increasing stability. In contrast to spore abundance, mycorrhizal colonization of roots was primarily affected by moisture availability. Assuming the

presence of adequate fungal inoculum (thus excluding the upper dune levels and the western dune sites), the levels of colonization achieved with the different grass species was determined by the duration of adequate moisture availability for plant and fungal growth. Fungi growing in association with annual plants in regions of low moisture availability had short growth periods, and intermediate colonization levels (e.g. annual plants sampled on the plains at Sites C and D). In contrast, isolates of the same fungal species growing with perennial plants that had access to deep moisture continued growing throughout the dry season. As a result, mycorrhizal colonization levels of perennial plants at the mid to lower plinth at Site C and D were the highest. The presence of arbuscules (structures involved in the active transfer of nutrients between plant and fungus (Hirrel *et al.*, 1978)) in *Stipagrostis lutescens* roots from the dry season sampling suggests that fungi associated with the roots of these plants also continue to grow throughout the dry season.

The seedling phenology study further demonstrated that moisture availability not only determines the level of mycorrhizal colonization achieved, but is also the primary factor controlling mycorrhizal functioning. Fungal growth with *Stipagrostis ciliata* seedlings following single rain events at two of the dune sites continued as long as moisture was available and, thereafter, fungal sporulation was associated with declining moisture availability. This flexible phenology provides further evidence that moisture is indeed the primary factor controlling VA-mycorrhizal community functioning in the dunes of the Namib Desert.

While the present study clearly establishes moisture as the primary factor affecting fungal phenology, it is not clear whether this effect is controlled by the fungus or mediated by the plant symbiont. The phenology study revealed a close synchronization of symbiont germination, growth and reproduction, which suggests that either the symbionts are independently cued by similar moisture conditions, or that as a result of the symbiosis, their phenologies are indeed linked. VA-mycorrhizal fungi and grasses in a semi-arid grassland in Botswana showed a similar synchronization which was attributed to the response of the plant symbiont to moisture availability (Veenendaal, 1991; Veenendaal *et al.*, 1992). In contrast, the phenologies of VA-mycorrhizal fungi associated with *Atriplex gardneri* in a cold desert in Wyoming (Allen, 1983) and various plants in the Sonoran Desert (Bloss, 1985) were attributed to seasonal temperature and moisture regimes. In the present study, fungal reproduction did not occur when moisture was minimal but still sufficient for plant reproduction (at Site D: dune base). This suggests that the plant symbiont may control the flow of carbohydrates to the fungal symbiont, thus ensuring plant reproduction. Ongoing studies in the Namib grasslands are examining the synchronization of plant and fungal life cycles under different rainfall regimes more closely.

In conclusion, VA-mycorrhizal fungal communities are an integral part of the dune ecosystem of the Namib desert. Despite their obligate mutualistic association with grasses, community distribution and function, like that of other desert organisms, is primarily affected by abiotic factors. The pulse-reserve paradigm (Noy-Meir, 1973) provides a useful framework for explaining VA-mycorrhizal fungal community structure and functioning in this arid ecosystem. VA-mycorrhizal fungi in the Namib dune desert have a flexible phenology cued by moisture that allows effective utilization of perennial and annual plant symbionts, a broad non-specific plant association so that carbohydrate nutrition is not a limiting factor, as well as spores that serve as effective reserve structures in an arid unstable environment.

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