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Organic amendment increases arbuscular mycorrhizal fungal diversity in primary coastal dunes

Andrew Willis[1,2], Tim H. Sparks[3], Bernard Felinov Rodrigues[2], Phillip John Charles Harris[1]

[1] *Centre for Agroecology, Water and Resilience, Coventry University, CV1 5FB, UK*
Corresponding author, E-mail: andyewillis@gmail.com

[2] *Botany Dept., Goa University, Taleigao Plateau 403 206, India*

[3] *Faculty of Engineering, Environment and Computing, Coventry University, CV1 5FB, UK*

ABSTRACT

Plastic pots were inserted beneath seedlings of a shallow-rooted C_4 grass species, *Ischaemum indicum*, with and without a root-impenetrable nylon sachet filled with organic matter (OM) amendment, at seven stations along an interrupted belt transect in which plant community and soil chemistry had been previously surveyed. The transect was perpendicular to mean high-water mark (MH-WM) across a primary coastal dune system in Goa, India, where summer monsoon is the predominant weather feature. The Quadrat survey of plant frequency was made in stations when the above-ground biomass was estimated to be highest. Arbuscular mycorrhiza fungal (AMF) spore density and diversity were determined morphologically in amended and control pots soils, and in OM sachet residues, after host-plant desiccation when monsoon rains had ceased. Twenty-seven AM fungal spore morphotypes were isolated from the pots containing OM amended rhizosphere soils, 19 from controls and 14 from OM residues in the sachets. *Gigaspora margarita* proved to be the dominant spore in all treatments. Eight morphotypes recovered from amended pots were not recovered from the controls. There was an increasing trend in species diversity in amended pots away from MH-WM. Spore recovery from the three regimes showed variable distribution that indicated differing AMF species strategies.

KEYWORDS

Glomeromycota spores, Interrupted-belt transect, *Ischaemum indicum*, Simpson's diversity, vermicompost amendment, West-coast India

INTRODUCTION

A seven-station (St.) interrupted-belt transect had been previously established across an aggrading primary dune system on the west coast of India before the onset of 2010 summer monsoon (Willis et al. 2016a). A plant-community zonation pattern that had been visually identified was confirmed by the restricted randomization quadrat survey method (Greig-Smith 1983). Spatial and temporal soil chemistry data (9 variables) had also been examined. A decreasing pH gradient away from the MH-WM was revealed. Canonical correspondence analysis (CCA) had shown that there was no pattern in soil chemistry matching plant zonation. One of the grass species recorded, namely the shallow-rooted perennial *Ischaemum indicum* (Houtt.) Merr., was ubiquitous to all stations in varying densities. In this dry-tropical environment, with rains for half the year (June to November) and no rain, intense radiation and soil heat through the other half (Varkey 2007), *I. indicum* displays considerable plasticity by adopting an ephemeral strategy, propagating exclusively by seed before foliar and root desiccation occur. It was considered that the specimen might offer a uniform component of further investigation along the transect.

Coastal primary dunes are hostile environments where most plants species rely upon symbiosis with arbuscular mycorrhizal fungi (AMF) that belong to the phylum Glomeromycota. The fungus' soil- and root-inhabiting mycelium is adept in scavenging phosphorus (P) and nitrogen (N) in deficient soils, and in facilitating nutrient uptake in exchange for carbon (C) (Smith & Read 2008). Various attributes and ecological functions of AMF are reviewed by Willis et al. (2013). In less harsh environments, AM fungal mycelium may be active throughout the year. In contrast, shallow roots and integral AMF hyphae that are potential inoculum in the following monsoon are entirely desiccated during the dry season. Surface soil temperatures exceed 40° C, and soil moisture is evaporated to >0.5

depth (unpublished). Glomeromycota spores, however, can tolerate hot and arid ecosystems (Picone 2000, Tao & Zhiwei 2005) and may thus represent the mycorrhizal inoculum potential (MIP) of the dunes' inhabitant AM fungal community. It is not inconceivable that AMF could also adopt an 'ephemeral' life strategy, from spore to spore. There is concern, however, about spore counts denoting AM fungal population biology accurately and consistently (Kowalchuk et al. 2002). For example, the fundamental differences of sporulation rate in taxa have been reported, as has variation in AM fungal species temporal and spatial sporulation patterns (Bever et al. 1996, Pringle & Bever 2002, Rosendahl & Stukenbrock 2004). Nevertheless, evidence of similarity in AMF species diversity assessed by molecular analysis compared with morphological identification of spores in field-extracted rhizosphere soils was presented by Clapp et al. (1995) and Helgason et al. (1999). Furthermore, as Sieverding & Oehl (2005) pointed out, 'the spore number of individual AMF species certainly gives an indication on the fitness of the species'. In the present study, emphasis is placed on the premise that, in response to the hot and dry environmental conditions encountered after monsoon in the dune system, viable AM fungal biomass in shallow rhizosphere soils would be almost entirely invested in the resilient spores.

The 2010 survey had shown organic matter (OM) to be the principal plant growth limiting factor (Sprengel-Liebig Law of the minimum: see van der Ploeg et al. 1999) over the transect (<0.05% - 1.5%). Nevertheless OM, along with silt/clay fraction, may have been the majority of available soil nutrient resource in the system (Maun 2009). AMF facilitate the nutrient uptake from available resource, the symbionts' mycelium preferentially associating with decomposing OM (St. John et al. 1983, Cavagnaro et al. 2005, Hodge et al. 2010). It is hypothesized that amendment with OM that is made available only to AMF hyphae, in each of the stations along a transect across a primary dune system will alter AM fungal sporulation dynamics. The greater the mineral nutrient availability, the greater will be spore density, the wider will be fungal species diversity. To test the hypothesis, an experiment was designed where *I. indicum* seedlings were transferred to plastic pots, in the field, early during the rainy season, without and with OM amendment confined in a membrane that was accessible to AMF hyphae but not to roots.

1. MATERIALS AND METHODS

At the onset of 2012 monsoon, 25 µm nylon-membrane sachets (mean capacity 13.4 mL, n = 21, 5.58% of pot volume) were prepared in the laboratory. Each was filled to capacity with OM, a mature worm-composted cattle manure (vermicompost) that had been stored for more than 3 years, rich in readily available mineral nutrients (Domínguez & Edwards 2004). The material was not sterilized as it was thought unlikely that the AMF propagules would have entered the bovine digestive system, and if by chance they had entered, they would not have survived the ruminant gut followed by epigeal worm consumption during the ensuing vermicomposting process. A sample was examined for chemical characteristics. Analyses followed the procedures laid out by Singh et al. (2005). The pH was measured in soil-water suspension 1:2 ratio (Elico L1 120 pH meter, ELICO Ltd., Hyderabad, India), EC from the clear extract after pH measurement (Elico CM 180 conductivity meter), organic carbon (OC) by the Walkley & Black (1934) method, available P_2O_5 by Brays method (Bray & Kurtz 1945) using Bray's No. 1 solution, potassium (K_2O) and Na by the ammonium acetate method from Hanway & Heidel (1952), and magnesium (Mg) and calcium (Ca) by a modified ammonium acetate method from Barvah & Barthakur (1997) (Elico flame photometer Unit 21). Small (60 mm deep, 75 mm diam., 240 mL capacity) black plastic pots were selected for the experiment, a capacity adequate for unrestricted root growth during the monsoon and a shallow depth maximizing solar effects in the hot, dry months, desiccating plant foliage and roots, and AMF mycelium, encouraging AMF sporulation. Holes (10 mm diam.) were drilled through the lower side (4) and base (3) of all pots to simulate the drainage capacity of the surrounding soils. Filled sachets were inserted into three of six pots buried beneath developing *I. indicum* seedlings in each of the seven transect stations (see Figure 2 in Willis et al. 2016a), 16 days after the onset of the monsoon. The seedlings selected at each station, based on visual assessment of vigour (up to second primary-leaf stage), were carefully transferred to the pots on site, along with their respective rhizosphere soils. Selected samples were unevenly dispersed over station areas and the pots were inserted into the ground to rim level.

The pots were removed after 131 days, 23 days after the monsoon rains had ceased. Some were unrecovered, one control pot from each of St. 5 and St. 7 (anthropogenic disturbance), and two OM amended pots from St. 1 on the front face of the dunes that had been buried by storm-surge sand. One vermicompost sachet from a St. 3 replicate had been invaded by roots. Three 50 g soil samples from each of the OM amended pots were examined for spore abundance and species level diversity by wet-sieve and decant method (Gerdemann & Nicolson 1963). Only fresh, clean spores were collected from filter papers using fine tweezers (Du Mont, Switzerland) that allowed a test of structural integrity by applying slight pressure. Spores with wall elasticity and intact phospholipid content were considered viable. Each station's control pots soils were thoroughly homogenized and one 50 g sample was examined for reference against the amended pots data. Vermicompost sachet residues from each station's amended pots were combined and thoroughly wetted in a glass Petri dish and carefully picked through with the tweezers, as previous experience (unpublished) had shown that full spore recovery might not be accomplished using the sieve and decant method. The scrutiny of the residues elicited no viable AMF hyphae. The stereo-microscope used for propagule isolation was an Olympus SZ 61, 10 x 4.5 (Tokyo, Japan). Spores were identified by comparing them to the original descriptions in Morton & Benny (1990), Schenck & Pérez (1990), Morton, J.B. (2002) and Schüßler & Walker (2010). Plant

frequency was assessed by restricted randomization quadrat method (Greig-Smith 1983), n = 5 St.$^{-1}$ on 1.10.2012 (89 days after onset of monsoon) when above-ground plant biomass was maximal and before culm development. A 600 mm × 600 mm quadrat was sub-divided into 100 mm squares, and the diversity and abundance of plant species rooted within each of the 36 inner squares were recorded. Fungal species diversity was assessed by Simpson's index of diversity (1-D). Reference to correlation is Pearson's coefficient except where Spearman's rank order correlation was applied to assess Simpson's AM species diversity association with increasing stations distances from MH-WM, in amended and control pots. The confidence interval was 95%. ANOVAs testing plant frequency similarity in the two monsoon seasons, and differences between distributions of St. 2-7 amended pots AMF spores (excluding St. 1, where two of the three amended pots were unrecovered) were conducted in Minitab 16 (Minitab Inc., State College, Pennsylvania, USA). All passed the Levene's test for homogeneity of variance prior to ANOVA. The micrographs were imaged by an Olympus (Tokyo, Japan) U-CMAD 3 DP12 digital camera mounted on an Olympus (Tokyo, Japan) BX 41 compound microscope, the specimens mounted in polyvinyl alcohol-lactic acid-glycerol (PVLG).

2. RESULTS

The rooted frequency (Table 1) of *I. indicum* (2012) was significantly correlated (r = 0.927; *P* = 0.003) with that recorded in the 2010 survey. One-way ANOVA indicated there was no significant difference ($F_{(6,7)}$ = 13.38; *P* = 0.002) between distributions over the transect. The establishments in mobile foredune sands (St. 1) were correspondingly tenuous; the frequency in St. 7 was again 100%. Chemical analysis of vermicompost prior to the amendment indicated pH 6.0, EC 1.1 dS m^{-1}, OC 3.47%, P_2O_5 46 µg g^{-1}, K_2O 40 µg g^{-1}, and high concentrations of Mg (1440 µg g^{-1}) and Ca (3500 µg g^{-1}). Sodium concentration (75 µg g^{-1}) was above the mean soil concentrations indicated in the 2010 survey (59.5 µg g^{-1}; range 50 – 75 µg g^{-1}).

Table 1. Quadrat (n = 5 St.$^{-1}$) survey survey of Ischaemum indicum distribution in transect stations at maximal above-ground biomass, comparing two monsoon seasons.

St.	m from MH-WM	Rooted frequency (%) 2010	SD	Rooted frequency (%) 2012	SD
1	5	8.8	-	27.5	24.1
2	20	61.3	4.7	52.5	12.8
3	35	71.3	5.8	83.5	11.1
4	65	68.8	15.0	90.0	2.9
5	101	33.8	16.5	53.8	24.6
6	135	75.0	14.8	87.5	7.5
7	175	100	-	100	-

The spores of 27 AM fungi in seven families, Acaulosporaceae, Dendiscutataceae, Gigasporaceae, Glomeraceae, Racocetraceae, Scutellosporaceae, and a novel species in the Sacculosporaceae, were recovered from the OM amended rhizosphere soils. These included 19 from control, and 14 from residual vermicompost sachets. In all, the total number of AM fungal spore morphotypes recovered was 28. Eight spore morphotypes were recovered only from the OM amended pots soils. One unidentified spore morphotype was recovered from St. 5 residual vermicompost, suggesting that there may have been AM fungal propagules present in at least one of the vermicompost sachets before the addition to the pots. However, the spore was not detected in any other of the amended or control pots soils, nor in any other station vermicompost residue. In the amended pots (Figure 1), *Acaulospora spinosa* (Plate 1) accounted for 20.0% of the total (4640) spores recovered, *A. scrobiculata* (Plate 2) 20.8% (together 82.7% of all *Acaulospora* species), *Gigaspora margarita* (Plate 3) 23.4%, *Racocetra gregaria* (Plate 4) 15.2%, *Sacculospora felinovii* sp. nov. (Willis et al. 2016b) (Plate 5) 4.4% and *Claroideoglomus claroideum* (Plate 6) 1.2%. These six species together represented 85.0% of the total AM fungal spores recovered from the amended pots. One-way ANOVA ($F_{(5,102)}$ = 7.72; *P* = <0.001) indicated the distributions of the six species in stations over the transect (Figure 2) were significantly different. Of the remaining morphotypes, *Acaulospora* (11 spp.) accounted for 10% of the total spore numbers, followed by *Gigaspora* (2 spp.), *Dentistucata* (2 spp.) and *Scutellospora* (1 sp.) together 4.2%, *Glomus* (5 spp.) <1%, and *Rhizophagus* (1 sp.).

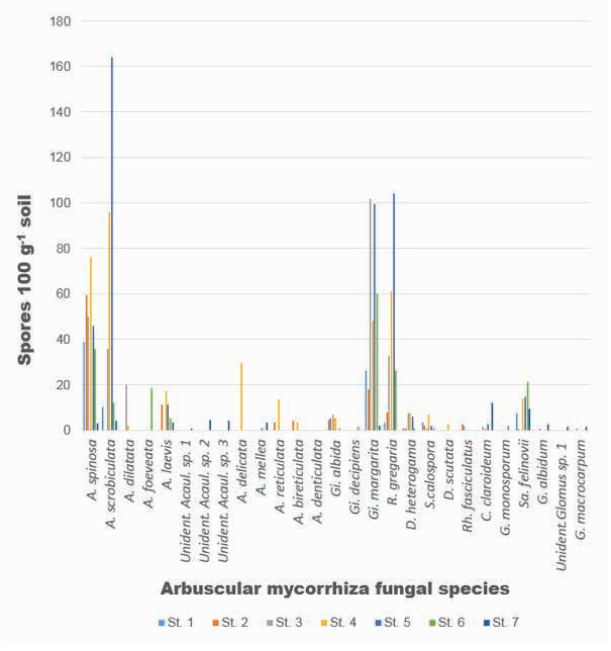
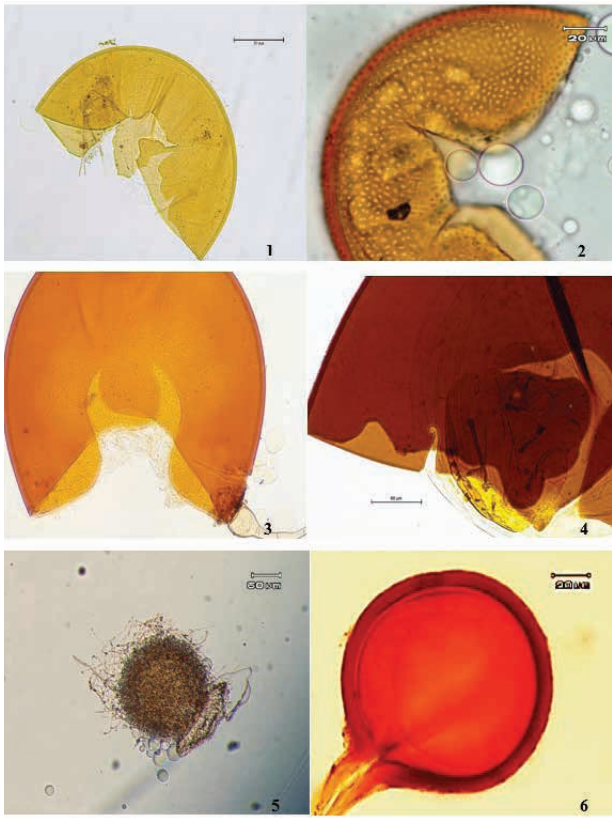


Figure 1. AM fungal spore abundance and diversity in vermicompost amended pots soils.



Plates 1-6. Micrographs of the 6 selected AMF spore morphotypes recovered from OM amended pots soils. 1. Acaulospora spinosa 2. A. scrobiculata (Courtesy Dr. James D'Souza, Goa University) 3. Gigaspora margarita 4. Racocetra gregaria 5. Sacculospora felinovii sp. nov. 6. Claroideoglomus claroideum. Specimens were mounted in PVLG.

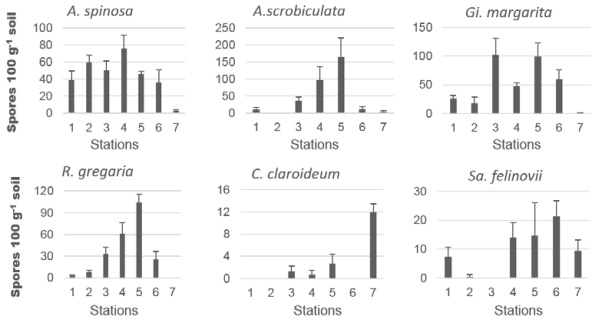


Figure 2. Distributions of the six most abundant species encountered over the transect in amended pots soils. Error bars = SD (n = 9).

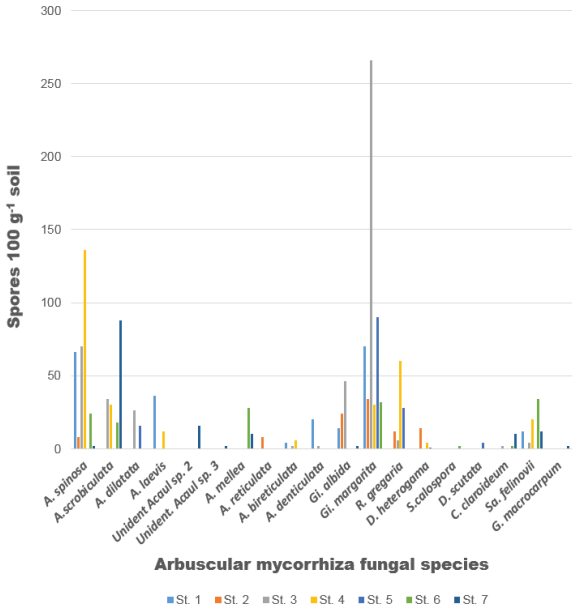


Figure 3. AM fungal spore abundance and diversity in control pots soils.

In control pots (Figure 3) the six species selected from the amended pots (Figure 2) together constituted 80% of the total (1463) spores recovered. *Acaulospora spinosa* spores were 20.9% of the total spore isolation and *A. scrobiculata* 11.6% (together 71.7% of all *Acaulospora* spp. recovered), *Gi. margarita* 24.5%, *R. gregaria* 16.4%, *Sa. felinovii* 5.6% and *C. claroideum* 1.0%. The remaining 20% comprised *Acaulospora* species (12.4%, 8), *Gigaspora*, *Scutellospra* and *Dentistucata* spp. (7.6%, 4), and *Glomus* (<1%, 1).

Of the 14 spore morphotypes extracted from the vermicompost residues (Figure 4), *Gi. margarita* and *R. gregaria* represented 52.2% and 12.5% respectively of the total (770) spores recovered. The distributions showed *Gi. margarita* to be dominant in all stations except St. 5, where the unidentified *Glomus* morphotype that was not recovered from either amended or control pots was the most abundant species. *Gi. margarita* spores were absent in the most acidic soils (mean pH 5.82; range 6.3-5.4 over the 2010 monsoon period) in amended and control pots soils at St. 7. In vermicompost residues, almost 15% of the total transect recovery was made from St. 7. *Racocetra gregaria* was not recovered from the first two stations at the front face of the foredunes. There were > 2× the number of *R. gregaria* spores in the amended pots soils than in controls soils, > 3× when the spores from vermicompost residues are included. The two dominant *Acaulospora* species that were extracted from amended and control pots soils were reduced in abundance in sachets, *A. spinosa* by 97% of both amended and control, and *A. scrobiculata* by 96% and 93% respectively. *Sacculospora felinovii* was recovered from St. 7 sachet residues only, and *C. claroideum* was not recovered from any sachet. There was significant positive correlation of spore distribution between sachets and control pots soils over the transect length ($r = 0.742$; $P = 0.046$) but no correlation between sachets and amended pots distribution.

Over the whole length of the transect, the calculation of 1-D for amended pots and controls showed that there was little difference between AM species spore diversities, 0.836 and 0.875 respectively, with sachets exhibiting the narrowest diversity of all. Controls' and sachets' diversities were wider than in the amended St. 1 and St. 2 pots, in contrast to St. 3 - 7 where the vermicompost amended pots consistently displayed greater AM fungal richness. There was no significant overall

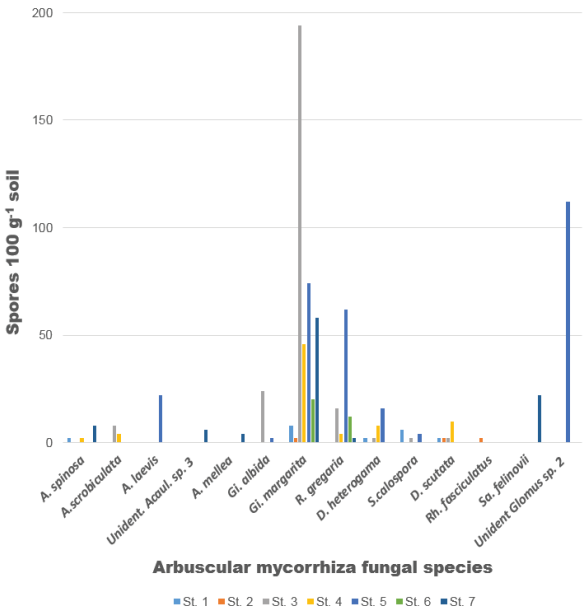


Figure 4. AM fungal spore abundance and diversity in vermicompost residues.

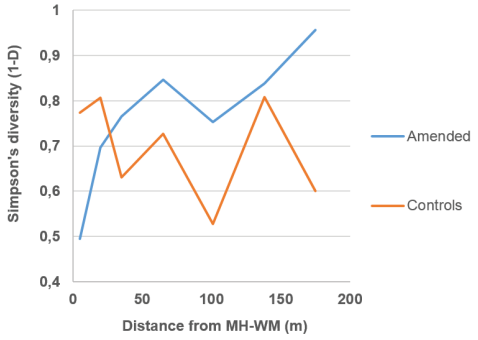


Figure 5. Simpson's diversity of arbuscular mycorrhiza fungal spores at species level in amended and control pots soils at stations on a transect across a west-coast India primary dune system.

transect correlation between datasets. A plot of Simpson's diversity (Figure 5) in control and amended pots against distance from MH-WM over the transect showed variation in controls but an increasing trend in amended pots diversity. Spearman's rank order correlation, detecting monotonic rather than linear pattern, between 1-D calculated in each station and increasing distance of the stations from the MH-WM in amended pots along the transect was statistically significant (r_s = 0.821; *P* = 0.023). There was no significant correlation between the control pots and increasing distance. There was significant negative correlation over the transect between the 2010 pH dataset and 2012 amended pots soils 1-D dataset (r = -0.780; *P* = 0.039).

3. DISCUSSION

The transplanted *I. indicum*, at harvest, had completed an unhindered life cycle, all with culm divested of seed. Those in OM amended pots were no less prostate than in control pots or surrounding specimens, despite access to greater concentrations of nutrients, equally affected by windshear (Maun 2009). The specimens observed growing in the lee of a *Vitex rotundifolia* L. thicket near St. 3, on the back slope of the foredunes, were observed to reach > 0.5 m in height. There were coiled roots only in St. 7 amended and control pots. When the pots were harvested, the foliage and roots in all plant specimens were too brittle, and decimated, to retrieve any meaningful plant properties data, or assess AMF colonization. Shallow pot size (6 cm depth) in the hot, dry conditions had contributed to desiccation, and to the absence of viable AM hyphae in residue vermicompost. This reinforces the premise that the remaining resilient spores thus represented the AMF biomass. The 2012 host plant frequency along the transect was closely aligned with that recorded in 2010 with only St. 2 showing greater frequency in the earlier set. Significant correlation between the 2010 and 2012 datasets, and no significant differences in ANOVA between transect frequencies suggest that the plant community component of the study may be relatively stable from year to year, in spite of the unstable nature of primary dune systems (Arens et al. 2005, Provoost et al. 2011).

Despite general concerns about the value of AM fungal spore counts, relative species data from the study have revealed interesting features of AMF population biology, albeit a small sample size for the field study, across a coastal dune habitat. Spore densities of *Acaulospora* species in the amended pots soils, and especially of the large-spored *Gi. margarita* in amended pots soils and vermicompost residues, were high (c.f. Camprubi et al. 2009, range 2-77 spores 100 mL^{-1} soil in Spanish Mediterranean dunes; Karthikeyan & Selvaraj 2009, range 38-580 spores 100 g^{-1} of all taxa recovered from coastal dune soils in South India; Ramos-Zapata et al. 2011, 23 spores 100 g^{-1} coastal dune soils in Mexico). Simpson's calculations showed that diversity was greater in OM amended than in control pots. Spearman's analysis showed a significant correlation of 1-D with distance from MH-WM only in amended pots.

The significant negative correlation between increasing AMF diversity and decreasing pH gradient suggests that the trend towards more acidic soil had a causative effect. Lower pH can enhance OM decomposition, increasing ion availability (Tipping and Woof 1990, Yan et al. 1996). Increasing soil acidity (range pH 7.0 to pH 3-4) was reported by Nierop and Verstraten (2003) to have increased lignin degradation in coastal dunes soil organic matter. Plants that obtain sufficient P nutrient *via* the direct pathway can reduce C contribution to the mycobiont, inhibiting growth and reducing biomass (Buwalda et al. 1985, Smith et al. 2011). This is reflected in reduced spore abundance in St. 7, a region of pedological transition from nutrient deficient psammic soil to humic soil (Willis et al. 2016a) where, in amended pots, 1% of the total transect abundance was recovered, but in controls 9.7% was recovered.

The distribution of the eight spore morphotypes isolated from amended pots soils but not control showed one was recovered from St. 3 (mean pH 2010 survey 6.6), one from St. 4 (pH 6.5), three from St. 6 (pH 6.3) and three from St. 7 (pH 5.8), all in low abundance (2, 29, 22 and 6 spores 100 g^{-1} soil respectively). There is significant negative correlation between the distribution and pH (r = -0.891; *P* = 0.024). None of the eight morphotypes was represented in vermicompost residues indicating that it was unlikely that the OM amendment was the source of inoculum. This suggests that the propagules were harboured in the soils that were transferred to pots prior to OM amendment, yet the morphotypes were not recovered from control pots soils. A similar phenomenon has been described often when field soils have been transferred to trap culture and spore morphotypes not recovered from field-soil samples were found in trap culture soils (e.g., Bever et al. 2001, Oehl et al. 2003). The comparative cultural conditions differ, of course, but the analogy does illustrate that AMF spore morphotypes can remain undetected in soils until there is a substantial change in the environment. Therefore, where there was a common host-plant species, similar rhizosphere soil volumes and a uniform vermicompost amendment against non-amended controls, the increasing trend revealed in AM species diversity along the transect is attributed to augmented mineral nutrient concentrations from vermicompost amendment, enhanced by a decreasing pH gradient. The hypothesis that OM amendment alters AM fungal spore diversity in this primary coastal dune system is supported.

Whether OM amendment is a determinant factor in regulating the AM fungal spore density is not clear. A number of the data interpretations have proved anomalous. One-way ANOVA of the six selected amended pots species depicted in Figure 2 showed disparate distributions over the transect. It can only be inferred that this indicates differing spatial strategies, but the variations are noteworthy. Of particular interest was the absence of the two dominant *Acaulospora* spp. in amended pots soils at St. 7, and a similar absence of *A. spinosa* in control pots soils. However, 52% of all *A. scrobiculata* spores in control pots soils were isolated from St. 7. *Dendiscutata*, *Gigaspora*, *Racocetra* and *Scutellospora* spp. in both amended and control pots soils were also absent from St. 7, and there was an emerging dominance of *Glomus* spp., albeit at low abundance, 6.3% of total transect abundance in amended pots soils, 7.0% in control pots soils. *Gigaspora margarita* spores were dominant in residual vermicompost in all but one of the seven stations. The greatest density, interestingly, was in the root-breached St. 3 sachet, where sporulation was not diminished despite the host plant having direct-pathway access to nutrients. Other than the unidentified *Glomus* morphotype uniquely encountered in St. 5 vermicompost residues, the remaining 11 species were represented at low abundance. Of particular note was the absence of *A. spinosa* and *A. scrobiculata* in the vermicompost residues whereas both taxa had occurred at high abundance in amended and control pots soils. *Racocetra gregaria,* and particularly *Gi. margarita*, on the other hand, readily sporulated in sachets. A functional explanation is not clearly evidenced.

Vermicompost sachets comprised *ca* 5.5% of amended pot volumes, a considerably greater OM component than in surrounding soils. Chemical analysis of the vermicompost revealed a rich source of readily available mineral nutrients. Directional root growth towards sachets was observed in OM amended pots, an indication of response to nutrient diffusion gradients (Brady 1974, Farrar et al. 2003). No *A. spinosa* spores were recovered from the single sachet that had been breached by roots, and few from sachets in the other stations. There was, however, parity in the total spore abundance of *A. spinosa* in amended and control pots soils (309 and 306 spores 100 g^{-1} soil respectively). Density was high, range 36-76 spores in 100 g of amended soils, 0-136 in control soils, up to St. 7 where numbers were negligible. This suggests vermicompost amendment had little or no effect on *A. spinosa* abundance. There was greater total abundance (almost 2×) of *A. scrobiculata* in amended pots soils than in control pots soils, also in high density up to St. 7 in amended pots soils (range 0-164 100 g^{-1} soil), but 51% of the transect total in control pots soils was retrieved from St. 7. The literature reports acidic soils are preferred by some *Acaulospora* species (e.g., Morton 1986, Porter et al. 1987). The distribution over the transect was uneven, 59% of the amended treatment spores retrieved from St. 4 and St. 5 pots. This suggests vermicompost addition contributed to an increased abundance but again there was virtually no sporulation of the species in sachets, 12 spores in total, eight of those recovered from the sachet breached by roots.

There was a greater abundance of *R. gregaria* spores in the amended pots distribution than in control (2.3×), suggesting that vermicompost addition had an impact on the increased spore abundance. In contrast, however, the abundance in sachets was less than that in controls. Conversely, there was 1.5× greater abundance of *Gi*. *margarita* in control (522) than in amended (358) pots soils, range in control pots 0-266 spores 100 g^{-1} soil, 51.0% of those recovered from St. 3, and range 2-102 spores 100 g^{-1} soil in amended pots, 28.5% recovered from St. 3 and 27.9% from St. 5. This indicated that the OM amendment did not affect the spore abundance, in contrast to Douds & Schenck (1990) who found that increase in N concentrations increased the *Gi. margarita* sporulation. In residual vermicompost in this study however, *Gi. margarita* spore abundance was 52.2% of the total spore recovery, range 2-194 spores, 48.3% (194 of total 402) of those found in St. 3, where one of the sachets was breached by roots. This is an intriguing puzzle. The vermicompost was rich in nutrients, concentrations more than sufficient for the plant to complete its life-cycle by direct pathway uptake. Yet St. 3 sachets recorded the highest *Gi. margarita* spore density of all stations, including amended and controls soils, a not inconsiderable C drain on small plants. However, the data showed the taxon had more readily accessed the nutrient-rich OM amendment, a distinctly different strategy to that adopted by *A*. *spinosa* and *A. scrobiculata*.

The study has shown a little of the complexity of interactions between plants and AMF in the field. The host plant *I. indicum* that has been restricted to a prostate and small stature has nevertheless contributed sufficient C to support a high sporulation rate in the associated AMF, particularly in *Gi. margarita* where a rich source of readily available mineral nutrients was provided in organic amendment. The spore number clearly indicates the fitness of the species. It further suggests that *Gi. margarita*, on the parasitism-mutualism continuum (Johnson et al 1997), may have tended towards the parasite end. The host plant, in the nutrient deficient and hostile dune environment was, of necessity, an obligate partner, whereby the inherently obligate fungus has gained advantage. A greater nutrient allocation, mineral and C, was apportioned to the production of a greater number of spores in the vermicompost. Consistent with the findings by Antunes et al. (2012) in long-term deficient agricultural soils, AMF may have reduced plant growth.

4. CONCLUSIONS

Desiccation of plant roots during the dry season of the west-coast India monsoon weather system has presented an opportunity in the study of AMF ecology where spore abundance was representative of AM fungal biomass. The assessment of spore abundance and diversity in a controlled OM amendment experiment along a perpendicular transect in primary coastal dune soils showed an increasing trend in species diversity away from MH-WM. Variation in species strategies was also indicated where *Gi. margarita* invaded the OM amendment and species of *Acaulospora* did not, which implies that there was a differing taxon function. Experimental research into AMF strategy and function, in the field, is inherently fraught with difficulties. Nevertheless, until these processes are fully understood, the concept of AMF species as efficient bio-fertilizers, whether in landscape reclamation and re-instatement, or in sustainable agriculture, is not fully formed.

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