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# Coastal mycology and invasive species: Boundary conditions for arbuscular mycorrhizal (AM) fungi in incipient sand dunes



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## ABSTRACT

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Arbuscular mycorrhizal (AM) fungi are ubiquitous in soil, and are associated with some 90% of terrestrial vascular plants, aiding plants to access water and nutrients the plant roots alone cannot, in exchange for photosynthates from their host. AM fungi were first found in the dune system in the 1960s, and many of the described species have been found in dune ecosystems, where they form symbiotic associations with psammophilic plants including dune grasses. The ephemeral environment of incipient sand dunes prevents long-term colonization by plants, and little research has been undertaken to examine the contribution of AM fungi to plant survival in the disturbed environment of incipient sand dunes, or what role, if any, they play in exotic plant species outcompeting native species. A first step to understanding these roles is to examine the edaphic and biological conditions of incipient dunes. Our findings quantify the boundary conditions that surround and support AM fungi and their host plant roots in incipient sand dunes on the southern coast of Victoria, and include the chemical and geomorphological characterizations of the dunes studied. We found the nutrient levels (TOC, P, and N) to be low, in contrast to the higher levels of N found on the Atlantic coast, and pH levels such that Al would be toxic for the majority of plants, whilst Fe is limited. Additionally, we found that the incipient dune sand was not saline, and that chemical characteristics between the toe and the crest of the incipient dune did not differ greatly.

ADDITIONAL INDEX WORDS: *Ephemeral environments, soil microbes, beach sand nutrients.*

## INTRODUCTION

Dune erosion is a fundamental part of beach adjustment during storms (Pye and Blott. 2008) and the role of vegetation in releasing sand into the littoral system is critical. Incipient dunes form closest to the swash, when pioneer vegetation (Hilton and Konlechner. 2011), or obstructions such as wrack trap windblown sand (Kennedy and Woods. 2012). Furthermore, the critical role of plants in the initiation and development of dunes has long been recognized (*e.g.* Cowles 1899).

In Victoria, there are three main dune grass species, *Ammophila arenaria* (marram grass), *Thinopyrum junceiforme* (sea wheatgrass) (Figure 1), and *Spinifex sericeus* (hairy spinifex) (Cousens *et al.*, 2012), but only the latter is native. Marram is mainly found in the foredunes, and spinifex and sea wheatgrass dominate the incipient dunes (Cousens *et al.*, 2012). Sea wheatgrass is an erect, rhizomatous, perennial grass, growing to 0.5 m in height, and is endemic across a wide range of the European coasts (Hanlon and Mesgaran. 2014). It has spread rapidly along Victoria's coast since it was first recorded in 1933.



Figure 1. *Thinopyrum junceiforme* on the incipient dune at the base of the foredune on Thirteenth Beach, Victoria (Source: L.M. Hanlon).

Grasses are known to have mutualistic associations, or symbioses, with arbuscular mycorrhizal (AM) fungi (Ramos-Zapata *et al.*, 2011). However, little is known about the

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symbioses of AM fungi and vegetation in incipient dunes, despite a substantial variety of AM fungi being found in sand dunes (Koske *et al.*, 2004). AM fungi are obligate biotrophs, forming symbioses with some 90% of terrestrial vascular plants (Young, 2015). They can substantially enhance their host's ability to uptake water and nutrients (Koske *et al.*, 2004) thereby aiding rapid establishment of plants in disturbed ecosystems. Additionally, glue-like polysaccharides in AM fungal mycelium bind sand grains together (Koske *et al.*, 2004) (Figure 2).

Incipient dunes present a challenging environment in which to study AM fungi, which we hypothesize have a role in the rapid spread of sea wheatgrass. Little is known about the physical and chemical environment of incipient sand dunes in relation to the vegetation and fungal flora it supports.



Figure 2. Root system of a 12-week old sea wheatgrass plant, grown from a 5 cm node in a pot in natural beach sand. Note the grains of sand adhering to the root hairs, forming a 'mycorrhizal necklace' of sand aggregates (Source: L.M. Hanlon).

The aim of this study is to define boundary conditions on a section of the southern Victorian coast where AM fungi are found in association with hairy spinifex and sea wheatgrass. We present results from field research on the geomorphology of the research site, and the edaphic, or chemical and biological conditions in the sand that surround and support AM fungi and their host plant roots. We quantify the total carbon, total organic carbon (TOC), and labile carbon percentages of the sand, and the nitrogen and extractable phosphorus percentages. Additionally, we report on the pH, EC, water content, mean grain size and calcium carbonate content of the sand of the incipient dune.

### Background

The research site is approximately 370 m long, situated between The Hole and The Corner at the eastern end of Thirteenth Beach, a 4.5 km stretch along the 7 km length of coast from Black Rocks to Barwon Heads (Figure 3). The beach comprises an aeolinite cliff dating from 90,000 year B.P. which is now submerged by modern sand (Alsop, 1983).

The site faces Bass Strait and experiences waves averaging 1.2-1.5 m (Water Technology, 2004). The height of the foredune is 9.37 m above mean sea level (MSL). The height of the incipient dune toe where it meets the beach is consistent along its length, being 5 m above MSL at either end of the site, and 4

m above MSL in the middle. The width of the incipient dune ranges from 5-9.5 m.

Annual mean minimum and maximum temperatures for March (when the data were collected) are 13.5°C and 24.7°C respectively (Bureau of Meteorology, 2015), with a mean annual rainfall of 549.2 mm (Bureau of Meteorology, 2015). Annual wind roses show due westerly winds of 21 km/h at 9.00 am and due southerly winds of 23 km/h at 3.00 pm, with a mean annual wind direction of due south (Bureau of Meteorology, 2015). Sand movement is greatest in summer months, and least during winter months (Alsop, 1973).

### METHODS

A Trimble R6 GNSS Surveying System was used to survey the site, with a vertical accuracy of  $\pm 3$  cm. Soil moisture readings were taken at 26 locations along the crest and the toe of the incipient dune using a MEA ThetaProbe, HH2 Moisture Meter. In the 24 hours prior to taking moisture readings, 0.6 mm of rainfall occurred (Bureau of Meteorology, 2015). Sand samples were taken from the top 10 cm of the dune/beach surface, which is where most microbial activity and nutrient circulation takes place (Queensland Government and G.R.D.C., 2015). Samples were collected by hand at 10 locations along the dune crest and toe. The samples were placed in individual plastic bags within a cool bag, for transport back to the laboratory.

In the laboratory, samples were stored in a refrigerator at 4°C. Particle size was analysed with a Beckman Coulter LS 13 320 Laser Diffraction Particle Size Analyzer. Air-dried, homogenized samples were analysed for pH and electrical conductivity (EC) in a 1:5 soil/0.01 M CaCl<sub>2</sub> (pH<sub>Ca</sub>) solution at 22.3°C. The EC of the saturation extract (EC<sub>e</sub>) was calculated by multiplying the EC by the soil multiplier factor for sand (13) (State Government of Victoria, 2015). Oven-dried moisture content was calculated by homogenizing 10 20g samples from the crest and toe of the incipient dune, and drying at 80°C for 48 hours. The carbonate content of the dune sand was determined through LOI (Kennedy and Woods, 2013). Five 3g sub-samples of sand from across the crest of the incipient dune were finely ground, dried at 105°C and then heated for 24 hours at 400°C, followed by 1000°C for one hour. Chemical analyses were conducted by Environmental Analysis Laboratory (EAL), whereby samples were dried at 60°C for 48 hours prior to crushing and analysis. Total N and total P were measured as the two main elements transferred from the fungi to their symbionts (Smith and Smith, 2011), from three locations across the dune face. Total carbon and labile carbon were also measured. EAL in-house protocols included extractable P in a 1:3 nitric/HCl digest, total organic carbon percentage (TOC) (LECO CNS2000 Analyser), labile C percentage using the protocol of Blair *et al.* (1995); and total N percentage (LECO TruMAC CNS Analyser) using the protocol of Rayment and Lyons (2011).



Figure 3. Map of Thirteenth Beach, Barwon Heads, showing research site between The Hole and The Corner (Cartographer: C. Jayasuriya).

### RESULTS

Dune morphology was characterized by a DEM of the research site. The incipient varies in elevation from 8.23-8.31 m (Table 1), and in slope from 0.33° at the western end (T0) to 0.40° at the eastern end (T6), with slopes between 0.18-0.24° through the remaining transects.

Table 1. Elevations (m) (AHD) of the foredune and incipient dune along seven perpendicular transects.

Transect	Top	Bottom	Incipient
T0	14.27	1.39	8.31 – 4.69
T1	13.03	1.22	5.85 – 4.17
T2	12.99	0.91	5.75 – 3.82
T3	12.78	1.29	5.48 – 3.75
T4	12.29	1.70	5.25 – 3.92
T5	13.16	1.49	4.64 – 3.95
T6	13.38	1.18	8.23 – 4.82

The dunes are composed of medium, moderately well-sorted, fine grade sand, with a mean grain size of 1.46 Ø for the toe of the dune and 1.451 Ø for the crest (Table 2). The average CaCO<sub>3</sub> content is 15% (Table 2). The crest of the incipient dune is pH<sub>Ca</sub> 6.09 and the toe pH<sub>Ca</sub> 6.06, making Fe concentrations less than optimum, and Al concentrations toxic for the majority of plants. However, EC<sub>1:5Ca</sub> indicates that the sand is not saline (Table 2). Overall, edaphic conditions between the incipient dune crest and the toe did not differ greatly except in relation to volumetric soil water content (%), with the toe being more saturated due to its proximity to the high tide mark. This agrees with global estimates of typical dune volumetric soil water ranges of 1.5-6.0% (Van der Valk. 1974).

Table 2. Edaphic conditions (0-10cm), Thirteenth Beach incipient dune crest and toe.

Procedure	(n)	crest	toe
pH <sub>1:5Ca</sub>	6	6.09	6.06
EC <sub>1:5Ca</sub> (dS/m)	6	0.003	0.003
EC <sub>e</sub> (*13)	6	0.040	0.040
Volumetric soil water (%)	26	3.00	5.18
Oven dried moisture (%)	10	0.04	0.04
Mean grain size (Ø)	2	1.451	1.456
CaCO <sub>3</sub> content (%)	5	15.0	

All the elements measured at the research site are low (Table 3), which is characteristic of dune sands (Maun 2009) including low TOC (average 2.15%) and limited P (272-340 ppm). Results for labile carbon (<0.02%) are lower, for example, than those on the east coast of Scotland (Wall *et al.*, 2002).

Table 3. Chemical analysis (0-10 cm), Thirteenth Beach incipient dune crest.

Location	acid extractable P (ppm)	TOC (%)	labile C (%)	total N (%)
The Hole	340	2.30	<0.02	0.021
Mid-way	272	2.12	<0.02	0.019
The Corner	287	2.04	<0.02	0.021

### DISCUSSION

Literature on the nutritional status of Australian dune sands is lacking, but as with other beach systems, it is assumed there would be substantial spatial variation (Perumal and Maun. 2006). For example, Welsh dune soils were found to contain as little as 0.006-0.008% N, but had 'appreciable' amounts of P and K (Hassouna and Wareing. 1964). By comparison, the surface soils of New Zealand range from 0.09-0.87% TSN (Rayment and Lyons. 2011). Nonetheless, dune soils are generally poor in the macronutrients N, P and K (Hawke and Maun. 1988). They are also alkaline due to CaCO<sub>3</sub> sourced from marine environments, which may cause nutrient deficiencies, albeit reduce the toxicity of sodium chloride (McLachlan and Brown. 2006).

The availability of the majority of nutrients is reduced at <pH<sub>Ca</sub> 3.5-4, with the exception of Fe which becomes limited at <pH<sub>Ca</sub> 6.5-7.0 additionally, exchangeable Al becomes toxic at pH<sub>Ca</sub> >4.7 (Hazelton and Murphy. 2007). The pH<sub>Ca</sub> of the sand (Table 3) in a sand:silt:clay soil would allow all of the major nutrients to be available for plants, however as sand lacks cation exchange capacity (CEC) due to its lack of electrostatic charge and buffering capacity (Ashman and Puri. 2002), it tends to be nutrient poor (Maun. 2009). Nonetheless, particles of OM between the sand grains have a variable electrostatic charge which allows some exchange of nutrients, depending upon the chemicals in the soil solution (Ashman and Puri. 2002). Furthermore, the deposition of wrack from wave action would



periodically raise nutrient levels (Kennedy and Woods. 2013). Notwithstanding this, the Fe and Al levels would not suit the majority of plants.

The two main plants that occupy the incipient dune at Thirteenth Beach are hairy spinifex and sea wheatgrass. Literature on the Al tolerance of these plants is lacking, most likely as they are wild species that are generally not cultivated. However, if the plants are surviving the concentration of Al, they are likely to be tolerant to it. Wheat is tolerant to Al at pH<sub>Ca</sub> 4.0-4.5 (Hazelton and Murphy. 2007), and as sea wheatgrass is a wild relative of wheat, Al levels would not be a limiting factor in its growth. Additionally, although acidic soils can impair the functioning of most microbial processes such as the breakdown and cycling of organic matter from which plants access nutrients and carbon (Gazey and Ryan. 2014), mycorrhiza have shown enhanced metal sorption capacity compared to other microorganisms (Joner *et al.*, 2000). For example, mycorrhizal plants successfully inhabit many environments where soil acidity results in elevated levels of metals, such as in mine spoils and other heavy-metal contaminated sites (Göhre and Paszkowski. 2006).

Coastal dunes contain little or no clay or silt, therefore salts are easily leached down the profile, and thus the sand at Thirteenth Beach is not saline (Table 3), in agreement with previous literature on coastal sand salinity levels. For example, Barbour *et al.* (1976) found that along the leading edge of vegetation of 34 beaches on the Pacific Coast in the USA, the concentration of soluble salt at a depth of 10 cm, was 0.008-0.280%.

The average CaCO<sub>3</sub> of the sand is 15% (Table 3), and although rates of 10 to 20% CaCO<sub>3</sub> are regarded as high by Nordstrom *et al.* (1990), it is not the dominant component at Thirteenth Beach.

Although few beach systems have been researched sufficiently to provide nutrient budgets (McLachlan and Brown. 2006), the periodic deposition of OM (Kennedy and Woods. 2013), and rainfall and nutrients in sea spray (Maun 2009) aid in supplying nutrients. Total organic carbon (TOC) is largely the result of plant decay and the low levels of TOC at the site are reflected in the lack of woody plants and shrubs along the incipient dune. Labile carbon levels (<0.02%) are lower than those of Wall *et al.* (2002) who found levels of 0.1-0.6% in the beach and foredune at Tentsmuir Point, on the east coast of Scotland.

In general, pioneer plants require low quantities of N and P, and the limited level of phosphorus at the site (272-340 ppm) is offset by pioneer plants gaining access to P via by the extra-radical mycelium of AM fungi. Mycelia work like an extension to the plant roots, growing beyond the phosphorus depletion zone around the roots to take up P, as well as some trace elements from the edaphic environment (Koske *et al.*, 2004). The N percentage at Thirteenth Beach (average 0.020%) is higher than on beaches along the Atlantic Coast of Maine and New Hampshire USA for example, where levels are 0.004-0.012% N by weight (Croker *et al.*, 1975).

The toe of the incipient dune sits above MHSW but is not protected from storm surges and is therefore subject to scarping and plant loss. Although gradual or minor disturbances do not necessarily lead to decreases in AM hyphal abundance (Abbott and Robson. 1991), hyphal networks in soil are easily disrupted by rapid environmental changes such as storm events. Such

events prevent long-term plant colonization (Koske *et al.*, 2004), and as a result, the contribution of AM fungi to plant survival in incipient dunes has been little studied.

## CONCLUSIONS

This study has sought to define the boundary conditions under which AM fungi are found in association with the invasive, exotic sea wheatgrass and the native hairy spinifex. The nutrients measured are low for most plant requirements, however pioneer plants require low quantities of N and P, surviving through adaptations to resource stresses, and through their symbioses with AM fungi and other soil microbes (Cockcroft and McLachlan. 1993). The data from our research will enable us to predict the how close to MHSW sea wheatgrass, hairy spinifex and AM fungi are likely to be found.

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