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Short Communication: Occurrence of arbuscular mycorrhizal fungi associated with *Casuarina equisetifolia* in saline sandy environment, North Sumatra, Indonesia

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Abstract. *Delvian, Hartanto A. 2022. Short Communication: Occurrence of arbuscular mycorrhizal fungi associated with* Casuarina equisetifolia *in saline sandy environment, North Sumatra, Indonesia. Biodiversitas 23*: *2520-2525*. Arbuscular mycorrhizal fungi (AMF) form a mutualistic association with plant roots to cope in the extreme environments including the saline and sandy soils in the coastal areas. In this study, the occurrence of AMF associated with *Casuarina equisetifolia* that has been planted for coastal rehabilitation located at Cermin Beach, North Sumatra was investigated including its abiotic-biotic interactions from topsoils (0-20 cm) to subsoils (20-80 cm). A total of 10 AM fungal morphotypes were documented with Glomeraceae as the dominant AM fungal taxa (9 morphotypes) and a Glomoid morphotype exists in all soil depths. The number of AMF spores decreased as soil depth increased following other environmental conditions such as pH, soil P availability and soil moisture based on the result of Pearson's correlation test. The results showed a moderate-to-low level of biodiversity of AMF based on Shannon's diversity index. The findings revealed the vertical distribution of AMF in a saline environment and supported the planting of *C. equisetifolia* which successfully recruited the indigenous AMF to form a symbiosis to thrive in the stressful environment.

Keywords: Arbuscular mycorrhizal fungi (AMF), coastal dune, subsoil

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) is a group of soil fungi forming a beneficial symbiosis to plant roots involved in the soil-microbe-plant system that is crucial to the soil ecology (Hestrin et al. 2019). AMF belongs to the monophyletic taxa or Glomeromycotina and colonized most land plants to initiate a symbiotic relationship (Spatafora et al. 2016). The role of AMF to the plant emerged as a set of beneficial phenotypic alteration of their hosts such as improvement in plant productivity (Ishaq 2017; Begum et al. 2019), improvement of nutrient uptake (Wang et al. 2017), the establishment of soil aggregation (Leifheit et al. 2014), provision of defense system against phytopathogens (Schouteden et al. 2015) and other related important features in agriculture.

AMF and plant symbiosis can be found in a variety of terrestrial habitats, although AMF distribution is determined by a multitude of ecological processes and soil physicochemical conditions (Neffar et al. 2015). The distribution of AMF is driven by a range of factors, including available nutrient content, particularly P, soil pH, soil temperature and moisture, soil disturbances, land use type, and vegetation above the soil surface (Treseder and Cross 2006; Liang et al. 2016; Liu et al. 2020; Delvian 2021). Furthermore, AM fungi are known to colonize plant species thriving in harsh environment such as saline to hypersaline environments (Silvani et al. 2017).

High soil salinity has a detrimental effect on AMF colonization and distribution in coastal areas (Juniper and Abbott 1993; Delvian and Rambey 2019). The occurrence and role of AMF in salt-tolerant plants in sites with high soil salinity have been reported and appreciated, especially those colonizing the whistling pine tree, *Casuarina equisetifolia* L (Zhang et al. 2010; Diagne et al. 2017; Djighaly et al. 2018). *Casuarina equisetifolia* is a perennial tree, a monoecious and an actinorhizal plant with the ability to fix atmospheric nitrogen and is generally planted for rehabilitation purposes in coastal areas (Jin et al. 2021). *Casuarina equisetifolia* has been planted to prevent the shift of coastal dunes due to afforestation and to maintain the soil environment through the construction of stable soil aggregates and modification of micro-climate (Dinh 1998; Harjadi 2017).

The majority of ecological studies on the distribution of AMF primarily focused on the plant rhizosphere, which is located in a 20-cm region underneath the soil surface which structures the enormous plant root biomass (Saia et al. 2011; Becerra et al. 2014; Thiem et al. 2017). Few studies have investigated the dispersion of AMF in subsoils (>20 cm). The physicochemical conditions of the subsoils are different than topsoils so the distribution and presence of

AMF in the subsoils might be altered. AMF colonization in plant roots, the number of infective propagules, and the number of spores were all reduced following the depth of soil layers (Becerra et al. 2014).

There has been limited report on the vertical distribution of AMF on sandy soils in coastal areas in Indonesia. The present study deals with the possibility of finding AMF assemblage colonizing the subsoils region adjacent to the area inhabited by *C. equisetifolia*. The AMF survey will be critical in improving our understanding of dune biodiversity in North Sumatra and to supporting the impact of coastal rehabilitation by planting *C. equisetifolia*.

MATERIALS AND METHODS

Study area

Field sampling was conducted at a tropical dune area inhabited by numerous stands of *C. equisetifolia* in Cermin Beach, Serdang Bedagai Regency, North Sumatra, Indonesia. The coastal ecosystem is located in the eastern part of the North Sumatra Coastline which lies in 2º0′0″ N, 98º0′0″ E. The average humidity per month was recorded at 79%. The precipitation ranged between 120 and 331 mm per month with some rainy days per month of 8 to 20 days. The average evaporation rate was about 3.9 mm/day with a minimum air temperature of 22.2ºC and a maximum at 31.9ºC.

Field sampling

Soil sampling for AMF spore isolation was conducted in Cermin Beach in August 2017 which corresponded to the end of dry season. Sampling area was situated in an area of 50 × 50 m grown by *C. equisetifolia.* Five plants of *C. equisetifolia* were considered as replicates while the soil sampling was randomly collected at four different soil depths, namely 0-20, 20-40, 40-60, and 60-80 cm using a metal corer. Soils (100 g) were collected at a distance of 0-15 cm from the base of the stem (*C. equisetifolia*) from each soil depth. Soil samples were analyzed for the soil physicochemical properties and the occurrence of AMF spores.

Soil analysis

Physicochemical characteristics of sandy soil collected from Cermin Beach were analyzed including its pH, electrical conductivity, moisture, soil P availability, soil N content, and total organic carbon content. Soil pH, moisture (%) and electrical conductivity (µS/cm) was recorded *in situ* using a digital instrument. Soil P availability (ppm) was determined following the protocol of Bray-1 using a series of reactions between HCl, NH_4F and molybdate-ascorbic acid solution. The formation of blue color indicated a positive result of P availability measured at 880 nm using a spectrophotometer (Bray and Krutz 1945). Soil N content (mg/100 g dry soil) was determined following the protocol of Kjeldahl using a series of steps such as digestion with concentrated H_2SO_4, the distillation of ammonia product, and volumetric analysis or titration of used HCl using methyl red as an indicator (Wang et al. 2016). Total organic carbon content (%) was determined following the protocol of Degtjareff using a chromic acid titration technique (Walkley and Black 1934).

Isolation and microscopical examination of AMF spores

AMF spores were extracted from a soil subsample from each soil sample by wet-sieving and decanting technique (Gerdemann and Nicolson 1963) followed by a 40%-sucrose centrifugation method (Daniel and Skipper 1982). Briefly, 50 g of soil was mixed with 500 mL of water in the 1 L-conical flask. The soil mixture was agitated vigorously to release the AMF spores from soil particles and settled from 15 to 45 min. The mixture was filtered using a series of sieves of different mesh sizes (450, 150, 45 µm). The final filtrate was purified in the 40% sucrose solution and then centrifuged at 1750 rpm for 5 min. The supernatant was collected and transferred to petri dishes. The solution of AMF spores was placed on a clean glass slide stained with polyvinyl-lacto-glycerol (PVLG) and Melzer's reagent (1%, v/v) to identify the species under dissecting microscope. The AMF spores were documented and identified based on morphological criteria provided in an online reference at the West Virginia University, USA (http://invam.caf.wvu.edu) supported by available references (Schenck and Perez 1990; Blaszkowski 2012).

Data analysis

All data are presented in means as a result of three replication. The effect of soil depth and physicochemical conditions of the sand dune in Cermin Beach on AMF population was evaluated based on Pearson' s product moment correlation test using Minitab ver. 17.0. Shannon's diversity index (*H'*) of the AMF community was generated using the PAST 4.02 program. Relative abundance and vertical profile of AMF isolates were visualized as a heatmap using GraphPad Prism ver. 8.0.2.

RESULTS AND DISCUSSION

Soil physicochemical parameters were varied among soil depths (Table 1). The pH was documented as neutral (6.7) at topsoils to alkaline (8.5) at 40-cm depth or subsoils. The electrical conductivity remained constant following the soil depths as a type parameter which indicated the salinity of sandy soils that lay within the range of 86.36-97.24 µS/cm. Total organic carbon in the soils and N content decreased as the soil depth increased. The highest extractable phosphorus (P) was documented from the deepest soil layer (80 cm) similar to the soil moisture profile.

A total of 10 AM fungal morphotypes were recorded with the majority of isolates that could only be attributed to the general level of identification namely *Acaulospora* and *Glomus* (Figure 1). The community of AMF was structured by Glomoid morphotypes with an even relative abundance recorded in the topsoils (0-20 cm) and altered as the soil depth increased. Glomoid morphotypes sp. 3 and sp. 7 were recorded from topsoils (0-20 cm) to subsoils (60-80 cm) revealing their absolute frequency of occurrence (100%) in

the study region (Figure 2). The highest number of AMF spores was recorded at the topsoils and decreased as the soil depth increased as presented in Figure 3. The biodiversity index for topsoils (0-20 cm) and subsoils at 20-40 cm, 40-60 cm, 60-80 cm were 2.25, 1.72, 1.52, and 0.66, respectively. The results indicated a medium-to-low diversity of AMF in the coastal region of Cermin Beach. Various local environmental conditions, such as climate, soil type, and soil depth, inevitably impact on the diversity and community assemblage of AM fungal species (Gong et al. 2012; Wang et al. 2018).

Interaction between physicochemical condition or abiotic factors in the study site and AMF assemblage in *C. equisetifolia* was weighted based on Pearson's correlation coefficient for each parameter (Table 2). The occurrence of AMF in the sandy soils was negatively correlated with the soil acidity (r = -0.762), soil P availability (r = -0.936), soil moisture (r = -0.978, $p < 0.05$), and soil depth (r = -0.982, $p < 0.05$).

Information on the plant diversity and associated AM fungal species on coastal dunes is of significant importance for their efficient utilization in the rehabilitation and management of the marginal ecosystems. The inventory of AM fungal species originating from marine dunes in Indonesia is scanty. Mycorrhizal symbiosis is a crucial facet in helping plants to cope with adverse environmental conditions. The occurrence of arbuscular mycorrhizal fungi in coastal dunes at Cermin Beach, North Sumatra was evaluated at different soil depth to reveal the AMF vertical distribution and possible limiting factors in the soil environment to the number of AMF spores. Environmental differences, on the other hand, have an important role in AMF spore production. Fungal species typically have a pH optimum that ranges from 5 to 9 pH units without substantial growth inhibition with an exception to highly acidic soils (Liu et al. 2020). Although environmental gradients have been predicted to affect the number of AMF spores in our study, other abiotic factors may also involve. AMF growth and sporulation require stable conditions for example light and temperature variations that could be negated at the deeper soil layers and roots which were not measured in this study (Brundrett 1991). The results showed that the saline sandy soils displayed favorable conditions for the initiation and development of AMF with plant host since they were deficient in phosphorus (P) at deeper soil layers or subsoils (Ranwell 1972). The theory is supported by our findings through the negative correlation between soil P content and the number of AMF spores in the study area and the record of AMF in the deepest soil layer. The results indicated the possible contribution of AMF in facilitating P transport and assimilation to the host although direct evidence must be elaborated by studying the root colonization in *C. equisetifolia*.

Table 1. Soil physicochemical characteristics at different depths of *Casuarina equisetifolia* grown at Cermin Beach

Soil depth (cm)	pH	Electrical conductivity (µS/cm)	Total organic carbon (%)	Available P (ppm)	N content (mg/100 g dry soil)	Soil moisture (%)
0-20	6.7	97.24	0.15	9.18	0.22	2.78
20-40	8.5	87.73	0.10	11.51	0.20	3.10
40-60	8.3	86.36	0.08	12.68	0.21	3.47
60-80	8.1	91.34	0.12	17.57	0.17	3.65

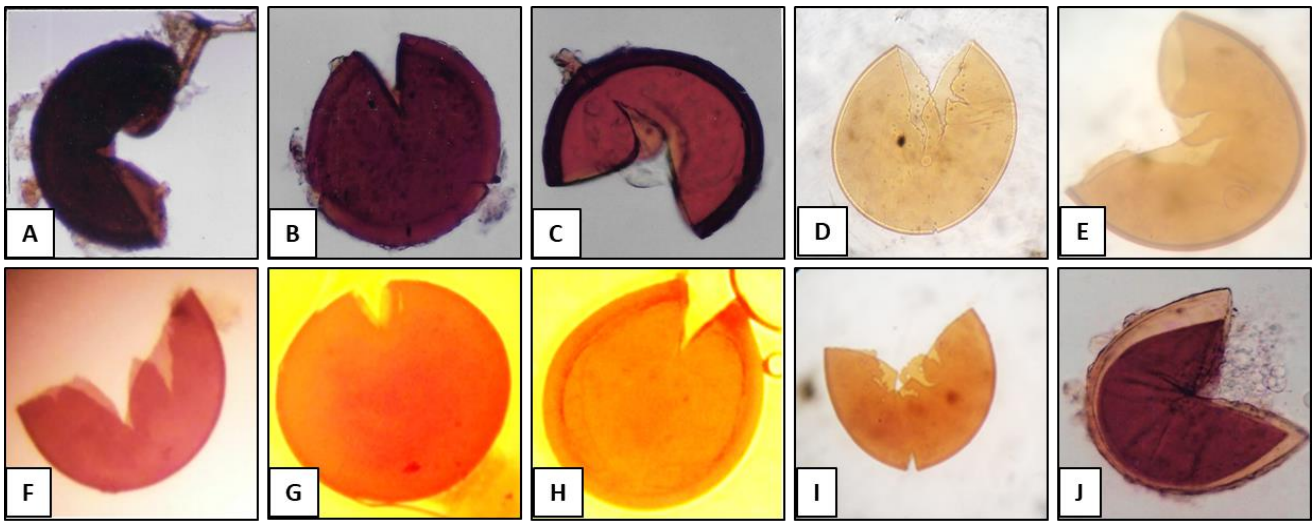

Figure 1. Spores of AMF found in the saline sandy soils of Cermin Beach, North Sumatra associated with *Casuarina equisetifolia*. Glomoid morphotypes (A-I), Acauloid morphotype (J). Magnification at 40×.

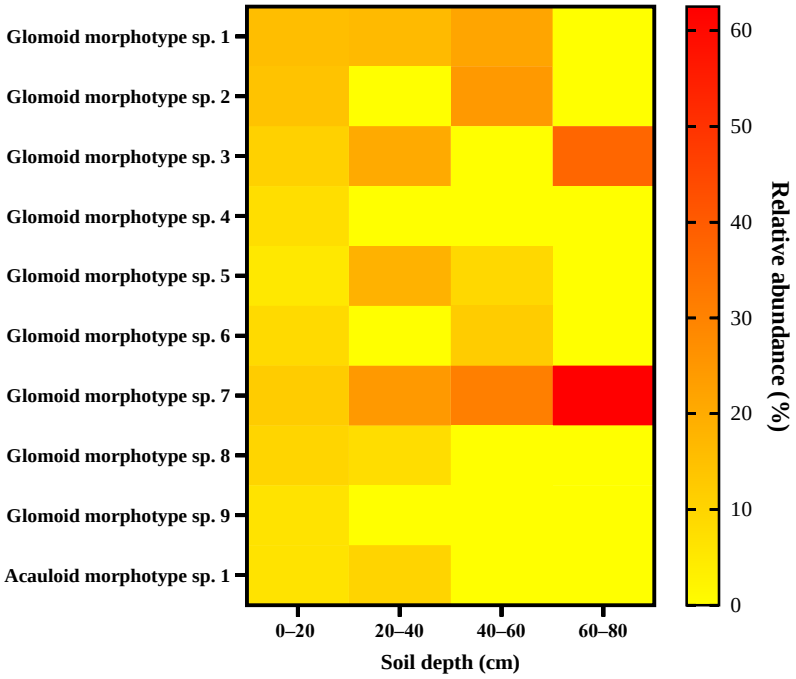

Figure 2. Heatmap distribution of relative abundance of AMF spores per fungal morphotype at different soil depths in sandy soils associated with *Casuarina equisetifolia*

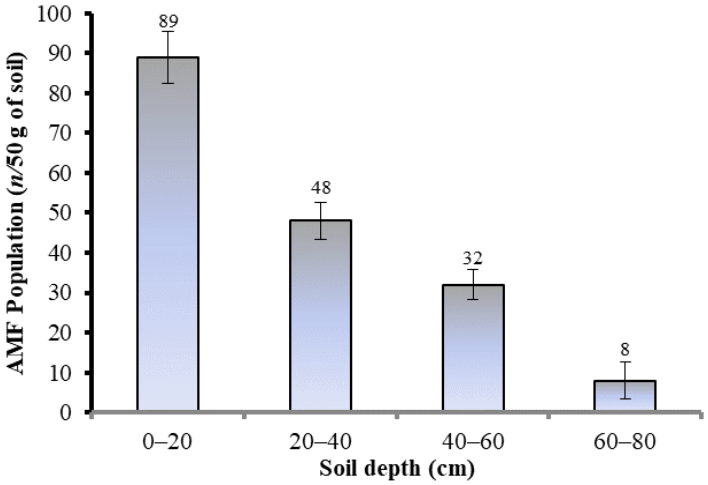

Figure 3. Number of AMF spores at different sandy soil depths associated with *Casuarina equisetifolia*

Table 2. Pearson's correlation coefficient between AMF population (*n*/50 g of soil) in *Casuarina equisetifolia*, soil depth (cm) and physicochemical conditions of sandy soils in Cermin Beach

Parameter (s)	AMF	pH	EC	TOC	P	N	Moisture
pH	-0.762						
EC	0.627	-0.944					
TOC	0.576	-0.875	**0.984**				
P	-0.936	0.517	-0.316	-0.252			
N	0.843	-0.491	0.217	0.103	-0.946		
Moisture	**-0.978**	0.675	-0.588	-0.572	0.917	-0.763	
Soil depth	**-0.982**	0.632	-0.507	-0.476	**0.962**	-0.837	**0.991**

AMF = arbuscular mycorrhizal fungi population, EC = electrical conductivity, TOC = total organic carbon, P = phosphorus, N = nitrogen. Data in bold showed a statistical significance at the 0.05 level (2-tailed)

AM fungal spore numbers in subsoils was similar in trend to other studies in saline soils. Becerra et al. (2014) reported AM fungal spores ranged between 3 and 1162 per 100 g of dry soils which were decreased in numbers as depth increased (0-50 cm). The AM fungal species were only identified to a morphotype level of identification revealing the dominance of Glomeraceae. Although the AM fungal species was recorded for the first time in the region, we still need to conduct a comprehensive survey and utilize a DNA barcoding technique to obtain a better understanding and exact species identification of the AM fungal isolates. Sosa-Hernandez et al. (2018) have conducted a metagenome analysis using high-throughput Illumina sequencing on the vertical distribution profile of AMF in 52-year agricultural fields revealing a collection of AM fungal species as indicators for topsoils and subsoils. The structure of the AMF community altered as soil depth increased. Glomeraceae species are the most common AM fungal species recorded in harsh habitats including saline environments (Djighaly et al. 2018).

Furthermore, Glomoid morphotypes sp. 3 and sp. 7 were shown to increase their occurrence or relative abundance in deeper soil layers but failed to sporulate predominantly in the topsoils (0-20 cm). The results may indicate the suboptimality of AM fungal species to survive in the topsoils regardless of supporting abiotic components and abundant sources of nutrients provided by the host. The possible explanation for this was formulated by Valyi et al. (2016) through two hypotheses namely abiotic filtering and biotic interactions. In this study, the possible biotic interaction that occurred in the topsoils was a competitive exclusion in which each AM fungal taxa competes to obtain access to rhizospheric region of *C. equisetifolia* hence reducing the number population of Glomoid morphotype sp. 3 and sp. 7 as a result.

In conclusion, the occurrence of AMF in the sandy soils inhabited by *C. equisetifolia* revealed the successful recruitment of the indigenous AM fungal species by the host species. In addition, the AM fungal isolates are well-adapted to adverse environmental conditions in the coastal environment while more exhaustive samplings may be considered to obtain a complete picture of AMF in the region.

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