



Effects of Dual Symbiotic Interactions Performed by the Exotic Tree Golden Wreath Wattle (*Acacia cyanophylla* Lindl.) on Soil Fertility in a Coastal Sand Dune Ecosystem

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The present study aims to evaluate the effects of the exotic shrub *Acacia cyanophylla* Lindl. on soil fertility by studying 1) its ability to modify the soil physicochemical composition, 2) its contribution to the soil mycorrhizal potential and its impact on the richness and diversity of the arbuscular mycorrhizal fungi (AMF) community in the rhizospheric soil (RS), and finally 3) its atmospheric nitrogen fixation potential. The physicochemical analysis of the RS has shown that soil invasion by *A. cyanophylla* has a beneficial effect on its fertility; this advantage is demonstrated by the increase of the organic matter and the nutrient contents (N, P, K, Na, Ca) in the RS. Furthermore, the roots of this shrub exhibited broad AMF colonization, which confirms its high mycotrophic aspect. Four differentiated morphotypes of mycorrhizal spores were isolated from the RS of *A. cyanophylla* by use of the wet sieving method. In addition, the most probable number method showed that *A. cyanophylla* was capable of dramatically increasing the mycorrhizal potential of the soil. Indeed, more than 1,213 infectious propagules per one hundred grams of soil were detected in the RS of *A. cyanophylla*. Moreover, *A. cyanophylla* roots showed a significant presence of nodules indicating an active atmospheric nitrogen fixation. Counting revealed the presence of at least 130 nodules in the root fragments contained in 1 kg of soil. In conclusion, the biological invasion of sand dunes by the exotic shrub *A. cyanophylla* exhibited beneficial effects on the soil's chemical composition and functioning, the activity of rhizobacteria in fixing atmospheric nitrogen, and phosphate bioavailability under the action of the native AMF community.

Keywords: *Acacia cyanophylla* Lindl, arbuscular mycorrhizal fungi, rhizobacteria, nitrogen fixation, sand-dune ecosystem

INTRODUCTION

A plant species is said to be “invasive” when its proliferation in natural environments induces significant changes in the composition, structure, and functioning of ecosystems. These species are categorized by their ability to adapt to a wide range of environmental conditions and resist disturbances (Blackburn et al., 2011; Sfairi et al., 2012; Baumel et al., 2018). In Morocco, several phylode plant species native to Australia have been introduced to enrich the local flora, stabilize soils, constitute green belts and produce fuelwood and fodder (Lahdachi et al., 2015). *Acacia cyclops*, *Acacia mollissima* and *Acacia cyanophylla* are among the most widely used species (Benbrahim et al., 2014). *A. cyanophylla* is used almost everywhere for reforestation, ornamentation, production of soil tannins and coastal dune fixation (Marzialetti et al., 2019). Its tolerance to cold, drought, salinity and fires has enabled it to be classified among the most forest species used in the reforestation of arid and semi-arid zones (Derkaoui et al., 2016; Kheloufi et al., 2019). In addition, this hardy competitive legume species is nodulating with a vast number of *Rhizobium* strains, fixing atmospheric nitrogen (Amrani et al., 2010; Pathak et al., 2017). Furthermore, the species can establish a symbiotic partnership with many soil arbuscular mycorrhizal fungi (AMF). These fungal symbionts are the most widespread on the surface of the globe and adapted to many environments and different host plants (Vaishnav et al., 2017; Jacobs et al., 2020); they can form mutualistic associations with the roots of about 80% of terrestrial plants (Smith and read 2008). Golden wreath wattle (*A. cyanophylla* Lindl or *A. saligna* Labill) is a species native to the temperate region of southwestern Australia, where it has a naturally widespread distribution (Millar and Byrne, 2012) in various habitats, especially on soils with a high proportion of sand, such as dunes, sandy plains, or rocky ridges where it forms open forests (Griffin et al., 2011). Regarding climatic requirements, *A. cyanophylla* grows under average temperatures close to 13°C in winter and 30°C in summer (Thompson et al., 2015). This shrub prefers sandy soils receiving more than 250 mm annual rainfall (El-Euch, 2000; Derbel et al., 2009). Outside of its natural range, *A. cyanophylla* has become an invasive species due to several factors, including its ability to regenerate after cutting or burning, its rapid growth in low-nutrient soils, its ability to fix nitrogen and its early reproductive maturity, and finally, the seeds’ ability to survive in fire (St-Denis et al., 2017; Badalamenti et al., 2018). *A. cyanophylla* presents direct socio-economic interests by the production of wood and edible seeds (Le Maitre et al., 2011); this shrub is also a source of high-quality fodder and complementary food for livestock due to its richness in proteins (Ee and Yates, 2013; Gebreyohannes, 2016). Regarding the ecological interests of *A. cyanophylla*, this species contributes to the softening of the climate of arid and semi-arid zones and ensures the fixation of sandy and coastal dunes. The lateral root system favors better maintenance of the cohesion of the soil particles (Boukhatem et al., 2012; Martínez et al., 2013; Lozano et al., 2020).

In addition, this shrub exhibits an arbuscular mycorrhizal symbiotic association receptiveness, particularly in phosphorus-deficient soils (Belay et al., 2013; Ilahi et al., 2021). The mycorrhizal fungi symbiosis is a mutualistic collaboration between a soil fungus and the roots of a host plant. This symbiosis is established by the development of a network of hyphae that can reach up to 1 km per 1 m of the root system (Fortin et al., 2015). The fungus removes sugars from the plant while the plant receives minerals and water from the fungus (Jadrane et al., 2021). Moreover, without this association, the AMF cannot complete its life cycle (Barea et al., 2005; Ouahmane et al., 2007b; He and Nara, 2007). The AMF develop long extra-root hyphae that allow the plant to exploit a large soil area. They can therefore have access to additional resources of water and mineral elements, which are then transmitted to the host plant through the roots (Smith and read 2008; Bouskout et al., 2022). Mycorrhizal plants receive phosphate from AMF and this most often leads to an increase in biomass compared to non-colonized plants (Balzergue et al., 2013). This difference can be explained by several mechanisms, among others, the expansion of the plant’s root absorption surface thanks to the extra-root network (Duponnois et al., 2009) and consequently, better exploitation of the phosphate of the soil beyond the zone of exhaustion of the root (Tisdall, 1991; Wahid et al., 2020). Additionally, the AMF hyphae possess phosphatases that promote the release of immobile phosphorus in the soil and allow the mineralization of organic sources of phosphates (Wahid et al., 2020). The phosphorus thus mobilized becomes available in the soil. On the other hand, several studies have reported an improvement in nitrogen nutrition provided by AMF, which has the necessary enzymatic equipment for the use of ammonium and nitrates (Nakmee et al., 2016). So, mycorrhizae play an important role in the plant’s uptake of certain forms of nitrogen (Beltrano et al., 2013). According to better use of the soil by the extra-root network, AMF increases the absorption of other mineral elements such as K, Ca, Mg and certain trace elements such as Zn, S and Cu (Abbaspour et al., 2012; Lehmann et al., 2014). AMF also have direct effects on soil quality, the extra-root mycelium produced by mycorrhizal roots, which constitutes a three-dimensional network that connects the plant to the surrounding soil, contributes to the formation of stable aggregates in the soil. The stability of these soil aggregates can be explained by the production of glomalin, a glycoprotein that acts by its hydrophobic properties to stabilize the aggregates (Singh et al., 2013). The stabilization of soil aggregates is also a result of the fixation of soil particles by hyphae and roots and the exudation of polysaccharides (Kohler et al., 2017).

The current study examined the effects of the invasive plant *Acacia cyanophylla* on the soil’s physicochemical parameters, on the soil’s richness and diversity of mycorrhizal fungi morphotypes, on the rhizospheric soil’s mycorrhizal potential, and finally on atmospheric nitrogen fixation in the sand dune ecosystem of Essaouira, Morocco.

TABLE 1 | pH, electrical conductivity and mineral contents of the rhizospheric soils of *Acacia cyanophylla* and the bare soil (control) in the sand dunes of Essaouira.

Physicochemical characteristics of soils	Bare soil	Site 1	Site 2	Site 3
pH	9.1 ^a	8.39 ^b	8.32 ^b	8.43 ^b
Electric conductivity ($\mu\text{s cm}^{-1}$)	0.41 ^b	0.48 ^a	0.47 ^a	0.48 ^a
Total organic carbon (%)	0.5 ^b	1.35 ^a	1.28 ^a	1.3 ^a
Organic matter (%)	0.86 ^b	2.33 ^a	2.21 ^a	2.24 ^a
Total nitrogen (%)	0.1 ^b	0.3 ^a	0.4 ^a	0.4 ^a
C/N	5 ^a	4.5 ^a	3.2 ^b	3.25 ^b
Available Phosphorus (mg/kg)	48 ^b	82 ^a	86 ^a	86 ^a
Total K ⁺ (mg/kg)	100 ^b	180 ^a	170 ^a	180 ^a
Total Na ⁺ (mg/kg)	220 ^b	410 ^a	420 ^a	420 ^a
Total Ca ²⁺ (mg/kg)	580 ^b	820 ^a	800 ^a	840 ^a

In the same line, values of each physical or chemical parameter with the same letter are not significantly different according to Tukey test (HSD) ($p < 0.05$).

MATERIAL AND METHODS

Study Site and Soil Sampling

The Essaouira region in Morocco seems to be a suitable study area due to the importance of the living dune ecosystem (latitude of 31° 30' 0.00" N; longitude of -9° -45' -36, 00" W) and the heavy invasion process conducted by *A. cyanophylla*. Three sites were randomly chosen to find out how *A. cyanophylla* changes soil chemical and biological fertility. They were named site 1, site 2 and site 3. On 4 March 2019, five *A. cyanophylla* plants were randomly picked at each site. At a depth of 10–20 cm, soils directly influenced by the roots (rhizosphere) were collected. Additionally, the bare soil (served as a control) was gathered well away from any plant roots at each location. Fifteen rhizosphere soil samples and three bare soil samples were collected. A composite soil was produced for each of the three sites by mixing the five samples homogeneously. The bare soils were also combined to generate a homogeneous sample (control).

Soil pH and Electric Conductivity

The physicochemical properties of the examined soils are given in **Table 1**. Initially, all soil samples were air-dried and sieved at 2 mm. Afterward, an aliquot of soil was suspended in distilled water (1:2.5, w/v) before being stirred for 30 min using a mechanical agitator. The pH of each soil was measured by a calibrated digital pH meter (JP Selecta pH-2006). The electric conductivity of the soil was measured after magnetic stirring for 20 min of 10 g of soil in 50 ml of distilled water; the measurement was carried out using a conductivity meter calibrated with a KCl solution (0.001N).

Total Organic Carbon and Soil Organic Matter

The organic matter was determined according to Anne's method as described by Genin et al. (2017), which is based on the

oxidation of soil organic carbon by potassium dichromate in an acidic medium and the back titration of the excess potassium dichromate by a solution of Mohr's salt in the presence of diphenylamine sulfonate as an indicator. After titration, the percentage of total organic carbon (%TOC) was calculated according to the following formula:

$$\% \text{TOC} = 3.9 \frac{V_c - V_s}{m * V_c} \quad (1)$$

where: V_c (cm^3) = Control volume, V_s (cm^3) = sample volume and m (g) = soil dry mass.

The soil organic matter content (%OM) was calculated as follows:

$$\% \text{OM} = \frac{\% \text{TOC} * 100}{58} \quad (2)$$

Soil Mineral Content

To assess the mineral content of *A. cyanophylla* soil, 2 g of rhizospheric and bare soil were digested in sulfuric acid solution at 600°C for 6 h. Ash was mixed with 2 ml of HCl (10N), agitated, evaporated on a hot plate, and collected in 100 ml of distilled water. The contents of K⁺, Na⁺ and Ca²⁺ were determined according to the method of Brown and Lilleland, (1946) using a flame spectrophotometer. The total nitrogen was determined using the Kjeldahl method (Bremner, 1960). The available phosphorus was determined using the molybdenum blue colorimetric technique (Wei et al., 2009). The optical densities of the solutions were measured at 880 nm using an S-22 UV/Vis spectrophotometer (Boeco, Germany).

Enumeration of Arbuscular Mycorrhizal Fungi Spores in Soils

Isolation of arbuscular mycorrhizal fungi spores from rhizosphere soil was performed according to the method described by (Brundrett et al., 1996), based on the extraction of spores by wet sieving and decanting of soil followed by centrifugation in a sucrose solution. So, 100 g of rhizosphere soil was suspended in 1 L of tap water. The mixture was then stirred and then left to settle. After decantation, the supernatant was poured through a series of sieves ranging in size from 800 μm to 50 μm to remove the largest particles of organic matter from the soil while still retaining spores of different sizes. The operation was repeated several times to recover as many spores as possible. Spores suspension retained in sieves was suspended in distilled water and centrifuged for 5 min at 2000 rpm, the supernatant and floating debris were removed to add 20 ml of sucrose solution (65%) to the pellet for 1 min centrifugation at 2000 rpm to separate the spores of denser soil components. The supernatant was filtered through a filter paper under a vacuum, and the spores were collected on Petri dishes for enumeration using a binocular magnifying glass (Magnification:×40).

Morphological Description of Arbuscular Mycorrhizal Fungi Spores Associated With *Acacia cyanophylla*

Spore extraction was based on the wet sieving and decantation of the soil samples (Gerdemann and Nicolson, 1963) and the concentration of the spores by centrifugation on a sucrose solution (Brundrett et al., 1996). The isolated spores were sorted under the binocular (Magnification $\times 40$) according to morphological characters (color, size, shape). Other morphological parameters were used under a microscope after mounting the spores between slide and coverslip, such as the diameter of the spores, the lamellar structure of the spore wall, and the form of attachment of the hyphae to the spore. Finally, recovered spores were stored in a polyvinyl-lacto-glycerine (PVLG) medium until use (Estaún et al., 1997). Spores with similar morphological traits were grouped in a homogeneous group called a morphotype. The relative abundance of each morphotype was calculated as the number of spores of that morphotype divided by the total number of spores from each soil (El kinany et al., 2018).

Determination of Mycorrhizal Traits in the Roots of *Acacia cyanophylla*

Thinning and coloring of roots were carried out following the modified method of Phillips and Hayman (1970). The roots were washed under a gentle stream of water to remove attached soil, organic matter, and foreign roots. A 10% KOH solution was used to bleach the roots for 60 min at a temperature of 90°C. The samples were then rinsed and acidified by adding a few drops of 5% lactic acid, which neutralized the remaining KOH. The thinned roots were stained with a 0.05% acidic trypan blue solution, diluted in lactoglycerol (1/3 water, 1/3 glycerol and 1/3 lactic acid) for 15 min at 90°C. The colored roots were then mounted between the slide and coverslip and observed using a Microscope. The mycorrhizal frequency represents the number of mycorrhizal roots in the total examined roots. While the mycorrhizal colonization provides information on the total volume of the root colonized by different fungal structures. It was estimated according to five classes of colonization 0 (non mycorrhized at all), 1 (trace of mycorrhization), 2 (less than 10% colonization), 3 (between 11 and 50% colonization), 4 (between 51 and 90%), 5 (more than 91% colonization) (McGonigle et al., 1990; Brundrett et al., 1996). The volume occupied by each different mycorrhizal structure (Mycelium, vesicles, arbuscules) was also estimated by microscopic observations.

$$M\% = (I n_5 + II n_4 + III n_3 + IV n_2 + V n_1 + VI n_0) \quad (3)$$

where n_5 is the number of fragments with index 5, n_1 is the number of fragments with index 1, and I is the frequency assigned to the index 5.

The absolute mycorrhization intensity (Mycorrhization intensity among mycorrhized fragments) is calculated as follows:

$$m\% = \frac{M\%}{Mf} * Tf \quad (4)$$

where Tf = Total fragments and Mf = Mycorrhized fragments.

Determination of the Mycorrhizal Rhizospheric Soil Infectivity of *A. cyanophylla* by the Method of the MPN of Fungal Propagules

The Most Probable Number (MPN) method was performed to determine the mycorrhizal infectious potential of a soil which exhibits the ability of that soil to initiate the formation of mycorrhizal associations in a test plant from a quantity of inoculum present in rhizosphere soil in the form of spores, mycelium and root debris bearing vesicles (propagules) (Ouahmane et al., 2012). For this, the five samples of rhizospheric soils taken were mixed to have a homogeneous composite, and the latter was diluted in sterile soil (121°C, 2 h) in proportion (1, 1/4, 1/16, 1/64, 1/256, 1/1,024) with 5 repetitions for each dilution level. In parallel, the control soil was diluted in the same way. Maize was used as an endophytic plant to trap the native mycorrhizal complex associated with *A. cyanophylla*. Corn seeds were pre-germinated in Petri dishes at an ambient temperature of 25°C and then planted in plastic cups containing 100 g of dilution soil. After 4 weeks of cultivation, the plants were harvested and their root systems were carefully prepared for staining. During reading, roots representing at least one point of infection were classified as positive and negative ones were presented by lack of colonization (Ouahmane et al., 2007a; Ramos-Zapata et al., 2010). The estimation of the MPN was performed using the table of Fisher and Yates (1970) and the following formula:

$$\text{Log } 10 \text{ MPN} = (x \log a - K) \quad (5)$$

where x is the average number of mycorrhized pots, a is the dilution factor and k is a constant from table VIIItbl of Fisher and Yates for fivefold dilutions.

The standard deviation of log MPN is estimated and given by the formula (Cochran, 1950).

$$\sigma \log 10 \text{ MPN} = 0.55 \sqrt{\frac{\log a}{n}} \quad (6)$$

where n is the number of samples per dilution and a is the dilution ratio.

Nodulation Intensity

The evaluation of modulation of *A. cyanophylla* shrubs was carried out by direct counting of the functional nodules formed on the root system encountered in 1-kg rhizosphere soil.

Statistical Analysis

The results were processed with XL Stat software and SPSS software using the analysis of variance test (ANOVA) at a threshold of statistical significance set at 5% and the Tukey's HSD test for the comparison of the means.

RESULTS

Soil pH and Electrical Conductivity

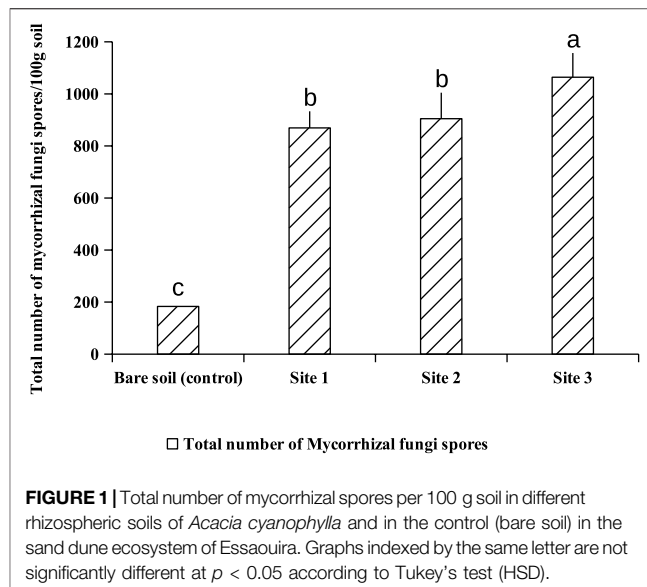
The soil pH and electric conductivity analysis showed significant differences between the rhizosphere soil and the control (bare soil) (Table 1). The introduction and establishment of golden wreath wattle (*A. cyanophylla*) in the sand dune of Essaouira resulted in widespread acidification of the soil in the study area. The reading of pH showed that the rhizospheric soil samples had values that were 7.4–8.6% lower than those of bare soil samples, which range from 9.10 to 8.3–8.4. Similarly, the presence of *A. cyanophylla* had shown a significant influence on electrical conductivity, which raised by 14.6–17.1% in rhizospheric soil (sites 1, 2 and 3) compared to bare soil (Table 1).

Total Organic Carbon and Mineral Content

A significant increase in the carbon and consequently in the organic matter contents of the sampled soils in the rhizosphere of *A. cyanophylla* was recorded. In comparison to adjacent bare soil, the amount of carbon in the rhizosphere rose approximately more than twice in the presence of trees at all sample locations. The pleading restitution of carbon to soil is certainly linked to the massive production of foliage by this legume (Table 1). The analysis of the rhizospheric soils under the crown of *A. cyanophylla* shrubs at the three sites studied showed organic matter contents two to three times greater than those in the bare soil. Mineral analysis of the soils showed a substantial accumulation of the main nutrients in the rhizosphere of *A. cyanophylla*. A significant difference was recorded for all the analyzed elements (N, P, K⁺, Ca²⁺, and Na⁺) compared with non-rhizosphere soil without the influence of the shrub. Enrichment of the soil by the golden wreath wattle was noticed for all the analyzed elements and which testified their contents doubling (P, K⁺, Ca²⁺, and Na⁺), particularly the nitrogen contents were three (site 1) to four (sites 2 and 3) times higher in rhizospheric soil than in control soil (Table 1). The quantity of available phosphorus and total potassium in the rhizosphere enhanced roughly 1.7–1.8 times in the presence of tree roots when compared to bare soil at all study sites as compared to bare soil.

Enumeration and Morphological Description of AMF Spores Associated With *A. cyanophylla*

Extraction and counting of spores from subjacent soils of *A. cyanophylla* showed at least 800 spores per 100 g of rhizosphere soil, while non-rhizosphere soil showed 160 spores per 100 g of soil only (Figure 1). *A. cyanophylla* had shown a greater capacity to establish a symbiosis with arbuscular mycorrhizal fungi and higher production of fungal spores in the soil. The community of AMF associated with *A. cyanophylla* was described by the relative abundance of different morphotypes of encountered spores. Observation of the spores under the binocular microscope at high magnification revealed the presence of four different morphotypes depending on their color: black, dark brown,



light brown, yellow and grey (Figure 2). As demonstrated in Figure 3, each AMF morphotype is represented by its relative abundance in *Acacia cyanophylla* rhizospheric soils as well as in control (bare soil).

Morphotype 1: dark brown spores; this is the most common group in different plants-sites with a relative abundance of 40–50%.

Morphotype 2: light brown spores which exhibit a relative abundance of 30–47%.

Morphotype 3: black spores with a relative abundance of 15–23%.

Morphotype 4: greyish transparent spores with a relative abundance of only 3–7%.

Determination of Mycorrhizal Traits in *Acacia cyanophylla* Plant Roots

Microscopic observation of the roots of *A. cyanophylla* in the various sites showed that all the plants presented an arbuscular mycorrhizal fungus infection, and at least the mycorrhizal frequency reached 90%. The mycorrhizal colonization assessment showed rates between 40 and 50% (Table 2). The intensity of mycorrhizal infection (Ma %) varied between 40, 44, and 50% of roots collected in rhizospheric soil at sites 3, 1, and 2, respectively. It was discovered that the levels of vesicles and hyphae varied according to the location of the plants and rhizospheric soil collected; for example, *Acacia cyanophylla* roots from site 1 had the highest proportion of vesicles (60%), while those from sites 2 and 3 had the highest percentage of hyphae (70%). The proportion of arbuscules, on the other hand, is almost identical (10%) throughout the three sampling sites. *A. cyanophylla* roots showed higher levels of colonization by arbuscular mycorrhizal fungi, indicating that *A. cyanophylla* is one of the symbiotic plants that weave a strong relationship with AMF in their natural habitats.

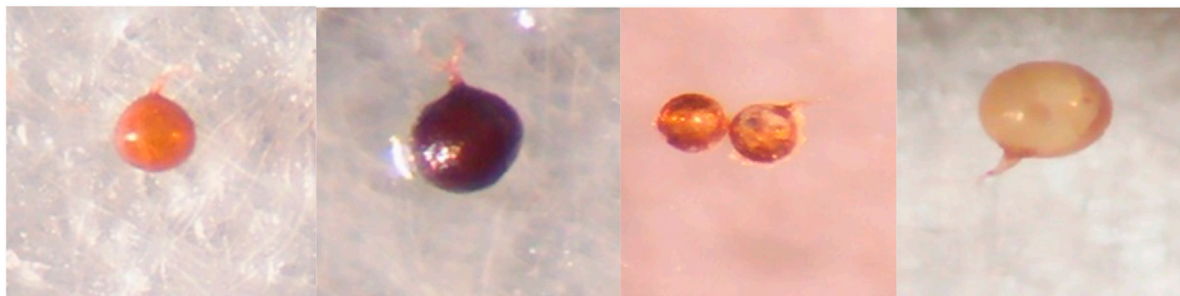


FIGURE 2 | Photos of the main representative mycorrhizal morphotypes encountered in the rhizosphere soil of *Acacia cyanophylla* in the sand dune ecosystem of Essaouira, Morocco.

TABLE 2 | Mycorrhizal and nodulation traits in roots of *Acacia cyanophylla* collected in different sites in the sand dune ecosystem of Essaouira.

	Mycorrhizal frequency (Fa %)	Mycorrhizal infection intensity (Ma %)	Mycorrhizal colonization (ma %)	Vesicles (%)	Arbuscules (%)	Hyphae (%)	Nodulation frequency (%)	Total number of nodules/kg soil
Site 1	90 ^a	44 ^a	50 ^b	60 ^a	10 ^a	30 ^b	100 ^a	124 ^a
Site 2	100 ^a	50 ^a	60 ^a	20 ^b	10 ^a	70 ^a	100 ^a	89 ^b
Site 3	100 ^a	40 ^a	60 ^a	20 ^b	10 ^a	70 ^a	100 ^a	111 ^a

In the same column, the values of each parameter of mycorrhizal and nodulation traits indexed by the same letter are not significantly different according to the Tukey test (HSD) ($p < 0.05$).

Assessment of Mycorrhizogenic Infectious Potential of *A. cyanophylla* Rhizospheric Soil Using the MPN of Fungal Propagules Test

Maize plants have been used as an endophytic plant to trap the native mycorrhizal complex naturally associated with *A. cyanophylla*. Corn seedlings were grown on different dilutions of rhizosphere soils (1; 1/4; 1/16; 1/64; 1/256; 1/1,024) from different sites under *A. cyanophylla*. A seedling is considered to be mycorrhizal when it has at least one point of infection. Only one among five corn seedlings was mycorrhizal in the control soil, which confirms the lower number of spores in this soil (Figure 1). However, all the roots of Maize seedlings grown in rhizospheric soils of *A. cyanophylla* exhibited a mycorrhizal infection with different colonization rates. The number of fungal propagules (viable spores, vesicles, root fragments) in each soil was determined using the table of Fisher and Yates. Based on the calculation, the MPN per 100 g was: MPN (IC 95%) = 1,240 for rhizosphere soil and was MPN (95% CI) = 48 for non-rhizospheric soil (Figure 4). The mycorrhizal fungal community of *A. cyanophylla* rhizosphere soil was assessed for its self-regeneration by testing the most probable number of fungal propagules in the soil without distinguishing between these propagules (viable spores, vesicles, root fragments).

Nodulation Activity

The direct counting of the total number of nodules formed by symbiotic rhizobacteria on *A. cyanophylla* roots showed that all

the rhizosphere soil samples contained nodulated root fragments. All the root fragments at the level of the rhizosphere soil samples were examined and approximately 130 nodules per kilogram of soil were noted (Table 2).

DISCUSSION

In this study, golden wreath wattle (*Acacia cyanophylla*), an exotic shrub/tree species, was selected to examine the soil's chemical and biological fertility in the sand dunes of Essaouira, Morocco. Generally, soils are the result of the activities of plants, which provide organic matter and play an important role in the weathering of rocks and mineral resources (Liu et al., 2022; Qin et al., 2019). In our study, the results of the physicochemical analysis suggest that *A. cyanophylla* has an advantage on chemical soil fertility, explained by the increase in the total organic matter and the content of mineral elements in the soil surrounding roots of *A. cyanophylla*. These results can be attributed to the fact that this shrub species is able to produce considerable amounts of biomass (Guarino and Sciarrillo, 2017). The roots and aerial biomass turn into the soil and undergo the degradation and mineralization process. This phenomenon leads to the soil enrichment in organic matter and mineral elements (C, N, P, K, Ca, Mg, N) (Yusoff et al., 2019). The environmental conditions of the study site (precipitations, temperature and sandy nature of the soil) allow the rapid transformation of organic matter and its incorporation into the soil, especially under the crown of

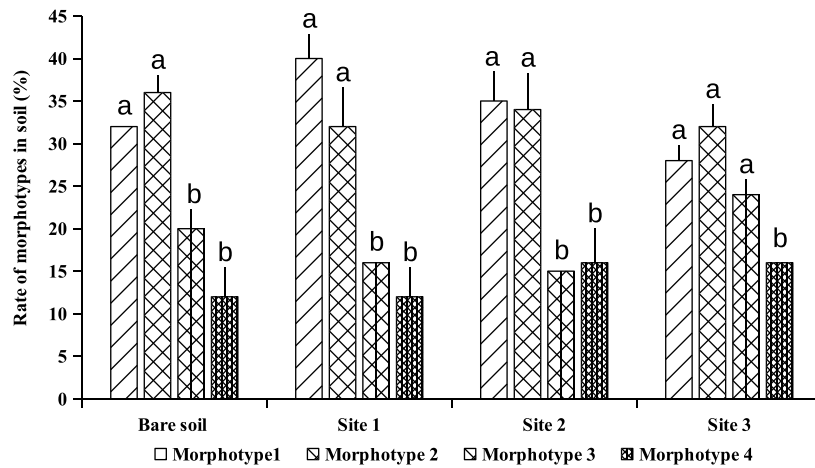


FIGURE 3 | Relative abundance of each AMF morphotype in rhizospheric soils of *Acacia cyanophylla* and in the control (bare soil) in the sand dune of Essaouira. For each soil sampling site, the bar-graphs (rate of each morphotype) indexed by the same letter are not significantly different at $p < 0.05$ according to the Tukey's test (HSD).

shrubs. In addition, *A. cyanophylla* is a legume species capable of conducting dual symbiosis relationships with arbuscular mycorrhizal fungi and atmospheric nitrogen-fixing rhizobacteria (Birnbaum et al., 2017). These two types of symbiosis are traditionally known for their undeniable respective roles in phosphate and nitrogen nutrition. The levels of available phosphorus and total nitrogen in rhizospheric soils bear witness to these effects (Amira et al., 2012; Yusoff et al., 2019). In the current work, the phosphorus contents are twice as high and the total nitrogen contents are four times higher compared to a non-rhizosphere soil without the influence of the roots of *A. cyanophylla*. On the other hand, it is possible that soil type, climate, and plant species have an impact on the species composition of AMF fungal communities. Our findings suggest that *A. cyanophylla* is associated with a diversified fungal community, and at least we could distinguish four different morphotypes among the mycorrhizal community

(Figure 3). The study of the morphological diversity of mycorrhizal fungi associated with the rhizospheric soil of *A. cyanophylla* has shown the existence of several morphotypes which show significant diversity (Afaf et al., 2015; Bouazza et al., 2015). This community of AMF adapted to these environments forms a mycelial network around the root system and allows the exploitation of a large surface at ground level, hence their capital role in the supply of water and phosphate for the plant. Otherwise, the self-regeneration capacity of the mycorrhizal fungal community in *A. cyanophylla* rhizosphere soil was determined by counting the most probable number of fungal propagules in the soil. We discovered that all the roots of Maize seedlings grown in *A. cyanophylla* rhizosphere soils had a mycorrhizal infection with varying colonization rates. These results confirmed the existence of an active community and a large number of viable propagules around the roots of *A. cyanophylla*, which provides information on the adaptation and functioning of these symbionts in the sand dune ecosystem of Essaouira (Guarino and Sciarrillo, 2017). This effect was also noticed in the rhizospheric soil of other invasive plants such as *Retama monosperma*, which presented a greater mycorrhizal potential, as well as *Nicotiana glauca*, which is a very mycotrophic shrub capable of increasing the stock of mycorrhizal propagules and thus serving as an effective source of mycorrhizal inoculum in severely disturbed and degraded soils characterized by very low mycorrhizal potential (Maier et al., 2000). Similar results were recently proved in mixed-species plantations of *Eucalyptus* and *Acacia* (Tchichelle et al., 2017). Moreover, *A. cyanophylla* was previously reported to exhibit a large number of nodules on the root system and to form symbioses with a higher number of *Rhizobia* strains (Amira et al., 2012; Bouazza et al., 2015; Birnbaum et al., 2017). The exotic shrub *A. cyanophylla* has shown its primordial role in maintaining and improving the chemical and biological fertility of the soil in the sand dunes ecosystem of Essaouira (Kavroulakis et al., 2020; Lozano et al.,

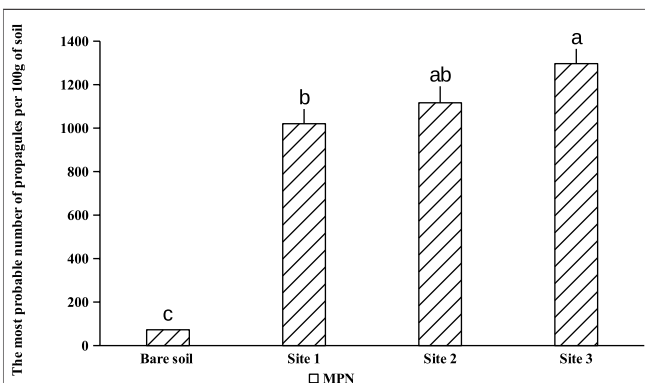


FIGURE 4 | The most probable number of fungal propagules in different rhizospheric soils of *Acacia cyanophylla* in the sand dune ecosystem of Essaouira. Bar-graphs indexed by the same letter are not significantly different according to the Tukey test (HSD) ($p < 0.05$).

2020; Tigka and Ipsilantis, 2020). These ecological and nutritional functions are ensured thanks to the rapid growth of the species, its enormous production of biomass, and its symbiotic status as a host plant for the rhizobacteria fixing atmospheric nitrogen and for arbuscular mycorrhizal fungi, which are known for their intervention in biogeochemical cycles of major nutrients such as phosphorus and nitrogen as well as their crucial role in the water nutrition of the host plants. According to our collected data, the total nitrogen level in the rhizosphere was three to four times greater than the nitrogen content in bare soil (Table 1). These interesting findings are in line with some previous studies and directly comparable to those published recently by Jian et al. (2022), who revealed the beneficial effects of *Leymus secalinus* and *Carex praeclara* on soil characteristics and microbial community composition in a Zoige desertified alpine grassland.

Indeed, all these potentialities and biological qualities highlighted and possessed by *A. cyanophylla* allow it to be advocated and recommended as an exotic plant in the programs aiming to combat soil degradation and to protect natural resources. Hence, the association of *A. cyanophylla* with fungal and rhizobial symbionts in reforestation programs would be of great importance to guarantee the success of Atlantic dunes fixation and to fight against the desertification phenomenon.

CONCLUSION

A. cyanophylla as an exotic plant has shown a vast ability to establish a dual symbiotic association with arbuscular mycorrhizal fungi and with atmospheric nitrogen-fixing rhizobacteria. *A. cyanophylla* showed beneficial effects on the quality and the fertility of the sand dune ecosystem soil of Essaouira. The rhizosphere microbial communities work in harmony with the plant to improve its nutrition and growth.

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Indeed, the fungal and bacterial partners provide the plant with sufficient amounts of water, phosphorus, nitrogen and other nutrients elements. On the other hand, *A. cyanophylla* is a fast-growing plant, and the large quantities of biomass produced by the plant are returned to the soil after their degradation and mineralization, which induces high levels of organic and mineral matter in the rhizosphere soil. *A. cyanophylla* can therefore modulate and maintain a sustainable balance between the different abiotic and biotic components involved in these interactions. The high potential of the rhizosphere soil to regenerate mycorrhizal and rhizobial associations testifies to the ecological balance established between the plant and its microbial symbionts. Hence, using golden wreath wattle in reforestation programs to rehabilitate and fix sand dunes is strongly recommended.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

HD, MB, EO, and MB: methodology, writing—original draft, formal analysis, reviewing, and editing. H-AN, ME-S, GA-A: reviewing, visualization, and editing. LO: visualization, supervision and validation.

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