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Research Article

Mycorrhizal status of *Plantago coronopus* L. in relation to edaphic parameters in a coastal dune of Oran

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Abstract

The present study was conducted in order to contribute to a better understanding of the psammo-halophytes adaptation to their hostile environments through the determination of their mycorrhizal status. It concerned the mycorrhizal status evaluation of *Plantago coronopu*s L. in relation to edaphic parameters of its rhizosphere in a dune on Oran coast of Northwestern Algeria. Rhizospheric soil and root samples were collected from three sites on dunes of Bomo-beach, in the west of Oran. Mycorrhizal colonization parameters of the plant and the physicochemical parameters of the substrate were determined. The results showed that the substrate had a sandy-silty texture, it was not saline, very poor in water and nutrients, with a high load of total limestone and low organic carbon and total nitrogen and assimilable phosphorus. Roots were colonized by Arbuscular mycorrhizae fungi type with a high average frequency (61.34%). Principal Component Analysis results revealed that mycorrhizal parameters were positively correlated with soil salinity, organic carbon and organic matter, total nitrogen and silt. However, the correlation between mycorrhizal parameters and pH, active and total limestone was negative.

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Introduction

Plantago coronopus L. belonging to *Plantaginaceae* family is one of the characteristic Mediterranean plant species of low-altitude dunes and sub-halophilic lawns. It is found in coastal cliffs, coastal dunes, and salt marshes, where it grows on dry or wet, acid to basic, rocky, or sandy soils. It is a native species to Eurasia and North Africa, but it is introduced in Australasia and North America, where it is used in the nutraceutical and pharmaceutical industries (Pereira et al., 2017; Schmidt and Maslo, 2020).

It is one of the coastal halophytes with phytochemical, ecological and physiological peculiarity. The efficiency of water use and the accumulation of compatible solutes under saline conditions are considered among the strategies of adaptation to its environment (Koyro, 2006). It has high phenotypic plasticity which increases its chance to survive on saline soils with variable salinity levels and under other environmental conditions (Smekens and van Tienderen, 2001).

Most of the plants, including halophytes, form a communication pathway with mycorrhizal fungi, which is one of the most remarkable and durable adaptations (Strullu-Derrien et al., 2018). Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs, colonizing the roots of nearly 90% of higher plants benefiting from photo-assimilates (Brundrett and Tedersoo, 2018). They promote tolerance to abiotic and biotic stresses through enhanced mineral nutrition (Diagne et al., 2020). They play a key role in the functioning of terrestrial ecosystems, affecting plant productivity and diversity, improving soil structure and regulating nutrient cycles (Koziol and Bever, 2016; Powell and Rillig, 2018).

There is no doubt that the mutualistic interactions between halophytes and mycorrhizal fungi are very important and have recently attracted the attention of scientists (Saxena et al., 2017). Among the studies carried out on mycorrhization, we mention those conducted on halophytes of interest such as *Asteriscus maritimus* (Estrada et al., 2013), *Aeluropus littoralis* (Hajiboland et al., 2015) and *Limbarda crithmoides* (Sidhoum and Fortas, 2018). Although *P. coronopus* is known to occur in a variety of habitats, very little research has been conducted on its mycorrhization (Mason, 1928; Hildebrandt et al., 2001; Dodd et al., 2002; Oliveira et al., 2005) and no specimens from coastal dunes have been studied. While recently, a study was conducted on the sporal morphology as well as the identification of AMF species associated with *P. coronopus* growing on a coastal dune in rugged Algeria (Tabti and Bendimered-Mouri, 2022). Thus, the objective of the present study was to complete the one previously carried out on the same study sites and consisted of the evaluation of the rate of mycorrhizal colonization of this species in relation to the physico-chemical characteristics of its substrate in the same region. We hypothesized that there is a relationship between the parameters of mycorrhizal colonization and the main physicochemical parameters of the substrate.

Materials and Methods

Study area

The study area is located at the dune of Bomo beach in the Commune of Bousfer, Daira of Ain El Turk, Wilaya of Oran (Western Algeria) (Figure 1). Individuals of *P. coronopus* population growing spontaneously in the lower fringe of the dune, in the semi-fixed part of the dune complex of Cap Falcon, located about 20 km west of Oran, were chosen. The geographical and ecological characteristics of the studied environment are mentioned in Table 1.

Sampling of rhizospheric soils and roots

Three sampling sites (S1, S2, and S3), ten meters apart, were chosen to compare the mycorrhizal load of the plant studied.

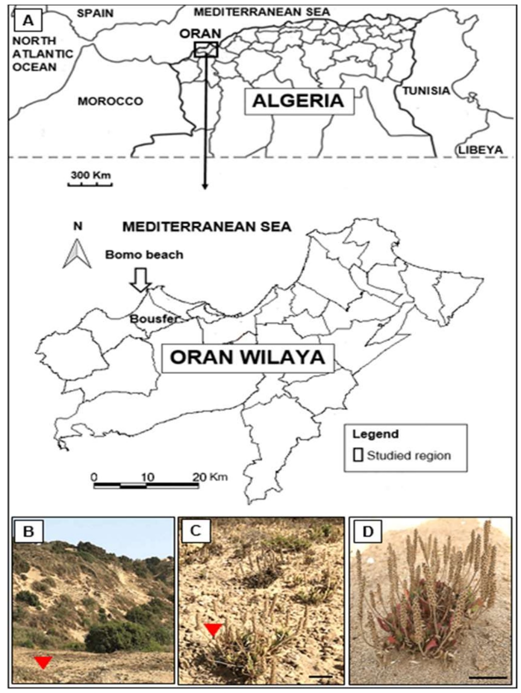

Figure 1. Location of the study site and *Plantago coronopus* photos in its natural site. A- Geographical location of the studied region (Bomo beach in the Commune of Bousfer, Wilaya of Oran, in northwest Algeria). B- Populations of *P. coronopus* at the bottom of the dune. C- and D- *P. coronopus* in its natural site (the scale bar corresponds to 5 cm).

Table 1: Geographical and ecological characteristics of the study area.

Geographical Parameters	
Latitude	35° 45’ 10’’ North
Longitude	0° 49’ 47’’ West
Altitude	7 m
Distance from the sea	200 m
Climatic and bioclimatic characteristics	
Climatic type	Mediterranean
Bioclimatic stage	semi-arid with worm winter
Annual average precipitations	364.5 mm
Average temperatures	18.33 °C
Annual average relative humidity	80%
Substrate and vegetation characteristics	
Substrate	Sandy, light, rich in shell debris and poor in nutrients
Type of dune	Foredune
Vegetation	Natural, sparse, heterogeneous, low and characterized by the presence of herbaceous plants such as: *Ammophila arenaria (*L.) Link., *Plantago coronopus* L., *Malcomia arenaria* (Desf.) DC., *Echium arenarium* Guss., *Medicago littoralis* Rohd. ex Loisel*,* *Lotus creticus* L.*, Orlaya maritime (*Gouan) W.D.J. Koch *, Ononis variegata* L.*, Cyperus kalli* (Forssk.) Murb.

Soil samples were collected in spring 2018. At each site, five rhizospheric soil and root samples of *P. coronopus* were collected at a depth of 0-20 cm. Soil samples were air-dried and sieved using a 2 mm diameter mesh screen. Physicochemical analysis of the soils and an estimation of mycorrhizal colonization were performed.

Physicochemical characterization of soils

The physic-chemical analysis of the soils concerned the following parameters: the Soil moisture (SM), by the gravimetric method; the granulometry by the Robinson pipette method (Gee and Or, 2002); the pH and the electrical conductivity (EC) by the electrometric method; the soil organic carbon (SOC) by the Walkley and Black titrimetric method (Nelson and Sommers, 1996) to calculate the soil organic matter (SOM) content; the total limestone (TCaCO3) by the Bernard calcimeter method and active limestone (ACaCO3) by the Drouineau-Galet method (1942); the Assimilable phosphorus (AP) by the Olsen method (Sims, 2000) and the total nitrogen (TN) by the Kjeldahl method (Bremner, 1996).

Estimation of mycorrhizal colonization

For the observation of mycorrhizal structures, thirty fine root fragments per sample were examined under an optical microscope (Axio Scope A1, Carl Zeiss, Germany), after staining, according to the method of Phillips and Hayman (1970). To evaluate the degree of mycorrhizal colonization of these fragments, five parameters were calculated: *F, M, m, a*, and *A*, according to the method of Trouvelot et al. (1986). *F*: Frequency of mycorrhizae in the root system; *M*: Intensity of the mycorrhizal colonization in the root system; *m*: Intensity of the mycorrhizal colonization in the root fragments; *a*: Arbuscules abundance in mycorrhizal parts of root fragments; *A*: Arbuscules abundance in the root system. Their respective formulas are:

$$F = \frac{n}{N} \times 100$$

N: total number of root fragments observed, n: Number of mycorrhizal fragments.

$$M = (95n5 + 70n4 + 30n3 + 5n2 + n1)/N$$

n5, n4, n3, n2, and n1 are number of mycorrhized fragments rated 5, 4, 3, 2, and 1, respectively.

Class 5 represent more than 91%; class 4; between 51% and 90%; class 3, between 11% and 50%; class 2, less than 10%; class 1, traces and class 0, no mycorrhization.

m = M x (total number of fragments/ the number of mycorrhized fragments

a = (100mA3 + 50mA2 + 10mA1)/100

mA3, mA2, and mA1 are the percentages of m, rated A3, A2, and A1, respectively.

mA3 = ((95n5A3 + 70n4A3 + 30n3A3 + 5n2A3 + n1A3)/n) x 100/m (and the same for A2 and A1)

A0, is no arbuscules; A1, some arbuscules: 10%; A2, moderately abundant arbuscules: 50%; A3, very abundant arbuscules: 100%.

$$A = a \times (M/100)$$

A and *a* are the specific parameters for evaluating the presence of the AMF type.

Statistical analysis

One-way analysis of variance (One-Way ANOVA) and Fisher's Least Significant Difference (LSD) test were used to test the difference in soil and mycorrhizal parameters between the three selected sites. A PCA (principal component analysis) was applied to determine the correlation that may exist between edaphic parameters and mycorrhizal colonization parameters. The data were treated with *Statistica* version 6.0 software.

Results and Discussion

Physicochemical characterization of rhizospheric soil

The rhizospheric soil samples analyzed showed that it was a sandy-loam soil texture for all three sites (Table 2). They also showed very low water content (<2%), low organic matter (<1.5%), assimilable phosphorus (<11 ppm) and total nitrogen (< 0.05%). They were alkaline (pH >7.5) and not or saline (<500 µs/cm). In contrast, they were rich in total limestone (≥25%) and active limestone (≥5%). Most of the soil parameters, such as moisture, pH, electrical conductivity, organic carbon, organic matter, assimilable phosphorus, total nitrogen, total limestone, and active limestone contents, showed a highly significant difference between the three sites (F = 0.92, p<0.01). Then, soil samples from site S3 showed higher values of electrical conductivity, soil organic carbon, soil organic matter, and total nitrogen than the other sites. Site S1 showed high percentages of soil moisture (which is still very low), assimilable phosphorus, total limestone and clay compared to the other sites. The percentages of active limestone and silt were high in site S2.The high pH is due to alkaline sediments of marine origin and low soil organic matter (Pan et al., 2016). This high alkalinity could also be explained by several other factors, such as lower precipitation (Wezel et al., 2000) and high temperatures that contribute to increased evapo-transpiration and low leaching. Dune soils often have low availability of mineral elements due to the particulate texture of the soil, which results in high leaching and influences the chemical and biological composition (Gilbert et al., 2008). The sparse and annual vegetation that characterizes the study site limits the availability of soil organic matter.

Table 2. Results of soil physicochemical analysis of the three study sites.

Soil Parameters	Sites		
	S1	S2	S3
SM (%)	1.87 ± 0.03[a]	1.23 ± 0.03[b]	1.31 ± 0.03[c]
pH	8.43 ± 0.08[a]	8.29 ± 0.08[b]	8.15 ± 0.05[c]
EC (µs/ cm)	219.6 ± 1.73[a]	212.7 ± 2.86[b]	224.3 ± 0.97[a]
SOC (%)	0.49 ± 0.03[a]	0.36 ± 0.08[b]	0.56 ± 0.05[c]
SOM (%)	0.84 ± 0.06[a]	0.62 ± 0.04[b]	0.91± 0.01[c]
AP (ppm)	8.19 ± 0.5[a]	7.9 ± 0,5[b]	7.79 ± 0.7[c]
TN (%)	0.04 ± 0.04[a]	0.033 ± 0.03[b]	0.05 ± 0.01[c]
$TCaCO_3$ (%)	27.4 ± 0.11[a]	25 ± 0.15[b]	25.8 ± 0.07[b]
$ACaCO_3$ (%)	4.8 ± 0.03[a]	6 ± 0.06[b]	5 ± 0.03[a]
Granulometry (%)			
Sand (%)	76 ± 2.88[a]	77 ± 1.2[a]	78.2 ± 1.11[a]
Silt (%)	15.44 ± 1.78[a]	17.2 ± 0.64[a]	15.3 ± 0.85[a]
Clay (%)	8.54 ± 0.55[a]	5.8 ± 0.57[a]	6.5 ± 1.04[a]
Textural classification	Sandy loam	Sandy loam	Sandy loam

Note: Data are presented as mean ± standard error (SE) of five replicates per sample.Mean values in columns followed by the same letter do not differ significantly ($p < 0.05$) by LSD test. SM: soil moisture; EC: electrical conductivity; SOC: soil organic carbon; SOM: soil organic matter; AP: assimilable phosphorus; TN: total nitrogen; $TCaCO_3$: total limestone; $ACaCO_3$: active limestone.

Assessment of mycorrhizal colonization of roots

Different endomycorrhizal structures were observed in *Plantago coronopus* roots, vesicles, arbuscules, spores and hyphae (Figure 2). The characteristics of this colonization are presented in Figure 3.

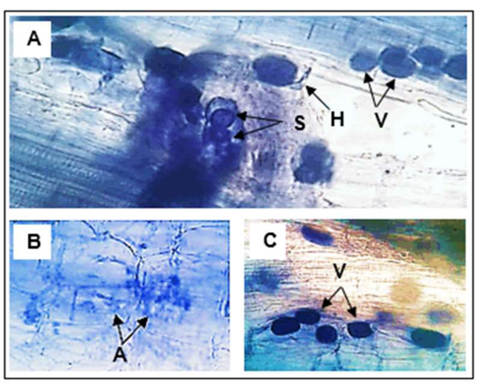

Figure 2. Microscopic photographs of mycorrhizal structures observed in the root fragments of *P. coronopus* stained with Trypan blue. A and C: 100x and B: 40x, V: Vesicles; H: Hyphae; S: Spores; A: Arbuscules.

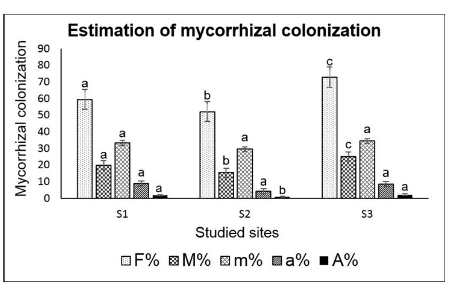

Figure 3. Mycorrhizal colonization rate of *P. coronopus* roots from the three sites considered (S1, S2 and S3).

Data are presented as mean ± Standard Error (SE) of five replicates per sample. Means followed by the same letter are not significantly different ($p < 0.05$) by LSD test, F: frequency of mycorrhizae in the root system; M: intensity of the mycorrhizal colonization in the root system; m: intensity of the mycorrhizal colonization in the root fragments; a: arbuscule abundance in mycorrhizal parts of root fragments; A: arbuscule abundance in the root system

The ANOVA shows that there is a highly significant difference between the three sites ($F = .92$, $p< 0.01$) in frequency (F) and intensity of mycorrhizal colonization (M). Both of these mycorrhizal colonization parameters were higher in site S3 (Figure 3). The mycorrhizal colonization parameters evaluated showed a variation from one individual to another; this can be explained by the phonological characteristics of the individuals. In a study carried out in a highly alkaline anthropogenic sedimentation basin in northern Portugal (Oliveira et al., 2005), it was reported that the same mycorrhizal structures were observed in *P. coronopus* and that the length of roots colonized by the mycorrhizal arbuscular fungus varied from 52% to 65% (depending on the season).

Various bibliographic information indicate that AMF-type mycorrhizae can establish effective symbiotic relationships with this species. Then, Dodd et al. (2002) found a percentage of mycorrhizal colonization of 84% at Samphire Hoe, Kent, UK. Hildebrandt et al. (2001) obtained a colonization rate that varied from 44% to 46% in *P. coronopus* in the salt marshes of Schiermonnikoog (Netherlands). Our results showed a low abundance of arbuscules compared to vesicles and which is higher than that found by Hildebrandt et al. (2001). The low abundance of arbuscules could be explained by their short lifespan (Jakobsen et al., 2003), in contrast to vesicles which have a longer lifespan (Birhane et al., 2017). Experimental evidence shows the crucial role of arbuscular forms of mycorrhizae in nutrient exchange (Jakobsen et al., 2003) and that of vesicles and spores in lipid carbon storage (Rich et al., 2017) and reproduction. Based on morphotypic identification of spores, recent work showed that *P. coronopus* rhizosphere from the same region was rich in endomycorrhizal species of type (AMF) (Tabti and Bendimered-Mouri, 2022). Thus, identified species belonged to the following genera: *Glomus, Septoglomus, Rhizophagus, Diversispora, Funneliformis, Dentiscutata, Claroideoglomus, Scutellospora,* and *Entrophospora* and *Glomeraceae* family were the most represented. The spore density evaluated was 107.94 spores per 100g of dry soil. The dominance of *Glomeraceae* allows a better adaptation of the plant to stressful conditions, such as aridity and drought (Panwar and Tarafdar, 2006). On the other hand, more studies have shown the effect of abiotic factors such as soil physicochemical properties on mycorrhizal parameters (Lekberg et al., 2007; Alguacil et al., 2016). The number of spores recorded in the rhizosphere of *P. coronopus* from the coastal dune of Oran was 115.2, 83.2 in site 2 and 125.4 in site 3, respectively, the mean value was 107.94 spores per 100 g dry soil (Tabti and Bendimered-Mouri, 2022).

Correlation between mycorrhizal parameters and edaphic variables

PCA results showed that spore density (SD) and mycorrhizal parameters were correlated with edaphic variables (Figure 4). Positive and significant correlations were found between electrical conductivity and mycorrhizal parameters (r=0.98). Despite the slight elevation of salinity in Site S3, which remains low by standards compared to the other sites, it does not appear to negatively influence mycorrhizal parameters. Liu et al. (2016) showed that the rate of AMF colonization was positively correlated with EC in two halophytes *Lycium barbarum* L. and *Elaeagnus angustifolia* L. in saline soils in Ningxia, China. Similar results were reported in mining soils in northwest China, where the variables OC and TN were positively correlated with mycorrhizal colonization (Qiu et al., 2019). The results found were also consistent with those found by Silva-Flores et al. (2019), who reported that TN was positively correlated with *Lithrea caustica* spore density in San Vicente, Chile. While Zhang et al. (2020) showed that TN was negatively correlated with mycorrhizal colonization and spore density in the halophyte *Kosteletzkya virginica* from Chinese coastal saline soils.

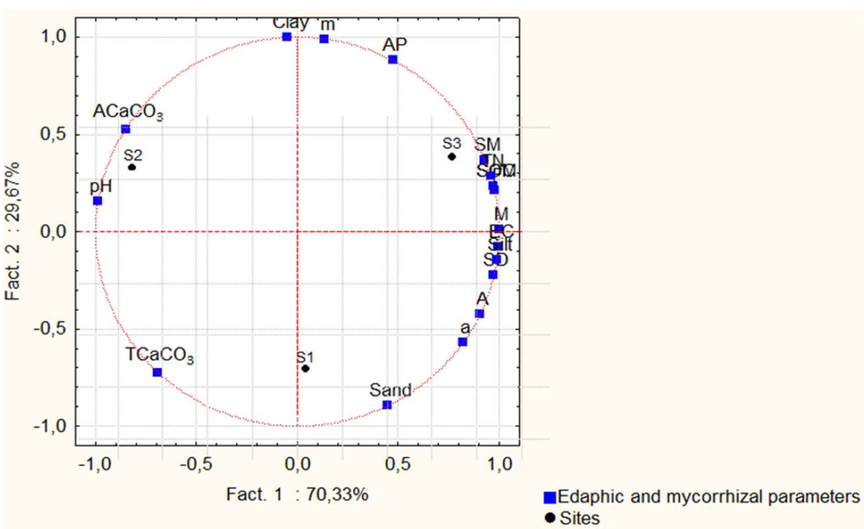

Figure 4. Results of PCA between mycorrhizal parameters and edaphic parameters of the three study sites (S1, S2, and S3). SD: spore density, F: frequency of mycorrhizae in the root system; M: intensity of the mycorrhizal colonization in the root system; m: intensity of the mycorrhizal colonization in the root fragments; a: arbuscule abundance in mycorrhizal parts of root fragments; A: arbuscule abundance in the root system; SM: soil moisture; EC: electrical conductivity; SOC: soil organic carbon; SOM: soil organic matter; AP: assimilable phosphorus; TN: total nitrogen; $TCaCO_3$: total limestone; $ACaCO_3$: active limestone.

It should be noted that the elevated levels of mycorrhizal parameters appeared to be related to the slight improvement in organic parameter levels in site S3 compared to the other sites. It was reported that mycorrhizal activity became important under these total nitrogen limiting conditions (Corrêa et al., 2015). Available water content was positively and strongly correlated with F, M, and SD (r=0.98, r=0.93, and r=0.82, $p<0.01$), and moderately correlated with arbuscular abundances (A and a) (r=0.55, $p<0.05$). The mycorrhization tends to increase under drought conditions, Becerra et al. (2005) affirmed that high soil moisture reduces colonization by AMF

Low levels of assimilable phosphorus in the three sites, especially in site S3 (Table 2) showed a positive correlation with F and m% (r=0.93, $p<0.01$ and r=0.64, $p<0.05$, respectively). Studies have shown that increasing phosphorus seems to have a significant negative influence on spore production, AMF diversity, and mycorrhizal root colonization rate (Panwar and Tarafdar, 2006; Cheng et al., 2013). The results obtained suggest that P deficiency conditions favoured mycorrhizal colonization.

Highly significant positive correlations were found between percent silt, which was low in sites S3 and S1 compared to site S2 (Table 2), and high rates of mycorrhizal parameters (r=0.99 and r=0.89) and between clay and mycorrhizal intensity m% with (r=0.98), while negative correlations were found between sand and the majority of mycorrhization parameters, including arbuscular abundances (a and A) and SD spore density (r=-0.87, r=-0.78, and r=-0.63), respectively. Zarei et al. (2008) showed that mycorrhizal parameters were positively correlated with clay and silt, but negatively correlated with sand. While Mohammad et al. (2003) found no correlation. Some researchers reported that sandy soils, due to their porosity and good aeration, stimulated the development of mycorrhizal association, while fine soil fractions prevented it (Carrenho et al., 2007). While others reported that fine fractions might have a positive effect on spore production due to water retention (Silva-Flores et al., 2019). pH had negative and significant correlations with SD, F, M, a, and A (between r=-0.99 and r=-0.90). This result is in agreement with that obtained by Zhang et al. (2020) and in contradiction with that obtained by Panwar and Tarafdar (2006) and Liu et al. (2016). While Zarei et al. (2008) found no correlation. Landwehr et al. (2002) reported that mycorrhization reaches a maximum level in highly alkaline soils such as salt marshes (pH ≥11).

The negative effect of active limestone had been confirmed on all mycorrhizal parameters (r=-0.99), (Figure 4). The low levels of mycorrhizal parameters in site S2 appear to be directly related to the high percentage of active limestone. Total limestone is negatively correlated also with mycorrhizal parameters, including F, m, and M (r=-0.82, r=-0.81 and r=-0.70, respectively) but weakly with spore density (r=-0.51). Labidi et al. (2012) reported that mycorrhizal colonization and AMF spore production were affected by high concentrations of $CaCO_3$ but without complete inhibition.

Conclusion

This study has shown that *P. coronopus* roots are colonized by AMF, with significant mycorrhizal frequencies. Rhizospheric soils were rich in limestone and poor in water and minerals. It was demonstrated that there was a relationship between the edaphic variables and the mycorrhizal colonization parameters. The mycorrhizal colonization depended on the contents of organic carbon, organic matter, total nitrogen, active limestone, silt and pH of the substrate. Further studies are needed to better understand the effect of substrate characteristics on the AMF community, the responses of *Plantago coronopus,* as species with important ecological and ethnobotanical roles, to microbiotic factors and the role of mycorrhizae in the nutritional status of this plant.

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