Root and soil fungal communities of Lycium intricatum Boiss. in Sicilian coastal dunes

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Abstract

This study investigates the fungal communities and mycorrhizal status of *Lycium intricatum* Boiss. in the coastal area of Torre Manfria (Gela, Sicily). These aspects remain largely understudied, particularly through analyses conducted in the species' natural habitat. To address this gap, we combined a morphological assessment of root samples to detect arbuscular mycorrhizal fungi (AMF) with high-throughput sequencing of roots and rhizosphere soils from plants growing on the bright and fixed dunes of the Torre Manfria coastline. Fungal communities associated with *L. intricatum* showed clear differences linked to elevation and sample type. ITS rRNA metabarcoding identified Ascomycota as the dominant phylum, followed by Basidiobolomycota and with minor contributions from Basidiomycota, Glomeromycota and other fungal taxa. In the 18S dataset, Glomeromycota communities in roots were primarily composed of *Rhizophagus*, *Claroideoglomus*, and *Glomus*, with genus-level abundances varying across sites. The presence of AM structures confirms the mycotrophic nature of the species, supporting its capacity to establish and thrive in nutrient-poor, saline dune environments. Given these traits, *L. intricatum* may represent a valuable candidate for dune stabilization and for the conservation of coastal habitats in Torre Manfria, an area threatened by erosion and the loss of natural dune systems. Overall, the species may play an important ecological role by forming buffer zones between dune landscapes and anthropized areas, contributing to vegetation connectivity, increased plant cover, and enhanced site naturalness.

Introduction

Shrubs play a disproportionately large role in supporting the functioning and stability of dryland ecosystems. By retaining sediments, limiting both wind- and water-driven erosion, and buffering microclimatic extremes, shrubs protect soils and create conditions that favour vegetation development. They may form "fertility islands" or "resource islands", namely vegetation patches that promote tree development partly by shaping soil microbial communities, particularly Plant Growth Promoting Organisms and, among them, mycorrhizal fungi (Ochoa-Hueso et al., 2018; Lombardo et al., 2020). Indeed, mycorrhizal symbioses facilitate the establishment of key plant species and thereby sustain vegetation cover in natural habitats (Azcón et al., 1997); at the same time, through interactions with other microorganisms in the rhizosphere, they improve soil fertility and quality (Maurice et al. 2023).

Overall, these interconnected processes strengthen plant establishment and resistance to stressors such as drought, nutrient limitation and soil disturbance (Herrera et al., 1993), with mycorrhizal symbiosis being particularly influential. This is especially critical in the Mediterranean region, where high sensitivity to climate change and human-driven degradation represents a major threat to ecosystem sustainability (López-Bermúdez & Albaladejo, 1990). In this framework, *Lycium intricatum* Boiss offers a representative example. Within the Solanaceae family, *Lycium* is recognised as one of the genera with the greatest species richness. Indeed, *Lycium* L. (tribe Lycieae Hunz.) is distributed almost worldwide, encompassing roughly 70 to 92 species (Fukuda et al., 2001; Miller et al., 2008; Levin et al., 2007, 2011). In most instances, these are medium to large shrubs or small trees with an upright growth habit and

varying levels of thorniness, distinguished by their notable morphological similarity. The highest species richness is found in South Africa, North America and South America (Fukuda et al., 2001), whereas the Ponic-Mediterranean region, considering only native species, includes just nine species, mainly concentrated in the eastern part. Among these, the native taxa occurring in the central and western Mediterranean are *L. europaeum* L., *L. intricatum* Boiss. and *L. schweinfurthii* Dammer (Monteleone et al., 2022). *Lycium intricatum* is a subshrub that grows in open thermophilic halo-nitrophilous scrublands near coastal dunes, on calcareous and saline soils (Sánchez et al., 2008; Gómez-Zotano et al., 2017), ranging from 0 to 500 m a.s.l., within the Infra-thermomediterranean belt under a dry subhumid ombrotype (Pedrotti, 2012). The plant is characterised by overlapping twigs and leafy spines; the stem is 0.3 to 2 metres long, strongly branched, stout and spiny; leaves are fleshy, glaucous and narrowly linear; flowers are solitary or arranged in clusters of two to three; the corolla is narrowly infundibuliform and may be blue-violet, purple, lilac, pink or white; fruits are berries that become reddish at maturity (Bendjedou et al., 2020).

From a geographical perspective, the taxon is indigenous to northwestern Africa, with its native range reaching into the Balearic Islands (Stafforini et al. 2001) as well as the southern and southeastern sectors of the Iberian Peninsula, spanning both Spain and Portugal (Gallego, 2012). Additionally, records from Tunisia indicate that it occurs there both as a cultivated plant and in sub-spontaneous populations (Pottier-Alapetite, 1981; Le Floc'h et al., 2010). Taxonomically, the species is split into two distinct subspecies (Dobignard & Chatelain 2013; Rankou et al., 2013): *L. intricatum* subsp. *pujosii* Sauvage, which is strictly endemic to Morocco and *L. intricatum* subsp. *intricatum*, whose native distribution encompasses Mauritania, Morocco, Algeria, the Balearic Islands, southeastern Spain, and southern Portugal (Carazo-Montijano & Fernández-López, 2006; Dobignard & Chatelain, 2013). This subspecies has also been documented within Italian territory. Regarding its Italian distribution, Galasso et al. (2018) mapped *L. intricatum* subsp. *intricatum* across three distinct regions. In Sardinia, its status is categorized as a casual and uncertain alien (Puddu et al., 2016); in Calabria, it is currently considered no longer recorded; and it is also established in Sicily. On the Sicilian territory, where it is classified as a cryptogenic species, its presence has been confirmed across mainland coastal sites, namely Gela, Scoglitti, Punta Braccetto, and Sampieri (Pozzallo), as well as several circum-Sicilian islands, including Favignana, Pantelleria, Lampione, Lampedusa, and Linosa (Minissale & Sciandrello, 2005; Giardina et al., 2007; Lo Cascio & Pasta, 2012; Monteleone et al., 2022). Along the coasts of southern Sicily and southern Spain, it is characteristic of the *Periplocion angustifoliae* alliance, of the *Periploco angustifoliae-Euphorbietum dendroidis*, *Periploco angustifoliae-Rhoetum tripartitae* and *Ephedro fragilis-Pistacietum lentisci* associations (Brullo et al., 2008), as well as of the *Artemision arborescentis* alliance, notably the *Lycio intricati-Salsoletum oppositifoliae* association (Brullo et al., 2013). Within these associations, *Lycium intricatum* plays a key ecological role as a mid-successional species, with functional traits that are highly relevant for the success of restoration programs in arid environments (Lombardo et al., 2020). Indeed, in associated plant communities, it significantly reduces photosynthetically active radiation and soil moisture in the understorey (Pugnaire et al., 2004; Hortal et al., 2017). Furthermore, it mitigates drought stress by shedding leaves to reduce water loss through evaporation and tends to develop deeper roots

during the seedling stage (Padilla et al., 2007; Padilla et al., 2009; Miranda et al., 2010). Due to these characteristics, it is frequently used as a hedge and windbreak species (Sánchez et al., 2008; La Mantia et al., 2012; Boulila & Bejaoui 2015). However, the literature provides limited information on biological drivers of *L. intricatum* growth and of its occurrence across different Sicilian areas, including the role of microbial associations as a key ecological factor. Indeed, the diversity and abundance of mycorrhizal fungi associated with this species have not yet been studied in natural habitats. Only a few studies report the presence of AMF, VAM (arbuscular and vesicular mycorrhizae), and endophytic fungi such as *Microdiplodia* sp. (Honrubia et al., 1992; Kumar & Kausic, 2012), although detailed investigations are lacking. Conversely, for another species of the genus *Lycium*, *L. europaeum*, which forms vegetation structures similar to *L. intricatum*, the presence of arbuscular mycorrhizae has been shown to be essential for species establishment and dune stabilisation (Touati et al., 2013). As highlighted by Duponnois et al. (2007), mycorrhizal associations play a major role in plant succession in nutrient-poor soils and in relation to mycorrhizal spore presence. In this perspective, mycorrhizae could be utilized for the restoration of degraded soils. Some highly mycotrophic species establish early during vegetation succession on degraded soils and subsequently facilitate the development of other plant species. Most mycotrophic species then become dominant, showing a strong positive correlation between fungal and plant biodiversity (Touati et al., 2013). Based on these premises, the aim of this study is to investigate AM fungi in the roots of *L. intricatum* in natural habitats, providing a screening of fungal diversity present in the rhizosphere and in the soil surrounding the roots, while considering the positive influence of mycotrophic shrubs on soil microbiota. To assess AM fungi in *L. intricatum*, a morphological analysis was conducted on root samples collected from the coastal dunes of Torre Manfria (Gela, Italy), while fungal diversity in roots and associated soils was evaluated through high-throughput sequencing of soil samples collected near the roots. This approach also aims to evaluate how mycorrhization in *L. intricatum* influences species establishment on the coastal dunes of Torre Manfria and supports its suitability as a candidate for dune stabilisation, which is threatened by erosion and the loss of sandy areas due to excessive construction in natural environments.

Material and Methods

Experimental site

The study area, where samples were collected, is the Site of Community Importance “Torre Manfria” (ITA050011), (Gela, Sicily). The area includes a significant variety of coastal habitats. Indeed, the area is characterised by stretches of sandy beaches, consisting of quartzarenitic sand, interrupted by steep coastal slopes, formed by Pliocene-Pleistocene evaporitic deposits and clayey-calcarenitic deposits. The area presents one of the driest climates in Sicily, with a dry period of about 5 months and average annual rainfall of 409 mm concentrated in the winter months, with a maximum in October. Average annual temperatures are around 18.3°C, with winter temperatures between 17°C (average maximum) and 8.5°C (average minimum), and summer temperatures between 30°C and 21°C. The bioclimate is oceanic

pluvial, such as the annual temperature range is less than 20°C, with a lower thermomediterranean thermotype and semi-arid ombrotype (Guarino et al., 2008).

The prevailing vegetation consists of closed thermophilic scrub belonging to *Ephedro-Pistacietum lentisci*, an association of *Oleo-Ceratonion*, whose characteristic species include *Pistacia lentiscus, Ephedra fragilis, Teucrium fruticans and Phillyrea latifolia*. The constant presence of *Ephedra fragilis* and *Lycium intricatum* highlights the thermoxerophilous nature of the vegetation. This association is now quite rare in the study area due to repeated anthropogenic disturbance linked to agricultural and pastoral practices involving periodic burning and cutting of shrubs, particularly where erosion has caused the bedrock to emerge, leading to the establishment of garrigue vegetation (Guarino et al., 2008). In addition, many dunes and the vegetation behind them have been compromised by the presence of beach establishments. This makes it more urgent than ever to implement a serious conservation and management programme for the site (Branca et al., 2010). In fact, coastal dunes are the transition element between sandy coastlines and the continental environment, performing various buffering functions, both physical and ecological-naturalistic. The ecological importance of coastal dunes lies in their plant communities, which are responsible for the most significant mechanisms of consolidation and growth of wind-blown deposits. From a faunal point of view, dune ecosystems also represent unique habitats, to which must be added their role as ecological corridors in the coastal environment. Specifically, the creation of buffer zones separating dune areas from anthropized areas, thanks in part to the planting of *Lycium intricatum*, is of fundamental importance for demarcating the boundaries of natural areas, mitigating the landscape impact of adjacent structures and expanding ecotonal strips, helping to reconnect the various fragments of vegetation to promote an increase in vegetation cover and the naturalness of the site (Carullo et al., 2016).

Sample collection

Root and soil samples were collected in January 2020. Specifically, in the study area of Torre Manfria (Gela, Sicily), four sampling points were considered (Fig. 1) in relation to different altitudes: 103 m s.l.m (37°6'0.04", 14°8'19.82"), 92 m s.l.m (37°5'59.4", 14°8'21.45"), 85 m s.l.m (37°5'57.3", 14°8'20.28"), 65 m s.l.m (37°5'55.71", 14°8'25.13"). Sampling consisted of digging to a depth of 10–30 cm and collecting fine feeder roots of *L. intricatum* and a small portion of soil surrounding the roots (Lumini et al., 2024). During the digging, the main lateral roots were identified, and the young lateral roots were collected. From each sampling point, two to three plants were sampled, and the corresponding rhizospheric soil was collected simultaneously. The soil samples (~ 300 mg) were sieved immediately at 2 mm, frozen and stored until molecular analysis. Root fragments from each plant were washed free of soil, air-dried at room temperature and immediately used for morphological analyses. The roots (~ 150 g) were stored at − 20°C until used for molecular analyses.

Morphological analysis of roots

Roots were harvested, rid of topsoil, cleaned and stained with 0.1% (w/v) cotton blue in 80% lactic acid overnight, then destained three times with lactic acid for 18 h, cut into 1-cm-long segments and placed on microscope slides for morphological analysis. Approximately 30 fragments were observed under a light microscope.

DNA extraction and sequencing

DNA extraction and metabarcoding analyses were conducted following the protocol outlined by Aranguren et al. (2023). Total DNA was isolated from 150 mg of soil substrates using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany). For root tissues, a 100 mg starting sample was processed via the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), strictly adhering to the respective manufacturer guidelines. DNA yields were verified spectrophotometrically on a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), after which all extracts were uniformly adjusted to a working concentration of 2 ng/µL prior to downstream PCR amplification.

To characterize fungal biodiversity patterns across both root and soil matrices (Supplementary Table S1), the nuclear ribosomal ITS2 region was targeted. Concurrently, the specific 18S rRNA barcode region was targeted exclusively in root samples to profile arbuscular mycorrhizal fungal (AMF) assemblages (Supplementary Table S2). All amplification reactions were performed using the Invitrogen Platinum HotStart PCR Master Mix (Thermo Fisher Scientific).

Assays utilized the primer combination fITS7 (5’–GTGARTCATCGAATCTTTG–3’) and ITS4 (5’–TCCTCCGCTTATTGATATGC–3’) (Ihrmark et al. 2012) to amplified ITS2 region of total fungal community. The cycling parameters consisted of an initial denaturation at 95°C for 5 min; followed by 35 cycles of 95°C (45 s), 56°C (45 s), and 72°C (45 s); with a final 7 min elongation phase at 72°C. In addition, the specific 18S rRNA barcode region was used to profile AMF communities using a nested PCR framework based on Berruti et al. (2017a, b). First round was performed with the AML1–AML2 primer pair (Lee et al., 2008) under the following conditions: 95°C for 15 min; 39 cycles of 95°C (60 s), 57°C (60 s), and 72°C (60 s); and a final 7 min hold at 72°C. The resulting amplicons served as templates to amplify a ~ 420 bp segment using the AMADf (5’-GGGAGGTAGTGACAATAAATAAC-3’) and AMDGR (5’-CCCAACTATCCCTATTAATCAT-3') primers (Berruti et al. 2017a, b). The nested thermal profile was set to 95°C for 15 min; 27 cycles of 95°C (40 s), 57°C (40 s), and 72°C (45 s); ending with 7 min at 72°C. Both sets of locus-specific primers incorporated standard Illumina overhang adapter sequences: Forward overhang: 5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[target primer], Reverse overhang: 5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[target primer].

The resulting PCR amplicons (~ 350 bp) were verified visually via 1% agarose gel electrophoresis and subsequently cleaned using the Wizard SV Gel and PCR CleanUp System (Promega). Cleaned products were quantified on a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) according to the provided instructions. Prepared amplicon libraries were shipped to IGA Technology Services S.R.L.

(Udine, Italy) for high-throughput sequencing on an AVITI platform (Element Biosciences, San Diego, CA) utilizing a 300-bp paired-end sequencing strategy.

ITS and 18S rRNA raw sequences processing

Raw sequencing reads were processed using DADA2 (version 1.36.0) implemented in RStudio (R version 4.5.1). For ITS analyses, only forward reads were processed, following the approach described by Pauvert et al. (2019). In contrast, for 18S rRNA analyses, both forward and reverse reads were used and subjected to barcode and primer removal, quality filtering, trimming, and read merging. Subsequently, chimeric sequences were identified and removed, and taxonomic assignment was performed. ITS rRNA sequences were classified using the UNITE dynamic reference database (version 19.02.2025). For 18S rRNA sequences, taxonomic annotation was inferred manually through comparative analyses across multiple reference databases. The number of reads lost at each step of the DADA2 processing for both ITS and 18S samples is reported in Supplementary Table S3, while the 18S taxonomic dataset generated for this analysis is reported in Supplementary Table S4. The resulting datasets were further refined using the phyloseq package (version 1.52.0) to retain only fungal ASVs with a relative abundance greater than 0.0001. The vegan package (version 2.7.1) was used to rarefy the datasets in a size-dependent manner, to a depth of 54,185 reads for the ITS dataset and 7,466 reads for the 18S dataset. At the selected sequencing depths, all samples reached a saturation plateau. The final datasets comprised 2,681 ASVs across 18 ITS samples and 223 ASVs across 10 18S samples. The rarefied datasets were subsequently merged into a phyloseq object and used to compute α- and β-diversity indices.

Statistical analysis

The phyloseq, vegan, reshape2 (version 1.4.4), and ggplot2 (version 3.5.1) packages in R were used to generate stacked bar plots of community composition and to perform both β-diversity analyses, based on the Bray–Curtis distance matrix, and α-diversity analyses using the Shannon and Observed indices. Statistical differences in α-diversity among phenotypic response groups and in ITS phyla across different elevations and root and soil samples were assessed using Dunn’s post hoc test and are reported in Supplementary Tables S5-S6.

To assess the shared Glomeromycotina between the 18S and ITS datasets across different taxonomic levels, the ggVennDiagram package (version 1.5.4) was used.

All the codes used in this work are provided as Supplementary scripts.

Results

Composition and Structure of ITS rRNA Fungal Communities

Fungal communities associated with *Lycium intricatum* roots and rhizosphere soils exhibited distinct patterns across four coastal elevations (65 m, 85 m, 92 m, 103 m a.s.l.) in Torre Manfria, Sicily, with clear differences both among elevations and between compartments (root vs. soil).

ITS rRNA metabarcoding revealed clear elevation-related shifts in phylum-level relative abundances (significant differences marked by asterisks; $p < 0.05$; e.g. Mortierellomycota, and Mucoromycota; Supplementary Table S5).

In both roots and rhizosphere soils, communities were consistently dominated by Ascomycota (67.4–68.8% relative abundance), followed by Basidiobolomycota (27.5–27%), with only minor contributions from Basidiomycota (1.9–1.1%), Glomeromycota (0.4–0.8%), and other fungal taxa.

Significant phylum-level variations between Glomeromycota members occurred between sample compartments (Dunn *post-hoc*, $p < 0.05$; Fig. 2A; Supplementary Table S6).

α- and β- diversity indexes revealed significant differences in fungal community composition between soil and root samples, with soil samples showing a higher Shannon diversity index (Dunn *post-hoc*, $p = 0.02$; Figs. 2A-B). ITS rRNA β-diversity, assessed via Bray-Curtis dissimilarity (Fig. 3A), showed strong separation by elevation (PERMANOVA, $p = 0.0002$) and sample type (PERMANOVA, $p = 0.00015$), with altitude and sample type explaining 30.8% and 14.7%, respectively, of community variation. Observed α-diversity index varied significantly across elevations (Dunn *post-hoc*, $p = 0.049$; Fig. 3B, left side), increasing from 65 m to 85 and 92 m. The same trend of increasing fungal richness with altitude was also detected by the Shannon α- diversity index (Fig. 3B, right side). In both cases, the diversity dropped at the highest altitude (103 m).

Root-associated 18S rRNA's AMF detection

Regarding the presence of the AMF, the typical intraradical structures (intraradical mycelium, arbuscules, and vesicles) were found in the root system of *L. intricatum* plants (Fig. 1E). Molecular analyses using 18S rRNA marker revealed that root-associated AMF communities were dominated by *Rhizophagus* (50%), followed by *Glomus* s.l. (15.6%), *Claroideoglomus* (11.2%) and *Funneliformis* (10.6%) (Fig. 4A). β-diversity differed significantly across elevations (PERMAVOVA, $p = 0.00375$, $R^2 = 59\%$; Fig. 4B). As for the ITS rRNA fungal community, the 18S AMF community showed significant differences in observed α-diversity indexes between increasing altitudes, especially between 85 and 92 meters (Dunn *post-hoc*, $p = 0.027$; Fig. 4C leftside). The same trend was revealed by the Shannon index, even if no significant difference was detected in this case (Dunn *post-hoc*, p = ns; Fig. 4C right side).

Taxonomic Overlap between ITS2 and 18S-based AMF communities

To assess the degree of overlap between the *Glomeromycotina* taxa detected using the two molecular markers (ITS and the more specific 18S rRNA), we compared the datasets using Venn diagrams. Results,

shown in Fig. 5, revealed 75% overlap (3 taxa) at class level, 67% (4 taxa) at order level, 38% overlap (3 taxa) at family level, 39% (7 taxa) at genus level, and 6% overlap (2 taxa) at the species level (*Funneliformis mosseae* and *Septoglomus viscosum*). Shared taxa included Glomeraceae and *Rhizophagus*, with colour intensity reflecting the degree of overlap. These patterns underscore the contribution of mycorrhizal communities to the adaptation of *Lycium intricatum* in coastal dunes.

Discussion

This study provides the first integrated morphological and metabarcoding characterization of fungal communities associated with *Lycium intricatum* in its natural Mediterranean coastal habitat. By combining ITS-based community profiling with AMF-targeted 18S sequencing and microscopic root observations, we demonstrate that *L. intricatum* hosts diverse fungal assemblages structured by elevation and compartment (roots vs rhizosphere) and forms consistent arbuscular mycorrhizal associations dominated by Glomeraceae taxa. These findings support the hypothesis that belowground fungal symbioses contribute significantly to the persistence of this shrub in nutrient-poor, saline dune environments and may partially explain its ecological role in semi-natural coastal vegetation.

Fungal Community Structure in Coastal Dune Systems

Across all elevations and compartments, ITS metabarcoding revealed strong dominance of Ascomycota and Basidiobolomycota, with Glomeromycota representing only a small fraction of total reads. This pattern is consistent with fungal assemblages reported from Mediterranean and arid coastal soils, where saprotrophic and stress-tolerant taxa often prevail due to low organic matter, salinity, and intense temperature and moisture fluctuations (Camprubí et al. 2010; Ontivero et al. 2020). Members of Ascomycota frequently dominate dune ecosystems (Egidi et al. 2019; Cao et al. 2024) because of traits linked to stress tolerance and resource uptake (Egidi et al. 2019), while Basidiobolomycota and other early-diverging fungal lineages such as Zoopagomycota have also been found in sandy or disturbed substrates (Cao et al. 2024).

Despite their low proportional abundance in ITS datasets, Glomeromycota were consistently detected and were abundant in roots when analysed using the AMF-specific 18S marker. This discrepancy reflects well-known primer and database biases inherent to ITS-based surveys, which tend to under-represent AM fungi (Berruti et al. 2017b). The taxonomic overlap between ITS and 18S datasets at higher ranks, and especially the shared detection of Glomeraceae and *Rhizophagus*, confirms the complementarity of the two approaches and underscores the importance of marker choice when assessing mycorrhizal communities in ecological studies (Ontivero et al. 2020, Lumini et al. 2024)

The significantly lower alpha diversity observed in roots compared to rhizosphere soils suggests selective recruitment and persistence of fungal taxa within host tissues (Berruti et al. 2017a). This compartmentalization is commonly observed in Mediterranean shrubs and psammophilous species and likely reflects plant-mediated filtering processes that favour symbiotic or endophytic fungi over free-

living soil assemblages. Such differentiation between root and rhizosphere communities may be particularly advantageous in nutrient-limited dune systems, where tight plant–fungus associations enhance resource acquisition efficiency (Read, 1989; Tsiknia et al. 2021).

Elevational Gradients and Community Turnover

Although the four sampling sites covered a relatively narrow altitudinal range (65–103 m a.s.l.), fungal communities showed marked turnover along this gradient, with elevation explaining a large proportion of β-diversity variation in both ITS and 18S datasets. This indicates that even subtle changes in coastal topography, likely associated with differences in soil development, salinity intrusion, wind exposure, and moisture retention, can strongly influence belowground fungal assemblages. The increase in total fungal alpha diversity at higher elevations may reflect progressively reduced salinity stress and greater organic matter accumulation inland, a trend observed in Mediterranean dune chronosequences and stabilized coastal systems (Camprubí et al. 2010).

In contrast, AMF richness remained relatively constant along the gradient, while community composition shifted significantly, indicating that *L. intricatum* maintains arbuscular mycorrhizal associations across contrasting microhabitats but recruits different fungal partners depending on local conditions. Comparable patterns of AMF turnover without major changes in richness have been reported for Mediterranean drylands and coastal dune ecosystems (Camprubí et al. 2010; López-Angulo et al. 2023), suggesting functional continuity across environmentally heterogeneous landscapes. Such taxonomic turnover without major changes in richness may reflect functional redundancy within Glomeraceae-dominated assemblages, ensuring continued nutrient acquisition and stress mitigation under varying coastal conditions.

Arbuscular mycorrhizal associations and ecological significance

Microscopic observations confirmed the widespread presence of arbuscules, vesicles and intraradical hyphae in *L. intricatum* roots, demonstrating that this species is consistently mycotrophic under natural conditions. Molecular analyses further revealed that AMF communities were dominated by *Rhizophagus*, *Glomus sensu lato*, *Claroideoglomus* and *Funneliformis*, genera frequently reported from Mediterranean dunes and other stressful substrates.

Similar assemblages have been described in coastal populations of *Lycium europaeum*, where AM fungi were shown to facilitate establishment on mobile sands and contribute to dune stabilization (Touati et al. 2013). The recurrence of Glomeraceae in congeneric species occupying analogous habitats suggests that these symbionts play a conserved functional role within Mediterranean *Lycium* communities.

Members of these genera are known to enhance phosphorus acquisition, water uptake and tolerance to osmotic stress, traits that are particularly relevant in Torre Manfria, characterized by semi-arid conditions and nutrient-poor quartzarenitic soils. In addition, AM fungi may indirectly influence geomorphological processes through effects on soil aggregation and root anchorage, thereby contributing to the

stabilization of incipient or semi-fixed dunes. Through such mechanisms, *L. intricatum* may act as a structuring species within thermoxerophilous scrub communities, promoting local soil improvement and facilitating the establishment of other plants, as previously reported for Mediterranean shrubs used in restoration contexts (Castro et al. 2004; Lombardo et al. 2020, Sciandrello et al. 2024).

Implications for conservation and management

The strong spatial structuring of fungal assemblages and the consistent presence of AM symbioses indicate that *L. intricatum* interacts closely with locally adapted soil microbial communities. This finding has practical relevance for conservation actions in coastal Natura 2000 sites such as Torre Manfria, where dune systems are increasingly threatened by erosion and human disturbance.

The use of *L. intricatum* in revegetation programmes may benefit from maintaining native soil microbiota or from employing locally sourced AMF inocula rather than generalized commercial formulations. Such approaches could enhance plant establishment, improve soil stability and contribute to the development of buffer strips between natural dune habitats and adjacent anthropogenic areas.

Conclusions

This study provides the first detailed characterization of fungal communities associated with *Lycium intricatum* in Mediterranean coastal dunes, integrating morphological evidence of mycorrhization with high-throughput sequencing of roots and rhizosphere soils. The study revealed that:

-Fungal assemblages are strongly structured by elevation and plant compartment, even across narrow coastal gradients, reflecting the sensitivity of dune microbiota to microenvironmental variation.

-Roots host distinct and diverse fungal communities, indicating active host filtering and specialization.

-Arbuscular mycorrhizal symbiosis is widespread and functionally important in *L. intricatum*, with dominance of Glomeraceae taxa such as *Rhizophagus*, *Glomus*, and *Claroideoglomus*.

-These symbioses likely contribute to drought and salinity tolerance, nutrient acquisition, and soil stabilization, supporting the role of *L. intricatum* as a mid-successional shrub and a keystone species for dune restoration.

Taken together, our findings highlight the importance of considering belowground fungal diversity when planning conservation and revegetation programs in Mediterranean coastal systems. The integration of native mycorrhizal communities into restoration strategies could substantially enhance the resilience of *L. intricatum* plantings and promote the long-term stabilization and ecological recovery of threatened dune habitats.

Declarations

Conflict of interest disclosure:

none

Author Contribution

Author contribution: Conceptualization: ErL, EmL; Data curation: ErL, EmL, AC; Formal analysis: AC, ErL; Fundraising: ErL; Research: ErL, EmL; Methodology: ErL, MG; Management of the project: ErL; Resources: ErL; Software: AC, MG; Supervision: ErL, MG; Visualization: ErL, EmL, AC; Writing first draft: ErL; EmL, AC, Writing, review and editing: ErL, EmL, AC, MG. All authors have read and agreed to the final version of the manuscript. Arianna Capparotto (AC); Emanuela Lombardo (EmL); Marco Giovannetti (MG); Erica Lumini (ErL).

Data Availability

The datasets generated during the current study are available in the NCBI SRA repository as Bioproject PRJNA1397343

References

1. Aranguren, R., Voyron, S., Ungaro, F., Cañón, J., & Lumini, E. 2023. Metabarcoding reveals impact of different land uses on fungal diversity in the South-Eastern Region of Antioquia, Colombia. Plants. 12: 1126. https://doi.org/10.3390/plants12051126.
2. Azcón, R. & Barea, J.M. 1997. Mycorrhizal dependency of a representative plant species in mediterranean shrublands (Lavandula spica L.) as a key factor to its use for revegetation strategies in desertification-threatened areas. Appl. Soil. Ecol. 7: 83–92. https://doi.org/10.1016/S0929-1393(96)00159-3
3. Bendjedou, H., Maggi, F., Bennaceur, M., Mancinelli, M., Benamar, H. & Barboni, L. 2020. A new ionone derivative from Lycium intricatum Boiss. (Solanaceae). Nat. Prod. Res. 36(3): 687–694.
4. Berruti, A., Demasi, S., Lumini, E., Kobayashi, N., Scariot, V. & Bianciotto, V. 2017a. Wild Camellia japonica specimens in the Shimane prefecture (Japan) host previously undescribed AMF diversity. Appl. Soil Ecol. 115: 10–18. https://doi.org/10.1016/j.apsoil.2017.03.004
5. Berruti, A., Desirò, A., Visentin, S., Zecca, O. & Bonfante, P. 2017b. ITS fungal barcoding primers versus 18S AMF-specific primers reveal similar AMF-based diversity patterns in roots and soils of three mountain vineyards. Environ. Microbiol. Rep. 9: 658–667. https://doi.org/10.1111/1758-2229.12574
6. Boulila, A. & Bejaoui, A. 2015. Lycium intricatum Boiss.: An unexploited and rich source of unsaturated fatty acids, 4-desmethylsterols and other valuable phytochemicals. Lipids Health Dis. 14: 59. https://doi.org/10.1186/s12944-015-0059-y

7. Branca, F., Brullo, S., Guarino, R., Guglielmo, A., Mascara, R., Minissale, P., Mulè, N., Petalia, A., Ronsisvalle, F. & Russo, C. 2010. Il litorale di Manfria (Gela). Natura e storia da proteggere. CASPUR-CIBER Publishing.
8. Brullo, S., Gianguzzi, L., La Mantia, A. & Siracusa, G. 2008. La classe Quercetea ilicis in Sicilia. Boll. Accad. Gioenia Sci. Nat. 41(369): 1–80.
9. Brullo, S., Giusso del Galdo, G., Guarino, R., Minissale, P., Sciandrello, S. & Spampinato, G. 2013. Syntaxonomic survey of the class Pegano harmalae–Salsoletea vermiculatae Br.-Bl. & O. Bolos 1958 in Italy. Plant Biosyst. 147(2): 472–492. https://doi.org/10.1080/11263504.2012.753134
10. Camprubí, A., Calvet, C., Cabot, P., Pitet, M. & Estaún, V., 2010. Arbuscular mycorrhizal fungi associated with psammophilic vegetation in Mediterranean coastal sand dunes. Span. J. Agric. Res. 8: 641–650. https://doi.org/10.5424/sjar/201008S1-1227
11. Cao, C., Zhang, Y. & Cui, Z. 2024. Response of soil fungal community to reforestation on shifting sand dune in the Horqin Sandy Land, Northeast China. Microorganisms 12: 1545. https://doi.org/10.3390/microorganisms12081545
12. Carazo Montijano, M.M. & Fernández López, C. 2006. Catálogo De Las Plantas Vasculares De Andalucía Y Marruecos. Jaén: Herbario Jaén; p. 350.
13. Carullo, L., Sciandrello, S. & Tomaselli, G., 2016. LIFE11 NAT/IT/000232 LEOPOLDIA Ripristino degli habitat dunali nel paesaggio serricolo del golfo di Gela per la salvaguardia di Leopoldia gussonei. ISBN 979-12-200-0993-5.
14. Castro, J., Zamora, R., Hódar, J.A., Gómez, J.M. & Gómez-Aparicio, L. 2004. Benefits of using shrubs as nurse plants for reforestation in Mediterranean mountains: a 4-year study. Rest. Ecol. 12: 352–358. https://doi.org/10.1111/j.1061-2971.2004.0316.x
15. Dobignard, A. & Chatelain, C., 2013. Index synonymique de la flore d'Afrique du nord, Vol. 5, Dicotyledoneae: Oleaceae–Zygophyllaceae. Genève: Conservatoire et Jardin Botaniques; p. 451.
16. Duponnois, R., Ba, A.M., Prin, Y., Baudoin, E., Galiana, A. & Dreyfus, B. 2007. Les champignons mycorhiziens: une composante majeure dans les processus biologiques régissant la stabilité et la productivité des écosystèmes forestiers tropicaux. Le projet majeur africain de la Grande Muraille Verte; p. 421-440.
17. Egidi, E., Delgado-Baquerizo, M., Plett, J.M., Wang, J., Eldridge, D.J., Bardgett, R.D., Maestre, F.T. & Singh, B.K. 2019. A few Ascomycota taxa dominate soil fungal communities worldwide. Nat. Commun. 10: 2369. https://doi.org/10.1038/s41467-019-10373-z
18. Fukuda, T., Yokoyama, J. & Ohashi, H. 2001. Phylogeny and biogeography of the genus Lycium (Solanaceae): inferences from chloroplast DNA sequences. Mol. Phylogenet. Evol. 19: 246-258. https://doi.org/10.1006/mpev.2001.0930
19. Galasso, G., Conti, F., Peruzzi, L., Ardenghi, N.M.G., Banfi, E., Celesti-Grapow, L., Albano, A., Alessandrini, A., Bacchetta, G., Ballelli, S., Bandini Mazzanti, M., Barberis, G., Bernardo, L., Blasi, C., Bouvet, D., Bovio, M., Cecchi, L., Del Guacchio, E., Domina, G., Fascetti, S., Gallo, L., Gubellini, L., Guiggi, A., Iamonico, D., Iberite, M., Jiménez-Mejías, P., Lattanzi, E., Marchetti, D., Martinetto, E.,

Masin, R.R., Medagli, P., Passalacqua, N.G., Peccenini, S., Pennesi, R., Pierini, B., Podda, L., Poldini, L., Prosser, F., Raimondo, F.M., Roma-Marzio, F., Rosati, L., Santangelo, A., Scoppola, A., Scortegagna, S., Selvaggi, A., Selvi, F., Soldano, A., Stinca, A., Wagensommer, R.P., Wilhalm, T. & Bartolucci, F. 2018. An updated checklist of the vascular flora alien to Italy. Plant Biosyst. 152(3):556–592. https://doi.org/10.1080/11263504.2018.1441197

20. Gallego, M.J. 2012. Lycium. In: Castroviejo S., Aedo C., Laínz M., Muñoz Garmendia F., Nieto Feliner G., Paiva J. & Benedí C. (eds). Flora iberica Vol. 11. Madrid: Real Jardín Botánico, CSIC; p. 238–240.
21. Giardina, G., Raimondo, F.M. & Spadaro, V. 2007. A catalogue of plants growing in Sicily. Bocconea. 20:147.
22. Gómez-Zotano, J., Olmedo-Cobo, J.A. & Martínez-Ibarra, E. 2017. Propuesta de creación de una microreserva en el Peñón de Salobreña para la protección de los hábitats del litoral de Granada (España). Invest. Geogr. Alicante 67: 143–154.
23. Guarino, R., Minissale, P. & Sciandrello, S. 2008. La biodiversità vegetale e relativa cartografia del S.I.C. “Torre Manfria” (Gela- CL). Quad. Bot. Ambientale Appl. 19:37-66.
24. Herrera, M.A, Salamanca, C.P. & Barea, J.M. 1993. Inoculation of woody legumes with selected arbuscular mycorrhizal fungi and rhizobia to recover desertified Mediterranean ecosystems. Appl. Environ. Microbiol. 59: 129–133.
25. Honrubia, M., Torres, P., Daaz, G. & Cano, A. 1992. Manual de micorrizar plantas en viveros forestales. Madrid: ICONA; p. 46.
26. Hortal, S., Lozano, Y.M., Bastida, F., Armas, C., Moreno, J.L., Garcia, C. & Pugnaire, F.I. 2017. Plant-plant competition outcomes are modulated by plant effects on the soil bacterial community. Sci. Rep. 7: 17756. https://doi.org/10.1038/s41598-017-18103-5
27. Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M. & Clemmensen, K.E. 2012. New primers to amplify the fungal ITS2 region —evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82: 666–677. https://doi.org/10.1111/j.1574-6941.2012.01437.x
28. Jesús López-Angulo, Matesanz, S., Illuminati, A., Pescador, D.S., Sánchez, A.M., Pías, B., Chacón-Labella, J., de la Cruz, M. & Escudero, A. 2023. Ecological drivers of fine-scale distribution of arbuscular mycorrhizal fungi in a semiarid Mediterranean scrubland. Ann. Bot. 131: 1107–1119. https://doi.org/10.1093/aob/mcad050
29. Kumar, S. & Kaushik, N. 2012. Metabolites of endophytic fungi as novel source of biofungicide: a review. Phytochem. Rev. 11:507–522. https://doi.org/10.1007/s11101-012-9263-0
30. La Mantia, T., Messana, G., Billeci, V., Dimarca, A., Del Signore, M.B., Leanza, M., Livreri Console, S., Maraventano, G., Nicolini, G., Prazzi, E., Quatrini, P., Sanguedolce, F., Sorrentino, G. & Pasta, S. 2012. Combining bioengineering and plant conservation on a Mediterranean islet. iForest 5(6): 296–305. https://doi.org/10.3832/ifor0638-005
31. Le Floc’h, É., Boulos, L. & Véla, E. 2010. Catalogue synonymique commenté de la Flore de Tunisie. Tunis: République tunisienne, Ministère de l’Environnement et du Développement Durable, Banque

Nationale de Gènes.

32. Lee, J., Lee, S. & Young, J.P.W. 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiol. Ecol. 65: 339–349. https://doi.org/10.1111/j.1574-6941.2008.00531.x
33. Levin, R.A., Bernardello, G., Whiting, C. & Miller, J.S., 2011. A new generic circumscription in tribe Lycieae (Solanaceae). Taxon 60: 681-690. https://doi.org/10.1002/tax.602018
34. Levin, R.A., Shak J.R., Miller J.S., Bernardello G. & Venter A.M., 2007. Evolutionary relationships in tribe Lycieae (Solanaceae). Acta Hort. 745: 225–239. https://doi.org/10.17660/ActaHortic.2007.745.28
35. Lo Cascio, P. & Pasta, S. 2012. Lampione, a paradigmatic case of Mediterranean island biodiversity. Biodivers. J. 3(4):311–330.
36. Lombardo, E., Bancheva, S., Domina, G., & Venturella, G., 2020. Distribution, ecological role and symbioses of selected shrubby species in the Mediterranean Basin: a review. Plant Biosyst. 154(4): 438–454. https://doi.org/10.1080/11263504.2020.1727988
37. López-Bermúdez, F. & Albaladejo, J. 1990. Factores ambientales de la degradacion del suelo en el area mediterranea, In Albadalejo J, Stocking MA, Dıaz E. editors, Soil degradation and rehabilitation in Mediterranean environmental conditions. Murcia: CSIC; p. 15–45.
38. Lumini, E., Ghignone, S. & Voyron, S. 2024. Improved DNA extraction methods and PCR primers for assessing AMF diversity and distribution in agroecosystems. In: Parihar M., Rakshit A., Adholeya A. & Chen Y. (eds). Arbuscular Mycorrhizal Fungi in Sustainable Agriculture. Singapore: Springer; p. 57–101. https://doi.org/10.1007/978-981-97-0296-1_3
39. Maurice, K., Laurent-Webb, L., Dehail, A., Bourceret, A., Boivin, S., Boukcim, H., Selosse, M.-A., & Ducousso, M., 2023. Fertility islands, keys to the establishment of plant and microbial diversity in a highly alkaline hot desert. J. Arid Environ., 219: 105074. https://doi.org/10.1016/j.jaridenv.2023.105074
40. Miller, J. S., Levin, R. A., & Feliciano, N. M. 2008. A tale of two continents: Baker's rule and the maintenance of self-incompatibility in Lycium (Solanaceae). Evol. 62: 1052–1065. https://doi.org/10.1111/j.1558-5646.2008.00358.x
41. Minissale, P. & Sciandrello, S. 2005. La vegetazione di Piano Stella presso Gela (Sicilia meridionale), un biotopo meritevole di conservazione. Quad. Bot. Amb. Appl. 16: 129–142.
42. Miranda, J., Padilla, F.M., Martínez-Vilalta, J. & Pugnaire, F.I. 2010. Woody species of a semi-arid community are only moderately resistant to cavitation. Funct. Plant Biol. 37: 828–839. https://doi.org/10.1071/FP10044
43. Montoleone, E., Longo, D., Cassanego, E., & Lazzeri, V. 2022. Lycium schweinfurthii Dammer (Solanaceae, Lycieae), specie mal compresa confermata per il meridione d'Italia. Acta Pl. Notes. 8: 11–16.
44. Ochoa-Hueso, R., Eldridge, D. J., Delgado-Baquerizo, M., Soliveres, S., Bowker, M. A., Gross, N., Le Bagousse-Pinguet, Y., Quero, J. L., García-Gómez, M., Valencia, E., Arredondo, T., Beinticinco, L., Bran,

D., Cea, A., Coaguila, D., Dougill, A. J., Espinosa, C. I., Gaitán, J., Guuroh, R. T., Guzman, E., Gutiérrez, J. R., Hernández, R. M., Huber-Sannwald, E., Jeffries, T., Linstädter, A., Mau, R. L., Monerris, J., Prina, A., Pucheta, E., Stavi, I., Thomas, A. D., Zaady, E., Singh, B. K., & Maestre, F. T., 2018. Soil fungal abundance and plant functional traits drive fertile island formation in global drylands. J. Ecol., 106: 242–253. https://doi.org/10.1111/1365-2745.12871

45. Ontivero, R.E., Voyron, S., Allione, L.V.R., Bianco, P., Bianciotto, V., Iriarte, H.J., Lugo, M.A. & Lumini, E. 2020. Impact of land use history on arbuscular mycorrhizal fungal diversity in arid soils of Argentinean farming fields. FEMS Microbiol. Lett. 367: fnaa114. https://doi.org/10.1093/femsle/fnaa114
46. Padilla, F.M., Miranda, J. & Pugnaire, F.I. 2007. Early root growth plasticity in seedlings of three Mediterranean woody species. Plant Soil. 296:103–113. https://doi.org/10.1007/s11104-007-9292-3
47. Padilla, F.M., Ortega, R., Sanchez, J. & Pugnaire, F.I. 2009. Rethinking species selection for restoration of arid shrublands. Basic Appl. Ecol. 10:640–647. https://doi.org/10.1016/j.baae.2009.04.002
48. Pauvert, C., Buée, M., Laval, V., Edel-Hermann, V., Fauchery, L., Gautier, A., Lesur, I., Vallance, J. & Vacher, C. 2019. Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. Fungal Ecol. 41: 23–33. https://doi.org/10.1016/j.funeco.2019.03.005
49. Pedrotti, F. 2012. Plant and Vegetation Mapping. New York: Springer Science & Business Media; p. 294. https://doi.org/10.1007/978-3-642-30235-0
50. Pottier-Alapetite, G. 1981. Flore de la Tunisie Angiospermes-Dicotylédones, Apétales Dialypétales. Tunis: Imprimerie Officielle de la République Tunisienne; p. 535.
51. Puddu, S., Podda, L., Mayoral, O., Delage, A., Hugot, L., Petit, Y. & Bacchetta, G. 2016. Comparative analysis of the alien vascular flora of Sardinia and Corsica. Not. Bot. Horti Agrobot. Cluj Napoca 44(2): 337–346. https://doi.org/10.15835/nbha44210567
52. Pugnaire, F.I., Armas, C. & Valladares, F. 2004. Soil as a mediator in plant-plant interactions in a semi-arid community. J. Veg. Sci. 15: 85–92. https://doi.org/10.1111/j.1654-1103.2004.tb02242.x
53. Rankou, H., Culham, A., Jury, S.L. & Christenhusz, M.J.M. 2013. The endemic flora of Morocco. Phytotaxa. 78(1):1–69. https://doi.org/10.11646/phytotaxa.78.1.1
54. Read, D.J., 1989. Mycorrhizas and nutrient cycling in sand dune ecosystems. Proc. R. Soc. Edinb. B Biol. Sci. 96: 89–110. https://doi.org/10.1017/S0269727000010873
55. Sánchez Martínez, J.J., Leemhuis, J.A.F., Vicente Colomer, M.J., Muñoz Muñoz, M., Bañón Arias, S., Conesa Gallego, E., Fernández Hernández, J.A., Valdés Illán, R., Miralles Crespo, J., Ochoa Rego, J. & López Marín, J. 2008. Especies silvestres mediterráneas con valor ornamental. Selección, producción viverística y utilización en jardinería. Murcia: Servicio de Protección y Conservación de la Naturaleza Dirección General de Patrimonio Natural y Biodiversidad.Consejería de Agricultura y Agua; p. 224.

56. Sciandrello, S., Ranno, V., & Tomaselli, V. 2024. The Role of Vegetation Monitoring in the Conservation of Coastal Habitats N2000: A Case Study of a Wetland Area in Southeast Sicily (Italy). Land, 13(1): 62. https://doi.org/10.3390/land13010062
57. Stafforini, M., Torres, N., Sáez, U., González, J.M., Duñó, J. & Puget, G. 2001. Notes florístiques de les Illes Balears (XIII). Boll. Soc. Hist. Nat. Balears 44: 57–66. https://doi.org/
58. Touati, J., Chliyeh, M., Ouazzani Touhami, A., Benkirane, R. & Douira, A. 2013. Mycorrhizal status of Lycium europaeum in the coastal dunes of Mehdia (Northwest of Morocco). J. Appl. Biosci. 71:5728–5741. https://doi.org/10.4314/jab.v71i1.98843
59. Tsiknia, M., Skiada, V., Ipsilantis, I., Vasileiadis, S., Kavroulakis, N., Genitsaris, S., Papadopoulou, K.K., Hart, M., Klironomos, J., Karpouzas, D.G., Ehaliotis, C. 2021. Strong host-specific selection and over-dominance characterize arbuscular mycorrhizal fungal root colonizers of coastal sand dune plants of the Mediterranean region. FEMS Microbiol. Ecol. 97:fiab109. https://doi.org/10.1093/femsec/fiab109

Figures

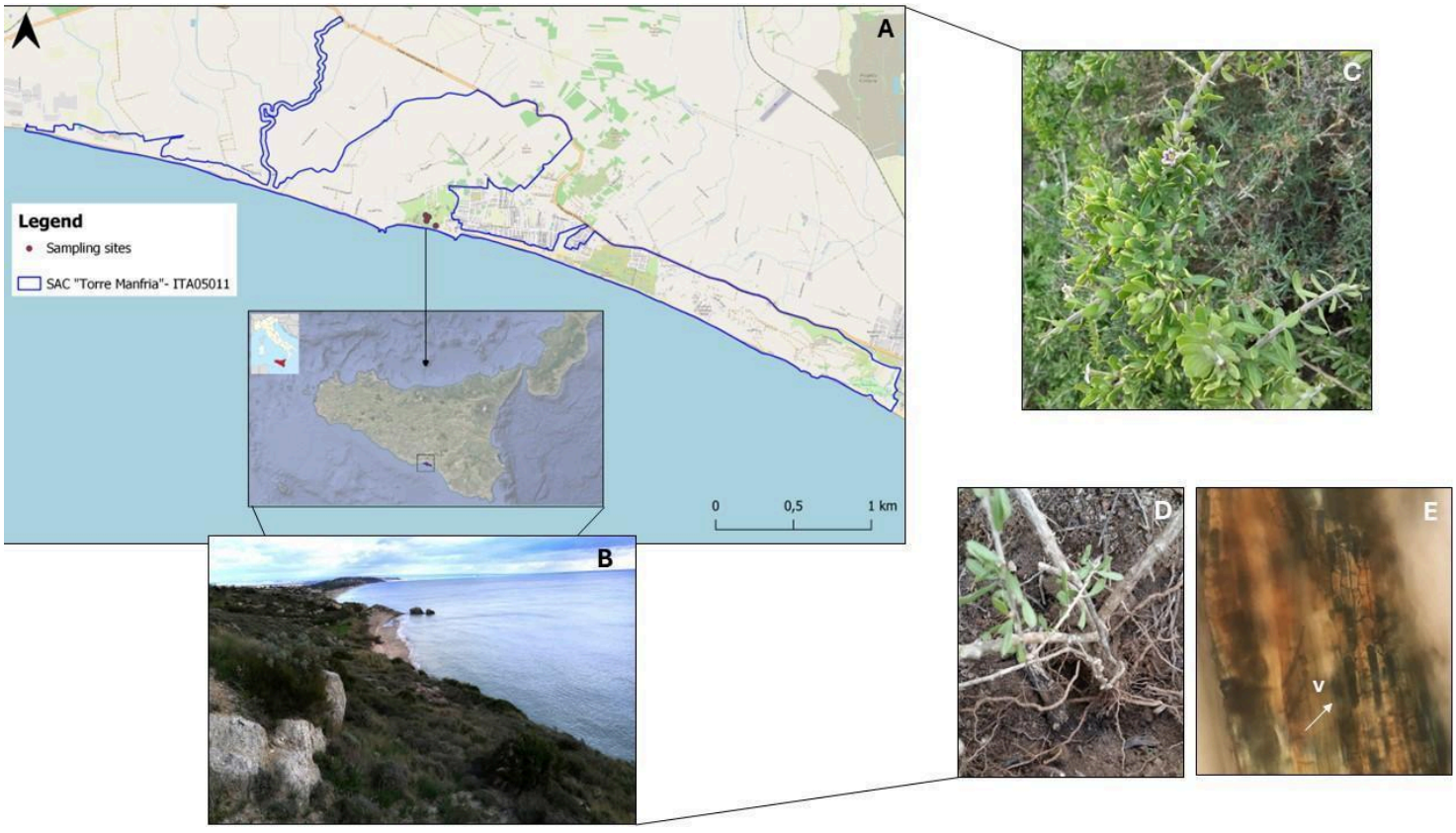

Figure 1

Experimental sites and sample examples. A-B) Geographical location of “Torre Manfria” (Gela, IT) and its environmental habitat. C-D) *Lycium intricatum* aerial and root apparatus with E) morphological characterization reporting AMF’s arbuscules presence (pointed by the arrow).

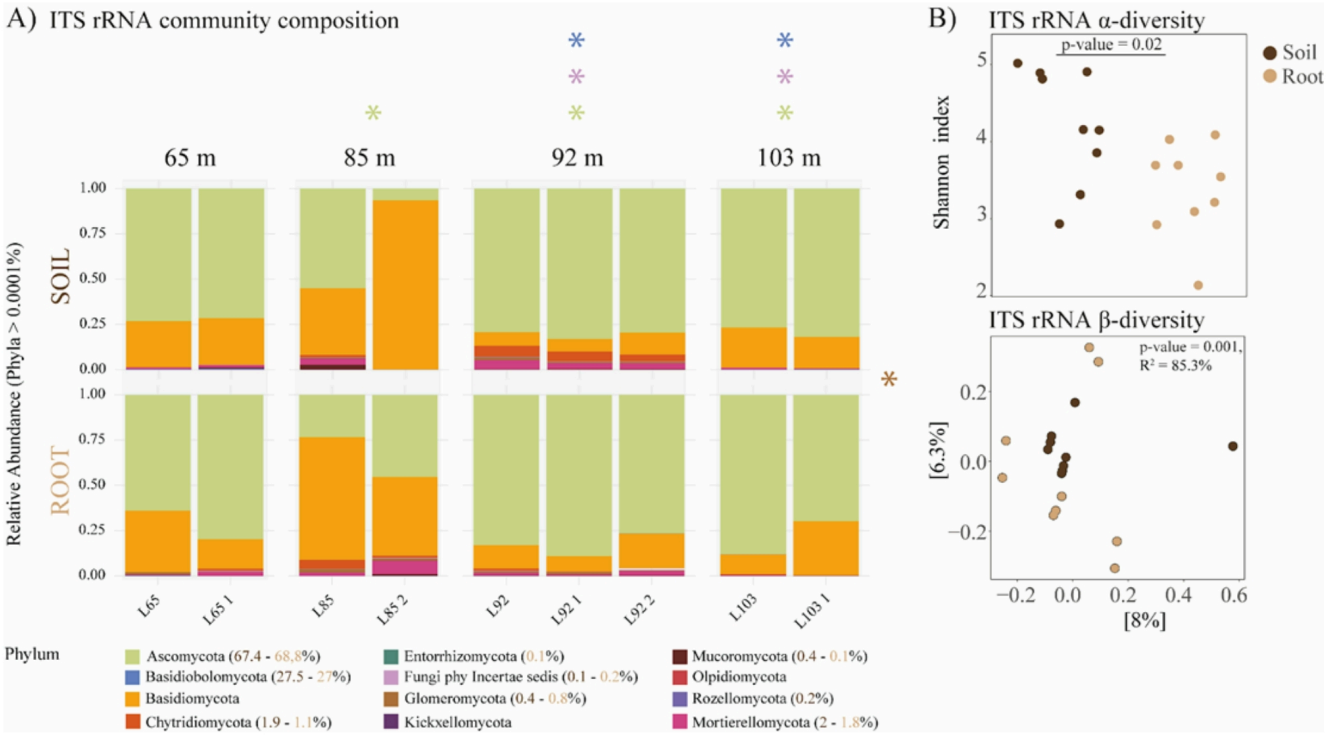

Figure 2

Fungal ITS communities associated with soil and roots of *Lycium intricatum*. (A) Relative abundance profiles of soil (upper panel) and root (lower panel) fungal communities across the sampled elevations. Asterisks indicate statistically significant differences in specific fungal phyla (coloured by phylum) across elevations and between root and soil samples. Phylum-level relative abundances within soil and root samples are shown next to each phylum label. (B) Fungal diversity metrics, including Shannon alpha diversity (upper panel) and β-diversity (lower panel), compared between soil and root samples.

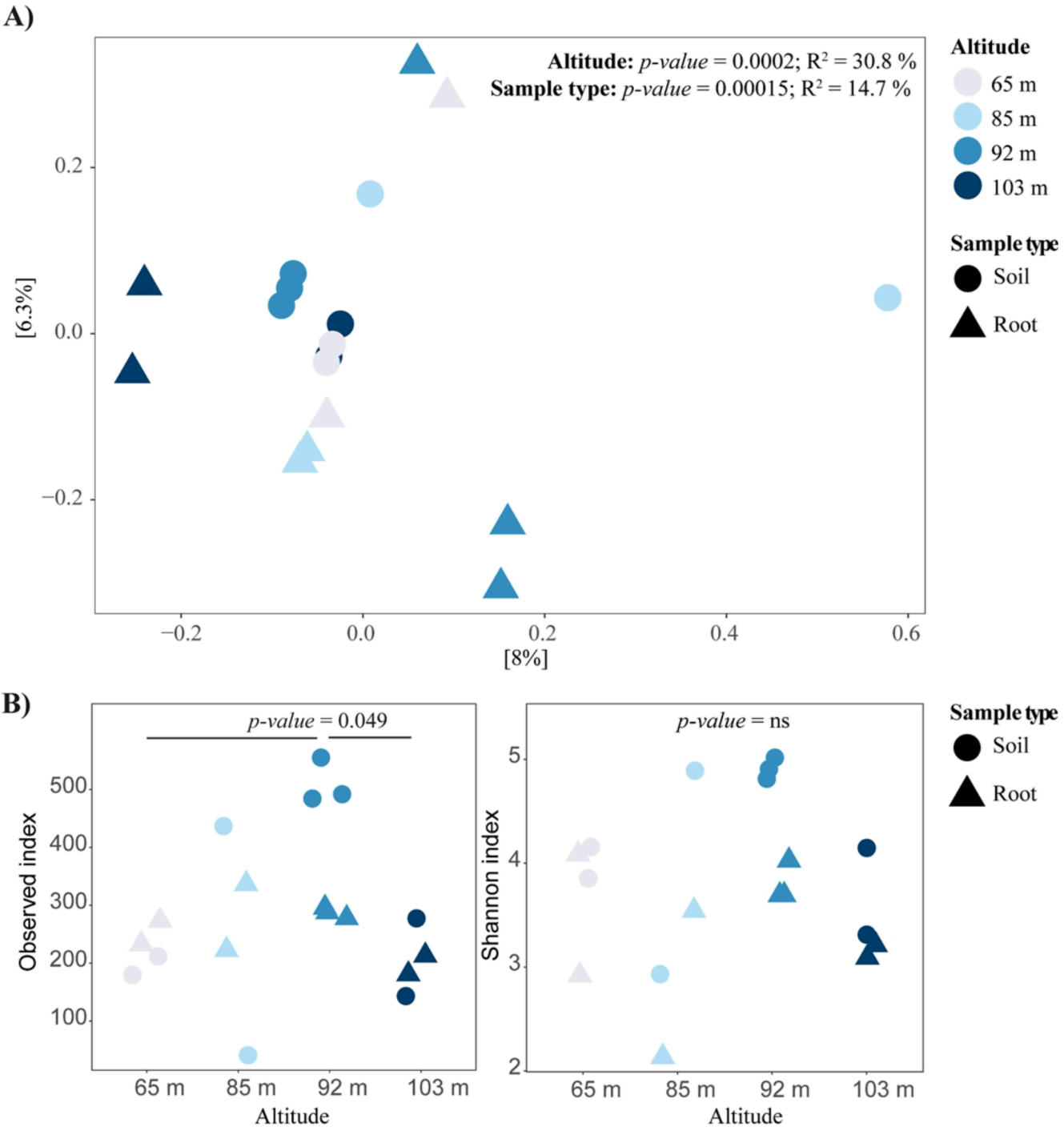

Figure 3

Fungal ITS diversity in soil and root samples of *Lycium intricatum* across an elevational gradient. **(A)** Bray-Curtis dissimilarity matrix visualizing fungal ITS community variation across elevations (indicated by colours) and sample types (soil and roots, indicated by shapes). **(B)** Observed (left panel) and Shannon (right panel) alpha diversity indices across samples from different elevations (represented by

colours). Statistical differences in alpha diversity between groups were assessed using Dunn's *post hoc* test.

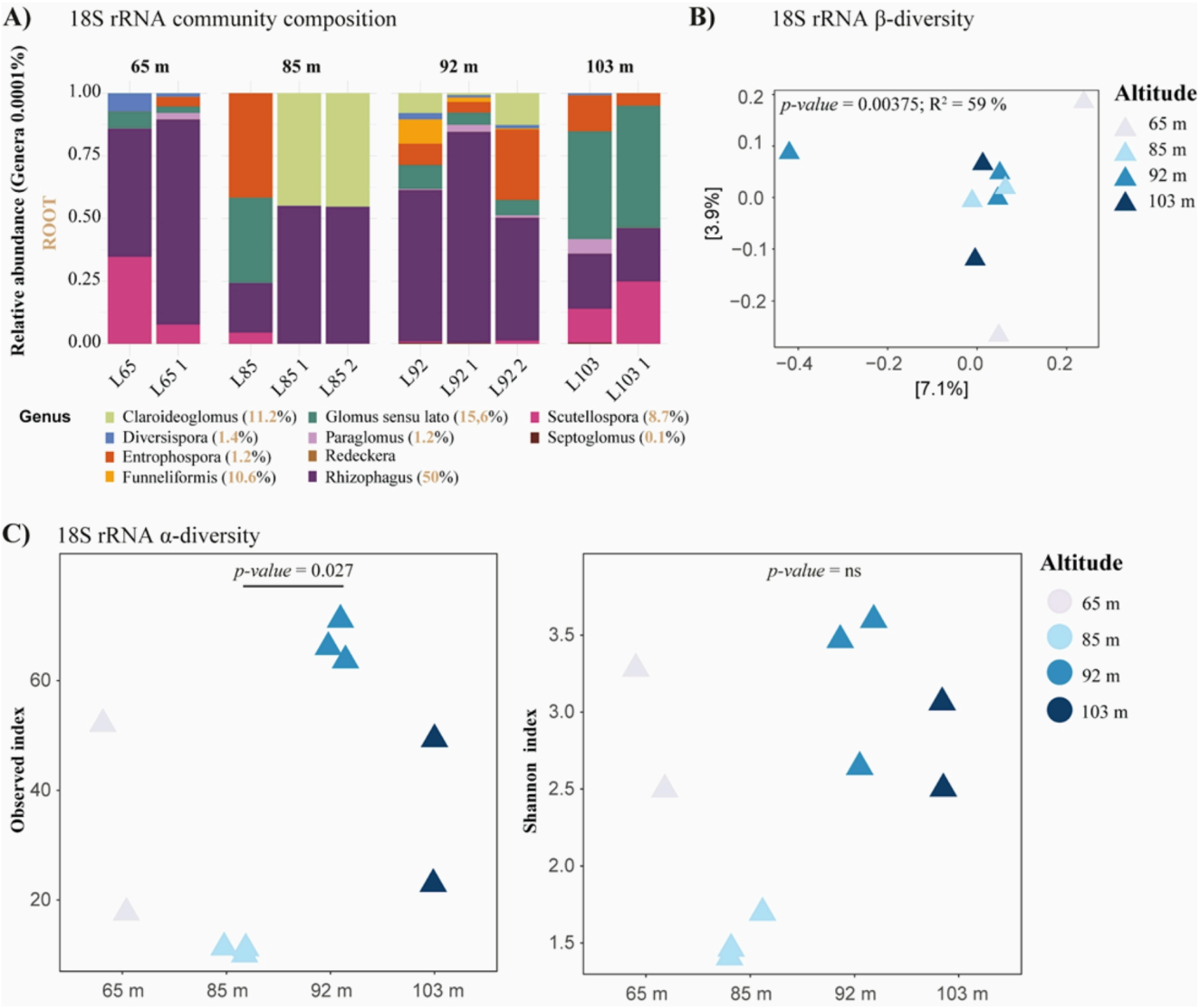

Figure 4

Glomeromycota(18S rRNA) communities associated with the roots of *Lycium intricatum*across an elevational gradient. **(A)** Genus-level relative abundance profiles of root-associated fungal communities inferred from 18S rRNA sequencing across the sampled elevations. Relative abundances within root samples are reported next to each genus label. **(B)** Bray–Curtis dissimilarity matrix illustrating variation in fungal 18S community composition among root samples, with colours indicating elevation. **(C)** Alpha diversity of root-associated fungal communities across elevations, expressed as Observed richness (left panel) and Shannon diversity (right panel). Differences in alpha diversity among elevation groups were evaluated using Dunn's post hoc test.

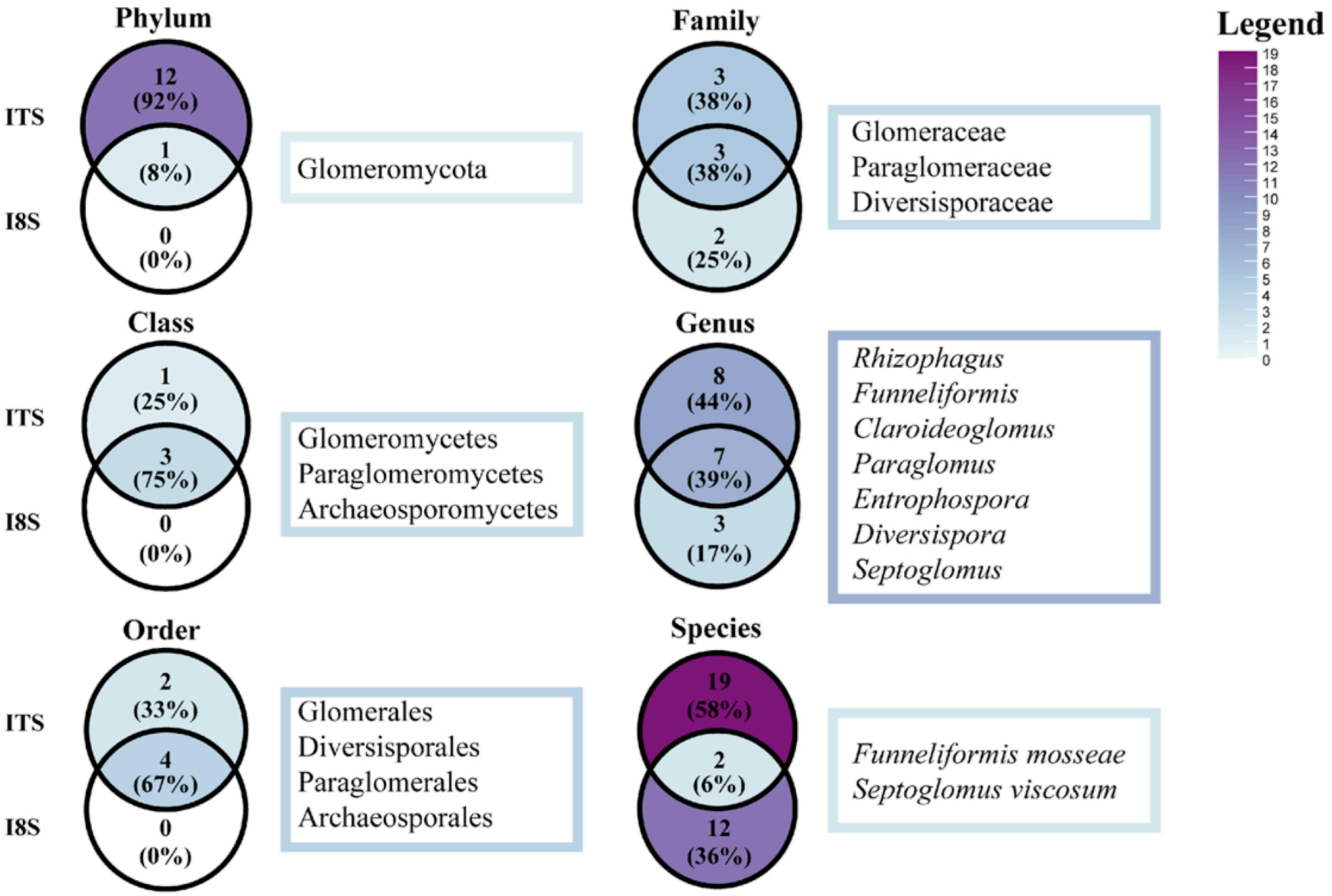

Figure 5

Venn diagrams showing the number of Glomeromycotina shared between ITS rRNA (top) and 18S rRNA (bottom) datasets at each taxonomic level. The degree of overlap is represented by colour intensity, while the names of shared taxa are displayed adjacent to each Venn diagram.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.xlsx
- SupplementaryscriptLycium.docx