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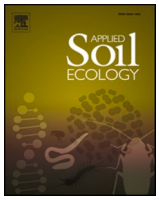

Soil microbial communities dynamics in response to invasive groundcover *Carpobrotus*: Insights into native species interactions and edaphic influence

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ABSTRACT

Alien invasive plant species of the genus *Carpobrotus* pose a major threat to coastal dune ecosystems. These human-introduced species have quickly expanded throughout the Mediterranean basin and other regions due to their high reproduction rates and adaptability. *Carpobrotus* invasion alters soil properties due to necromass and the release of allelopathic compounds, hindering the regrowth of native flora, with anticipated impacts on the native vegetation-associated soil microbial communities. While some studies have described changes in microbial communities between native and *Carpobrotus*-impacted areas, none have specifically addressed the responses of microbial communities associated with single native species. In this light, the bacterial and fungal communities specifically associated with different native species in both natural and *Carpobrotus*-impacted plots were examined in three locations along the middle Tyrrhenian Italian coast. Microbial communities responses to the *Carpobrotus* invasion varied greatly depending on the native species and the edaphic characteristics of the study locations. Microbial communities associated with *Pancratium maritimum* were the most affected ones and those associated with *Cakile maritima* the less sensitive to the invasion, which may be correlated to the different characteristics of these plant species. Furthermore, fungal communities exhibited a greater degree of disruption compared to bacterial ones. In invaded plots, fungal species that comprise plant pathogens were notably more abundant. This suggests that patterns in microbial communities response to this invasion phenomenon cannot be generalized, and that recovery strategies should consider various local conditions and be adjusted for the various native vegetation species. Finally, the possible spread of fungal plant pathogens as a mechanism of defence of *Carpobrotus* should be considered.

1. Introduction

Coastal dunes are dynamic ecosystems of high conservation value, strongly impacted by human disturbances and invasion of alien plant species worldwide (Pinna et al., 2015). Organisms in these environments face severe environmental conditions, including salt spray, soil oligotrophy, and intense solar irradiance that determine high-temperature values at the soil surface. These environmental constraints allow the growth of highly specialized (often endemic) plant species and associated microbial communities (Rajaniemi and Allison, 2009; Parsons et al., 2020; Souza-Alonso et al., 2022).

The invasion of fast-growing alien plants is among the main threats to the conservation of coastal dune ecosystems. In particular, species of the genus *Carpobrotus*, *C. acinaciformis* (L.) L.Bolus and *C. edulis* (L.) N.E. Br., native to South Africa, are among the major plant invaders of coastal dunes worldwide (Vilà et al., 2006; Traveset et al., 2008; Jucker et al., 2013; Novoa et al., 2013). *Carpobrotus* spp. are succulent perennial groundcover plants belonging to the Aizoaceae family, with three-sided leaves forming thick mats covering large areas. Some species of *Carpobrotus* have been intentionally introduced by humans to stabilize dunes or for decorative purposes. As a result, these species have now spread and become established in dune ecosystems across various regions

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outside of their native range. These regions include Australia, New Zealand, California, and Chile, as well as both Northern and Southern Europe (Campoy et al., 2018; Novoa et al., 2020; Parsons et al., 2020). The invasiveness of *Carpobrotus* spp. in many ecological niches, such as coastal dunes is due to their high vegetative reproduction ability, as well as the production of high numbers of fruits and seeds (Campoy et al., 2018). Moreover, the litter decomposition releases secondary metabolites inhibiting native plant species, reducing seed germination and seedling establishment rates (Novoa et al., 2012; Jucker et al., 2013). Among the different strategies implemented to control *Carpobrotus* spp. diffusion, physical removal and herbicide application on leaves have proven to be the most efficient (Campoy et al., 2018). However, the recovery of native vegetation resulted very problematic. Indeed, even though 14 projects funded by the European Commission were dedicated to eliminating *Carpobrotus* spp. from the coasts of France, Portugal, and Italy in recent years, all of them proved to be ineffective. This is because the allelopathic compounds released and persisting in soils inhibited the growth of native plants. Meanwhile, *Carpobrotus* spp. were able to grow quickly again, thanks to the abundance of their seeds in the litter (Campoy et al., 2018). *Carpobrotus* spp. diffusion dramatically impacts soil properties due to its abundant necromass, which improves soil water-holding capacity and nutrient availability, thus altering soil chemistry (Conser and Connor, 2009; Vilà et al., 2006; Santoro et al., 2011). Major leaf phytoconstituents include high levels of tannins and antibacterial compounds that may reduce litter decomposition rates and increase soil acidity (Vilà et al., 2006; Conser and Connor, 2009; de la Pena et al., 2010; Santoro et al., 2011; Novoa et al., 2013, 2014; Parsons et al., 2020). Soil acidification caused by the invasion of *Carpobrotus* spp. can, in turn, affect the availability of different elements, such as calcium (Ca) and magnesium (Mg) (D'Antonio and Haubensak, 1998) and, in particular, phosphorus (P), which is generally increasingly available in invaded areas (Novoa et al., 2014).

Moreover, invasion processes can be mediated and influence plant-soil microbe interactions at both the organism and community level, with critical roles in the invasion outcome (Inderjit and Cahill, 2015; Dawson and Schrama, 2016). The success of alien plant invasions and their impacts on the diversity and functioning of ecosystems depends on the characteristics of the invaded habitat (Hierro et al., 2005). In this regard, many studies have documented changes in soil microbial communities induced by *Carpobrotus* spp. An increase in total microbial biomass, a decrease in the bacteria/fungi ratio, which is generally used as an indicator of ecosystem health (De Vries et al., 2006; Strickland and Rousk, 2010), and a shift of microbial species relative abundances were found in coastal sites of Linosa Island (Mediterranean Sea, Italy) invaded by *C. acinaciformis* (Badalamenti et al., 2016). Bacterial communities composed of large numbers of rare taxa, mainly belonging to the phyla Actinobacteria and Proteobacteria, were recorded in natural coastal dunes on the north-western coast of Spain, while the presence of the alien *C. edulis* increased the diversity of soil bacteria and changed bacterial community structure (Novoa et al., 2020). Moving from the Mediterranean basin to California, a lower abundance of nitrifiers, fermentative bacteria, fungal parasites, and fungal dung saprotrophs, and a higher abundance of cellulolytic bacteria were observed in sites heavily invaded by *Carpobrotus* spp. (Parsons et al., 2020). Here, the increased abundance of cellulolytic bacteria driven by increasing plant biomass and changes in nitrification caused by bacteria competing with plant roots for available ammonium and/or acidification were postulated. Moreover, it was hypothesized that a significant increase in arbuscular mycorrhizal fungi in invaded soils may facilitate the invader development (Parsons et al., 2020). Finally, it was observed that changes in soil properties in *Carpobrotus*-invaded dune systems significantly impacted the diversity and functionality of soil microbial communities at both Mediterranean and Atlantic sites (Rodríguez-Caballero et al., 2020; Caravaca et al., 2022; Souza-Alonso et al., 2022). The changes observed in bacterial and fungal communities due to the spread of *Carpobrotus* followed a geographic pattern and were correlated with soil parameters (Rodríguez-Caballero et al., 2020). Additionally, changes in microbial communities were correlated with changes in their metabolic activities, including carbon metabolism and other nutrient cycling and catabolic activities (Caravaca et al., 2022; Souza-Alonso et al., 2022). Despite numerous studies reported significant changes in microbial communities of coastal dunes invaded by *Carpobrotus* spp., the response of soil bacterial and fungal communities associated with different native species has never been studied so far. The identification of the selective impact of *Carpobrotus* spp. on microbial taxa associated with each plant species could provide new tools for developing specific strategies for the recovery of native species based on their different responses.

In this light, the diversity and structure of soil bacterial and fungal communities associated with different native plant species in both uninvaded and *Carpobrotus*-invaded coastal dune systems of 3 localities of central Italy using a DNA metabarcoding approach were studied. The following hypotheses were tested: i) does the colonization of *Carpobrotus* spp. in coastal dunes alter the structure of microbial communities associated with native species?; ii) are the response patterns of microbial communities associated with native species taxon-related?; iii) are specific microbial taxa affected by the expansion of *Carpobrotus* spp.?; iv) do edaphic conditions of different study sites drive different responses of microbial communities?.

2. Methods

2.1. Sampling activity

Three study sites were chosen in central Italy: Saline (salt pans) di Tarquinia, Sant'Agostino, and Santa Severa (fig. S1). At each site, both natural dune habitats and areas invaded by *Carpobrotus* spp. were identified, with *C. acinaciformis* present at Saline di Tarquinia and Santa Severa, and *C. edulis* at Sant'Agostino. For each site the presence of *Carpobrotus* spp. is documented for >10 years. In each site, soil samples were collected for chemical and DNA analysis in correspondence with root systems of dominant native plant species present (Saline di Tarquinia: *Anthemis maritima* L., *Cakile maritima* Scop., *Centaurea sphaerocephala* L., and *Pancratium maritimum* L.; Sant'Agostino: *A. maritima*, *C. maritima*, and *C. sphaerocephala*; Santa Severa: *A. maritima*, *C. sphaerocephala*, and *P. maritimum*), both in their natural habitats and in association with *Carpobrotus* spp. (Fig. S1). Three replicates for each vegetation type were collected under sterile conditions and stored at −20 °C for subsequent analysis.

2.2. Soil properties

Air-dried soil samples (about 500 g each) were < 2 mm sieved. Chemical properties were analyzed according to standard methods of SISS (Società Italiana della Scienza del Suolo) (Colombo and Miano, 2015). Cation exchange capacity (CEC) was determined by barium chloride ($BaCl_2$) extracts method; soil exchangeable bases (Na^+, Ca^{2+}, K^+, and Mg^{2+}) were analyzed by flame atomic absorption spectrometry (AAS 1100B, PerkinElmer, Waltham, MA, USA); pH was measured in a 1:2.5 soil:water suspension; soil available phosphorus (P_{av}) was determined by Olsen and (P_{tot}) by a colorimetric method after acid digestion. Total soil organic carbon (OC) and total N were measured by NA 1500 CHNS Analyzer (Carlo Erba, Milan, Italy) and soil inorganic C (IC) as carbonate by using the procedure reported by Santi et al. (2006).

2.3. DNA extraction, amplification, and sequencing

Total DNA was extracted from 500 mg of all the samples through the E.Z.N.A. Soil DNA kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturing protocol. The V4 hypervariable region of the 16S rDNA gene was amplified using 515F (Parada et al., 2016) and 806R (Apprill et al., 2015) primers. The ITS1 region was amplified using the ITS1F (Gardes and Bruns, 1993) and ITS2 (White et al., 1990) primers.

Libraries were prepared following the protocol of Minich et al. (2018). The equimolar pools of uniquely barcoded amplicons were paired-end sequenced (2 × 250 bp) on an Illumina MiSeq platform at Macrogen, Inc. (Seoul, South Korea).

2.4. Bioinformatic analyses

Bcl files were converted to Fastq files and demultiplexed using bcl2fastq (v 2.20). Demultiplexed sequences were processed with the Amplicon ToolKit (AMPtk) for NGS data (formally UFITS) v.1.3.0 (Palmer et al., 2018). Starting reads were quality trimmed and PhiX screened using USEARCH v. 11.0.667 (Edgar, 2010) with default parameters. Reads with <100 bp were removed, the others were trimmed to the length of 250 bp, and paired-end reads were merged in one step. 16S amplicon reads were clustered in Amplicon Sequence Variants (ASVs), using DADA2 v1.20.0 (Callahan et al., 2016), which includes phiX reads removal and chimera detection. ITS1 amplicon reads were clustered into Operational Taxonomic Units (OTUs) using VSEARCH v 2.7.0 (Rognes et al., 2016), simultaneously removing putative chimeras. Rare ASVs (with less than two reads) and rare OTUs (with <5 reads) were removed from the datasets. Taxonomy was assigned to ASVs and OTUs with a hybrid approach that combines the results of a Global Alignment (USEARCH/VSEARCH), UTAX, and SINTAX (Edgar, 2010). Alignment was performed on the RDP11 database for bacteria and on the UNITE database for fungi. ASVs and OTUs with no match in the databases or showing <70 % identity with any known bacterial and fungal taxa were removed. Also, ASVs identified as Chloroplasts were excluded from the final dataset. Before diversity analyses, the number of reads per sample was rarefied to the lowest library size (30,493 reads for bacteria and 25,660 reads for fungi). Fungal OTUs with an identity higher than 90 % of any known fungal genera have been assigned to different functional guilds through comparisons within the FungalTraits database (Põlme et al., 2020).

2.5. Statistical analyses

All statistical analyses were carried out in R v. 4.2.1 (R Core Team, 2018).

The total richness and the relative richnesses (i.e. the richness of a specific group in a sample in relation to the total richness of the sample) and abundances of dominant bacterial phyla and fungal orders and functional groups were compared between samples in the plots of native vegetation and invaded by *Carpobrotus* (both considering all localities together and independently) with a Kruskal-Wallis test (McKight and Najab, 2010) followed by Dunn multiple comparisons (Dunn, 1964), with *p*-values adjusted with the Benjamini-Hochberg method.

Constrained Analysis of Principal (CAP) coordinates were used to identify patterns in microbial composition with the Bray-Curtis dissimilarity distance. The best set of explanatory variables was chosen through forward selection, using a Monte Carlo permutation test with 9999 randomizations and a significance level of $p < 0.05$. Finally, a Monte Carlo permutation test with 999 permutations was conducted to evaluate the significance of each environmental variable and the ordination axes in the CAP.

A Multinomial Species Classification Method (CLAM) statistical approach was used for classifying shared and specialist (i.e. sequences with statistically different read abundances in two conditions) ASVs or OTUs in both plots of native vegetation and invaded by *Carpobrotus* spp. as described in Chazdon et al. (2011). Genera differentially associated with the plots of native vegetation and invaded by *Carpobrotus* spp. were retrieved with a Linear Discriminant Analysis (LDA) effect size (LEfSe) based on LDA scores of >2 and p-values of <0.05 (Segata et al., 2011). The correlations between the richness of the total community and the relative richness and abundance of the dominant phyla and the parameters measured for soil samples were tested with Spearman's rank correlation coefficient.

3. Results

3.1. Dataset composition and taxonomic diversity

The sequencing resulted in 5,831,096 and 7,867,729 total reads for bacteria and fungi, respectively. After quality filtering 5,794,193 valid bacterial reads were retained, merged in 30,090 ASVs (after chimera removal), corresponding to 4,592,905 reads (79 % of the total). Two singleton ASVs were then removed. For fungi, after quality filtering 7,276,465 valid reads were retained that were clustered in 6480 OTUs (after chimera removal), corresponding to 6,464,930 reads (89 % of the total). 1423 rare fungal OTUs (<5 reads in the dataset) were removed. After the removal of ASVs or OTUs not assigned to bacteria and fungi in the specific datasets and of those showing an identity lower than 70 % to any match in the specific databases, a total of 10,956 bacterial ASVs and 4029 fungal OTUs were obtained. After the rarefaction, the two final datasets were composed of 10,811 bacterial ASVs and 3882 fungal OTUs.

From a taxonomic point of view, bacterial communities were dominated by 10 phyla, which represented 98.54 ± 0.34 total reads per sample. Fungal communities were dominated by few phyla and 6 more abundant classes, which together represented 74.32 ± 16.25 reads per sample, and that were considered for further analyses. Additionally, 1121 fungal OTUs were assigned to different functional guilds, namely animal parasites (64 OTUs), plant-associated fungi (88 OTUs; which also included 37 OTUs assigned to arbuscular mycorrhizal fungi and 33 OTUs to ectomycorrhizal fungi), plant pathogens (200 OTUs), and saprotrophs (727 OTUs). Within this last group, some sub-categories were also identified: dung saprotrophs (31 OTUs), litter saprotrophs (142 OTUs), soil saprotrophs (204 OTUs), and wood saprotrophs (172 OTUs). Finally, 42 OTUs were assigned to some low-represented groups, such as algal parasites and mycoparasites, that were not considered in subsequent analyses due to their low representativeness in the dataset.

The total bacterial and fungal diversity showed not statistically significant ($p < 0.05$) differences between soil samples associated with the native vegetation and those associated with *Carpobrotus* spp. invaded plots (fig. S2 and S3). Regarding the main bacterial phyla, fungal classes and fungal functional groups, very few significant ($p < 0.05$) differences in their relative richness and abundance were found between invaded and uninvaded plots (fig. S2, S3, S4, and S5). The richness of Actinobacteria increased in invaded plots compared with uninvaded, while the richness of Acidobacteria and the relative abundance of Gemmatimonadetes decreased (fig. S2 and S4). For fungi, the richness and the abundance of Leotiomycetes and the abundance of plant associated and litter saprotrophs increased in invaded plots, while the abundance of plant pathogens decreased (fig. S3 and S5). When considering the single native vegetation species independently, significant ($p < 0.05$) results were observed. In particular, we found that the colonization of *Carpobrotus* spp. of coastal dunes led to an increase in the richness of both bacterial and fungal communities associated with *P. maritimum* (Fig. 1a, b).

Looking at the most represented bacterial phyla, the invasion by *Carpobrotus* spp. reduced the Acidobacteria diversity in communities associated with roots of *C. sphaerocephala* and *P. maritimum*. Furthermore, a decrease in Gemmatimonadetes diversity and an increase in Proteobacteria diversity in communities associated with *C. sphaerocephala* were found (Fig. 1c, d, e; fig. S6). Additionally, an increase in the relative abundance of Actinobacteria in *P. maritimum*-invaded plots and Verrucomicrobia in *C. sphaerocephala*-invaded plots and a decrease in the relative abundances of Bacteroidetes associated with *A. maritima* invaded plots was observed (fig. S8). Instead, considering the fungal taxa, *A. maritima* was the most affected plant species, exhibiting a significant ($p < 0.05$) increase in both richness and relative abundance of Leotiomycetes in invaded plots (Fig. 1f; fig. S7). A significant ($p < 0.05$) decrease in the abundance of Dothideomycetes and Sordariomycetes was also observed in invaded plots (fig. S9). Moreover,

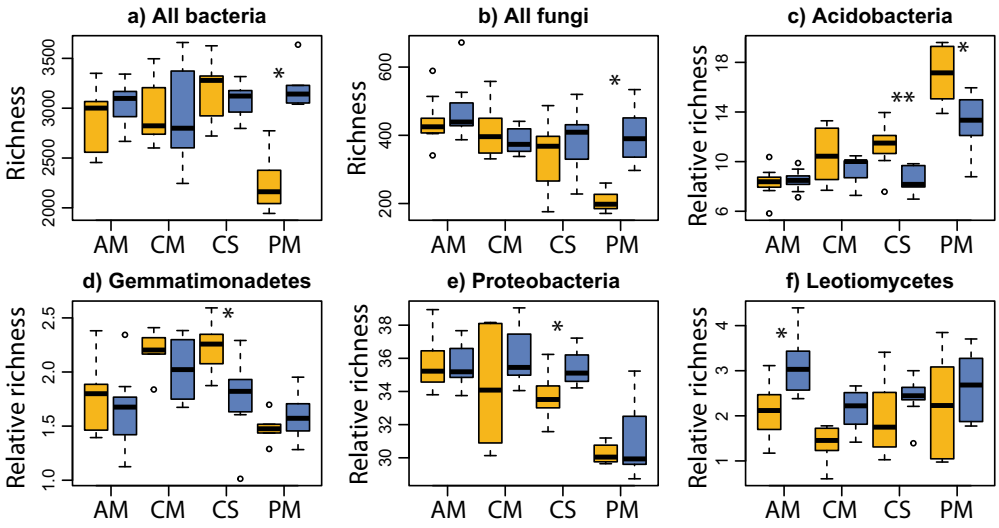

Fig. 1. Differences in the richness of the total (a) bacterial and (b) fungal communities and the relative richness of the bacterial phyla (c-e) and a fungal class (f) between native (yellow) and *Carpobrotus*-invaded (blue) plots considering the different native species independently (AM: *Anthemis maritima*; CM: *Cakile maritima*; CS: *Centaurea sphaerocephala*; PM: *Pancratium maritimum*). Asterisks indicate statistically significant differences in a Kruskal-Wallis test followed by Dunn multiple comparisons (* *p*-value <0.05; ** p-value <0.01). Only the groups showing significant differences are reported (all the comparisons are shown in figs, S6 and S7). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

regarding the abundance of identified functional guilds, no significant ($p < 0.05$) effect of *Carpobrotus* spp. colonization was found. In addition, the abundance of plant pathogens decreased in the presence of the alien species, with significance found only in soils associated with *A. maritima* (fig. S9).

3.2. Communities structure

At the community level, significant ($p < 0.05$) differences were observed in the structure of bacterial and fungal communities between native plots and those invaded by *Carpobrotus* spp. (Fig. 2). Specifically, the differences were statistically significant ($p < 0.05$) when considering each of the four species of native vegetation independently, except for bacterial communities associated with *C. maritima* (Table S1). Additionally, significant ($p < 0.05$) differences in the structure of the bacterial and fungal communities associated with *A. maritima* and of the fungal communities associated with *C. sphaerocephala* were observed among the different study sites.

3.3. Specialist and cosmopolitan taxa

The CLAM analysis of the distribution of bacterial ASVs and fungal OTUs confirmed the previous trends observed for communities structure (Fig. 3; Table S2). The percentages of taxa specialists in *Carpobrotus* spp.-invaded plots were always higher than those observed in native plots. Additionally, the effect of *Carpobrotus* spp. invasion of coastal dunes seemed to be more pronounced for fungal communities than for bacterial ones. Indeed, a higher proportion of specialist taxa in both native and invaded plots for fungal communities compared to bacterial ones was always observed (Fig. 3). Additionally, in this case, the communities associated with *P. maritimum* were the most affected by the invasion of *Carpobrotus* spp., with a higher proportion of both bacterial and fungal specialists taxa for native and invaded plots (Fig. 3).

Additionally, a high number of bacterial and fungal genera was described as significantly ($p < 0.05$) differentially enriched between invaded and uninvaded plots. *A. maritima* and *P. maritimum* associated bacterial and fungal communities had the highest numbers of genera with significantly different abundances in the two conditions (Fig. 4).

3.4. Effect of edaphic parameters

Soil samples exhibited an IC content between 0.4 and 4.0 %, with very low contents of OC ranging from 0.04 to 0.32 % and N from 0.0004 to 0.0238 %. The P_{tot} varied from 327.4 to 857.4 ppm with only a small proportion resulting in P_{av}, ranging from 1.9 to 22.1 ppm. Cation exchange capacity (CEC) ranged between 1.7 and 5.6 cmolkg-1, with exchangeable Na between 0.10 and 0.98 cmolkg-1 and exchangeable K between 0.0004 and 0.0614 cmolkg-1. The soil pH was slightly basic, ranging between 7.69 and 8.28 (Table S3). No significant ($p < 0.05$) differences in soil parameters were found when invaded and native soil plots were compared independently from the individual site (fig. S10). Instead, few site-dependent significant ($p < 0.05$) differences were observed between native and invaded plots, with increased OC content at Santa Severa and P_{tot} at Sant'Agostino in invaded plots compared to native ones (fig. S11).

The richness of bacterial phyla showed few significant correlations ($p < 0.05$) with the different parameters measured, except for Firmicutes, which showed a significant ($p < 0.05$) negative correlation with N content (Fig. 5). Regarding fungi, the classes Eurotiomycetes and Sordariomycetes exhibited the highest number of significant ($p < 0.05$) correlations, being negatively correlated with C and N contents and positively correlated with P_{tot}. Considering functional groups, plant-associated fungi were strongly correlated with edaphic parameters, showing significant ($p < 0.05$) positive correlations with C and N contents and negative correlations with both forms of P (Fig. 5). For the abundance of the mentioned groups, a lower number of significant ($p < 0.05$) correlations was observed, following the same trends of the correlations for richness (fig. S12). Regarding community composition, few of the parameters measured were correlated with the observed variance, with organic C and both forms of P being the most influential parameters (Fig. 3; table S1).

4. Discussion

A deep understanding of the ecological impact given by the invasion of natural habitats (e.g. coastal dunes) by alien plant species is crucial for developing successful control and environmental recovery strategies, also in view of the increasing evidence that climate change will stimulate biological invasions (Hellmann et al., 2008; Bellard et al., 2018;

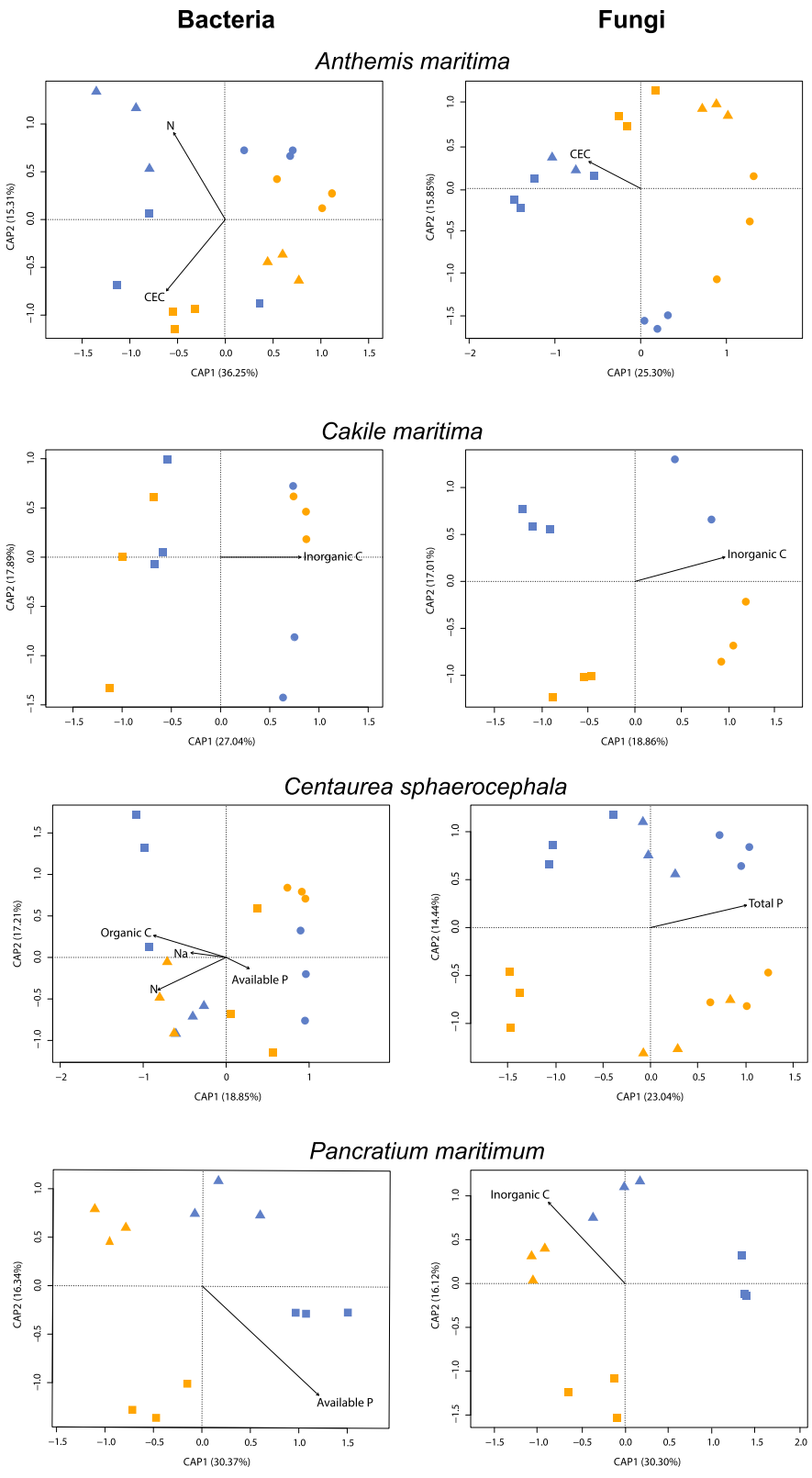

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Fig. 2. Constrained Analysis of Principal (CAP) coordinates using Bray-Curtis dissimilarity of the structure of the bacterial (left column) and fungal (right column) communities associated with the four different species of native vegetation when present in uninvaded (yellow) and invaded (blue) plots for the three study sites. Significant variables ($p < 0.05$) retained by the model (table S1) are reported as arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Bartz and Kowarik, 2019; Weidlich et al., 2020; Rai and Singh, 2020). Among alien species, those belonging to the genus *Carpobrotus* have been widely studied for their significant impacts on neighbour native vegetation. In addition to the widely described allelopathic effects that *Carpobrotus* spp. exert on soils, induced changes in soil properties may result in shifts of soil microbial communities that, in turn, can alter the ability of native species to grow in coastal dunes and compete with the invaded species. In the present study, the invasion of *Carpobrotus* spp., despite having minimal effects on the diversity of soil microbial communities associated with native vegetation, strongly affected their composition. Specifically, we observed alterations in the structure of soil bacterial and fungal communities associated with the root ecosystem of native species, following a trend related to the different study sites and the soil parameters. This suggests that the impact of *Carpobrotus* spp. colonization on microbial communities associated with the roots of native plants of coastal dunes can be difficult to generalize because it may be highly dependent on local factors. Indeed, it is well known that alien and invasive plants, including *Carpobrotus* spp., exhibit high levels of plasticity (Davidson et al., 2011; Geng et al., 2016; Liao et al., 2016) and/or high genetic diversity (Estoup et al., 2016; Roman and Darling, 2007), enabling them to tolerate a wide range of conditions and dominate in highly diverse habitats. On the other hand, the great diversity of the different environments in which this genus can spread can be linked with highly differentiated responses of resident microbial communities. Indeed, it is widely recognized that the impact of invasive alien species is highly variable both in time and space, as invasion is a dynamic process based on the interactions between the alien species and the endangered ecosystem (Shackleton and Gambiza, 2008; Vilà et al., 2011). In this light, the results reported in the present study confirm the existence of different responses of soil microbial communities in *Carpobrotus*-invaded plots in different sites, in correlation to local environmental conditions, in agreement with a previous study (Rodríguez-Caballero et al., 2020) and in close analogy with the response to other alien and invasive species, such as *Acacia dealbata* Link (Kamutando et al., 2017) and *Phragmites australis* (Cav.) Trin. ex Steud. (Farrer et al., 2021).

Microbial communities associated with native *P. maritimum* exhibited the strongest response to the invasion of coastal dunes by *Carpobrotus* spp., both in terms of diversity and structure, in agreement with the study of Caravaca et al. (2022), which documented a similar trend. The higher impact of the colonization on microbial communities associated with *P. maritimum* could be explained by the presence of traction or contractile roots, very common in various families of monocots, that penetrate deeply into the soil to reach water sources (Voliotis and Drossos, 1983). Due to runoff from rainfall (differently from the other plant species herein studied, which exhibit more surface root systems capable of efficiently evading salt stress), *P. maritimum* root system has to cope with higher salt concentrations and possibly higher concentrations of the allelopathic substances released by *Carpobrotus*, affecting in turn the soil associated microbial communities.

The microbial communities associated with *C. maritima* were less affected by the presence of *Carpobrotus* spp., with no statistically significant differences in bacterial community composition between native and invaded plots. A lower proportion of specialist bacterial and fungal species in both uninvaded and invaded plots, and a lower number of differentially abundant bacterial and fungal genera were also described for the communities associated with this species compared to the other three species of the native vegetation. *C. maritima* is strongly resistant to many sources of contamination, such as heavy metals (Taamalli et al., 2015) or polycyclic aromatic hydrocarbons (Shiri et al., 2015); this justifies its strong antioxidant activity and its high ability to accumulate osmoprotectants (Ksouri et al., 2007). The peculiar resistance of this species to contaminants could be a secondary result of its adaptation to the high salinity levels that the plant experiences, being a pioneer species of the foreshore, where it can tolerate water spray and seawater inundation (Barbour, 1970). These adaptations could also allow the species to tolerate better the detrimental effects of *Carpobrotus* spp. on soil characteristics. Indeed *C. maritima* is also described as a second invader in areas where *Carpobrotus* spp. is widely diffused on California coasts (Parsons et al., 2020). The strong resistance of *C. maritima* and its ability to cope with *Carpobrotus* spp. allelopathic effect may lead to a reduced impact on microbial communities associated with the roots of this species when it grows in the presence of the invader.

Interestingly, a stronger differentiation of fungal communities than bacterial ones was observed for all the native species studied (Fig. 2); this evidence was confirmed by a higher proportion of specialist fungal taxa of both native and invaded plots than bacterial ones (Fig. 3). While never specifically documented in the case of *Carpobrotus* spp., a stronger influence on fungi than bacteria has been previously reported in the analysis of soil communities associated with other invasive plant species when compared to native ones, such as *Eupatorium adenophora* (Spreng.) R.M.King & H.Rob. *Chromolaena odorata* (L.) R.M.King & H.Rob. (Xiao et al., 2014), and *Wedelia trilobata* (L.) Pruski (Si et al., 2013). This suggests the hypothesis that fungi may have a stronger relationship with the roots of native plants and, therefore they may be subjected to a stronger response to the invasion of coastal dunes by alien species.

Another important aspect to consider is that the colonization of coastal dunes by *Carpobrotus* spp. resulted in an increased abundance of fungal taxa including plant pathogenic species, namely Didymellaceae, *Knufia* spp., *Cyphellophora* spp., *Ulocladium* spp., *Myrothecium* spp., *Fusicladium* spp., *Curvularia* spp., and *Plectosphaerella* spp.. The possibility of accumulating plant pathogenic species has been hypothesized as an additional mechanism reinforcing the ability of alien species to colonize a given ecosystem. This phenomenon is usually referred to as "spillback": it was described as the ability of an alien species to host microbial pathogens towards native species, thus potentially exacerbating the impact of the colonization (Flory and Clay, 2013). Such phenomenon was observed for some alien species, which has also been described as the "enemy of my enemy hypothesis" (Colautti et al., 2004; Flory and Clay, 2013). *Chromolaena odorata* can accumulate soil fungal pathogens (i.e. *Fusarium incarnatum*) that selectively inhibit native plants (Mangla and Callaway, 2008). Similarly, the invasive *Bromus tectorum* (L.) Nevski is a potential reservoir of *Pyrenophora semeniperda* (Beckstead et al., 2010), while the invasive shrub *Rhododendron ponticum* L. hosts the plant pathogens *Phytophthora ramorum* and *Phytophthora kernoviae* (Purse et al., 2013), in both cases increasing the vulnerability of invaded ecosystems to disease spread.

Furthermore, some black yeasts, such as *Capronia* spp., *Knufia* spp., *Saxophila* spp., *Cyphellophora* spp., and *Neodevriesia* spp. were highly represented among those enriched in *Carpobrotus* spp.-invaded plots. Fungi of this polyphyletic group are well known for their aptitude to withstand multiple stressors, such as extreme temperatures, salinity, and pH, excessive radiation, oligotrophy, desiccation, and different chemicals and pollutants (Gostinčar et al., 2012). Therefore, they could exploit their extremophilic aptitude to increase their presence thanks to their tolerance to *Carpobrotus* spp. allelopathic molecules. Finally, species of *Knufia* and *Cyphellophora* have also been described as dark septate endophytic symbionts (Santos et al., 2021; Vanó et al., 2011), which are a group of fungi involved in C, N, and P uptake (Della Monica et al., 2015; Narisawa, 2018) and protecting plants against pathogens and abiotic stresses (for example heavy metals and drought) (Likar and

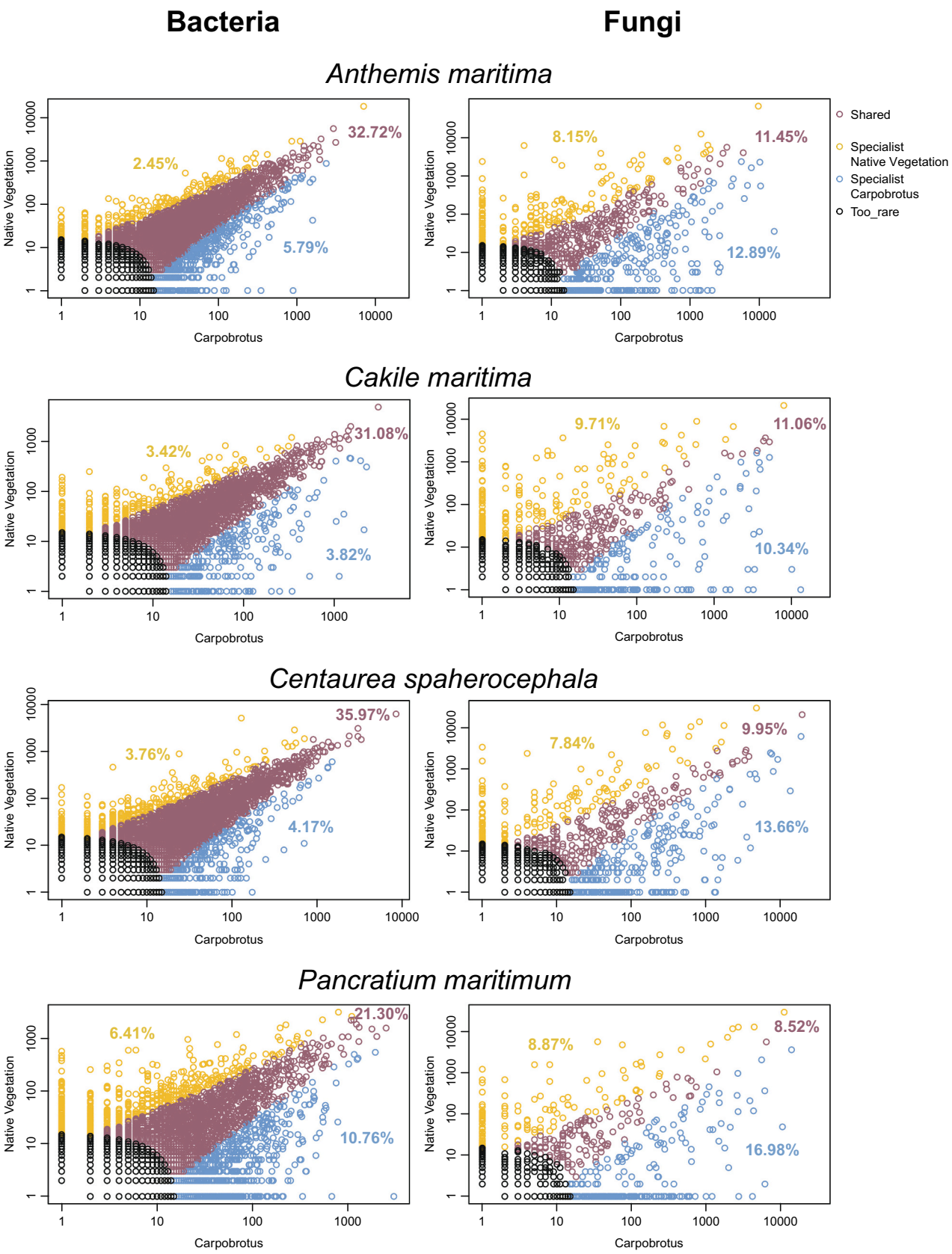

Fig. 3. Biplots showing the results of CLAM tests based on pairwise comparisons between native and invaded plots for bacterial (left column) and fungal (right column) communities associated with different native plant species. Percentages values of ASVs (for bacteria) and OTUs (for fungi) belonging to the shared or specialist groups are reported with the same colours.

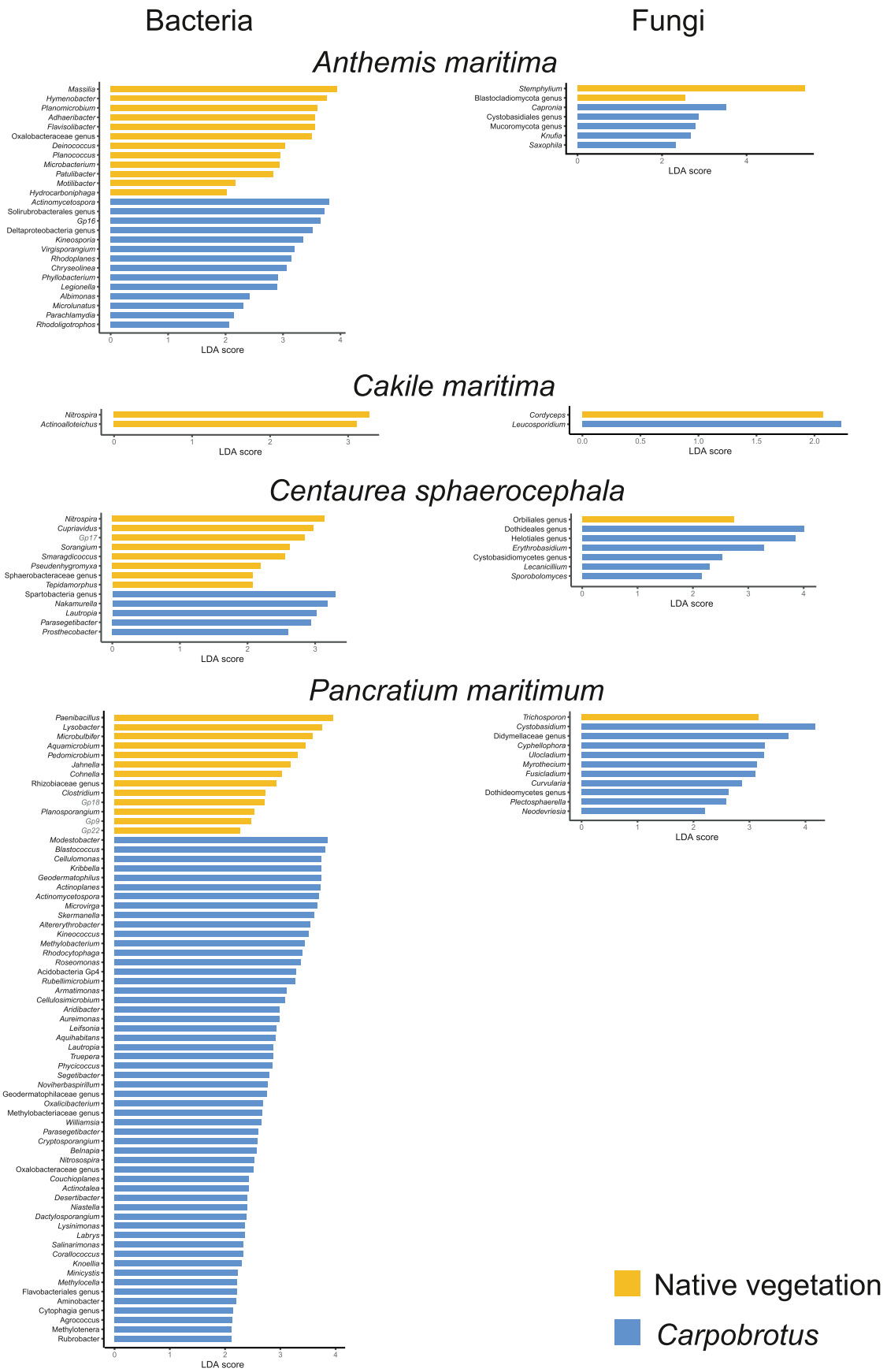

Fig. 4. Linear discriminant analysis (LDA) effect size (LEfSe) method for differentially abundant bacterial (left column) and fungal (right column) genera for the communities associated with different native plant species.

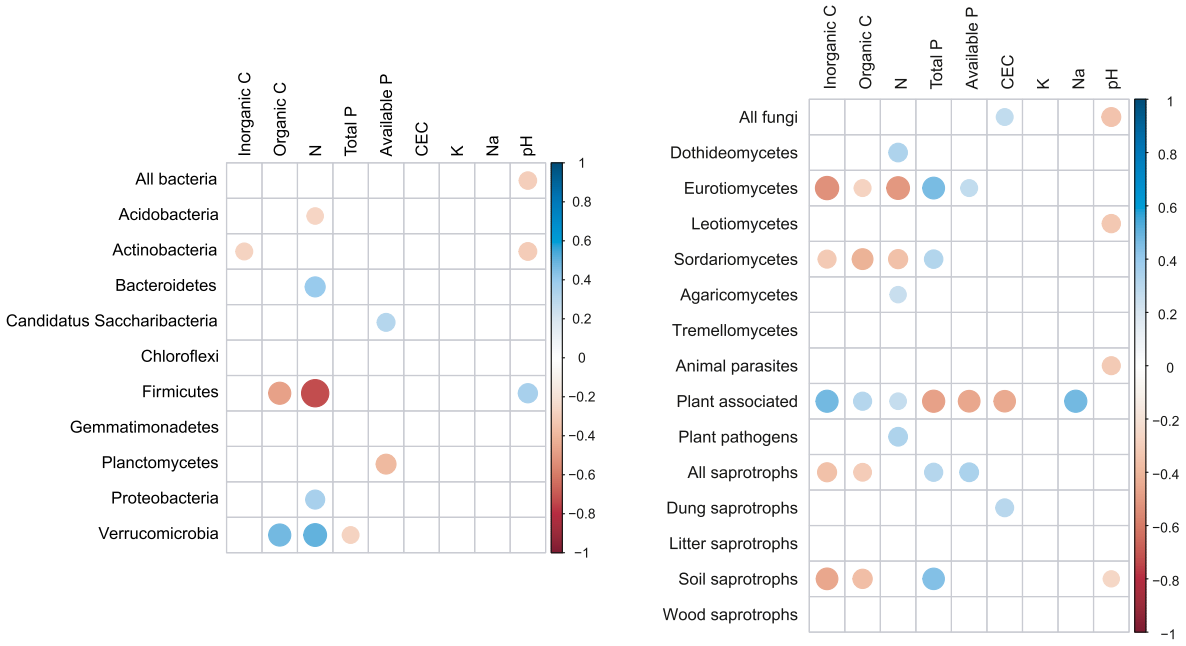

Fig. 5. Spearman's rank correlation of the richness of the total bacterial and fungal communities and of the relative richness of most represented bacterial phyla and fungal classes and functional groups with the soil physicochemical parameters measured. Rho values are represented in the correlogram corresponding to the colours and size of the circles. Only significant correlations are shown (p-value <0.05).

Regvar, 2013; Santos et al., 2021; Narisawa, 2018). Therefore, the positive selection of these taxa could be a further mechanism implemented by *Carpobrotus* spp. to increase its persistence in invaded coastal dunes.

Regarding bacteria, the genera more significantly enriched in *P. maritimum* invaded plots belonged to Geodermatophilaceae (*Geodermatophilus*, *Blastococcus*, and *Modestobacter*), which are known for their ability to withstand several environmental stressors (Hongmin et al., 2015).

5. Conclusion

The colonization of coastal dunes by *Carpobrotus* spp. strongly alters the structure of both bacterial and fungal communities associated with the roots of native vegetation (especially *P. maritimum*). Such alterations were strongly correlated to both local edaphic conditions and native species, making it very difficult to elucidate a general trend on the microbiota responses to these invaders. It also suggests the need to study different restoration strategies for the different native species.

In particular, a higher impact has been observed in the structure of soil fungal communities, probably determining strong changes in soil functions and feedback, especially in soil nutrient dynamics and in an increased possibility of spreading plant diseases caused by pathogenic fungi. The alteration of the soil fungal community may impact the native plant communities for years after removing invasive alien plants (a possible legacy effect). In particular, these changes can be more severe in areas where plants have been eradicated after long-term invasions which can take months or decades to reverse, even with active management, making restoration particularly difficult (Pickett et al., 2018).

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CRediT authorship contribution statement

F. Canini: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **L. Borruso:** Writing – review & editing, Visualization, Formal analysis. **S. Magrini:** Writing – review & editing, Project administration, Conceptualization. **L.P. D'Acqui:** Writing – review & editing, Investigation. **P. Buzzini:** Writing – review & editing. **G. Cavallini:** Writing – review & editing. **L. Zucconi:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated and analysed during the current study are available in the NCBI SRA repository, BioProject ID: PRJNA1081671

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