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# The invader *Carpobrotus edulis* promotes a specific rhizosphere microbiome across globally distributed coastal ecosystems

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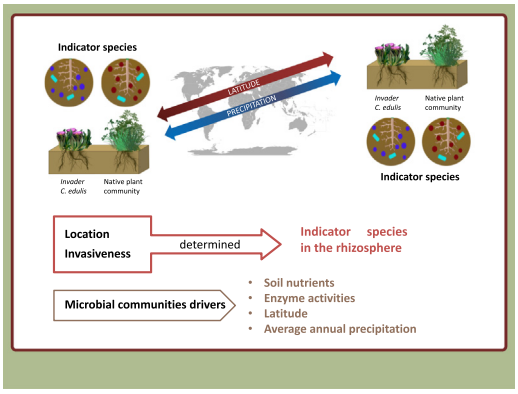
## HIGHLIGHTS

- Carpobrotus edulis is an aggressive invader in coastal areas around the world.
- The invader shifted local bacterial and fungal community structure and composition.
- The geographical location had a significant effect on the soil microbiota.
- The invader altered microbial activities linked to nutrient cycling depending on site.
- The invader rhizosphere promoted a specific microbiome over the entire invaded range.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The importance of plant-microbe interactions to the success of invasive plants has rarely been studied at a global scale. *Carpobrotus edulis* (L.) N. E. Br is an aggressive invader in many areas around the world, forming dense mats in coastal environments. In an approach at a large geographical scale, over a wide latitudinal and climatic range, we tested the ability of *C. edulis* to alter the local bacterial and fungal community structure and microbial activity in eight invaded coastal locations. The factors invasiveness and geographical location had a significant effect on the soil microbiota, the microbial community composition and structure from the rhizosphere of native and *C. edulis* plants being distinct in every location. The effect of the invader on all the chemical, physico-chemical, and microbiological properties studied depended on the invaded location. The soil bacterial and fungal community composition and structure were related to the soil available nutrients and mean annual rainfall, and those of the soil bacterial community were also linked to the soil respiration and latitude. Overall, our results reveal that the ability of the invader *C. edulis* to alter soil microbial community structure harboring a specific microbiome was widespread across a large invaded range - leading to concurring changes in the rhizosphere microbial functioning, such as nutrient cycling.



## 1. Introduction

Biological invasions pose a major threat to the conservation of biodiversity and ecosystem stability and functions, and also cause important

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economic losses (Pimentel et al., 2005; Secretariat of the Convention on Biological Diversity, 2014). Currently, this environmental problem is worsening due to increased trade and transportation, which has originated a spread of invasive exotic plants around the world (Pyšek et al., 2010). Predictably, the geographical distribution of plant species is expected to shift along with climate change, favoring their establishment at new sites and further spread from existing sites (Walther et al., 2009). The fact that some geographic areas are more easily invaded than others is generally poorly understood (Catford et al., 2012). The establishment and spread of an introduced plant species are largely determined by the climate of the new habitat, ranges with favorable climatic conditions for plant growth and/or with a climate similar to that of the native range being more susceptible to invasion (Thuiller et al., 2005). Disturbed areas with high resource availability and weak pressure from enemies (predators, parasites, and pathogens) are generally vulnerable to invasions (Hierro et al., 2005), which has been observed at intermediate (Stohlgren et al., 2003) to large spatial scales (Lonsdale, 1999). The invasion success can also be mediated by direct (absence of virulent pathogens) and indirect (enhanced growth of pathogens of native plants, enhanced mutualists presence and resource availability, supression of mycorrhiza related to native hosts) feedbacks established between the exotic plant and the native soil microbiota (van der Putten et al., 2013; Inderjit and Cahill, 2015). Among the soil microbiota involved in plant-soil feedback are rhizosphere bacteria and saprophytic fungi that perform key functions in relation to plant establishment and soil fertility - such as biological control of plant pathogens, nutrient cycling, solubilization of mineral phosphates and other nutrients, resistance to stress, and stabilization of soil aggregates (van der Putten et al., 2007). The net effects of plant-microbe feedbacks and their impact on the invasion process change spatially across the geographical ranges of the invader and over time, after its introduction, because the underlying mechanisms are dependent on the local conditions, edaphic features, plant species, and soil microbial community diversity and composition (Inderjit and Inderjit and Cahill, 2015).

*Carpobrotus edulis* (L.) N.E. Br. (*Aizoaceae*), a ground-creeping plant with succulent leaves, is an aggressive invader in many areas around the world (Campoy et al., 2018). This species is native to South Africa (Albert, 1995) and has been introduced for ornamental use and landscaping in coastal areas, as well as for sand dune stabilization. It prefers a warm, temperate-to-dry climate, being sensitive to frost. It is resistant to drought and has an inducible CAM metabolism when subjected to drought or salt stress (Earnshaw et al., 1987). *Carpobrotus edulis* is an invader of coastal habitats in America, Australasia, and Europe and one of the major invaders of Mediterranean coastal ecosystems (GISD, 2018). It spreads readily and rapidly to form deep, dense mats over 50 cm deep, decreasing the availability of space, light, and nutrients as well as inhibiting the germination of seeds of other plants (Conser and Connor, 2009). It can also decrease plant species diversity by preventing sand movement, which hinders the natural processes of disturbance and change in dune environments (Delipetrou, 2009). In particular, this invader competes aggressively with native plant species, threatening endemic and endangered species of Mediterranean coastal environments, one of the ecosystems valued most highly around the globe. When *C. edulis* invades a coastal dune ecosystem it can significantly modify soil properties and nutrient dynamics by increasing soil salinity, available P, organic C, and water availability (Novoa et al., 2014) and can differently affect soil pH depending on the invaded substrate (Vieites-Blanco and González-Prieto, 2018). The changes in the physico-chemical features and soil substrate conditions following invasion by *C. edulis* can affect the soil microbial community structure and activity, as has been observed previously for other invasive plants (Yang et al., 2016; Rodríguez-Caballero et al., 2017). Also, alterations in the litter quality and in the inputs of root exudates and litter entering the invaded soil can shift soil microbiota (Rodríguez-Caballero et al., 2017). Despite the extensive literature on this invader, only one study, at a local scale, has examined the effect of *C. edulis* on soil microbial communities within an insular Mediterranean ecosystem (Badalamenti et al., 2016). For this global environmental threat, it is essential to know the contribution of soil microbial communities to the facilitation of and resistance to plant invasion, preferably through studies at broad geographical scales.

We hypothesized that the invader *C. edulis* may have promoted unique soil microbial communities with distinct functional abilities, in comparison with those of the microbiota of native plant species. We also questioned if the changes mediated by this invader are dependent on the invaded biogeographical range. To address these questions we compared the bacterial and fungal communities harbored in the rhizosphere of *C. edulis* with those of the rhizosphere of the co-occurring native plants growing in coastal environments, over a wide latitudinal and climatic range. We used a DNA-metabarcoding approach for high-throughput monitoring of bacterial and fungal communities and determined soil basal respiration and enzymatic activities to analyze the soil microbial activity, at eight coastal locations that are geographically distributed at a global scale. Also, we attempted to determine the most important environmental factors (location, climatic and edaphic variables) involved in driving the soil microbial community assemblages of the invaded ecosystems.

## 2. Materials and methods

### 2.1. Study locations and sampling

Different coastal localities, where *C. edulis* is considered as an invasive species for a long-time (>20 years) were sampled, that include 5 locations with Mediterranean climate characterized by warm and dry summers, moderately wet and cool winters, and average annual rainfall ranging from 200 to 600 mm, and 3 Atlantic locations with contrasted temperature and average rainfall (temperate oceanic and subtropical oceanic climates). All soils are classified as Albic Arenosols with sandy texture, except the soil from Tenerife Island that is an Andic Cambisol (IUSS Working Group WRB, 2015). The composition of the native coastal plant communities and climatic parameters of each location have been described in Table 1. The sampling was conducted as a two-factor factorial with four replicates, randomly arranged, the first factor being "Location" with eight levels, and the second factor being the "Invasiveness" character of the plant with two levels: invasive and non-invasive plants. At each location, four (3 m by 3 m) sampling plots long-invaded by *C. edulis*, 10 m apart, were established where invasive and native species were growing. Within each plot, we collected one composite sample comprised of samples from the rhizospheres of *C. edulis* under a mature mat and one composite sample comprised of samples of the rhizospheres of the most abundant native species. The selected individuals of the native species were separated from the invasive plant by about 1.5 m to minimize the influence among rhizospheres. Thus, four replicates for plant invasive character were established in each location (64 samples in total). Sampling was conducted during the spring of 2017. The samples, including the soil adhering firmly to the roots (rhizospheric soil), were transported to the laboratory in polyethylene bags. One portion of the rhizospheric soil was stored at −20 °C, for DNA sequencing, and the remaining soil was divided into two subsamples. One soil subsample was sieved through a 2-mm mesh and stored for up to 3 weeks at 4 °C for biochemical analysis (Kandeler, 2015) and another was sieved through a 2-mm mesh and allowed to dry at room temperature for physico-chemical and chemical analyses.

### 2.2. Soil physico-chemical, biochemical, and biological analyses

Soil pH and electrical conductivity were measured in a 1:5 (*w*/*v*) aqueous extract. In this aqueous extract, water soluble carbohydrates (WSCH) were determined as reported by Brink et al. (1960). A Euro EA elemental analyzer (EuroVector, Milan, Italy) was used for

**Table 1**
Sampling points location, geographical coordinates, climatic parameters and native vegetation.

| Location | Abbreviation (invasive, native) | Geographical coordinates | Mean annual rainfall (mm) (*) | Mean annual temperature (°C)(*) | Dominant native plant species |
|---|---|---|---|---|---|
| Valparaíso, Chile (V) | VC, VN | 33° 00′ 40.3″ S/71° 33′ 10.9″ W | 424 | 14.8 | *Baccharis concava* (Ruiz & Pav.) Pers.; *Eriosyce subgibbosa* (Haw.) Katt.; *Leptocarpha rivularis* DC.; *Nolana paradoxa* Lindl. |
| Almería, Spain (A) | AC, AN | 36° 50′ 6.5″ N/2° 23′ 5.9″ W | 196 | 18.7 | *Launaea fragilis* (Asso) Pau; *Lotus maritimus* L.; *Brachypodium retusum* (Pers.) P.Beauv.; *Cakile maritima* Scop.; *Asteriscus maritimus* L.; *Fagonia cretica* L.; *Beta vulgaris* L. subsp. *maritima* (L.) Arcang. |
| La Llana, Spain (L) | LC, LN | 37° 49′ 40.1″ N/0° 45′ 55.9″ W | 299 | 18.2 | *Paronychia argentea* Lam.; *Trachynia distachya* (L.) Link; *Aizoon hispanicum* L.; *Beta vulgaris* L. subsp. *maritima* (L.) Arcang.; *Lysimachia arvensis* (L.) U.Manns & Anderb.; *Erodium malacoides* (L.) L'Hér.; *Reichardia tingitana* (L.) Roth |
| El Prat de Llobregat, Spain (P) | PC, PN | 41° 17′ 18.7″ N/2° 06′ 31.9″ E | 622 | 16.6 | *Pistacia lentiscus* L.; *Brachypodium retusum* (Pers.) P.Beauv.; *Crithmum maritimum* L. |
| Santoña, Spain (S) | SC, SN | 43° 28′ 22.6″ N/3° 30′ 15.8″ W | 1128 | 14.0 | *Beta maritima* L.; *Aetheorhiza bulbosa* (L.) Cass.; *Ononis natrix* subsp. *ramosissima* (Desf.) Batt. |
| Tunisia (T) | TC, TN | 36° 53′ 20.3″ N/10° 19′ 38.0″ E | 448 | 18.1 | *Beta maritima* L.; *Pancratium maritimum* L.; *Echium vulgare* L. |
| Tenerife, Canary Islands, Spain (CI) | CIC, CIN | 28° 20′ 30.6″ N/16° 55′ 25.3″ W | 336 | 19.8 | *Maytenus canariensis* (Loes.) G.Kunkel & Sunding; *Lycium intricatum* Boiss.; *Limonium thouinii* (Viv.) Kuntze |
| Cádiz, Spain (CA) | CAC, CAN | 36° 31′ 22.6″ N/6° 17′ 12.9″ W | 597 | 17.9 | *Ammophila arenaria* (L.) Link.; *Eryngium maritimum* L.; *Echium maritimum* Willd.; *Pancratium maritimum* L. |

(*)Mean annual rainfall and mean annual temperature have been calculated considering values from 1982 to 2012 (Data source: https://es.climate-data.org)

measuring soil total organic carbon (TOC) and nitrogen (TN) concentrations. Available phosphorus, extracted with 0.5 M NaHCO3 and available K, extracted with ammonium acetate, were analyzed by ICP/OES iCAP 6500-duo spectrometry (Thermo Elemental Co. Iris Intrepid II XDL).

Urease and protease activities were determined as described by Kandeler et al. (1999) using as substrates urea or N-α-benzoyl-L-arginine amid, respectively (Nannipieri et al., 1980). β-Glucosidase and alkaline phosphomonoesterase activities were quantified colorimetrically as the p-nitrophenol produced during the incubation of soil with the substrates *p*-nitrophenyl-β-D-glucopyranoside (Tabatabai, 1982) and *p*-nitrophenyl phosphate disodium, respectively (Naseby and Lynch, 1997).

Soil respiration (SR) in soil samples moistened to 60% of its water holding capacity was estimated by measuring the amount of $CO_2$ emitted with a μ-Trac 4200 automatic (SY-LAB Microbiology, Vienna, Austria).

### 2.3. DNA extraction, PCR amplification, and sequencing

To extract the total genomic DNA, 0.25 g of rhizospheric soil from each sample was subjected to the DNeasy PowerSoil DNA Isolation kit (Qiagen), following the manufacturer protocol. DNA was eluted in a final volume of 100 μL. To avoid possible microbial DNA contaminations originating from DNA extraction kits, negative controls have been included (Vestergaard et al., 2017). The selected primers for DNA metabarcoding library preparation were ITS86F and ITS4 to amplify the complete fungal ITS2 region; and 341F and 805R to amplify a bacterial 16S rRNA fragment from the V3-V4 region. The Illumina sequencing primers were attached to their 5′ ends. Each PCR mixture contained 2.5 μL of the DNA template, 0.5 μL of the corresponding primers and 12.5 μL of Supreme NZYTaq 2× Green Master Mix (NZYTech) and was diluted to a total volume of 25 μL with ultrapure water. The PCR incubation program consisted in an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30s, 72 °C for 30s and a final extension step at 72 °C for 10 min. To multiplex different libraries in the same sequencing pool, the barcodes identifiers were attached in a second PCR round with identical conditions but only five cycles and 60 °C as the annealing temperature. Equimolar amounts of amplified DNA from each library were pooled according to the Qubit dsDNA BR Assay (Thermo Fisher Scientific, Waltham, MA, USA) results. Previously, each library was purified using the Mag-Bind RXNPure Plus magnetics beads (Omega Biotek Inc., Norcross, GA, USA) and following the manufacturer indications. Finally, the pool was sequenced using an Illumina MiSeq PE300 sequencing platform (Illumina Inc., San Diego, CA, USA) at AllGenetics & Biology S.L (A Coruña, Spain).

### 2.4. Sequencing data processing

After the sequencing, the quality of the obtained and demultiplexed reads was checked with the software FASTQC. Paired-end assembly of the forward and reverse reads was performed with FLASH (Magoč and Salzberg, 2011). Sequences that did not contain the PCR primers (allowing up to 2 mismatches) and sequences shorter than 300 nucleotides (bacteria) or 200 nucleotides (fungi) were discarded using the CUTADAPT software 1.3 (Martin, 2011) and Geneious 10.2.3. The processing of the FASTQ files, containing the reads, was carried out with Qiime 1.9.0 (Caporaso et al., 2010). After quality-filtering of the reads, using the criteria of a minimum Phred score of 20, chimeras were detected and removed using the UCHIME algorithm implemented in VSEARCH (Edgar et al., 2011). The reference databases used for the chimera detection procedure were the UCHIME dataset (based on UNITE) (REF) and the Greengenes database (REF) for fungal and bacterial sequences respectively. The remaining filtered and cleaned ITS2 and 16S reads were then clustered into OTUs through the open-reference approach in Qiime. More specifically, the BLAST algorithm with the UNITE reference database was used for the picking of the fungal OTUs whereas the UCLUST algorithm with the Greengenes reference database was used in the case of the bacterial reads clustering procedure into OTUs.

Once the two "OTU tables" were obtained, a new quality-filtering was carried out in order to remove the singletons and the OTUs with a number of sequences lower than 0.005% of the total number of sequences. To avoid mistagging, the low abundance OTUs (0.1% threshold for fungal and bacterial OTUs) of each sample were also discarded.

The sequence files were submitted to the NCBI Sequence Read Archive repository (www.ncbi.nlm.nih.gov/sra) and are accessible in the BioProject PRJNA472435.

### 2.5. Statistical analyses

The Kolmogorov-Smirnov and the Levene's tests were applied to test the normality of data and homogeneity of their variance, respectively. Data that not follow normality assumptions were transformed using a log transformation. The effect of the two factors (location and invasiveness) and their interaction on measured variables was analyzed using a two-way analysis of variance and comparisons among means were made using the Tukey HSD (Honestly Significant Difference) test calculated at $p < 0.05$.

Concerning microbial communities' datasets, a normalization procedure of the total number of sequences per sample had to be undertaken in order to appropriately compare communities from different samples. Thus, the number of sequences per sample was rarefied to the lowest one (1315 reads in TN4 sample for the bacterial communities and 2151 reads in LN2 for the fungal communities). Rarefaction curves were calculated to guarantee that the number of sequences per sample after rarefying was enough to cover the true species diversity (Figs. S1 and S2, Supplementary material).

Multivariate statistics methods were used to assess beta-diversity. In order to obtain the spatial distribution of samples according to the structure of their communities, a 3-D non-metric multidimensional scaling (NMDS) was performed for rhizosphere bacterial and fungal communities independently. The NMDS was calculated on a Bray-Curtis dissimilarity matrix so that communities that are more alike are closer together on the plot. The analysis was carried out using the "metaMDS" function implemented in "vegan" package for R, which automatically applied square-root and Wisconsin transformations on the data. To confirm the effect of the two experimental factors, invasiveness and location, on the rhizosphere microbial communities composition and structure, a permutational multivariate analysis (perMANOVA) was conducted on the same Bray-Curtis matrix with the "adonis" function in vegan and using 999 permutations.

Canonical correspondence analyses (CCA) were carried out to assess the relationships between the structural variability of bacterial or fungal communities and soil physicochemical and biochemical properties (constraining variables). To avoid multicollinearity, the variance inflation factor (VIF) was calculated for each constraining variable and those with a VIF > 10 were sequentially removed. The remaining variables were then subjected to a forward selection procedure to select the subset of constraining variables that better explain the communities' variation in the CCA final model. The significance of the CCA final models, for bacterial or fungal communities, was tested by Monte-Carlo permutational test (999 permutations).

In order to find indicator taxa, meaning those which are characteristic of a group of samples or category, an indicator species analysis (ISA) was conducted with the "indicspecies" package for R. The relative abundances of microbial families and OTUs in each sample were used to calculate the Indicator Value (IndVal) and its significance.

## 3. Results

### 3.1. Rhizosphere soil chemical and physico-chemical properties

The factor invasiveness had a significant effect on all chemical and physico-chemical properties except TOC and available K (Tables 2 and 4). The interaction location x invasiveness was significant for all chemical and physico-chemical properties. The presence of *C. edulis* either increased or decreased significantly the contents of WSCH, TN, TOC, and available P, depending on the locality.

### 3.2. Rhizosphere soil microbiological properties

The invasive status of the plant influenced all microbiological parameters except urease activity and soil respiration (Tables 3 and 4). The interaction location x invasiveness had a significant effect on all microbiological properties. The presence of the invasive plant significantly modified basal respiration and the alkaline phosphomonoesterase, protease, and β-glucosidase activities in some of the invaded locations. However, no general pattern of change for these microbiological properties arose in response to the invader in these locations.

### 3.3. Rhizosphere soil bacterial communities

The total number of raw sequences, obtained through sequencing of the 16S rRNA V3-V4 fragment, was 11,340,741. From these, a total of 4,457,285 sequences passed the quality filters criteria. Subsequently, the OTU filtering reduced the number of useful sequences to 533,173, corresponding to a final number of 1933 OTUs. The number of total bacterial OTUs was affected by both factors, location and invasiveness, as well as their interaction (Table 4). Significant differences in richness of bacterial OTUs were observed between the rhizospheres of invasive plant and native plant community in the locations Túnez and Almería,

**Table 2**
Chemical and physico-chemical properties of the rhizospheres of invasive (C = *C. edulis*) and native plants (N = native) grown in eight different coastal locations globally distributed. When the interaction between factors (location and invasiveness) was significant, values in column sharing the same letter do not differ significantly ($P < 0.05$) as determined by the Tukey HSD test. Mean ± standard error, $n = 4$. WSCH = Water soluble carbohydrates, TN = total nitrogen; TOC = total organic carbon; EC: electrical conductivity; Avail. P = available phosphorus; Avail. K = available potassium.

| Location | Sample | WSCH | TN | TOC | pH | EC | Avail. P | Avail. K |
|---|---|---|---|---|---|---|---|---|
| | | (mg kg$^{-1}$) | (g kg$^{-1}$) | (g kg$^{-1}$) | ($H_2O$, 1:5) | (1:5, μS cm$^{-1}$) | (mg kg$^{-1}$) | (mg kg$^{-1}$) |
| **V** | VC | 18 ± 2 bcd | 0.18 ± 0.02 a | 1.07 ± 0.21 a | 8.51 ± 0.05 def | 188 ± 14 c | 7 ± 0 a | 76 ± 17 bc |
| | VN | 22 ± 2 cde | 0.19 ± 0.04 a | 1.64 ± 0.51 a | 8.39 ± 0.04 cde | 211 ± 15 c | 12 ± 1 ab | 152 ± 9 d |
| **A** | AC | 25 ± 0 ef | 0.79 ± 0.14 c | 8.37 ± 0.62 c | 8.43 ± 0.08 cde | 202 ± 28 c | 47 ± 9 d | 92 ± 11 c |
| | AN | 14 ± 1 ab | 0.36 ± 0.08 ab | 4.61 ± 0.41 ab | 8.65 ± 0.03 fg | 132 ± 3 ab | 4 ± 0 a | 52 ± 1 abc |
| **L** | LC | 29 ± 1 fg | 0.74 ± 0.31 c | 13.12 ± 4.77 d | 8.56 ± 0.10 ef | 172 ± 16 bc | 12 ± 3 ab | 56 ± 3 abc |
| | LN | 24 ± 1 de | 1.91 ± 0.04 e | 31.63 ± 0.23 f | 7.86 ± 0.02 a | 962 ± 43 f | 19 ± 1 bc | 74 ± 1 abc |
| **P** | PC | 17 ± 0 bc | 0.72 ± 0.03 c | 8.97 ± 0.25 cd | 8.39 ± 0.19 cde | 124 ± 2 ab | 5 ± 1 a | 63 ± 1 abc |
| | PN | 26 ± 3 efg | 1.43 ± 0.04 d | 20.34 ± 1.23 e | 8.33 ± 0.03 bcd | 136 ± 9 ab | 8 ± 0 a | 65 ± 2 abc |
| **S** | SC | 20 ± 1 cde | 0.79 ± 0.13 c | 7.82 ± 0.65 c | 8.39 ± 0.01 cde | 139 ± 5 ab | 5 ± 0 a | 33 ± 1 a |
| | SN | 19 ± 1 bcd | 0.66 ± 0.12 bc | 4.97 ± 0.21 abc | 8.76 ± 0.03 g | 133 ± 8 ab | 7 ± 0 a | 39 ± 2 ab |
| **T** | TC | 18 ± 2 bcd | 0.45 ± 0.09 abc | 6.67 ± 0.79 bc | 8.28 ± 0.01 bc | 138 ± 2 ab | 19 ± 2 bc | 61 ± 3 abc |
| | TN | 17 ± 2 bc | 0.45 ± 0.08 abc | 8.65 ± 0.68 cd | 8.37 ± 0.06 cde | 126 ± 7 ab | 8 ± 1 a | 65 ± 7 abc |
| **CI** | CIC | 49 ± 2 h | 4.20 ± 0.02 f | 49.88 ± 2.74 g | 8.16 ± 0.00 b | 483 ± 5 e | 75 ± 7 e | 1363 ± 4 e |
| | CIN | 31 ± 4 g | 0.78 ± 0.07 c | 13.10 ± 0.28 d | 9.16 ± 0.03 hi | 263 ± 12 d | 25 ± 0 c | 1405 ± 42 f |
| **CA** | CAC | 10 ± 0 a | 0.15 ± 0.01 a | 3.11 ± 0.20 ab | 9.03 ± 0.05 h | 131 ± 10 ab | 6 ± 0 a | 89 ± 4 c |
| | CAN | 9 ± 0 a | 0.14 ± 0.03 a | 3.04 ± 0.11 ab | 9.23 ± 0.01 i | 102 ± 4 a | 6 ± 0 a | 77 ± 3 bc |

**Table 3**
Biochemical and biological properties and number of total bacterial and fungal OTUs of the rhizospheres of invasive (C=*C. edulis*) and native plants (N = native) grown in eight different coastal locations globally distributed. When the interaction between factors (location and invasiveness) was significant, values in column sharing the same letter do not differ significantly ($P < 0.05$) as determined by the Tukey HSD test. Mean ± standard error, n = 4. BGL = β-glucosidase activity; ALP = alkaline phosphomonoesterase activity; URE = urease activity; PRT = protease activity; SR = Soil respiration.

| Location | Sample | BGL (μmol PNF $g^{-1}$ $h^{-1}$) | ALP (μmol PNF $g^{-1}$ $h^{-1}$) | URE (μmol N-$NH_4^+$ $g^{-1}$ $h^{-1}$) | PRT (μmol N-$NH_4^+$ $g^{-1}$ $h^{-1}$) | SR (mg C-$CO_2$ $h^{-1}$ $kg^{-1}$) | Total bacterial OTUs | Total fungal OTUs |
|---|---|---|---|---|---|---|---|---|
| V | VC | 0.12 ± 0.02 a | 0.34 ± 0.05 a | 0.58 ± 0.20 a | 0.06 ± 0.02 a | 1.8 ± 0.1 a | 197 ± 11 ab | 62 ± 8 ab |
| | VN | 0.39 ± 0.11 abc | 0.31 ± 0.05 a | 1.77 ± 0.73 b | 0.08 ± 0.02 a | 4.9 ± 0.9 cd | 181 ± 8 a | 62 ± 7 ab |
| A | AC | 0.60 ± 0.11 abcd | 1.40 ± 0.16 bcd | 1.11 ± 0.07 ab | 0.73 ± 0.10 cde | 8.1 ± 0.5 ef | 180 ± 19 a | 53 ± 5 a |
| | AN | 0.23 ± 0.03 ab | 0.32 ± 0.02 a | 0.48 ± 0.13 a | 0.26 ± 0.01 ab | 2.6 ± 0.2 ab | 243 ± 5 cde | 66 ± 3 abc |
| L | LC | 0.96 ± 0.29 de | 1.19 ± 0.21 bc | 0.68 ± 0.20 a | 0.55 ± 0.09 bc | 4.7 ± 1.5 bcd | 251 ± 17 cde | 62 ± 7 ab |
| | LN | 1.89 ± 0.11 f | 2.03 ± 0.08 fg | 1.02 ± 0.17 a | 0.87 ± 0.08 de | 11.7 ± 0.1 g | 260 ± 1 de | 67 ± 3 abc |
| P | PC | 0.75 ± 0.09 cd | 1.85 ± 0.12 ef | 2.58 ± 0.46 c | 0.99 ± 0.06 e | 6.6 ± 0.6 de | 235 ± 8 bcde | 70 ± 9 abc |
| | PN | 1.36 ± 0.08 e | 2.36 ± 0.09 g | 3.37 ± 0.10 d | 0.79 ± 0.06 cde | 11.8 ± 0.4 g | 240 ± 2 bcde | 81 ± 6 bcd |
| S | SC | 0.99 ± 0.06 de | 1.63 ± 0.17 de | 0.86 ± 0.07 a | 1.03 ± 0.04 e | 7.2 ± 0.2 e | 225 ± 21 abcde | 67 ± 10 abc |
| | SN | 0.73 ± 0.05 bcd | 1.82 ± 0.13 ef | 0.91 ± 0.07 a | 1.74 ± 0.08 f | 4.9 ± 0.3 cd | 220 ± 11 abcd | 68 ± 8 abc |
| T | TC | 1.04 ± 0.18 de | 1.51 ± 0.23 cde | 0.81 ± 0.08 a | 0.53 ± 0.07 bc | 9.5 ± 0.1 f | 209 ± 36 abc | 66 ± 5 ab |
| | TN | 1.06 ± 0.10 de | 1.10 ± 0.10 b | 0.88 ± 0.12 a | 0.63 ± 0.14 cd | 8.3 ± 1.6 ef | 268 ± 15 e | 85 ± 3 bcd |
| CI | CIC | 3.52 ± 0.43 g | 6.87 ± 0.16 h | 3.69 ± 0.21 d | 2.45 ± 0.31 g | 18.5 ± 0.1 h | 216 ± 4 abcd | 96 ± 20 d |
| | CIN | 0.67 ± 0.03 bcd | 1.73 ± 0.09 def | 0.62 ± 0.03 a | 0.30 ± 0.04 ab | 13.6 ± 0.4 g | 249 ± 5 cde | 93 ± 7 cd |
| CA | CAC | 0.37 ± 0.09 abc | 0.60 ± 0.04 a | 0.32 ± 0.02 a | 0.30 ± 0.02 ab | 2.8 ± 1.0 abc | 221 ± 4 abcd | 70 ± 6 abc |
| | CAN | 0.28 ± 0.04 abc | 0.64 ± 0.06 a | 0.29 ± 0.03 a | 0.29 ± 0.01 ab | 1.8 ± 0.2 a | 223 ± 5 abcde | 75 ± 3 abcd |

being the bacterial community of native plant community considerably richer than that associated with the invasive plant.

A perMANOVA analysis was applied to test for significant differences between bacterial communities. Its results showed that both factors, invasiveness ($F = 5.26$, $P = 0.001$) and location ($F = 12.90$, $P = 0.001$), as well as their interaction ($F = 4.42$, $P = 0.001$), significantly influenced the composition and structure of the rhizosphere bacterial communities.

The spatial ordination of the bacterial communities, resulting from the NMDS, returned a stress value of 0.098. The NMDS showed that the rhizosphere bacterial communities clustered by sampling location and invasiveness (Fig. 1). It is worth noting the visual differences between the bacterial communities of invasive and native plants in the locations Tenerife (CI), Valparaíso (V), Almería (A), El Prat de Llobregat (P) and Tunisia (T).

The OTUs were assigned to 17 different bacterial phyla (Table S4, Supplementary material). The most abundant phylum was *Actinobacteria* (43% of the total relative abundance), closely followed by *Proteobacteria* (42%). The presence of the other phyla was consequently much lower: *Firmicutes* (4%), *Bacteroidetes* (4%), *Acidobacteria* (2%), *Chloroflexi* (2%), *Gemmatimonadetes* (1%), and the remaining phyla (total relative abundance below 1%). The relative abundances of copiotrophic bacterial phyla (e.g. *Firmicutes*, *Bacteroidetes* and *Proteobacteria*) were higher in the native rhizosphere (Table S4, Supplementary material), whereas bacterial phyla exhibiting oligotrophic attributes (e.g. *Verrucomicrobia* and *Acidobacteria*) showed higher relative abundances in the invasive rhizosphere.

When the indicator species analysis (ISA) was performed at the taxonomic level of the family, the number of indicator taxa was eight for both the native and the invasive plant rhizospheres, *Glycomycetaceae* (0.39%) and *Rhodospirillaceae* (3.44%) being the most frequent indicator families of the *C. edulis* and native rhizospheres, respectively (Table 5).

The ISA at the OTU level revealed a total of 60 bacterial indicator OTUs of the *C. edulis* rhizosphere (Table S1, Supplementary material), whereas the number of bacterial indicators of the native plant mixture was 103 (Table S2, Supplementary material). The most abundant indicator OTUs of the *C. edulis* rhizosphere were otu00008 (total relative abundance 0.29%), which was assigned to the family *Sphingomonadaceae*, and otu01744 (0.29%), which belongs to the genus *Glycomyces*. In the case of the native plant rhizospheres, the indicator OTU with the highest presence was otu01846 (1.82%), identified as *Skermanella*.

**Table 4**
Two factor ANOVA (location and invasiveness) for all parameters studied. F values (*P* values).

| | Location (L) | Invasiveness (I) | Interaction (L × I) |
|---|---|---|---|
| Water soluble carbohydrates | 44.92 (<0.001) | 11.36 (0.001) | 10.11 (<0.001) |
| Total N | 107.73 (<0.001) | 25.05 (<0.001) | 86.13 (<0.001) |
| Total organic C | 102.03 (<0.001) | 3.54 (0.066) | 61.03 (<0.001) |
| pH ($H_2O$) | 37.93 (<0.001) | 14.59 (<0.001) | 27.17 (<0.001) |
| EC | 216.21 (<0.001) | 62.79 (<0.001) | 195.01 (<0.001) |
| Available P | 50.54 (<0.001) | 50.79 (<0.001) | 28.53 (<0.001) |
| Available K | 2859.97 (<0.001) | 3.81 (0.057) | 4.08 (0.001) |
| β-glucosidase | 32.74 (<0.001) | 8.10 (0.007) | 27.92 (<0.001) |
| Alkaline phosphomonoesterase | 198.06 (<0.001) | 103.62 (<0.001) | 117.01 (<0.001) |
| Urease | 25.49 (<0.001) | 1.69 (0.200) | 14.02 (<0.001) |
| Protease-BAA | 44.78 (<0.001) | 17.42 (<0.001) | 36.56 (<0.001) |
| Soil respiration | 75.94 (<0.001) | 0.02 (0.897) | 21.50 (<0.001) |
| Total bacterial OTUs | 4.24 (0.001) | 7.35 (0.009) | 2.30 (0.042) |
| Total fungal OTUs | 3.87 (0.002) | 2.56 (0.116) | 0.46 (0.861) |

### 3.4. Rhizosphere soil fungal communities

The sequencing of the ITS2 region yielded a total of 14,438,696 raw sequences. After the preliminary filtering process, the number of sequences was reduced to 6,317,509. The filtering of the OTUs obtained resulted in a final number of 2,762,153 useful sequences, which were clustered into 1106 OTUs. Only the factor location had a significant effect on the number of total fungal OTUs, being the highest richness of fungi reached in the location Tenerife (Tables 3 and 4).

As with the rhizosphere bacterial communities, the rhizosphere fungal communities differed significantly among locations ($F = 9.41$, $P = 0.001$) and also between *C. edulis* and the native co-occurring plants in each location ($F = 6.29$, $P = 0.001$). An interaction between the two factors was also revealed by the perMANOVA ($F = 4.89$, $P = 0.001$). As observed in the NMDS (stress value = 0.140), the rhizosphere fungal communities seemed to cluster according to the sampling location and the invasiveness of the plant (Fig. 2).

A total of nine fungal phyla were detected when considering the whole dataset. *Ascomycota* was the most represented phylum, with a total relative abundance of 75%, followed by *Basidiomycota* (17%), an unidentified phylum (5%), and *Chytridiomycota* (1%). The remaining fungal phyla were present in abundances below 1%.

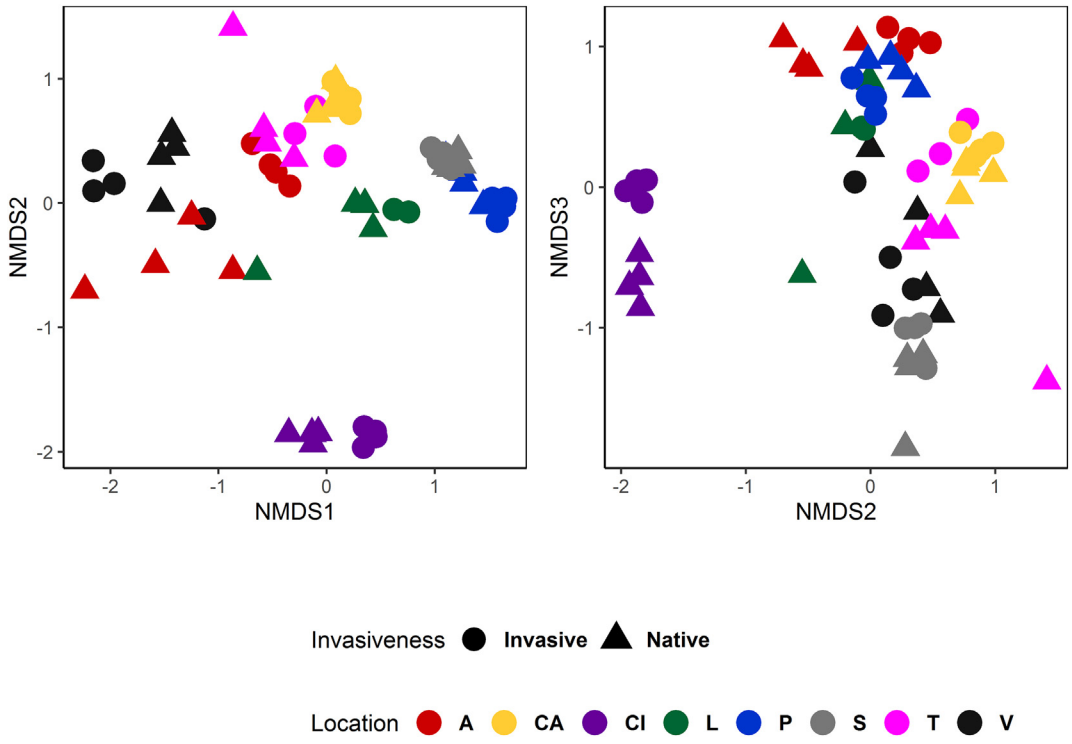


**Fig. 1.** Three-dimensional non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarities of rhizosphere bacterial community (at OTU level) of invasive (*C. edulis*) and native plants from eight different coastal locations. Plots of NMDS axis 1 vs. 2 (left) and NMDS axis 2 vs. 3 (right) are shown.

**Table 5**
Indicator Species Analysis (ISA) at family level of the rhizosphere from *C. edulis* (invasive) and co-occurring native plants.

| Bacterial families | IndVal | *p*-Value |
|---|---|---|
| ***C. edulis*** | | |
| *Unidentified_family35* | 0.728 | 0.001 |
| *f__Methylocystaceae* | 0.676 | 0.008 |
| *f__Piscirickettsiaceae* | 0.634 | 0.014 |
| *f__Glycomycetaceae* | 0.615 | 0.037 |
| *Unidentified_family13* | 0.468 | 0.012 |
| *Unidentified_family49* | 0.451 | 0.032 |
| *Unidentified_family26* | 0.433 | 0.024 |
| *Unidentified_family9* | 0.395 | 0.044 |
| ***Native plants*** | | |
| *f__Rhodospirillaceae* | 0.750 | 0.039 |
| *f__Acetobacteraceae* | 0.709 | 0.008 |
| *f__Nitrospiraceae* | 0.660 | 0.006 |
| *Unassigned_family7* | 0.458 | 0.025 |
| *f__Moraxellaceae* | 0.415 | 0.023 |
| *f__Patulibacteraceae* | 0.404 | 0.034 |
| *Unidentified_family1* | 0.371 | 0.046 |

| Fungal families | IndVal | p-Value |
|---|---|---|
| ***C. edulis*** | | |
| *f__Trichocomaceae* | 0.871 | 0.003 |
| *f__Helotiales_fam_Incertae_sedis* | 0.593 | 0.018 |
| *f__Montagnulaceae* | 0.545 | 0.008 |
| *f__Wallemiaceae* | 0.530 | 0.007 |
| *f__Myxotrichaceae* | 0.433 | 0.019 |
| *f__Valsaceae* | 0.395 | 0.047 |
| ***Native plants*** | | |
| *f__Lasiosphaeriaceae* | 0.782 | 0.002 |
| *f__Sporormiaceae* | 0.769 | 0.010 |
| *f__Phaeosphaeriaceae* | 0.735 | 0.001 |
| *f__Pyronemataceae* | 0.705 | 0.015 |
| *f__Bulleribasidiaceae* | 0.659 | 0.001 |
| *f__Rhizopodaceae* | 0.593 | 0.006 |
| *f__unidentified.14* | 0.508 | 0.004 |
| *f__Ophiocordycipitaceae* | 0.500 | 0.015 |
| *f__Polyporaceae* | 0.359 | 0.038 |
| *f__Xylariales_fam_Incertae_sedis* | 0.359 | 0.038 |

At the taxonomic level of the family, six indicator taxa were found for the invader's rhizosphere. *Trichocomaceae* (4.8%) was the most abundant family among these indicators, followed by *Wallemiaceae* (1.63%), as shown in Table 5. Regarding the rhizospheres of the native plants, 10 indicator families were found: *Pyronemataceae*, the most represented taxon (5.61%), was followed in abundance by *Sporormiaceae* (2.04%) and *Lasiosphaeriaceae* (1.22%).

Twenty-five fungal OTUs were revealed as indicators of the *C. edulis* rhizosphere (Table S3, Supplementary material) and 46 OTUs were assigned as indicators of the native plants rhizospheres (Table S5, Supplementary material). Among the OTU indicators of the *C. edulis* rhizosphere, the most abundant was otu00216, classified as *Lepista sordida*, a member of *Basidiomycetes*, with a total relative abundance of 3.7% in the whole dataset. Moreover, at the taxonomic level of the OTU, several of the *C. edulis* indicators obtained belong to the family *Trichocomaceae*, including *Aspergillus austroafricanus*, *Aspergillus sydowii*, *Aspergillus niger*, and two members of the *Penicillium* genus: *P. thomii* and *P. raperi*. The presence of *Aspergillus* as an indicator was exclusive to the *C. edulis* rhizosphere, but this was not the case for *Penicillium*, which was also present among the indicator OTUs of the native rhizospheres. The most abundant indicator OTU of the native plants rhizospheres was otu00083 (1.01%), identified as *Chalastospora ellipsoidea*.

### *3.5. Relationships between rhizosphere soil physico-chemical and biochemical properties and microbial communities*

After a forward selection of the constraining variables, the CCA final model for the rhizosphere bacterial communities' dataset included the following environmental variables: soil available potassium, mean annual rainfall, latitude, urease activity, available phosphorus, water soluble carbohydrates, β-glucosidase activity, protease, and soil respiration (Fig. 3). The percentage of the total inertia explained by the CCA constrained axes was 34%. The first two axes accumulated 36% and 12% of the constrained and unconstrained inertia, respectively. The first CCA axis was predominantly related to available potassium, followed by the gradients of available phosphorus, water soluble

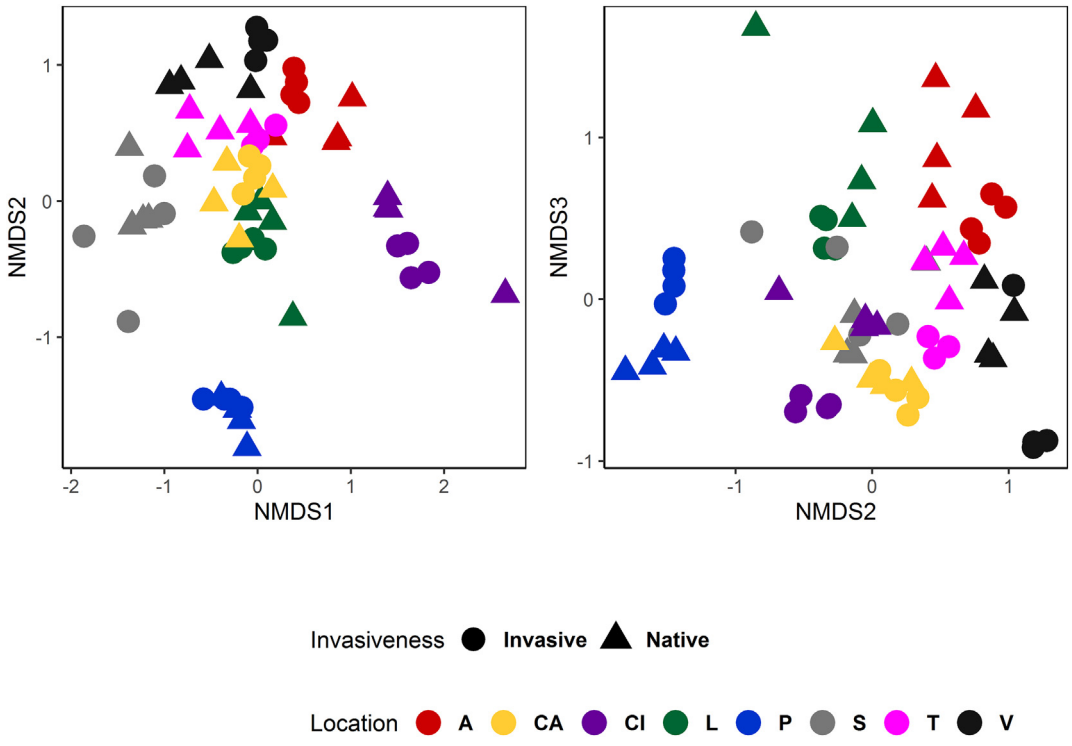


**Fig. 2.** Three-dimensional non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarities of rhizosphere fungal community (at OTU level) of invasive (*C. edulis*) and native plants from eight different coastal locations. Plots of NMDS axis 1 vs. 2 (left) and NMDS axis 2 vs. 3 (right) are shown.

carbohydrates, and soil respiration, whereas the second CCA axis was mainly defined by the gradients of latitude and mean annual rainfall.

Concerning the rhizosphere fungal communities, the forward selection procedure reduced the constraining variables to available potassium, mean annual rainfall, available phosphorus, urease activity, latitude, water soluble carbohydrates, electrical conductivity, protease activity, β-glucosidase activity, and pH (Fig. 4). The CCA final model explained 31% of the total inertia, the first two axes accumulating 28% and 9% of the constrained and unconstrained inertia, respectively. As happened for the bacterial communities, the gradient of available potassium was principally represented by the first axis. Similarly, available phosphorus and water soluble carbohydrates were also related to this axis, but to a lesser degree. The constraining variable showing the most important relationship with the fungal community distribution along the CCA second axis was the mean annual rainfall. In contrast to the CCA results for the bacterial community dataset, the influence of the latitude on the fungal community ordination along the vertical axis was less evident.

## 4. Discussion

The invader *C. edulis* was able to colonize a diverse range of coastal ecosystems, competing with a large variety of native coastal plant communities. The occurrence of this invader was associated with shifts in the below-ground microbial communities. In fact, the invasion of these coastal ecosystems by *C. edulis* was accompanied by the prevalence of specific microbial groups in its rhizosphere, in comparison to those harbored by the native and co-occurring plant species.

The influence of plant invasion on soil microbial communities has been studied for other invasive plant species. For instance, Johansen et al. (2016) also used the Illumina MiSeq platform and found similar rhizosphere fungal communities for the exotic *Ammophila arenaria* and the native *Leymus mollis* coexisting in an invaded Californian foredune habitat. However, in a more recent study by the same authors and following a similar methodology, the rhizosphere fungal communities harbored by *A. arenaria* were richer than those in the co-occurring native grasses *Leymus arenarius* and *Spinifex sericius* (Johansen et al., 2017). Besides, the influence of regional characteristics - such as the temperature, soil pH, and nitrogen - on the soil microbial community composition was also reported in this latter study.

At a large scale, Gibbons et al. (2017) found that the diversity of soil bacterial, archaeal, and fungal communities in plots invaded by three exotic species (*Centaurea stoebe, Euphorbia esula*, and *Bromus tectorum*) was similar to that in plots dominated by native plant communities. Nevertheless, in their study, the abundance of some microbial groups - such as copiotrophs or oligotrophs for bacteria, and symbionts or pathogens for fungi - varied between the types of plant community. Similarly, Carey et al. (2015) conducted research in experimental semiarid grassland plots subjected to plant invasion and other environmental changes, and reported no significant differences between the bacterial and archaeal soil communities from invaded and non-invaded plant communities. In line with our results, the bacterial communities found in the rhizosphere of the invasive *Pennisetum setaceum* differed from those of the native *Hyparrhenia hirta*, in five Mediterranean semiarid locations with different edaphic characteristics (Rodríguez-Caballero et al., 2017).

Despite the great interest in *C. edulis* (and other *Carpobrotus* spp.) invasion processes, the impact of *Carpobrotus* species on soil microbial communities invading non-native ecosystems has only been addressed by Badalamenti et al. (2016), through a PLFA-based approach. Following the invasion by *C. acinaciformis*, they reported an increase in the soil microbial biomass as well as changes in the community structure, the ratio of bacteria to fungi being decreased, compared to that of the native flora.

Our high-throughput sequencing approach allowed us to obtain a more complete and detailed characterization of the microbial communities and to identify indicator taxa for both invasive and native plant rhizospheres. The occurrence of *C. edulis* tended to enrich for oligotrophic bacterial taxa that are characterized by slower growth rates and adapted to conditions of low nutrient availability (Gibbons et al., 2017), while the abundance of copiotrophs was decreased. Meanwhile, *Trichocomaceae* was one of the most abundant fungal families overall in our study, and it stood out as an indicator of the *C. edulis* rhizosphere.

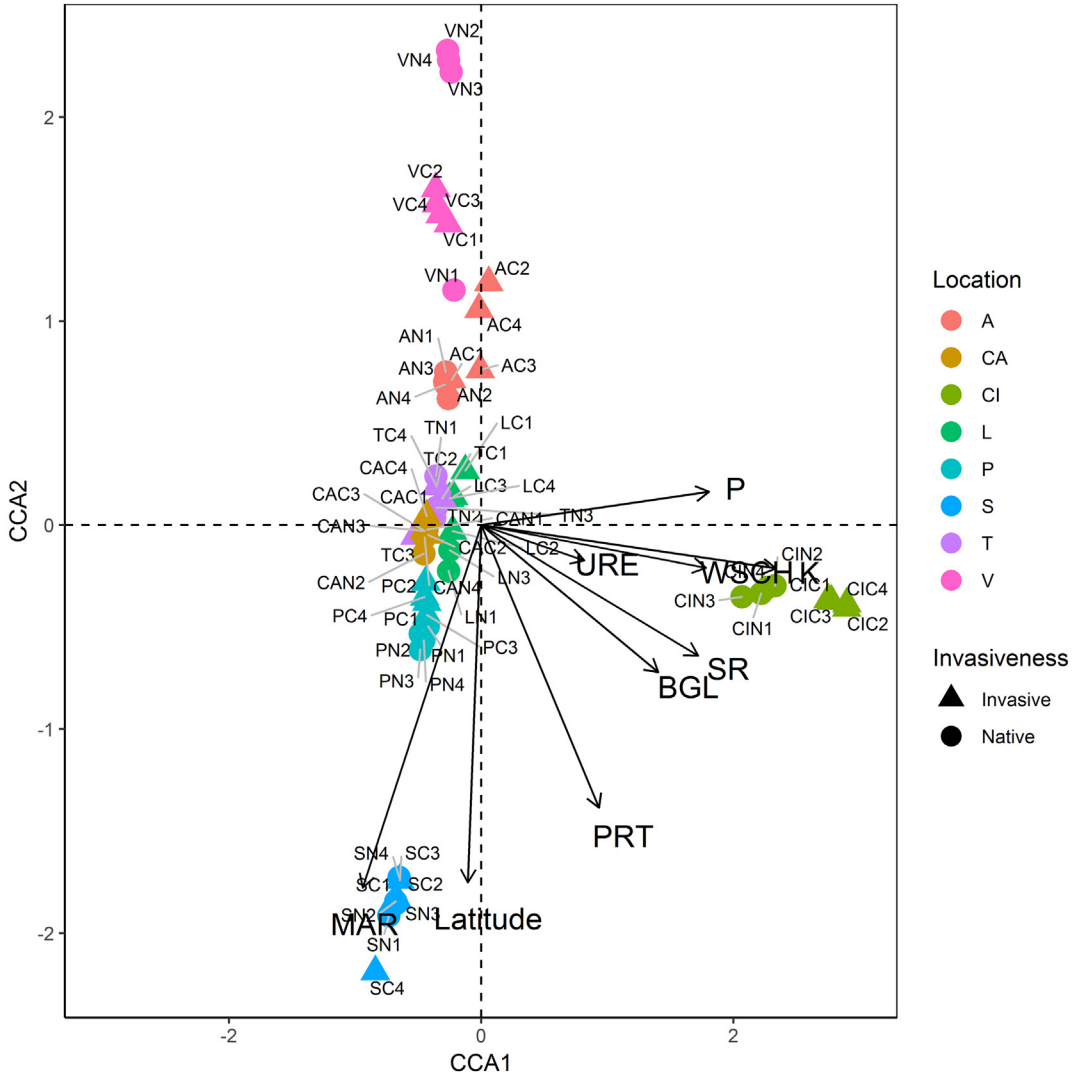


**Fig. 3.** Canonical correspondence analysis (CCA) on rhizosphere bacterial communities at OTU level. The explanatory variables (arrows) are those from a larger set of environmental variables which better explain the communities variance after a forward selection procedure (K = available potassium, MAR = mean annual rainfall, URE = urease activity, P = available phosphorus, WSCH = water soluble carbohydrates, BGL = β-glucosidase activity, PRT = protease activity, SR = soil respiration).

The presence of several members of the genus *Aspergillus* (family *Trichocomaceae*) as indicators of the *C. edulis* rhizosphere, including *A. niger*, might contribute to greater acquisition of phosphorus by the invader, considering that members of this genus have recognized phosphate-solubilizing ability (Caravaca et al., 2005; Mengual et al., 2016). This assumption could be supported by the fact that *C. edulis* increased the content of soil available P in several of invaded locations, highlighting the importance of these fungal indicators in soil P processes as well as their potential involvement in the invasion success of invader. *Wallemiaceae* was the second most abundant fungal indicator of the *C. edulis* rhizosphere at the taxonomic level of the family and is characterized by its xerophilic character. In fact, one of the *C. edulis* indicator OTUs was identified as *Wallemia tropicalis*, probably the most halophilic species described for this genus (Jančič et al., 2015).

Regarding the bacterial communities, *Glycomycetaceae*, a member of *Actinomycetales*, was the most abundant indicator of the invasive plant rhizosphere. Also, *Methylocystaceae* (*Rhizobiales*), a family of methanotrophic and nitrogen-fixing bacteria, was found to be an indicator of *C. edulis*. Both of these families may act as Plant Growth Promoting Rhizobacteria (PGPR), favoring the establishment and growth of this invasive plant.

Structural and compositional shifts in rhizosphere microbial communities, as observed in our survey, might be explained by the allelopathic potential of *C. edulis* tissues. It has been demonstrated that *C. edulis* litter has inhibitory effects on the germination, establishment, and growth of some native species (Novoa and González, 2014). Moreover, several compounds with antibacterial activity, including tannins and flavonoids, have been isolated from *C. edulis* tissues and identified (van der Watt and Pretorius, 2001).

Soil physico-chemical properties can be relevant to the assemblage of the rhizosphere microbiome, as has been shown in several surveys performed with different types of soil and plant species (Andrew et al., 2012; Kim et al., 2016). In studies on invaders, Souza-Alonso et al. (2015) reported that the decrease in pH after *Acacia dealbata* invasion could favor the prevalence of a community dominated by fungi. Previous research focused on the impacts of *Carpobrotus* spp. on soil physico-chemical properties revealed a general trend. Overall, soil pH generally decreased (Conser and Connor, 2009; Novoa et al., 2013; Badalamenti et al., 2016) and salinity increased in the presence of the invader (Novoa et al., 2013). In our study this trend in physico-chemical properties in response to plant invasion was observed in the soils from the locations Tenerife and Almería.

The microbial community composition and structure can also change in response to variations in nutrient availability. In general, invasion by *Carpobrotus* spp. has been linked to an increase in soil nutrients, such as soil organic carbon (Vilà et al., 2006; Conser and Connor, 2009;

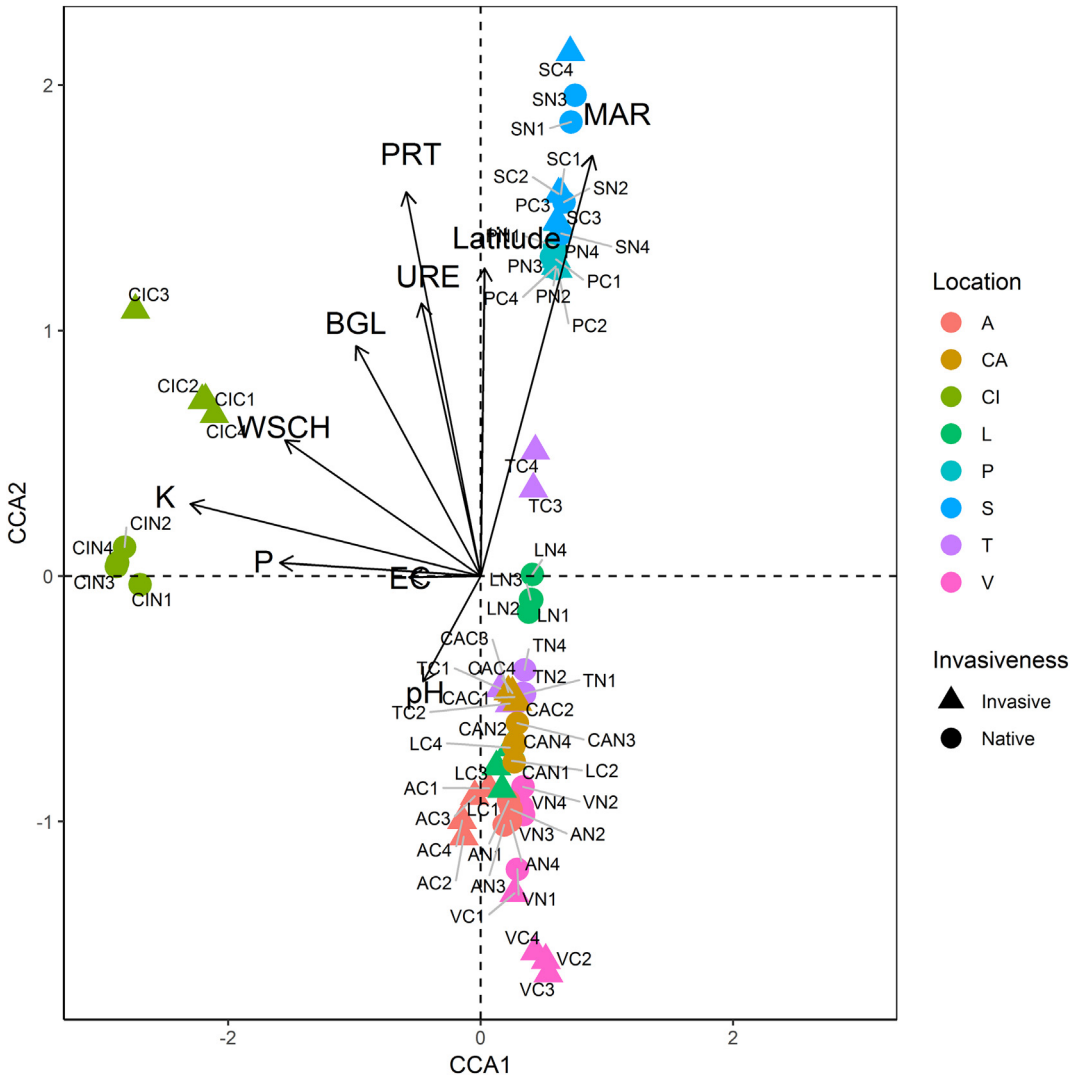


**Fig. 4.** Canonical correspondence analysis (CCA) on rhizosphere fungal communities at OTU level. The explanatory variables (arrows) are those from a larger set of environmental variables which better explain the communities variance after a forward selection procedure (K = available potassium, MAR = mean annual rainfall, P = available phosphorus, URE = urease activity, WSCH = water soluble carbohydrates, EC = electrical conductivity, PRT = protease activity, BGL = β-glucosidase activity).

de la Peña et al., 2010; Novoa et al., 2013, 2014; Badalamenti et al., 2016), nitrogen (Novoa et al., 2013; Badalamenti et al., 2016), and phosphorus (Novoa et al., 2013, 2014). These shifts have been largely attributable to the recalcitrant litter produced by *Carpobrotus* spp., which accumulates for years and supposes an increase in the organic matter supply (Conser and Connor, 2009; Novoa et al., 2014). However, soil nutrient contents can be affected by soil microorganisms because they play a key role in decomposition and nutrient cycling (van der Putten et al., 2007). In our study, the pattern described above was only clearly observed in the non-sandy soil samples from the location Tenerife, whereas the response of these soil properties to plant invasion in the soils at the other locations, with sandy texture, was highly variable. This could be related to differences in the leaching of nutrients caused by the distinct average rainfall amounts at the locations.

Shifts in the microbial activities related to nutrient cycling can be explained by alterations in the soil microbial community (Nannipieri et al., 2003). In our study *C. edulis* caused changes in soil enzymatic activities but no clear patterns of change could be detected at all locations. Soil microbial respiration also showed a variable trend in response to the invader. In this respect, our findings are in agreement with those reported by Novoa et al. (2014).

In our study, potassium availability was strongly related to the structural variability in both the bacterial and fungal communities. In a study by Li et al. (2016), the variations among microbial communities were largely related to the soil available potassium. We also found a significant relationship between available phosphorus, water soluble carbohydrates, and soil respiration (the latter only for the bacterial communities) and the microbial assemblages.

Not only the soil physico-chemical and biochemical characteristics were related to the changes in the rhizosphere microbial communities; their composition and structure also followed biogeographical patterns. Especially, the rhizosphere bacterial communities were strongly driven by the gradients of latitude and mean annual rainfall. In fact, the compositional and structural differences between bacterial or fungal communities from different locations were greater as the geographic distance increased. It is worth noting that, in spite of the geographical differences in the assemblages of soil microbial communities, the rhizosphere of the invasive plant shared a number of indicator species, including taxa with plant growth-promoting activities, at all the invaded locations.

The relationship between latitude and the fungal communities was, in contrast, less important than for the bacterial communities. The structural differences between rhizosphere fungal communities from different locations were principally related to changes in mean annual rainfall. In this line, research conducted by Glynou et al. (2016) revealed that the community composition of non-mycorrhizal root fungal

endophytes was determined by latitudinal gradients of precipitation (as well as temperature) rather than geographic distance.

Therefore, the variability in the bacterial communities seemed to be more dependent on latitudinal gradients than that of the rhizosphere fungal communities. Fierer and Jackson (2006), in a continental-scale survey across North and South America, suggested that the bacterial community composition was independent of the geographic distance. However, they did not sample rhizospheric soil and they used a T-RFLP methodology, which greatly underestimates the total bacterial community. On the other hand, bacterial and fungal communities from the rhizosphere of the invasive *Acacia dealbata* were characterized through the use of Illumina MiSeq sequencing in recent research by Kamutando et al. (2017). In agreement with our results they reported that the microbial communities from the *A. dealbata* rhizosphere also varied depending on the biogeography and soil nutritional status.

In conclusion, the results of this study show the ability of the invader *C. edulis* to promote unique soil microbial communities with distinct functional abilities, in comparison with those of the microbiota of native plant species. The soil microbial communities followed a biogeographical pattern related to soil abiotic and biotic properties, such as soil available nutrients and rhizosphere metabolic activity, but was influenced also by more global parameters such as climatic and geographic variables. Over the entire invaded range, *C. edulis* harbored a specific microbiome in its rhizosphere, which could favor the establishment and growth of this invasive plant.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.137347.

## Declaration of competing interests

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.

## CRediT authorship contribution statement

**A. Rodríguez-Caballero:**Formal analysis, Visualization, Writing - original draft, Validation, Resources, Data curation.**G. Caravaca:**Writing - review & editing, Resources, Data curation, Funding acquisition.**F. Díaz:**Resources, Funding acquisition.**G. Torres:**Resources, Funding acquisition.**P. Roldán:**Conceptualization, Investigation, Resources, Supervision, Project administration, Funding acquisition.

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