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RESEARCH ARTICLE

Urbanization and *Carpobrotus edulis* invasion alter the diversity and composition of soil bacterial communities in coastal areas

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One sentence summary: Invasion by *Carpobrotus edulis* alters the abundance, diversity and composition of soil bacterial communities, while urbanization only impacts their composition.

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ABSTRACT

Coastal dunes are ecosystems of high conservation value that are strongly impacted by human disturbances and biological invasions in many parts of the world. Here, we assessed how urbanization and *Carpobrotus edulis* invasion affect soil bacterial communities on the north-western coast of Spain, by comparing the diversity, structure and composition of soil bacterial communities in invaded and uninvaded soils from urban and natural coastal dune areas. Our results suggest that coastal dune bacterial communities contain large numbers of rare taxa, mainly belonging to the phyla Actinobacteria and Proteobacteria. We found that the presence of the invasive *C. edulis* increased the diversity of soil bacteria and changed community composition, while urbanization only influenced bacterial community composition. Furthermore, the effects of invasion on community composition were conditional on urbanization. These results were contrary to predictions, as both *C. edulis* invasion and urbanization have been shown to affect soil abiotic conditions of the studied coastal dunes in a similar manner, and therefore were expected to have similar effects on soil bacterial communities. Our results suggest that

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other factors (e.g. pollution) might be influencing the impact of urbanization on soil bacterial communities, preventing an increase in the diversity of soil bacteria in urban areas.

Keywords: biological invasions; coastal dune ecosystems; ecological impacts; enzymatic activities; invasive species; next-generation sequencing; urban areas

INTRODUCTION

Coastal dunes are unique ecosystems that are highly threatened by anthropogenic disturbances. They are dynamic ecosystems and are exposed to extreme environmental conditions such as salt spray, intense solar irradiance and soil nutrient deficiencies (Maun 2009). These conditions impose unique selection pressures that often result in a high degree of specialization of coastal dune plants. As a consequence, coastal dune ecosystems usually support a large number of threatened and endemic plant species that are of high conservation value (Acosta, Carranza and Izzi 2009). Coastal dunes are threatened by several anthropogenic drivers of global change such as biological invasions, climate change and habitat degradation (Sax and Gaines 2003; Dawson *et al*. 2017). In particular, plant invasions are considered to be one of the main threats to native plant diversity and ecosystem structure and functioning of coastal dunes (Mack, Cole and Treviño 2000; Millenium Ecosystem Assessment 2005). Coastal dunes are particularly vulnerable to plant invasion, since shifting sand dunes essentially represent disturbed systems, and thus constantly produce open spaces susceptible to colonization by pioneer alien species capable of tolerating coastal conditions (Ley *et al*. 2007; Dawson *et al*. 2017). The effects of other anthropogenic disturbances on coastal dune ecosystems may further facilitate the establishment, performance and survival of alien plants (Nobis, Jaeger and Zimmermann 2009; Kowarik 2011; Lechuga-Lago *et al*. 2017). Environmental conditions associated with urban areas (i.e. areas with high population density such as cities or towns, as opposed to non-urban areas, in which there are none or only few houses or buildings and low population density), for example, high levels of pollution and altered nutrient dynamics, generally benefit invasive plants (Cadotte *et al*. 2017). However, interactions between the presence of urban areas (hereafter 'urbanization') and plant invasions have rarely been tested.

A major plant invader of coastal dunes worldwide is *Carpobrotus edulis* (L.) N.E. Br., a South African succulent species that currently dominates millions of hectares of dune ecosystems in 71 regions outside its native range, including along the coasts of Australia, California, Chile and Southern Europe (Pyšek *et al*. 2017; Campoy *et al*. 2018; van Kleunen *et al*. 2019). In invaded areas, *C. edulis* has long been recognized as an ecosystem engineer (Cuddington *et al*. 2011). This is due, in part, to the ability of *C. edulis* to produce a considerable amount of litter, which increases soil water holding capacity, and, during its decomposition, adds nutrients to the soil (e.g. nitrogenous compounds, organic matter and phosphorus) and decreases soil pH (Novoa *et al*. 2014). These changes create positive plant–soil feedbacks (van der Putten *et al*. 2013), facilitating the establishment and growth of *C. edulis* and that of several opportunistic native weeds (Novoa *et al*. 2013), which can compete with and displace native coastal dune vegetation (Novoa and González 2014). Similar changes to the characteristics of pristine (natural) dune soils have been reported as a result of urbanization. Urban soils have been shown to have higher moisture and nutrient content and lower pH levels than natural soils (Lechuga-Lago *et al*. 2017). This suggests that coastal areas invaded by *C. edulis* and urban coastal areas may be more prone to *C. edulis* invasion than natural coastal areas, as both plant invasion and urbanization may make soils more suitable for invasion.

Soil abiotic factors such as soil pH, nutrient input and moisture are also important drivers of soil bacterial community diversity and composition (Zhou *et al*. 2002; Lauber *et al*. 2009; Fierer *et al*. 2012; Van Horn *et al*. 2014). For example, increases in soil nutrient levels and moisture and decreases in soil pH, resulting from the invasion of *Spartina alterniflora* in eastern Chinese coastal wetlands, were linked to increases in soil bacterial diversity and changes in soil bacterial community composition (Yang *et al*. 2019). We expect that the changes in soil conditions caused by the invasion of *C. edulis* and urbanization (Lechuga-Lago *et al*. 2017) will also alter the diversity and composition of soil bacterial communities (Wardle *et al*. 2004; Le Roux *et al*. 2018). Moreover, urbanization and *C. edulis* invasion in coastal dune ecosystems have already been shown to change the functioning of soil microbial communities by enhancing the secretion of extracellular enzymes by soil microorganisms (Lechuga-Lago *et al*. 2017). These enzymes initiate the decomposition and nutrient mineralization of complex organic and non-organic substrates such as carbon, nitrogen and phosphorus (Allison and Vitousek 2004). Changes in soil bacterial community composition linked to alterations in soil functioning (Kourtev, Ehrenfeld and Häggblom 2002, 2003). For example, in south-eastern Spain, the native plant *Hyparrhenia hirta* is being displaced by invasive fountain grass *Pennisetum setaceum*. The presence of fountain grass alters the composition and structure of soil bacterial communities, which leads to changes in soil functioning, mainly through an increase in protease activity (Rodríguez-Caballero *et al*. 2017).

Soil bacterial communities play a key role in the decomposition of organic matter, nutrient cycling and development of soil structure, especially in sand dune environments (McLachlan, Kerley and Rickard 1996). Thus, changes in soil bacterial community diversity and composition due to urbanization and/or *C. edulis* invasion could significantly affect the biotic (e.g. plant communities) and abiotic (e.g. nutrient composition) conditions of coastal dune ecosystems (Wasserstrom *et al*. 2017). However, to our knowledge, whether such changes occur in coastal dune ecosystems, or what their nature or direction is, remains unknown.

Here, we determine the impact of urbanization and invasion of *C. edulis* on coastal dune soil bacterial communities. We use next-generation sequencing data to compare the diversity and composition of bacterial communities in uninvaded and invaded soils from both urban and natural coastal dune ecosystems. We also use data on soil physicochemical properties obtained from a previous study by Lechuga-Lago *et al*. (2017) to explore the influence of *C. edulis* and urbanization on soil bacterial communities. We hypothesize that soil bacterial diversity and community composition will be influenced by the changes in soil conditions and elevated enzyme activity levels caused by the invasion of *C. edulis* and urbanization. At the same time, we predict, because of the similar impacts by invasion and urbanization on soil abiotic conditions, that soil microbial

Table 1. Location and characteristics, including beach type, of our study sites along the coast of Galicia, Spain.

Beach	Lat/Long	Average annual temperature (°C)	Average annual precipitation (mm)	Beach type
Ardia	42°28′14.304″N 08°51′22.758″W	14.8	1211	Natural
Baldaio	43°18′5.603″N 08°39′26.632″W	11.5	1318	Natural
Cariño	43°44′20.874″N 7°52′11.464″W	14.2	969	Urban
Cedeira	43°39′5.316″N 08°03′2.812″W	14.2	985	Urban
Corrubedo	42°34′36.056″N 09°04′1.972″W	14.8	1146	Natural
Samil	42°12′27.5″N 8°46′36.5″W	14.9	1303	Urban

communities of invaded urban coastal dunes and invaded natural dunes will be similar. We expect that, since both urbanization and *C. edulis* invasion alleviate the extreme environmental conditions of coastal dune soils and enhance the activity of soil microorganisms (Lechuga-Lago *et al.* 2017), they will also modify the diversity and community composition of soil bacteria.

MATERIALS AND METHODS

Study sites and soil collection

We conducted our study in Galicia, Spain, an area with 1720 km of coastline that has a mild temperate climate with dry, warm summers, and cool, wet winters. We selected a total of six coastal foredunes with areas invaded by *C. edulis* in close proximity (i.e. < 10m) to uninvaded areas (Table 1). Three of the selected coastal dunes were in urban beaches (*sensu* Jiménez *et al.* 2011) and three along natural beaches with minimal human-mediated disturbance. The former was characterized by high levels of urban infrastructure (e.g. housing, parking spaces, streets and boardwalks) with fragmented habitats, and intense human traffic during the summer season. These factors led to high levels of disturbances such as waste disposal and trampling. Natural beaches were well-preserved dune systems belonging to the Red Natura 2000 Network; these sites are not fragmented and contain no manmade infrastructure. Uninvaded areas in both urban and natural dunes were dominated by native species, including *Ammophila arenaria* (L.) Link, *Eryngium maritimum* L., *Euphorbia paralias* L., *Malcolmia littorea* (L.) R. Br. and *Pancratium maritimum* L. In contrast, a visual assessment of invaded dunes indicated that dunes were covered by monocultures of *C. edulis*.

We collected four soil samples from 12 sites at each of the six selected coastal dunes in both invaded and uninvaded areas ($n = 6$ beaches $\times$ 2 invasion statuses [invaded vs uninvaded] $\times$ 4 replicates = 48 total). In each of the 12 sites, we randomly established four 0.5 x 0.5 m plots between 29 March and 4 April 2016, situated at least 10 m apart from each other. In each plot, we removed all plants and leaf litter and took five soil subsamples from the top 10 cm of soil using an ethanol-sterilized shovel. Soil subsamples for each plot were subsequently homogenized into a single replicate and kept on ice during transport and stored at −80°C as soon as possible.

DNA extraction and next-generation sequencing

We extracted whole genomic DNA from 0.25 g of soil from each sample using the PowerSoil® DNA extraction kit (MO BIO Laboratories Inc., Carlsbad, CA, USA), following the manufacturer's protocol. We checked DNA quality using the NanoDrop ND-1000 UV–Vis Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). The use of next-generation sequencing approaches for studying bacterial diversity in soils has the advantage of identifying 'hidden' soil diversity, since conventional methods of bacterial culturing are not able to detect the vast majority of these free-living soil bacteria (Birnbaum *et al.* 2016). We amplified the 16S rRNA gene, which is frequently used for the identification of bacterial taxa and consists of nine hypervariable regions (V1–V9) that differ in variability (Lane *et al.* 1985; Tringe and Hugenholtz 2008). Higher ranking taxa (e.g. families) can be identified using the more conservative regions, while more variable regions can be used for genus- or species-level identifications (Bukin *et al.* 2019). We targeted the V5–V7 hypervariable regions using primers 799F (5′-AAC MGG ATT AGA TAC CCK G-3′) and 1391R (5′- GAC GGG CGG TGW GTR CA-3′). These primers are known to display low non-target DNA amplification (i.e. low non-specificity), accurately differentiate species, and are able to give reproducible results while still capturing high bacterial diversity (Chakravorty *et al.* 2007; Beckers *et al.* 2016; Thijs *et al.* 2017). Amplification of the V5–V7 16S rRNA gene regions was done with sample-specific barcodes in the forward primer, using a 30 cycle PCR and the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, USA) under the following PCR conditions: 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, followed by a final elongation at 72°C for 5 min. After amplification, we checked the PCR products on a 2% agarose gel to determine the success of amplification and the relative intensity of bands. We then pooled multiple PCR samples together in equal proportions based on their molecular weight and DNA concentrations. We purified the pooled samples using calibrated Ampure XP beads (Agencourt Bioscience Corporation, Beverly, MA, USA) and used the samples to prepare DNA libraries by following Illumina TruSeq DNA library preparation protocol. We sequenced the samples using the Molecular Research LP next-generation sequencing service (www.mrdnalab.com, Shallowater, TX, USA) on an Illumina MiSeq instrument (Illumina, San Diego, CA, USA) following the manufacturer's guidelines.

Bioinformatics and taxonomic identification

We processed all raw MiSeq DNA sequence data following standard procedures as described in Schloss, Gevers and Westcott (2011) using mothur version 1.37.1 (Schloss *et al.* 2009). First, we removed low quality sequences and optimized the sequence lengths (to between 385 and 395 bp). Then, we aligned the unique sequences to the SILVA-ARB reference database (release 123) to the same region of the 16S rRNA gene and removed those columns that only contained gaps. Independent of a reference database, we removed all the chimeric sequences using the uchime algorithm (Edgar *et al.* 2011) and the template as self, i.e. *de novo* removal. Subsequently, we clustered the operational taxonomic units (OTUs) at the 97% sequence similarity level. The most abundant OTUs in each cluster were selected as representative sequences. We determine the taxonomic identity of each OTU with the ribosomal database project Classifier (Wang *et al.* 2007), and all sequences classified as chloroplast, mitochondria and archaea were removed. In order to standardize the number of reads across all samples, we subsampled equivalent reads from each of the 48 samples (equivalent to the read count of the smallest sample size present in the dataset). There is a debate on whether or not subsampling (i.e. rarefaction) should be done on microbiome datasets, since it is believed to increase the false discovery rate (McMurdie and Holmes 2014). However, this has been demonstrated to be invalid (Weiss *et al.* 2017); instead, rarefying can lower sensitivity (false negatives) as a result of data discarding. This is a well-known problem, and recommendations are to rarefy to the highest depth possible (de Cárcer *et al.* 2011), as we have done here. Thus, rarefying is still considered a useful normalization technique, especially for very uneven library sizes between groups (as in our case), and results in a higher PERMANOVA R^2 for studied biological effects. Nevertheless, there is still a chance that OTUs are discarded in the process, and this is a potential shortcoming.

Soil physicochemical and microbial enzymatic activity data

We incorporated soil physicochemical and microbial enzymatic activity properties in our analyses. These data were obtained from a previous study by Lechuga-Lago *et al.* (2017) who analyzed the same 48 samples collected here for pH, conductivity, water content, available soil nutrients (Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, P, NO_2^{-}, NO_3^{-}, NH_4^{+} and total organic carbon) and three soil enzymatic activities that play key roles in soil nutrient cycling (i.e. ß-1,4-glucosidase—E.C. 3.2.1.21, involved in carbon metabolism through the release of glucose from cellulose; urease—E.C 3.5.1.5, involved in the release of nitrogen by degrading urea to ammonium; and phosphatase—E.C. 3.1.3.1, involved in the release of phosphate from organic matter by hydrolyzing phosphate ester bonds).

Statistical analyses

All statistical analyses were conducted in the R statistical environment (version 3.5.1), unless otherwise specified. To visualize the abundances of individual bacterial genera across our study sites, we created a heat map using the package `gplots` and function `heatmap.2` (Warnes *et al.* 2015). In order to determine whether sampling was adequate to detect all OTUs present, we calculated OTU accumulation curves with the function `speccacum` in `vegan` package version 2.3–3 (Oksanen *et al.* 2016).

We calculated four diversity metrics from the sample × OTU matrix: OTU richness (total number of OTUs), the exponent of Shannon diversity (taking into account abundance differences between dominant and rare OTUs), inverse Simpson diversity (weights abundances of dominant OTUs more than rare ones) and Pielou's evenness (OTU abundance equality) (Jost 2006; Jost *et al.* 2010). The exponent of Shannon and Inverse Simpson diversities were chosen since these metrics represent true diversities (i.e. 'effective species'), which is not the case with other diversity indices (Jost 2006; Jost *et al.* 2010). Evenness was calculated as $\frac{H}{\ln(S)}$ (Hill 1973), where H is the Shannon diversity and S is the total number of OTUs. We calculated these various metrics with the function `renyi` in `vegan`. To determine the effect of invasion status (i.e. invaded vs uninvaded) and beach type (i.e. urban vs natural) on the four calculated diversity metrics, we performed two-way ANOVAs.

To visualize soil bacterial community composition, we performed non-metric multidimensional scaling (NMDS) using function `metaMDS` in the package `vegan` (Oksanen *et al.* 2016) based on Horn similarity values (Jost 2007) for the 97% OTU table, created with the function `sim.table` in the `vegetarian` package (Charney and Record 2012). We used permutation multivariate analysis of variance (PERMANOVA) (Anderson 2001) with 9999 permutations to test for significance in soil bacterial community composition differences using the function `adonis`. We also investigated the influence of soil chemistry (see the section 'Soil physicochemical data') on bacterial communities. For this, we first selected the least correlated soil variables for our analysis in order to reduce collinearity, while simultaneously retaining soil variables that are known to influence soil bacterial community composition (Zhou *et al.* 2002; Lauber *et al.* 2009; Fierer *et al.* 2012; Van Horn *et al.* 2014). We thus retained pH, phosphorous and nitrate as explanatory variables. These variables were used in a canonical correspondence analysis (CCA) with function `cca` in the `vegan` package, while fractionating out the effect of site (i.e. conditional variance).

To investigate whether invasion influenced associations between soil bacterial community composition and enzyme function, we calculated the mean functional difference between invaded and uninvaded samples from each site for each studied enzyme (i.e. Δphosphatase, Δglucosidase and Δurease; see the section 'Soil physicochemical data'), and the soil bacterial community composition. A measure of community composition was obtained from the first axis (which captured maximal variation) of a principle coordinates analysis (PCoA), performed with the function `cmdscale` in R `base` package based on Horn similarity values (Jost 2007) for the OTU table, created with the function `sim.table` in `vegetarian` R package (Charney and Record 2012). We used this mean difference in first PCoA axis in a Pearson's r^2 correlation analysis with mean difference in enzyme activity. If invasion status induced soil bacterial community compositional changes that lead to elevated enzyme activity levels, a significant correlation was expected.

Finally, we identified bacterial taxa that characterize soil communities of the various treatments, which we define as biomarker taxa. For this, we performed a linear discriminant analysis (LDA) effect size (LEfSe) (Segata *et al.* 2011) using the mothur software (Schloss *et al.* 2009). Such biomarker taxa are those that are highly specific to the various 'treatments', although such taxa might not necessarily be the most abundant in such treatments. Biomarker taxa plots were created using their relative abundance and frequency of occurrence. We controlled for the effect of site during analyses. Biomarkers were

identified using an alpha value of 0.05 and an effect size threshold (i.e. LDA score) of 2.

RESULTS

We obtained a total of 679 008 high-quality sequences comprising 25 602 OTUs, after data cleaning. Relatively few OTUs made up the bulk of the sequences (i.e. 1806 OTUs accounted for 80% of sequence reads; Figure S1, Supporting Information), and thus many rare OTUs were present. Although we could not classify 56% (378,745) of the representative sequences to genus level, we identified a total of 491 genera (Figure S2, Supporting Information), belonging to 179 families, 93 orders, 44 classes and 20 phyla (Fig. 1). The most abundant phyla were Proteobacteria (48.4%) and Actinobacteria (29.4%). At class level, Actinobacteria (29.1%) was the most abundant, followed by Alphaproteobacteria (24.4%) and Betaproteobacteria (15.2%). The order Actinomycetales was the most abundant (17.00%), followed by Rhizobiales (13.47%) and Burkholderiales (9.87%). The most abundant families were Streptococcaceae (5.65%), Sphingomonadaceae (3.58%) and Flavobacteriaceae (2.85%). Finally, the most abundant genera were *Lactococcus* (5.02%), *Ralstonia* (2.53%) and *Mycobacterium* (2.10%).

Invaded soils harbored 19 844 OTUs, while uninvaded soils contained 18 810 OTUs. A total of 6792 OTUs (26.2% of all OTUs), comprising 15 230 sequences (2.24% of all sequences), were unique to invaded soils (Fig. 2), while 5758 OTUs (22.5% of all OTUs), comprising 12 262 sequences (1.81% of all sequences), were unique to uninvaded soils. Invaded and uninvaded soils shared 13 052 OTUs (50.98% of all OTUs), comprising 651 516 sequences (96% of all sequences). For beach types, urban soils had a total of 5951 unique OTUs (23.2% of all OTUs; 11 361 sequences or 1.67% of all sequences), while natural soils had 6598 unique OTUs (25.8% of all OTUs; 15 213 sequences or 2.24% of all sequences). Both beach types shared 13 053 OTUs (51.0% of all OTUs, 652 434 sequences or 96.1% of all sequences). Our OTU accumulation curves did not saturate (Figure S3, Supporting Information), indicating that there are still OTUs that remain to be discovered with increased sampling effort.

The two-way ANOVAs showed that all diversity metrics were significantly affected by invasion status, having higher values in invaded soils than in uninvaded soils, with no interaction between beach type and invasion status (Table 2, Fig. 3; Table S1, Supporting Information). There were no significant differences between urban and natural beaches for any of the diversity indices.

The NMDS analysis of the soil bacterial community composition had a good (low) stress coefficient (0.08; Figure S4, Supporting Information), which, since it is below 0.1, indicates that there is no risk of drawing false inferences from the plot (Clarke 1993). The CCA plots also indicated obvious clustering of samples (Fig. 4) by invasion status (invaded vs uninvaded). The PERMANOVA model confirmed these trends (Table 3), and indicated that beach type (urban vs natural) ($F_{PERMANOVA} = 3.73$, $p < 0.001$) and the presence of *C. edulis* ($F_{PERMANOVA} = 6.83$, $p < 0.001$) significantly altered soil bacterial community composition. However, the PERMANOVA model also showed a significant interaction between beach type and invasion status ($F_{PERMANOVA} = 1.83$, $p = 0.041$), indicating that the influence of *C. edulis* is conditional on the type of beach it invades. We found numerous taxa to be characteristic to each beach type and invasion status (Fig. 5; Table S2, Supporting Information). For example, the genera *Algoriphagus, Flavobacterium, Mucilaginibacter, Pedobacter, Rhodopseudomonas* and *Spirosoma* were characteristic of natural areas, while the genus *Hyphomicrobium* was characteristic of urban areas; and the genera *Adhaeribacter, Arenimonas* and *Devosia* were characteristic of invaded areas, while the genera *Bacillus, Massilia* and *Streptococcus* were characteristic of uninvaded areas.

Our CCA analysis showed that the selected soil variables (i.e. pH, NO_2^- and P) explained 10.8% of bacterial community variation (Fig. 4), while the conditional effect of site accounted for 22.5% of the variation. The fact that site explained the highest amount of variation is not surprising, since a multitude of processes, both stochastic and deterministic, could be present in some of the study sites and affect their bacterial communities (e.g. competition, predation, disturbance events, dispersal limitation, spatial isolation and plant community composition) (Borcard, Legendre and Drapeau 1992; Zhou *et al.* 2002; Gibbons and Gilbert 2015). A total of 66.8% of variance therefore remains unexplained. Although no clear pattern was observable for beach type, it was evident that the changes in pH, NO_2^- and P observed in areas invaded by *C. edulis* were correlated with changes in soil bacteria community composition. Moreover, we also found that the changes in community composition observed in invaded areas were correlated with changes in phosphatase activity (Fig. 6).

DISCUSSION

Only few of the identified bacterial taxa dominated the soil communities we analyzed and further sampling effort would likely identify even more taxa in our sampled coastal dunes. This suggests that bacterial communities of coastal dune ecosystems contain a large number of rare taxa. Further research on this topic is warranted to clarify the implications of these findings, since the currently available information on soil bacterial communities of coastal dune ecosystems remains very limited (Rajaniemi and Allison 2009), having been mostly restricted to cold or wet environments, such as the Arctic tundra or tropical rainforests (Rajaniemi and Allison 2009; Koyama *et al.* 2014; Petersen, Meyer and Bohannan 2019).

In agreement with our findings, the phyla Actinobacteria and Proteobacteria (especially the class Alphaproteobacteria) have been previously reported as the dominant groups of bacteria in sand dunes (Lin *et al.* 2014). Moreover, we found Actinomycetales to be the most abundant order. This is probably due to the fact that, unlike the majority of other soil bacteria, actinomycetes are generally adapted to the conditions of high pH, temperature and water stress characteristic of coastal dune ecosystems (Pepper and Gentry 2015).

Our results support our hypothesis that the presence of dense invasive *C. edulis* populations affects the diversity of soil bacteria. The reason for the observed increases in soil bacterial diversity may be related to the differences in soil characteristics between invaded and non-invaded areas (Lechuga-Lago *et al.* 2017). In particular, soil moisture is known to be the main environmental factor that influences soil microbial communities (Chen, Chiu and Tian 2005; Brockett, Prescott and Grayston 2012; Manzoni, Schimel and Porporato 2012), with bacterial diversity increasing with soil water availability (Wasserstrom *et al.* 2017). Invasive *C. edulis* forms thick layers of organic litter on the soil surface, which increases soil water content and decreases soil water loss (Novoa *et al.* 2012). This was also true for our sites, where, Lechuga-Lago *et al.* (2017) found higher soil moisture in *C. edulis*-invaded plots compared to uninvaded plots. The observed increases in soil bacterial diversity are likely to promote soil

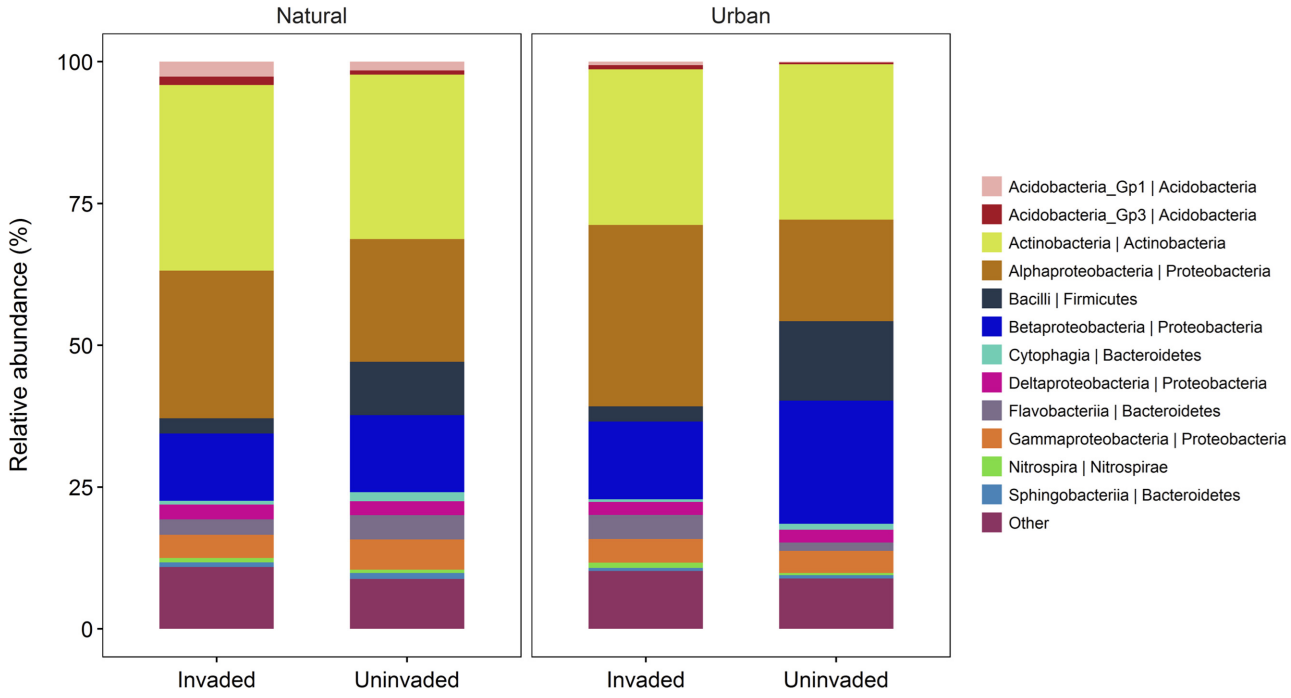

Figure 1. Relative abundances of taxa (class | phylum) for each beach type (natural vs urban) and invasion status (invaded vs uninvaded). Class-level relative abundances were calculated using the number of sequences for each taxon as a percentage of the total sequences for each beach type × invasion status combination. The 'Other' category includes taxa that were unclassified at class level together with classes representing <0.5% of the total number of sequences.

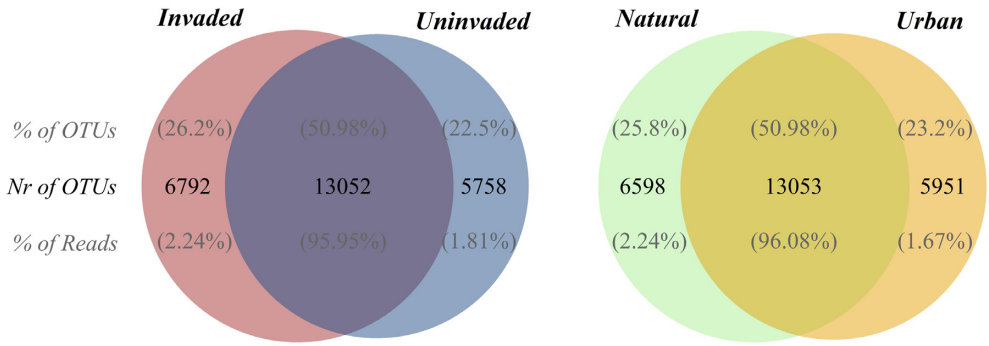

Figure 2. Distribution of OTUs between invaded and uninvaded (left), and natural and urban (right) beaches. Values in brackets indicate percentage of total read count.

ecosystem functioning, such as organic matter decomposition and nutrient cycling (Philippot *et al.* 2013; Maron *et al.* 2018).

Carpobrotus edulis also modifies other soil properties such as salinity, organic matter and nutrient content, especially in urban areas (Lechuga-Lago *et al.* 2017), which are all expected to affect the composition of soil bacterial communities (Rajaniemi and Allison 2009; Shen *et al.* 2013; Lin *et al.* 2014). Accordingly, our results suggest that increases in NO_2^- and P content and a decrease in soil pH under *C. edulis* invasion (Novoa *et al.* 2014) might be partially responsible for the observed differences in the composition of soil bacterial communities between invaded and uninvaded areas, which were larger in urban than in natural areas.

Our results showed that invasion induced changes in soil bacterial community composition, which correlated with the observed elevated levels of phosphatase activity (Lechuga-Lago *et al.* 2017). These are important results, since phosphatases are a large group of enzymes that are key in the metabolism of phosphate (Speir and Ross 1978) and phosphorus is often the most limiting soil nutrient for plant growth (Van Vuuren, Bouwman and Beusen 2010). Increases in phosphate in coastal dunes could facilitate the establishment of plant species that are not adapted to the generally low nutrient levels of coastal dune soils (Lechuga-Lago *et al.* 2017).

Contrary to our expectations, our results show no significant effect of urbanization (i.e. beach type) on the diversity of soil bacteria. These results are surprising, since urbanization has been shown to affect soil characteristics in a similar manner as *C. edulis* invasion (Lechuga-Lago *et al.* 2017). However, other factors associated with urbanization such as soil metal or polycyclic aromatic hydrocarbon concentration might prevent an increase in the diversity of soil bacteria in urban areas (Yan *et al.* 2016). Unfortunately, the effect of such factors on soil microbial communities has received relatively little attention to date (Pickett and Candenasso 2006), and more research is needed to unravel the mechanisms underlying these observations.

Interestingly, several of the biomarker taxa of urban beaches (i.e. taxa that were found to be highly specific to urban beaches)

Table 2. Results from two-way ANOVA showing the influence of invasion status (invaded vs uninvaded) and beach type (urban vs natural) on the various soil bacterial community diversity metrics. Significance indicated in bold as follows: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Diversity	Factor	MS	F	p
Richness	Invasion status	1 207 454	6.56	**0.014***
	Beach type	77 844	0.42	0.519
	Invasion status × beach type	33 761	0.18	0.670
	Error	184 009		
Exponent of Shannon	Invasion status	966 781	16.12	**<0.001*****
	Beach type	98 608	1.64	0.206
	Invasion status × beach type	89 022	1.48	0.230
	Error	59 973		
Simpson	Invasion status	290 767	42.73	**<0.001*****
	Beach type	3445	0.51	0.481
	Invasion status × beach type	13 939	2.05	0.159
	Error	6805		
Evenness	Invasion status	0.0399	10.66	**0.002****
	Beach type	0.0118	3.16	0.082
	Invasion status × beach type	0.0121	3.25	0.078
	Error	0.0037		

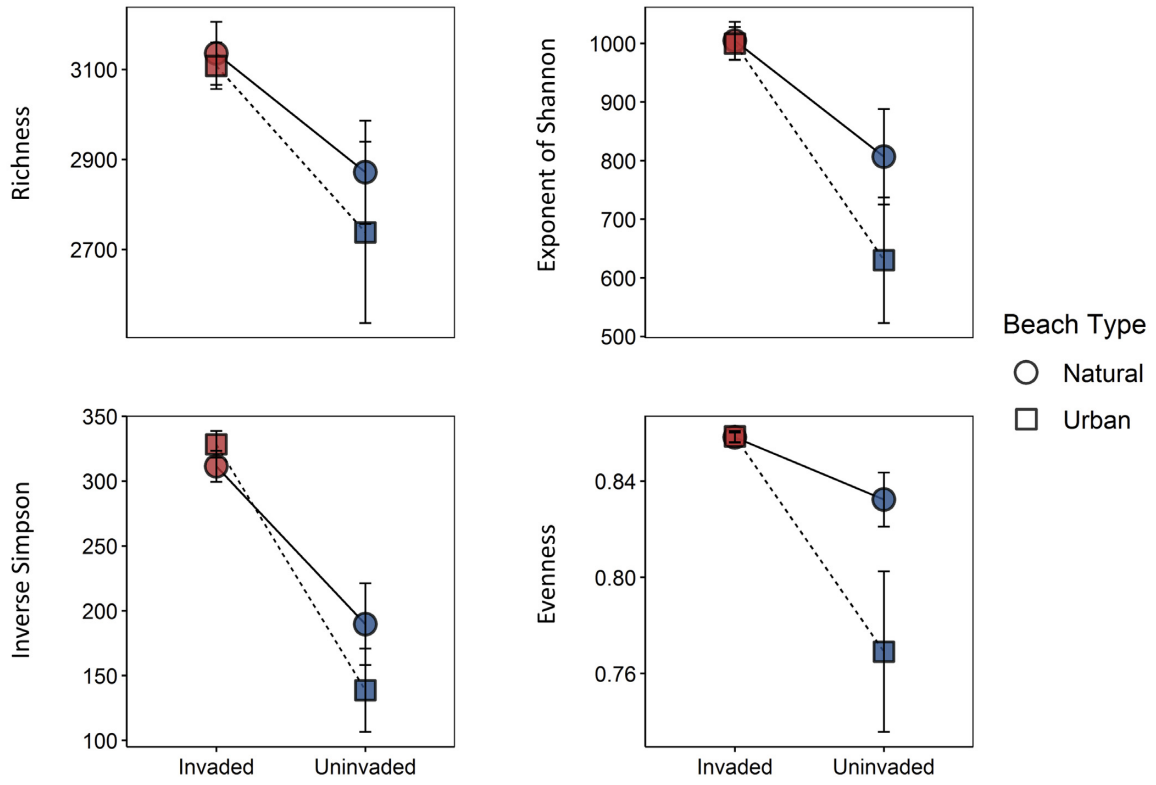

Figure 3. Soil community diversity metrics for *C. edulis* invaded and uninvaded soils in urban and natural areas. Error bars indicate the standard deviation of the mean. See Table 2 for related ANOVA results.

Table 3. Results from the PERMANOVA model showing the influence of invasion status (invaded vs uninvaded) and beach type (urban vs natural) on the soil bacterial community composition. Significance indicated in bold as follows: $^{*}p < 0.05$; $^{***}p < 0.001$.

Factor	df	MS	*F*-value	R^2	*P*
Beach type	1	0.546	3.73	0.066	**<0.001*****
Invasion status	1	0.999	6.83	0.121	**<0.001*****
Beach type × invasion status	1	0.267	1.83	0.032	**0.041***
Error	44	0.146		0.780	

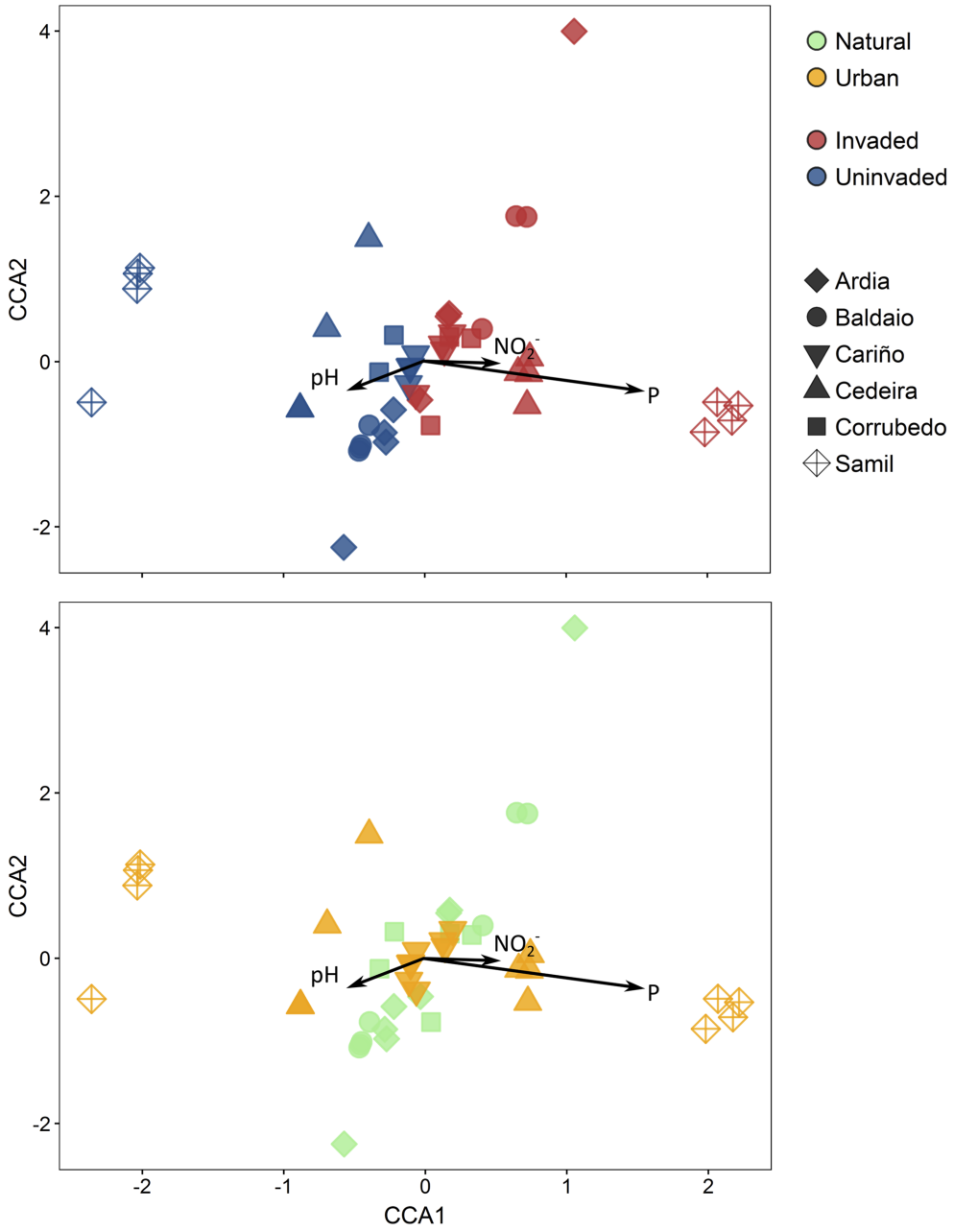

Figure 4. CCA plots colored according to invasion status (top) and beach type (bottom). Only variables that were not colinear and that are biologically significant were included in the model (i.e. those variables that are known to influence soil bacterial community composition). Individual sites are shown by using different symbols.

are also known for their ability to degrade organic pollutants. For example, species within the genera *Pseudomonas* and *Sphingomonas* have the ability to use contaminants as nutrients, and can even degrade plastic (Kawai 1995; Kyaw *et al.* 2012); *Sphingopyxis* spp. are able to degrade crude oil, diesel and kerosene (Kim *et al.* 2014); and *Rhodococcus* spp. are able to degrade a wide range of recalcitrant, toxic hydrocarbons (Laczi *et al.* 2015). These results suggest that pollution might be driving the observed changes in the soil bacterial communities of the studied coastal urban areas, which could further threaten the conservation of coastal dune ecosystems.

Overall, our study shows that dominance of a single invasive species and urbanization can alter the soil bacterial communities of coastal dune ecosystems in distinct ways. The impact of *C. edulis* invasion on soil physiochemical properties, such as moisture, pH, soil salinity, organic matter and nutrient contents (Novoa *et al.* 2014), is correlated with an increase in the diversity of soil bacterial communities and alters their composition. On the other hand, urbanization, possibly in synergy with anthropic pollution, impacts the composition of bacterial communities. While we did not examine a suite of invasive species, coastal areas or habitats and therefore cannot generalize our results beyond the invasion by *C. edulis* on the coast of Galicia (Spain), our findings provide evidence that anthropogenic drivers can have different and distinct impacts on soil microbial communities. Therefore, our results pave the way for urgently needed research on this topic to unravel the impacts of these anthropogenic drivers on soil microbial communities. Understanding

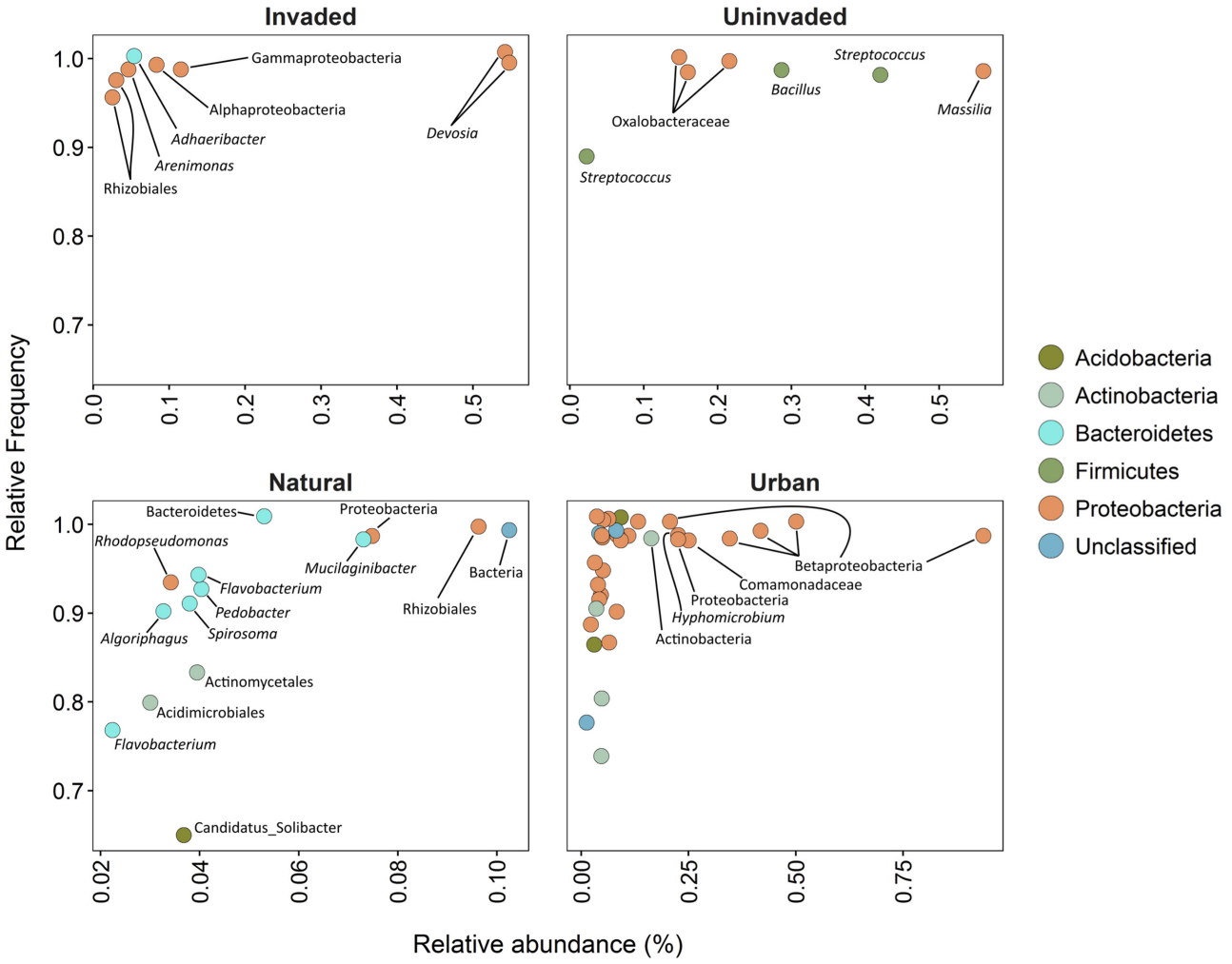

Figure 5. Relative frequency and abundance of the biomarker taxa (order, phylum, family, genus or species) that were most characteristic of the various factor combinations as determined by LEfSe. Biomarker taxa were those that have the highest LDA scores. The different colors indicate the phylum of the biomarker taxa. 'Unclassified' indicates biomarker taxa that were only classifiable as domain Bacteria.

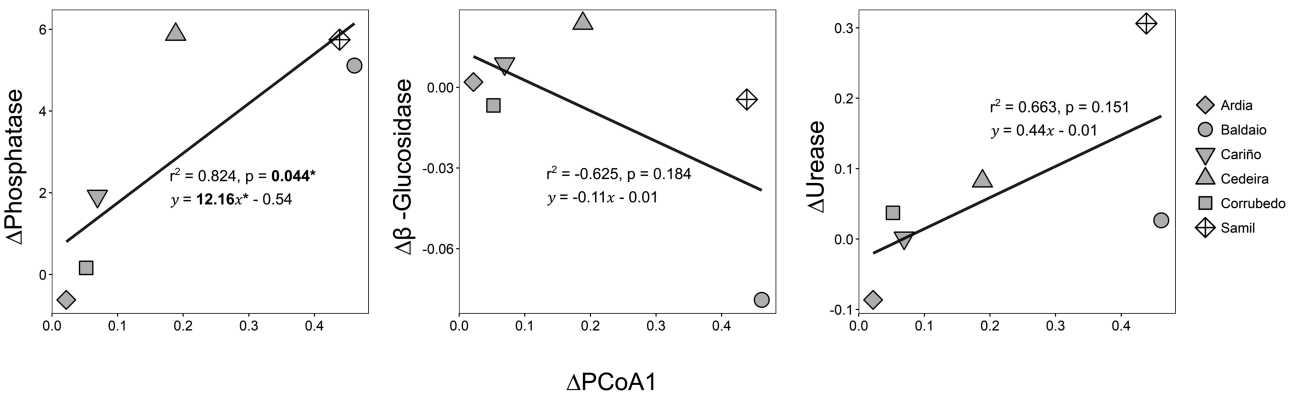

Figure 6. Relationships between mean differences in community composition (as expressed by the first axis of a PCoA; right panels), and phosphatase, β-glucosidase and urease activities. Values are expressed as mean differences between invaded and pristine areas of each site.

the multiple dimensions of the impacts of anthropogenic drivers on the soils of coastal dunes will have important implications for the conservation of these unique and highly threatened ecosystems.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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