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ORIGINAL PAPER

# Soil quality: a key factor in understanding plant invasion? The case of *Carpobrotus edulis* (L.) N.E.Br.

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**Abstract** The cycles of carbon, nitrogen and phosphorus are essential components of the processes and functioning of ecosystems. The functional capacity of the soil microbial community that drives these cycles varies among soils dominated by different plant species. This work aims to quantify changes in soil features of coastal ecosystems of the Iberian Peninsula caused by the invasion of *Carpobrotus edulis* by analysing soil chemical properties and extracellular soil enzymes. We also analyse the influence of these changes on the germination and early development of native species *Malcolmia littorea* (L.) R.Br. and *Scabiosa atropurpupea* L. and the alien *C. edulis*. Our results reveal that when *C. edulis* invades a dune ecosystem, it causes significant changes to pH, enzymatic activities, nutrients, salinity and moisture content of the soil (the level of the change depends on the initial characteristics of the invaded ecosystem). These changes alter the germination process of native and invasive plants in different ways. The results of this work suggest mechanism whereby *C. edulis* competes with native species at an early stage and breaks the initial abiotic resistance of newly invaded landscapes. This study highlights the importance of studying the effects of invasive plant-soil interactions on the germination and emergence of different plant species in order to fully understand the effects of invasion and to consider options for restoration activities in areas invaded by *C. edulis*.

**Keywords** Biological invasions · Enzymatic activities · Seed germination · Seedling development

## Introduction

Communities of soil microorganisms provide ecosystem services and control life-support functions such as decomposition, nutrient cycling, soil carbon storage, production of greenhouse gases, degradation of pollutants, and maintenance of soil structure. They release extracellular enzymes that allow them to access energy and nutrients present in complex substrates, catalysing the initial step of decomposition and nutrient mineralization (Allison and Vitousek 2004).

The functional capacity of soil's microbial community varies among soils dominated by different plant species (Kourtev et al. 2002). Invasion by alien plant species is a major threat to ecosystem stability (Mack et al. 2000). Altered species composition of a

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community caused by an invasive alien species often leads to marked changes in nutrient cycling processes (Ehrenfeld 2003). A given species may have very different effects at different sites, suggesting that the composition of the invaded community and/or environmental factors, such as soil type, influence the direction and magnitude of such ecosystem-level impacts of invasive plant species (Ehrenfeld et al. 2001).

Differences in plant size, spatial distribution, and tissue chemistry lead to changes in C and N cycling. Moreover, differences in the quantity and quality of inputs to the soil by different species of plants may reflect alterations in the soil microbial community (Grierson and Adams 2000). Nutrient dynamics may also be altered as a result of changes in the physical properties of the soil caused by the introduction of new species. Another qualitative change that may be important is the introduction of alien species with novel compounds in their tissues, as well as those with large litterfall masses (Boon and Johnstone 1997). These novel compounds can inhibit the microbial colonization of leaf litter and can be associated with very slow decomposition rates in the invaded habitat (Boon and Johnstone 1997). Changes may also result from alterations in the patterns of species dominance within the plant community, since the effects of a given species on ecosystem processes are modulated by its relative abundance in the community. Changes in plant functional types (e.g. changed relative abundance of herbaceous plants versus woody plants, N-fixing versus non-fixing species, C3 vs. C4 species) are also associated with changes on the distribution and dynamics of soil nutrients. There is thus a variety of mechanisms through which changes in the species composition of a community may alter nutrient cycling processes (Ehrenfeld 2003).

The cycles of carbon, nitrogen and phosphorus are essential components of the processes and functioning of ecosystems. It is necessary to understand how these cycles correspond to the invasion of alien species (Liao et al. 2008), since soil microorganisms or soil nutrient levels can have a significant effect on the success of invasive species (Levine et al. 2003; Reinhart and Callaway 2006; Jordan et al. 2008). The physicochemical characterization, together with the assessment of biological parameters related to soil microbial activity, forms a broad vision on which to assess the status of the invaded community in terms of soil quality (Schloter et al. 2003).

*Carpobrotus edulis*, a succulent chamaephyte that can also propagate vegetatively, is native to South Africa (Albert 1995) and one of the most intensively studied invasive plants globally (Pyšek et al. 2008; GEIB 2006). As with other invasive species that are particularly well studied (Pyšek et al. 2012), the reason for the strong focus on this species is the strong impact it has had on invaded ecosystems. Mediterranean ecosystems in the Iberian Peninsula are generally considered to be relatively resistant to invasion by alien plant species (Vilà et al. 2008). However, *C. edulis* readily invades coastal dune ecosystems. It grows between and on native vegetation, covering the substrate in a few years (D'Antonio and Mahall 1991). *C. edulis* differs markedly in terms of plant size, spatial distribution, litterfall dynamics, decomposition rates and plant functional type (CAM metabolism) compared to the native plants of the ecosystems it has invaded. Therefore, it has the potential to cause major changes to key features of invaded ecosystems, through its influence on the composition and structure of plant and microbial communities and trajectories of succession (Donath and Eckstein 2009). Changes in the properties of the soils invaded by *C. edulis* probably have major impact on seed germination and seedling emergence of native species. A detailed study of the changes in the quality of microsites due to the presence of *C. edulis* and the impact of these changes on the germination and establishment of native and invasive species is crucial for understanding the invasion strategies followed by the species and the challenges faced in restoration.

With the aim of assessing the impact that the alien species *C. edulis* causes on the invaded ecosystems and on the native plant community, we tested the following hypothesis: (1) *C. edulis* changes soil characteristics, nutrient availability and nutrient cycle differently, depending on the initial characteristics of the invaded ecosystem; (2) The residual effects of *C. edulis* on soils (changes in pH, salinity, nutrients…) improve the conditions for the emergence of *C. edulis* seedlings; and (3) Soil characteristics of dunes covered by native vegetation are more suitable for native plant germination than those of areas invaded by *C. edulis*.

## Methods

### Study sites

We conducted the experiment on the Iberian Peninsula, covering a range of different soil features and microclimatic regimes (Fig. 1; Table 1). We sampled soils in 6 different areas corresponding to spaces on the Natura 2000 system (Habitat Directive 92/43-Annex I: Natural habitat types of community interest whose conservation requires the designation of special areas of conservation). All sites corresponded to the category of Habitat 2230: *Malcolmietalia* dune grasslands, with the exception of the dunes of La Flecha (which corresponded to Habitat 2270: Wooded dunes with *Pinus pinea* and/or *Pinus pinaster*) and the dunes of Arriba Fósil-Mata dos Medos, Lisbon (Habitat 2250: Coastal dunes with *Juniperus* spp.).

We chose the particular locations mentioned above aiming to compare Mediterranean coastal dune ecosystems with different initial characteristics invaded by *C. edulis*. (1) Pontevedra. The primary dunes of Punta Ron, Pontevedra (approx. 10 m from the sea) was the first site invaded by *C. edulis* in Spain (GEIB 2006). *C. edulis* was introduced into the region for stabilizing the dunes; the first plantings in the area were performed in 1900 (GEIB 2006). (2) Bilbao. In the primary dunes of Playa de la Arena (Muskiz, Bilbao, 110 m from the sea), *C. edulis* was introduced as an ornamental plant (information by the Eusko Jaurlaritza). (3) Barcelona. The beach of Castelldefels, in Barcelona (approx. 130 m from the sea), is located in an urban area. *C. edulis* was introduced into this area as an ornamental plant (information by the local government of Castelldefels). (4) Alicante. In Guardamar del Segura, in Alicante, *C. edulis* was introduced at the beginning of the 20th century to stabilize the dunes. The sand of these dunes (approx. 200 m from the sea) came from the Segura River and not from the sea, as in the other areas (information by the Generalitat Valenciana). (5) Huelva. In contrast to the other areas, where *C. edulis* invasions were driven by seeds from local sites, invasions in the dunes of La Flecha del Rompido, in Huelva (200 m from the sea), were initiated by seeds dispersed from other sites in the sea (information by the Junta de Andalucia). These dunes are formed by moving sands (information by the Junta de Andalucia). (6) Lisbon. Finally, in the Mata dos Medos at the protected area Arriba Fóssil da Costa da Caparica, Lisbon, *C. edulis* invaded the stabilized interior dunes (approx. 400 m from the sea) after several fires in the area (information by the Câmara Municipal de Lisboa).

**Fig. 1** Distribution of sampling points along the Iberian Peninsula. 1: Punta Ron (Pontevedra). 2: Playa de la Arena (Bilbao). 3: Platja de Castelldefels (Barcelona). 4: Guardamar del Segura (Alicante). 5: La Flecha del Rompido (Huelva). 6: Mata dos medos (Lisbon)

**Table 1** Climatic ranges of sampling points (Ninyerola et al. 2005)

| County | | Climate (Köppen) | *AAT* (°C) | *AAMT* (°C) | *AAmT* (°C) |
|---|---|---|---|---|---|
| 1 | Pontevedra | *Cfa* | 15.0 | 10.0–12.5 | 20.0 |
| 2 | Bilbao | *Cfb* | 15.0 | 10.0 | 17.5–20.0 |
| 3 | Barcelona | *Csa* | 17.5 | 12.5–15.0 | 22.5 |
| 4 | Alicante | *Bsk* | 17.5–20.0 | 12.5–15 | 25.0 |
| 5 | Huelva | *Csa* | 20.0 | 15.0 | 22.5–25.0 |
| 6 | Lisboa | *Cfa–Csa* | 17.5 | 12.5 | 20.0–22.5 |

*AAT* Annual average temperature, *AAmT* annual average minimum temperature, *AAMT* annual average maximum temperature, *Cfa* humid subtropical climate, *Cfb* oceanic climate, *Csa* hot summer Mediterranean climate, *Bsk* cold semi-arid climate

## Soil collection

Soil samples were collected on invaded and non-invaded areas at the dunes of Punta de Ron, La Arena, Castelldefels, Guardamar del Segura, La Flecha and Arriba Fóssil-Mata dos Medos. All the studied soils correspond to the classification of Arenosols (FAO-ISRAC-ISSS 1998).

At each location, we chose 5 random points (0.5 × 0.5 m) to collect sandy soil in an area invaded by *C. edulis* (soil from an invaded dune) and 5 random points in an adjacent area without a historical presence of *C. edulis* (soil from the native dune). Five soil samples were taken from the top 10 cm at each point. Samples were pooled in two sets per location: invaded and native.

## Soil analysis

In the laboratory, the samples were air dried, passed through a 2 mm-sieve and homogenized in a vibratory homogeniser for solid samples (Fritsch Laborette 27 rotary sampler divider). Elemental soil analysis was performed on three replicates of the two soil types per location. Soil pH was determined in a soil solution rate of 1:2.5 and 1:5 (soil:distilled water) respectively (Guitián and Carballas 1976; Allen et al. 1974; Maun 2009). Salinity (mgNaCl per g) was analysed by the Mohr method (Jander 1961). Soil moisture was calculated as *% Moisture = (Fresh weight-Dry weight)/(Fresh weight)* × *100* by drying three replicate sub-samples of each soil sample at 70 °C for 48 h. The total C content was estimated after combustion at 1200 °C of 0.1 g of soil, previously powdered in LECO-CNS 2000 analyser (Comisión de Métodos Analíticos del Instituto Nacional de Edafología y Agrobiología 1973). The percentage of organic matter was calculated by multiplying the percentage of total carbon by the Van Bemmelen factor of 1.724 (Comisión de Métodos Analíticos del Instituto Nacional de Edafología y Agrobiología 1973). Nitrogen compounds such as ammonium, nitrate and nitrite were analysed using the method described by Kempers (1974). The available P was extracted using the method described by Jakmunee and Junsomboon (2009) for colorimetric determination using the method of Bray and Kurtz (1945).

## Enzyme assays

The enzymes assayed were β-1,4-glucosidase (EC 3.2.1.21), urease (EC 3.5.1.5) and phosphatase (EC 3.1.3.1.) (Table 2). The substrates for the β-glucosidase, urease and phosphatase assays were *p*-nitrophenol (*p*NP) β-d-glucopyranoside, urea and *p*NP-phosphate, respectively. The substrates were made in Tris-HCl 1 M, deionized water and Tris-HCl 1 M, respectively. There were five analytical replicates and five sample controls of each treatment.

For the analysis of β-glucosidase, urease and phosphatase, the methods followed were those described by Allison and Vitousek (2005), Kandeler and Gerber (1998) and Tabatabai and Bremner (1969), respectively.

Following the recommendation of German et al. (2011), the enzyme assays were run at the environmental pH, and assays on the freshest samples possible (less than 48 h of storage) were conducted. Assays at the soil pH provide a measure of the potential activity under field conditions (Turner 2010). All samples were analysed after the same time of storage (2 days), since we found during previous analyses that in 3-day

**Table 2** Function and enzymes assayed for potential soil activity

| Enzyme | EC | Abbreviation | Function |
|---|---|---|---|
| β-1,4-glucosidase | EC 3.2.1.21 | BG | Releases glucose from cellulose |
| Phosphatase | EC 3.1.3.1 | AP | Releases inorganic phosphate from O.M. |
| Urease | EC 3.5.1.5 | UR | Degrades urea to ammonium |
| Dehydrogenase | EC 1.1.1.1 | DH | Oxidises organic compounds |

old sand samples the storage method can affect the activities of these enzymes in different ways at each soil type, causing an increase in some and a decrease in others [Novoa et al. (in Press)].

### Germination experiment

Two native species, *Malcolmia littorea* (L.) R.Br. and *Scabiosa atropurpurea* L., were selected. *Malcolmia littorea*, an annual species native to South Europe, grows in coastal sand dunes (Tutin et al. 1993). It is distributed in France, Spain, Italy and Portugal (Albert 1995), where it grows in the same habitats invaded by *C. edulis* (Thuiller et al. 2005). It is endangered in some regions of Spain, though not in the study area (Allen et al. 1974), as well as in Italy, where it was recently suggested for listing as a threatened species (Del Vecchio et al. 2012). *S. atropurpurea* is a species typically present in pastures, road margins, fixed dunes and rocky terrain of the Iberian Peninsula and also throughout the Mediterranean region and Macaronesia (Webb et al. 1988). We chose these species as native target species since they are present in all the investigated areas, and in many other areas invaded by *C. edulis* in the Mediterranean Basin.

Seeds of the native and invasive species were collected between 10 September and 10 October 2010, from at least 15 plants of each species in the study area. The seeds were separated from the rest of the fruit and their accessory dispersion parts, and then stored at 4 °C until sowing. Based on the soil analysis results, we studied the germination response to different levels of nutrients, salinity, pH and moisture, and the early development of seeds of the study species. Different levels for each factor were established. Petri dishes were watered every 3 days with 4 ml of different solutions to test the effect of the nutrients, salinity and pH on seed germination. We chose two nutrient levels: distilled water or 11 ppm of N and 1.5 ppm of P (levels of Hogland Solution); four salinity levels: 0, 0.02, 0.04, and 0.06 gNaCl/L; and three pH levels: 6, 7, 8, and 9. We used three levels of moisture to test the effect of the moisture level: 2, 4, and 6 mL of irrigation solution every 3 days. A solution made of distilled water (pH 6) and with no salinity (0 gNaCl/L) constituted a common treatment. Overall, there were 10 different treatments (Table 3).

Cultures were performed in Petri dishes using 3 MM Whatman paper with a total of 5 replicates per treatment and 20 seeds per dish. Tests were carried out in germination chambers with a 12/12 light/dark photoperiod. Petri dishes were watered on a daily basis for 3 weeks. The number of germinated seeds was recorded daily. After 3 weeks, the length of the leaves, stems and roots of five random seedlings per plate were measured.

### Germination indices

The total germination rate (*Gt*) and the cumulative rate of germination (*AS*) were calculated using germination data. These indices are very representative of the germination pattern followed in each treatment and are two of the most widely cited and used in the literature (Chiapusio et al. 1997). The total germination (*Gt*) provides an overview of the germination process. It detects possible stimulatory or inhibitory effects on germination, and reports the germination capacity of each species in each situation (Chiapusio et al. 1997). $Gt = (Nt \times 100/N)$, where $Nt$ is the total number of seeds germinated when the last measurement took place and $N$ is the number of seeds used in the bioassay. The speed of cumulative germination index (*AS*) indicates the effect of treatment on the cumulative speed during each of the times (Bradbeer 1998; Dias 2001). $AS = (n_1/1 + n_2/2 + n_3/3 + \cdots + n_n/n)$, where $n_1$, $n_2$, $n_3$, ..., $n_n$ are the cumulative number of germinated seeds at time 1, 2… $n$ throughout the assay.

**Table 3** Treatments assayed for the germination experiment (nutrients, salinity, pH and moisture adjusted on distilled water)

| Treatment | Salinity (gNaCl/L) | pH | Moisture (mL/day) | Nutrients (PPM) | |
|---|---|---|---|---|---|
| | | | | N | P |
| 1 | **0** | **6** | **4** | **0** | **0** |
| 2 | 0 | 6 | 4 | **11** | **1.5** |
| 3 | **0.02** | 6 | 4 | 0 | 0 |
| 4 | **0.04** | 6 | 4 | 0 | 0 |
| 5 | **0.06** | 6 | 4 | 0 | 0 |
| 6 | 0 | **7** | 4 | 0 | 0 |
| 7 | 0 | **8** | 4 | 0 | 0 |
| 8 | 0 | **9** | 4 | 0 | 0 |
| 9 | 0 | 6 | **2** | 0 | 0 |
| 10 | 0 | 6 | **6** | 0 | 0 |

Bold: adjusted parameter

### Statistical analysis

Data were analyzed using the statistical program IBM-SPSS Statistics 19. The first exploratory analysis of the data was performed using box plots to detect and remove outliers. Then the Kolmogorov–Smirnov test was applied to check the normality of data, and the Levene's test of homogeneity of variances to test their homoscedasticity.

The data that presented normality and homoscedasticity were analysed using the parametric statistic, using a simple factorial analysis of variance (ANOVA). In cases where the ANOVA revealed significant differences, the results of the Tukey test were used for multiple comparison of means.

The data that were not normal or heteroscedastic were analysed using nonparametric statistics. The nonparametric Kruskal–Wallis and the *U* Mann–Whitney tests were used as post hoc tests.

The data on the substrate characteristics that did not show more than two treatments and that presented normally were analysed by the Student's *t* test.

## Results

### Soil characteristics

The data obtained from the elemental analysis of soils indicated significant differences ($P \leq 0.05$) between the non-invaded areas and the areas invaded by *C. edulis* at all study sites. The pH values were significantly lower in areas invaded by *C. edulis* than in non-invaded areas at the dunes of Pontevedra (22 %), Bilbao (1 %) and Barcelona (7 %), whereas in Lisbon the pH was higher in invaded areas (12 %). Our results showed an increase in the salinity level in invaded areas, significantly in Pontevedra (28.6 %), Bilbao (50 %), Barcelona (50 %) and Lisbon (40 %). Moisture levels are greater in invaded soils than in native soils in all locations except for Lisbon, where no significant differences were found. The organic matter calculated from the values of total carbon is also higher in invaded soils in Pontevedra (52.2 %), Barcelona (44.5 %) and Huelva (21.6 %). The available phosphorus was higher in invaded areas in Pontevedra (45.5 %), Bilbao (9.2 %) and Barcelona (79.6 %). Nitrogen levels were less affected than other soil characteristics. Sites at Barcelona and Lisbon were the most affected, but in different ways. Levels of $NH_4^+$ and $NO_3^-$ were higher in invaded areas of Barcelona, but significantly lower in invaded dunes in Lisbon. $NO_2^-$ levels were not significantly different between invaded and uninvaded sites except for Pontevedra, where levels were lower in invaded sites (Table 4).

### Enzyme activities

Glucosidase activity was significantly different ($P \leq 0.01$) in half of the studied dunes. Activity was always lower in invaded soils [Bilbao (9.8 %), Alicante (12 %) and Huelva (20.2 %)] than in uninvaded sites. Urease activity showed a different response, depending on the origin of the samples. It was significantly greater in invaded soils than in native soils of Pontevedra (65 %), Barcelona (77 %) and Alicante (95 %); however, the opposite trend was found at Huelva (50 %) and Lisbon (28 %). Phosphatase activity was greater in invaded soils except for Lisbon, where there were no significant differences (Table 5).

### Germination process

Germination tests (Table 6) revealed a clear dependence of native plants on salinity and humidity (germination is null at minimum moisture rate). pH is also crucial for germination of *M. littorea* and the best final germination was found where pH was greater than 6. Two factors strongly stimulate

**Table 4** Soil properties from the sampling points along the Iberian Peninsula

| County | Pontevedra | | Bilbao | | Barcelona | | Alicante | | Huelva | | Lisbon | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *N* | *I* | *N* | *I* | *N* | *I* | *N* | *I* | *N* | *I* | *N* | *I* |
| pH | 9.15 | 6.99** | 8.85 | 8.70** | 9.06 | 8.44** | 8.93 | 9.08 | 8.74 | 8.92 | 5.80 | 6.60** |
| | (0.05) | (0.16) | (0.02) | (0.01) | (0.03) | (0.01) | (0.07) | (0.09) | (0.08) | (0.12) | (0.06) | (0.04) |
| Salinity | 0.52 | 0.75** | 0.03 | 0.14** | 0.01 | 0.02** | 0.04 | 0.04 | 0.02 | 0.02 | 0.03 | 0.05** |
| (mgNaCl/ g) | (0.04) | (0.03) | (0.003) | (0.007) | (0.002) | (0.002) | (0.011) | (0.007) | (0.002) | (0.001) | (0.002) | (0.003) |
| Moisture | 0.09 | 0.74** | 0.07 | 0.08** | 0.06 | 0.07** | 0.03 | 0.05** | 0.04 | 0.05** | 0.53 | 0.49 |
| (%) | (0.002) | (0.004) | (0.001) | (0.001) | (0.002) | (0.003) | (0.002) | (0.001) | (0.001) | (0.003) | (0.023) | (0.032) |
| O.M. | 3.02 | 6.32** | 7.07 | 6.49 | 3.90 | 7.03** | 5.20 | 6.27 | 1.20 | 1.53** | 5.45 | 4.55 |
| (g/Kg) | (0.21) | (0.35) | (0.42) | (0.33) | (0.23) | (0.45) | (1.02) | (1.53) | (0.21) | (0.15) | (0.63) | (0.42) |
| $P_{available}$ | 22.08 | 38.37** | 23.40 | 25.80** | 42.69 | 209.20** | 21.40 | 19.20 | 43.20 | 48.8 | 10.20 | 8.25 |
| (mg/Kg) | (0.48) | (1.42) | (0.51) | (0.15) | (6.77) | (5.14) | (0.86) | (0.88) | (2.63) | (3.42) | (1.34) | (0.79) |
| $NH_4^+$ | 6.93 | 8.40 | 2.02 | 2.15 | 2.51 | 4.28** | 2.42 | 1.76 | 3.60 | 4.40 | 9.80 | 4.30** |
| (mg/Kg) | (0.55) | (1.03) | (0.33) | (0.11) | (0.13) | (0.85) | (0.68) | (0.16) | (0.43) | (0.38) | (0.17) | (0.11) |
| $NO_3^-$ | 0.87 | 1.10 | 9.68 | 18.40** | 8.36 | 28.60** | 8.60 | 7.30 | 9.41 | 9.44 | 41.94 | 24.60** |
| (mg/Kg) | (0.20) | (0.43) | (0.48) | (1.12) | (0.40) | (2.40) | (1.2) | (0.34) | (0.79) | (0.68) | (2.09) | (0.42) |
| $NO_2^-$ | 0.12 | 0.07** | 0.22 | 0.22 | 0.25 | 0.51 | 0.22 | 0.17 | 0.29 | 0.28 | 0.42 | 0.36 |
| (mg/Kg) | (0.01) | (0.00) | (0.11) | (0.18) | (0.16) | (0.21) | (0.05) | (0.02) | (0.04) | (0.06) | (0.01) | (0.05) |

Numbers in parentheses indicate the standard error

*N* non-invaded areas, *I* invaded areas

** Indicate significant differences at 1 % level

**Table 5** Enzyme activities from the sampling points along the Iberian Peninsula

| | County | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pontevedra | | Bilbao | | Barcelona | | Alicante | | Huelva | | Lisboa | |
| | *N* | *I* | *N* | *I* | *N* | *I* | *N* | *I* | *N* | *I* | *N* | *I* |
| β-1,4-glucosidase | 2.98 | 2.83 | 0.92 | 0.83** | 1.11 | 0.84 | 10.02 | 8.83** | 1.49 | 1.19** | 3.67 | 3.91 |
| (EC 3.2.1.21) | (0.19) | (0.20) | (0.02) | (0.02) | (0.42) | (0.15) | (0.22) | (0.12) | (0.04) | (0.04) | (0.13) | (0.11) |
| Urease | 1.73 | 5.66** | 3.23 | 4.18 | 0.90 | 3.08** | 0.20 | 3.75** | 1.88 | 0.56** | 3.34 | 2.43** |
| (EC 3.5.1.5) | (0.27) | (0.32) | (0.44) | (0.57) | (0.19) | (0.33) | (0.12) | (0.98) | (0.22) | (0.21) | (0.20) | (0.14) |
| Phosphatase | 1.09 | 8.45** | 0.34 | 0.73** | 0.71 | 3.25** | 0.47 | 0.95** | 1.00 | 1.17** | 1.16 | 1.17 |
| (EC 3.1.3.1.) | (0.06) | (0.30) | (0.04) | (0.07) | (0.04) | (0.11) | (0.01) | (0.04) | (0.02) | (0.09) | (0.09) | (0.06) |

Numbers in parentheses indicate the standard error

*N* non-invaded areas, *I* invaded areas

** Indicate significant differences at 1 % level

($P \leq 0.05$) the germination of *C. edulis:* the enrichment of nutrients and pH 8, which respectively triplicate and duplicate it. The level of nutrients did not affect the germination of native species and *C. edulis* had the same rate of final germination at different levels of salinity and moisture.

*Malcolmia littorea* showed a reduction of 52 % in the AS index with an increase in nutrient content, and of 32 % at the lowest pH (Table 7). However, the speed of germination of this species was also increased (72.3 %) by a low salinity level (0.02 g NaCl/L) and the highest moisture. *S. atropurpurea* showed a

**Table 6** Concentration effect of essential nutrients, salinity concentration, pH and moisture levels on the *Gt* index

| Species | Nutrients | | Salinity (gNaCl/L) | | | | pH | | | | Moisture (mL/day) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *DW* | *HS* | 0 | 0.02 | 0.04 | 0.06 | 6 | 7 | 8 | 9 | 2 | 4 | 6 |
| *M. littorea* | 33.0 | 35.0 | $33.0^{b}$ | $77.5^{a}$ | $53.0^{ab}$ | $35.0^{b}$ | $33.0^{c}$ | $63.0^{ab}$ | $48.8^{b}$ | $68.3^{a}$ | $0.0^{c}$ | $33.0^{b}$ | $56.7^{a}$ |
| | (3.7) | (5.7) | (3.7) | (5.9) | (12.0) | (7.9) | (3.7) | (5.4) | (2.4) | (4.4) | (0.0) | (3.7) | (3.3) |
| *S. atropurpurea* | 69.0 | 64.0 | $69.0^{b}$ | $80.0^{a}$ | $65.0^{bc}$ | $50.0^{c}$ | 69.0 | 62.0 | 55.0 | 61.0 | $0.0^{c}$ | $69.0^{a}$ | $54.0^{b}$ |
| | (2.9) | (3.6) | (2.9) | (2.0) | (0.0) | (7.3) | (2.9) | (7.5) | (4.1) | (6.2) | (0.0) | (2.9) | (8.4) |
| *C. edulis* | $16.3^{b}$ | $46.7^{a}$ | 16.3 | 21.2 | 21.2 | 23.7 | $16.3^{b}$ | $10.0^{b}$ | $35.0^{a}$ | $16.3^{b}$ | 20.0 | 16.3 | 16.3 |
| | (3.1) | (1.6) | (3.1) | (4.2) | (2.3) | (5.1) | (3.1) | (5.0) | (3.5) | (1.25) | (5.4) | (3.1) | (4.2) |

*N* = 4. Different letters mean significant differences at 5 % level. Numbers in parentheses indicate the standard error

Common treatment: *DW*, 0 gNaCl/L, pH 6 and 4 mL/day

*DW* distilled water, *HS* Hogland solution

**Table 7** Concentration effect of essential nutrients, salinity concentration, pH and moisture levels on *AS* index

| Species | Nutrients | | Salinity (gNaCl/L) | | | | pH | | | | Moisture (mL/day) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *DW* | *HS* | 0 | 0.02 | 0.04 | 0.06 | 6 | 7 | 8 | 9 | 2 | 4 | 6 |
| *M. littorea* | $13.0^{a}$ | $6.3^{b}$ | $13.0^{b}$ | $22.4^{a}$ | $17^{ab}$ | $7.4^{b}$ | $13.0^{b}$ | $18.1^{a}$ | $17.5^{a}$ | $19.2^{a}$ | $0.0^{b}$ | $13.0^{a}$ | $16.4^{a}$ |
| | (1.1) | (1.2) | (1.1) | (1.3) | (3.6) | (1.4) | (1.1) | (2.3) | (2.0) | (0.7) | (0.0) | (1.1) | (7.5) |
| *S. atropurpurea* | 15.8 | 18.3 | 15.8 | 16.5 | 14.8 | 12.4 | 15.8 | 14.4 | 14.7 | 17.3 | $0.0^{c}$ | $15.8^{a}$ | $12.3^{b}$ |
| | (1.1) | (1.1) | (1.1) | (1.9) | (1.4) | (2.3) | (1.1) | (0.3) | (1.2) | (0.7) | (0.0) | (1.1) | (2.4) |
| *C. edulis* | $5.6^{b}$ | $13.9^{a}$ | 5.6 | 5.8 | 6.4 | 7.9 | $5.6^{b}$ | $4.5^{b}$ | $6.6^{b}$ | $9.1^{a}$ | 4.6 | 5.6 | 7.0 |
| | (1.9) | (2.2) | (1.9) | (1.5) | (1.0) | (0.7) | (1.9) | (1.8) | (1.2) | (0.5) | (1.9) | (1.9) | (1.5) |

*N* = 4. Different letters mean significant differences at 5 % level. Numbers in parentheses indicate the standard error

Common treatment: *DW*, 0 gNaCl/L, pH 6 and 4 mL/day

*DW* distilled water, *HS* Hogland solution

significant reduction ($P \leq 0.05$) in the *AS* value just with the highest moisture level. In accordance with the *Gt* results, *C. edulis* showed lower *AS* values (60 %) when the rate of nutrients in the irrigation solution decreased, but these values were superior at the highest pH.

## Seedling growth

Table 8 shows that the radicle length of the native *M. littorea* seedlings increased depending on the quantity of salt present in the irrigation solution (85.3, 14.7 and 47.1 % respectively at 0.02, 0.04 and 0.06 gNaCl/L). With a higher nutrient level in the irrigation solution, a decrease of 69.4 % occurred, and also an increase of almost 3 cm ($P \leq 0.05$) with pH 7 regarding the common treatment. The radicle length of *S. atropurpurea* was only affected by nutrients and moisture level, showing a decrease in radicle length (30.4 %) with the presence of nutrients in the irrigation solution and an increase in accordance with the rise of moisture level. Finally, the radicle of *C. edulis* seedlings showed stimulated growth with an increase in the acidity (40 %) and water regime ($P \leq 0.05$).

The growth of *M. littorea* shoots (Table 9) was influenced by salinity levels (2 times lower when salinity levels are high). The growth of *S. atropurpurea* shoots was stimulated by the nutrient level, with an increase of 25 %, and by the moisture level, with an increase of 33 % at medium irrigation rates. The growth of *C. edulis* shoots decreased at the highest pH regimes (50 %) and with the presence of salt in the irrigation solution (66.7 %).

**Table 8** Concentration effect of essential nutrients, salinity concentration, pH and moisture levels on radicle length (cm)

| Species | Nutrients | | Salinity (gNaCl/L) | | | | pH | | | | Moisture (mL/day) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *DW* | *HS* | 0 | 0.02 | 0.04 | 0.06 | 6 | 7 | 8 | 9 | 2 | 4 | 6 |
| *M. littorea* | $3.6^{a}$ | $1.1^{b}$ | $3.6^{b}$ | $6.3^{a}$ | $3.9^{a}$ | $5.0^{a}$ | $3.6^{c}$ | $6.2^{a}$ | $5.3^{b}$ | $5.2^{b}$ | 3.1 | 3.4 | 3.7 |
| | (0.8) | (0.4) | (0.8) | (0.3) | (0.2) | (0.7) | (0.8) | (0.3) | (0.2) | (0.3) | (0.4) | (0.2) | (0.3) |
| *S. atropurpurea* | $5.6^{a}$ | $3.9^{b}$ | 5.6 | 5.8 | 5.4 | 4.8 | 5.6 | 6.0 | 6.1 | 5.9 | $4.5^{b}$ | $5.6^{a}$ | $6.5^{a}$ |
| | (0.3) | (0.1) | (0.3) | (0.3) | (0.1) | (0.3) | (0.3) | (0.2) | (0.2) | (0.1) | (0.3) | (0.3) | (0.3) |
| *C. edulis* | 2.6 | 2.7 | 2.6 | 2.0 | 1.7 | 1.7 | $2.6^{a}$ | $3.0^{a}$ | $1.2^{b}$ | $1.2^{b}$ | $1.7^{b}$ | $2.6^{b}$ | $5.1^{a}$ |
| | (0.3) | (0.3) | (0.3) | (0.1) | (0.2) | (0.1) | (0.3) | (0.5) | (0.09) | (0.1) | (0.08) | (0.3) | (0.3) |

$N = 4$. Different letters mean significant differences at 5 % level. Numbers in parentheses indicate the standard error

Common treatment: *DW*, 0 gNaCl/L, pH 6 and 4 mL/day

*DW* distilled water, *HS* Hogland solution

**Table 9** Concentration effect of essential nutrients, salinity concentration, pH and moisture levels on shoot length (cm)

| Species | Nutrients | | Salinity (gNaCl/L) | | | | pH | | | | Moisture (mL/day) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *DW* | *HS* | 0 | 0.02 | 0.04 | 0.06 | 6 | 7 | 8 | 9 | 2 | 4 | 6 |
| *M. littorea* | 0.2 | 0.2 | $0.2^{a}$ | $0.2^{a}$ | $0.2^{a}$ | $0.1^{b}$ | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| | (0.01) | (0.01) | (0.01) | (0.01) | (0.00) | (0.02) | (0.01) | (0.01) | (0.01) | (0.01) | (0.02) | (0.01) | (0.01) |
| *S. atropurpurea* | $0.3^{b}$ | $0.4^{a}$ | 0.3 | 0.3 | 0.2 | 0.2 | 0.3 | 0.3 | 0.3 | 0.3 | $0.2^{b}$ | $0.3^{a}$ | $0.2^{b}$ |
| | (0.01) | (0.01) | (0.01) | (0.01) | (0.01) | (0.01) | (0.01) | (0.01) | (0.01) | (0.01) | (0.01) | (0.01) | (0.01) |
| *C. edulis* | 0.6 | 0.6 | $0.6^{a}$ | $0.2^{b}$ | $0.2^{b}$ | $0.2^{b}$ | $0.6^{a}$ | $0.2^{b}$ | $0.2^{b}$ | $0.1^{c}$ | 0.5 | 0.6 | 0.5 |
| | (0.04) | (0.04) | (0.04) | (0.01) | (0.00) | (0.00) | (0.04) | (0.00) | (0.00) | (0.02) | (0.03) | (0.04) | (0.04) |

$N = 4$. Different letters mean significant differences at 5 % level. Numbers in parentheses indicate the standard error

Common treatment: *DW*, 0 gNaCl/L, pH 6 and 4 mL/day

*DW* distilled water, *HS* Hogland solution

## Discussion

### Soil characteristics

It is known that when *C. edulis* invades coastal habitats, it modifies certain parameters of the soil such as organic matter content and pH (D'Antonio and Mahall 1991; Vilà et al. 2006; Conser and Connor 2009). There is also evidence that density of *C. edulis* flowers is correlated with different levels of *N* and soil pH, suggesting that soil plays a role in the reproductive plasticity of this species (Traveset et al. 2008).

*Carpobrotus edulis* was introduced and widely planted because of its ability to form a solid mat covering the soil, thereby stabilizing the dunes. The study by Lichter (1998) showed a reduction of the pH (8.5–4.3) after 400 years of dune stabilization. Thus, the decrease of pH during the invasion was expected (especially in Pontevedra, the oldest area invaded by *Carpobrotus* in Spain). However, there is an interesting result showing that the pH in Lisbon, where it is not so high (5.8) in native soils, is greater on invaded areas (6.6). The pH in the dunes of Pontevedra, Bilbao and Barcelona descended rapidly probably due to the production of organic acids by the *C. edulis* mat. The influence of organic matter and its cycling on soil pH change is still unclear. Thus, significant nitrification could be leading the decreased pH in Barcelona and Bilbao (Rukshana et al. 2012). The roots of plants during the absorption of nutrient ions release $H^{+}$ or $OH^{-}$ to maintain electrical neutrality on their surfaces (Maun 2009). Therefore, these can be acidic or basic sources. The magnitude of the pH derived from $H^{+}$ association/dissociation reactions depends on the type of organic acids (pKa values) and the initial soil pH (Xu et al. 2006). So in Lisbon, where native pH is 5.8, *Carpobrotus* raises it.

Dune soil salinity is due to salt spray (facilitated by the presence of wind and distance to shoreline), to flooding by seawater or to light rain (it washes the salt from the surface of the plants and transfers it to the ground, while heavy rainfall decreases the salt content in soil by washing) (Maun 2009). In Pontevedra, Bilbao, Barcelona, and Lisbon, salt accumulation in invaded areas was found to be significantly higher than in non-invaded areas. These salinity levels can indicate an accumulation of seaweed and marine debris (with salt accumulation) between the leaves of the invasive plant (personal observation). These results are also in agreement with previous studies on another invasive species of the family Aizoaceae (*Mesembryanthemum crystallinum* L.), which showed that this species retains salt in its leaf tissue and trichomes and releases it into the soil after leaf senescence (Vivrette and Muller 1977). The leaching of salt into the soil changes soil water potential levels, affecting water availability for neighbouring plants. The values of soil salinity obtained a range from 0.005 to 0.05 mgNaCl/g, except for the soils in Pontevedra, where values are higher. These differences are due to the distance from the sea and the presence/absence of strong winds to the coast in the sampling area.

In sandy soils, moisture is one of the most limiting factors for plant growth. Arenosols have excessive permeability (FAO-ISRAC-ISSS 1998). After rains, these soils drain much of the water, and evaporation and wind in the dune systems also cause them to lose substantial amounts of water (Maun 2009). Except for Lisbon, low levels of moisture in non-invaded areas were found. However, in the area invaded by *C. edulis*, values were higher. One of the reasons could be the attenuation of temperature and radiation levels due to the presence of *C. edulis*, and wind reduction on the invaded dune compared to the open dune (Lortie and Cushman 2007). Moreover, higher contents of soil organic matter (as in most of the areas invaded by *C. edulis*) can increase the amount of water retained by the substrate, as shown by Gooding (1947) (who found increases in the moisture content of soils from 0.2 to 2 % in the dunes of Barbados) and Kononova (1982). This also explains the moisture values in Lisbon, were the organic matter content and vegetal cover is approximately the same in invaded and in non-invaded areas.

Significantly higher organic content was found in soils invaded by *C. edulis* in comparison to soils with native vegetation in Pontevedra, Barcelona and Huelva, as observed by Conser and Connor (2009). The increase in organic matter associated with *C. edulis* is consistent with the considerable litter amount produced by this species. *C. edulis* produces new branches over old ones, forming an inferior layer of necromass and an outer layer of live matter. Nevertheless, there were no significant differences between invaded and non-invaded sites in terms of organic matter content in the fixed coastal dunes in Lisbon, which had a relatively high plant cover and abundant litter. The high levels of litter production by native species in these habitats are probably comparable to the levels of *C. edulis* litter.

Most of the necessary and available nutrients for the growth of dune plants are contained in seawater (Min 2006), except for nitrogen and phosphorus, which occur at such small concentrations that coastal dunes show low concentrations of these macronutrients (Süβ et al. 2008; Pye and Tsoar 2009). Phosphorus is the second most important nutrient in the coastal dunes and pH has a direct influence on its availability: at high pH, phosphorus released precipitate as salts. As the pH decreases, phosphorus availability increases to a maximum between pH 6.8 and 7.2 (Grootjans et al. 2008). The results of the analysis of the available phosphorus indicate a greater value in invaded soils rather than in the native soils. This can be explained by both the input of organic matter from *C. edulis*, and by the influence of pH on the availability of this nutrient. Thus, no significant differences were found in Lisbon.

Our results showed a greater amount of nitrate and ammonium in invaded soils. This can also be explained by the input of organic matter from *C. edulis.* However, the area of Lisbon, characterized by relatively high plant cover and abundant litter, shows lower values of nitrate and ammonium in invaded soils. In these habitats, the high levels of litter production by native species are comparable to the levels of *C. edulis* litter. In this case, *Carpobrotus* could be accumulating nitrates and ammonium, reducing its levels in the soil. Moreover, native soils in Lisbon present a higher volatilization of $NO_3$ due to the greater pH levels (Jones et al. 2007). A decrease in nitrite levels in invaded soils was also found. The pH levels of native soils could prevent the transformation of the nitrite in nitrate due to the basic pH of the soils (Bothe et al. 2006).

Overall, the most dramatic effect of *C. edulis* in the soil quality was found in the primary dunes of Punta Ron (Pontevedra) and Castelldefels (Barcelona). These results are consistent with the contents enunciated by Santoro et al. (2011), who stated that particular attention is to be paid in the fore dune zone (pioneer habitats), where the invasion of *Carpobrotus* is more likely to affect the parameters of the soil.

## Enzyme activities

β-1,4-Glucosidase (BG) is an enzyme that contributes to the degradation of cellulose and other β-1,4 glucans (Ljungdahl and Eriksson 1985). The principal function of BG is the hydrolysis of cellobiose to glucose, but it is active against other substrates as well. Therefore, BG is one of the most important enzymes involved in the mineralization of carbon. BG activity varied only with soil pH, presumably because cellulose and other β-1,4-glucan polymers dominate the organic matter inputs. In our soil samples, pH is higher than optimum ($pH = 5–6$), except for invaded soils in Lisbon. Significantly lower BG activity in invaded soils of Bilbao and Huelva was found in comparison with soils with native vegetation. These results suggest that although *C. edulis* generates plenty of litter, it is not easily mineralized. Ehrenfeld showed in 2004 that some invasive plants have notably slow rates of decomposition, possibly due to the high concentrations of secondary plant substances. These are in agreement with Van der Watt and Pretorius (2001), whom showed that *C. edulis* leaves have high contents of tannin and antibacterial compounds, which may reduce the rate of litter decomposition. In Pontevedra, no significant BG activity differences between invaded and non-invaded soil were detected, but this area presents the highest levels of BG activity in comparison with the other areas. This could be due because the enzymatic activities devoted to organic matter degradation are higher in sites temporally covered by seawater (Misic and Fabiano 2005), like the sand dunes in Pontevedra. These results suggest that sea action enlivens the degradation processes.

Urease catalyses the hydrolysis of urea to $NH_3$ and $CO_2$. The content of organic matter does not seem responsible for the increase in urease activity, but the type of organic matter (Pancholy and Rice 1972). Thus, our results showed that the litter produced by *C. edulis* increases the urease activity. However, in Huelva (Habitat 2270) and Lisbon (Habitat 2250), where different native vegetation can be found, the native vegetation litter seems to increase this activity (Xu et al. 2006).

Phosphatases are a large group of enzymes that catalyse the hydrolysis of esters and anhydrides of phosphoric acid (Speir and Ross 1978). The phosphatase activity is influenced by various soil properties, soil-microorganism interactions, vegetation cover, leachate inputs and the presence of inhibitors or activators (Stege et al. 2009). Our results suggest that *C. edulis* generally increases the phosphatase activity in almost all soil dune conditions.

Overall, and although they are important for soil–plant relationships, the changes observed in soil enzymatic activity are not very important for restoration, since after the removal of the invasive plant, soil characteristics and enzymatic rates follow a trajectory toward pre-invasion conditions (Novoa et al. 2013).

## Germination process

A sufficient amount of water of sufficient quality should be available to begin the germination process. The water softens the seed coat, so the root can emerge more easily, and also solubilises nutrients (Khurana and Singh 2004). A high salt content can block the germination process by the osmotic effect, drawing water from seeds (Bubel 1988). As Necajeva and Ievinsh (2008) had already reported, our results show a reduction in the germination of dune plants due to high concentrations of NaCl. The salinity of the substrate can act as a major selective force determining the seed germination, the establishment and the survival of seedlings in coastal areas (Necajeva and Ievinsh 2008). Dune species germinate in autumn, the rainy season, when there is a higher presence of water in the soil and the salt content decreases (Barbour et al. 1985; Pemadasa and Lovell 1975), although there may be a second germination period in spring (Balestri and Cinelli 2004). Our results showed that increasing moisture conditions and a lower level of salinity stimulate the germination process of *M. littorea* and *S. atropurpurea* (although higher salinity contents are detrimental for the germination process of these species).

In dune ecosystems, seedlings growing in the vicinity of other plants may benefit from them (Maun

2009 and Tielbörger and Prasse 2009), e.g. the effect of shade cast by adult plants onto the surface of the sand. This positive influence is caused by a significant decrease in the insolation and a lower evaporation in the shaded habitat (Maun 2009). *M. littorea* seems to reduce the timing and percentage of germination with an increase in nutrient levels and a decrease of pH. This response to the percentage of nutrients and pH rank, according to the level of ecological specialization of *M. littorea,* could have evolved to detect the presence of other plants in the surrounding area. In fact, the selection process has favoured the mechanisms of emergence that allow seeds to sense their neighbours (Tielbörger and Prasse 2009).

The seeds of *S. atropurpurea* have greater plasticity in order to adapt to different conditions. In fact, *M. littorea* is found exclusively on coastal dunes in the Mediterranean region, Italy and the Atlantic coast of the Iberian Peninsula, but *S. atropurpurea* is a species typical of pastures, road margins, fixed dunes and rocky terrain of the Iberian Peninsula, and throughout the Mediterranean region and Macronesia (Webb et al. 1988). In addition, *S. atropurpurea* is found as an invasive plant in Chile, Australia and the USA (National Herbarium of New South Wales 2010; U.S. Government 2010, Dalton et al. 2006). S. *atropurpurea* could therefore have a greater phenotypic plasticity. In fact, the germination process of *S. atropurpurea* seems to be influenced only by moisture and salinity levels.

The results of the germination process showed that *C. edulis*, like most invasive plants, has a high plasticity. The germination process of this invasive species is only influenced by the nutrient content of the irrigation solution and the pH, two factors that *C. edulis* modifies in its own favour. In accordance with Conser and Connor (2009), *C. edulis* modifies the conditions of the substrate when it invades coastal habitats in the Iberian Peninsula. This feature could have evolved as a mechanism to facilitate the germination process and the recolonization when the clones die.

### Seedling growth

There is usually an inverse linear relationship between dune seedling growth and increased salinity (Seneca 1972; Hesp 1991; Rodgers and Parker 2003). Although, as Seneca (1972) observed in some specialist species, *M. littorea* stimulates radicle growth with relatively low salinity levels. The seedling growth of S. *atropurpurea* and *C. edulis* did not show any response to salinity probably because of their plasticity.

The nutrient content of sand (especially nitrogen and phosphorus) positively or negatively affects the growth of plant dune species depending on the species (Maun 2009). In addition, high doses of ammonium negatively affect the length of the radicle (Maun 2009). Our results showed that the radicle length of *M. littorea* and *S. atropurpurea* decreases if the nutrient level increases. However, the shoot and leaf growth of *S. atropurpurea* and the leaf growth of *C. edulis* increase in line with the nutrient level. As Gagné and Houle (2002) noted, the amount of nutrients did not increase the survival rate of seedlings, although it can increase the growth of the survivors, especially when water is available in the substrate (Gagné and Houle 2002).

The pH level influences the growth of both native species in different ways. The pH level is one of the most important factors limiting the quantity of available soil nutrients: as its presence in the soil increases, the availability of microelements decreases, thereby causing a negative effect on seedling growth (Okay et al. 2011). High levels of pH decreased the shoot growth of *C. edulis* and the radical length of *M. littorea* and *C. edulis*. However, pH 6 is detrimental for the root growth of *M. littorea* (this pH is non-typical of coastal dune ecosystems, where *M. littorea* is a specialist species). The leaf and radicle growth of *C. edulis* and *S. atropurpurea* was influenced by the moisture content, while *M. littorea* did not show any response. As previously mentioned, *M. littorea* is found exclusively in coastal dunes, so this native species could be better adapted to low moisture levels in the early stages of growth.

Nevertheless, there are some aspects that need further consideration. Only germination tests in Petri dishes were used. But as there is not another option to test separately the effect of salinity, nutrients, or pH in the field, the extrapolation of the present results should only be done with caution.

## Conclusions

Residual effects on soils caused by invasive species are a key factor to be considered when planning restoration actions in invaded areas. Up to now, soils invaded by *C. edulis* were considered to have a lower pH and a higher percentage of organic matter and available nitrogen (D'Antonio and Mahall 1991; Vilà et al. 2006; de la Pe ña et al. 2010; Santoro et al. 2012). This study reports the fact that depending on the initial characteristics of the invaded ecosystems, the invasive plant will cause different responses in the physico-chemical and biological features of the soil that affect the establishment of native flora in diverse ways. This fact provides some insights into difficulties encountered by managers dealing with this species invasion. As it was reported before, in order to restore areas invaded by *C. edulis,* removal projects may not be sufficient due to the allelopathic effect of its litter (Novoa et al. 2012), to its negative effects on plant biodiversity (Novoa et al. 2013) and on both the bryophyte and the lichen cover (Cogoni et al. 2011; Zedda et al. 2013), and to the colonization of ruderal species under the new conditions created by the iceplant (Santoro et al. 2011) that persist after the removal of the invasive plant (Novoa et al. 2013). Moreover, our results show that the new conditions created by *C. edulis* depend on the ecosystem-type, which further constrains eradication efforts. These results should serve not only to enhance restoration strategies in areas invaded by *C. edulis*, but also to give more importance to soil residual effects when developing restoration strategies that deal with other invasive plant species.

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