Vieites-Blanco, C.^{1,2}, González-Prieto, S.J.^{1*} 2018. Effects of *Carpobrotus edulis* invasion on main litter and soil characteristics in backdune and rocky coastal habitats with oceanic climate. *Plant and Soil* 425, 363-374.

¹Instituto de Investigaciones Agrobiológicas de Galicia, IIAG-CSIC. Apartado 122. E-15780 Santiago de Compostela, Spain.

²Departamento de Biología Funcional, Universidade de Santiago de Compostela. R/ Lope Gómez de Marzoa, s/n, 15782 Santiago de Compostela, Spain

* author for correspondence: serafin@iiag.csic.es.

Abstract

Background and aims: *Carpobrotus edulis* invades coastal areas throughout the world, decreasing plant diversity and hampering restoration efforts by changing soil properties. Some of its effects on soils are known but there is a knowledge gap about the effects in rocky areas and micronutrients that we aimed to fill for dunes and rocky habitats with temperate-humid climate.

Methods: We compared invaded vs non-invaded paired plots in two backdune and two rocky areas by measuring 18 variables in litter and 24 in soils (0-5 and 5-10 cm layers).

Results: Invasion effects increased with the accumulated alien necromass, decreased with soil depth and are substrate-dependent: soil pH, Al, Fe and P increased in dunes, while these variables and Mg, Cu and Zn decreased in rocky sites. *Carpobrotus* necromass is richer in Mg and Ca and poorer in Al, Co, Cu, Fe, Ni and Zn than native necromass.

Conclusions: Invader effects on soils are largely mediated by its necromass, which has contrasting characteristics with the autochthonous necromass. *Carpobrotus edulis* ability to discriminate against Al-uptake, while favouring Mg and Ca uptake, and its lower requirement (or higher resorption) of key micronutrients (Co, Cu, Fe, Ni, Zn) than native vegetation could partly explain its invasiveness.

Keywords Alien plants · Macronutrients · Micronutrients · Nitrogen · Carbon · Ecosystem engineer

Introduction

Carpobrotus edulis (L.) N.E.Br. (Hottentot fig or common iceplant), a succulent *Aizoaceae* plant native from South Africa ([Campos et al., 2004](#)), and its hybrid *Carpobrotus aff. acinaciformis* (L.) L. Bolus have been introduced in many countries with ornamental purposes, as well as for dunes or slopes stabilization and for medical use ([Campos et al., 2004](#); [Malan and Notten, 2006](#); [Maltez-Mouro et al., 2010](#)). It is present in western USA ([D'Antonio et al., 1993](#)), Northern Africa, West temperate Asia, South Central Pacific, Australia, New Zealand, Southern America ([Delipetrou, 2006](#)), and Mediterranean Europe ([de la Peña et al., 2010](#)), the first record of *C. edulis* in Spain being in the NW coast, in Baiona in 1892 ([Lázaro-Ibiza, 1900](#)). *Carpobrotus edulis* colonizes dunes, cliffs and disturbed environments ([Campos et al., 2004](#); [D'Antonio et al., 1993](#); [Maltez-Mouro et al., 2010](#)), including burnt areas ([D'Antonio et al., 1993](#)). Nowadays both *Carpobrotus* species are widely considered highly invasive plants in coastal areas with Mediterranean and temperate humid climates throughout the world ([Global-Invasive-Species-Database, 2016](#); [Vilà et al., 2006](#)).

Although sometimes disregarded, the study of how invasive plants affect soil properties is a highly important research topic because soils are the base of all terrestrial ecosystems. This research is particularly needed for *C. edulis*, which has been considered an ecosystem engineer ([Conser and Connor, 2009](#); [Molinari et al., 2007](#)), as the changes it produces in abiotic properties [soil chemistry and water use ([Molinari et al., 2007](#))] impact other biota and benefit itself ([Conser and Connor, 2009](#)). *Carpobrotus edulis* has been proved to decrease germination, survival, reproduction and growth of native species ([Conser and Connor, 2009](#); [de la Peña et al., 2010](#); [Novoa and González, 2014](#)), and by that decrease plant ([Carta et al., 2004](#); [Jucker et al., 2013](#); [Vilà et al., 2006](#)) and community net productivity ([Maltez-Mouro et al., 2010](#)). Moreover, the structure of native plant communities in dunes changes towards the one associated to transition dunes ([Santoro et al., 2012](#)). Both the shift produced in soil communities after the invasion ([de la Peña et al., 2010](#)) and the allelopathic compounds released by *C. edulis* necromass ([Novoa et al., 2012](#)) can facilitate the invasion. Changes in soil remain after *C. edulis* removal, hindering restoration ([Novoa et al., 2013](#)).

and leading to secondary invasions by ruderal nitrophilous plants ([Novoa et al., 2013](#); [Santoro et al., 2012](#); [Santoro et al., 2011](#)).

The most studied soil properties in *C. edulis* invaded areas have been organic matter, available P, salinity and humidity, which increase; as well as pH and inorganic N, which were differently affected depending on the invaded area ([Conser and Connor, 2009](#); [Novoa et al., 2013](#); [Novoa et al., 2014](#); [Vilà et al., 2006](#)). Less studied have been other macronutrients, for which increases (Ca, Na) or no significant changes (Mg) have been observed ([Novoa et al., 2013](#)). As far as we know, the effect of *C. edulis* on micronutrients and trace element availability has not been studied until now, despite their importance for the soil-plant system derived from their role as essential nutrients or due to their toxicity above a given threshold ([Williams and Fraústo da Silva, 2000](#)). While Cr, I, Se, Si, V and W can also be essential for some organisms, the most widespread and important micro-nutrients, listed in a decreasing order of concentration in living organisms, are: Fe > Mn > Zn, B > Cu >> Co, Mo and Ni ([Grusak et al., 2016](#); [Hoppert, 2011](#); [Williams and Fraústo da Silva, 2000](#)). As constituents of metalloenzymes and other proteins, these eight elements are essential in metabolic processes as respiration, photosynthesis or N₂ fixation ([Grusak et al., 2016](#); [Hoppert, 2011](#); [Williams and Fraústo da Silva, 2000](#)), which play a key role even at the ecosystem level. On the other hand, plant and soil ¹³C and ¹⁵N isotopic signatures are a basic and powerful tool in environmental sciences as they provide valuable information on water, C and N cycles ([Dawson et al., 2002](#); [Robinson, 2001](#)). Although these analyses have been used in studies of other invasive species ([Li et al., 2017](#); [Msanne et al., 2017](#)), and despite being *C. edulis* a facultative CAM species with a ¹³C isotopic signature different from that of C-3 plants ([Herrera, 2009](#)), we have not found references on C and N isotopic signatures in *C. edulis* invaded areas.

Habitat characteristics seem to condition the impact of *C. edulis* over soil properties, since induced changes of the invasive plant on some soil characteristics vary between sites ([Molinari et al., 2007](#); [Novoa et al., 2014](#)), as it has also been reported for other invasive species ([Vilà et al., 2006](#)). Poorer soils, with low content in nutrients and organic matter, appear to be more sensitive and where soil properties will change more ([Novoa et al., 2014](#); [Santoro et al., 2011](#)), and most studies of *C. edulis* effects on soil properties focus on dunes. However, the other frequently invaded habitats, coastal cliffs and rocky areas, have been less studied.

The aim of the present study was, therefore, to

evaluate the effect of *C. edulis* invasion by comparing the necromass and topsoil (0-5 and 5-10 cm depth) characteristics in invaded and non-invaded dunes and rocky areas along the Atlantic coastline of NW Iberia. In soils, 24 variables were measured: WHC, humidity, pH, EC, NH₄⁺-N, NO₃⁻-N, δ¹³C, δ¹⁵N, organic C, organic N, and available Al, B, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P and Zn. In necromass, the total content of the latter 16 elements was measured as well as their amounts on a surface basis and also the δ¹³C and δ¹⁵N isotopic signatures.

Material and methods

Site and sampling description Among 23 sites with well established *C. edulis* populations (8 on dunes; 15 on rocks), two dune and two rocky representative study sites of the areas invaded by *C. edulis* were selected across the Atlantic coastline of NW Iberia (Table 1).

In September 2015, for each location, necromass and soil (0-5 and 5-10 cm depth) were separately sampled in 10 randomly distributed 15x15 cm squares under native vegetation and another 10 under *C. edulis*. Soil subsamples were mixed into a composite sample per site and depth and the same was done with the necromass. In the laboratory, necromass was dried at 55 °C, weighted, triturated and homogenized. The soils were sieved (< 2mm), homogenized and then divided into fresh subsamples, which were kept at 4 °C for inorganic N measurements, and air-dried subsamples for the other analyses. Sub-samples of dried necromass and soils were finely ground (< 100 μm) for chemical analysis in a planetary ball mill (Retsch PM100, Germany, with cups and balls of zirconium oxide)

Soils and necromass analysis Soil water-holding capacity (WHC) was measured in a Richards' membrane-plate extractor at a pressure of 10 kPa. Soil pH was measured in a 1:2.5 soil:solution ratio, both in water and 0.1 M KCl, with a pH-meter (Metröhm, Switzerland). Electrical conductivity (EC) was measured in soil extracts (1:5 soil:water ratio) with a conductometer (Metröhm, Switzerland). Soil humidity was determined by drying soil samples at 105 °C for 5 h.

Total C and total N of soils and necromass, as well as their ¹³C and ¹⁵N isotopic signatures, were measured in ground samples with an elemental analyser (Carlo Erba, Milano, Italy) coupled on-line with an isotopic ratio mass spectrometer (Finnigan Mat, delta C, Bremen, Germany). In the backdune soils, organic C and organic δ¹³C were also determined after CaCO₃ removal with the 'capsule method' using 20% HCl ([Brodie et al., 2011](#)). An elemental reference material (Soil 3 from Eurovector, Milano, Italy) and isotopic standards [IAEA-C-6 and

IAEA-CH-7 (for $\delta^{13}\text{C}$) or IAEA-N1 and IAEA-N2 (for $\delta^{15}\text{N}$), alternately, from the International Atomic Energy Agency, Vienna, Austria] were

included in each set of 10 samples to check the accuracy of the results; if necessary, drift correction was made against internal standards during the run.

Table 1. Main characteristics of the four study sites.

	Punta Nariga	Sálvora Island	Pragueira	Moledo
Country	Spain	Spain	Spain	Portugal
Province	A Coruña	A Coruña	Pontevedra	Viana do Castelo
UTM coordinates (29T grid zone)	0507346-4796386	0498894-4701427	0511201-4695464	0511011-4632650
Latitude	43°19'13" N	42°27'55" N	42°24'41" N	41°50'44" N
Longitude	8°54'34" W	9°0'49" W	8°51'50" W	8°52'3" W
Altitude	35-40 m asl	20-25 m asl	15-20 m asl	3-5 m asl
Distance to the sea	70-80 m	65-95 m	70-85 m	110-130 m
Parent material	Coarse grained two micas granitoid		Coastal dune	
Soil type (WRB, 2014)	Umbric Leptosol		Eutric Arenosol	
Dominant natural vegetation	<i>Ulex</i> sp., <i>Erica</i> sp. and <i>Armeria</i> sp.	<i>Armeria</i> sp.	<i>Cistus</i> sp.	Gramineae and <i>Cistus</i> sp.
EU Habitats Directive Site	Yes	Yes	No; in the border	Yes
Time from <i>C. edulis</i> invasion	15-20 years	80 years	6-8 years	> 15 years
Mean annual temperature	----- 14-16 °C -----			
Mean annual precipitation	----- 1400-1800 mm -----			

Inorganic N species were extracted with 2 M KCl (1:5 soil:solution ratio). The mixture was shaken for 1 h and filtered through glass microfiber filters (Whatman GF/A, Ø 125 mm). Ammonium and nitrate were sequentially liberated with two consecutive microdiffusions (55 °C, 72 h) from 50 mL aliquots placed in 500 mL glass jars, by adding respectively MgO (0.2 g) and MgO (0.2 g) plus Devarda's alloy (0.4 g). Both N forms were trapped as NH_3 into 10 mL of 0.004 M H_2SO_4 in a Teflon bottle suspended in the glass jar. Measurement was made by back titration of the H_2SO_4 excess with 0.004 M NaOH. Three blanks and three standards (NH_4NO_3) were included in each batch to subtract N from reagents and to check for N recovery.

Soil available nutrients and trace elements (Al, B, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P and Zn) were extracted with a mixture of 1 M NH_4Ac and 0.005 M DTPA (soil:solution ratio of 1:5). The mixture was shaken for 2 h, filtered through cellulose paper (Filter-Laboratory 1242, Ø 90 mm) and analysed with a simultaneous ICP-OES (Varian Vista Pro, Mulgrave, Australia). A calibration curve prepared with certified standards of all elements was measured beforehand and one of the calibration solutions was routinely included in each set of 30 samples as a quality control and, when necessary, the calibration curve was measured again. In order to measure the total nutrient and trace element content of necromass, aliquots of 500 mg were

digested in a high performance digestion unit (Milestone 1200 Mega, Sorisole, Italy) for 55 min with 8 mL of 65% HNO_3 and 25 mL of 30% H_2O_2 . Blanks and reference materials (hay powder No. 129, Community Bureau of References, EU; apple leaves No. 1515, National Institute of Standards and Technology, USA) were also included in each digestion batch to subtract elements from reagents and to check for element recovery. Once cooled, the solutions were filtered through quantitative filter paper (Filter-laboratory 1242, Ø 90-mm), transferred to 25 mL volumetric flasks, made to volume with water and analysed by ICP-OES as previously described for soils.

Samples analyses were always done in duplicate (in triplicate for inorganic N analysis), and the mean was used for the statistical analysis.

Statistical analysis Due to the highly contrasting characteristics of soils from backdune and rocky habitats, the differences in soil properties between invaded (X_{inv}) and non-invaded (X_{nat}) sites were standardized by the value of the non-invaded site (X_{nat}), as:

$$X_{\text{stand}} = \frac{X_{\text{inv}} - X_{\text{nat}}}{X_{\text{nat}}}$$

and significant differences between the standardized variable (X_{stand}) and 0 were tested with a one-

sample *t*-test. Data on necromass characteristics were analysed by the paired *t*-test. The normality assumption was tested in both cases with the Shapiro-Wilk test, and when not accepted, data were subjected to Cox-Box transformations or to the Tukey's ladder of powers. Statistically significant differences were established at $P < 0.05$. A Principal Component Analysis (PCA, based on the correlation matrix, to extract the factors, plus Varimax rotation with Kaiser normalization) was performed to assess the relationships among the studied variables, in soils and necromass separately, and whether samples are grouped together according to vegetation cover (native plants or *C. edulis*). The anti-image correlation matrix (comprising the negative values of the partial correlation coefficients) was analysed to detect and discard the variables less suitable for the factor analysis, aiming to improve the Kaiser-Meyer-Olkin measure of sampling adequacy and the Bartlett's test of sphericity. The statistical package IBM SPSS Statistics 23 was used in all analysis.

Results

Soil characteristics Substrate seemed to determine the effect of *C. edulis* on soil pH, measured either in water or KCl, which increased around 0.6 units in invaded dune soils and decreased more than 1.1 units in invaded rocky soils ($P < 0.05$, Fig. 1 and ESM Table 1). Compared to soils under native vegetation, the NO_3^- -N content decreased by 60-74% in all invaded soils ($P < 0.001$, Fig. 1 and ESM Table 1), whilst the other SOM related variables (organic C, total N and NH_4^+ -N) decreased by 34-39% in dune soils ($P < 0.01$) but not in soils over rocks, where NH_4^+ -N even increased ($P = 0.057$, Fig. 1 and ESM Table 1). Regarding nutrients and trace elements, *C. edulis* invasion led to a decrease in available Co (-55 to -95%, significant only in dune soils), as well as in Mg, Cu and Zn (-35 to -54%, significant only in soils over rocks) (Fig. 1 and ESM Table 1). Depending on the substrate, *C. edulis* invasion had significant contrasting effects on available B (+14% in invaded dunes; -15% in invaded rocks) and Fe (-45% in invaded dunes; +45% in invaded rocks). Besides, *C. edulis* invaded soils over rocks became depleted in Na (-12%) and enriched in Ni (+51%). We found no significant differences between *C. edulis* soils and native vegetation soils in WHC, humidity at sampling, EC, $\delta^{15}\text{N}$, $\delta^{13}\text{C}_{\text{organic}}$, C/N ratio, and available Al, Ca, K, Mn, Mo and P.

The best PCA with soil properties (KMO measure of sampling adequacy = 0.754; Bartlett's test of sphericity $P < 0.001$) included $\text{pH}_{\text{H}_2\text{O}}$, EC, WHC, total N, NH_4^+ -N, organic C and available Al, B, Fe, K, Mg,

Mo and P concentrations (ESM Table 2). This PCA extracted two factors that jointly explained 86% of the variance (76.3% by Factor 1 and 9.9% by Factor 2). Factor 1 was mostly determined by total N, organic C, WHC, and available B, K and Mg, with factor loadings higher than +0.71. The positive arm of Factor 2 was mostly determined by available Al, Fe, Mo and P with factor loadings higher than +0.76, and to a lesser extent by EC and NH_4^+ -N (factor loadings of +0.69 and +0.67, respectively), whilst $\text{pH}_{\text{H}_2\text{O}}$ (-0.837) was the only variable in the negative arm. The plane of these two factors discriminated the samples according to substrate, plant cover and depth (Fig. 2): a) soils from rocky areas and from dunes formed two differentiated groups in the graph, and wider differences between invaded and non-invaded soils were found for rocky areas; b) in rocky areas, invaded soils showed lower loadings on Factor 1 and higher loadings in Factor 2 than non-invaded ones; c) invaded dune soils showed also lower loadings on Factor 1 (except for the Moledo topsoil) but lower loadings in Factor 2 than non-invaded ones; and d) for each sampling site and vegetation, soils from 0-5 cm showed higher loading in Factor 1, except Pragueira soil, and bigger effects of *C. edulis* were found in the topsoil.

Several elements were significantly correlated with soil pH, either positively (available Ca) or negatively (WHC, EC, total C, total N, $\delta^{15}\text{N}$, NH_4^+ and available Al, Fe, K, Mo, Na, Ni and P) (Table 2).

Necromass characteristics After *C. edulis* invasion, an alien litter layer was deposited over the native litter layer leading to an average necromass accumulation under *C. edulis* in dunes and rocky areas two and four times higher respectively ($P < 0.05$, Fig. 1 and ESM Table 1) than under native vegetation. Both the amount of necromass and its C content showed a significant strong negative correlation with soil pH ($r = -0.739$ and -0.808 , respectively; $P < 0.01$).

In the necromass of the invaded areas, the concentration of Al ($P = 0.052$), Fe and Cu were significantly lower than in native necromass, while that of B, Ca and Na were higher (Fig. 3); levels of K were also higher in necromass from invaded rocky habitats, although lower in dunes (ESM Table 3).

When expressed in amount of nutrients per surface unit (ESM Table 4), compared to non-invaded areas, necromass under *C. edulis* accumulated significantly more B in backdunes, more Al and Na in rocky habitats and more Ca and Mn in both habitats, these differences being completely explained by the higher necromass amount. No significant differences were found for the other studied elements.

Fig. 1. Standardized value $[(I-A)/A] \pm$ standard error and significance of differences between the standardized variable and 0 (one-sample *t*-test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) for some of the most representative studied soil properties for dunes and rocky sites. Key: A, autochthonous vegetation; I, invasive vegetation.

The best PCA with necromass properties (KMO= 0.614; Bartlett's test of sphericity $P < 0.01$) included the Al, Co, Cu, Fe, Mg, Ni and Zn concentrations (ESM Table 5). This PCA extracted two factors that jointly explained 83% of the variance (66.3% and 16.9%, respectively, by Factor 1 and 2). Factor 1 was determined by all trace elements considered, with factor loadings higher than +0.70, whereas Factor 2 was determined by Mg and to a lesser extent by Co and Cu (factor loadings of +0.99, -0.42 and -0.42, respectively). The plane of these two factors

discriminate the samples according to both substrate and plant cover (Fig. 4): a) except native necromass in Nariga site, necromass over rocky substrate has positive loadings on Factor 2 while those over dunes have ever negative loadings; and b) in all cases, necromass from invaded sites had more negative loadings on Factor 1 and (except for Moledo site) more positive loadings on Factor 2 than native necromass.

Fig. 2. Score plots from the principal component analyses (PCA) performed with soil data for each habitat type and deepness. Key:

Moledo 0-5 cm layer (MS),
Moledo 5-10 cm layer (MD),
Nariga 0-5 cm layer (NS),
Nariga 5-10 cm layer (ND),
Pragueira 0-5 cm layer (PS),
Pragueira 5-10 cm layer (PD),
Sálvora 0-5 cm layer (SS),
Sálvora 5-10 cm layer (SD).

Discussion

Soil acidification, as we found in rocky habitats, is the most usually reported effect of *C. edulis* invasion in soil pH (Badalamenti et al., 2016; Conser and Connor, 2009; Molinari et al., 2007; Novoa et al., 2013; Novoa et al., 2014; Santoro et al., 2011; Vilà et al., 2006; Winsemius, 2013). Conversely, to our knowledge, an increase of soil pH as that we observed in invaded dunes has only been reported in a burnt area (Novoa et al., 2014).

The habitat dependent effect of *C. edulis* invasion on soil pH we found, as well as the relationship between acidification and accumulated necromass, agree with the results of Molinari et al. (2007). In invaded rocky locations, where pH decreases, the accumulated necromass largely quadruplicate that in invaded dunes, where pH increases, leading to strong negative correlations of soil pH with the necromass quantity and its C content. These relationships were likely due to the higher production of organic acids by *C. edulis* (Novoa et al., 2014; Santoro et al., 2011).

Soil pH increase in our studied invaded dune environments could be related to the deposition of Ca from sea spray on plants (Schlesinger and Hasey, 1980). However, taking into account the

distances to the sea and the altitudes above sea level (Table 1), differences in Ca deposition from sea spray between our dune and rocky sites are not expectable. A more likely explanation for the pH increase in invaded dune soils could be a higher Ca uptake from deeper soil layers by *C. edulis* than by the native vegetation and, therefore, greater necromass-Ca inputs to the invaded dune topsoils. In fact, Ca content of *C. edulis* necromass in dunes was 4.5 times higher compared to native necromass in dunes and 2 times higher than invaded rocky areas. Moreover, a positive correlation was found in soils between available Ca increase and pH. These results agreed with the correlation between the content of basic cations in residues additions and the increase in soil pH (Pocknee and Sumner, 1997; Tang and Yu, 1999). As dune soils tend to acidify over the years (Anwar-Maun, 2009), *C. edulis* invasion seemed to have an opposing effect in pH than natural succession. Conversely, in rocky sites natural acidification seemed to be accelerated in *C. edulis* invaded soils, as also was found in other studies for dunes (Conser and Connor, 2009). Changes in pH due to *C. edulis* invasion could therefore lead to long-term effects at an ecosystem level, acting by this as an ecosystem engineer (Levin and Crooks, 2011).

Fig. 3. Mean value $\pm$ standard error for some of the most representative studied necromass properties under autochthonous (A) and invasive (I) vegetation. Different letters (a, b) indicate significant differences (paired *t*-test; $P < 0.05$) within type of vegetation. For Al, the significance ($P = 0.052$) was close to the considered threshold.

Table 2. Pearson Correlation values and significance (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; $n = 16$) between soil pH and accumulated necromass and the analyzed soil properties.

	pH _{H2O}	pH _{KCl}	Necromass
Necromass	-0.739**	-0.613*	1.000
WHC	-0.771**	-0.770**	0.421
Humidity	-0.073	-0.088	-0.238
EC	-0.902***	-0.819***	0.710**
Total N	-0.829***	-0.759***	0.581*
$\delta^{15}\text{N}$	-0.607*	-0.565*	0.369
$\text{NH}_4^+\text{-N}$	-0.687**	-0.531*	0.722**
$\text{NO}_3^-\text{-N}$	-0.234	-0.213	-0.356
Organic C	-0.808***	-0.766**	0.528*
$\delta^{13}\text{C}$	-0.128	-0.148	0.031
Available Al	-0.862***	-0.750***	0.756***
Available B	-0.372	-0.225	0.370
Available Ca	0.852***	0.932***	-0.492
Available Co	0.163	0.145	-0.456
Available Cu	-0.147	-0.127	-0.339
Available Fe	-0.832***	-0.647**	0.702**
Available K	-0.731**	-0.673**	0.536*
Available Mg	-0.488	-0.440	0.081
Available Mn	0.053	-0.039	-0.418
Available Mo	-0.834***	-0.706**	0.669**
Available Na	-0.668**	-0.603*	0.456
Available Ni	-0.505*	-0.505*	0.131
Available P	-0.696**	-0.456	0.582*
Available Zn	-0.063	-0.100	-0.411

Fig. 4. Score plots from the principal component analyses (PCA) performed with necromass data for each habitat type and deepness. Key: Moledo (M), Nariga (N), Pragueira (P) and Sálvora (S) sites.

Most studies showed an increase in organic matter content in soils invaded by *C. edulis* (Novoa et al., 2013; Novoa et al., 2014; Santoro et al., 2011; Vilà et al., 2006). However, in dunes, despite an increase in necromass amount, organic C and N significantly decreased in invaded soil, what could be related to a reduction in the activity of microbial decomposer due to the higher content in antibacterial compounds (van der Watt and Pretorius, 2001). As high C/N ratios also reduce OM mineralization (Packham et al., 2001), another explanation of our result could be the higher C/N of *C. edulis* necromass than that of native necromass (61 vs. 38), also found by Badalamenti et al. (2016). Unfortunately, neither the ^{13}C nor the ^{15}N isotopic signatures in our soils and plants provide useful information to clarify the C and N dynamics in the invaded soil-plant system. The lack of significant differences in the soil and plant ^{13}C natural abundances of the invaded plots was likely due to the low contribution of CAM-fixed CO_2 to total C fixation in *C. edulis* (around 3%; (Herrera, 2009)); in the case of ^{15}N isotopic signatures, the lack of significant differences was probably caused by the high data variability. The decrease in dunes of both soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ seems to derive from the

decrease in organic matter in invaded soils. In other studies, the effect of *C. edulis* on soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ largely varied within sites (Novoa et al., 2013; Novoa et al., 2014), and the same goes with $\text{NH}_4^+\text{-N}$ when dunes and rocky areas were compared. The decrease in soil total N and organic C produced by the invasion of dunes contrasts with the accumulation of this nutrient that is produced by natural succession in dunes (Anwar-Maun, 2009; Jones et al., 2008) and is likely due to the accumulation of undecomposed alien necromass over the soil surface we found.

While ideal soil pH for nitrification is around 7.5-8.0 (Prosser, 1990), optimum pH for indigenous nitrifying communities has been suggested to be near soil pH (Bramley and White, 1990). Consequently, the decrease in $\text{NO}_3^-\text{-N}$ in invaded rocky soils could be due to the decrease in nitrifying activity because of soil acidification triggered by *C. edulis*, leading to an accumulation of $\text{NH}_4^+\text{-N}$ in invaded soils ($P = 0.057$).

The effect of *C. edulis* in soil pH has been considered the cause of variations in the soil availability of several nutrients, including Ca decrease (Conser and Connor, 2009; Molinari et al., 2007) and N decrease (Conser and Connor, 2009).

However, as far as we know, no studies have been made on its effect on micronutrients availability despite their dependency on pH ([Fageria et al., 2002](#)). As a rule, the availability of B, Cu, Fe, Mn and Zn usually decreases, and Mo increases as soil pH increases; Ni availability is usually higher in acidic than in alkaline soils and Co availability decrease with extreme soil pH ([Fageria et al., 2002](#)).

Some of the studied elements that were found to be highly correlated with soil pH showed opposed variations in dunes and rocky coasts, likely as a result of the habitat-dependent effect of *C. edulis* in soil pH. Thereby, soil Fe availability, which was negatively correlated with pH, significantly decreased in invaded dune soils and increased in the invaded rocky environments. Other elements which were also negatively correlated ($P < 0.005$) with soil pH – available Al, K, Ni and P – showed similar trends in invaded areas (decrease in dunes and increase in rocks), although the changes were not statistically significant. The correlation of soil available K with pH is reflected in the K content of necromass from invaded areas that is lower in dunes and higher in rocky sites. This was corroborated by the soil PCA, where invaded soils in dunes and rocky sites showed contrasting tendencies along Factor 2 (with high positive loadings of Al, Fe, and P) compared to non-invaded soils. The effect of *C. edulis* on soil available B does not correspond to the expected effect of pH, but care must be taken when interpreting these results because B levels were very low in all cases and close to the detection limits.

In dunes, soil pH under natural vegetation (mean value 7.15) falls within the optimum range for nutrient availability [6.5-7.5; ([Jensen, 2010](#))] and in *C. edulis* invaded areas (mean value 7.74) it remains close to this range. This small change seemed enough to significantly decrease Co availability by more than one order of magnitude, but had no significant effects on Cu, Mg and Zn availability which also tended to decrease. The strong decrease of Co availability [very sensitive to high pH; ([Macías-Vázquez and Calvo-de-Anta, 2009](#))], could lead to a deficiency of this essential trace element with a broad range of biological functions ([Kamnev et al., 2002](#)), including key steps in protein synthesis ([Williams and Fraústo da Silva, 2000](#)), N_2 fixation by *Azotobacter* and nitrate reductase activity, crucial for microbial NO_3^- -N assimilation ([Nicholas et al., 1962](#)). Conversely, in rocky areas, where soil pH (6.14) under natural vegetation was already below the optimum range, the acidification triggered by *C. edulis* invasion (down to pH 4.97) was strong enough to significantly decrease Cu, Mg and Zn availability. If these elements become limiting to living organisms (necromass-Cu already decreased in invaded areas), their metabolic processes could be

seriously affected as these nutrients play crucial roles in protein synthesis and stabilization (Mg, Zn), N metabolism (Zn), as well as redox reactions and energy transfer (Cu, Zn) ([Williams and Fraústo da Silva, 2000](#)).

As a result of its opposite effect on the soil pH in dunes and rocky sites, *C. edulis* induces different changes in nutrients availability in both type of habitats. Consequently, *C. edulis* affects in different ways native plant species depending on the habitat, and even alters natural succession patterns differently. The invaded site initial characteristics can, therefore, condition the invasion effects on soil properties. Further studies on the relation between ecosystem characteristics and the effect of *C. edulis* in soils would be useful to foresee the invasion effects on soil properties and consequently being able to adapt restoration methods to the different environments.

In opposition to what [Novoa et al. \(2013\)](#) and [Molinari et al. \(2007\)](#) found in invaded soils, but coinciding with [Winsemius \(2013\)](#) results, available Na significantly decreases in soils of rocky sites, despite its increase in necromass. Sodium inputs in soils of humid regions are mainly from marine sources ([Schlesinger and Hasey, 1980](#)). Salt accumulation in *C. edulis* tissue could be a salt-tolerance adaptation of this species, similar to that found in other species of the same Family as *Mesembryanthemum crystallinum* L. and *Carpobrotus chilensis* (Molina) N.E. Br. ([Agarie et al., 2007](#); [Delnavaz Hashemloian et al., 2010](#); [Weber and D'Antonio, 1999](#)).

The best PCA with soil properties showed a bigger effect of *C. edulis* in the more nutrient rich rocky areas than in backdunes, contrarily to other studies which found larger effects on the poorer foredune than in backdune soils ([Novoa et al., 2014](#); [Santoro et al., 2011](#)). The much higher accumulation of necromass in the studied rocky areas, which could be derived from the higher mean duration of the invasion in the studied rocky sites, could explain that these soils are more affected by *C. edulis*. This PCA also indicated that the most affected soil layer was the uppermost (0-5 cm), presumably through the effect of necromass; being the only significant effect on the 5-10 cm soil layer the decrease in NO_3^- -N (probably because of its high mobility with water).

Irrespectively of the changes induced by *C. edulis* in soil pH (acidification in rocks, alcalinization in dunes), the PCA that better differentiates native and invader necromass showed that *C. edulis* tends to have lower concentration of most trace elements in its tissues (Al, Mo, Ni, Fe and Cu; differences significant for the two latter), but higher

concentration of Mg (except for Moledo site). Besides, compared to native necromass, the results indicated that *C. edulis* necromass has significantly more Ca and B. Moreover, *C. edulis* also tends to have less P and more Mn. Taking into account the strong soil acidification triggered by *C. edulis* in the rocky sites, its surprising ability to keep low the tissues levels of Al (potentially deleterious) while being able to uptake more key macronutrients (Mg, Ca) than native vegetation may be a decisive competitive advantage. Our results also showed a lower necromass content on P and some micronutrients that are key for plant metabolism and growth as Co, Cu, Fe, Ni and Zn; which could be due to a lower requirement of these nutrients by *C. edulis* or, as indicated for other species (Brant and Chen, 2015), to a higher resorption of them from senescing tissues induced by their lower availability in soils. Both explanations suggest a competitive advantage of *C. edulis* over native vegetation in nutrient poor soils, although more research on this topic is needed.

Conclusions

Our results showed that the effect of *C. edulis* on soil properties was higher in rocky sites (where more alien necromass was found) and in the uppermost soil layer studied, suggesting that the invasion mostly affects soil characteristics through necromass production. This fact, together with the strong relationship between alien necromass accretion and soil pH, suggest that the amount of necromass accumulated is one of the main drivers of both the intensity and the type of the effects of *C. edulis* over soil properties. Thus, restoration of invaded ecosystems with *C. edulis* should be preferentially done in the first stages of the invasion, when soil effect would be lighter, and should include the elimination of necromass to avoid legacy effects on soil.

The differences in necromass characteristics from invaded and non-invaded areas could be reflecting some competitive advantages related to the physiology of this invasive plant, such as a restriction of Al uptake, higher macronutrient uptake and less requirements (or higher resorption from senescing tissues) of micronutrients. Therefore, to better understand the invasive potential of *C. edulis*, further research on these topics would be useful.

Acknowledgements We thank Dr. Margarita Lema for field assistance and Leticia Bravo and Jorge Benítez for their technical assistance in the laboratory. The participation of Cristina Vieites-Blanco was supported by a pre-doctoral fellowship by Xunta de Galicia. The isotopic ratio mass

spectrometer was partly financed by the European Regional Development Fund (EU).

References

- Agarie S, Shimoda T, Shimizu Y, Baumann K, Sunagawa H, Kondo A, et al. Salt tolerance, salt accumulation, and ionic homeostasis in an epidermal bladder-cell-less mutant of the common ice plant *Mesembryanthemum crystallinum*. *Journal of Experimental Botany* 2007; 58: 1957-1967.
- Anwar-Maun M. The sand dune environment. In: Anwar-Maun M, editor. *The Biology of Coastal Sand dunes*. OUP Oxford, Wiltshire, UK, 2009, pp. 23-39.
- Badalamenti E, Gristina L, Laudicina VA, Novara A, Pasta S, La Mantia T. The impact of *Carpobrotus* cfr. *acinaciformis* (L.) L. Bolus on soil nutrients, microbial communities structure and native plant communities in Mediterranean ecosystems. *Plant and Soil* 2016; 409: 19-34.
- Bramley RGV, White RE. The variability of nitrifying activity in field soils. *Plant and Soil* 1990; 126: 203-208.
- Brant AN, Chen HYH. Patterns and Mechanisms of Nutrient Resorption in Plants. *Critical Reviews in Plant Sciences* 2015; 34: 471-486.
- Brodie CR, Casford JSL, Lloyd JM, Leng MJ, Heaton THE, Kendrick CP, et al. Evidence for bias in C/N, delta C-13 and delta N-15 values of bulk organic matter, and on environmental interpretation, from a lake sedimentary sequence by pre-analysis acid treatment methods. *Quaternary Science Reviews* 2011; 30: 3076-3087.
- Campos JA, Herrera M, Biurrun I, Loidi J. The role of alien plants in the natural coastal vegetation in central-northern Spain. *Biodiversity & Conservation* 2004; 13: 2275-2293.
- Carta L, Manca M, Brundu G. Removal of *Carpobrotus acinaciformis* (L.) L. Bolus from environmental sensitive areas in Sardinia, Italy In: Arianoutsou, Papanastasis, editors. *Proceedings 10th MEDECOS Conference*. Millpress, Rhodes, Greece, 2004, pp. 1-4.
- Conser C, Connor EF. Assessing the residual effects of *Carpobrotus edulis* invasion, implications for restoration. *Biological Invasions* 2009; 11: 349-358.
- D'Antonio CM, Odion DC, Tyler CM. Invasion of Maritime Chaparral by the Introduced Succulent *Carpobrotus edulis* the Roles of Fire and Herbivory. *Oecologia* (Berlin) 1993; 95.
- Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP. Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics* 2002; 33: 507-559.
- de la Peña E, de Clercq N, Bonte D, Roiloa S, Rodríguez-Echeverría S, Freitas H. Plant-soil feedback as a mechanism of invasion by *Carpobrotus edulis*. *Biological Invasions* 2010; 12: 3637-3648.
- Delipetrou P. *Carpobrotus edulis*. Delivering Alien Invasive Species Inventories for Europe (DAISIE), 2006.
- Delnavaz Hashemloian B, Ataei Azimi A, Nasiri Semnani S. The determination of salt tolerance and storage degree of sea fig (*Carpobrotus chilensis*) in liquid culture. *The Quarterly Journal of Animal Physiology and Development* 2010; 3: 61-69.

- Fageria NK, Baligar VC, Clark RB. Micronutrients in Crop Production. In: Donald LS, editor. *Advances in Agronomy*. Volume 77. Academic Press, 2002, pp. 185-268.
- Global-Invasive-Species-Database. Species profile: *Carpobrotus edulis*, 2016.
- Grusak MA, Broadley MR, Philip J. Plant macro- and micronutrient minerals. eLS. John Wiley & Sons Ltd, Chichester, 2016.
- Herrera A. Crassulacean acid metabolism and fitness under water deficit stress: if not for carbon gain, what is facultative CAM good for? *Annals of Botany* 2009; 103: 645-653.
- Hoppert M. Metalloenzymes. In: Reitner J, Thiel V, editors. *Encyclopedia of Geobiology*. Springer, Heidelberg, 2011, pp. 558-563.
- Jensen TL. Soil pH and the Availability of Plant Nutrients. *Plant Nutrition Today*. IPNI, 2010.
- Jones MLM, Sowerby A, Williams DL, Jones RE. Factors controlling soil development in sand dunes: evidence from a coastal dune soil chronosequence. *Plant and Soil* 2008; 307: 219-234.
- Jucker T, Carboni M, Acosta ATR. Going beyond taxonomic diversity: deconstructing biodiversity patterns reveals the true cost of iceplant invasion. *Diversity and Distributions* 2013; 19: 1566-1577.
- Kamnev AA, Antonyuk LP, Smirnova VE, Serebrennikova OB, Kulikov LA, Perfiliev YD. Trace cobalt speciation in bacteria and at enzymic active sites using emission Mossbauer spectroscopy. *Analytical and Bioanalytical Chemistry* 2002; 372: 431-435.
- Lázaro-Ibiza B. Contribuciones a la flora de la Península Ibérica. Notas críticas acerca de la flora española. *Anales de la Sociedad Española de Historia Natural* 1900; 29: 125-176.
- Levin LA, Crooks JA. Functional Consequences of Invasive Species in Coastal and Estuarine Systems. In: Middelburg JJ, Philippart CJM, editors. *Treatise on Estuarine and Coastal Science*. . 7. Academic Press, 2011, pp. 17-51.
- Li H, Wei Z, Huangfu C, Chen X, Yang D. Litter mixture dominated by leaf litter of the invasive species, *Flaveria bidentis*, accelerates decomposition and favors nitrogen release. *Journal of Plant Research* 2017; 130: 167-180.
- Macías-Vázquez F, Calvo-de-Anta R. Niveles genéricos de referencia de metales pesados y otros elementos traza en suelos de Galicia: Xunta de Galicia, 2009.
- Malan C, Notten A. *Carpobrotus edulis*(L.) L.Bolus, 2006.
- Maltez-Mouro S, Maestre FT, Freitas H. Weak effects of the exotic invasive *Carpobrotus edulis* on the structure and composition of Portuguese sand-dune communities. *Biological Invasions* 2010; 12: 2117-2130.
- Molinari N, D'Antonio C, Thomson G. 7 - *Carpobrotus* as a Case Study of the Complexities of Species Impacts. In: Cuddington K, Byers JE, Wilson WG, Hastings A, editors. *Theoretical Ecology Series*. 4. Academic Press, 2007, pp. 139-162.
- Msanne J, Awada T, Bryan NM, Schacht W, Drijber R, Li Y, et al. Ecophysiological responses of native invasive woody *Juniperus virginiana* L. to resource availability and stand characteristics in the semiarid grasslands of the Nebraska Sandhills. *Photosynthetica* 2017; 55: 219-230.
- Nicholas JD, Wilson PW, Kobayashi M. Cobalt requirement for inorganic nitrogen metabolism in microorganisms. *Proceedings of the National Academy of Sciences of the United States of America* 1962; 48: 1537-1542.
- Novoa A, González L. Impacts of *Carpobrotus edulis* (L.) N.E.Br. on the Germination, Establishment and Survival of Native Plants: A Clue for Assessing Its Competitive Strength. *PLOS ONE* 2014; 9: e107557.
- Novoa A, González L, Moravcová L, Pyšek P. Effects of Soil Characteristics, Allelopathy and Frugivory on Establishment of the Invasive Plant *Carpobrotus edulis* and a Co-Occurring Native, *Malcolmia littorea*. *PLOS ONE* 2012; 7: e53166.
- Novoa A, González L, Moravcová L, Pyšek P. Constraints to native plant species establishment in coastal dune communities invaded by *Carpobrotus edulis*: Implications for restoration. *Biological Conservation* 2013; 164: 1-9.
- Novoa A, Rodríguez R, Richardson D, González L. Soil quality: a key factor in understanding plant invasion? The case of *Carpobrotus edulis* (L.) N.E.Br. *Biological Invasions* 2014; 16: 429-443.
- Packham JR, Harding DJL, Hilton GM, Stuttard RA. Decomposition and renewal. In: Packham JR, Harding DJL, Hilton GM, Stuttard RA, editors. *Functional Ecology of Woodlands and Forests*. Kluwer Academic Publishers, Dordrecht, The Netherlands, 2001, pp. 245-268.
- Pocknee S, Sumner ME. Cation and nitrogen contents of organic matter determine its soil liming potential. *Soil Science Society of America Journal* 1997; 61: 86-92.
- Prosser JI. Autotrophic Nitrification in Bacteria. In: Rose AH, Tempest DW, editors. *Advances in Microbial Physiology*. Volume 30. Academic Press, 1990, pp. 125-181.
- Robinson D. d ¹⁵N as an integrator of the nitrogen cycle. *Trends in Ecology & Evolution* 2001; 16: 153-162.
- Santoro R, Jucker T, Carboni M, Acosta ATR. Patterns of plant community assembly in invaded and non-invaded communities along a natural environmental gradient. *Journal of Vegetation Science* 2012; 23: 483-494.
- Santoro R, Jucker T, Carranza M, ACosta A. Assessing the effects of *Carpobrotus* invasion on coastal dune soils. Does the nature of the invaded habitat matter? *Community Ecology* 2011; 12: 234-240.
- Schlesinger WH, Hasey MM. The Nutrient Content of Precipitation, Dry Fallout, and Intercepted Aerosols in the Chaparral of Southern California. *The American Midland Naturalist* 1980; 103: 114-122.
- Tang C, Yu Q. Impact of chemical composition of legume residues and initial soil pH on pH change of a soil after residue incorporation. *Plant and Soil* 1999; 215: 29-38.
- van der Watt E, Pretorius JC. Purification and identification of active antibacterial components in *Carpobrotus edulis* L. *Journal of Ethnopharmacology* 2001; 76: 87-91.
- Vilá M, Tessier M, Suehs CM, Brundu G, Carta L, Galanidis A, et al. Local and regional assessments of the impacts of plant invaders on vegetation structure

- and soil properties of Mediterranean islands. *Journal of Biogeography* 2006; 33: 853-861.
- Weber E, D'Antonio CM. Germination and growth responses of hybridizing *Carpobrotus* species (Aizoaceae) from coastal California to soil salinity. *American Journal of Botany* 1999; 86: 1257-1263.
- Williams RJP, Fraústo da Silva JJR. The distribution of elements in cells. *Coordination Chemistry Reviews* 2000; 200-202: 247-348.
- Winsemius SD. Plant-Soil Interactions and Implications for Restoration of Coastal Sand Dunes in Point Reyes National Seashore. *Coastal Dune Restoration 2013*: 21.

ESM Table 1a Raw and standardized [(I-A)/A] values (mean $\pm$ s.e), *t*-test value and significance (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) of the statistical analysis for the studied soil properties under autochthonous (A) and invasive (I) vegetation, globally (d.f.=7) and separately for dunes and rocky sites (d.f.= 3). Data are given in the following units: accumulated necromass, kg m⁻²; water holding capacity (WHC), g H₂O kg⁻¹ dw; humidity, %; electrical conductivity (EC), μ S cm⁻¹; organic C and N, g kg⁻¹ dw; C/N ratio, g g⁻¹; $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, ‰; and other elements, mg kg⁻¹ dw.

	Globally				Dune				Rock			
	A	I	(I-A)/A	<i>t</i> -value	A	I	(I-A)/A	<i>t</i> -value	A	I	(I-A)/A	<i>t</i> -value
Necromass	1.38 $\pm$ 0.25	4.52 $\pm$ 1.12	2.38 $\pm$ 0.46	3.39*	0.864 $\pm$ 0.322	1.66 $\pm$ 0.13	1.88 $\pm$ 0.86	2.20	1.9 $\pm$ 0.0	7.37 $\pm$ 0.61	2.88 $\pm$ 0.29	9.81**
WHC	306 $\pm$ 70	287 $\pm$ 65	-0.09 $\pm$ 0.06	1.15	148 $\pm$ 52	155 $\pm$ 69	-0.08 $\pm$ 0.13	0.66	465 $\pm$ 60	420 $\pm$ 59	-0.10 $\pm$ 0.03	2.85
Humidity	13.5 $\pm$ 3.0	13.5 $\pm$ 3.6	-0.12 $\pm$ 0.10	1.23	15.3 $\pm$ 5.1	16.9 $\pm$ 6.09	-0.27 $\pm$ 0.15	0.00	11.8 $\pm$ 3.72	10.1 $\pm$ 3.7	0.03 $\pm$ 0.10	2.40
pH _{H2O}	6.65 $\pm$ 0.28	6.36 $\pm$ 0.56	-0.05 $\pm$ 0.05	1.00	7.16 $\pm$ 0.40	7.74 $\pm$ 0.35	0.08 $\pm$ 0.01	6.56**	6.14 $\pm$ 0.21	4.98 $\pm$ 0.27	-0.19 $\pm$ 0.02	7.72**
pH _{KCl}	4.73 $\pm$ 0.41	4.47 $\pm$ 0.72	-0.09 $\pm$ 0.08	1.13	5.50 $\pm$ 0.60	6.10 $\pm$ 0.74	0.11 $\pm$ 0.03	3.12	3.96 $\pm$ 0.15	2.83 $\pm$ 0.25	-0.29 $\pm$ 0.06	5.08*
EC	114 $\pm$ 29	145 $\pm$ 47	0.20 $\pm$ 0.20	1.01	54.5 $\pm$ 27.0	28.6 $\pm$ 10.6	-0.15 $\pm$ 0.25	0.60	174 $\pm$ 30	261 $\pm$ 37	0.55 $\pm$ 0.20	2.70
Total N	3.53 $\pm$ 0.96	3.45 $\pm$ 1.10	-0.13 $\pm$ 0.10	1.30	1.47 $\pm$ 0.64	0.915 $\pm$ 0.434	-0.37 $\pm$ 0.05	6.92**	5.60 $\pm$ 1.04	5.99 $\pm$ 1.07	0.10 $\pm$ 0.11	0.90
$\delta^{15}\text{N}$	3.64 $\pm$ 1.01	3.95 $\pm$ 0.93	1.39 $\pm$ 1.34	1.04	1.76 $\pm$ 1.25	2.46 $\pm$ 1.38	2.79 $\pm$ 2.65	1.05	5.52 $\pm$ 0.93	5.44 $\pm$ 0.81	0.00 $\pm$ 0.06	0.01
NH ₄ ⁺ -N	2.45 $\pm$ 0.64	5.41 $\pm$ 2.68	0.66 $\pm$ 0.47	1.40	1.28 $\pm$ 0.18	0.776 $\pm$ 0.090	-0.38 $\pm$ 0.07	5.55*	3.62 $\pm$ 0.98	10.0 $\pm$ 4.4	1.69 $\pm$ 0.56	3.01
NO ₃ ⁻ -N	13.4 $\pm$ 3.3	4.16 $\pm$ 0.90	-0.65 $\pm$ 0.03	20.30***	10.8 $\pm$ 4.8	4.23 $\pm$ 1.79	-0.60 $\pm$ 0.01	64.21***	16.0 $\pm$ 4.8	4.1 $\pm$ 0.8	-0.70 $\pm$ 0.05	13.24**
Organic C	61.6 $\pm$ 16.1	60.9 $\pm$ 17.9	-0.12 $\pm$ 0.11	1.12	30.6 $\pm$ 15.3	20.2 $\pm$ 11.3	-0.38 $\pm$ 0.06	6.69**	92.5 $\pm$ 17.9	101 $\pm$ 16	0.14 $\pm$ 0.10	0.95
$\delta^{13}\text{C}$	-27.1 $\pm$ 0.3	-27.1 $\pm$ 0.2	0.00 $\pm$ 0.01	0.19	-27.0 $\pm$ 0.5	-27.2 $\pm$ 0.3	0.01 $\pm$ 0.03	0.40	-27.1 $\pm$ 0.4	-27.0 $\pm$ 0.3	-0.01 $\pm$ 0.01	1.95
C/N	18.8 $\pm$ 1.3	18.7 $\pm$ 1.8	-0.01 $\pm$ 0.05	0.13	21.2 $\pm$ 2.2	20.4 $\pm$ 3.6	-0.05 $\pm$ 0.10	0.51	16.4 $\pm$ 0.3	17.1 $\pm$ 0.7	0.04 $\pm$ 0.03	4.44
Available Al	33.7 $\pm$ 10.4	44.9 $\pm$ 18.7	0.07 $\pm$ 0.22	0.33	10.8 $\pm$ 4.8	4.15 $\pm$ 0.97	-0.36 $\pm$ 0.20	1.83	56.6 $\pm$ 11.6	85.6 $\pm$ 23.0	0.50 $\pm$ 0.26	0.81
Available B	0.089 $\pm$ 0.018	0.084 $\pm$ 0.016	0.29 $\pm$ 0.36	4.73**	0.056 $\pm$ 0.019	0.064 $\pm$ 0.005	0.77 $\pm$ 0.67	3.55*	0.122 $\pm$ 0.019	0.103 $\pm$ 0.029	-0.20 $\pm$ 0.13	1.08*
Available Ca	1442 $\pm$ 352	1429 $\pm$ 424	-0.06 $\pm$ 0.13	0.44	2158 $\pm$ 429	232 $\pm$ 535	0.06 $\pm$ 0.16	0.36	726 $\pm$ 226	536 $\pm$ 156	-0.17 $\pm$ 0.22	32.83
Available Co	0.049 $\pm$ 0.012	0.007 $\pm$ 0.003	-0.66 $\pm$ 0.19	3.38*	0.076 $\pm$ 0.009	0.005 $\pm$ 0.002	-0.93 $\pm$ 0.02	38.39***	0.021 $\pm$ 0.009	0.009 $\pm$ 0.005	-0.38 $\pm$ 0.35	6.59
Available Cu	0.578 $\pm$ 0.195	0.334 $\pm$ 0.106	-0.31 $\pm$ 0.07	4.40**	0.75 $\pm$ 0.36	0.403 $\pm$ 0.194	-0.29 $\pm$ 0.15	1.90	0.406 $\pm$ 0.166	0.265 $\pm$ 0.108	-0.34 $\pm$ 0.01	1.65***
Available Fe	167 $\pm$ 68	224 $\pm$ 98	0.07 $\pm$ 0.17	0.40	40.1 $\pm$ 19.2	22.1 $\pm$ 9.7	-0.36 $\pm$ 0.07	4.98*	293 $\pm$ 103	427 $\pm$ 132	0.50 $\pm$ 0.08	7.47**
Available K	80.6 $\pm$ 22.9	87.3 $\pm$ 29.8	-0.03 $\pm$ 0.11	0.25	32.8 $\pm$ 13.2	22.3 $\pm$ 10.3	-0.24 $\pm$ 0.13	1.84	128 $\pm$ 27	152 $\pm$ 35	0.19 $\pm$ 0.11	1.69
Available Mg	294 $\pm$ 91	176 $\pm$ 51	-0.32 $\pm$ 0.07	4.61**	154 $\pm$ 70	93.1 $\pm$ 47.1	-0.29 $\pm$ 0.14	2.03	432 $\pm$ 145	259 $\pm$ 71	-0.36 $\pm$ 0.05	0.36**
Available Mn	8.23 $\pm$ 2.57	6.49 $\pm$ 2.75	-0.19 $\pm$ 0.10	1.84	9.48 $\pm$ 4.62	9.64 $\pm$ 5.24	-0.06 $\pm$ 0.06	1.03	6.98 $\pm$ 2.93	3.35 $\pm$ 1.10	-0.32 $\pm$ 0.19	3.31
Available Mo	0.093 $\pm$ 0.036	0.104 $\pm$ 0.042	553 $\pm$ 553	-0.67	0.004 $\pm$ 0.004	0.011 $\pm$ 0.011	1106 $\pm$ 1107	-	0.182 $\pm$ 0.030	0.198 $\pm$ 0.046	0.05 $\pm$ 0.14	5.04
Available Na	219 $\pm$ 75	190 $\pm$ 67	-0.12 $\pm$ 0.08	1.63	49.2 $\pm$ 12.7	40.5 $\pm$ 10.8	-0.12 $\pm$ 0.16	0.73	388 $\pm$ 84	339 $\pm$ 76	-0.13 $\pm$ 0.04	2.02*
Available Ni	0.119 $\pm$ 0.037	0.144 $\pm$ 0.039	79.1 $\pm$ 78.8	1.53	0.13 $\pm$ 0.07	0.124 $\pm$ 0.070	157 $\pm$ 158	0.79	0.108 $\pm$ 0.029	0.163 $\pm$ 0.040	0.56 $\pm$ 0.11	3.45*
Available P	4.93 $\pm$ 0.68	4.89 $\pm$ 0.94	-0.05 $\pm$ 0.07	0.76	3.85 $\pm$ 0.46	3.19 $\pm$ 0.58	-0.19 $\pm$ 0.10	1.77	6.01 $\pm$ 1.07	6.59 $\pm$ 1.35	0.08 $\pm$ 0.04	2.02
Available Zn	8.12 $\pm$ 3.40	3.37 $\pm$ 1.61	-0.41 $\pm$ 0.11	3.78**	11.5 $\pm$ 6.6	4.60 $\pm$ 3.26	-0.38 $\pm$ 0.19	1.94	4.79 $\pm$ 1.85	2.18 $\pm$ 0.71	-0.45 $\pm$ 0.13	3.45*

ESM Table 1b Raw and standardized [(I-A)/A] values (mean $\pm$ s.e), *t*-test value and significance (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) of the statistical analysis for the studied soil properties under autochthonous (A) and invasive (I) vegetation, for the soil 0-5 cm and soil 5-10 cm layers (d.f.= 3). Data are given in the following units: water holding capacity (WHC), g H₂O kg⁻¹ dw; humidity, %; electrical conductivity (EC), mS cm⁻¹; organic C and N, g kg⁻¹ dw; C/N ratio, g g⁻¹; $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, ‰; and other elements, mg kg⁻¹ dw.

	0 - 5 cm soil layer				5 - 10 cm soil layer			
	A	I	(I-A)/A	<i>t</i> -value	A	I	(I-A)/A	<i>t</i> -value
WHC	369 $\pm$ 122	351 $\pm$ 112	-0.07 $\pm$ 0.11	0.66	244 $\pm$ 74	224 $\pm$ 68	-0.11 $\pm$ 0.07	1.48
Humidity	12.1 $\pm$ 5.8	10.7 $\pm$ 5.6	0.00 $\pm$ 0.16	2.39	14.9 $\pm$ 2.7	16.2 $\pm$ 4.7	-0.25 $\pm$ 0.10	0.21
pH _{H2O}	6.60 $\pm$ 0.35	6.26 $\pm$ 0.83	-0.06 $\pm$ 0.09	0.65	6.69 $\pm$ 0.50	6.46 $\pm$ 0.88	-0.05 $\pm$ 0.07	0.68
pH _{KCl}	4.70 $\pm$ 0.45	4.56 $\pm$ 0.96	-0.05 $\pm$ 0.13	0.41	4.76 $\pm$ 0.75	4.37 $\pm$ 1.21	-0.13 $\pm$ 0.11	1.13
EC	130 $\pm$ 52	174 $\pm$ 83	0.33 $\pm$ 0.32	1.03	97.8 $\pm$ 33.9	116 $\pm$ 56	0.07 $\pm$ 0.26	0.27
Total N	4.60 $\pm$ 1.68	4.38 $\pm$ 1.93	-0.17 $\pm$ 0.12	1.36	2.47 $\pm$ 0.86	2.52 $\pm$ 1.13	-0.10 $\pm$ 0.19	0.56
$\delta^{15}\text{N}$	3.40 $\pm$ 1.68	3.02 $\pm$ 1.77	0.07 $\pm$ 0.15	0.45	3.88 $\pm$ 1.39	4.89 $\pm$ 0.56	2.72 $\pm$ 2.67	1.02
NH ₄ ⁺ -N	2.77 $\pm$ 1.17	8.04 $\pm$ 5.26	1.02 $\pm$ 0.84	1.21	2.13 $\pm$ 0.67	2.82 $\pm$ 1.13	0.30 $\pm$ 0.50	0.61
NO ₃ ⁻ -N	17.8 $\pm$ 5.4	4.89 $\pm$ 1.59	-0.70 $\pm$ 0.05	13.21**	9.00 $\pm$ 2.83	3.43 $\pm$ 0.95	-0.60 $\pm$ 0.02	28.90***
Organic C	81.3 $\pm$ 27.3	78.0 $\pm$ 31.0	-0.18 $\pm$ 0.14	1.27	41.8 $\pm$ 13.7	43.8 $\pm$ 18.4	-0.07 $\pm$ 0.19	0.23
$\delta^{13}\text{C}$	-27.5 $\pm$ 0.4	-27.5 $\pm$ 0.3	0.00 $\pm$ 0.03	0.09	-26.7 $\pm$ 0.3	-26.7 $\pm$ 0.1	0.00 $\pm$ 0.01	0.10
C/N	17.8 $\pm$ 1.7	17.7 $\pm$ 2.7	-0.02 $\pm$ 0.07	0.27	19.9 $\pm$ 2.2	19.8 $\pm$ 2.7	0.01 $\pm$ 0.09	3.03
Available Al	26.1 $\pm$ 9.5	37.6 $\pm$ 22.0	0.17 $\pm$ 0.39	0.44	41.3 $\pm$ 19.5	52.2 $\pm$ 33.4	-0.03 $\pm$ 0.26	0.72
Available B	0.101 $\pm$ 0.033	0.105 $\pm$ 0.028	0.53 $\pm$ 0.67	3.40*	0.077 $\pm$ 0.017	0.062 $\pm$ 0.008	0.04 $\pm$ 0.37	1.66
Available Ca	1600 $\pm$ 379	1661 $\pm$ 536	0.02 $\pm$ 0.21	0.08	1284 $\pm$ 646	1199 $\pm$ 720	-0.13 $\pm$ 0.18	2.09
Available Co	0.050 $\pm$ 0.023	0.006 $\pm$ 0.004	-0.74 $\pm$ 0.23	3.24*	0.048 $\pm$ 0.012	0.009 $\pm$ 0.004	-0.57 $\pm$ 0.35	0.28
Available Cu	0.684 $\pm$ 0.310	0.444 $\pm$ 0.191	-0.32 $\pm$ 0.04	8.78**	0.472 $\pm$ 0.271	0.224 $\pm$ 0.089	-0.31 $\pm$ 0.15	0.49
Available Fe	205 $\pm$ 128	261 $\pm$ 173	0.06 $\pm$ 0.23	0.25	129 $\pm$ 66	187 $\pm$ 117	0.08 $\pm$ 0.28	2.27
Available K	107 $\pm$ 41	121 $\pm$ 53	0.03 $\pm$ 0.15	0.22	54.5 $\pm$ 17.8	53.8 $\pm$ 24.5	-0.09 $\pm$ 0.19	0.67
Available Mg	422 $\pm$ 160	258 $\pm$ 83	-0.33 $\pm$ 0.06	5.72*	165 $\pm$ 46	94.9 $\pm$ 26.6	-0.32 $\pm$ 0.14	0.21
Available Mn	12.3 $\pm$ 3.9	9.21 $\pm$ 5.13	-0.32 $\pm$ 0.17	1.82	4.15 $\pm$ 2.20	3.78 $\pm$ 2.01	-0.07 $\pm$ 0.10	1.35
Available Mo	0.108 $\pm$ 0.063	0.110 $\pm$ 0.070	-0.23 $\pm$ 0.26	-0.90	0.078 $\pm$ 0.046	0.099 $\pm$ 0.056	1107 $\pm$ 1107	2.75
Available Na	291 $\pm$ 138	261 $\pm$ 121	-0.06 $\pm$ 0.06	0.93	147 $\pm$ 60	120 $\pm$ 53	-0.19 $\pm$ 0.14	0.58
Available Ni	0.142 $\pm$ 0.059	0.155 $\pm$ 0.058	0.06 $\pm$ 0.23	0.27	0.096 $\pm$ 0.051	0.133 $\pm$ 0.059	158 $\pm$ 157	1.70
Available P	5.69 $\pm$ 0.91	5.67 $\pm$ 1.33	-0.03 $\pm$ 0.08	0.40	4.17 $\pm$ 0.96	4.11 $\pm$ 1.38	-0.08 $\pm$ 0.13	0.58
Available Zn	10.5 $\pm$ 5.6	5.31 $\pm$ 3.01	-0.46 $\pm$ 0.08	5.44*	5.70 $\pm$ 4.35	1.44 $\pm$ 0.73	-0.37 $\pm$ 0.22	1.70

ESM Table 2 Factor loadings of the rotated component matrix from the principal component analysis (PCAs) performed with soil data for each locality and deepness. Note: WHC, water holding capacity; EC, electrical conductivity.

	Component	
	1	2
pH _{H2O}	-0.398	-0.837
WHC	0.849	0.447
EC	0.648	0.687
Total N	0.803	0.582
NH ₄ ⁺ -N	0.459	0.672
Organic C	0.839	0.497
Available Al	0.101	0.951
Available B	0.705	0.345
Available Fe	0.480	0.835
Available K	0.857	0.405
Available Mg	0.934	0.133
Available Mo	0.535	0.800
Available P	0.435	0.761

ESM Table 3 Mean values $\pm$ standard error for the studied necromass properties under autochthonous (A) and invasive (I) vegetation, globally (d.f.=3) and separately for dunes and rocky sites (d.f.=1), and *t*-test value and significance (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) of the correspondent statistical analysis. Data are given in the following units: total C and N, g kg⁻¹ dw; C/N ratio, g g⁻¹; $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, ‰; and other elements, mg kg⁻¹ dw.

	Globally			Dune			Rock		
	A	I	<i>t</i> -value	A	I	<i>t</i> -value	A	I	<i>t</i> -value
Total N	12.2 $\pm$ 1.4	9.51 $\pm$ 2.00	1.65	10.6 $\pm$ 1.9	6.37 $\pm$ 1.00	4.95	13.9 $\pm$ 1.5	12.6 $\pm$ 1.8	0.37
$\delta^{15}\text{N}$	2.00 $\pm$ 2.54	3.49 $\pm$ 1.76	0.98	-0.99 $\pm$ 2.08	1.83 $\pm$ 1.09	0.89	4.99 $\pm$ 4.06	5.14 $\pm$ 3.45	0.26
Total C	414 $\pm$ 26	384 $\pm$ 7	1.4	394 $\pm$ 1	377 $\pm$ 10	2.14	433 $\pm$ 58	391 $\pm$ 8	0.83
$\delta^{13}\text{C}$	-27.5 $\pm$ 1.2	-27.9 $\pm$ 0.3	0.01	-26.4 $\pm$ 2.5	-28.3 $\pm$ 0.1	0.57	-28.5 $\pm$ 0.5	-27.5 $\pm$ 0.2	2.94
C/N	34.8 $\pm$ 3.5	46.3 $\pm$ 9.8	1.70	38.4 $\pm$ 6.9	60.8 $\pm$ 11.0	5.35	31.1 $\pm$ 0.8	31.7 $\pm$ 5.2	0.13
Al	2138 $\pm$ 582	1304 $\pm$ 332	3.13	2335 $\pm$ 1398	1368 $\pm$ 792	1.60	1940 $\pm$ 12	1239 $\pm$ 167	4.51
B	15.0 $\pm$ 4.9	26.0 $\pm$ 3.8	3.47 *	8.79 $\pm$ 1.06	19.5 $\pm$ 0.3	13.95 *	21.1 $\pm$ 8.2	32.5 $\pm$ 0.5	1.47
Ca	7158 $\pm$ 1183	29533 $\pm$ 6441	4.21 *	9024 $\pm$ 644	40560 $\pm$ 1331	45.96 *	5293 $\pm$ 1012	18506 $\pm$ 1979	13.66 *
Co	0.536 $\pm$ 0.060	0.501 $\pm$ 0.149	0.35	0.588 $\pm$ 0.094	0.623 $\pm$ 0.318	0.16	0.484 $\pm$ 0.085	0.378 $\pm$ 0.046	2.72
Cu	8.43 $\pm$ 2.12	3.67 $\pm$ 1.40	6.37 **	10.8 $\pm$ 4.0	5.09 $\pm$ 2.79	4.72	6.08 $\pm$ 0.31	2.24 $\pm$ 0.11	9.03
Fe	1164 $\pm$ 281	698 $\pm$ 165	3.73 *	1228 $\pm$ 603	771 $\pm$ 373	1.99	1100 $\pm$ 317	625 $\pm$ 114	2.35
K	1531 $\pm$ 135	1641 $\pm$ 219	0.45	1638 $\pm$ 266	1323 $\pm$ 280	22.72 *	1424 $\pm$ 125	1960 $\pm$ 87	14.14 *
Mg	2122 $\pm$ 362	2690 $\pm$ 521	2.11	1674 $\pm$ 222	1832 $\pm$ 283	2.59	2570 $\pm$ 580	3549 $\pm$ 270	3.17
Mn	116 $\pm$ 23	213 $\pm$ 91	1.65	118 $\pm$ 57	295 $\pm$ 192	1.72	114 $\pm$ 0	131 $\pm$ 3	6.57
Mo	3.67 $\pm$ 0.95	2.12 $\pm$ 0.51	2.72	3.63 $\pm$ 2.28	1.68 $\pm$ 1.09	1.63	3.71 $\pm$ 0.43	2.56 $\pm$ 0.03	2.52
Na	1085 $\pm$ 251	1991 $\pm$ 309	4.61 *	699 $\pm$ 184	1470 $\pm$ 24	3.72	1471 $\pm$ 215	2512 $\pm$ 176	2.67
Ni	2.55 $\pm$ 0.54	1.90 $\pm$ 0.23	1.12	2.95 $\pm$ 1.03	1.70 $\pm$ 0.37	1.90	2.15 $\pm$ 0.59	2.11 $\pm$ 0.33	0.05
P	749 $\pm$ 61	571 $\pm$ 82	2.73	815 $\pm$ 51	540 $\pm$ 107	4.96	682 $\pm$ 104	601 $\pm$ 164	1.37
Zn	61.3 $\pm$ 18.2	64.5 $\pm$ 20.8	0.43	74.2 $\pm$ 40.5	67.7 $\pm$ 50.7	0.63	48.4 $\pm$ 3.4	61.3 $\pm$ 3.4	1.90

ESM Table 4 Mean values $\pm$ standard error for the studied necromass properties under autochthonous (A) and invasive (I) vegetation, globally (d.f.=3) and separately for dunes and rocky sites (d.f.=1), and *t*-test value and significance (***P* < 0.001; **P* < 0.01; **P* < 0.05) of the correspondent statistical analysis. Except for total N and C (g m⁻²), all data are expressed in mg m⁻².

	Globally			Dune			Rock		
	A	I	<i>t</i> -value	A	I	<i>t</i> -value	A	I	<i>t</i> -value
Total N	17.2 $\pm$ 5.6	52.7 $\pm$ 26.9	1.56	8.11 $\pm$ 4.31	10.3 $\pm$ 0.4	0.55	26.3 $\pm$ 2.6	95.2 $\pm$ 26.9	2.34
Total C	581 $\pm$ 170	1753 $\pm$ 668	2.15	341 $\pm$ 221	628 $\pm$ 139	3.49	821 $\pm$ 100	2877 $\pm$ 357	4.5
Al	2459 $\pm$ 706	5487 $\pm$ 2037	2.23	1237 $\pm$ 96	2012 $\pm$ 867	0.80	3680 $\pm$ 24	8961 $\pm$ 77	99.90 **
B	24.2 $\pm$ 11.6	136 $\pm$ 62	2.18	8.18 $\pm$ 5.81	32.4 $\pm$ 6.8	23.75 *	40.3 $\pm$ 16.1	240 $\pm$ 38	9.18
Ca	9108 $\pm$ 2492	103157 $\pm$ 25547	3.89 *	8152 $\pm$ 5590	67741 $\pm$ 15442	6.05	10065 $\pm$ 2047	138574 $\pm$ 34189	4.00
Co	0.686 $\pm$ 0.178	1.83 $\pm$ 0.54	2.49	0.456 $\pm$ 0.247	0.930 $\pm$ 0.325	0.83	0.916 $\pm$ 0.150	2.74 $\pm$ 0.06	8.75
Cu	9.30 $\pm$ 1.67	12.1 $\pm$ 3.2	0.92	7.08 $\pm$ 2.56	7.53 $\pm$ 2.97	0.08	11.5 $\pm$ 0.4	16.7 $\pm$ 3.2	1.41
Fe	1409 $\pm$ 476	2943 $\pm$ 1209	2.02	725 $\pm$ 165	1158 $\pm$ 368	0.81	2094 $\pm$ 627	4727 $\pm$ 1504	3.00
K	1983 $\pm$ 506	8232 $\pm$ 3585	1.95	1266 $\pm$ 684	2105 $\pm$ 33	1.17	2699 $\pm$ 203	14359 $\pm$ 1432	7.13
Mg	3105 $\pm$ 1173	14702 $\pm$ 7181	1.91	1322 $\pm$ 742	2948 $\pm$ 128	2.65	4889 $\pm$ 1161	26457 $\pm$ 5750	4.70
Mn	143 $\pm$ 43	695 $\pm$ 186	3.53 *	70.1 $\pm$ 16.6	426 $\pm$ 222	1.49	217 $\pm$ 3	963 $\pm$ 118	6.43
Mo	4.46 $\pm$ 1.54	10.6 $\pm$ 4.9	1.85	1.86 $\pm$ 0.05	2.43 $\pm$ 1.26	0.44	7.06 $\pm$ 0.90	18.9 $\pm$ 2.5	7.44
Na	1648 $\pm$ 693	10395 $\pm$ 4627	2.22	501 $\pm$ 231	2447 $\pm$ 519	6.77	2795 $\pm$ 443	18343 $\pm$ 1365	16.88 *
Ni	3.02 $\pm$ 0.81	9.30 $\pm$ 4.27	1.57	1.98 $\pm$ 0.76	2.69 $\pm$ 0.06	0.88	4.07 $\pm$ 1.07	15.9 $\pm$ 4.7	2.06
P	986 $\pm$ 261	2735 $\pm$ 1318	1.54	676 $\pm$ 411	861 $\pm$ 1	0.45	1297 $\pm$ 214	4609 $\pm$ 1844	2.03
Zn	66.6 $\pm$ 14.9	276 $\pm$ 113	2.06	41.5 $\pm$ 6.4	95.8 $\pm$ 62.1	0.79	91.7 $\pm$ 5.3	456 $\pm$ 90	3.81

ESM Table 5 Factor loadings of the rotated component matrix from the principal component analysis (PCA) performed with necromass data for each locality and deepness.

	Component	
	1	2
Total Al	0.966	-0.072
Total Co	0.696	-0.418
Total Cu	0.870	-0.422
Total Fe	0.901	0.031
Total Mg	-0.004	0.992
Total Ni	0.827	-0.078
Total Zn	0.897	0.031