

Research Article

# Comparative invasion ecology of *Carpobrotus* from four continents: responses to nutrients and competition

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## Abstract

Two key hypotheses in invasion biology are that certain traits underlie invasiveness in introduced species, and that these traits are selected for during or after introduction. We tested these hypotheses by focusing on two traits likely to confer invasiveness, high increase in growth in response to increase in nutrients and low decrease in growth in response to competition. We compared four species of *Carpobrotus* that differ in invasiveness, using species from four continents: Africa, Australia, Europe and North America. To test for selection for these traits in *Carpobrotus edulis*, a highly invasive species, we compared plants from its native range in South Africa to plants from the other three regions, where *C. edulis* has been introduced. Plants were propagated in a common garden. Offspring were then grown alone with or without added nutrients, and together with another species of *Carpobrotus* or with the grass *Ammophila arenaria* (a co-occurring native species in Europe) without added nutrients. Response to nutrients did not differ between species of *Carpobrotus*, nor was competitive response less negative in more invasive species. However, increase in growth in response to added nutrients was greater in introduced than in native *C. edulis*. Moreover, fresh mass per ramet at the start of treatments was higher in the two invasive species than in the two non-invasive ones. We provide new evidence that introduction can select for response to nutrient enrichment in invasive species and add to the evidence for an association between size and invasiveness in introduced plants.

**Keywords** *Carpobrotus*, coastal sand dune, competitive response, invasive introduced plant, nutrient availability, rapid evolution

## 四大洲食用昼花属的比较入侵生态学：对养分和竞争的响应

摘要：入侵生物学的两个关键假设是，某些特征是外来物种入侵的基础，且这些特征是在引入期间或之后选择的。我们通过关注两个可能赋予入侵性的特征来检验这些假设，即营养增加引起的较高生长加速和竞争引起的较低生长减速。我们对采自非洲、澳洲、欧洲和北美洲的4个食用昼花属(*Carpobrotus*)品

种进行了比较。为了测试高入侵物种莫邪菊(*Carpobrotus edulis*)对这些特征的选择,我们将原产于南非的植株与入侵其他3个地区的植株进行了比较。在一个同质园中繁殖植株。然后,在添加或不添加养分的情况下单独培育子代,并在不添加养分的情况下将子代与另一个食用昼花属品种一起、或与欧洲的一种共生本土物种马兰草(*Ammophila arenaria*)一起培育。不同品种的食用昼花属对营养物质的反应并无差异,在入侵性更强的物种中,竞争反应的负面性也没有减弱。然而,与本土莫邪菊相比,添加营养物质引起外来莫邪菊更高的生长加速。此外,在处理开始时,两种入侵物种每分株的鲜质量高于两种非入侵物种。我们的研究表明,引种可以选择入侵物种对营养富集的反应进行选择,也证明了外来植物的体积和入侵性之间的关联。

关键词:食用昼花属(*Carpobrotus*),海岸沙丘,竞争反应,外来入侵植物,养分有效性,快速进化

## INTRODUCTION

Biological invasions are one of the main threats to biodiversity worldwide and an increasingly important subject of research needed to promote the conservation of biodiversity and ecosystem function (Mack *et al.* 2000; Richardson and Pyšek 2008; Strayer 2012; Vitousek *et al.* 1996). A key objective of this research is to determine if certain traits favor invasiveness in introduced species (Blackburn *et al.* 2011; Ordonez 2014; Richardson and Pyšek 2006; Thuiller *et al.* 2006; van Kleunen *et al.* 2011). Determinants of plant invasiveness are expected to be complex and often context-specific, as different factors can act at different stages in an invasion (Levine *et al.* 2003; Pyšek and Richardson 2007).

Previous work suggests that an ability to increase growth rates in response to greater availability of soil nutrients may be an important contributor to the invasiveness of introduced plant species (Alpert *et al.* 2000; Blossey and Nötzold 1995; Davidson *et al.* 2011; Keser *et al.* 2014; Pichancourt and van Klinken 2012). For example, a relatively large increase in growth in response to greater nutrient availability is one of the most commonly observed characteristics of invasive, introduced species. This is of particular concern because agriculture and industry have raised soil nutrient availability in many habitats.

High competitive ability is a second factor likely to increase invasiveness (Burke and Grime 1996; Callaway and Ridenour 2004; D'Antonio and Mahall 1991; Gioria and Osborne 2014; Schultheis and MacGuigan 2018). Introduced species that outcompete natives are more likely to rapidly expand their range, invade established vegetation, and resist recolonization by native, i.e. to be more invasive. Increase in competitive ability could also be a mechanism by which high response to nutrients promotes invasiveness.

Evolution during or following introduction has been widely implicated as a frequent contributor to invasiveness (Bossdorf *et al.* 2005; Colautti and Lau 2015; Felker-Quinn *et al.* 2013; Lavergne and Molofsky 2007; Maron *et al.* 2004; Willis *et al.* 2010). Both intentional and unintentional introduction may select for traits that increase invasiveness, such as high reproductive output and tolerance of environmental conditions in the introduced range. Rapid evolution through selection after introduction may likewise enhance invasiveness.

Two powerful approaches for assessing determinants of invasiveness are comparison between invasive and non-invasive introduced species within genera, known as the target-area approach (van Kleunen *et al.* 2010), and comparison between native and introduced populations within species, which we refer to as the inter-range approach. The target-area approach is especially useful for identifying traits that contribute to invasiveness (Mack 1996; Nijs *et al.* 2004; Pyšek *et al.* 2004). The inter-range approach helps detect selection for these traits during or following introduction (Hierro *et al.* 2005; Lee 2002).

The genus *Carpobrotus* offers an excellent set of species in which to apply these approaches to test whether competitive ability and a relatively large increase in growth in response to increased nutrients underlie invasiveness in introduced plant species and whether these traits are selected for in introduced populations. Nutrient availability is known to influence performance of *Carpobrotus*, such as by playing a key role in germination (Novoa *et al.* 2014). Response to nutrients is also known to differ between species. For example, Campoy *et al.* (2019) found that *Carpobrotus edulis* appears to be more responsive to an increase in soil nutrients than the hybrid *C. aff. acinaciformis*. Previous studies have demonstrated the capacity of

introduced *Carpobrotus* to successfully compete with native species and affect local community structure and diversity (D'Antonio 1993; Fried *et al.* 2014; Roiloa *et al.* 2010; Vilà *et al.* 2006). It has also been recently shown that biomass partitioning in response to nutrient availability in *C. edulis* differs between populations from the native and introduced ranges, indicating that this plasticity could be under selection during the invasion process (Portela *et al.* 2019).

To test the specific hypotheses that a large increase in growth with increase in nutrients and a low decrease in growth in response to competition are associated with invasiveness and that introduction selects for both responses, we compared four morphologically similar species in the genus *Carpobrotus* that occur in similar

habitats but differ in invasiveness, using comparisons in four regions, each on a different continent (Fig. 1; Table 1). We used the target-area approach to address association between traits and invasiveness and the inter-range approach to address selection for traits during or after introduction in a large common garden experiment designed to test eight predictions (Fig. 1).

## MATERIALS AND METHODS

### Species, collection and propagation

*Carpobrotus* is a genus of clonal plants in the Aizoaceae with approximately 20–25 species found in Mediterranean-type climates; several species

Approach	Hypothesis	Prediction	Schematic representation
Target-area	Higher nutrients increase performance more in invasive than non-invasive introduced species.	<i>C. edulis</i> from California will increase growth more with higher nutrients than <i>C. chilensis</i> from California.	
Target-area	Higher nutrients increase performance more in more widespread invasive introduced species.	<i>C. edulis</i> from Iberia will increase growth more with higher nutrients than <i>C. acinaciformis</i> from Iberia.	
Target-area	Invasive introduced species outcompete non-invasive ones.	<i>C. edulis</i> from California will outcompete <i>C. chilensis</i> from California.	
Target-area	More widespread invasive introduced species outcompete less widespread ones.	<i>C. edulis</i> from Iberia will outcompete <i>C. acinaciformis</i> from Iberia.	
Target-area	More widespread invasive introduced species show less negative competitive response to unrelated dominant native species.	<i>C. edulis</i> from Iberia will show a less negative competitive response to <i>A. arenaria</i> from Iberia than will <i>C. acinaciformis</i> from Iberia.	
Inter-range	High nutrients increase performance more in invasive introduced than in native populations of a species.	<i>C. edulis</i> from Iberia, California, and Australia will increase growth more with higher nutrients than <i>C. edulis</i> from South Africa.	
Inter-range	Invasive introduced populations show less negative competitive response than native populations to closely related natives.	<i>C. edulis</i> from Australia will show a less negative competitive response to <i>C. virescens</i> from Australia than will <i>C. edulis</i> from South Africa.	
Inter-range	Invasive introduced populations show less negative competitive response than native populations to unrelated natives.	<i>C. edulis</i> from Iberia will show a less negative competitive response to <i>A. arenaria</i> than will <i>C. edulis</i> from South Africa.	

**Figure 1:** Experimental design. Ranges: AU = Australia, CA = California, USA, IB = Iberia (Spain and Portugal), SA = South Africa. Species: *Ce* = *Carpobrotus edulis*, *Cc* = *Carpobrotus chilensis*, *Ca* = *Carpobrotus acinaciformis*, *Cv* = *Carpobrotus virescens*, *Aa* = *Ammophila arenaria*. Nutrient treatment: white pot = low nutrients; black pot = high nutrients. See text for details.

**Table 1:** Native and introduced ranges, invasiveness in the introduced range and sampled regions of the studied species

Species	Native range	Introduced range	Apparent invasiveness in the introduced range	Regions sampled
<i>Carpobrotus edulis</i>	South Africa	Southern Europe, California, Chile, Southern Australia	High <sup>ab,c</sup>	Cape Region (South Africa), northwestern Iberia, northern California, southwestern Australia
<i>Carpobrotus acinaciformis</i>	South Africa	Southern Europe	High but less widespread <sup>d,e</sup>	Northwestern Iberia
<i>Carpobrotus chilensis</i>	Southern Africa	California	Low <sup>fg</sup>	Northern California
<i>Carpobrotus virescens</i>	Western Australia	Britain	None observed <sup>h</sup>	Southwestern Australia
<i>Ammophila arenaria</i>	Europe and northern Africa	California, Oregon	High <sup>i</sup>	Northwestern Spain

See Fig. 2 and Supplementary Table S1 for collection sites. <sup>a</sup>D'Antonio and Mahall (1991). <sup>b</sup>Traveset et al. (2008). <sup>c</sup>Campoy et al. (2018). <sup>d</sup>Lambinon (1995). <sup>e</sup>Suehs et al. (2001). <sup>f</sup>Vilà and D'Antonio (1998). <sup>g</sup>Vivrette (2012). <sup>h</sup>Preston and Sell (1988). <sup>i</sup>Alpert (2016).

have been introduced into regions where they now co-occur with native congeners (Campoy 2018; Hartmann 2012; Vivrette 2012; Wisura and Glen 1993). We compared the widely introduced, invasive species *C. edulis* (L.) N.E. Br.; the invasive, less widespread species *C. acinaciformis* (L.) L. Bolus; the extensively naturalized but apparently non-invasive species *C. chilensis* (Molina) N. E. Br. and the infrequently naturalized, non-invasive species *C. virescens* (Haw.) Schwantes (Fig. 1; Table 1). All four species grow on coastal sand dunes and bluffs, reproduce sexually via fleshy, many-seeded fruits and asexually via monopodial, creeping stems that root and grow short, ascending to vertical shoots at the nodes that bear succulent leaves. Nodes can function as ramets, i.e. potentially physiologically independent units within a clone, and connected ramets of *C. edulis*, *C. acinaciformis* and *C. chilensis* have been shown to be physiologically integrated (Portela and Roiloa 2017; Roiloa et al. 2019), which is a common trait of clonal plants (Pitelka and Ashmun 1985).

*Carpobrotus edulis* and *C. acinaciformis* are native to the Cape Region of South Africa and introduced in southern Europe (Campoy et al. 2018; Suehs et al. 2001; Wisura and Glen 1993); *Carpobrotus edulis* also has large, introduced populations in all three of the other main regions with Mediterranean-type climate: California, southern Australia and Chile (D'Antonio and Mahall 1991; Campoy et al. 2018; Traveset et al. 2008). *Carpobrotus chilensis* is probably also native to southern Africa (Vivrette 2012), though it was previously thought to have been native to South America or to both California and South America (Bicknell and Mackey 1998; Vilà and D'Antonio 1998). *Carpobrotus virescens*, native to the coast of western Australia, is sold horticulturally and reported to have become naturalized in Britain (Preston and Sell 1988; cf. Campoy et al. 2018). The presence of a hybrid taxon between *C. edulis* and *C. acinaciformis* has been described in southern Europe, with an introgression of part of the *C. edulis* genome into that of *C. acinaciformis* (Suehs et al. 2004). This hybrid taxon is described as close to *C. acinaciformis* and generally referred to in the scientific literature as *C. aff. acinaciformis* (Campoy et al. 2018). To identify *C. edulis* and *C. acinaciformis*, we used three diagnostic characters included in the literature (Campoy et al. 2018; Gonçalves 1990; Wisura and Glen 1993), petal color and leaf color and shape: yellow petals and green leaves that form an equilateral triangle in cross section in *C. edulis*, and purple petals and glaucous



leaves that form an isosceles triangle in cross section in *C. acinaciformis*. To exclude *C. aff. acinaciformis*, we avoided plants with any combination of characters from both species. However, we are not completely sure that none of the fragments of *C. acinaciformis* contained any genetic material derived from *C. edulis* since we did not conduct genetic analyses.

*Carpobrotus edulis* was collected in its native range in South Africa and in three regions in its introduced range, northwestern Spain and Portugal (which we refer to collectively as Iberia), northern California and southwestern Australia, allowing comparison between native *C. edulis* and non-native *C. edulis* from three different continents (Supplementary Table S1; Fig. 2). *Carpobrotus acinaciformis* was collected within its introduced range in northwestern Iberia, *C. chilensis* within its introduced range in northern California and *C. virescens* within its native range in southwestern Australia, allowing comparison between *C. edulis* and each of the other species within a region. Each species was collected from 3 to 4 populations in each region. In each population, at least 20 sets of connected ramets, or clonal fragments, with at least two unrooted, apical ramets were collected at least 2 m apart from each other to increase the likelihood that fragments belonged to different clones. The goal of sampling was to collect a representative sample of each species in each region. Plants were collected in the winter of 2015 in South Africa and Iberia, in January 2016 in California, and in November 2016 in Australia. Collections were transported to an experimental garden at the University of A Coruña in northwestern Spain and vegetatively propagated in regularly watered trays of sand from local coastal sand dunes for at least 4 months to reduce any differential effects of source environments. Only plants produced after at least two vegetative generations, i.e. production of an offspring ramet that then became a parent ramet, in the garden were used in the experiment.

To compare the ability of introduced *C. edulis* and *C. acinaciformis* to compete with a frequently co-occurring native species, we collected *Ammophila arenaria* (L.) Link (Poaceae, marram grass) in northwestern Spain. *Ammophila arenaria* is a rhizomatous perennial native to coastal sand dunes in Europe and northern Africa (Purser 1942). The species was intentionally introduced in the 1800s to northern California and is now highly invasive on coastal sand dunes in California and Oregon, where it co-occurs with introduced *C. edulis* (Alpert 2016). Except in wet swales or slacks between dunes, *A. arenaria* mainly

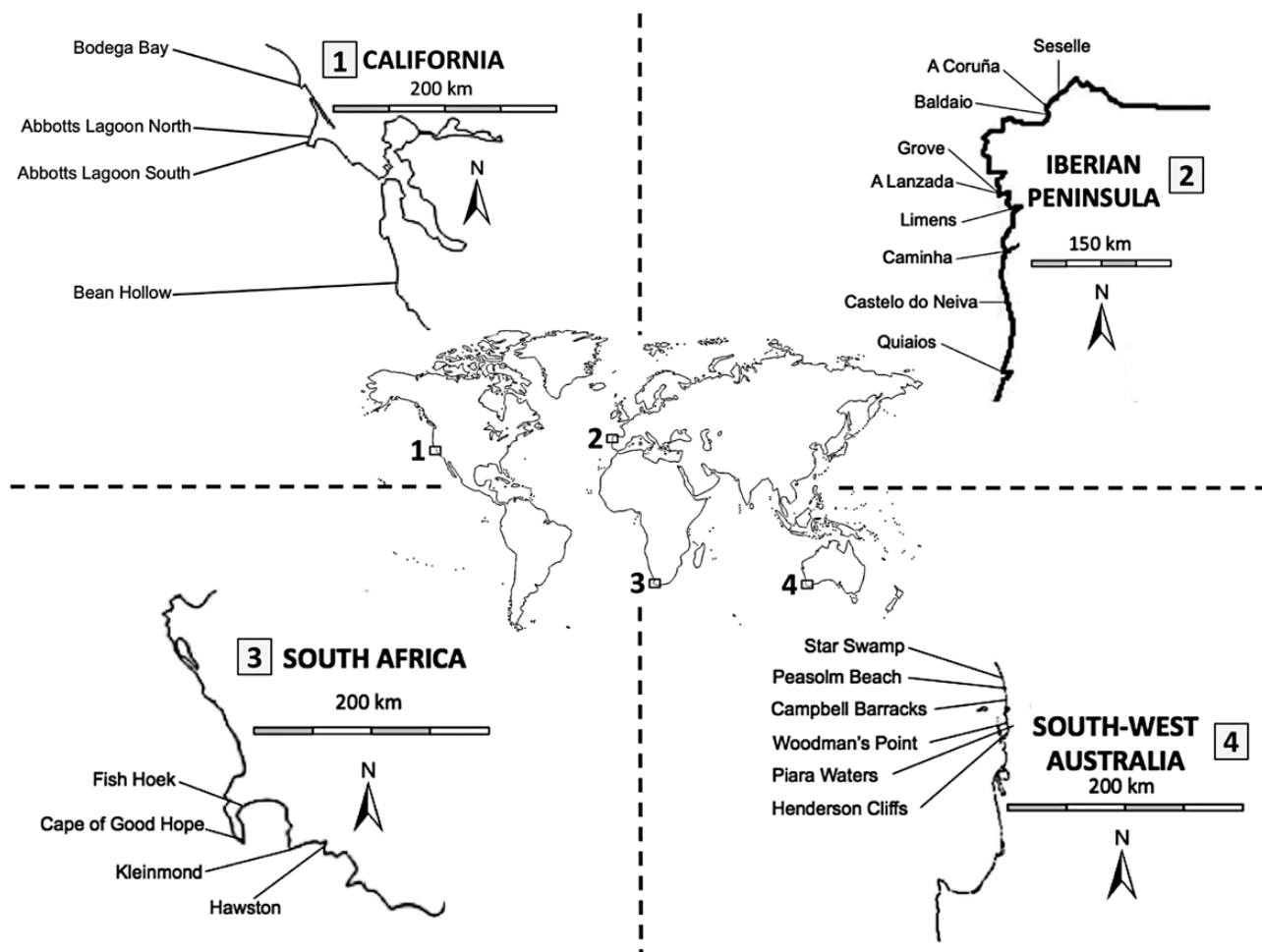
reproduces clonally (Huiskes 1977) via rhizomes that produce an extensive root system that holds the plant and the surrounding sand in place (Chergui *et al.* 2017). *Ammophila arenaria* was collected on 9 March 2017 from a single native population using the same protocol as for other species except that the criterion for selecting plants was that each have at least 3 cm of rhizome. Plants were kept in water for 2 weeks before planting directly into experimental pots.

## Experimental design

The experiment included 21 treatments designed to compare response to soil nutrient availability (low or high) and response to competition (grown alone or with a congener or *Ammophila*) between species of *Carpobrotus* within regions (target-area approach) and between native and introduced populations of *C. edulis* (inter-range approach; Fig. 1). Some treatments were used in multiple comparisons. To compare species within regions, we used *C. edulis* (widespread invasive) and *C. chilensis* (widespread non-invasive) from California, and *C. edulis* (widespread invasive) and *C. acinaciformis* (less widespread invasive) from Iberia. To compare native and introduced populations of *C. edulis*, we used native plants from South Africa and introduced populations from California, Australia, and Iberia.

For the high-nutrient treatment, 8 g of 3–4 month, slow-release fertilizer was added to each pot at the start of treatment (Osmocote Bloom, ICL Specialty Fertilizers Iberia, containing, in mg L<sup>-1</sup>, 212 NO<sub>3</sub>, 268 NH<sub>4</sub>, 280 P<sub>2</sub>O<sub>3</sub>, 720 K<sub>2</sub>O, 14 Fe, 2 Mn, 1.8 Cu, 1.8 Mo and 0.4 Zn). For the low-nutrient treatment, no fertilizer was added. Ramets were grown alone in both the high- and low-nutrient treatments. For the treatments with competition, a ramet of *Carpobrotus* was grown in the same pot as a ramet of a different species with no added fertilizer, i.e. the low-nutrient treatment. To compare competitive response between introduced species within a region, we grew *C. edulis* with *C. chilensis* from California, *C. edulis* with *C. acinaciformis* from Iberia, and *C. edulis* and *C. acinaciformis* from Iberia each with *A. arenaria* from Iberia (Fig. 1). To compare competitive response between native and introduced populations of *C. edulis*, *C. edulis* from South Africa and from Australia were each grown with *C. virescens* from Australia, and *C. edulis* from South Africa and *C. edulis* from Iberia were each grown with *A. arenaria* from Iberia (Fig. 1).

Plants were selected for use in the experiment on 20 March 2017 and placed in 1.5-L plastic, square



**Figure 2:** Collection sites for the *Carpobrotus* species used in this study. See [Supplementary Table S1](#) for the latitude and longitude of sites and for the species collected at each site.

pots (10 cm × 10 cm × 15 cm) filled with the type of sand used for propagation. To provide 10 replicates for each of the treatments, a total of 240 ramets of *Carpobrotus* and 40 plants of *A. arenaria* were chosen. For all *Carpobrotus* species, the youngest ramet with a well-developed shoot along a stem was used to minimize any effects of initial developmental stage. Ramets were unrooted. Care was taken to include as equal a number from each population within a region and species as possible and to select ramets of similar developmental stage. Ramets within species and region were randomly assigned to replicates and treatments, and pots were randomly arranged in a single array. Each ramet of *Carpobrotus* and plant of *A. arenaria* was weighed to determine initial fresh mass just before treatments began. During the experiment, plants were watered as necessary with tap water to maintain soil near field capacity and avoid water stress. The experiment was conducted in the same experimental garden used for propagation, under ambient light and temperature.

### Measurements and statistical analyses

Treatments were ended after 110 days, on 7 July 2017, when plants started to show crowding of roots at the bottom of pots. Each ramet of *Carpobrotus* plus its new roots, stems, and offspring ramets was weighed for fresh mass and then separated into shoots (i.e. leaves plus stems) and roots, dried at 70 °C for 72 h, and weighed again for dry mass. Fourteen plants died during the experiment and were excluded from analyses. These were three *C. edulis* from South Africa grown alone in low nutrients; two *C. edulis* from South Africa in competition with *A. arenaria*; two *C. edulis* from South Africa in competition with *C. virens*; one *C. edulis* from South Africa grown alone in high nutrients; one *C. edulis* from California in competition with *C. chilensis*; one *C. chilensis* grown alone in low nutrients; one *C. chilensis* in competition with *C. edulis*; one *C. chilensis* grown alone in high nutrients; one *A. arenaria* grown alone in low nutrients and one *A. arenaria* in competition with *C. edulis*.

Relative growth rate (RGR, biomass gained per unit of biomass and time,  $(\text{g}\cdot\text{g}^{-1}\cdot\text{day}^{-1})$ ) was calculated as  $[\text{Ln}(\text{FWt}_2) - \text{Ln}(\text{FWt}_1)] / (t_2 - t_1)$ , where  $\text{FWt}_1$  and  $\text{FWt}_2$  are, respectively, fresh mass at the start ( $t_1$ ) and the end ( $t_2$ ) of the experiment, and  $(t_2 - t_1)$  is the duration (days) of the experiment. Root to shoot ratio (RSR) was calculated as dry mass of roots divided by dry mass of shoots. Response to high nutrients was measured by comparing final dry mass between low- and high-nutrient treatments and also by subtracting mass of each replicate in the low-nutrient treatment within a species and region from a randomly chosen replicate in the high-nutrient treatment of the same species and region. Response to competition was calculated using a relative interaction index (RII, Armas *et al.* 2004,  $[\text{B}_w - \text{B}_o] / [\text{B}_w + \text{B}_o]$ ), where  $\text{B}_w$  is total final dry mass when grown with another plant, and  $\text{B}_o$  is total final dry mass when grown alone. This index is symmetrically distributed around zero and ranges from  $-1$  to  $1$ ; negative values indicate competition and positive values indicate facilitation. Replicates were obtained by randomly matching a plant grown alone to one grown with another plant from the same species and region in the low-nutrient treatment.

Data were analyzed using one-ANOVAs and two-way ANCOVAs with species of *Carpobrotus*, soil nutrient availability (low or high) and region of collection as fixed effects depending upon the analysis. Initial fresh mass was included as a covariable except in analyses of RGR, RII and change in dry mass. We used Kolmogorov–Smirnov and Levene tests ( $P > 0.05$ ) to test whether data met the assumptions of normality and homocedasticity, and transformed data when necessary, as indicated in figures. When an effect with more than two levels was significant ( $P < 0.05$ ), a Tukey test was used to detect differences among individual means. Analyses were conducted with IBM SPSS Statistics, version 23 (IBM Corp., Armonk, New York, USA).

## RESULTS

### Differences between species of *Carpobrotus*

Despite selection of ramets of similar developmental stage in all *Carpobrotus* species, initial fresh mass was one-fourth to two times greater in the invasive species *C. edulis* and the less widespread invasive species *C. acinaciformis* than in the non-invasive species *C. chilensis* and *C. virescens* (Fig. 3). Although RGR was significantly lower in *C. edulis* than in

*C. chilensis* (Fig. 4a: effect of species (S)  $P = 0.025$ ), final total and shoot dry masses were significantly greater in *C. edulis* even when tested with initial fresh mass as a covariate (Fig. 4b and d: effect of species (S), each  $P < 0.05$ ). Both *C. chilensis* and *C. edulis* increased RGR much more and accumulated more final mass when given high nutrients relative to low nutrients (Fig. 4a–e: effect of nutrients (N), each  $P < 0.01$ ). Effects of nutrient treatments did not differ between the two species (Fig. 4a–e: interactive effects of species and nutrients (X), each  $P > 0.4$ ), failing to support the prediction that an invasive, introduced species would respond more to high nutrients than a non-invasive, introduced one (Fig. 1). The effects of increased nutrients on growth did not differ between *C. edulis* and *C. acinaciformis* (Fig. 4f–j), supporting the prediction that invasive introduced species would respond similarly to high nutrients (Fig. 1). Final root/shoot mass (RSR) was greater at high than at low nutrients in both species (Fig. 4e and j; see discussion).

*Carpobrotus edulis* showed a negative response to the presence of *C. chilensis* as measured by a relative response index (RII) based on final total dry mass (Fig. 5), indicating competition. In contrast, *C. chilensis* showed a positive response to the presence of *C. edulis*, indicating facilitation. That is, *C. edulis* had lower biomass in the presence of *C. chilensis*, whereas *C. chilensis* had greater biomass in the presence of *C. edulis*. This failed to support the prediction that an invasive, introduced species would outcompete a non-invasive, introduced one (Fig. 1). Responses of *C. edulis* and *C. acinaciformis* to each other were marginally more negative in *C. acinaciformis* (Fig. 5;  $P = 0.05$ – $0.1$ ), failing to support the prediction of similar competition response of invasive introduced species to each other (Fig. 1). However, the responses of the two species to the presence of the co-occurring, dominant, unrelated native species *A. arenaria* did not differ (Fig. 5), supporting the prediction that invasive introduced species would show a similar negative competitive response to an unrelated native species (Fig. 1).

### Differences between native and introduced *C. edulis*

Initial fresh mass was significantly lower in *C. edulis* from the native than the introduced range and did not differ significantly between the three regions in the introduced range (Fig. 3). Effects of nutrient treatments on RGR and on final dry mass adjusted for initial fresh mass did not differ significantly ( $P > 0.1$ )

between *C. edulis* from different regions (Fig. 6a–d: interactive effect of region and nutrients), except that the positive effect of high nutrients on root/shoot mass was marginally greater ( $0.05 < P < 0.1$ ) in introduced than in native plants (Fig. 6). However, response to nutrients was highly significantly greater ( $P < 0.01$ ) in introduced than in native plants (Fig. 7). Taken together, these results were partly consistent with the prediction that introduced plants of an invasive species would respond more to high nutrients than native plants of the species (Fig. 1).

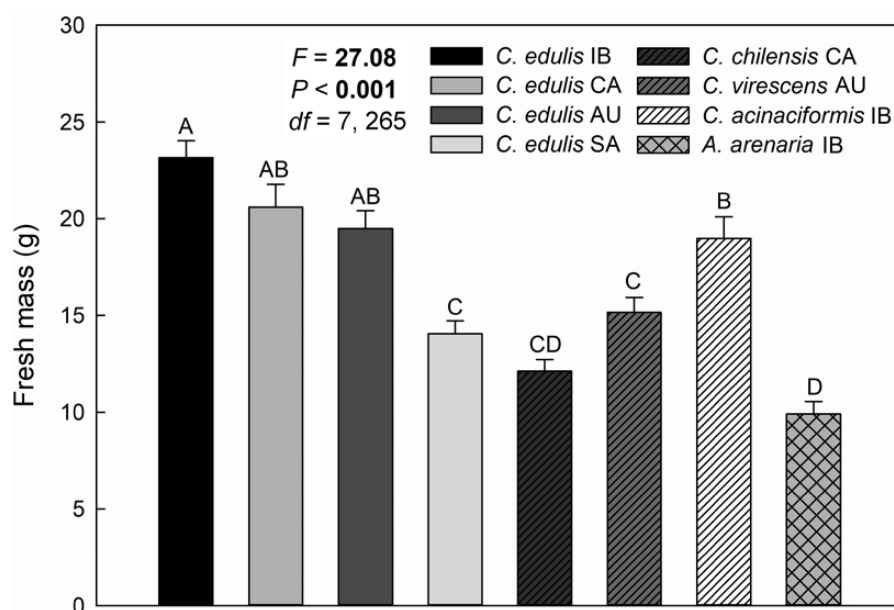
As measured by final total dry mass, the responses of native South African and introduced Australian *C. edulis* to the presence of native Australian *C. virescens* were negative and did not differ (Fig. 8a). Similarly, the responses of native South African and introduced Iberian *C. edulis* to the presence of native Iberian *A. arenaria* were negative and did not differ (Fig. 8b). This did not support the predictions that invasive introduced plants would have greater ability than native plants of the same species to compete with a congener or with an unrelated native species (Fig. 1).

## DISCUSSION

Our findings provided little evidence that competitive ability in introduced plants contributes to invasiveness or is selected for during or after introduction. For example, the highly invasive, introduced species *Carpobrotus edulis* showed a more negative response

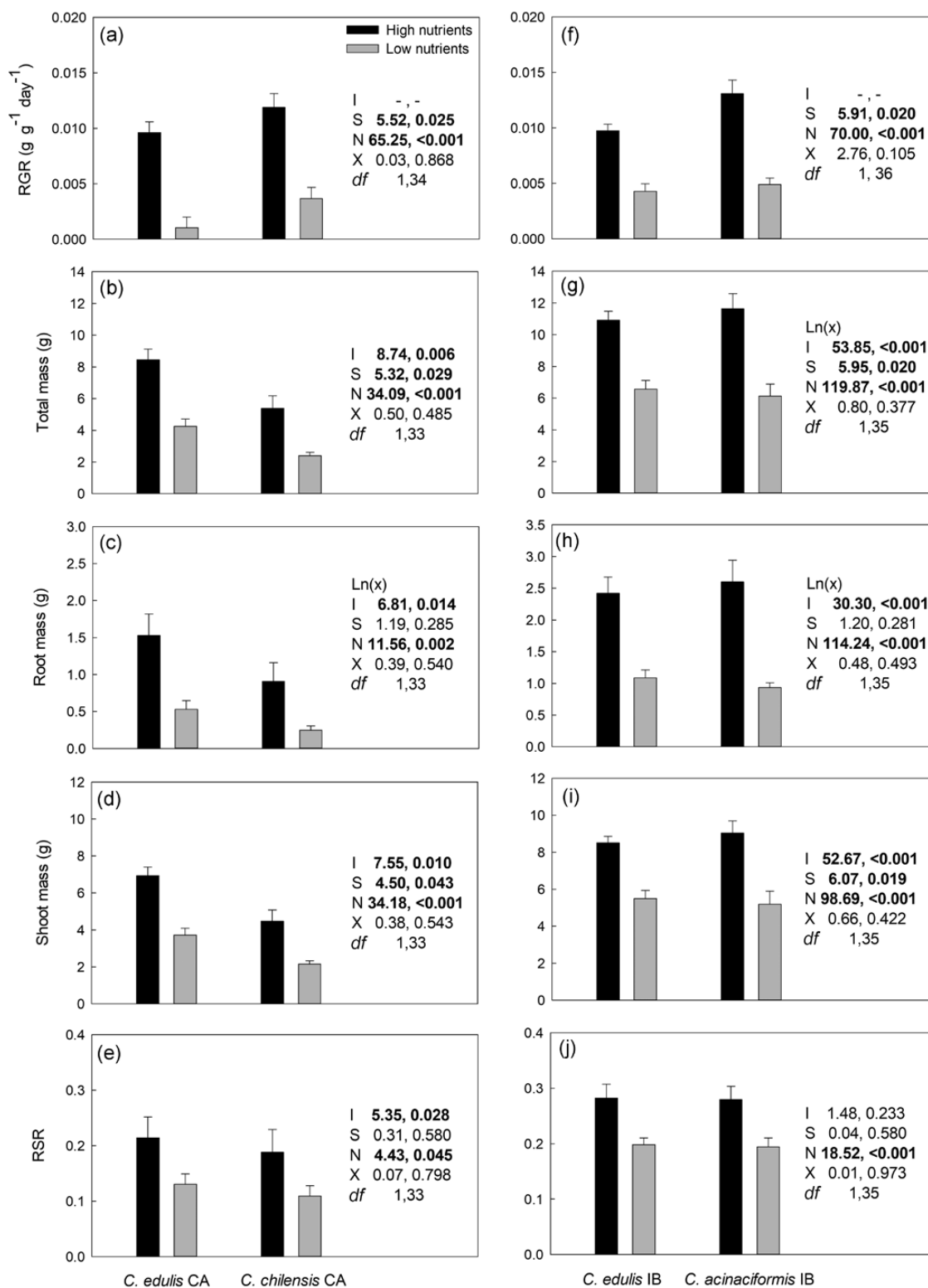
to co-occurring, introduced, non-invasive *C. chilensis* than vice versa. In fact, performance of *C. chilensis* was apparently facilitated by the presence of *C. edulis*, possibly due to the high ability of *C. edulis* to add organic matter to soil and ameliorate soil conditions (Conser and Connor 2008; Fenollosa *et al.* 2016). Introduced and native *C. edulis* did not differ in competitive response to either a native congener or the co-occurring, unrelated, native species *Ammophila arenaria*.

Results likewise provided little evidence that invasiveness is associated with response to increased nutrient availability. Response to high nutrients did not differ between *C. edulis* and either *C. chilensis* or *C. acinaciformis*. The latter comparison helps confirm that *C. acinaciformis* is potentially as invasive as *C. edulis* in Iberia despite being less widespread so far. Both species were introduced to the region by the beginning of the twentieth century for soil stabilization and as ornamentals (Campoy *et al.* 2018; Gonçalves 1990). For management purposes in Iberia, it may be wise to consider the two species as equally invasive; in Portugal, only *C. edulis* is currently listed as a problem species. It may be that the invasiveness of the two species has been increased by hybridization. Spread of introduced *Carpobrotus* in southern Europe has likely been increased by rapid expansion of the hybrid *C. aff. acinaciformis* (Campoy *et al.* 2018; Suehs *et al.* 2004). Campoy *et al.* (2019) found that *C. aff. acinaciformis* showed greater relative growth rate and water-use

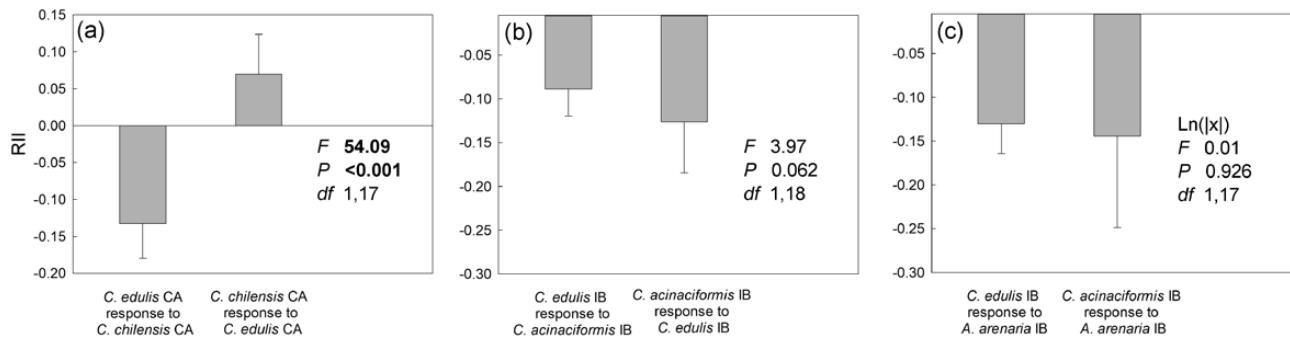


**Figure 3:** Initial fresh mass (mean + SE). AU = Australia, CA = California, IB = Iberia, SA = South Africa. Letters show which means differed (Tukey test,  $P < 0.05$ ).





**Figure 4:** Effect of nutrient treatments (mean + SE) on *Carpobrotus edulis* and *C. chilensis* from California (CA, **a–e**) and on *C. edulis* and *C. acinaciformis* from Iberia (IB, **f–j**): **a, f**—relative growth rate (RGR); **b, g**—final total dry mass; **c, h**—final dry root mass; **d, i**—final dry shoot mass; **e, j**—final dry root/shoot mass (RSR). Inset tables give ANOVA and ANCOVA results ( $F$  followed by  $P$ ); effects: I—initial fresh mass (covariate); S—species; N—nutrients; X—S  $\times$  N; Ln(x) means data were log-transformed.



**Figure 5:** Competitive response (relative interaction index, RII; mean + SE) of (a) *Carpobrotus edulis* and *C. chilensis* from California (CA) to each other, (b) *C. edulis* and *C. acinaciformis* from Iberia (IB) to each other, and (c) *C. edulis* and *C. acinaciformis* to *Ammophila arenaria* from Iberia. Inset tables give one-way ANOVA results.

and photochemical efficiency than *C. edulis*, while *C. edulis* was more responsive to nutrient availability than the hybrid. Future research is needed to test whether the hybrid is more invasive than the parental species.

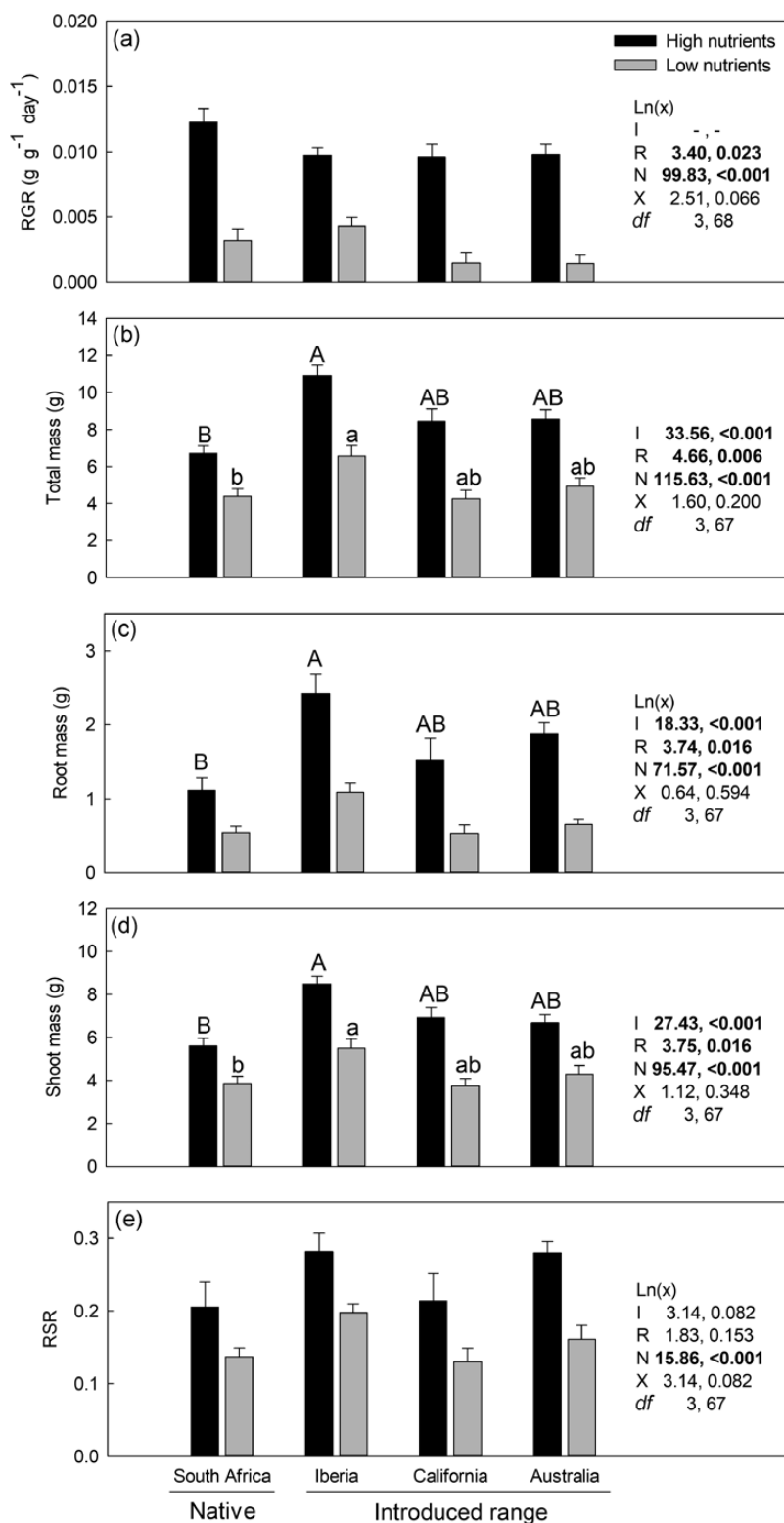
Results did provide evidence of selection for increased response to high nutrients during or after introduction. Analysis of final total dry mass without adjustment for initial fresh mass showed a greater difference between net accumulation of mass at high and low nutrient levels in introduced *C. edulis* from Australia, California and Iberia than in native *C. edulis* from South Africa. Adjusted final total dry mass showed the same pattern, but it was not statistically significant. Previous studies have found a greater benefit of physiological integration between connected ramets in Iberian than South African *C. edulis* (Roiloa *et al.* 2016) and differences between native and introduced populations of the species in allocation of mass (Portela *et al.* 2019), likewise suggesting selection during or after introduction.

Introduced and native plants within species might differ due to genetic drift or founder effects, rather than selection pressure (Keller and Taylor 2008; Lachmuth *et al.* 2011). However, this seems unlikely to explain the consistent difference found here between native *C. edulis* and introduced plants on each of three continents. Further genetic studies could help confirm that this finding represents selection in association with introduction. One other important caveat is that apparent differences in invasiveness between introduced species may be due to different invasion histories, particularly time since introduction (Larkin 2012; van Kleunen *et al.* 2010). Similarly, lack of differences in apparent invasiveness could be due to insufficient time since introduction. The species in this study were all introduced at least

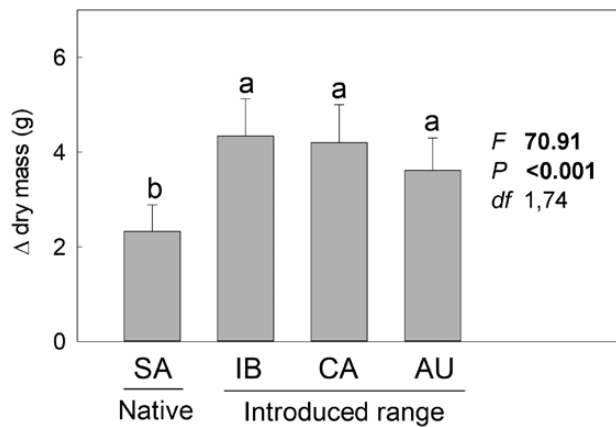
a century ago to each of the introduced regions in the study, but time might still be a factor.

Although not included in predictions, initial fresh mass per ramet was the measure most consistently associated with introduction and invasiveness. Ramets propagated for the experiment from introduced populations of the two invasive species, *C. edulis* and *C. acinaciformis*, had significantly greater fresh mass at the start of treatments than ramets from native populations of *C. edulis* and the non-invasive species *C. chilensis* and *C. virescens*, despite the experiment using standardized ramet morphology across species. Because we did not randomly select ramets for inclusion in the experiment, but rather used a consistent morphological definition, this measure may not have been free of bias. However, previous studies overall have found a positive relationship between size and invasiveness in introduced plant species (van Kleunen *et al.* 2010). Roiloa *et al.* (2019) likewise reported that comparable ramets of *C. edulis* had more mass than those of *C. chilensis* in California. Numerous studies have also found that plants from introduced populations of an invasive species are larger than plants from native populations of the same species when grown in a common garden (e.g. Getman-Pickering *et al.* 2018; Sun and Roderick 2019).

One unexpected result was that the proportion of final dry mass in roots was higher at high than at low nutrients. This was true for all species and regions tested. It was unexpected because allocation to roots typically decreases with increasing soil resource availability. One possible explanation is that plants produced more ramets when given the higher level of nutrients but that these ramets did not develop extensive roots before harvest and instead largely imported nutrients from the original, connected ramet. Previous studies show



**Figure 6:** Response (mean + SE) of relative growth rate and final dry mass to nutrient treatments in native and introduced *Carpobrotus edulis*: (a) relative growth rate (RGR); (b) total mass; (c) root mass; (d) shoot mass; (e) root/shoot mass (RSR). Letters above bars show which means differed (Tukey test,  $P < 0.05$ ) within the high nutrient treatment (uppercase) and within the low nutrient treatment (lowercase); no letter indicates that means did not differ. Inset tables give ANOVA and ANCOVA results ( $F$  followed by  $P$ ); effects: I = initial fresh mass (covariate); R = region; N = nutrients; X = R  $\times$  N; Ln(x) means data were log-transformed before analysis.



**Figure 7:** Effect of region (SA = South Africa; IB = Iberia; CA = California; AU = Australia) on difference ( $\Delta$ , mean + SE) in final total dry mass between high and low nutrient treatments in *Carpobrotus edulis*. Letters above bars show which means differed (Tukey test,  $P < 0.05$ ). Inset tables give one-way ANOVA results.

that connection to a ramet with relatively low direct access to nutrients can increase allocation to roots in a connected ramet with higher access in various species of clonal plants, an instance of what has been termed division of labor (e.g. [Alpert and Stuefer 1997](#); [Roiloa et al. 2007](#)). Subsequent work has shown capacity for division of labor in *C. edulis* ([Campoy et al. 2017](#); [Roiloa et al. 2014, 2016](#)). However, no studies appear to have specifically tested whether connection to juvenile unrooted ramets increases greater relative allocation to roots in established ramets.

Identification of mechanisms underlying invasiveness of introduced species is complex

because invasion depends on the means and history of introduction as well as context relating to the new habitat and its organisms. Moreover, invasiveness as an outcome—the rapid range extension of a population—is not readily quantified at an individual level via plant-level traits and interactions between individuals. Comparison of similar, closely related species with strong apparent differences in invasiveness and repeated introductions to different regions, such as species of *Carpobrotus*, is one promising way to overcome some of these difficulties.

### Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

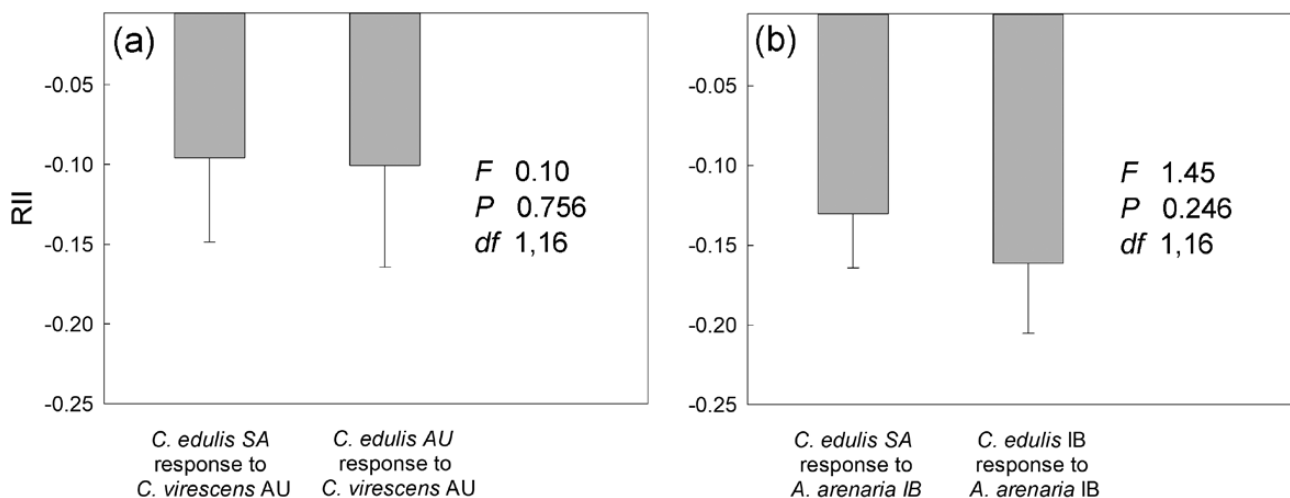
Table S1: Specifications of the collection sites and species collected.

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**Figure 8:** Competitive response (relative interaction index, RII; mean + SE): (a) native South African (SA) and introduced Australian (AU) *Carpobrotus edulis* to native Australian *C. virescens*; (b) native and introduced Iberian (IB) *C. edulis* to native Iberian *Ammophila arenaria*.



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*Conflict of interest statement.* The authors declare that they have no conflict of interest.

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