4   **Field comparison of ecophysiological traits between an invader and a native**
5   **species in a Mediterranean coastal dune**

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15   **ABSTRACT**
16   Photochemical efficiency, gas exchange, leaf water potential and pigment and free proline

17   content of *Oenothera drummondii* subsp. *drummondii* Hook (an invasive non-native

18   species) and *Achillea maritima* (L.) Ehrend. & Y.P. Guo, (an ecologically similar native

19   species) were explored to understand the success of invasive non-native species in

20   Mediterranean coastal dunes of southwest Spain. We have conducted a field study during

21   a complete annual cycle, comparing both species. Fifteen pairs of neighbouring plants of

22   the two study species of similar size were selected and measured seasonally. The results

23   show that in spring and summer, assimilation rates of *O. drummondii* were significantly

24   higher than those of the native, *A. maritime,* even though the native species had higher

25   photochemical efficiency. Additionally, the non-native species presented better water

26   content regulation than the native one, surely related to better water use efficiency and

27   may be linked to greater root development. The differences in leaf dry matter content

28   values for both species might indicate a different strategy of resource use; with *A.*

29   *maritima* displaying a more conservative strategy and *O. drummondii* presenting a rapid

30   resource acquisition and use strategy as predictors of rapid growth and soil fertility. We

31   conclude that *O. drummondii* utilizes light, water and probably nutrients more efficiently

32   than the native *A. maritima* and suffers lower stress in Mediterranean coastal dunes where

33   water availability is reduced (44 mm from May to October in the study area) and light

34   radiation levels are high.

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36   **Keywords:** *Achillea maritima;* Invasiveness; Leaf water potential; Mediterranean-type

37   ecosystems; *Oenothera drummondii*; Photosynthesis.

Introduction

Coastal dunes are complex and dynamic ecosystems where vegetation is subjected to many stressors including salinity, episodic overwash, water stress, high temperature, high winds, sand burial, substrate instability, low field capacity, and nutrient scarcity as the most common abiotic stressing factors that influence plant community assembly processes in dune systems (Hesp, 1991; Maun, 2009). Additionally, physiological plant activity in Mediterranean-type ecosystems is limited during summer by high temperatures, water scarcity and high radiation levels (Cowling, et al. 2005; Godoy et al., 2011), but it is also limited in winter through the combination of low temperatures with high radiation (Oliveira and Peñuelas, 2004; Flexas et al., 2014). Therefore, in the coastal dunes of the Mediterranean type climate, these stressors reinforce each other intensifying the vegetation stress level. As a consequence of the notable selection pressure imposed by these environmental conditions, the coastal dune vegetation has a high degree of specialization (Novoa et al., 2012), particularly in Mediterranean type climate. In beach and embryo dunes frequent occurrence of intense disturbance events provide invasive species with the opportunity to colonize novel areas (Lortie and Cushman 2007), additionally, in inland dunes, intermediate levels of disturbance and stress can also offer good conditions for the settlement of invasive species (Carboni et al., 2010). That is, the patterns of species invasions on coastal dunes depend on the environmental conditions and the species involved (Gallego-Fernández et al., 2019).

Coastal dune environmental conditions impose adaptations to plants that stablish in these areas. For instance, to withstand salt, plants increase leaf thickness and the degree of succulence, while to cope with burial they produce larger seeds and have stolons or rhizomes (Hesp, 1991). Water-limited resources are a primary stressor in coastal dune ecosystems; therefore, many coastal dune plants have adaptations to resist dryness. The adjustments to dryness occur at different plant levels: physiological, reproductive, morphological at the leaf, roots, or the whole plant structure. At the leaf level, for example, plants can adjust leaf rolling, thickness, orientation, hairiness, size or epicular wax. At physiological levels, plants can have osmotic adaptations to increase water use efficiency or different photosynthetic pathways such as C4 and CAM (Hesp, 1991).

As a result of the high specialization grade imposed by these harsh environmental conditions, these ecosystems support many threatened and endemic species which are especially sensitive to invasions by non-native plants. One of the greatest threats of

natural habitats around the world is the introduction of exotic species (Sax and Gaines, 2003) and on coastal dune ecosystem in particular, this has been assessed as high and still increasing (Millennium Ecosystem Assessment, 2005; Novoa et al., 2012), mainly due to the frequent stressor and the existence of open patches free of plant competition (Carboni et al., 2010; Antunes et al., 2018).

The success of invasive plants on novel environments depends on several factors, such as the adaptive plasticity of their morphological or physiological traits and the environmental conditions of the new area. Alternatively, numerous studies (Oduor et al., 2016; Liao et al., 2016) support that pre-adaptation or rapid adaptive evolution to new ecological niches could be as important as plasticity to explain the ability of invasive plants to persist and expand in new-introduced habitats. These two processes would not be mutually exclusive. Thus to maximize fitness in response to new environmental conditions a combination of plasticity and rapid genotypic adaption could occur. According to Liao et al. (2016) plasticity plays an important role in invasiveness, but also local genotypic variation in plasticity does.

Besides, also the biological interactions (such as plant-plant competition or plant-herbivore interaction) have an ecological impact on original communities and in the ecosystem function which is crucial for the success of plant invasion (Ehrenfeld, 2010; Bottollier-Curtet et al., 2013). Invasive plants commonly compete so effectively in new ecosystems that they displace native species and alter ecosystem function and ecosystem services. Van Kleunen et al. (2010) studied what functional traits were associated with invasiveness (physiology, allocation, growth, size or fitness), considering invasiveness as the capacity of plants to spread through other biota and ecosystems. They found that invasive non-native species had higher values for traits related to performance than non-invasive species. The question of what kinds of traits promote plant invasiveness is of utmost significance for understanding plant success in general, and particularly for understanding the mechanisms of non-native plant invasions. However, studies that suggest that invasive species have advantages over native ones have had irregular results and moreover, the available data of the positive effect of physiological traits on invasive species' fitness are inconsistent.

Nonetheless, invasive species expected to have physiological traits (such as higher photosynthetic rates or better stomatal control) than allow them to take advantage of a better physiological status and overcome natives. A comparative study of physiological

plant activity in co-occurring invader and native species is a useful tool to improve understanding of the invasiveness of exotic plants. Plant ecophysiological characteristics must be well adapted to their local environmental conditions (Ackerly et al., 2000) so that, the physiological response of plants to local conditions can play a fundamental role in species' ecological distribution (Körner and Diemer, 1987; Brodersen et al., 2008). Physiological adjustments allow species to maintain their fitness under different environmental conditions (Becklin et al., 2016). Among these ecophysiological characteristics, photosynthesis and water status-related traits have been proven to have a considerable influence on the invasive success of non-native species (McDowell, 2002).

Oenothera drummondii subsp. *drummondii* is a non-native invasive species of coastal dunes around the world; native to coastal dunes of the Gulf of Mexico and in the last century has colonized coastal dunes all over the world (Dietrich and Wagner, 1988). Nowadays it has spread over coastal areas of different continents, being considered invasive in Spain, Israel, China and Australia and naturalized in many places of the world, shifting to regions with different climatic characteristics (Dietrich 2000; Heyligers 2008; Campos and Herrera 2009; Xu et al. 2012, Dufour-Dror 2013; García de Lomas et al. 2015).

In coastal dunes of southwestern Spain, *O. drummondii* has produced a strong impact on native communities by modifying the composition and abundance of the species (García de Lomas et al., 2016; Gallego-Fernández et al., accepted). To understand some of the causes of the success of this non-native species we have compared our observations of *O. drummondii* with a species of similar characteristics that has been displaced after the invasion and that used to be highly abundant, *Achillea maritima*. Both are small-sized plants (40-50 cm tall) with reproduction mainly by seeds. The purpose of this study is to establish whether the success of *O. drummondii* can be explained in part by differences from native species in the physiological response to Mediterranean coastal dune environment. To answer this question, we compared the physiological performance of both species measuring the photochemical efficiency, gas exchange, leaf water potential and free proline and pigment content under field conditions over the whole year. All these ecophysiological variables have proven to have a considerable influence in the invasive success of the non-native species (McDowell, 2002) while free proline in leaves has been widely proved to accumulate as a response to numerous stressors (such as drought or cold temperatures). Additionally, weather conditions also induce changes in

138 the pigment content (Hoffmann and Parsons, 1997; Gratani, 2014, Zunzunegui et al.,

139 2016). We hypothesized that *O. drummondii* would exhibit a high capacity to adapt to

140 Mediterranean environmental conditions presenting higher photochemical efficiency,

141 assimilation rates and water use efficiency than the native species

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143 **Material and methods**

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145 Study site

146 The study was accomplished on a coastal dune system located in the protected area

147 "Marismas del Odiel" close to Huelva city, SW Spain (37º09'N, 6º54'W). The climate is

148 Mediterranean with hot summer; the type is Csa, according to the Köppen-Geiger

149 classification (Peel et al., 2007). The mean annual temperature is 18.1 °C while the mean

150 minimum and maximum temperatures are 13.3 and 21.7° C, respectively. Average annual

151 rainfall is 467 mm, with a pronounced drought period in summer (data from Agencia

152 Estatal de Meteorología at Huelva Meteorological Station, 30-year record from 1971 to

153 2000). The year of the study, with precipitation of 296 mm, was 36% dryer than the annual

154 average (Fig. 1), with remarkably low precipitation during the warmest period of the year

155 in which the accumulated rainfall from May to October was 44 mm.
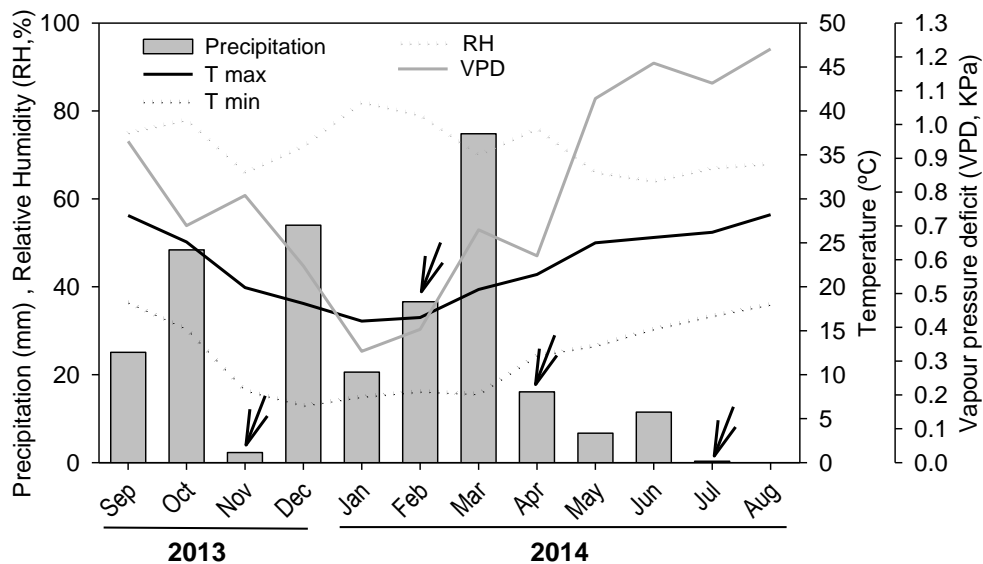
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159 **Fig. 1** Monthly precipitation, daily maximum and minimum mean temperatures and air

160 relative humidity during the study period (2013-14 hydrological cycle). Arrows indicate

161 sample periods.

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163   Study species

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165   *Oenothera drummondii subsp. drummondii Hook.* (*Onagraceae*), is a short-lived leaf

166 perennial species. The main stems are erect to procumbent while the basal side stems are

167 prostrate or ascending to about 50 cm in height, with a strong taproot. Flowers are self-

168 compatible, outcrossing and pollinated by hawkmoths in their native habitat (Wagner et

169 al., 2007). The species reproduces and spreads through small and numerous seeds. In its

170 native range area, the species grows in coastal dunes with humid subtropical and wet

171 tropical climates (Cfa, Aw, Am) according to the Köppen-Geiger classification (Peel et

172 al., 2007). The first records of *O. drummondii* in the southwest of the Iberian Peninsula

173 date from 1957 (Silvestre, 1980) and in the study area it was registered for the first time

174 in 1996 (García Mora com. pers).

175   *Achillea maritima* (L.) Ehrend. & Y.P. Guo is a pioneering herbaceous perennial

176 species, with 20-40 cm of height, from the *Asteraceae* family (Ehrendorfer and Guo,

177 2005). This species grows in sand coast dunes from Europe and Morocco and exerts a

178 stabilizing action on the sandy soils (García Novo and Merino, 1993). In the Iberian

179 Peninsula, the species is in regression, having disappeared, or almost, in some regions

180 (Silván and Campos, 2002; Mayoral, 1999) mainly because of the loss of its natural

181 habitat due to anthropic pressure. Thick white fuzz covers both the stems and the small

182 oval, alternate leaves. The globose yellow flowers are entomophilous and visible from

183 June to September. The selection of this species was based on the fact that it is one of the

184 most abundant native plants in the studied site and has similar functional traits to the

185 invasive species. Moreover, Gallego-Fernandez et al. (2019) found evidence that this

186 species is out-competing *A. maritima* since in dunes invaded by *O. drummondii* the cover

187 of *A. maritima* is 91% lower than in non-invaded dunes.

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189 Experimental design

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191 Field measurements were made in a 50 x 50 m plot located inside the dune zone. In this

192 plot, we randomly selected 15 pairs of neighbouring mature plants of *Oenothera*

193 *drummondii* and *Achillea maritima* of similar size. All pairs were separated more than 2

194 meters from each other. The measurements were carried out with a seasonal periodicity

195 during an annual cycle: November (autumn, with mild temperatures and when the first

196 rains after the summer drought allow plants to recover water status), February (winter,

197 the period of the wet season with the coldest month), April (spring, period with mild but

increasing temperatures and at the end of the rainy season) and July (summer, with the highest temperatures and radiation coupled with drought).

Photochemical efficiency of photosystem II: Chlorophyll fluorescence kinetics was determined in the field on healthy fully expanded leaves using a pulse-amplitude modulation technique through a portable fluorometer (mini-PAM, Walz, Effeltrich, Germany). Fluorescence was excited by a saturating pulse of red light (approximately 3000 µmol m^{-2}s^{-1} for 0.8 s) from a diode (type H-3000 LED, Stanley) attached to a fibre optic. Leaves were exposed to a weak modulated measuring beam from a LED at a frequency of 600 Hz to determine F_0 and F and then to the pulse of saturating red light to assess F_m and F'$_m$.

Maximum photochemical efficiency of PSII (F_v/F_m) was measured on dark-adapted leaves (leaves were artificially maintained 20 minutes in darkness by means of leaf-clips, a time interval considered sufficient to complete the reoxidation of all the reaction centres of photosystem II) from the ratio of variable fluorescence to maximum fluorescence as $F_v/F_m = (F_m-F_0)/F_m$, where F_0= initial and F_m= maximal fluorescence (Genty et al., 1989). Effective photochemical efficiency of PSII (Φ_{PSII}) was estimated on light-adapted leaves as $\Phi_{PSII}= (F'_m-F)/F'_m$, where F'$_m$= maximal and F steady-state fluorescence under actinic irradiance (Genty et al., 1989).

Three leaves were measured per plant at 9:30-11:30 h solar time (mean values per plant were used for statistical analysis).

Gas exchange measurements: Measurement of net photosynthetic or net CO_2 assimilation rate (A_n, µmol CO_2 m^{-2}s^{-1}), transpiration rate (E, mmol H_2O m^{-2}s^{-1}), stomatal conductance rate (g_s, mol H_2O m^{-2}s^{-1}) and leaf intercellular CO_2 concentration (Ci, vpm) were taken on mature leaves during clear sunny days, using portable open system, compact infra-red gas exchange analyser (LCi-Portable Photosynthesis, ADC, UK). From these set of data, we calculated instantaneous water use efficiency (WUE$_i$, mmolCO_2 mol$^{-1}H_2O$) as the ratio of net photosynthesis to transpiration (A_n/E) indicative of the moles of CO_2 assimilated per mol of H_2O lost, and instantaneous carboxylation efficiency (A_n/C_i, molCO_2 m^{-2}s^{-1}) as the ratio of net assimilation to leaf intercellular CO_2 concentration.

Three leaves per plant were measured and the average values per plant calculated. The surface of leaves used for gas exchange measurements was calculated in the laboratory using the image area analyser software Midebmp (Ordiales, Spain, 2000).

230 Measurements were made from 8:30 to 10:30 h, (solar time), to avoid possible midday

231 depression of stomatal conductance and to get maximum net photosynthetic rates.

232 *Leaf water potential:* Plant water status was evaluated through midday leaf water

233 potential (Ψ_{md}) and was determined with a pressure chamber (Manofrigido, Lisbon,

234 Portugal) in the field using terminal shoots which were excised and immediately

235 measured. The readings were made between 12:30 and 14:00 (solar time), when the

236 greatest water deficit of the day occurs and when the minimum values are reached.

237 *Leaf parameters:* Leaves used and scanned for photosynthesis measurements were

238 dried at 70 °C for 48 h and weighted (Leaf mass area (LMA) was calculated as the ratio

239 of dry leaf mass (Md) to fresh leaf area (g m^{-2}).

240 New, fully expanded, healthy leaves were collected from all plants selected for

241 physiological measurements. Leaves for relative water content (RWC) and leaf dry matter

242 content (LDMC) measurements were kept refrigerated and stored in plastic bags until

243 fresh mass (Mf) was recorded within 3 h. After weight, leaves were then hydrated with

244 distilled water to saturation for 24 h at 5°C in the plastic bags. Leaves were dried with

245 tissue paper to remove any surface water, and then immediately weighed to obtain

246 saturated mass (Ms). Finally, leaf samples were then dried at 70 °C for 48 h and weighted

247 (Md). We estimated RWC (%) as follows: $\frac{Mf-Md}{Ms-Md} x100$

248 The ratio LDMC (mg g^{-1}), also known as tissue density was calculated as Md/Ms.

249 The rest of the collected leaves were kept in liquid nitrogen in the field and preserved

250 in the laboratory at −24 °C until analysis. Photosynthetic pigments were quantified

251 following Lichtenthaler (1987) on a dry mass basis. Chlorophyll a (Chl a), b (Chl b) and

252 total carotenoid (Car) pigments were extracted with 100 % acetone and determined

253 spectrophotometrically. From these data were calculated the ratio chlorophyll a/b (Chl

254 a/b) and the ratio of total carotenoids to total chlorophylls (Car/Chl),

255 Free proline content was determined colourimetrically by the ninhydrin acid method

256 of Bates et al. (1973).

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258 Statistical analyses

259 Two-way ANOVAs were used to investigate the effects of species and season

260 (independent factors) on ecophysiological variables (dependent factor). Post-hoc Tukey

261 tests were then used for appropriate pair-wise comparisons. Bivariate correlation tests

262 were made between the different variables. A multivariate principal component analysis

263 (PCA) was performed with the physiological and leaf parameters matrix (21 variables x

264   4 seasons x 2 species x 15 plants) to identify which are the variables responsible for the

265   different seasonal response between species. The normality of all measured variables was

266   first checked with the Kolmogorov–Smirnov test. The statistical tests were considered

267   significant at the $P < 0.05$ level. All statistical tests were made with the software package

268   IBM SPSS Statistics for Windows, Version 24 (Armonk, NY, USA).

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270   **Results**

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272   Photosynthetic rates ranged close to 20 μmol m^{-2}s^{-1} in the non-native species throughout

273   the year, whereas in the native this variable was seasonal dependent, with pronounced

274   drops in spring and summer (Fig. 2A). *O. drummondii* assimilation rates were

275   significantly higher than those of *A. maritima* in spring and summer ($P < 0.008$), while

276   no differences occurred in autumn and winter. As observed for A_n, and responding to

277   summer stressful conditions, native plants had lower A_n/C_i values in spring ($P < 0.016$)

278   and summer ($P < 0.001$) than non-native plants (Fig. 2B). Native plants showed lower g_s

279   in spring and summer but both species showed similar g_s values after the first rains in

280   autumn and winter (Fig. 2C). Non-native species showed greater WUE_i than native plants

281   during the drought period (Fig. 2D).

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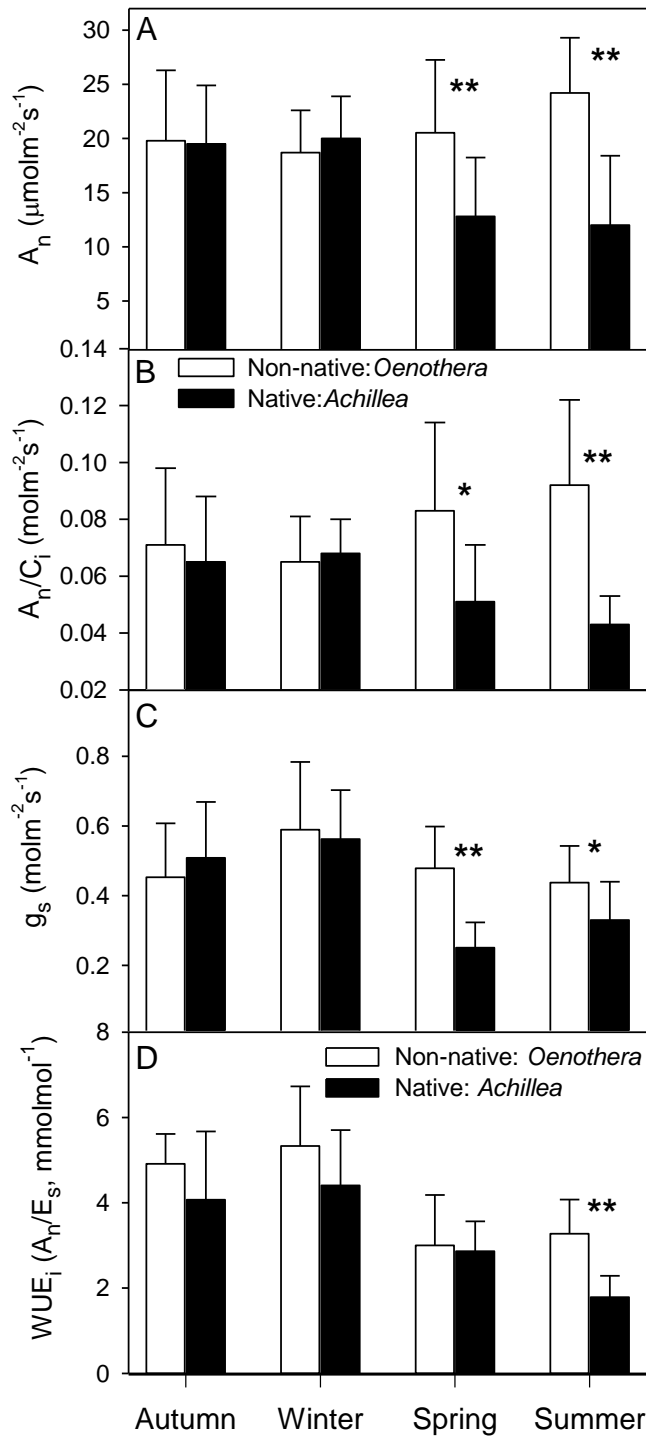290

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Fig. 2 Mean values (±sd) of A_n: photosynthesis rate (A), A_n/C_i: instantaneous carboxylation efficiency (B), g_s: stomatal conductance (C), and WUE_i: instantaneous water use efficiency (D) in the two study species, over the 2013-14 annual cycle. Asterisks denote significance levels for the comparisons between the two species by Student's t-tests (**$P < 0.001$; *$P < 0.05$).

Maximum photochemical efficiency (F_v/F_m) was lower in the non-native than in the native species, these differences being significant in spring, summer and autumn (Fig. 3). Also, Φ_{PSII} was higher in the native species in winter and spring.
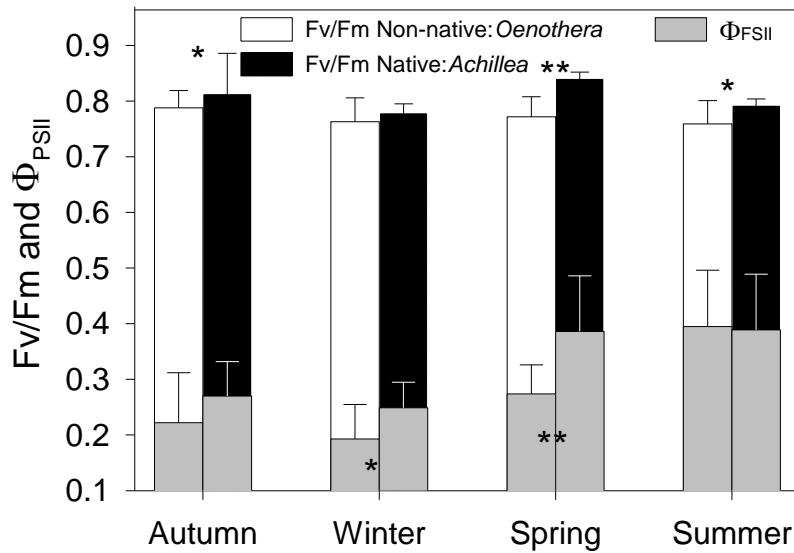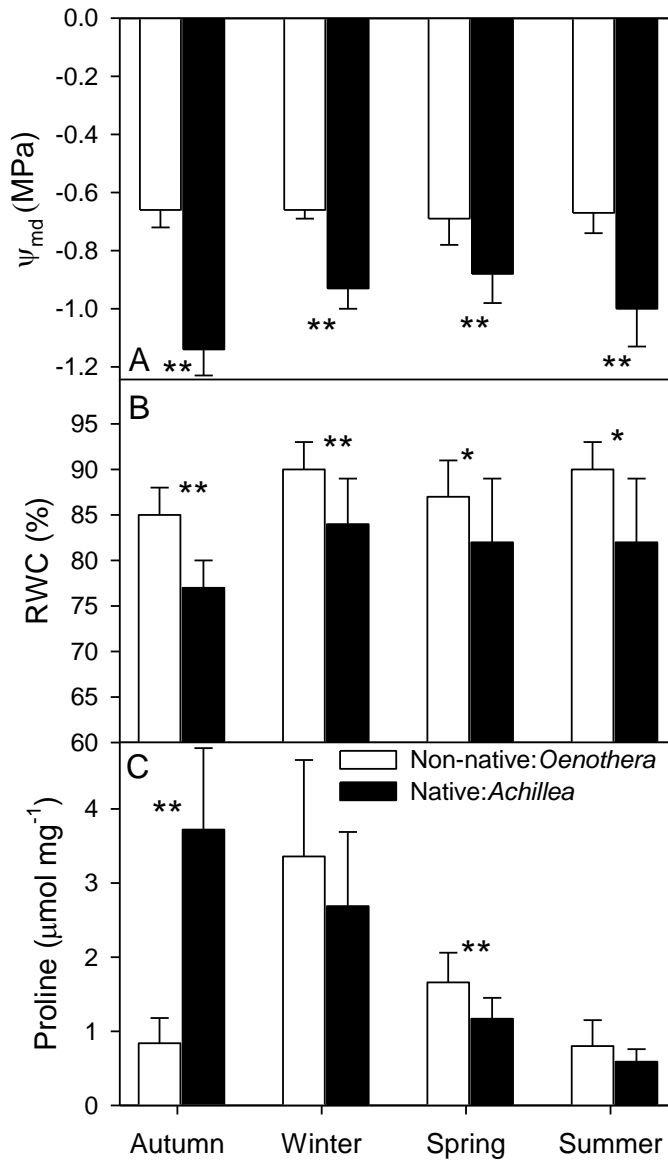

Fig. 3 Mean values (±sd) of maximum (F_v/F_m), and effective (Φ_{PSII}) photochemical efficiency in the two study species, over the 2013-14 annual cycle. Asterisks denote significance levels for the comparisons between the two species by Student's t-tests (**$P < 0.001$; *$P < 0.05$).

Overall, the highest Ψ_{md} of the year of both species were recorded in winter and spring, while summer water deficit decreased, Ψ_{md} reaching the lowest values in autumn (Fig. 4A). The non-native species maintained higher Ψ_{md} than the native one throughout the whole year. This difference was also observed in the RWC values recorded throughout the year, which were always significantly higher in the non-native species (Fig. 4B). Proline content in leaves, as a measure of stress, or an osmotic regulator in plants (Ain-Lhout et al., 2001), was not elevated in any season even in winter when the highest quantities were accumulated. Significant differences between the two species were observed in autumn and spring (Fig. 4C).

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Fig. 4 Mean values (±sd) of midday leaf water potential (Ψ_{md}), Relative water content (RWC) and proline content, of the two study species, over the 2013-14 annual cycle. Asterisks denote significance levels for the comparisons between the two species by Student's t-tests (**$P < 0.001$; *$P < 0.05$).

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The lowest LDMC values occurred always in *O. drummondii,* while annual dynamics were similar in both study species, increasing from winter to summer with a marked drop in autumn (Fig. 5A, B). Compared to the native species, *O. drummondii* had significantly lower LMA in summer and autumn, while the maximum values in both species were recorded in spring and summer. LMA and LDMC were correlated, with *P* < 0.001.
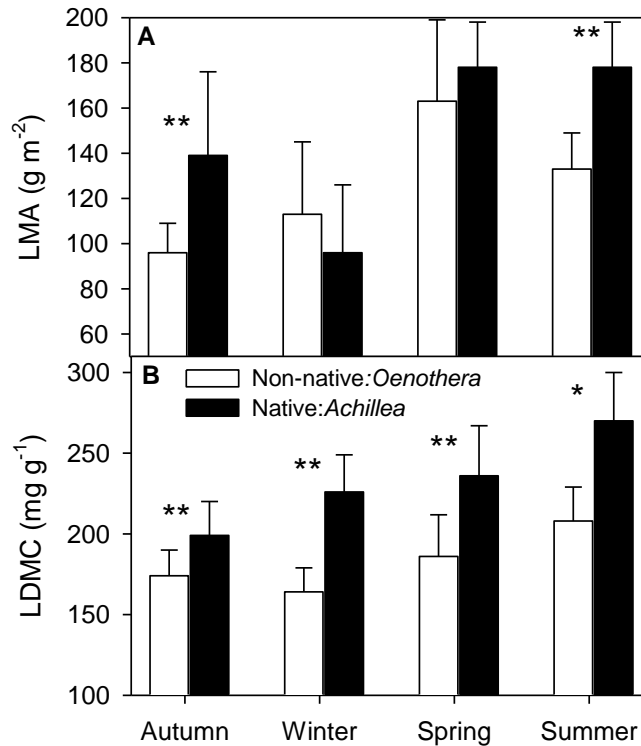
Fig. 5 Mean values (±sd) of LMA: leaf mass area (A), and LDMC: leaf dry matter content (B) in the two study species, over the 2013-14 annual cycle. Asterisks denote significance levels for the comparisons between the two species by Student's t-tests (**P < 0.001; *P < 0.05).

The most stressful seasons of Mediterranean climate, winter and summer, were the periods in which *O. drummondii* accumulated significantly more Chl a+b than the native species (Fig. 6A). On the contrary, ratio Chl a/b was significantly higher than in the native in autumn and spring (Fig. 6B). As for the carotenoids, non-native species accumulated more pigments than the native one, in winter and spring, and also presented higher Car/Chl ratio (Fig. 6 C, D).
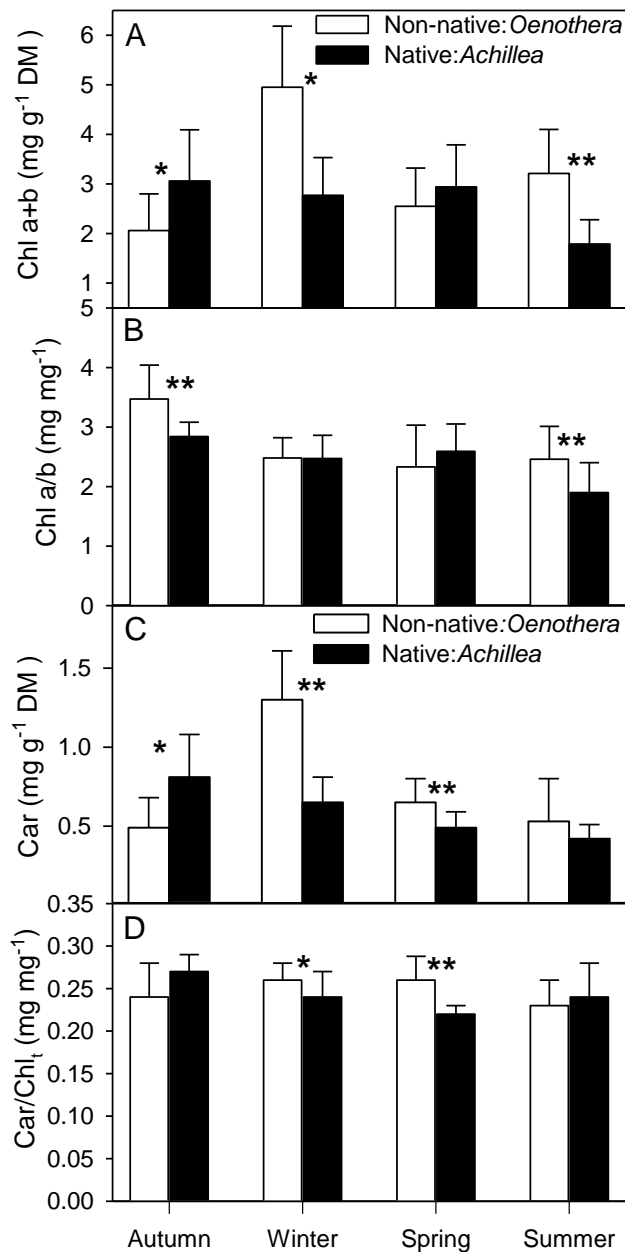
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391 **Fig. 6** Mean values (±sd) of the 2 study species in the 4 sampling periods for total

392 chlorophyll content (A, Chl a+b), chlorophyll a/b ratio (B, Chl a/b), carotenoid content

393 (C, Car), and total chlorophyll content to total carotenoids ratio (D, Chl/Car), over the

394 2013-14 annual cycle. Asterisks denote significance levels for the comparisons between

395 the two species by Student's t-tests (**$P < 0.001$; *$P < 0.05$).

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The PCA identified two main clusters in the data cloud differentiating the native from the non-native species, reinforcing the idea of a different physiological annual strategy to respond to Mediterranean coastal sand habitat (Fig. 7). The PCA provided an overview of the seasonal physiological pattern of the twenty one-measured variables. The plane defined by the first two axes of the analysis accounted for 44% of the variation. The variables more strongly correlated with PCA 1 axis ordered by importance were: LDMC, Car, Chl a, Chl a+b, Chl b, LWP (leaf water potential), Proline, Φ_{PSII}, LMA; while the second PCA axis was associated with the variables WUE_i, Ci, A_n/C_i, A_n, E. The PCA showed that the four seasons and two species segregate according to the two first axes; this implies that each species presents a different ecophysiological strategy throughout the year. The first PCA axis runs from *O. drummondii* leaves tending to have a better water status and larger pigment content than the native species, tending to exhibit higher LDMC. PCA 2 runs from summer *O. drummondii* leaves ending to have better WUE_i and carboxylation efficiency.
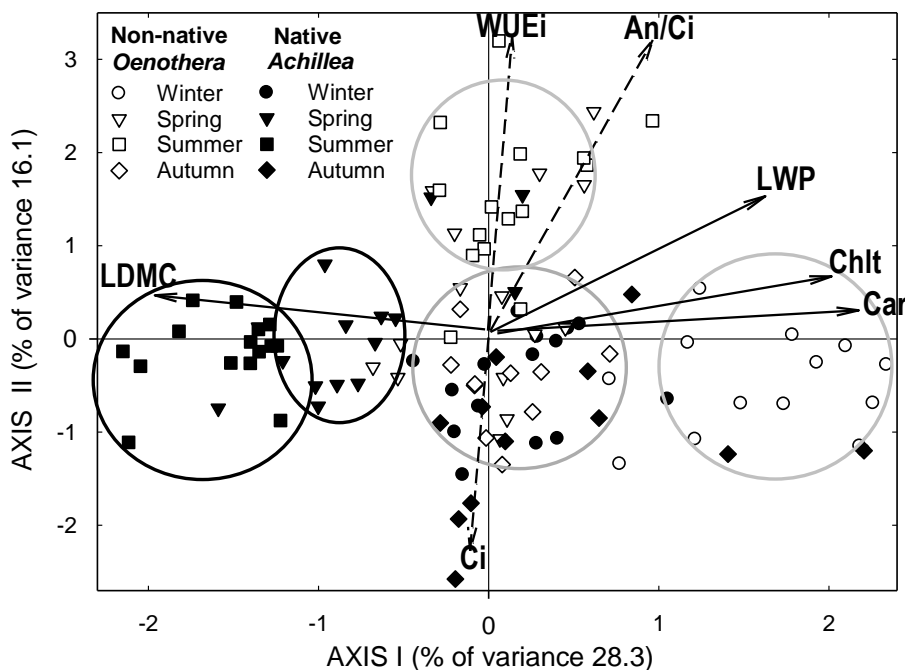
Fig. 7 Projection on the plane defined by principal component axes (PC) 1 and 2 of 30 individual plants (dots) of *Oenothera drummondii* and *Achillea maritima* from the 21 variables considered. Solid arrows indicate direction and weighing of vectors representing the more strongly correlated variables with PC1 while short dash arrows

indicate the variables correlated with PC2. A_n/C_i: instantaneous carboxylation efficiency, Car: total carotenoid content, C_i: leaf intercellular CO_2, Chlt: total chlorophyll content; LDMC: leaf dry matter content, LWP: leaf water potential, WUEi: instantaneous water use efficiency.

Discussion

Our main outcome in this study is that in spite of having higher photosynthetic rates in spring and summer and better water status through the year, the non-native species *O. drummondii* had lower photochemical efficiency than the native, *A. maritima.* The better ecophysiological performance of *O. drummondii* appears to be related mostly to capture of resources, such as CO_2 assimilation, and water-use strategy.

Respecting maximum photochemical efficiency, and considering the optimum values between 0.75–0.85 in normal plants (Bolhàr-Nordenkampf and Öquist, 1993, Maxwell and Johnson, 2000), the Fv/Fm values in *A. maritima* denote that the native species, with values through the year higher to 0.760, was not under particularly stressing conditions. These data agree with those recorded by Scorce et al. (2019) in the same species in a coastal dune system in Italy. In contrast, Φ_{PSII} was affected by the meteorological conditions, pointing out the species underwent certain dynamic photoinhibition, especially in winter when the lowest values were recorded. When compared with the invasive species, *A. maritima* was photochemically more efficient. Leaf pubescence in *A. maritima* (Ciccarelli et al., 2009), an adaptation of plants to the Mediterranean climate, reflect photosynthetically active radiation and provides protection against UV-BA radiation and inhibits the reduction in photochemical efficiency (Grammatikopoulos et al., 1994; Bisba et al., 1997). Pubescence increases the reflectance of the leaves, improving plant energy balance and therefore diminishing the risk of photoinhibition (Díaz Barradas et al., 1999; Zunzunegui et al., 1999; 2005). So the higher photochemical efficiency values found in *A. maritima* agree with higher F_v/F_m values in pubescence leaves as it has been found in other studies (Savé et al., 2000).

The fact that the lowest photochemical efficiency values were measured in winter in the non-native species could be due to the tropical origin that makes it more sensitive to low temperatures as it has been proven in other species with a tropical origin (Zunzunegui et al., 2005). In these species, the risk in photoinhibition might be higher in winter than in summer, as lower temperature might inhibit the photosynthetic electron

452   chain. Nevertheless, despite the lower photochemical efficiency in the invasive species,

453   the photosynthetic rates recorded for this species in spring and summer, were higher than

454   for the native one. This, therefore, indicates the sensitivity of *O. drummondii* to cold but,

455   simultaneously that it must be a species of great photosynthetic capacity because it is able

456   to overcome the native species despite its lower photosynthetic efficiency. That fact

457   would help to explain a 91% lower cover of *A. maritima* in dunes invaded by *O.*

458   *drummondii* (Gallego et al., 2019)

459   The meteorological data show that the harsh summer meteorological conditions,

460   (high temperature and radiation together with low relative humidity and water

461   availability) represent an important element in regulating the seasonal photosynthetic

462   activity. But above all, these results are an important differentiator of the best competitive

463   capacity of the invasive species. We may argue that increasing VPD (0.34 to 1.22 KPa)

464   from winter to summer caused the decreasing trend of g_s and the lower photosynthetic

465   rate of *A. maritima* in summer; since VPD has been considered the main environmental

466   variable controlling stomatal behaviour and causing the decrease of the photosynthetic

467   rate in Mediterranean woody vegetation (Tenhunen et al., 1987; Tognetti et al., 1998). On

468   the contrary, the spring and summer VPD effect on gs was not patent in the invasive

469   species.

470   Summer conditions affected the variables A_n and A_n/C_i in an opposite way to both

471   species; while in the non-native species the figures increased and the highest annual

472   values were registered, in the native plants they decreased, and the lowest values were

473   recorded. Reductions in instantaneous A_n/C_i suggest the occurrence of a non-stomatal

474   limitation of photosynthesis and some mesophyll limitation on the photosynthesis of

475   studied plants (Silva et al., 2010). Although photosynthesis rate and stomatal conductance

476   have been described to have a linear correlation in response to environmental conditions

477   (Ball et al., 1987) other factors may be acting to increase An, as Rubisco kinetic or

478   mesophyll conductance that could explain that the highest annual An values recorded in

479   *O. drummondii* were not linked to the highest stomatal conductance values.

480   The high carboxylation efficiency values recorded in *O. drummondii* in summer

481   were supported by higher photosynthetic activity with no significant variations of C_i while

482   the low values recorded in *A. maritima* were supported by high C_i values and low

483   photosynthetic rates.

484 The WUE_i is a major trait of the water economy of plants and of their performance,

485 survival capacity and functioning in arid sites (Damesin et al., 1997; Bacon, 2004).

486 Summer conditions similarly affected WUE_i in both species, as shown by the parallel

487 decline in this variable in spring and summer, especially for *A. maritima*, when the plants'

488 water status decreased to -1.1 MPa. A high evapotranspiration rate in summer was

489 responsible for the reduction in WUE_i especially in the case of *O. drummondii,* where the

490 photosynthetic rate increased during summer. Scorce et al. (2019) also studying *A

491 maritima*, recorded summer values of Ψ_{md} as low as -2 MPa, Thus the Ψ_{md} values

492 registered in this study are moderately high. The neighbourhood of the sea might provide

493 moisture to these species during the night and early morning, causing the recorded values

494 in LWP were not as negative as in other Mediterranean species. Nonetheless, *O.

495 drummondii* always displayed higher RWC values along with higher Ψ_{md} values than *A.

496 maritima*. This fact denoted a different strategy in water use or access and would be

497 indicative of better water status of the invasive species.

498 Under winter conditions both species presented similar photosynthetic rates but

499 significant differences in hydric status. Photosynthetic activity varies with environmental

500 conditions, and although plants can acclimate to these changes, given the tropical origin

501 of the non-native species, lower photosynthetic rates could be expected in the cold season

502 compared with the native one. Leaves can have different photosynthetic rates under the

503 same environmental conditions due to different stomatal conductance caused by internal

504 or external factors. It has been suggested that changes in the response of cell-wall

505 conductance may be an important factor in temperature acclimation (Makino et al., 1994).

506 The high stomatal conductance in *O. drummondii* leaves along the year could be the

507 underlying factor in its optimal winter response and better water status.

508 Furthermore, the maintenance of high intercellular CO_2 concentration (data not

509 shown) associated with the lowest annual photosynthetic rate in *O. drummondii* suggests

510 the occurrence of non-stomatal limitation of photosynthesis in winter, while in *A.

511 maritima*, high intercellular CO_2 concentration values combined with the highest annual

512 photosynthetic rate recorded suggests the occurrence of stomatal limitation in the native

513 species. Also, high A_n/C_i values recorded in *A. maritima* indicate a non-mesophyll

514 limitation on photosynthesis.

515 The highest LDMC and LMA values recorded in the native species leaves in all

516 seasons indicate that this species would be relatively tougher and more resistant to

physical stresses than *O. drummondii,* whose leaves have lower LDMC and LMA. Leaf mass area can be interpreted as the cost of light interception at the leaf level (Gutschick and Wiegel, 1988; Poorter et al., 2009). Increases in LMA are often associated with drought exposure and high irradiance (Gratani and Bombelli, 1999). According to these results, higher fitness could be expected in the native *A. maritima*, and although the species presented significant higher photochemical efficiency in the most stressful period of the year, the highest photosynthetic rate was always measured in the invasive species *O. drummondii.* On the other hand, these higher photosynthetic rates in the invasive species are consistent with lower LDMC values recorded, since this functional trait indicates the species' resource use strategy and represents the compensation between assimilation and rapid growth *versus* efficient conservation of resources (Wilson et al., 1999; Hodgson et al., 2011). The results in LDMC values would indicate a different strategy of resource use for both species; with *A. maritima* displaying a more conservative strategy as a predictor of poor environments and *O. drummondii* presenting a rapid resource acquisition and use strategy as a predictor of rapid growth and soil fertility. These outcomes would reinforce the concern that should accompany the invasion by this species due to its rapid resource assimilation and growth which promote its expansion (Garnier et al., 2001; Díaz et al., 2004).

Pigment study is important from an ecophysiological perspective as it provides information about productivity, stress or limiting nutrients. Across the most stressful seasons of the year, winter and summer, the invasive species has the advantage over the native of its higher chlorophyll content but also higher Chl a/b ratios in autumn and summer and higher Car/Chl ratios in winter. These variables are both representative of stress. The Chl a/b ratio is indicative of the light absorption capacity of photosystem I to II; declines in this variable are indicative of differential degradation of Chl a concerning Chl b. Under stress conditions, a more rapid destruction of Chl a than Chl b might occur; whereas Car/Chl decreases point out to damage in the photosynthetic apparatus, which is expressed by a faster breakdown of chlorophylls than carotenoids (Lichtenthaler and Buschmann, 2001). This higher pigments concentration can be a response to avoid photochemical damage and an increase in reactive oxygen species due to the combination of low temperatures with high radiation in Mediterranean winter. When the incident light intensity is higher than necessary for photosynthesis reactive intermediates can be produced in excess, leading to oxidative damage (Aro et al., 1993). The presence of

photoprotective carotenoids under this combination of winter stressful conditions is believed to be a feature of the photoprotective function of carotenoid pigments (Havaux and Kloppstech, 2001) and consequently, the invasive species could have better stress tolerance or better maintenance of physiological functions under unfavourable environmental conditions.

Principal component analysis established the presence of three groups corresponding to seasons (summer of both species and *O. drummondii* winter). According to Wilson et al. (1999), LDMC seems to be the best variable for locating plant species on a resource use on the first axis and also as predictors of the different strategies for the invasive and native species on resources use. The accumulation of Chl and Car, the other variables defining this first axis, can be also related to resources used as optimal growth conditions, as well as sufficient nutrients are positively correlated with pigment contents and levels (del Campo et al., 2000; He et al., 2013). In contrast, axis two seems to separate the two species and seasons according to its water use strategy.

In summary, even though *O. drummondii* has a tropical/subtropical origin, our data show that this species is well acclimated to Mediterranean coastal dunes, exhibiting a higher photosynthetic rate and better water performance than the native species *A. maritima*. We conclude that *O. drummondii* utilizes light, water and probably nutrients more efficiently than the native *A. maritima* and suffers lower stress in environments with reduced water availability and high light radiation. Our results seem to indicate that the underlying mechanism that confers *O. drummondii* its competitive capacity may be its ability in water uptake, which makes it an effective non-native invasive species; the only limiting factor to control its expansion in the Mediterranean coastal dune could be low temperatures. In the study area, removal projects have been carried out to restore invaded dunes (García-de-Lomas et al., 2016, personal observation) and they have proven the difficulties to maintain the population level below an impact threshold.

As the authors, Flores-Moreno and Mole (2013), sustain it is evident that all non-native species are not super plants with high invasive capacity and capable of maintaining a higher fitness than the co-occurring native species in their new ranges. Furthermore, studies comparing non-native and native species performance are contradictory (Daehler, 2003; Palacio-López and Gianoli, 2011). However, in the case of *O. drummondii* our findings suggest that it is particularly successful in the Mediterranean range (including other distribution areas with Mediterranean climate, Csa, where this species is expanding

583 such as the coast of Israel and the west coast of Australia). Therefore we would advise
584 managers to take measures for their eradication in the early stages of their appearance in
585 the Mediterranean dune ecosystems since it is extremely successful in these habitats.
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