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Leaf morpho-physiological comparison between native and non-native plant species in a Mediterranean island

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Abstract Islands tend to be more prone to plant invasions than mainland regions, with the Mediterranean ones not being an exception. So far, a large number of studies on comparing leaf morphological and physiological traits between native and non-native plants in Mediterranean environments have been performed, although none of them on Mediterranean islands. To fill this gap, this study focuses on 14 plant species grown in a controlled growth chamber in the absence of stress. The goal was (1) to differentiate leaf morpho-physiological traits between native and non-native plants on a Mediterranean island and (2) to deepen in the underlying causes of the differential photosynthetic traits displayed by non-native species.

Results showed that in Mediterranean islands, non-native plant species show on average larger values of net CO_2 assimilation, stomatal conductance (g_m), photosynthetic nitrogen-use efficiency, among others, and lower leaf mass per area (LMA) and leaf thickness, compared to the native species. Among the assessed traits, this study reports for the first time larger g_m, and lower mesophyll conductance limitation in non-native species, which seems to be linked to their lower LMA. These novel traits need to be added to the 'leaf physiological trait invasive syndrome'. It was also found that on a Mediterranean island, native and non-native species are placed on opposite sides of the leaf economics spectrum, with non-native species being placed on the ''fast-return'' end. In conclusion, this study demonstrates that non-native species inhabiting a Mediterranean island possess distinct leaf morphological and physiological traits compared to co-occurring native species, at least during the favorable growth season, which increases the chances of a successful invasion.

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Introduction

Plant invasions are considered to be one of the strongest global ecological threats during the twenty-first century, having a wide range of impacts on ecosystems and their functioning (Vilà and Hulme 2017; Castro-Díez et al. 2019). It has been shown that the level of invasions varies among regions, biomes, and habitats (Chytrý et al. 2008). Notably, islands tend to be more prone to invasions than mainland regions (Dawson et al. 2017) with this pattern being also observed in Mediterranean islands (Gimeno et al. 2006; Celesti-Grapow et al. 2016; Guarino et al. 2021).

There are multiple characteristics that confer a given non-native species its invasive capacity, ranging from biotic interactions to many other ecological features (Abdallah et al. 2021; Traveset and Richardson 2021). Among those suggested to play an important role in plant invasiveness are the physiological traits. Non-native plants have shown to possess higher rates of leaf net CO_2 assimilation (A) (van Kleunen et al. 2010; Oliveira et al. 2014; Le et al. 2019), stomatal conductance (g_s) (Durand and Goldstein 2001; Caplan and Yeakley 2010), photosynthetic nitrogen-use efficiency (PNUE) (Durand and Goldstein 2001; Heberling and Fridley 2013; Petruzzellis et al. 2021), among others. Moreover, leaf morphological traits have also been suggested to be responsible for the success of non-native plants. Several studies have demonstrated that non-native species generally possess lower leaf mass per area (LMA). These studies have also found correlations between lower LMA and some physiological traits, indicating that non-native species with lower LMA tend to have higher leaf nitrogen (N), nitrogen concentration per unit mass (N_{mass}), and higher PNUE (Peñuelas et al. 2010; Morais et al. 2021). Another important morphological trait is leaf area (LA), which tends to be higher in non-native plants than co-occurring native species (Daehler et al. 2004; Petruzzellis et al. 2021). To the present, none of the studies comparing leaf morphological and physiological traits variations between native and non-native plants in Mediterranean environments (Zunzunegui et al. 2020; Morais et al. 2021; Petruzzellis et al. 2021) have been performed in Mediterranean islands.

Combining leaf morphological and physiological traits, Wright et al (2004) described a universal spectrum of leaf economics reflecting coordinated changes in leaf structure and function relationships. This leaf economics spectrum (LES) was described as running from a "slow-return" end, encompassing species characterized by high LMA, low nutrient contents (N_{mass} and P_{mass}, respectively), low respiration rates, and lower maximum net assimilation rate per unit dry mass (A_{mass}) to a "fast-return" end with the opposite suite of traits. Surprisingly, its use in the field of invasion ecology is rare. The very few studies that applied the LES have suggested that non-native and native species are aligned on its opposite sides, placing non-native species cluster on the ''fast-return'' end, with higher values of N_{mass} and A_{mass}, and lower values of LMA relative to native species (Peñuelas et al. 2010; Ordonez and Olff 2013; Petruzzellis et al. 2021).

Based on the above evidences, it appears that photosynthesis and photosynthesis-related parameters may have a crucial role in favoring the invasive character of non-native species. Mechanistically, photosynthesis can be limited by either diffusional (i.e., stomatal and/or mesophyll conductance) or photobiochemical limitations. Grassi and Magnani (2005) proposed a method to quantify photosynthetic limitations by separating the relative controls on the net CO_2 assimilation rate (A) resulting from stomatal (l_s), mesophyll conductance (l_m) and biochemical limitations (l_b)—the latter pooling together potential photochemical and biochemical factors. To the best of our knowledge, no study has previously quantified the photosynthetic limitations of native and non-native species. Quantifying these limitations is important, as a different sharing of photosynthetic limitations could confer some advantages to the latter. In particular, it has been shown that increased photosynthetic capacity along the land plant's phylogeny is associated to a progressive reduction from a predominant l_m in bryophytes to an almost matched co-limitation by l_s, l_m, and l_b in angiosperms (Gago et al. 2019). Decreased l_m over l_s is associated with increasing both water-use efficiency (WUE) and PNUE along plant's phylogeny (Flexas and Carriquí 2020), but also with increased WUE within angiosperms themselves (Flexas et al. 2013). Large WUE and PNUE are to be important traits conferring competitive advantage in source-poor environments, such as those encountered in Mediterranean islands, especially if achieved by means of reducing stomatal conductance and

transpiration on one hand and nitrogen content on the other, rather than by increasing photosynthesis itself. Since a high mesophyll conductance is often associated with a low LMA, it could happen that non-native species have large photosynthesis due to low l_m.

Based on the described gaps in the current knowledge, the aims of this study (1) to differentiate leaf morpho-physiological traits between native and non-native plants on a Mediterranean island and (2) to deepen in the underlying causes of the differential photosynthetic traits displayed by non-native species. To do so, 14 plant species were grown in a controlled growth chamber in the absence of stress. The specific hypotheses studied were:

1. On a Mediterranean island, non-native plant species present advantageous leaf physiological traits as compared to co-occurring native species.
2. In association with leaf morphological traits, decreased l_m may contribute to larger A, WUE and PNUE in non-native species.
3. Native and non-native plant species are aligned on the opposite sides of the leaf economics spectrum, placing non-native species cluster on the ''fast-return'' end of the spectrum (in accordance with previous studies on non-island species).

Materials and methods

Species selection

14 plant species were chosen: eight natives and six non-natives (see details Table 1). Those plant species were chosen based on their abundant presence as coexisting native and non-native species growing side by side across different sites of the island of Mallorca (Balearic Islands, Spain, Western Mediterranean Sea). Sites differed in their environmental conditions and topographic positions, including mountain areas, riparian sites of intermittent streams, and coastal areas. Therefore, plant species were not randomly selected, but chosen to represent a diversity of habitats inhabited by both native and non-native plant species. Seeds of the selected species were collected during summer-autumn 2018 and preserved in paper bags in a dry, cool place to be later grown under controlled conditions.

Experimental site and growth conditions

The study was carried out in the laboratory of the plant physiology deparment at the University of the Balearic Islands (UIB). For this study, six individuals per species (84 plant individuals in total, 14 species × 6 replicates), were grown individually, each in a plastic pot (11 cm diameter top; 8.8 cm depth; capacity: 0.52 Liters) containing peat (Silver Torf, Agrochimica, Bolzano, Italy) and Agriperlite® (70:30 v: v). Each pot was irrigated with 0.2 L of 100% Hoagland solution, three times a week. Plants were grown for 90 days in a plant growth chamber under controlled growing conditions (60% air humidity, 400 μmol m^{-2} s^{-1} of photosynthetic active radiation (white light), 12/12 h light/dark regime and temperature fixed at 27/22 °C day/night) before starting measurements.

Leaf morphological traits and Carbon/Nitrogen contents and stable isotopes

Choosing one young fully expanded leaf per individual (n = 6 leaves per species), leaf morphological traits and Carbon/Nitrogen contents and isotopes were fully characterized by implementing the following steps. Leaf area (cm^2) was scanned using Canon CanoScan LiDE 220 where its projected leaf area was determined using IMAGEJ software (Schneider et al. 2012), leaf thickness (cm) was assessed using a caliper. Thereafter, the same leaf was dried to calculate the dry leaf mass per unit leaf area (LMA, g m^{-2}) by placing it in a paper envelope inside a ventilation oven (70-80°C) until constant dry weight (g) was reached. After estimating LMA, the maximum net assimilation rate per unit dry mass (A$_{mass}$, nmol g^{-1} s^{-1}) was calculated as the ratio of net CO$_2$ assimilation (A) (see details below) to LMA.

On the other hand, the obtained dry leaves were grinded into fine and uniform powder using a mixer mill (MM-200; Retsch, Haan, Germany) from which 2 mg were used to analyze Carbon and Nitrogen isotopes ratios. Carbon isotope ratios (δ^{13}C) has been used as a proxy for long-term WUE (Farquhar et al. 1989), while nitrogen isotope ratios (δ^{15}N) are an integrated measure of nitrogen fluxes, assimilation, and allocation in plants, allowing to distinguish N$_2$-fixing from non-fixing species (Kalcsits et al. 2014). Isotope ratios were determined with

Table 1 Family, status, and description of the 14 studied plant species

Species	Family	Status	Description
Physalis peruviana	Solanaceae	Non-native	Herbaceous, perennial small shrub common in the subtropical zones of the Andes (South America). It has been introduced as a cultivated plant in different geographical regions (Cedeño and Montenegro 2004)
Nicotiana glauca	Solanaceae		Evergreen shrub native to central northwest Argentina and Bolivia. It has successfully invaded disturbed areas of semi-arid habitats worldwide by forming dense monodominant stands (Ollerton et al. 2012)
Tropaeolum majus	Tropaeolaceae		Fast growing climbing annual plant, native to the Andes Mountains. It can form extensive carpet-like growths in sunny torrent banks and beds (Garzón and Wrolstad 2009)
Solanum linnaeanum	Solanaceae		Perennial shrub, native to southern Africa, is found occasionally in many Mediterranean countries and considered a serious invasive alien in parts of Australia and New Zealand (Vorontsova and Knapp 2012)
Arundo donax	Poaceae		Perennial, herbaceous grass, occurring over a wide range of climatic habitats. It is originally from East Asia but was introduced and diffused widely across the Mediterranean area and around the world through human activity (Corno et al. 2014)
Mesembryanthemum crystallinum	Aizoaceae		Annual to biennial succulent herb, native to South Africa, mostly found on coasts. It is a salt accumulator that leaches salts upon death, increasing soil salinity in the vicinity. Such increase reduces the competitive ability of associated native plant species, increasing its germination and establishment (Abd El-Gawad and Shehata 2014)
Withania somnifera	Solanaceae	Native	Perennial small shrub that inhabits disturbed sunny and dry areas. Native to a wide range of areas, spreading from the Mediterranean region to regions like India and Sri Lanka (Gaurav et al. 2015)
Hyoscyamus albus	Solanaceae		Annual to biennial herb, native to the coastal areas in southern Europe and the Near East. This plant is an inhabitant of cities, which grows next to walls, and in very remote places
Convolvulus arvensis	Convolvulaceae		Climbing perennial plant, native to the Mediterranean Basin. It grows in a wide range of conditions from full sun to full shade, on all soils (Culhavi and Manea 2011)
Solanum nigrum	Solanaceae		A predominantly Eurasian species, ranging from being annual to biennial herb sometimes. It appears to be distributed throughout the world, from Finland in the northern hemisphere to New Zealand in the southern hemisphere (Jagatheeswari et al. 2013)
Phragmites australis	Poaceae		Perennial grass from Eurasian origin, present in a wide range of habitats, from cold temperate regions to the wetlands of hot and moist tropics in almost all continents, especially Asia and Europe (Vymazal 2013)
Glaucium flavum	Papaveraceae		Perennial herb, native to the Mediterranean Basin that occurs along its shores and the coasts of Western Europe (Thanos et al. 1989)
Mesembryanthemum nodiflorum	Aizoaceae		Annual succulent herb, native to the Mediterranean, always living along the coast. Occasionally it forms a kind of carpet or lawn on soils that used to be bare ground (Soliman et al. 2014)
Crithmum maritimum	Apiaceae		Facultative halophyte growing on maritime cliffs and sometimes in sand. It is widely distributed along coastal areas of the Mediterranean Sea and of the Atlantic Ocean (Meot-Duros and Magné 2009)

a Continuous-Flow Isotope Ratio Mass Spectrometry (CF-IRMS) combined with gas chromatography (Thermo Delta XPlus, Bremen, Germany) to analyze gas of mass 44/45/46 (for CO_2) and 28/29 (for N_2) after separation on a GC column (Flash EA 1112 Thermo Finnigan elemental analyzer). Each measurement of the isotope ratios ($\delta^{13}C$ and $\delta^{15}N$, %) of a given sample with the CF-IRMS was referred to PDB following the known value of PLS standards run in parallel every 6 samples during the analysis (here Peach-leaf control samples), obtained from the National Institute of Standards & Technology (NIST 1547). For determination of mass-based leaf carbon and nitrogen content (C_{mass} and N_{mass}, %), a linear relationship was established between the area of the peak of Carbon 44 or Nitrogen 28 and the weight of C or N present in the standards (Peach-Leaf) used during the measurements. From these obtained weights, the ratio of Carbon to Nitrogen (C:N) was then estimated. Finally, to assess the photosynthetic nitrogen-use-efficiency (PNUE) (see details below), the area-based nitrogen content (N_{area}, g m^{-2}) was calculated as: N_{mass} X LMA.

Leaf physiological traits

Leaf physiological traits were conducted using a portable photosynthesis system (Li-6400; Li-Cor, Inc., Lincoln, NE, USA) with an infrared gas analyzer (IRGA) coupled with a 2 cm^2 leaf fluorescence chamber (Li−6400–40 leaf chamber fluorometer; Li-Cor, Inc.) Choosing one young fully expanded leaf per individual (n = 6 leaves per species), instantaneous measurements were carried out between 09:00 to 13:00 h (Central European summer time), at 400 μmol CO_2 mol^{-1} air and saturating photosynthetic photon flux density (PPFD) at 1200 μmol m^{-2} s^{-1}, and 90:10 red:blue light. This light intensity was used to ensure saturation and was chosen after performing light response curves for all species (Supplementary Fig. S1). Net CO_2 assimilation (A, μmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}) were taken from instantaneous measurements. Combining these obtained data with the data recorded for (N_{area}), both the intrinsic water-(WUE) and photosynthetic nitrogen-use efficiency (PNUE) were assessed. Intrinsic WUE (μmol mol^{-1}) was calculated as the ratio of

A to g_s and PNUE (μmol mol^{-1} s^{-1}) as the ratio of A to N_{area}. Despite very different leaf morphologies, no significant differences were observed for either leaf temperature or leaf-to-air vapor pressure deficit among species (Supplementary Table 1), making all measurements comparable.

The electron transport rate (ETR) was estimated as ETR = PPFD × ΦPSII × αβ (Genty et al. 1989), ΦPSII being the quantum efficiency of photosystem II, α the leaf absorbance, and β the partitioning of absorbed photons between photosystems I and II. ΦPSII was estimated as ΦPSII = (Fm′-Fs)/Fm′ (Genty et al. 1989), with Fm′ as maximum fluorescence under illumination, and Fs as steady-state fluorescence. The αβ parameter was estimated from A/Ci curves preformed under low oxygen (1% O_2), following Valentini et al. (1995). The ratio ETR to A was calculated (μmol mol^{-1}) after previous correction of ETR. Finally, mesophyll conductance (g_m) was estimated following Harley et al. (1992), as:

$$g_m = \frac{A}{C_i - \frac{\Gamma* \times [ETR + 8 \times (A+R_d)]}{ETR - 4 \times (A+R_d)}}$$

where Γ* is the CO_2 compensation point in absence of R_d (here assumed a value of 37.4 μmol mol^{-1} (following Sharkey et al. 2007), and R_d (μmol m^{-2} s^{-1}) is the non-photorespiratory respiration during the day, which was estimated as half the respiration rate measured after 2 h of darkness (Niinemets et al. 2005; Martins et al. 2013; Veromann-Jürgenson et al. 2017). The ratio between net CO_2 assimilation rate and respiration was also calculated (A/R_d, Pattison et al. 1998; Galmés et al. 2007). After calculation, g_m data were filtered following the reliability criterion established by Harley et al. (1992):

$$\partial C_c / \partial A = 12 \times \Gamma * \times \frac{ETR}{(ETR - 4 \times (A + R_d))^2}$$

Data in the range of $10 < \partial C_c/\partial A < 50$ were considered as reliable. Aberrant values of g_m following this criterion were discarded, together with all the other photosynthetic parameters of that replicate. Photosynthetic limitations—i.e., stomatal limitation (l_s), mesophyll conductance limitation (l_m) and biochemical limitation (l_b)—were estimated following Grassi and Magnani (2005), as follows:

$$l_s = \frac{g_{tot}/g_s + \partial A/\partial C_c}{g_{tot} + \partial A/\partial C_c}$$

$$l_m = \frac{g_{tot}/g_m + \partial A/\partial C_c}{g_{tot} + \partial A/\partial C_c}$$

$$l_b = \frac{g_{tot}}{g_{tot} + \partial A/\partial C_c}$$

where g_{tot} is the total CO_2 diffusion conductance, calculated as $g_{tot} = 1(1/g_s + 1/g_m)$ and $\partial C_c/\partial A$ is considered as the initial slope of the $A - Cc$ response curve.

Data analysis

Differences in leaf morphological traits, Carbon/Nitrogen contents and stable isotopes, and leaf physiological traits between native and non-native plant species, were tested with a non-parametric Kruskal–Wallis test performed using each trait as response variable and status (native and non-native) as explanatory variable. In order to identify general trends in those variables that were significantly affected by the plant 'status, and to understand how these traits were related in a multivariate context, a Principal Component Analysis (PCA) was performed. Lastly, correlations among parameters were performed using Spearman correlation. All data have been analyzed using R v.3.4.3 (R Core Team 2017).

Results

Leaf morphological traits and Carbon/Nitrogen contents and stable isotopes

Statistically significant differences between native and non-native species were found for all leaf morphological traits ($p < 0.001$; Table 2). The present results show that on a Mediterranean island, non-native plants possess on average larger values of leaf area (LA), while native ones registered on average the highest values for both leaf thickness (LT) and leaf mass per area (LMA) (Fig. 1). The largest values of LA corresponded to the non-native *Solanum linnaeanum*, whereas the smallest to the native *Mesembryanthemum nodiflorum*. Both largest and smallest LT values were reported for native species, *Crithmum maritimum* and *Solanum nigrum,* respectively. *C. maritimum* also had the largest LMA, whereas the

Table 2 Statistics (Chisq and P values) of the Kruskal–Wallis test performed using each trait (leaf morphological traits, Carbon/Nitrogen contents and stable isotopes, and leaf physiological traits) as response variable and plant species status (native and non-native) as explanatory variable

	Status	
	Chisq	*P*-value
Leaf morphological traits		
LA	19.47	< 0.001
LT	6.32	< 0.001
LMA	50.17	< 0.001
Carbon/Nitrogen contents and stable isotopes		
C_{mass}	5.78	< 0.05
N_{mass}	0.73	0.391
C:N	0.08	0.775
$\delta^{13}C$	8.80	< 0.05
$\delta^{15}N$	13.60	< 0.001
Leaf physiological traits		
A	11.41	< 0.001
A_{mass}	11.30	< 0.001
ETR	4.02	< 0.001
g_s	9.33	< 0.001
g_m	12.52	< 0.001
WUE	0.53	0.467
PNUE	4.45	< 0.001
R_d	0.04	0.833
A/R_d	1.10	< 0.05
l_s	0.89	0.348
l_m	7.54	< 0.001
l_b	0.19	0.661

Leaf morphological traits (leaf area (LA), leaf thickness (LT), leaf mass per area (LMA)), Carbon/Nitrogen contents and stable isotopes (mass-based carbon content (C_{mass}), mass-based nitrogen content (N_{mass}), carbon:nitrogen (C:N) ratio, carbon isotope ratios ($\delta^{13}C$), nitrogen isotope ratios ($\delta^{15}N$)), and leaf physiological traits (net CO_2 assimilation (A), maximum net assimilation rate per unit dry mass (A_{mass}), electron transport rate (ETR), stomatal conductance (g_s), mesophyll conductance (g_m), intrinsic water-use efficiency (WUE), photosynthetic nitrogen-use efficiency (PNUE), respiration (R_d), photosynthetic CO_2 assimilation over respiration (A/R_d), stomatal limitation (l_s), mesophyll conductance limitation (l_m) and biochemical limitation (l_b))

smallest values were found in non-native *Physalis peruviana* (Table 3).

Regarding to Carbon/Nitrogen contents and their stable isotopes, plant status had a significant effect on most of this category traits except for mass-based
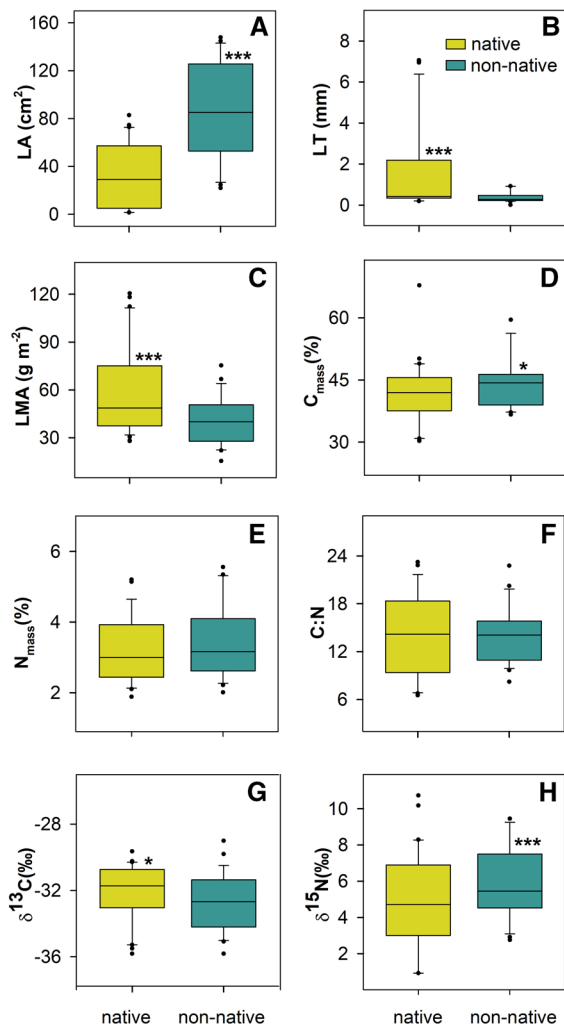
Fig. 1 Box plots of the leaf morphological traits and Carbon/Nitrogen contents and stable isotopes. **A** Leaf area (LA), **B** leaf thickness (LT), **C** leaf mass per area (LMA), **D** mass-based carbon content (C_{mass}), **E** mass-based nitrogen content (N_{mass}), **F** carbon:nitrogen ratio (C:N), **G** carbon isotope ratios ($\delta^{13}C$), and **H** nitrogen isotope ratios ($\delta^{15}N$) for native (in yellow) and non-native (in green) plant species studied. *Denotes statistically significant difference at $p < 0.05$ and *** $p < 0.001$

nitrogen content (N_{mass}) and the carbon:nitrogen (C:N) ratio ($p > 0.05$; Table 2). Non-native species showed the highest values for both mass-based carbon content (C_{mass}) and nitrogen isotope ratios ($\delta^{15}N$), whereas the carbon isotope ratios ($\delta^{13}C$) was highest for native ones, although all the species showed values typical of non-N_2-fixing species (Fig. 1). Non-native *P. peruviana* showed the highest values of C_{mass}, while native *M. nodiliforum* presented the lowest. Both the largest

and the lowest LT values were reported for non-native species, *S. linnaeanum* and *Arundo donax,* respectively. C:N, $\delta^{13}C$ and, $\delta^{15}N$ followed the same trend in having their highest and lowest values for native species exclusively. C:N was highest for *Withania somnifera* and lowest for *M. nodiflorum*, $\delta^{13}C$ was highest for *Glaucium flavum* and lowest for *P. peruviana* and, $\delta^{15}N$ was highest for *S. nigrum* and lowest for *G. flavum* (Table 3).

Leaf physiological traits

Plant status had a significant effect on most leaf physiological traits except on intrinsic water-use efficiency (WUE), respiration (R_d) and both stomatal (l_s) and biochemical limitations (l_b) ($p > 0.05$; Table 2). All traits differing significantly, except for the mesophyll conductance limitation (l_m), presented on average larger values in non-native plants (Fig. 2). Native species, *S. nigrum* and *C. maritimum* showed the largest and smallest values for both the net CO_2 assimilation (A) and the electron transport rate (ETR). Maximum net assimilation rate per unit dry mass (A_{mass}), stomatal conductance (g_s), mesophyll conductance (g_m) and photosynthetic nitrogen-use efficiency (PNUE) were highest in non-native species (*P. peruviana* (for both A_{max} and PNUE), *Tropaeolum majus* (for g_s), and *A. donax* for (g_m)), while the lowest values were observed in native species (*P. australis* (for both A_{max} and g_s) and *C. maritimum* (for both g_m and PNUE)) (Table 4). The highest values for R_d were observed in the native *C. maritimum,* whereas the smallest were for the non-native *Nicotiana glauca*. Lastly, the largest and smallest values of WUE, the photosynthetic CO_2 assimilation over respiration (A/R_d), l_s, l_m and l_b were reported in non-native species exclusively. WUE was highest for *Mesembryanthemum crystallinum* and lowest for *P. peruviana*, $\delta^{13}C$ was highest for *A. donax* and lowest for *M. crystallinum*. *N. glauca* presented the highest l_s values, while *M. crystallinum* presented the lowest values. l_m was highest for *M. crystallinum* and lowest for both *N. glauca* and *A. donax*, while l_b was highest for *T. majus* and lowest for *N. glauca* (Table 4).

Principal component analysis and correlations among traits

Native and non-native species showed different patterns in the bidimensional space generated by the

Table 3 Values of leaf morphological traits

Species	Status	LA (cm^2)	LT (mm)	LMA (g m^{-2})	C$_{mass}$ (%)	N$_{mass}$ (%)	C:N	δ^{13}C (‰)	δ^{15}N (‰)
Physalis peruviana	Non-native	106.54 ± 20.74	0.29 ± 0.16	23.95 ± 6.61	57.83 ± 17.09	3.92 ± 1.25	14.85 ± 0.61	−30.39 ± 1.18	5.50 ± 0.74
Nicotiana glauca		98.51 ± 21.29	0.47 ± 0.03	40.38 ± 3.32	39.28 ± 4.51	2.69 ± 0.37	14.67 ± 0.97	−31.57 ± 0.70	4.72 ± 2.64
Tropaeolum majus		38.32 ± 14.77	0.23 ± 0.04	28.58 ± 6.23	42.94 ± 2.73	2.85 ± 0.49	15.55 ± 3.44	−34.96 ± 0.57	5.48 ± 0.98
Solanum linnaeanum		138.02 ± 9.35	0.21 ± 0.01	48.14 ± 2.94	46.23 ± 6.72	4.72 ± 0.70	9.84 ± 1.00	−32.97 ± 0.55	9.64 ± 2.40
Arundo donax		27.67 ± 3.14	0.22 ± 0.01	72.07 ± 4.64	45.77 ± 2.93	2.29 ± 0.26	20.13 ± 2.37	−32.22 ± 0.36	3.53 ± 0.24
Mesembryanthemum crystallinum		74.30 ± 6.37	0.91 ± 0.01	44.76 ± 16.74	41.23 ± 4.13	3.61 ± 0.47	11.47 ± 0.69	−32.12 ± 0.99	5.26 ± 0.95
Withania somnifera	Native	26.18 ± 2.65	0.38 ± 0.04	63.90 ± 13.35	50.12 ± 12.06	2.47 ± 0.44	20.25 ± 2.62	−31.47 ± 0.77	7.13 ± 0.75
Hyoscyamus albus		39.68 ± 8.01	0.43 ± 0.04	54.92 ± 13.12	40.35 ± 2.17	2.30 ± 0.14	17.58 ± 1.04	−32.19 ± 0.61	7.15 ± 1.09
Convolvulus arvensis		4.88 ± 0.44	0.19 ± 0.01	43.05 ± 7.92	45.38 ± 2.77	2.75 ± 0.54	17.15 ± 4.93	−32.49 ± 1.58	6.07 ± 1.27
Solanum nigrum		72.88 ± 2.28	0.19 ± 0.01	33.67 ± 7.75	44.67 ± 3.33	3.47 ± 0.45	12.93 ± 0.72	−33.05 ± 0.76	10.45 ± 0.40
Phragmites australis		10.49 ± 1.16	0.22 ± 0.04	73.13 ± 31.34	47.27 ± 2.35	2.51 ± 0.14	18.81 ± 0.15	−33.11 ± 0.75	1.13 ± 0.22
Glaucium flavum		65.20 ± 14.94	0.75 ± 0.06	39.69 ± 4.66	42.35 ± 3.98	3.89 ± 0.91	11.23 ± 2.16	−35.45 ± 0.28	0.24 ± 2.04
Mesembryanthemum nodiflorum		1.46 ± 0.15	2.18 ± 0.01	37.62 ± 10.09	31.56 ± 1.79	4.53 ± 0.41	7.00 ± 0.49	−30.20 ± 0.27	4.02 ± 0.93
Crithmum maritimum		63.15 ± 9.70	6.46 ± 0.76	110.81 ± 8.95	40.60 ± 3.75	2.88 ± 0.19	14.12 ± 0.99	−31.55 ± 0.64	3.31 ± 0.56

Carbon/Nitrogen contents and stable isotopes for each studied species. Leaf area (LA), leaf thickness (LT), leaf mass per area (LMA), mass-based carbon content (C$_{mass}$), mass-based nitrogen content (N$_{mass}$), carbon:nitrogen (C:N) ratio, carbon isotope ratios (δ^{13}C), and nitrogen isotope ratios (δ^{15}N). Values are average ± SD (n = 6 per species)

horizontal and vertical axes. These axes stood for the first and second Principal Components of the PCA, which account for 57.72 percent of the variance of the assessed parameters (Fig. 3). The first axis of PCA (PC1) represented by the leaf physiological traits, morphological traits, and Carbon/Nitrogen contents and stable isotopes, characterized by high A/R$_d$, PNUE, g$_m$ and A on the upper right and higher LMA and LT on the upper left. The second (PC2) was represented as well by all these traits characterized by high ETR, A$_{mass}$, g$_s$ on the lower right and high l_m on the lower left. Non-natives presented high PNUE, A,

A$_{mass}$ and g$_m$, while native species had higher LMA and LT and were more represented on the upper left of the PCA.

While the PCA suggests some positive and negative relations between some leaf physiological traits and morphological traits, Spearman correlation only showed a significantly negative correlation between A$_{mass}$ and LMA ($r = -0.75$; $p < 0.001$), placing non-native and natives species at a different end, with non-natives possessing larger values in A$_{mass}$ and lower values in LMA, in contrast to natives (Fig. 4A). However, no significant
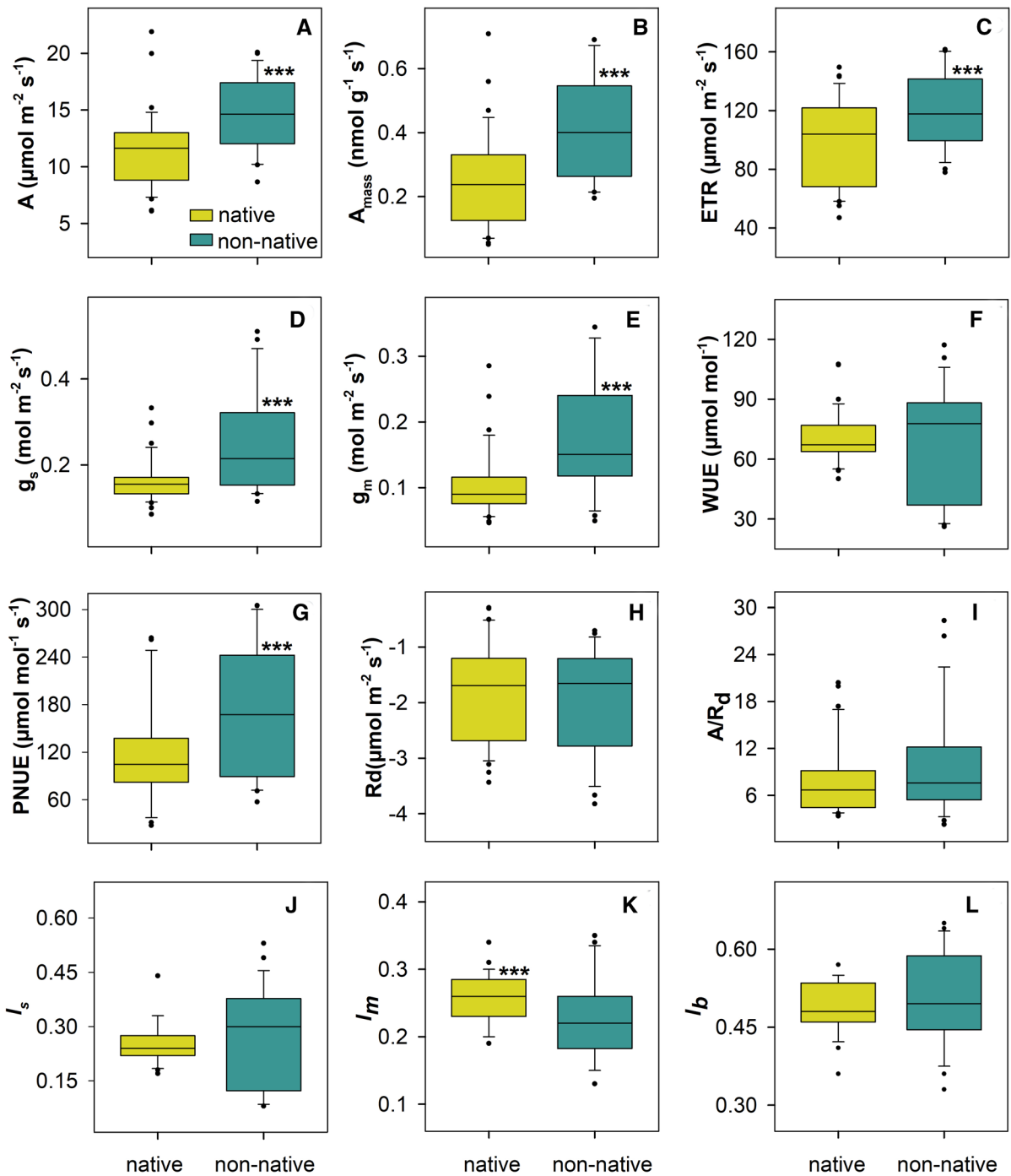
Fig. 2 Box plots of the leaf physiological traits. **A** net CO_2 assimilation (A), **B** maximum net assimilation rate per unit dry mass (A_{mass}), **C** electron transport rate (ETR), **D** stomatal conductance (g_s), **E** mesophyll conductance (g_m), **F** intrinsic water-use efficiency (WUE), **G** photosynthetic nitrogen-use efficiency (PNUE), **H** respiration (R_d), and **I** photosynthetic CO_2 assimilation over respiration (A/R_d), **J** stomatal limitation (l_s), **K** mesophyll conductance limitation (l_m) and **L** biochemical limitation (l_b) for native (in yellow) and non-native (in green) plant species studied. *Denotes statistically significant difference at $p < 0.05$ and *** $p < 0.001$

**Table 4** Values of leaf physiological traits for each studied species

Species	Status	A (μmol m^{-2} s^{-1})	A$_{mass}$ (nmol g^{-1} s^{-1})	ETR (μmol m^{-2} s^{-1})	g$_s$ (mol m^{-2} s^{-1})	g$_m$ (mol m^{-2} s^{-1})	WUE (μmol mol^{-1})
Physalis peruviana	Non-native	15.08 ± 3.74	0.70 ± 0.37	133.02 ± 22.65	0.17 ± 0.05	0.21 ± 0.08	91.57 ± 9.03
Nicotiana glauca		17.38 ± 1.28	0.43 ± 0.04	147.02 ± 11.38	0.18 ± 0.04	0.29 ± 0.05	98.51 ± 18.07
Tropaeolum majus		15.49 ± 1.08	0.56 ± 0.11	111.40 ± 11.97	0.40 ± 0.09	0.13 ± 0.01	40.87 ± 11.37
Solanum linnaeanum		12.14 ± 0.62	0.25 ± 0.02	96.32 ± 4.56	0.15 ± 0.02	0.15 ± 0.02	80.19 ± 8.91
Arundo donax		20.89 ± 1.55	0.29 ± 0.02	155.15 ± 9.96	0.23 ± 0.01	0.34 ± 0.14	89.10 ± 4.36
Mesembryanthemum crystallinum		10.67 ± 1.94	0.27 ± 0.12	99.25 ± 23.52	0.35 ± 0.13	0.06 ± 0.01	33.34 ± 12.33
Withania somnifera	Native	10.89 ± 1.91	0.18 ± 0.06	89.59 ± 21.31	0.15 ± 0.01	0.11 ± 0.02	73.09 ± 11.82
Hyoscyamus albus		10.83 ± 1.29	0.20 ± 0.04	106.58 ± 13.02	0.16 ± 0.01	0.08 ± 0.02	67.94 ± 8.42
Convolvulus arvensis		13.57 ± 0.61	0.32 ± 0.07	125.74 ± 5.37	0.14 ± 0.02	0.16 ± 0.04	98.20 ± 15.85
Solanum nigrum		20.93 ± 1.35	0.63 ± 0.11	146.24 ± 4.56	0.31 ± 0.02	0.26 ± 0.03	66.54 ± 0.91
Phragmites australis		7.78 ± 0.17	0.12 ± 0.04	59.56 ± 2.10	0.12 ± 0.01	0.08 ± 0.01	65.09 ± 1.91
Glaucium flavum		13.09 ± 1.45	0.34 ± 0.07	121.77 ± 16.23	0.22 ± 0.03	0.09 ± 0.01	61.16 ± 7.72
Mesembryanthemum nodiflorum		11.92 ± 0.67	0.33 ± 0.09	115.85 ± 9.06	0.16 ± 0.01	0.10 ± 0.01	76.40 ± 8.05
Crithmum maritimum		7.40 ± 1.36	0.07 ± 0.02	59.47 ± 8.78	0.12 ± 0.03	0.06 ± 0.01	62.19 ± 8.92

Species	Status	PNUE (μmol mol^{-1} s^{-1})	R$_d$ (μmol m^{-2} s^{-1})	A/R$_d$	l_s	l_m	l_b
Physalis peruviana	Non-native	291.46 ± 225.65	-2.57 ± 0.94	6.88 ± 3.89	0.38 ± 0.05	0.20 ± 0.04	0.42 ± 0.03
Nicotiana glauca		226.33 ± 17.5	-2.87 ± 0.39	6.15 ± 1.13	0.42 ± 0.10	0.16 ± 0.02	0.41 ± 0.08
Tropaeolum majus		277.34 ± 45.87	-1.15 ± 0.26	14.02 ± 3.56	0.14 ± 0.04	0.26 ± 0.01	0.60 ± 0.04
Solanum linnaeanum		76.50 ± 13.80	-1.43 ± 0.28	8.77 ± 2.10	0.31 ± 0.04	0.21 ± 0.02	0.48 ± 0.03
Arundo donax		176.81 ± 13.41	-0.77 ± 0.07	27.01 ± 1.15	0.37 ± 0.04	0.17 ± 0.05	0.48 ± 0.02
Mesembryanthemum crystallinum		101.64 ± 33.74	-2.84 ± 0.74	4.00 ± 1.31	0.10 ± 0.04	0.32 ± 0.03	0.58 ± 0.06
Withania somnifera	Native	109.98 ± 22.62	-1.49 ± 0.21	$7.31 \pm .085$	0.27 ± 0.05	0.23 ± 0.02	0.50 ± 0.05
Hyoscyamus albus		122.96 ± 28.78	-2.31 ± 0.50	4.90 ± 1.37	0.23 ± 0.04	0.29 ± 0.02	0.48 ± 0.02
Convolvulus arvensis		174.64 ± 77.80	-1.42 ± 0.19	9.60 ± 1.14	0.39 ± 0.09	0.22 ± 0.03	0.39 ± 0.05

Table 4 (continued)

Species	Status	PNUE (µmol mol^{-1} s^{-1})	R_d (µmol m^{-2} s^{-1})	A/R_d	l_s	l_m	l_b
Solanum nigrum		255.27 ± 9.43	− 2.73 ± 0.31	7.74 ± 1.39	0.26 ± 0.01	0.20 ± 0.01	0.55 ± 0.01
Phragmites australis		60.34 ± 32.75	− 0.83 ± 0.33	10.42 ± 4.21	0.24 ± 0.01	0.23 ± 0.02	0.53 ± 0.01
Glaucium flavum		123.99 ± 31.54	− 2.30 ± 0.51	5.87 ± 1.20	0.21 ± 0.03	0.30 ± 0.01	0.50 ± 0.04
Mesembry-anthemum nodiflorum		106.07 ± 36.33	− 2.95 ± 0.38	4.09 ± 0.62	0.27 ± 0.04	0.27 ± 0.02	0.46 ± 0.03
Crithmum maritimum		35.02 ± 7.17	− 0.59 ± 0.41	15.92 ± 6.08	0.21 ± 0.03	0.27 ± 0.01	0.52 ± 0.04

Net CO_2 assimilation (A), maximum net assimilation rate per unit dry mass (A_{mass}), electron transport rate (ETR), stomatal conductance (g_s), mesophyll conductance (g_m), intrinsic water-use efficiency (WUE), photosynthetic nitrogen-use efficiency (PNUE), respiration (R_d), photosynthetic CO_2 assimilation over respiration (A/R_d), stomatal limitation (l_s), mesophyll conductance limitation (l_m) and biochemical limitation (l_b). Values are average ± SD (n = 6 per species)

Fig. 3 Principal Component Analysis (PCA) of the significant variables for the leaf morphological traits, Carbon/Nitrogen contents and stable isotopes, and leaf physiological traits between for native (in yellow) and non-native (in green) plant species studied. The 2 big points represent the centroids of both native and non-native plant species
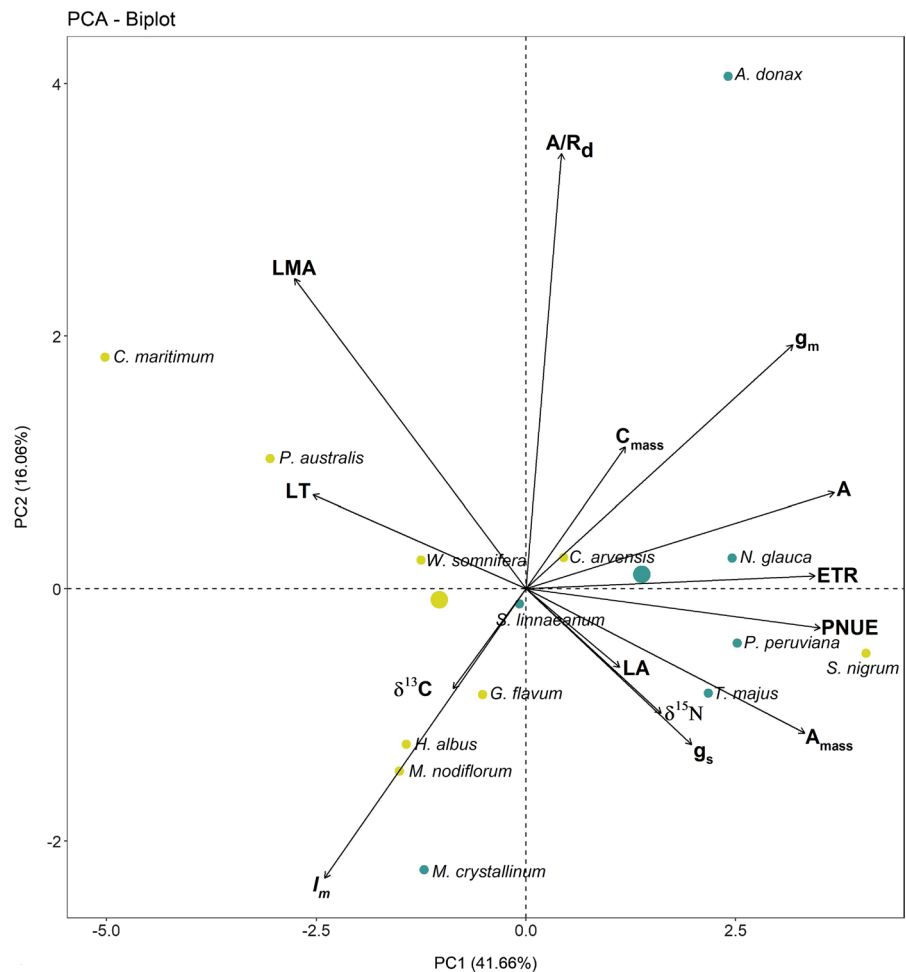
correlation was found between both A_{mass} and N_{mass} ($r = 0.08$; $p = 0.55$) (Fig. 4B) or A and R_d ($r = -0.21$; $p = 0.11$) (Fig. 4C) and, in fact, no other significant correlation was found between any of the studied parameters, except the obvious ones (e.g., A vs g_s and g_m, A vs PNUE, etc.—data not shown).
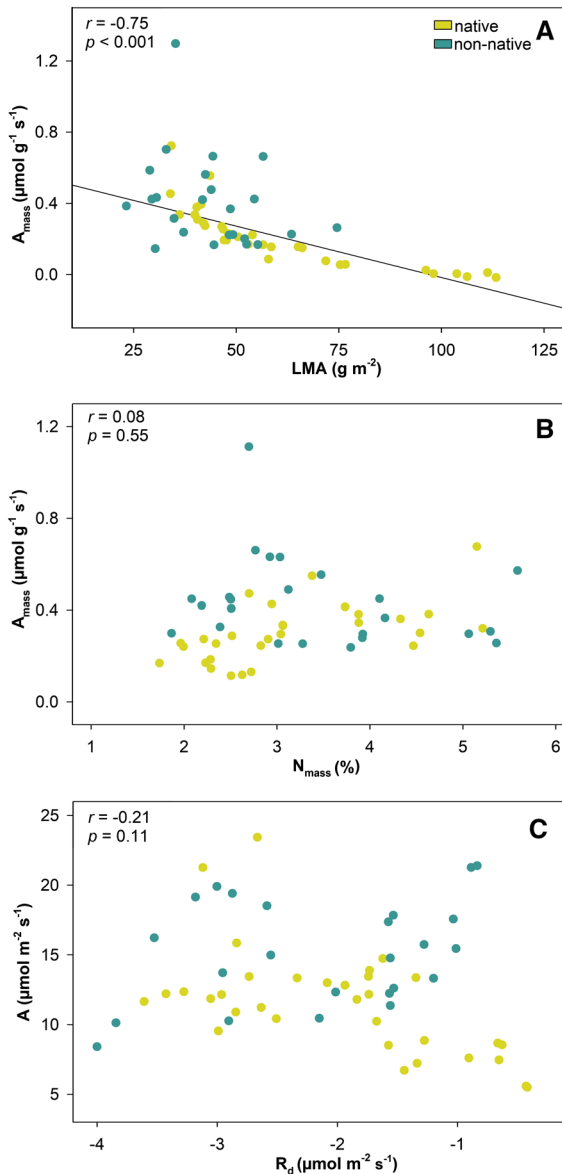

Fig. 4 Relationship between the maximum net assimilation rate per unit dry mass (A_{mass}) versus leaf mass per area (LMA) (**A**), mass-based nitrogen content (N_{mass}) (**B**), and net CO_2 assimilation (**A**) versus respiration (R_d) (**C**) for native (in yellow) and non-native (in green) plant species studied

Discussion

Our results showed that non-native plant species present in a Mediterranean island tend to have advantageous values in their leaf physiological traits, such as large A, A_{mass}, g_s, PNUE, among others (Fig. 2), while possessing lower leaf construction cost in terms of LT and LMA, when comparing them to co-occurring native species (Fig. 1). Many of these traits partly explain the success of non-native plant species (Baruch and Goldstein 1999; Funk and Vitousek 2007) since they may contribute to a faster growth rates and confer a competitive advantage over native species (Peñuelas et al. 2010; Matzek 2011; Funk 2013; Kunstler et al. 2015), at least under favorable weather conditions which, in the Mediterranean, usually coincide with the plant's growing season. Of course, traits conferring tolerance to environmental stresses occurring especially in summer may also contribute to different species fitness, yet this is out of the scope of the present study. The results presented here are in accordance with previous views for native and non-native plants comparisons from other environments. Peñuelas et al. (2010) have reported that alien species throughout Oahu Island (Hawaiʻi) had enhanced capacities in terms of productivity (photosynthetic capacity) and lower LMA, causing such species to have higher photosynthetic returns from their foliar biomass investment. More recent studies also showed that the high photosynthetic rates of non-native species reflect their potential to accumulate more biomass, which convey higher competitive ability leading to invasion success (Tordoni et al. 2019; Zunzunegui et al. 2020; Morais et al. 2021).

Along with the long list of distinguished physiological traits between native and non-native plants, the present study reveals a novel trait which is mesophyll conductance (g_m), being on average higher in non-native species. It can be observed that g_m plays a major role in setting the difference of the photosynthetic capacity between native Mediterranean plants and non-native species. Such difference is usually correlated with leaf anatomy (Flexas et al. 2008; Terashima et al. 2011). Lower g_m appears to be related to a significantly higher LMA observed in native species (Fig. 1C). Large LMA is associated to high leaf thickness, which appears to be an adaptation to stressful environments like those in the Mediterranean climate (Niinemets 1999). Besides

LMA, sub-cellular anatomical traits, not studied here, had been shown to determine low g_m, such as thick cell walls and low chloroplast coverage of intercellular air spaces (Tomás et al. 2013; Tosens et al. 2016; Carriquí et al. 2019; Gago et al. 2019). When performing the photosynthetic limitations analysis, only mesophyll conductance limitation (l_m) differed significantly, being on average higher in native species (Fig. 2K). Such finding is in accordance with the fact that l_m tends to be larger in native Mediterranean species (Flexas et al. 2014). Regardless of the mechanisms, which remain unknown, this study reports, for the first time, that a lower l_m may be a significant physiological trait for contributing to the 'invasive syndrome' of non-native species. Despite this, these results were not in full accordance with the 2nd hypothesis, as WUE did not significantly differ between native and non-native species. This result is in contrary to the findings of Zunzunegui et al. (2020) and Morais et al. (2021). Such result with significantly lower $\delta^{13}C$ for non-native plants (Fig. 1G), suggest lower WUE in the long term. Considering this, it could be argued that under water stress non-native species may not perform as well as natives, making them less adaptive to substantial warmings and prolonged periods of water shortage predicted to occur in Mediterranean ecosystems (Cramer et al. 2018). To test this, however, further studies should be performed under stress conditions.

When it comes to leaf structure and function relationships described in the leaf economics spectrum, the present results were in accordance with a few other studies that used it in the field of invasion ecology (Ordonez and Olff 2013; Petruzzellis et al. 2021). We report that non-native species on a Mediterranean island, as a group, differ from natives in both individual traits and their positioning along the LES, locating them predominantly at the "fast-return" end of it, with faster investment strategies than co-occurring native species, associated with higher values of A, PNUE and lower values of LMA. This high PNUE in 'fast' strategy plants may be due to a greater proportional allocation of C and N to structural than metabolic components of the leaf in low LMA leaves (Poorter et al. 2009). Such finding highlights the importance of the particular ecological-abiotic background a species faces once introduced. Consequently, introduction success is not only dependent of area of origin, but also on introduced species having a suite of traits that enable them to use the new habitat (Thompson and Davis 2011). However, and surprisingly, only the relationship between A_{mass} vs LMA was significant, while A_{mass} vs N_{mass} and A vs R_d relationships were not, contrarily to what was described for LES for plants in general (Wright et al. 2004) and native/non-native comparisons in particular (Petruzzellis et al. 2021). McDowell (2002) attributed the higher PNUE observed in non-native species to their lower N content, and this is confirmed in our study since leaf N value in our assessed non-native plants was significantly lower ($p < 0.05$) to that in native species when expressed on an area basis, although not significant when expressed on a mass basis ($p = 0.39$).

Conclusion

A leaf physiological trait syndrome is described for non-native species invading a Mediterranean island that is similar to that previously described for non-native species in other environments, consisting of larger A, A_{mass}, g_s, PNUE and lower LMA and LT, among others. While the vast majority of the assessed traits followed the same trend in non-native species possessing advantageous values, WUE was not significant. This lack of advantage on a key parameter under semi-arid conditions may perhaps reduce the success of non-native species when subjected to water stress. Hence, this should be confirmed in further experiments. Among the assessed traits, we report for the first-time larger g_m, and lower l_m in non-native species, which seems to be linked to their lower LMA. These novel traits need to be added to the 'leaf physiological trait invasive syndrome'. Finally, our results confirm that non-native plants on a Mediterranean island are also positioned on the "fast-return" end of the LES. However, that only one significant LES relationship (A_{mass} vs LMA) was found here might be explained by the fact that these plants were not grown in the field but in a growth chamber with addition of nutrient solution containing large amounts of N. This is interesting although it deserves confirmation. If so, it would mean that the A_{mass} vs LMA relationship is the most robust among studied LES traits, for which it possibly is a central or primary one, while others being secondary or consequences of it.

Declaration

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

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Leaf morpho-physiological comparison between native and non-native plant species in a...

2611

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