

1 Title: **Multi-species comparison reveals that invasive and native plants**
2 **differ in their traits but not in their plasticity.**

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18 Running headline: **Phenotypic plasticity and invasiveness**

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1 **Summary**

2 1. Plastic responses to spatio-temporal environmental variation strongly influence
3 species distribution, with widespread species expected to have high phenotypic
4 plasticity. Theoretically, high phenotypic plasticity has been linked to plant
5 invasiveness because it facilitates colonization and rapid spreading over large and
6 environmentally heterogeneous new areas.

7 2. To determine the importance of phenotypic plasticity for plant invasiveness, we
8 compare well-known exotic invasive species with widespread native congeners. First,
9 we characterized the phenotype of 20 invasive-native ecologically and phylogenetically
10 related pairs from the Mediterranean region by measuring 20 different traits involved in
11 resource acquisition, plant competition ability and stress tolerance. Second, we
12 estimated their plasticity across nutrient and light gradients.

13 3. On average, invasive species had greater capacity for carbon gain and enhanced
14 performance over a range of limiting to saturating resource availabilities than natives.
15 However, both groups responded to environmental variations with high albeit similar
16 levels of trait plasticity. Therefore, contrary to the theory, the extent of phenotypic
17 plasticity was not significantly higher for invasive plants.

18 4. We argue that the combination of studying mean values of a trait with its
19 plasticity can render insightful conclusions on functional comparisons of species such as
20 those exploring the performance of species co-existing in heterogeneous and changing
21 environments.

22

23 **Keywords:** Exotic species, phylogenetically independent contrast, light, nutrient,
24 ecophysiological traits, resource use efficiency, phylogenetic conservatism.

25

1 **Introduction**

2 Linking phenotypic plasticity to invasiveness of exotic species is an important topic in
3 the study of biological invasion (Hulme, 2008, Richards, Bossdorf, Muth *et al.*, 2006,
4 Funk, 2008). Phenotypic plasticity, measured as the ability to express different
5 phenotypes in different environments (Sultan, 1995, Valladares, Wright, Lasso *et al.*,
6 2000, Pigliucci, 2001, West-Eberhard, 2003) has been widely predicted to be a key trait
7 for explaining why exotic invasive species are so successful in their recipient
8 communities (e.g. Williams, Mack & Black, 1995, Sakai, Allendorf, Holt *et al.*, 2001,
9 Sexton, McKay & Sala, 2002, Niinemets, Valladares & Ceulemans, 2003, Funk, 2008).
10 Theoretically, high plasticity is likely to influence the potential invasiveness of species
11 because it may enable them to express advantageous phenotypes over a broad range of
12 environments (detailed in Matesanz, Gianoli & Valladares, 2010), potentially enhancing
13 their ecological success and their impact across ecosystems (Alpert, Bone & Holzapfel,
14 2000, Daehler, 2003, Hulme, 2008). For instance, it has been postulated that plasticity
15 would reduce the risk of stochastic local extinction after the arrival of a few genotypes
16 and augment the likelihood of species to become invasive under environmental
17 conditions to which they were not pre-adapted (Sultan, 2001, Ghalambor, McKay,
18 Carroll *et al.*, 2007, Bossdorf, Lipowsky & Prati, 2008). High plasticity can be due
19 either to an adaptive strategy to cope with spatio-temporal resource fluctuation in the
20 native region (Williams *et al.*, 1995, Atkin, Loveys, Atkinson *et al.*, 2005) or to rapid
21 evolution in novel sites after colonization (Agrawal, 2001, Yeh & Price, 2004).
22 Although it still remains to be tested, the higher the capacity to vary a broad number of
23 morphological and ecophysiological traits the higher the likelihood of invaders to be
24 pre-adapted to invasion (Hulme, 2008), especially under low resource environments
25 (Davis, Grime & Thompson, 2000).

1 Richards, Bossdorf, Muth *et al.*, (2006) classified invasive species into three
2 categories according to the fitness outcome due to plasticity as compared with a control
3 group of native/non-invasive species: two main categories “Jack-of-all-trades” and
4 “Master-of-some”, and a third category “Jack-and-Master”, which is a combination of
5 the first two. Under the “Jack-of-all-trades” strategy, successful invasive species are
6 hypothesized to have homeostasis of fitness (i.e. fitness remains constant along a
7 resource gradient), because they are better able to maintain higher fitness in
8 unfavourable environments. Several studies have shown evidence for this strategy. For
9 example, invasive species (two trees, one shrub and two grasses) in Hawaiian Islands
10 varied their specific leaf area to maximize light capture in response to a decrease of
11 light availability (Funk, 2008). In response to increased temperature, *Ailanthus altissima*
12 and *Acer platanoides*, two widespread invasive tree species, shifted their biomass
13 allocation from transpiring tissues to roots and water transporting tissues (Säumel,
14 2006). High plasticity in root/shoot ratio resulted in enhanced water uptake under
15 drought conditions in species such as, *Alternanthera philoxeroides* and *Taraxacum*
16 *officinale* (Brock & Galen, 2005, Geng, Pan, Xu *et al.*, 2006). In contrast to the “Jack-
17 of-all-trades” strategy, the “Master-of-some” strategy encompasses those successful
18 invasive species able to obtain higher fitness in response to an increase in resources
19 availability via phenotypic plasticity. Finally, “Jack-and-Master” strategy includes those
20 successful invasive species which are better able to maintain and to increase fitness in
21 unfavourable and favourable environments, respectively.

22 Although arguments in favor of linking phenotypic plasticity to plant invasion
23 seem reasonable, no general pattern between phenotypic plasticity and invasiveness
24 have emerged so far. While some studies support higher plasticity of invaders
25 (Niinemets, Valladares & Ceulemans, 2003, Schumacher, Kueffer, Edwards *et al.*,

1 2009, Davidson, Jennions & Nicotra, 2011) others do not (Bossdorf, Auge, Lafuma *et*
2 *al.*, 2005, Peperkorn, Werner & Beyschlag, 2005, Funk, 2008). Nevertheless and despite
3 the fact that trait plasticity and trait mean value covary, only trait plasticity has been
4 taken into account (but see Stinchcombe & Schmitt, 2006, Callahan & Pigliucci, 2002
5 for selection analyses of plasticity). With the same adaptive extent of phenotypic
6 plasticity one species may display higher fitness compared to another if the value of a
7 given trait that account for fitness is significantly higher in the former. Accordingly,
8 even low plasticity may be advantageous for the former species if the difference in the
9 mean value of this trait is proportionally higher than the difference in plasticity
10 displayed between the two species. This may explain why some studies have found that
11 invasive species outperform native species even when the two have the same level of
12 plasticity (e.g. Peperkorn, Werner & Beyschlag, 2005, Funk, 2008). Therefore, if we
13 want to assess the importance of phenotypic plasticity to the invasiveness of exotic
14 species, the question is not only how plastic an invasive species is, but how is the trait
15 mean value related to fitness.

16 The aim of this study was to explore phenotypic plasticity in exotic invasive
17 species using a common garden experiment. 20 phylogenetically related pairs of
18 invasive-native species covering a diversity of growth forms (trees, shrubs, perennial
19 and annual herbs) that co-occur in Mediterranean ecosystems were compared measuring
20 20 different traits in a common garden experiment across one nutrient and one light
21 gradient. Specifically, we ask: (1) whether exotic invasive species and native species
22 differ in their trait mean values (2) whether exotic invasive species have higher levels of
23 plasticity than native species, (3) whether differences of particular trait-plasticity and
24 overall mean trait-plasticity between both groups are dependent on the level of the
25 resource gradient considered or not.

1 Studies at both species- and genotype-level are useful approaches to explore the
2 relationship between plasticity and invasiveness (Richards, Bossdorf, Muth *et al.*,
3 2006). Genotype-level approaches provide a precise study of plasticity, its mechanisms
4 and its evolutionary potential (Sultan, 2000). However, the species-level approach
5 allows for broader generalizations if many species and traits are included (Schlaepfer,
6 Glättli, Fischer *et al.*, 2010), and for the development of risk-assessment protocols
7 because species level is the most frequent taxonomic level for coping with invasions in
8 practice (van Kleunen, Weber & Fischer, 2010). Also, multispecies comparison allows
9 us to explore whether plasticity is phylogenetically conserved (Kembel & Cahill, 2005)
10 which may increase our capacity to predict potential invasiveness from phylogenetic
11 information of the species. Despite their importance, studies involving multi-species
12 comparison are scarce and most functional studies of invasive organisms are restricted
13 to only a few species (but see Schlaepfer, Glättli, Fischer *et al.*, 2010) and a limited
14 number of traits.

15

16 **Material and Methods**

17 *Species selection*

18 From January to October 2005 and 2006, plants from twenty exotic and twenty native
19 species (Table 1) were grown from seeds in the Botanical Garden of the University of
20 Alcalá (40° 28'N, 3° 22'W, 588 m). The exotic species set was comprised of introduced
21 species clearly invasive in the Iberian Peninsula (*sensu* Pyšek, Richardson, Rejmánek *et*
22 *al.*, 2004), local dominants in some native ecosystems (Valéry, Fritz, Lefeuvre *et al.*,
23 2008), and species with potential impact on native ecosystems (transformer species,
24 *sensu* Richardson, Pyšek, Rejmanek *et al.*, 2000). Overall, they represent the broad
25 range of taxonomy, invaded habitats (woodlands, scrublands, grasslands and riparian

1 areas) and growth forms (annual and biannual herbs, shrubs and trees) of invasive
2 exotic species in the Iberian Peninsula (Sanz Elorza, Dana Sanchez & Sobrino
3 Vesperinas, 2004). Nomenclature follows the Iberian Flora (Castroviejo, 1986-2008),
4 which is in agreement with the Missouri Botanical Garden, VAST nomenclatural
5 database (W3Tropicos, <http://mobot.mobot.org/W3T/Search/vast.html>).

6 When assessing differences between invasive and native species, it is important
7 to take phylogenetically independent contrast controls into account as well as to ensure
8 that within pairs both species occur in similar ecosystems (Lambdon & Hulme, 2006).
9 Thus, we paired each invader with one closely related native species based on the
10 following phylogenetic and ecological guidelines: a) the native species had to belong to
11 the same family as the invasive species (that was achieved in 17 of 20 pairs), b) they
12 had to have the same growth form (achieved in all pairs except number 15 and 16 (See
13 Table S1 in Supporting Information), in which invasive species were trees and natives
14 shrubs), c) they had to co-exist in the same habitat-type in the Iberian Peninsula and the
15 same successional community stage, and d) they had to be recorded as co-occurring at
16 least once in Spain. We consulted the extensive Herbarium database at Universidad
17 Complutense de Madrid (MACB, founded 1968) to check for co-occurrence within
18 pairs. Native species with small distribution ranges, rare or with endangered status were
19 excluded.

20

21 *Experimental design*

22 Several resource concurrent gradients influence plastic responses of plants (Portsmouth
23 & Niinemets, 2007). However, in order to make useful predictions we need to quantify
24 potentially adaptive plastic traits to one resource at a time using a realistic resource
25 gradient (e.g. Funk, 2008, Quero, Villar, Marañón *et al.*, 2006, Sánchez-Gómez,

1 Valladares & Zavala, 2006, Poorter, 1999), despite the limitations of this approach
2 (Hulme, 2008). Accordingly, we designed a non-factorial experiment with two resource
3 gradients using two different greenhouses due to logistical limitations: Nutrient
4 Gradient (Low-Medium-High) under the same sun light conditions and Light Gradient
5 (Shade-Sun) under the same medium nutrient conditions. Within the “nutrient
6 greenhouse” radiation was kept constant at 50% full radiation ($950\text{-}1050\mu\text{molm}^{-2}\text{s}^{-1}$)
7 and light quality red:far red ratio (R:fR) =1. Within “light greenhouse” plants were
8 subjected to Medium-nutrient growth environment with “Shade” being 20% of full
9 sunlight radiation ($350\text{-}500\mu\text{molm}^{-2}\text{s}^{-1}$) and light quality modified to R:fR=0.8, which is
10 the most common shade under Mediterranean ecosystems (Valladares, 2004). Although
11 we did not use low light levels (e.g. 1-15% full radiation) that will potentially show the
12 non-linear plant responses to light variation (Poorter, 1999), this design enabled us to
13 reasonably frame our hypothesis across nutrient and light availabilities where exotic
14 species invade in Spanish Mediterranean ecosystems.

15 Half of the species pair were grown during 2005 and the other half during 2006
16 (see Table S1). In each year 144 plants per species were grown from seeds in individual
17 1 L pots (QP 12T/18, PROJAR, Spain) with vermiculite (0-3 mm grain, $80\text{-}100\text{kg/m}^3$,
18 PROJAR, Spain). Seeds were obtained from commercial supply or field collection and
19 sown in March of the corresponding year and grown for a full growing season. In both
20 cases, seeds came from locations where the exotic species are clearly invasive. For
21 commercial supply, seeds were certified to come from one single location. For field
22 collection, we collected seeds from 15-20 haphazardly chosen plants within one
23 population. Population delimitation was according to Schlaepfer, Glättli, Fischer *et al.*,
24 (2010) criteria.

1 In each greenhouse, plants were divided into 3 different blocks (12 plants per
2 block*species*treatment) to control for possible variation in measurements due to
3 microclimatic gradients. In addition, plants were randomly positioned in their block and
4 rotated every month. We fertilized plants at the beginning of the experiment with a
5 Plantacote mix 6 month slow-release fertilizer 14-9-15 N-P-K, (Aglukon Spezialdünger
6 GMBH & Co.KG, Dusseldorf, Germany). We used a slow-release fertilizer to ensure
7 that plants had available nitrogen throughout the experiment. The main nitrogen
8 compound was ammonium nitrate (NH_4NO_3) (85%). Plants received one dose, over the
9 six months, equivalent to 0.010g N in the Low-nutrient level, 0.085g N in the Medium-
10 nutrient level and 0.245g N in the High-nutrient level. Pure vermiculite was used as
11 substrate in the experiments to ensure that the fertilizer was the only source of nutrient
12 supply. The gravimetric soil water content in the pots was maintained at >30%. Local
13 air temperature and available photosynthetic photon flux density (PPFD) were recorded
14 every 5 min throughout the growing season with a data logger (HOBO model H08- 006-
15 04; Onset, Pocasset, MA, USA) and self-made external sensors that were cross-
16 calibrated with a Li-Cor 190SA sensor (Li-Cor, Lincoln, NB, USA). Mean daily PPFD
17 (400–700 nm) over the summer was $41 \text{ mol m}^2\text{d}^{-1}$, which is equivalent to full sunlight.

18

19 *Phenotypic traits*

20 We measured 20 variables related to plant- and leaf-level traits (see Table 1 for
21 abbreviations). Traits were selected because of their functional significance for resource
22 acquisition (e.g. high LAR and RWR are associated with light and nutrient acquisition,
23 respectively), plant competition (e.g. high rate of maximum photosynthesis and F_v/F_m
24 are associated with fast growth and good physiological status), and stress tolerance (e.g.
25 high PNUE is associated with high plant performance in nitrogen limited environments

1 and high SLA in light limited environments). Finally, we measured survival (n=36 per
2 species*treatment) and total biomass (above + below ground biomass) after 6 months
3 since germination as surrogates of fitness. These variables are commonly used in short-
4 term studies of perennial species when reproductive measures are difficult to obtain
5 (Sultan, Wilczek, Bell *et al.*, 1998, Funk, 2008).

6 Plant-level traits were recorded for nine replicate plants per treatment and
7 species (plants were arranged in 3 blocks). Each individual plant was separated into
8 leaves, stems and roots, oven-dried at 60°C for 3 days and weighed to calculate weight
9 ratio of leaf (LWR), stem (SWR) and root (RWR) per total biomass. Before oven-
10 drying the material, leaf area of each whole individual was measured using a Delta-T
11 leaf area meter device (Delta-T devices, Cambridge), to calculate leaf area ratio (LAR=
12 leaf area/plant dry mass) and specific leaf area (SLA=leaf area/leaf dry mass).

13 Because the selected plant-level traits vary with ontogeny (Evans, 1972,
14 Poorter, 1999), we used a non-destructive method for measuring plant volume over time
15 that partially corrected for possible ontogenetic development drift between treatments
16 (e.g. usually plants exhibit faster development under higher nutrient availability). To
17 satisfy both needs, we first followed the growth dynamics measuring plant volume as a
18 function of height and crown cover for each species and nutrient treatment in each
19 month. For this, we used the semi-sphere equation ($V=(2/3)*\pi*r^2*H$) where H is height
20 and r is the mean radius (r) of plant cover (maximum radius + minimum radius/2).
21 Later, during August, we fitted for the mean plant volume of invasive and native species
22 a sigmoidal function to predict their volume increase. Results revealed that plants
23 growing under the high nutrient treatment developed faster (i.e. ontogenetic drift).
24 Therefore, we partly correct for differences on ontogeny across treatments by collecting
25 plants grown in the higher nutrient treatment one month before the end of the

1 experiment (October of 2005 or 2006) (see Appendix S2). This procedure also ensured
2 that the harvesting period was short enough to avoid an effect on the results.

3 Leaf-level traits were measured using a LI-6400 portable photosynthesis system
4 with a fluorescence chamber (LI-COR, Lincoln, NE) in one mid-height undamaged
5 fully expanded leaf (n=3 plants randomly selected per species and treatment). We
6 constructed light response curves at 10 light intensities of PAR following the order 0,
7 800, 1100, 1500, 1900, 500, 250, 150, 100, 50, 0 $\mu\text{molm}^{-2} \text{s}^{-1}$ and with the following
8 constant conditions: CO₂ concentration 400 ppm, flow 400 $\text{cm}^3 \text{min}^{-1}$, air humidity 40-
9 60% and block temperature 25°C. First, we adapted the leaf to dark for 30 minutes to
10 measure respiration (R_{dark}). Then, the leaf was irradiated with saturating and non-
11 inhibitory light (800 $\mu\text{molm}^{-2} \text{s}^{-1}$) for 10 minutes to be sure that plants were
12 photosynthetically active. We then changed light intensity and recorded maximum
13 photosynthetic rate (A_{max}) at each light level when it was stable (i.e. every 3 minutes on
14 average). At maximum light intensity (1900 $\mu\text{molm}^{-2} \text{s}^{-1}$), transpiration rate (T) was also
15 recorded to subsequently calculate instantaneous water use efficiency ($i\text{WUE} = A_{\text{max}}/T$).
16 Instantaneous WUE was calculated instead of intrinsic $\text{WUE} = A_{\text{max}}/g_s$ because we were
17 interested in the gas exchange ratio between carbon acquisition and water release. From
18 dark to maximum light intensity, we measured a set of fluorescence parameters (F_v/F_m ,
19 Φ_{PSII} , qP, qN, NPQ and ETR) (see Appendix S3). Additionally, we selected 3 random
20 plants per species and treatment to measure A_{max} and WUE in a total of 6 plants per
21 species and treatment.

22 We obtained photosynthetic parameters from light response curves using
23 Photosyn Assistant software version 1.1.1 (Richard Parsons, Dundee, U.K). This
24 software models the photosynthetic response of leaves to variation in light level by a
25 rectangular hyperbola following the quadratic equation presented by Chartier & Prioul

1 (1976), where the light compensation point (Γ) is estimated from intercept to x-axis, the
2 light saturation point (I_c) is the light level at which the leaf reaches its maximal
3 photosynthetic capacity and the convexity light curve factor (Θ) describes the
4 progressive rate of bending between the linear gradient and the maximum value.

5 We measured organic leaf nitrogen concentration per mass (N_{mass}) and per area
6 (N_{area}) at Nutrilab (University Rey Juan Carlos, Móstoles, Madrid, Spain) with
7 segmented flux autoanalyzer (S-F.A.S. Skalar San ++), after digestion with H_2SO_4 and
8 $Cu-KSO_4$, which converts all organic nitrogen into ammonium (NH_4^+-N). Previously,
9 leaves of each species and treatment had been pooled within blocks and ground in a
10 Culatti mill to 1 mm particle size. After that, N_{area} was calculated by dividing N leaf
11 content by the leaf area mean and photosynthetic nitrogen use efficiency (PNUE) as the
12 division of A_{max} by N_{area} . Mean values for each trait and species are shown in Table S4.

13

14 *Phenotypic plasticity*

15 We calculated trait variation for each plant species with the plasticity index (PI) created
16 by Valladares, Wright, Lasso *et al.*, (2000).

$$17 \quad PI = \frac{Mean(env1) - Mean(env2)}{Max(Mean(env1), Mean(env2))}$$

18 $Mean(env1)$ and $Mean(env2)$ are the mean values of a given trait for one species in the
19 environment 1 and 2, representing the mathematical expression of a reaction norm.

20 $Max(Mean(env1), Mean(env2))$ serves to standardize the index, which ranges from zero
21 (no plasticity) to one (maximum plasticity). PI also indicates the direction of the
22 change. For instance, a negative PI value indicates that the mean value of a given trait is
23 higher under environment 2. We preferred PI to other published plasticity indices (see
24 list of plasticity indices in Valladares, Sanchez-Gomez & Zavala, 2006) because it is the
25 index that better reflects a reaction norm and it is not sensitive to differences in variance

1 between two samples. *PI* was calculated for each trait and species along the two
2 resource gradients. We also calculated the mean plant-level and leaf-level plasticity, as
3 well as the overall mean plasticity.

4 Finally, it must be noted that we calculated *PI* for shade to sun under light
5 gradient (Sh-S) and for each experimental resource change under nutrient gradient (i.e
6 low to medium, medium to high nutrient level (L-M, M-H), instead of for the extreme
7 of the gradient (i.e low-high nutrient level L-H), in order to relate *PI* values to “Jack-of-
8 all-trades, Master-of-Some” framework.

9

10 *Statistical analysis*

11 We evaluated the effect of invasiveness (two levels, invasive vs. native), block
12 (three levels), and phylogenetic distance within pairs on phenotypic traits, on different
13 trait-plasticity scales (each trait-plasticity, mean plant-level and leaf-level plasticity, and
14 overall mean plasticity) and, finally, on fitness in the two resource gradients using a
15 non-parametric analysis of variance (PERMANOVA, Anderson, 2001, Anderson,
16 2005). We selected a PERMANOVA approach because it permits pairwise comparison
17 at different phylogenetic levels. This type of analysis was also selected because it does
18 not make assumptions of normality or homocedasticity of the data and its residuals. We
19 initially performed an analysis with all 20 phenotypic traits included, considering
20 invasive/native status and nutrient/light levels as fixed factors, block as a random factor
21 and phylogenetic distance within pairs as a co-variable. Next, a series of models
22 including one trait at a time as the dependent variable were performed to search for the
23 possible differences found in the first model including all variables. The same statistical
24 procedure was performed for the plasticity of the 20 measured traits, for the mean plant-
25 level and leaf-level plasticity, as well as for the overall mean plasticity. However, we

1 took a slightly different approach when we analysed each trait-plasticity separately.
2 Because plant size can directly influence biomass partitioning and thus plant-level trait
3 plasticity (Poorter, 1999, Funk 2008), we also included biomass as a covariable to test
4 whether observed plasticity was a mechanistic consequence of an increase in plant size
5 (apparent plasticity *sensu* Dudley, 2004, Hulme, 2008) or a plastic strategy *per se*.
6 Analyses were conducted to compare data from L-M nutrient levels and M-H nutrient
7 levels and from Sh-S light levels. In all cases, significant results between invasive and
8 native pairwise comparisons and post-hoc comparisons were estimated from 9999
9 permutations using Bray-Curtis dissimilarity, which measures the distance between the
10 trait values that remains unique to one group (invasive or native) divided over the trait
11 values common to both groups. The phylogenetic distance from one species to another
12 for each of the species pairs was calculated through to the first common ancestor to both
13 species using the plant phylogenetic supertree described by Soltis, Soltis, Chase *et al.*,
14 (2000) and modifications by Bremer, Bremer, Chase *et al.*, (2003).

15

16 *Phylogenetic pattern of plasticity*

17 We investigated phylogenetic conservatism in plasticity at different taxonomic levels by
18 implementing the phylogenetic node-dated tree of our invasive and native species set
19 and plasticity values (mean plant-level and leaf-level plasticity, overall mean plasticity)
20 into the Analysis of Traits (AOT), module of Phylocom package (Webb, Ackerly &
21 Kembel, 2008). However, we did not correct for ontogenetic drift here since we used
22 mean plasticity values. To perform the analyses, we first built a pruned phylogenetic
23 tree with the study species as terminal tips using the maximally resolved seed plant tree
24 available in Phylomatic (<http://www.phylodiversity.net/phyloomatic/>). Next, we
25 calibrated the resulting tree by dating the nodes with the Branch Length ADJustment
26 function (BLADJ), another module of Phylocom, on the basis of clade age estimation of

1 Wikstrom, Savolainen & Chase, (2001). Once we obtained a node-dated tree, calibrated
2 in millions of years, we introduced plasticity values into the AOT to calculate
3 divergence-convergence degree at each internal node of the tree. The standard deviation
4 between trait means of daughter nodes was used as a proxy of the degree of divergence
5 at the focal node (i.e., divergence size). Significance of divergence size was estimated
6 by 20000 randomly permuting trait values across the tips of the phylogeny at a p-
7 value<0.05.

8 Furthermore, we dealt with polytomies in the input tree (Butler & King, 2004)
9 by randomly generating 100 fully resolved trees using MESQUITE (Maddison &
10 Maddison, 2009). We then re-sampled 50 of the 100 fully resolved trees randomly and
11 ran the analyses described above again separately for each of those 50 trees. None of the
12 results described in the following section changed with input tree which supports the
13 robustness of the analyses above to phylogenetic uncertainty.

14

15 **Results**

16 *Mean phenotypic values and performance*

17 Results of the PERMANOVA indicated significant differences from plant- to leaf-level
18 traits between invasive species and natives (Table 2, Table S4). Plant allometry was
19 similar in both groups, except that invasive species assigned more resources to above-
20 ground plant construction (higher SWR and lower RWR). Invasive species showed the
21 same maximum photosynthetic rate (A_{max}) as natives but surprisingly their nitrogen leaf
22 concentration per mass and per area was lower (N_{mass} , N_{area}). Thus, the photosynthetic
23 nitrogen use efficiency (PNUE) was higher for invasive species (Table 2). Despite both
24 groups exhibiting the same maximum photosynthetic rates, invasive species reached this
25 value at lower light intensities (light saturation point, I_c) and with faster light saturation

1 (light curve convexity, Θ). In addition, invasive species had lower instantaneous water
2 use efficiency (iWUE) than natives (Table 2). The different ways of leaf photo-
3 protection of invasive and native species led to convergent results in the physiological
4 status of the plants. (i.e. no significant differences were founded in Fv/Fm). Invasive
5 species protected their photosynthetic machinery against an excess of light through
6 higher non-photochemical quenching (NPQ) associated with a greater number of rich-
7 carbon photo-protective pigments (e.g. xanthophylls), whereas native species used
8 greater amounts of chlorophyll (photochemical quenching, qP) (Table 2). In summary,
9 differences on the light curve shape and the ability to produce photo-protective
10 pigments based on rich-carbon compounds reflected the fact that invasive species
11 possessed a fast-growth strategy due to faster carbon acquisition. Congruent with these
12 results, invasive species had higher biomass than natives at medium and high nutrient
13 levels but not at low nutrient level (Fig. 2). In the case of the light gradient, greater light
14 availability had a positive effect on biomass for both groups, but invasive species
15 always exhibited higher above-ground biomass for both light treatments (shade and
16 sun), and higher total biomass than natives under low light availability (shade).
17 PERMANOVA results revealed that plant survival did not differ between invasive and
18 native species along the nitrogen gradient ($F(\text{nutrient})_{2,119}=1.17$, $p=0.76$,
19 $F(I/N)_{1,119}=0.89$, $p=0.83$, $F(\text{nutrient}*I/N)_{1,119}=0.91$, $p=0.83$), whereas invasive species
20 had higher survival than natives under low light conditions ($F(\text{light})_{1,119}=14.22$ $p<0.01$,
21 $F(I/N)_{1,119}=2.15$ $p=0.73$, $F(\text{light}*I/N)_{1,119}=17.32$ $p<0.01$ (Fig. 2). Finally, analysis
22 including all variables showed that the effect of block and phylogenetic distance within
23 pairs on survival were not significant either for the nutrient greenhouse
24 ($F(I/N)_{1,119}=19.8$, $p<0.001$, $F(\text{block})_{2,119}=0.09$, $p=0.99$), $F(\text{phylogeny})_{1,119}=0.34$, $p=0.92$,

1 or for the light greenhouse ($F(I/N)_{1,119}=15.7$, $p<0.001$, $F(\text{block})_{2,119}=0.43$, $p=0.95$,
2 $F(\text{phylogeny})_{1,119}=0.55$, $p=0.89$).

3

4 *Phenotypic plasticity values: Invasive versus native*

5 Trait-plasticity in response to nutrient and light variation was highly variable
6 within traits (e.g. $N_{\text{area}}=0.07\text{-}0.66$ or $qP_{1900}=0.01\text{-}0.42$) and between traits from low trait
7 plasticity (e.g. $F_v/F_m=0.01\text{-}0.04$ or $qN_{1900}=0.01\text{-}0.06$) to high trait plasticity (e.g.
8 $LAR=0.11\text{-}0.70$) (Table 2). However, relatively few traits showed significant
9 differences in plasticity between invasive and native species. In most cases, variability
10 in plant-level trait plasticity was captured by plant size (biomass as a covariable) in
11 PERMANOVA analyses. Only after accounting for allometric effects, invaders did
12 show significantly higher LAR plasticity from medium to high nutrient. For leaf-level
13 traits, PNUE plasticity was consistently higher in invasive species from low to medium
14 nutrient and from shade to sun light. A_{max} followed the same pattern from low to
15 medium nutrient availability. On the other hand, nitrogen content per mass (N_{mass}) and
16 per area (N_{area}) varied less in invasive species from low to medium nutrient availability
17 and from shade to sun light conditions respectively (Table 2). The effective quantum
18 yield of Photosystem II and electronic transport rate under at high irradiances ($\Phi_{\text{PSII}1900}$,
19 ETR_{1900}) varied in opposite directions (i.e. the plasticity sign was different between
20 groups) from medium to high nutrient (Table 2). While invasive species showed a
21 positive increase in response to nutrient addition (i.e. negative PI_v values), native
22 species did the opposite. Finally, PERMANOVA results for all trait-plasticity
23 measurements, including into the analyses PI values from low to high nutrient,
24 indicated that the effect of block and phylogenetic distances within pairs were not
25 significant, either for nutrient gradient ($F(I/N)_{1,119}=22.8$, $p<0.001$,

1 F(nutrient)_{1,119}=19.44, p<0.001, F(block)_{2,119}=0.12, p=0.97), F(phylogeny)_{1,119}=0.30,
2 p=0.93, or for light gradient (F(I/N)_{1,119}=20.72 p<0.001, F(block)_{2,119}=0.66, p=0.90,
3 F(phylogeny)_{1,119}=0.55, p=0.89).

4 The main pattern of non-significantly higher plastic responses of invaders was
5 repeated for the mean of plant-level, leaf-level and overall plasticity in both nutrient and
6 light gradients (Fig. 2-3). In some cases the invasive species even had lower phenotypic
7 plasticity, as in the case of mean leaf physiology plasticity when nutrient availability
8 increased from medium to high. In summary, contrary to theory, our results showed that
9 invasive species did not display a higher phenotypic plasticity.

10

11 *Phylogenetic conservatism of plasticity*

12 Cross-species phenotypic plasticity differences were not explained by the phylogenetic
13 structure of the species selected when including together invasive and native species
14 into the node-dated tree. All the taxonomic families showed the same level of
15 phenotypic plasticity, across the phylogenetic tree, thus no divergence or convergence
16 between nodes was observed (Plant-level plasticity $r^2=0.09$ p=0.80, Leaf-level plasticity
17 $r^2=0.11$ p=0.77, Mean phenotypic plasticity $r^2=0.04$ p=0.91) (Fig. 4).

18

19 **Discussion**

20 *Mean phenotypic values: invasive versus native species*

21 Mean phenotypic values of traits related to growth and allocation are considered
22 important for explaining invasiveness (Pyšek & Richardson, 2007, van Kleunen, Weber
23 & Fischer, 2010). Several studies have shown that invasive species have lower
24 root/shoot ratio, higher SLA, and exhibit more efficient photosynthetic machinery
25 compared to native or non-invasive species (Daehler, 2003, Schlaepfer, Glättli, Fischer

1 *et al.*, 2010, Pyšek & Richardson, 2007 and references therein, van Kleunen, Weber &
2 Fischer, 2010). At the same time, high resource use efficiency (i.e. high carbon
3 assimilation per unit of resource) have been hypothesized as a decisive feature allowing
4 exotic species to become invasive in newly colonized ecosystems (Dukes & Mooney,
5 1999, Niinemets, Valladares & Ceulemans, 2003, Funk & Vitousek 2007). In support of
6 both hypotheses, we have found significantly lower RWR and higher PNUE in invasive
7 than in native species (Table 2). On the other hand, higher SLA and LAR as strong
8 correlates of invasiveness (Daehler, 2003, Hamilton, Murray, Cadotte *et al.*, 2005) were
9 not found in our data. Although both groups had a similar rate of net photosynthesis per
10 unit area (A_{\max}), invasive species achieved it with lower N investment in leaves (N_{area})
11 (Table 2). Investing less nitrogen in leaves may be inherent to the nitrogen resource use
12 strategy of invasive species (Godoy, Castro-Díez, Logtestijn *et al.*, 2010). For instance,
13 there were no significant differences in N_{mass} and N_{area} across the three nutrient levels
14 (data not shown). Niinemets, Valladares & Ceulemans, (2003) suggested that the origin
15 of higher PNUE of invaders was related to reduction in soil nitrogen availability upon
16 invasion, because invasive species slow down nutrient cycling due to the production of
17 litter with a high concentration of recalcitrant compounds (e.g. allelopathic and carbon-
18 based compounds, Godoy, Castro-Díez, Logtestijn *et al.*, 2010). By contrast, Liao,
19 Peng, Luo *et al.*, (2008) and Ehrenfeld, (2003) have found that most invasive species
20 tend to speed up nutrient cycling, specially nitrogen-fixing invaders. Irrespective of
21 what theory matches better with different empirical studies, our results support the idea
22 that high PNUE, is a key trait associated with invasiveness in both limiting and non-
23 limiting N environments.

24 Our results have documented for the first time the existence of a suite of traits
25 that allow invasive species to achieve a higher light-harvesting efficiency than that of

1 the natives and a better photosynthetic performance under a range of light
2 environments. Significantly higher light curve convexity (Θ) and lower light saturation
3 point (I_c) clearly enhanced carbon uptake of invasive species under limited irradiance
4 (Table 2 and Fig. 2). Additionally, invaders avoided an excess of irradiance by
5 producing higher amount of carbon-rich compounds, such as xanthophylls (significant
6 higher NPQ_{1900}), whereas native species tend to use photosynthesis pathway and thus
7 chlorophylls (significant higher qP_{1900}) (Table 2). These higher trait values associated
8 with carbon acquisition by invaders and quenching differences between both groups
9 suggest that invaders disposed enough carbon to diminish the carbon trade-off between
10 growth and tissue protection (Villar, Robleto, De Jong *et al.*, 2006). In contrast, native
11 species might have to trade off growth for leaf photo-protection by producing N-rich
12 pigments, such as chlorophylls, as suggested by their higher nitrogen leaf content (N_{area} ,
13 N_{mass}) (Table 2) . Although we did not find significant differences in the physiological
14 status of both groups (i.e. Fv/Fm was similar), this subtle difference between leaf-photo
15 protection strategies involves for native species an increased risk of damage of their
16 chlorophylls by photo inhibition, which in turn can decrease carbon gain and growth.

17 Collectively, our results reveal that the invasive species studied displayed high
18 tolerance to and high performance over a wide range of nutrient and light conditions.
19 This is in agreement with results for other ecosystems (Mooney & Hobbs, 2000, van
20 Kleunen, Weber & Fischer, 2010). The higher trait mean values for key aspects of
21 physiological performance of invasive species must positively influence their capacity
22 to outcompete natives. These higher trait means might be more important than
23 phenotypic plasticity to succeed under changing conditions because in Mediterranean-
24 type ecosystems (MTE) plasticity is not always advantageous. Under stochastic and
25 unpredictable resource fluctuations, plastic adaptive responses to a given abiotic factor

1 can turn out to be maladaptive when another abiotic factor is also fluctuating and
2 stressful (see discussion in Valladares, Gianoli & Gomez, 2007).

3 4 *Phenotypic plasticity: invasive versus native species*

5 Our results do not match with previous studies that suggest that invasiveness of
6 exotic species is related to an increased phenotypic plasticity (see for instance Gerlach
7 & Rice, 2003, Niinemets, Valladares & Ceulemans, 2003, Davidson, Jennions &
8 Nicotra, 2011). In general, the level of plasticity of invasive species measured at
9 multiple scales (trait, mean trait-level, and overall mean plasticity) was similar, and in
10 some cases even lower, to that of natives (Fig. 2-4, Table 2). In addition, both invasive
11 and native species had highly plastic responses across the entire resource gradient,
12 supporting the idea that trait plasticity is not constrained in low resource environments
13 (Funk, 2008). Still, our results should be corroborated at very low resource conditions
14 where species usually show non-linear plastic responses (e.g. light availability below
15 20% full radiation) (Poorter, 1999). The lack of evidences supporting high plasticity as
16 a determinant of invasiveness might indicate that their importance *per se* is low
17 compared to other mechanisms. For instance, invasiveness may be also attributed to the
18 benefits of escaping from natural enemies, such as pathogens or predators, as predicted
19 by the Enemy Release Hypothesis (ERH) and the Evolution of Increased Competitive
20 Ability (EICA). Since there are almost 20 different hypotheses in the literature trying to
21 explain invasiveness, future promise research may be channelled to distinguishing the
22 relative importance of different hypotheses.

23 Even so, invasive species did display higher plasticity for a few traits under
24 particular resource availabilities, providing some insights on plastic strategies that may
25 convey invasiveness. When resource availability changed from low to medium nutrient

1 and from shade to sun, invasive species displayed higher plasticity in only two leaf
2 physiological traits, PNUE and N leaf content (both N_{mass} and N_{area}) (Table 2). Funk,
3 (2008) investigated the plastic responses of 5 invasive-native pairs in low resource
4 environments of the Hawaiian Islands and found that A_{max} and N_{area} were positively
5 related to the invasive species fitness, although this pattern was observed in response to
6 nitrogen but not to light availability. Thus, it seems that high plasticity in leaf-level
7 nitrogen and light use traits is important for successful plant invasions, particularly in
8 low resource environments.

9 Surprisingly, when resource conditions change from medium to high nutrient
10 level, invasive species tracked the nutrient increase with a higher production of leaf area
11 per unit biomass (plasticity for LAR) than natives, rather than increasing N_{mass} in leaves
12 (Table 2). Perhaps, higher LAR plasticity entailed higher plasticity in Φ_{PSII} and ETR at
13 high light intensities, $1900 \mu\text{molm}^{-2}\text{s}^{-1}$ (Table 2) because new leaves have their
14 photosynthesis machinery intact. If this is the case, the production of new leaves to
15 maximize carbon acquisition may be a profitable strategy to grow faster when nitrogen
16 is abundant. Overall, these plastic responses support the idea of invader's fast-growing
17 dynamics as suggested by Niinemets, Valladares & Ceulemans, (2003), for example.

18

19 *Relationship between traits, plasticity and fitness*

20 Strong trait variation responses do not necessarily confer success to exotics; instead the
21 interaction of plasticity with certain trait values (typically high values) results in a
22 “general purpose phenotype” (i.e. high mean values of traits associated with a strong
23 ability to compete along broad ranges of environmental conditions). Following the
24 predictions of Richards, Bossdorf, Muth *et al.*, (2006), our results showed that invasive
25 species can be classified in different categories depending on how resource gradients

1 affect fitness traits. While in the nutrient gradient invasive species followed the
2 “Master-of-Some” strategy because they showed similar levels of survival to natives
3 across nutrient treatments but with higher biomass as nitrogen availability was
4 increased, in the light gradient invaders followed a “Jack-and-Master” strategy because
5 they always had higher levels of above-ground biomass and higher rate of survival than
6 natives in shade (Fig. 1).

7 Further attempts are needed to distinguish the mechanism by which invasive
8 species benefit more from plasticity than natives. Theoretically, two potential
9 mechanistic explanations (not mutually exclusive) can explain this result: (1) invasive
10 species display high trait plasticity resulting in significantly higher fitness than the
11 natives; and (2), invasive species have the same plasticity level but trait values
12 associated to fitness were always higher in invasive species. Our results of similar
13 plasticity between both groups but higher capacity for carbon acquisition by invasive
14 species suggest that the latter mechanism explains the higher fitness of invasive species.
15 However, this might not be always the case. Further studies applying multivariate
16 techniques such as structural equation modelling (SEM, see Shipley, 2004 for details)
17 should explicitly disentangle the relative importance for plant fitness of trait mean
18 value, phenotypic plasticity, and other important features not measured in this study
19 such as phenotypic integration (Murren, Pendleton & Pigliucci, 2002).

20

21 *Phylogenetic signal*

22 The lack of phylogenetic signal in trait plasticity across different plant scales (leaf-level,
23 whole plant-level, and mean phenotypic plasticity) suggests that there are no significant
24 phylogenetic constraints for wide trait variation (Fig. 4). In addition, phylogenetic
25 analyses suggest that higher levels of phenotypic plasticity are not related to a specific

1 growth form of invasive species, since no differences were found at the family-level
2 nodes where woody and herbaceous are nested to each taxonomic family. Therefore,
3 these results reflect the fact that plasticity is a convergent evolutionary strategy, at least
4 for the set of species studied here.

5

6 *Strengths and limitations of the study*

7 Multi-species comparisons enable more robust conclusions than single-species
8 studies, but they have been restricted to the evaluation of a reduced number of traits
9 (Goodwin, McAllister & Fahrig, 1999, Prizing, Durka, Klotz *et al.*, 2002, van Kleunen,
10 Johnson & Fischer, 2007). Very few studies have tackled the invasiveness of exotic
11 species experimentally with a large number of species and traits (van Kleunen &
12 Johnson, 2007, Schlaepfer, Glättli, Fischer *et al.*, 2010). However, species selection is
13 an important step in multi-species comparison studies (van Kleunen, Dawson,
14 Schlaepfer, Glättli, Fischer *et al.*, 2010) and our study has the limitation that three
15 native species, *Pinus pinaster*, *Achillea millefolium* and *Dittrichia viscosa*, were also
16 recorded as invasive species elsewhere (Beckmann, Erfmeier & Bruelheide, 2009,
17 Wacquant, 1990, Rejmánek & Richardson, 1996). Besides, methods to control for
18 ontogenetic drift were only partial because plant biomass did not overlap between
19 treatments. We acknowledge that both facts, invasiveness elsewhere and ontogenetic
20 drift, might have affected our results. Despite these limitations, our study provides novel
21 insights on invasiveness and an important methodological conclusion: phenotypic
22 plasticity should not be considered alone regarding invasiveness, but rather in
23 combination with trait mean values.

24

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article

Table S1 Invasive-Native pairs selected for the experiment

Appendix S2 Method for controlling partially plasticity with ontogeny

Appendix S3 Measurements of leaf fluorescence

Table S4 Trait mean values detailed for each species

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Table 1 Variables and descriptions of the traits measured. Effective quantum yield, photochemical and non-photochemical quenchings and electronic transportation rate were measured at non-saturating light level ($150 \mu\text{mol photon m}^{-2} \text{s}^{-1}$) and saturating light level ($1900 \mu\text{mol photon m}^{-2} \text{s}^{-1}$)

Variable	Description	Units
<u>Plant-level traits</u>		
LWR	Leaf weight ratio	$\text{g leaf g}^{-1} \text{ plant}$
SWR	Stem weight ratio	$\text{g stem g}^{-1} \text{ plant}$
RWR	Root weight ratio	$\text{g root g}^{-1} \text{ plant}$
LAR	Leaf area ratio	$\text{m}^2 \text{ leaf kg}^{-1} \text{ plant}$
<u>Leaf-level traits</u>		
A_{max}	Maximum photosynthetic rate at saturating light per unit area	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf s}^{-1}$
iWUE	Instantaneous Water use efficiency	$\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$
PNUE	Photosynthetic nitrogen use efficiency	$\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ N s}^{-1}$
N_{area}	Leaf nitrogen content per unit area	$\text{g N m}^{-2} \text{ leaf}$
N_{mass}	Leaf nitrogen concentration	$\text{mg N g}^{-1} \text{ leaf}$
SLA	Specific leaf area	$\text{cm}^2 \text{ leaf g}^{-1} \text{ leaf}$
R_{dark}	Plant respiration	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf s}^{-1}$
Curvature factor (Θ)	Light curve convexity	-
Compensation point (Γ)	Light compensation point	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf s}^{-1}$
Saturation point (I_c)	Light saturation point	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf s}^{-1}$
qP_{1900}	Photochemical quenching	-
qN_{1900}	Non-photochemical Quenching associated with radiant energy dissipation	-
NPQ_{1900}	Non-photochemical Quenching associated with non-radiant energy dissipation	-
F_v/F_m	Ratio of variable to maximum fluorescence	
$\Phi_{\text{PSII}1900}$	Effective quantum yield of PSII	$\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{ photon}$
ETR_{1900}	Electron transport rate	$\mu\text{mol e}^- \text{ m}^{-2} \text{ leaf s}^{-1}$
<u>Fitness related variables</u>		
Survival	Percentage of survival during growth	%
Total biomass	Total above and below ground biomass	g plant

Table 2 Mean±SE plant-level and leaf-level traits and their plasticities across nutrient and light gradients for twenty pairs of phylogenetically related invasive and native species across light and nutrient treatments. Woody pairs are n=11 and herbaceous pairs are n=9, d.f=39. Significantly higher values (p<0.05 or below) across invasive/native pairs, on the basis of a PERMANOVA analysis, are denoted by bold font. *PI* is the plasticity index of Valladares *et al.*, (2000). Units do not apply to *PI* values. Mean±SE trait values for each species are supplied in Table S4.

Variable	Trait Mean Value		<i>PI</i> Low to Medium nutrients		<i>PI</i> Medium to High nutrients		<i>PI</i> Shade to Sun	
	Invasive	Native	Invasive	Native	Invasive	Native	Invasive	Native
<u>Plant-level traits</u>								
LWR (g)	0.21±0.02	0.21±0.01	0.09±0.08	0.08±0.07	-0.01±0.06	-0.11±0.04	0.33±0.06	0.44±0.0
SWR (g)	0.28±0.02	0.24±0.02	-0.03±0.08	-0.01±0.08	-0.14±0.03	-0.11±0.06	0.23±0.07	0.22±0.1
RWR (g)	0.50±0.02	0.55±0.02	0.02±0.02	0.07±0.04	-0.08±0.02	-0.09±0.02	-0.34±0.05	-0.31±0.0
LAR (m ² /kg)	3.00±0.56	3.36±06.3	-0.14±0.08	-0.11±0.07	-0.18±0.07	0.01±0.05	0.60±0.06	0.70±0.0
<u>Leaf-level traits</u>								
A _{max} (μmolCO ₂ m ⁻² s ⁻¹)	8.97±0.40	8.88±0.41	-0.27±0.07	-0.09±0.07	-0.13±0.04	-0.21±0.04	-0.15±0.05	-0.12±0.0
iWUE (μmolCO ₂ mol ⁻¹ H ₂ O)	2.73±0.08	3.33±0.22	-0.14±0.06	-0.07±0.05	-0.08±0.02	-0.02±0.06	-0.17±0.03	-0.16±0.0
PNUE (μmolCO ₂ mol ⁻¹ Ns ⁻¹)	164.07±10.46	136.44±10.01	-0.31±0.08	-0.08±0.07	-0.12±0.03	-0.17±0.05	-0.46±0.05	-0.23±0.0
N _{area} (gNm ⁻²)	0.73±0.06	0.86±0.07	-0.66±0.11	-0.45±0.11	-0.50±0.06	-0.48±0.09	0.07±0.05	-0.21±0.0
N _{mass} (mgNg ⁻¹)	15.34±0.78	18.71±0.83	0.04±0.02	-0.07±0.03	-0.02±0.03	0.04±0.04	0.31±0.05	0.24±0.0
SLA (cm ² g ⁻¹)	210.7±18.9	218.2±16.7	0.02±0.04	0.07±0.05	0.10±0.05	0.06±0.04	0.39±0.06	0.46±0.0
R _{dark} (μmolCO ₂ m ⁻² s ⁻¹)	-1.30±0.09	-1.36±0.08	-0.01±0.09	-0.07±0.07	-0.02±0.08	-0.11±0.08	-0.17±0.10	-0.07±0.0
Curvature factor (Θ)	0.61±0.02	0.49±0.03	-0.09±0.09	-0.03±0.11	0.02±0.08	0.10±0.09	-0.05±0.04	0.17±0.0
Compensation point (Γ) (μmolCO ₂ m ⁻² s ⁻¹)	30.6±3.2	36.1±4.6	0.14±0.13	0.04±0.10	0.03±0.10	0.05±0.09	-0.29±0.10	-0.20±0.1
Saturation point (Ic) (μmolCO ₂ m ⁻² s ⁻¹)	226.9±9.8	269.3±14.1	0.02±0.08	0.06±0.08	-0.01±0.08	-0.01±0.09	-0.21±0.07	-0.22±0.0
qP ₁₉₀₀	0.26±0.01	0.28±0.01	0.01±0.05	-0.03±0.07	0.08±0.06	-0.03±0.06	-0.35±0.05	-0.42±0.0
qN ₁₉₀₀	0.88±0.01	0.86±0.001	0.02±0.01	-0.02±0.02	-0.01±0.01	-0.02±0.01	-0.06±0.01	-0.03±0.0
NPO ₁₉₀₀	2.76±0.07	2.42±0.07	-0.06±0.03	0.01±0.04	-0.02±0.03	0.01±0.04	-0.32±0.05	-0.11±0.0

Figure Legends

Figure 1 Representation of biomass and survival profiles, A) and B) respectively. Significant differences found between invasive and native species within each treatment and within above- or below-ground biomass are denoted with an asterisk. Differences across treatment and invasive or native species were assessed by Tukey Post-hoc tests and denoted by letters (above-ground biomass from a to e, below ground biomass from a to j).

Figure 2 Nutrient gradient. Mean phenotypic plasticity of invasive and native species for plant-level traits, leaf-level traits and mean plasticity of the whole phenotype from low to medium nutrient level (left) and from medium to high nutrient level (right). * p<0.05.

Figure 3 Light gradient. Mean phenotypic plasticity of invasive and native species for plant-level traits, leaf-level traits and mean plasticity of the whole phenotype from shade to sun. * p<0.05.

Figure 4 Phylogenetic tree of the mean plasticity across invasive and native species. Mean \pm SE for each species is also shown.

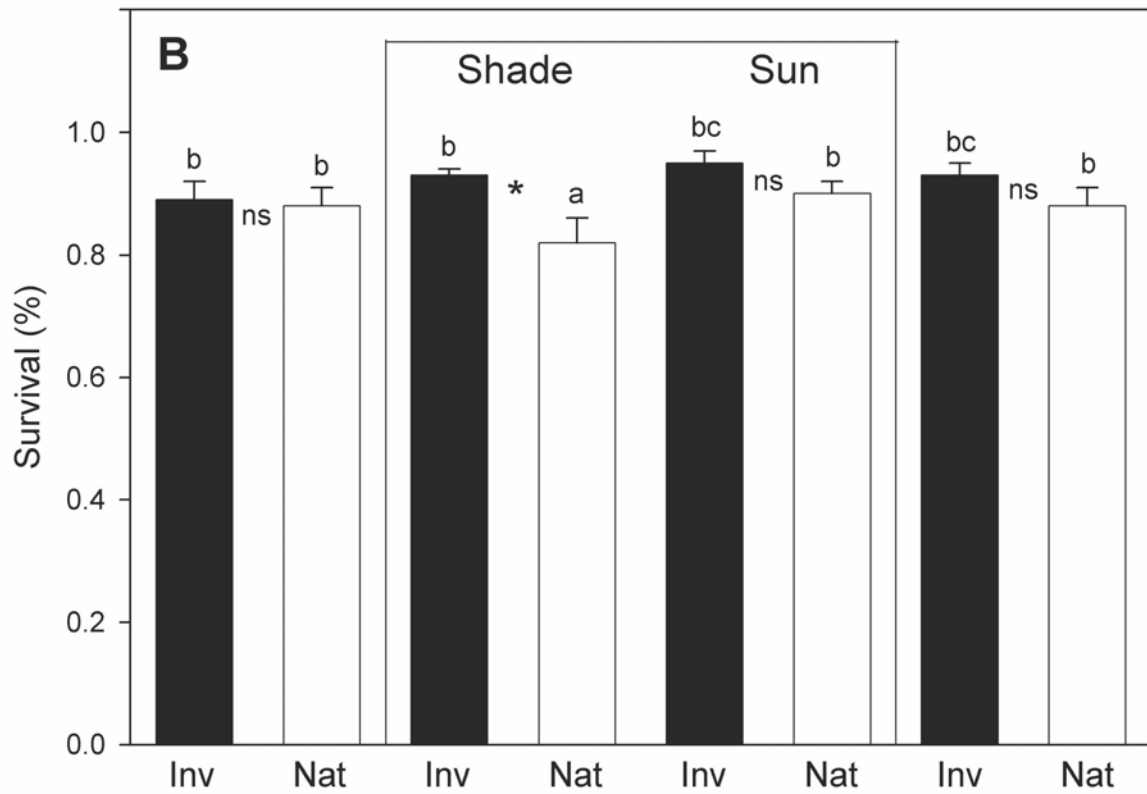
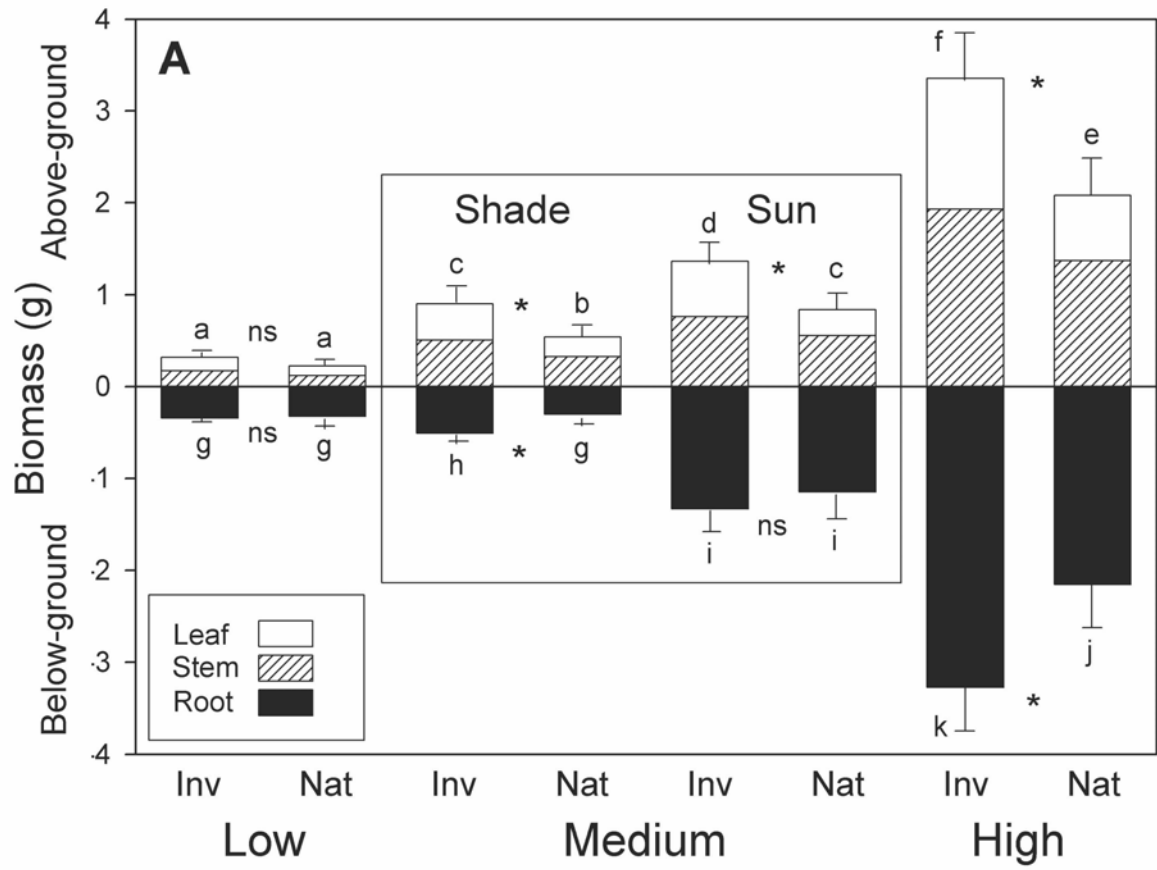


Figure 1

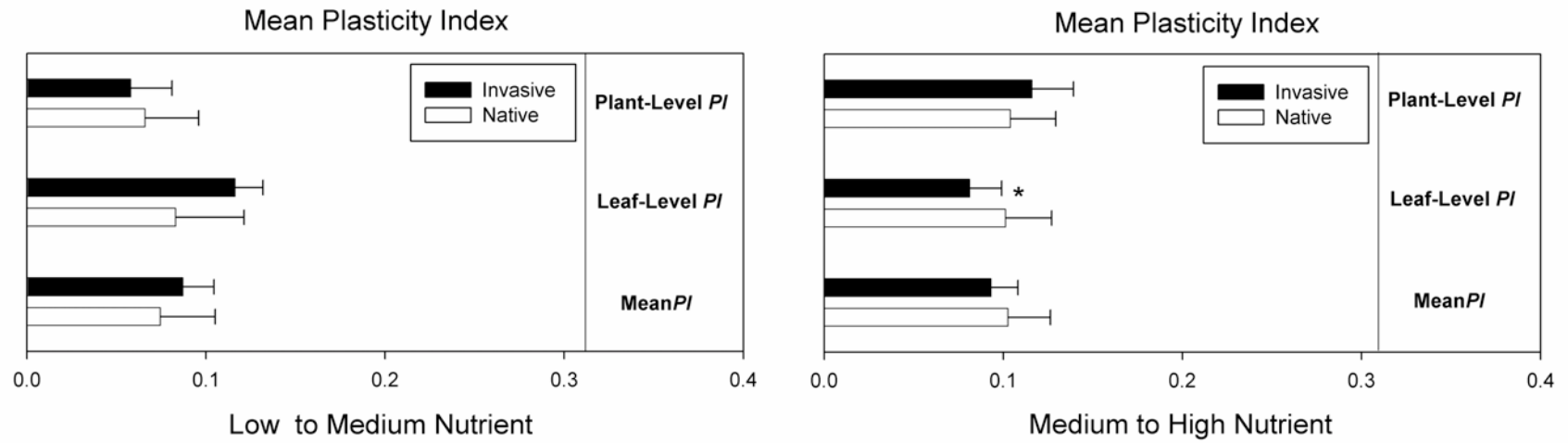


Figure 2

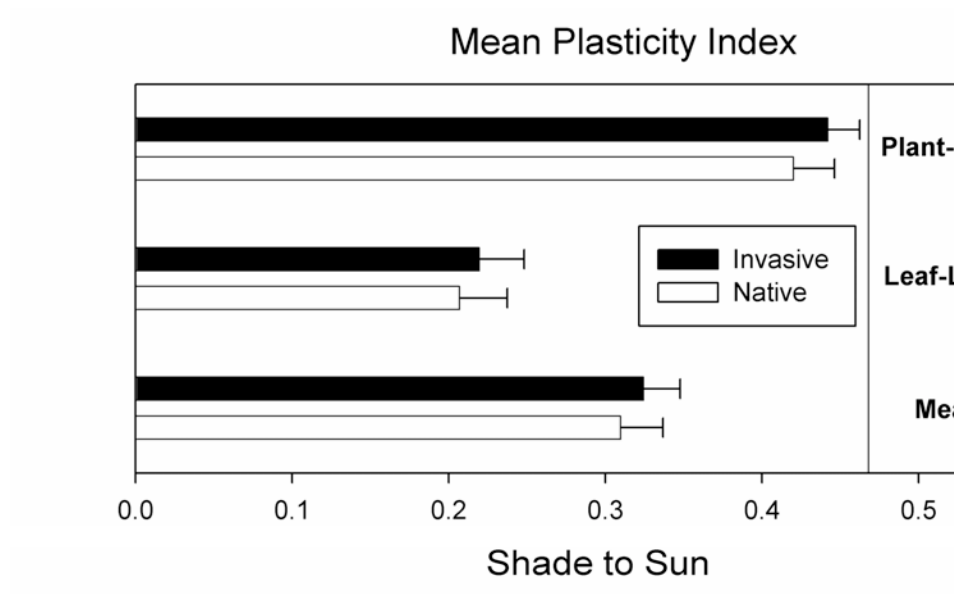


Figure 3

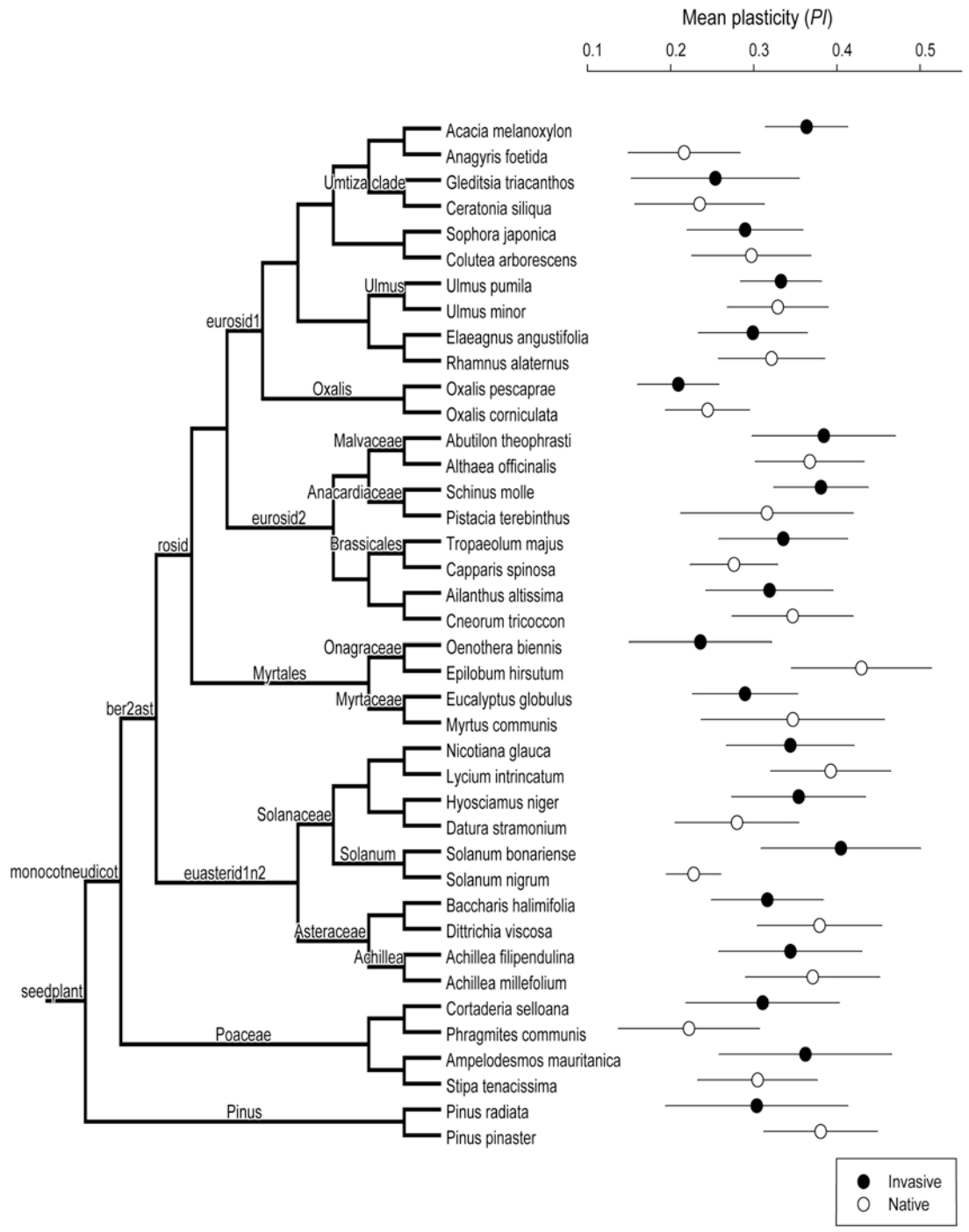


Figure 4