Research paper

Low among-provenance differences in structural and functional plasticity in response to nutrients in saplings of the circum-Mediterranean tree *Arbutus unedo* L.

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The Mediterranean region is an area of special interest for conservation where the incidence of multiple drivers of global change is expected to increase. One of the factors predicted to change is soil-nutrient availability, an essential factor for plant growth. Thus, study of the effects of variation in this parameter is especially relevant in species with a circum-Mediterranean distribution, such as *Arbutus unedo* L., in which the different provenances grow in different habitats, which must differ in nutritional conditions.

We aimed to determine the effect of provenance on plasticity, to establish whether structural and morphological traits differ in the level of plasticity and to assess how nutrients affect the photosynthetic light response. In a common garden experiment, we studied seven provenances from the circum-Mediterranean range of *A. unedo* and established two nutrient treatments (low and high nutrient availability). We measured physiological and structural traits in 1-year-old sapling and determined a phenotypic plasticity index (PPI) to quantify the level of plasticity, whereas the radiation effects were tested by construction and analysis of light response curves. Interestingly, provenance did not explain a significant amount of variance, but the plasticity was four times higher for the structural traits than for the physiological traits. Therefore, the plasticity to nutrient availability will not favour or prevent the expansion or contraction of the range of any of these provenances of *A. unedo*. Furthermore, the structural plasticity demonstrated the ability of the strawberry tree to optimize resource allocation, whereas the physiology remained stable, thus avoiding extra expenditure. The study findings also suggest that increased availability of nutrients would improve the performance of the species during the Mediterranean summer, characterized by high irradiance. These abilities will be key to the survival of saplings of the species under the future scenario of changes in nutrient availability.

**Keywords**: adaptation, canalization, global change, nutrient availability, phenotypic plasticity index (PPI).

Introduction

The Mediterranean ecosystems have been recognized as a prime target for conservation efforts (Myers et al. 2000), but the prevalence of multiple drivers of global change, including climate, atmospheric and land-use changes, will endanger its biodiversity (Sala et al. 2000, Loarie et al. 2009, Pereira et al. 2010, Bellard et al. 2012). Thus, changes in atmospheric deposition of nitrogen are one of the main drivers of change in Mediterranean-type ecosystems (Doblas-Miranda et al. 2015). Plants can respond to global change by adjustments in phenology, physiology or reproduction (Matesanz et al. 2010); they can even migrate to areas with more favourable environmental conditions, potentially over long distances (Hampe and Petit 2005, Williams et al. 2008, Hansen et al. 2012). Non-migratory changes can lead to adaptation, mainly via genetic evolution (local adaptation) through natural selection (Kandemir et al. 2010, Hoffmann and Sgró 2011, Ramirez-Valiente et al. 2011) or via phenotypic plasticity (Callaway et al. 2003, Williams et al. 2008, Lande...
2009), although adaptation to stationary fluctuations sometimes occurs via cananalization (Kawecki 2000, Le Rouzic et al. 2013). The mechanisms of adaptation that operate will depend on factors such as intensity and direction of the environmental change, life-history traits, standing genetic variation and interactions between coexisting species (Matesanz et al. 2010). Evolutionary adaptation can occur rapidly and potentially help species to counteract stressful conditions or to take ecological opportunities arising from global changes (Hoffmann and Sgrò 2011). However, Atkins and Travis (2010) used a simulation model to show that evolutionary adaptation may not be sufficient by itself to enable a species to survive a period of continuous changes. Phenotypic plasticity, or the capacity of a given genotype to render different phenotypes under different environmental conditions, may assist species, particularly sessile organisms, to cope with environmental heterogeneity (Sultan 2000).

In the short term, species can acclimate to shifting environmental conditions or adapt to the composition of their communities via phenotypic plasticity, developing and expressing particular traits in response to local environmental conditions, buffering the environmental change and increasing their tolerance to stress (Callaway et al. 2003, Lande 2009, Matesanz et al. 2010, Hansen et al. 2012). Indeed, forecasted changes in species range indicate less lost area and extinction probabilities in more adaptable species (Chevin et al. 2010, Valladares et al. 2014). Additionally, when environmental stresses are reversed, plastic phenotypic changes will also rapidly reverse, in contrast to genetic changes, which are more difficult to reverse (Hansen et al. 2012). Thus, in the short term, adaptability may be important for the persistence of species under continuous global change. In addition, separation of genetic and plastic contributions has shown that the latter often seem more important (Mutke et al. 2010, Hoffmann and Sgrò 2011). However, rather than being exclusive, plasticity and evolutionary adaptation may be complementary, because if plants maintain or achieve greater fitness in a stressful environment as a result of their ability to adapt, this will prevent the plant provenances from losing genetic variability (Matesanz et al. 2010), which may later promote further genetic adaptation, evolution and long-term survival (Lande 2009). Therefore, plasticity, acting alongside evolutionary change, is crucial for increasing survival under rapidly changing environmental conditions (Hoffmann and Sgrò 2011, Matesanz and Valladares 2014). However, in situations where the environmental conditions change periodically, canalization may be an alternative to buffering changes and avoiding maladaptation, i.e., adaptation to one situation that may lead to reduced fitness when the environment changes (Kawecki 2000).

Species habitats are expected to be modified as a result of the ongoing environmental changes and this will particularly affect soil systems and nutrient availability (IPCC et al. 2013). Soil-nutrient availability is one of the most important limiting factors affecting plant growth (Lammers et al. 2008), and it has been shown to be a fundamental abiotic factor in the structure and distribution of Mediterranean ecosystems (Mayor and Rodà 1992, Hanley and Fenner 1997). Soil-nutrient availability is highly heterogeneous, showing a strong spatial and temporal variation that is frequently associated with seasonal and climatic variations, as shown in a Mediterranean shrubland (Monokroussos et al. 2004). It is increasingly clear that changes in temperature or precipitation provoked by climate change will alter nutrient cycles and ecosystems (Sardans and Peñuelas 2007, Matías et al. 2011). Furthermore, soil processes can be affected by land-use change, nitrogen deposition, biotic exchange and elevated carbon dioxide concentration (Sala et al. 2000).

Some studies of nutrient-related issues in Mediterranean forests have shown correlations between soil-nutrient availability and plant growth, plant anatomy and physiological traits (Valladares et al. 2002, Sardans et al. 2005, 2006a, 2006b, Pulido et al. 2014). These studies provide evidence of the capacity of Mediterranean plants to adjust their morphology and physiology in response to variations in nutrient availability. Mediterranean plants are also able to respond to the environmental unpredictability of their ecosystems (Valladares et al. 2002) and are well suited to adapt to the ongoing changes they are facing (Sardans et al. 2006b). The degree of plasticity differs depending on the species, provenance and traits considered (Matesanz and Valladares 2014). Previous studies have shown differences in plasticity between physiological and structural traits (Mou et al. 2013), and that the capacity to exhibit structural and physiological plasticity may be complementary and, thus, negatively correlated (Derner and Briske 1999). Therefore, knowledge about the degree of plasticity of both types of traits is also necessary to determine the evolutionary strategy of a species.

The study of the degree of plasticity in widely distributed plants, living under different environments, will provide the opportunity to assess the role of plasticity in the evolution of plant distribution ranges. The circum-Mediterranean distribution of Arbutus unedo L. (strawberry tree) is of special interest (Torres et al. 2002), occupying a narrow coastal fringe from Tunisia to Morocco along the north of Africa and from Spain to Turkey along southern Europe (Figure 1). Along the Atlantic Ocean, it is distributed between northern Morocco and the Iberian Peninsula and western France, with outliers (disjunct provenances) in south-western Ireland, where the limit of distribution concurs with the isotherm of 4 °C for the mean temperature of January (Sealy 1949). Specifically, in relation to nutrient supply, we can expect a different degree of plastic response given that the different provenances inhabit climatically and geologically contrasted habitats that must produce variation in soil nutritional conditions (Crick and Grime 1987, Fitter and Stickland 1991).

Here, we applied the robust, simple and widely used phenotypic plasticity index (PPI) (Valladares et al. 2000, 2002, 2006, Balaguer et al. 2001, Gratani et al. 2003, 2006, Zhao et al. 2010) to determine the level of plasticity in response to nutrient availability in 10 physiological and four structural traits in saplings.
of seven provenances distributed throughout the Mediterranean distribution range of *A. unedo* established in a common garden. More specifically, we aimed to ascertain (i) whether plants of the different provenances differ in the level of plasticity to nutrient availability and (ii) whether structural and physiological traits differ in the level of plasticity. There is a lack of information about differences in levels of plasticity between provenances in *A. unedo*. However, the identification of these differences may be important for determining how the distribution of this species may be affected by global changes. Likewise, knowledge of the levels of plasticity in structural and functional traits will increase our ability to predict how this species will respond to ongoing global changes. In addition, given that plant responses to light constitute a key component of the survival strategies of species in Mediterranean ecosystems and potentially enable them to be influenced by nutrient availability (Demmig-Adams and Adams 1992, Bermúdez and Retuerto 2014), we determined how nutrient levels affected photosynthetic responses, which has not been previously reported.

**Materials and methods**

**The species**

*Arbutus unedo* belongs to the family Ericaceae, subfamily Arbutoideae, which includes evergreen, shrub-like woody taxa with sclerophyllous, laurel-like leaves (Torres et al. 2002). The genus *Arbutus* has several members in USA and four in Europe: *A. andrachne* L. (eastern Mediterranean), *A. pavani* Pampanini (Libyan coast), *A. canariensis* Veill. (Canary Islands) and *A. unedo* L. (Torres et al. 2002). Flowering spans from September to December and fecundation is entomophilous (Mitchell 1993); indeed, the nectar and pollen are the main food source for *Bombus terrestris* L. (Rasmont et al. 2005). Fruits take 12 months to ripe, and seeds are dispersed by animals (mainly birds); seed viability has been reported to be ~55% in favourable places, while seedlings loss was found to exceed 60% (Sealy 1949).

**Sample collection and experimental design**

We obtained samples from seven provenances of *A. unedo* L. growing in six countries on the indicated date: France Montpellier (FM) (17 December 2010) (31T 531757 4829416), Spain Balearic Islands (EI) (28 November 2010) (31S 461243 4392754), Morocco Debdou (MD) (30 October 2010) (30S 492658 3759875), Italy Tuscany and Italy Cagliari (IT and IC) (9 November 2010 and 12 November 2010) (32T 637192 4811775 and 32S 491121 4333556), Tunisia Kroumerie (TK) (19 November 2010) (32S 470929 4061547) and Turkey Izmir (TI) (35S 458841 4254352) (7 December 2010), i.e., throughout the circum-Mediterranean range of the species (Figure 1). Considering the range of climatic and geological conditions represented in our study, we assumed that the provenances studied...
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cover a wide range of soil/nutritional conditions. Likewise, the sampling design included trees growing in different and representative substrata of each provenance (valleys, riverbank, uplands, etc.). Thus, soil conditions within provenance were also well represented. We collected several fruits per tree and stored them at 4 °C. We extracted the seeds and kept them in a dry atmosphere before sowing in forestry trays with fifteen 1-l cavities, each filled with Tref substrate (Tref Substrates BV, Moerdijk, The Netherlands), using a 3 : 1 mixture of this potting compost and perlite. The average weight of seeds per provenance was as follows—FM: 1.7 mg; El: 1.8 mg; MD: 2.3 mg; IT: 2.2 mg; IC: 1.8 mg, TK: 1.5 mg and TI: 1.6 mg. To minimize the possible influence of maternal effects, we selected seeds (from different trees) with similar weight (around 2 mg) for each provenance and maintained the growing plants in the same environment (an outdoor plot) for 6 months (March–September 2012). We then selected 20 plants per provenance at random and transferred them to 7-l pots filled with the above-mentioned substrate. Ten plants of each provenance were randomly assigned to the low nutrient availability (LN) treatment (no nutrients added) and 10 plants were assigned to the high nutrient availability (HN) treatment (supplied with 6 g l⁻¹ of nutrient: 16-8-16 NPK, Osmocote, Serrablen, Marysville, OH). The plants were watered as required, to avoid stress, and were maintained for another 9 months, until June 2013. All of the traits described below were measured in each plant and all plants were then harvested to determine the biomass.

**Physiological traits**

**Leaf spectral reflectance** Measurements were made on all plants on the adaxial side of one leaf per plant, with a portable spectrometer (Unispec, PP Systems, Haverhill, MA, USA), which registers the leaf reflectance spectrum. The chlorophyll content index (CHL), calculated as \( R_{510} : R_{650} \), where \( R \) represents the reflectance at x nm, is positively correlated with the chlorophyll content of leaves (Lichtenthaler et al. 1996). The normalized difference vegetation index, computed as \( (R_{860} - R_{680}) / (R_{860} + R_{680}) \), has been related to the ‘greenness’ or general vigour of vegetation because it is correlated with leaf chlorophyll content, photosynthetic efficiency, and foliar nitrogen, phosphorus and potassium content (Gamon et al. 1995). The water index (WI), calculated as \( R_{900} : R_{970} \) is correlated with the plant water content, which is crucial for leaf expansion, photosynthesis and growth (Peñuelas et al. 1997). The photochemical reflectance index (PRI), determined as \( (R_{531} - R_{570}) / (R_{570} + R_{531}) \), is inversely correlated with the dissipation of excess radiation energy as heat (Peñuelas et al. 1995) and is indicative of a photoprotective mechanism that dissipates excess excitation energy. These traits, related to foliar physiology, constitute the main determinants of plant growth and survival (Chaerle and Van Der Straeten 2000).

**Chlorophyll fluorescence** Measurements were taken on every plant on the adaxial side of one leaf per plant, with a portable pulse amplitude modulated fluorometer (Mini-PAM; Heinz Walz, GmbH, Effeltrich, Germany). The effective quantum yield of Photosystem II (PSII) (ΦPSII) was calculated as \( (F_m - F_i) / F_m \), where \( F_m \) is the maximum fluorescence emitted by an illuminated leaf after a saturating pulse and \( F_i \) is the basal fluorescence emission. The ΦPSII provides information about the proportion of the light absorbed by the chlorophyll that is used photochemically, i.e., the radiation use efficiency, a main determinant of the net primary productivity (Genty et al. 1989). This parameter was also used to construct light response curves. The fluorometer recorded the ΦPSII after successive exposure of 3 min to increasing photosynthetic photon flux densities: 10, 75, 120, 180, 250, 375, 500, 800 and 1200 μmol m⁻² s⁻¹. These measurements were made in five plants per treatment. The maximum quantum yield of PSII (\( F_v/F_m \)) was determined as \( (F_m - F_o) / F_m \), where \( F_m \) and \( F_o \) are the maximum and the basal fluorescence emission, respectively, in dark adapted samples, when all reaction centres of PSII were fully open. Leaves were dark adapted by using dark leaf clips (Heinz Walz, GMBH) for 30 min before the measurement. \( F_v/F_m \) estimates the efficiency of capture of the excitation energy by open PS II reaction centres and represents the fraction of incident photon energy that is processed photochemically (Krause and Weis 1991).

**Infrared CO₂ analyser** The rate of photosynthesis (μmol CO₂ m⁻² leaf area s⁻¹), intercellular CO₂ concentration (C) (μmol CO₂ mol⁻¹), conductance to H₂O (mol H₂O m⁻² s⁻¹) and transpiration rate (mmol H₂O m⁻² s⁻¹) were measured in all plants, on one leaf per plant, by gas exchange, with a portable carbon dioxide analyser (LI-6400XT; LI-COR Inc., Lincoln, NE, USA), at an air temperature of 32.5 °C (±0.2) (± SE), which is the ambient air temperature at the experimental site, and chamber radiation of 500 μmol m⁻² s⁻¹, to avoid the photoinhibition effects. These measurements related to plant water use efficiency and photosynthetic efficiency are the main determinants of the tolerance to water stress, fecundity and plant survivorship (Arntz et al. 2000).

**Structural traits**

**Biomass** Plant biomass was separated into roots, stems and leaves. After washing the roots, all plant material was oven-dried to constant weight at 40 °C. Each part was weighed separately to the nearest 0.0001 g (Mettler AI100, Greifensee, Switzerland). Root biomass, shoot biomass (stem + leaf biomass) and root/ shoot biomass were used in the analyses. The root and shoot biomass perform essential functions for the plants, including absorption, structural support, storage and reproduction (Mokany et al. 2006), and the root : shoot ratio is related to precipitation, temperature and forest stand height (Mokany et al. 2006).

**Specific leaf area** The specific leaf area (SLA) (cm² leaf area (g leaf dry mass)⁻¹) was calculated as the average value for two
leaves per tree. The area was measured using a scanner (CanoScan LiDE 50; Canon, Tokyo, Japan) and an image analysis programme (ImageJ software; National Institutes of Health, Bethesda, MD). Specific leaf area has been associated with photosynthetic capacity and tolerance to drought (Sánchez-Vilas and Retuerto 2007).

Data analysis
For each variable and provenance, plasticity was determined by the PPI (Valadares et al. 2006). The PPI index (the values of which range from zero to one) was calculated as (maximum – minimum mean values)/maximum mean values, where the numerator is the difference between the mean values for the two nutrient treatments and the denominator is the mean value for the highest of the two nutrient treatments. We used a two-way analysis of variance (ANOVA) to test for effects of nutrient treatments and plant provenances on growth and physiological traits after log transformation of the data to meet the required assumptions of normality and homogeneity of variance. We also used a one-way ANOVA to check the effect of provenance on the PPI. We analysed the 10 physiological traits and the four structural traits separately. To determine the effects of the light curves, we used a two-way ANOVA with repeated measures, where ‘provenance’ and ‘nutrient availability’ were considered for the between-subject effect and ‘light’ (the different measures at each light level as a repetition) was the within-subject effect.

Before carrying out the tests, we checked that dependent variables were normally distributed (Shapiro–Wilks test) and that variances of the differences between related groups were homogeneous (Mauchly’s test of sphericity). When sphericity was violated, we used the Greenhouse–Geisser procedure to correct the degrees of freedom of the F-distribution. All analyses were carried out using IBM SPSS Statistics V.22 (IBM, Armonk, NY, USA), and differences were considered significant at P < 0.05, after sequential Bonferroni correction.

Results
Light response curves
The decreasing effect of light on the effective quantum yield of PSII was significant throughout the experiment and was also significantly higher for plants grown under LN than in those grown under HN (Table 1; Figure 2). Irrespective of the nutrient status, the decreasing effect of light on the effective quantum yield did not differ significantly between the plants of the different provenances.

Physiological and structural traits
Plants of the different provenances did not differ significantly in any traits (Figure 3). Furthermore, the level of plasticity across provenances (both in morphological and physiological traits) did not vary depending on the provenance (Figure 4). This result was confirmed by ANOVA (data not shown). Regardless of plant provenance, the addition of nutrients significantly increased the structural traits root and shoot dry weights but decreased root/shoot ratios (Figure 3). Regarding the physiological traits, and also irrespective of the plant provenance, the availability of nutrients significantly increased the leaf reflectance parameters CHL and PRI, and the effective quantum yield of PSII, but decreased transpiration and C4 (Figure 3). More detailed results are given in Table S1 available as Supplementary Data at Tree Physiology Online.

According to the PPI (Table 2), the structural traits were more plastic than the physiological traits (mean value 0.553 vs 0.125, respectively; Figure 4). Considering the structural traits, the plasticity index for SLA (0.101) was more than five times lower than the values for root, shoot and root : shoot biomass ratio (0.688, 0.849 and 0.574, respectively), which explains the high mean value. On the other hand, for the physiological traits, the plasticity values for normalized difference vegetation index (NDV) and WI were the lowest (0.011 and 0.010, respectively), and was the highest (0.280) for PRI.

Discussion
Our results clearly show that A. unedo responds to the addition of nutrients, as expected by the widely recognized pivotal role that nutrient availability play in determining plant growth (Lammers et al. 2008). The effect of HN in increasing CHL, PRI and PSII was expected, as high nutrient content has been associated with higher chlorophyll contents and light use efficiency (Tissue et al. 1993, Gamon et al. 1997). The effect of HN in decreasing transpiration may be indirect. High nutrient availability also reduced root/shoot allocation. Lower root allocation implies less water
within the plant, and a smoother plant-to-atmosphere vapour pressure gradient and less water to transpire (Ma et al. 2010). As a consequence, both transpiration and $C_i$ decrease. Regarding our hypothesis, the variations in the degree of plasticity in response to nutrient availability were non-significant for provenances, which showed a homogenous response. However, we found significantly higher plasticity in physiological than in structural traits.

**Do provenances differ in their plasticity to nutrient availability?**

Contrary to our expectations, the effect of nutrient availability on structural and physiological plant traits did not differ between the seven provenances studied, all of which showed very similar levels of phenotypic plasticity in response to nutrient availability. Thus, we conclude that provenance does not explain a significant amount of the variance in response to nutrient availability. Likewise, analysis of the levels of plasticity of the provenances, both in structural and physiological traits, enabled us to conclude that all provenances exhibit a common level of response to nutrient availability. Therefore, under scenarios of global change, any of the sampled provenances would be equally susceptible to being favoured or hindered on the basis of its ability to respond to nutrients. These findings contrast somewhat with those of previous studies showing that the extent of phenotypic plasticity of different herbaceous plant species in response to nutrients depended on the temporal nutrient availability in the original habitats (Fitter and Stickland 1991, Anderson and Gezon 2015, Freschet et al. 2015). Therefore, taking into account the diverse provenances of *A. unedo*, we would expect to find differences between them, but we did not. This may be partially explained by the fact that *A. unedo* is a woody species. The observation of very similar levels of plasticity among provenances suggests the existence in *A. unedo* of some degree of canalization in response to nutrients. According to Wagner et al. (1997), the canalization decreases the amount of genetic variation that is expressed by the phenotypes. This is related to a reduction in genetic variance (a decrease in the number of alleles) induced by natural selection when the stabilization of the phenotypes is advantageous (Gibson and Wagner 2000). Thus, when the direction of the natural selection changes frequently within short periods, it would be very expensive to undergo continuous adaptations and therefore canalization is favoured (Kawecki 2000, Le Rouzic et al. 2013). Examples of canalization in situations of seasonal environmental fluctuations have been described (Kawecki 2000, Picotte et al. 2009, Le Rouzic et al. 2013) and it has been reported as a common strategy in
the Mediterranean (Valladares et al. 2002). The existence of canalization in the strawberry tree would, therefore, genetically constrain the variability of the provenances and explain the homogeneous responses.

Do structural and physiological traits differ in plasticity to nutrient availability?

Knowledge of how traits of different nature, such as structural and functional traits, respond to nutrients is important for predicting how a species may evolve in the face of rapid environmental changes. Thus, the variation in plasticity between traits and the relationship between the plasticity in physiological and structural traits has previously been considered (Derner and Briske 1999, Mou et al. 2013, Matesanz and Valladares 2014). In the strawberry tree, we observed that both types of traits clearly differed in their plasticity, with higher values of PPI for structural traits (especially for the biomass traits) than for physiological traits. This is consistent with previous findings showing that the plasticity was higher for the structural traits (also mainly for dry weight traits) when the nutrient availability was constant over a long period of time (Mou et al. 2013). By way of explanation for this observation, Hodge (2006) has...
suggested that the construction cost of new biomass could be buffered over time, and, at the same time, the high cost of increasing the uptake rate under physiological plasticity could be avoided. Furthermore, other studies have reported that structural and physiological plasticity were negatively correlated due to their complementary nature (Derner and Briske 1999), as we also observed. The higher values of plasticity in structural traits in response to nutrients are also explained by the key role that biomass allocation plays in resource acquisition, whereas physiological plasticity would be favoured in response to other factors such as light (Gratani et al. 2006). Consequently, our results suggest that in a future scenario of increased nutrient availability, structural plasticity will determine the plant response. On the other hand, the lower plasticity in the physiological traits may be explained by stabilizing selection, which has been reported for A. unedo (Santiso et al. 2015). This behaviour leads to a strategy where trait variation is constricted, avoiding the cost associated with continuous responses (Kawecki 2000). Therefore, we conclude that A. unedo is able to adjust its biomass allocation for a more appropriate and efficient distribution of the nutrients, but without spending resources to modify its physiology.

Beyond the plastic response to nutrient availability, we must take into account that the simultaneous occurrence of several global change drivers in the Mediterranean area may limit plasticity if the environmental cues that elicit this response are not always reliable. This could occur because of the action of other factors and would produce costs in maintaining an induced phenotype (cost of plasticity) (Valladares et al. 2007, Matesanz et al. 2010). A reduction in root/shoot allocation under conditions of high availability of nutrients may have an important negative impact on the water status of the plant, which could limit plant growth in areas where increased intensity and frequency of drought are predicted (IPCC et al. 2013). Additionally, we must consider that the performance of A. unedo can be modified due to interaction with other species in the community, mainly plants that may function as physical ecosystem engineers, which modulate the availability of resources such as water or nutrients (Jones et al. 1996, Maltez-Mouro et al. 2005). Because our study considered A. unedo saplings, we must acknowledge that the performance of adult trees may well be different (Thomas and Winner 2002, He et al. 2005). Therefore, exploration of the interactions and age effects would be a promising area for future studies.

Another well-known route to adaptation is by genetic selection and evolutionary adaptation (Hoffmann and Größ 2011, Hansen et al. 2012), as reported for another Mediterranean species (Kandemir et al. 2010, Ramírez-Valiente et al. 2011). Regarding A. unedo, it has been shown that most of the traits evaluated, especially structural traits, have significant additive genetic variance and therefore the species has significant potential to undergo genetic evolution and adaptation (Santiso et al. 2015). Thus, this ability to evolve, acting together with the observed plasticity, will enhance the adaptability and survival of the strawberry tree under future scenarios of changes in the unpredictable environment of the Mediterranean ecosystems.

Figure 4. Mean values (±1 SE) for the index of phenotypic plasticity in response to nutrients for 10 physiological traits (grey bars) and four structural traits (black bars) in A. unedo plants of seven provenances.

Table 2. Index of phenotypic plasticity. (maximum mean – minimum mean)/maximum mean, in response to nutrient availability for physiological traits (CHL, chlorophyll content index; NDVI, normalized difference vegetation index; WI, water index; PRI, photochemical reflectance index; effective (ΦPSII) and maximum (Fm/Fo) quantum yield of photosystem II; Photo, photosynthesis rate; Transp, transpiration rate; C, intercellular CO2 concentration; Cond, conductance to water) and structural traits (Root, root biomass; Shoot, shoot biomass; Root : Shoot, root biomass/shoot biomass; SLA, specific leaf area) in plants of seven provenances (El, Spain Balearic Islands; FM, France Montpellier; IC, Italy Cagliari; IT, Italy Tuscany; MD, Morocco Debdou; Ti, Turkey Izmir; and TK, Tunisia Kroumerie) of A. unedo L.

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<th>Provenance</th>
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Are light response curves modified by nutrient availability?

It is well known that nutrient availability can modify plant responses to light (Demmig-Adams and Adams 1992, Bermúdez and Retuerto 2014), an essential factor determining plant growth. Furthermore, it has been widely demonstrated that light can typically produce plant stress in the Mediterranean, especially during the summer (Faria et al. 1998, Werner et al. 1999). Therefore, study of the interaction between nutrients and light is of great interest. Here, we observed that plant performance, as estimated by photochemical quantum yield, improved with nutrient supply under increasing radiation. This positive correlation has been observed in some studies (Fetcher et al. 1996, Martin and Jokela 2004), in which the interaction was key to improving plant growth, but not in another (Lawler et al. 1996), in which the high light counteracted the effects of nutrients. Consequently, under a scenario of increased nutrient availability, photoinhibition would be decreased in A. unedo, thus improving growth and survival of the plant.

In conclusion, considering the uniform plastic capabilities observed for A. unedo across provenances, none of the sampled provenances seem to be more capable of colonizing or more susceptible to becoming extinct than others as a consequence of predicted changes in nutrient availability. This is consistent with the existence of some degree of canalization, which constrains the variations in provenances. However, the response to nutrients depends on the type of trait and is considerably more marked for the redistribution of biomass (structural traits) than for physiological traits, indicating that the strategy of the strawberry tree is to optimize resource allocation by maintaining the physiology quite stably. This low physiological variation is consistent with the demonstrated stabilizing selection for the species. Stabilizing selection and canalization are both evolutionary strategies that reduce the variability in the responses and, consequently, reduce the costs of allowing unnecessary changes. Finally, we also observed that supply of nutrients improved the performance of A. unedo under high radiation conditions. Thus, the responses to addition of nutrients observed in the strawberry tree are associated with the capacity of the tree to modify the biomass structure and with the economy of expenditure provided by stabilizing selection and possible canalization. This, together with the demonstrated ability of the strawberry tree to evolve, explains how this species has been able to inhabit such a broad distribution range and may also be a determinant in enabling the future survival of the species.

Supplementary data

Supplementary data for this article are available at Tree Physiology Online.

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Conflict of interest

None declared.

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