

Genders in *Juniperus thurifera* have different functional responses to variations in nutrient availability

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Summary

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- Differences in reproductive investment can trigger asymmetric, context-dependent, functional strategies between genders in dioecious species. However, little is known about the gender responses of dioecious species to nutrient availability.
- We experimentally fertirrigated a set of male and female *Juniperus thurifera* trees monthly for 2 yr. Water potential, photosynthesis rate and stomatal conductance were measured monthly for 2 yr, while shoot nitrogen (N) concentration, carbon isotopic composition ($\delta^{13}\text{C}$), branch growth, trunk radial growth and reproductive investment per branch were measured yearly.
- Control males had lower gas exchange rates and radial growth but greater reproductive investment and higher water use efficiency (WUE; as inferred from more positive $\delta^{13}\text{C}$ values) than females. Fertirrigation did not affect water potential or WUE but genders responded differently to increased nutrient availability. The two genders similarly increased shoot N concentration when fertilized. The increase in shoot N was associated with increased photosynthesis in males but not in females, which presented consistently high photosynthetic rates across treatments.
- Our results suggest that genders invest N surplus in different functions, with females presenting a long-term strategy by increasing N storage to compensate for massive reproductive masting events, while males seem to be more reactive to current nutrient availability, promoting gas-exchange capacity.

Introduction

Male and female trees have to satisfy different reproductive demands and have to deal with different selective pressures in dioecious species. Female plants often invest more resources in reproduction than males, which results in different resource allocation trade-offs for male and female plants (Obeso, 2002). For instance, female trees of the dioecious tree *Juniperus thurifera* presented lower radial growth than males, but only after reaching sexual maturity (Montesinos *et al.*, 2006). However, reproductive costs are often not detected because genders might have different functional strategies to compensate for them (Delph, 1990). These include photosynthetic reproductive structures, delayed reproduction, nutrient resorption from senescing organs, module specialization and higher photosynthetic rates in females (Obeso, 2002).

Genders usually have functional differences that can be observed before reproductive maturity (Retuerto *et al.*, 2000; Wang & Curtis, 2001), indicating that many sex-specific differences have an evolutionary basis and are not only a consequence of resource allocation trade-offs between plant functions. Gender differences frequently arise or are greater under stressful conditions. Thus, males tend to be more drought-tolerant and have a

more conservative use of water than females under water shortage or low-temperature conditions (Dawson & Ehleringer, 1993; Delph, 1999; Retuerto *et al.*, 2000; Dawson *et al.*, 2004; Xu *et al.*, 2008; Chen *et al.*, 2010). Therefore, trade-offs between reproduction and growth might be detected under some environmental conditions but not in others (Stearns, 1989; Correia & Diaz Barradas, 2000; Verdú *et al.*, 2004). Most studies that have shown functional differences between sexes related to abiotic environmental factors have focused on drought, light, extreme temperatures and salinity (Dawson & Ehleringer, 1993; Delph, 1999; Retuerto *et al.*, 2000; Dawson *et al.*, 2004; Xu *et al.*, 2008; Chen *et al.*, 2010). However, studies on gender responses to nutrient availability are scarce and show disparate patterns (Wang & Curtis, 2001; Zhao *et al.*, 2011; Hesse & Pannell, 2011). Mineral nutrients, especially nitrogen (N) and phosphorus (P), are critical for plant performance. Plant reproduction is a particularly important N sink (Marschner, 1986). Soils in Mediterranean ecosystems are frequently poor in N and P (Di Castri, 1981; Rodà *et al.*, 1999) so plants have to cope not only with drought but also with low nutrient availability.

Plant physiology changes through time and ontogeny (Dawson *et al.*, 2004). Consequently, a more precise idea of a species'

ecology is obtained when its physiology is studied for extended periods to include seasonal and inter-annual variations or using long-term indicators of physiological performance, such as tissue isotope composition. For instance, tissue carbon (C) isotopic composition ($\delta^{13}\text{C}$) is a long-term proxy for potential water use efficiency (WUE) in C_3 species (Hubick *et al.*, 1986; Farquhar *et al.*, 1989; Zhang & Marshall, 1995) and more negative $\delta^{13}\text{C}$ values are usually associated with lower WUE (Hubick *et al.*, 1986; Zhang *et al.*, 1993).

We studied how variation in nutrients affected the physiology, growth and reproduction of sexes in the dioecious *J. thurifera*, by experimentally fertirrigating a set of male and female trees for 2 yr in the field. *Juniperus thurifera* is a masting, slow-growing species that inhabits semiarid Mediterranean mountains. Females usually present greater reproductive investment (RI) than males. Females' reproduction correlate with reductions in vegetative growth (Montesinos *et al.*, 2006). Water potential and gas exchange were measured monthly for 2 yr while other long-term integrative physiological variables, such as shoot N concentration and $\delta^{13}\text{C}$ isotope composition, together with growth and RI per branch were measured yearly. We addressed the following questions: do *J. thurifera* genders exhibit physiological differences, and how does an increase in resource availability influence the functional performance of the sexes? On the basis of previous studies and taking into account that *J. thurifera* lives in very harsh and oligotrophic environments, we expected functional differences between genders to diminish when nutrient availability increased.

Materials and Methods

Study species

Juniperus thurifera L. is a dioecious, long-lived tree with a relictual distribution from the Tertiary throughout the calcareous high mountains of the western Mediterranean Basin. Trees are 5–10 m high and form low-density stands in which *J. thurifera* can be the dominant species or can coexist with *Quercus ilex*, *Quercus faginea* and *Pinus nigra* (Bertaudière *et al.*, 1999). It is a masting species, that is, individuals present low or null reproduction for several years but every few years most individuals in a population present a massive reproduction event (Montesinos, 2007). Flowering and pollination occur during February and March and female trees bear two cohorts of fleshy cones. The first cohort (hereafter Fr1) consists of unripe green cones that grow from the last flowering period. The second cohort of cones (hereafter Fr2) originates from the flowers produced 22 months before and is dark blue when ripe (October–November). Ripe cones usually have 1–7 seeds (mean 3.5; $n = 2000$) and are typically dispersed by birds of the genus *Turdus*. The viability and germination of seeds of *J. thurifera* are extremely low (Ceballos & Ruiz de la Torre, 1979; Montesinos *et al.*, 2010). RI per branch during 2003, a typical nonmasting year, was 0.22 ± 0.34 g (mean \pm SD; $n = 17$) for males and 0.19 ± 0.19 g ($n = 23$) for females (the RI calculation is described later in this section). During 2001, a masting year, male and female RI increased to 0.52 ± 0.42 g ($n = 17$) and 1.42 ± 1.85 g ($n = 23$), respectively (Montesinos, 2007).

Study site

The study was performed at La Puebla de San Miguel (Valencia, eastern Spain). The study site is situated at 1500 m above sea level and the vegetation is dominated by *J. thurifera*. The study area has been used for agriculture, timber, and extensive livestock grazing for centuries. Narrow valleys were tilled for rye (*Secale cereale*) and barley (*Hordeum vulgare*) until land abandonment in 1960 (Rodrigo, 1999). In the intervening decades, forests have occupied many of these abandoned croplands (Lasanta, 1996), although livestock grazing is still an important activity. This forest represents a typical *J. thurifera* population in the Iberian Peninsula, and is managed by the Puebla de San Miguel Natural Park staff. The soils are limestones < 30 cm deep, although cracks in the rock allow roots to go deeper (Verdú *et al.*, 2004). The climate is Mediterranean, with cold, long winters (the duration of the freezing period is > 120 d yr^{-1}), and warm, dry summers. Mean annual precipitation is 486 mm, with October being the wettest month (58.6 mm) and July the driest (26.9 mm). The annual mean temperature is 13°C; August is the warmest month (22.8°C) and January the coldest (4.8°C).

Selection of trees and fertirrigation treatments

We selected 20 healthy and reproductive trees of each gender. Trees were considered healthy if they showed no signs of decay, defoliation or chlorosis. From April 2003 to March 2005, half of the male and female trees were randomly assigned to two fertilization treatments: fertilized and control. Fertilization was applied by fertirrigation which was applied monthly, except in June and July, the driest months, during which it was applied fortnightly. Fertirrigation was not applied between November and February, when the temperature is very low and precipitation is high. On each fertirrigation date, every tree was supplied with 100 l of a 200 ppm balanced fertilization solution (Universol Blue; 18 N : 11 P : 18 K plus oligoelements; Scotts, Marysville, Ohio, USA) according to the manufacturer's recommended concentrations. This resulted in a dose of 1000 l of water and 2 kg of fertilizer per tree and year. Water was distributed on a 20-m² area around trunks, resulting in an approximate increase on annual precipitation of 50 mm. Fertirrigation treatments were applied on the day following physiological measurements to avoid short-term interference with tree physiology. The aboveground biomass of selected trees was estimated from the equation described by Bertaudière *et al.* (2001) and Montès *et al.* (2002) for *J. thurifera*. No significant differences in biomass were found either between sexes ($F = 0.127$, $df = 1$, $P = 0.72$) or between the trees that were assigned to different fertilization treatments ($F = 0.009$, $df = 1$, $P = 0.92$). The effect of the interaction between sex and fertilization on aboveground biomass was not significant ($F = 0.157$, $df = 1$, $P = 0.69$). One of the males consistently presented disproportionately variable values and was removed from the study.

Physiological performance

Shoot water potential (ψ), net photosynthesis rate (A) and stomatal conductance (g_s) were measured monthly from April 2003 to

March 2005. As leaves are tiny and scale-like, physiological measurements had to be taken from shoots. Different shoots were randomly chosen on each measurement date. Predawn and midday (06:00–08:00 h and 12:00–14:00 h solar time, respectively) ψ values were determined with a Scholander chamber (SKPM 1400; Skye Instruments Ltd, Powys, UK) on one shoot per tree sampled on the south side of each tree. Gas exchange was measured with a portable infra-red gas analyzer (IRGA) (LCi, ADC BioScientific Ltd, Herts, UK) immediately after ψ measurements. Shoots used for gas-exchange measurements were very close to those sampled for ψ . From December 2004 to February 2005 we could not measure ψ , A or g_s because shoots were frozen and very low air temperatures hindered IRGA functioning. Other technical and logistical issues also prevented physiological data sampling in October 2003 and May 2004.

In January 2004 and 2005, we sampled five current-year twigs distributed all around each tree crown to avoid any effect of orientation. Newly grown twigs can be differentiated by their brighter green colour. Samples from each tree were mixed, dried at 50°C for 48 h, and ground with a mill. A 1-g subsample was used for measurement of the total N concentration following the standard Kjeldahl method. A second 20-mg subsample was analysed at the Laboratorio de Isótopos Estables (Universidad Autónoma de Madrid, Madrid, Spain) to determine the C isotopic composition. After combustion of the samples in an elemental analyser (EA 1108; CHNS; Carlo Erba, Milan, Italy), the $^{13}\text{C} : ^{12}\text{C}$ ratios were determined with a continuous flow isotope mass ratio spectrometer (Micromass CF-Isocrom, Micromass Ltd., Manchester, UK) with a measurement error of 0.1‰. The ^{13}C isotope composition was expressed in delta notation and calculated as:

$$\delta^{13}\text{C}(\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 \quad \text{Eqn 1}$$

(R_{sample} and R_{standard} , the $^{13}\text{C} : ^{12}\text{C}$ ratios of the sample and the Pee Dee Belemnite carbonate standard, respectively.)

Reproductive investment and growth

For 2 yr (February 2003 to October 2005) we counted the number of male or female cones (hereafter flowers or Fw), unripe female cones (hereafter Fr1 fruits; 10 months old) and ripe female cones (hereafter Fr2 fruits; 22 months old) on 10 branches randomly sampled all around each tree. The Fw, Fr1 and Fr2 mass was measured on 10 flowers (in February of each year) and in 10 Fr1 and Fr2 cones (in October of each year) randomly sampled from each tree after drying at 50°C for 48 h. Reproductive investment per branch was calculated as:

$$(\text{Fw}_{\text{mass}} \times \text{Fw}_{\text{frequency}}) + (\text{Fr1}_{\text{mass}} \times \text{Fr1}_{\text{frequency}}) + ((\text{Fr2}_{\text{mass}} - (\text{previous year Fr1}_{\text{mass}})) \times \text{Fr2}_{\text{frequency}})(\text{g}) \quad \text{Eqn 2}$$

Juniperus thurifera branches are defoliated at their insertion into the main trunk and leaves are concentrated in the distal ends.

Males (length \times width = 1095 \pm 419 mm²; n = 50) and females (1074 \pm 382 mm²; n = 50) had similar branch sizes (Kolmogorov–Smirnov Z = 1.00; P = 0.27). In March 2003 we marked the tips of five branches with gardening wire tags in each experimental tree. Resulting branch growth from the marks was collected in January 2006. Samples were dried for 48 h and weighed to the nearest 0.0001 g.

Dendrochronological cores were extracted at 1.5 m height from each experimental tree in July 2005. After mounting and polishing the samples, tree ring growth was measured to the nearest 0.01 mm for years 2003 and 2004 following standard dendrochronological methods.

Data analyses

Linear mixed models (LMMs) with an autoregressive covariance structure of order one permit the study of time series accounting for temporal autocorrelations among years and correlation between factors. LMMs with time of day (dawn or midday), month and year as repeated measures and as fixed factors were fitted to the data to analyse the effect of watering and sex on the tree's ψ , A and g_s . LMMs with year as the repeated measure were fitted to the data to analyse the effect of sex and fertirrigation on shoot $\delta^{13}\text{C}$, N concentration and reproductive investment. Finally, a general linear model and LMM were fitted to log-transformed data to test the effect of sex and fertirrigation on branch growth and tree ring growth, respectively. SPSS 19 was used for statistical analyses (SPSS, 2005).

Results

Physiological performance

Over 2 yr, control females presented slightly lower ψ than males and fertirrigated trees presented slightly higher ψ than control individuals (Fig. 1). However, these differences were not statistically significant (Table 1). Shoot ψ was more positive in mid spring and the most negative values were measured in mid-summer in both years, in concomitance with the reduction in natural rainfall (Fig. 1).

Gas exchange also showed seasonal variation but the seasonal pattern was less marked than for ψ . Thus, several A and g_s maximums were observed through the spring and the fall and minimum values tended to occur in summer, especially in the second year (Fig. 1). Over the 2 yr, control females had greater A and g_s than control males. Fertirrigation increased A in males but not in females, resulting in no significant differences between genders when fertirrigated (significant sex \times fertirrigation interaction; Table 1, Fig. 1).

Females consistently presented, across both years, more negative $\delta^{13}\text{C}$ values than males independently of fertirrigation treatments, which did not differ between the sexes (Table 1, Fig. 2a). Fertirrigation similarly increased shoot N concentration in trees of both sexes, (Table 1, Fig. 2b).

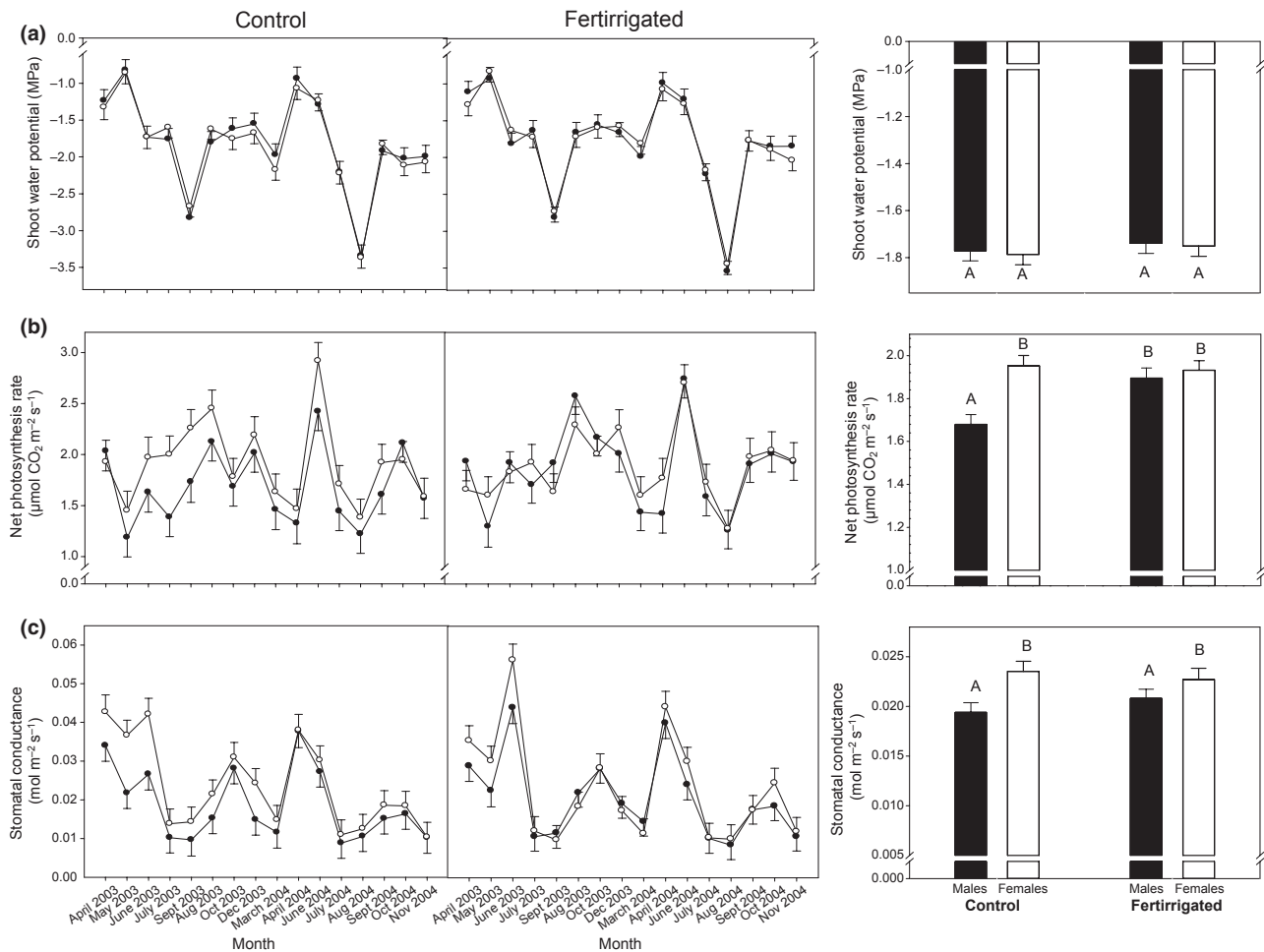


Fig. 1 The columns on the left show the monthly variation of (a) shoot water potential, (b) net photosynthesis rate and (c) stomatal conductance of control and fertirrigated males (closed circles) and females (open circles) of *Juniperus thurifera*. The column on the right shows the mean values for shoot water potential, net photosynthesis rate and stomatal conductance of control and fertirrigated male (closed bars) and female (open bars) trees during the studied period. Data are mean \pm 1 SE.

Table 1 Linear mixed model (LMM) F values

	Sex	Fertirrigation	Sex \times fertirrigation
ψ	0.70	1.69	0.05
A	11.42***	4.42*	3.81[†]
g_s	10.65***	0.12	1.43
$\delta^{13}C$	6.89*	0.61	0.63
N	0.05	33.48***	0.01
Reproductive investment	7.13*	2.97[†]	0.17
Branch growth	1.50	7.04**	3.42[†]
Ring growth	0.39	0.07	4.23*

ψ , water potential; A , net photosynthesis rate; g_s , stomatal conductance. Significant tests are indicated in bold († , $P \leq 0.09$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$). See Supporting Information Table S1 for additional interactions for ψ , A and g_s .

Reproductive investment and growth

Males presented greater reproductive investment than females in both years, and fertirrigation nearly doubled reproductive investments in both sexes (Fig. 3a). Fertirrigation increased branch

growth in females twice as much as in males (marginal sex \times fertirrigation interaction; $P = 0.066$; Table 1, Fig. 3b). By contrast, fertirrigation increased radial growth in males but reduced it in females (sex \times fertirrigation interaction; Table 1, Fig. 3c).

Relationship among variables

Photosynthesis rate was positively related to shoot N concentration in males ($F = 11.29$; estimate = 0.08 ± 0.02 ; $P = 0.002$) but not in females ($F = 1.76$; estimate = 0.09 ± 0.02 ; $P = 0.194$) (Fig. 4).

Discussion

This study demonstrates that males and females of the dioecious tree *J. thurifera* differ in their physiology and that such differences are asymmetrical and context dependent. Control female trees had higher A and g_s and more negative $\delta^{13}C$ values than control males. Similar findings have been reported in *Ilex aquifolium*, *Acer negundo* and other juniper species (Dawson & Ehleringer,

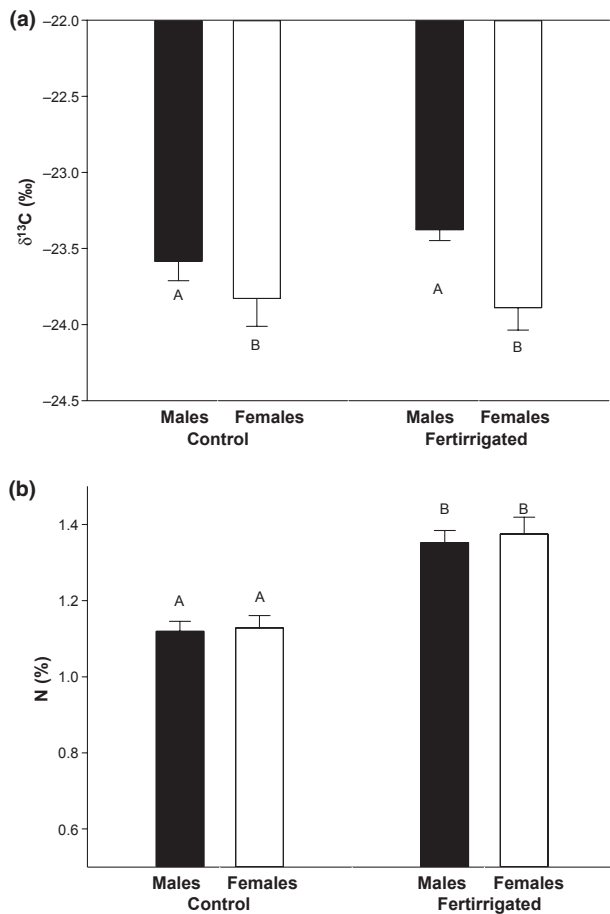


Fig. 2 Mean values for (a) shoot carbon isotopic composition ($\delta^{13}\text{C}$) and (b) branch nitrogen (N) concentration in male (closed bars) and female (open bars) *Juniperus thurifera* trees. Error bars represent + 1 SE. Different letters indicate significant differences (see statistical values in Table 1).

1993; Hill *et al.*, 1996; Retuerto *et al.*, 2000; He *et al.*, 2003; Dawson *et al.*, 2004; Verdú *et al.*, 2004). Physiological differences between genders have also been previously described for other species with sex-biased reproductive investments (e.g. Delp & Meagher, 1995; Saulnier & Reekie, 1995; Nicotra, 1999a,b; Wheelwright & Logan, 2004). Our results suggest that *J. thurifera* genders have different water relations. Higher *A* in females than in males was probably attributable to higher female g_s , which implies a higher transpiration rate. Moreover, control females also had a lower WUE than males, as inferred from their more negative $\delta^{13}\text{C}$ values. Tissue C isotopic composition is a long-term proxy for potential WUE in C_3 species and more negative $\delta^{13}\text{C}$ values are usually associated with lower WUE (Hubick *et al.*, 1986; Farquhar *et al.*, 1989; Zhang *et al.*, 1993; Zhang & Marshall, 1995). It has been suggested that females of *J. thurifera* have higher sensitivity to summer water stress than males based on long-term dendrochronological analyses (Rozas *et al.*, 2009). However, control trees in our study did not differ between genders in ψ during summer, when drought stress is maximal (Fig. 1). This suggests that females may have other functional mechanisms to override drought stress, such as higher

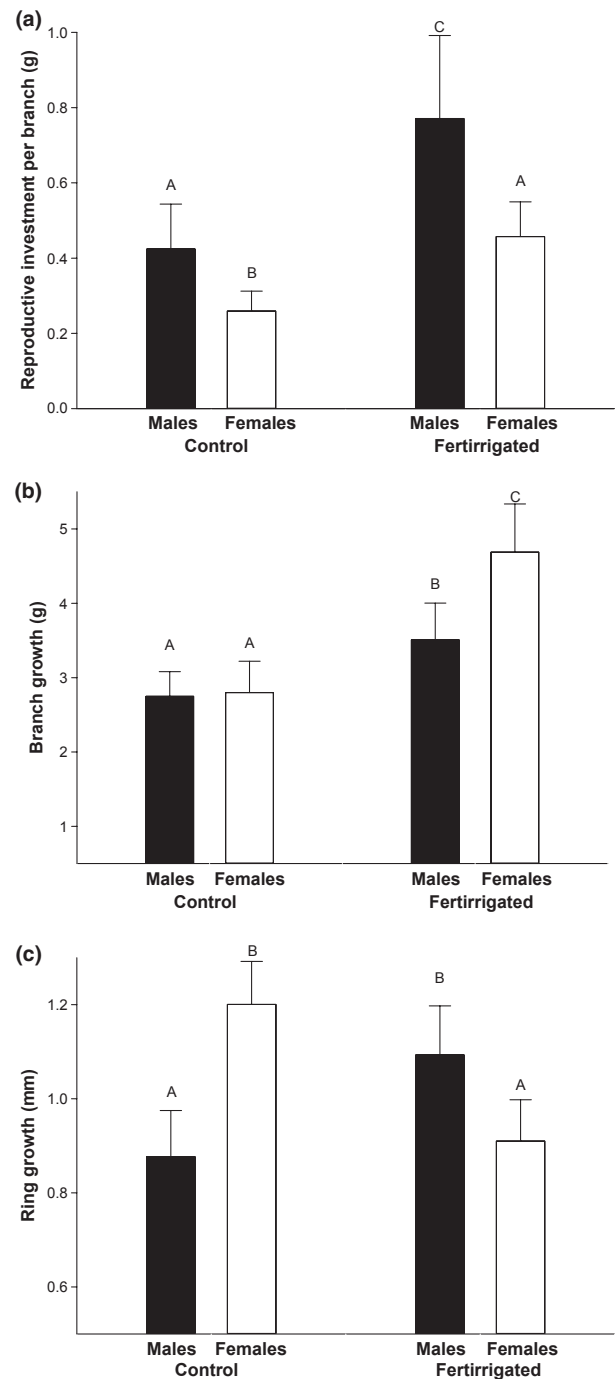


Fig. 3 Mean values for (a) branch reproductive investment, (b) branch growth and (c) tree ring growth of male and female *Juniperus thurifera* trees. For each fertirrigation treatment (watering and fertilization): closed bars, males; open bars, females. Error bars represent + 1 SE. Different letters indicate significant differences (see statistical values in Table 1).

sapwood to foliage area, lower tissue osmotic potential, deeper and more extensive root systems and higher root to aboveground biomass ratio (Turner, 1986; Padilla & Pugnaire, 2007; Martínez-Vilalta *et al.*, 2009). These adaptive mechanisms, however, may result in plant growth reduction (Lambers & Poorter, 1992).

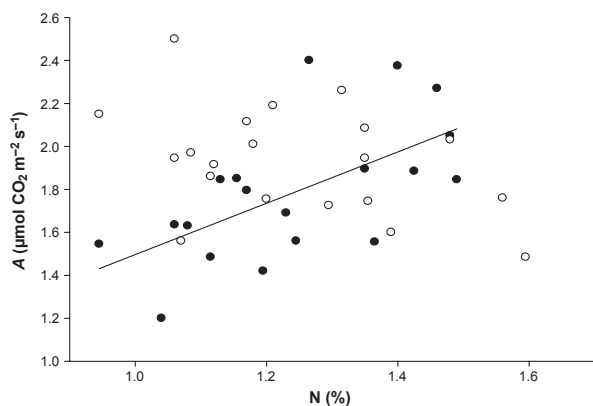


Fig. 4 Relationship of net photosynthesis rate (A) vs shoot nitrogen (N) concentration in male (closed circles) and female (open circles) *Juniperus thurifera* trees. The line shows a significant relationship for males (see text for statistical values).

An increase in photosynthesis has been suggested as a mechanism to compensate for higher reproductive investment (Dawson & Ehleringer, 1993; Obeso, 2002). However, during the studied nonmasting years, males presented a reproductive investment that was low but significantly higher than that of females, preventing us from finding evidence of this compensating mechanism in the short term. Alternatively, lower A in control males relative to control females might reflect a trade-off between A and reproduction, with greater resource investment to reproduction structures at the expense of other functions (Karlsson, 1994; Wheelwright & Logan, 2004). However, genders did not differ in shoot N , which is a key nutrient for photosynthesis (Field & Mooney, 1983; Zhao *et al.*, 2011). *Juniperus thurifera* females allocate up to threefold more biomass to reproduction during masting years, leading to an overall higher reproductive investment in female than in male trees (Montesinos, 2007), which is common among masting species (Herrera *et al.*, 1998; Kelly & Sork, 2002). Therefore, we suggest that the females' higher A relative to males could be a long-term response to higher inter-annual reproductive investment. This hypothesis should be tested during masting years.

Fertirrigation caused significant changes in several physiological variables. Interestingly, the sexes reacted differently to surpluses of nutrients and water. Males principally increased gas exchange, while fertirrigated females principally increased shoot growth. These results show that gender differences in *J. thurifera* are, at least in part, context dependent. Sex-specific physiological responses to abiotic factors have been described for other plant species, mainly as a response to water, light, and CO_2 availability (Dawson & Ehleringer, 1993; Geber *et al.*, 1999; Dawson *et al.*, 2004), and, overall, gender differences arise or are greater under drought stress (see for instance Dawson & Ehleringer, 1993). We suggest that nutrients, rather than water, seem to be the main explanatory factor for the different functional responses of *J. thurifera* genders to fertirrigation. This is evidenced by the increase in shoot N in both sexes in fertirrigated trees and the lack of differences in ψ between fertirrigation treatments. Therefore, our results indicate the importance of nutrients as a major factor

influencing gender functional response in plants and support the idea that nutrients also are an important limiting factor for plant life in Mediterranean environments. Nutrient use has been shown to differ between the sexual functions of monoecious and gynodioecious species (Havens *et al.*, 1995; Eckhart & Chapin, 1997), but only a handful of studies have experimentally studied the functional responses of genders of dioecious species to nutrient availability. Nutrient addition either had no effect on physiological performance (Wang & Curtis, 2001) or had a similar effect on both males and females (Zhao *et al.*, 2011). Similarly to our results, genders of a dioecious population of the otherwise androdioecious annual herb *Mercurialis annua* presented differential adjustments in plant biomass and reproductive investments in response to changes in levels of nutrient availability (Hesse & Pannell, 2011). Differences among the few works available are likely to be a result of the distinct functional demands and constraints of the different life forms studied, which range from herbs to trees.

Our results suggest that *J. thurifera* genders use N differently. On the one hand, control females seemed to invest a greater proportion of shoot N in photosynthesis than males, as control females had greater A than control males in spite of having the same shoot N concentration. Nitrogen is also an essential component of the photosynthetic machinery (Marschner, 1986) and A and N concentration are often positively correlated (Field & Mooney, 1983; Zhao *et al.*, 2011). This indicates that control females have higher photosynthetic N use efficiency than control males (Reich *et al.*, 1995). On the other hand, males increased A while females did not in response to an increase in shoot N following fertilization (Fig. 2a,d). Finally, A was positively correlated with N concentration in males but not in females (Fig. 4). The increase in A in *J. thurifera* males with fertilization was probably attributable to an increase in carboxylation capacity (Livingston *et al.*, 1999) rather than to an increase in g_s , as indicated by the greater $\delta^{13}\text{C}$ difference between sexes in the fertirrigated treatment.

Females did not increase A with an increase in shoot N concentration, indicating that females prioritize N storage over photosynthesis. Evergreen trees store great amounts of N in foliage and shoots, which can be translocated to support future reproduction or growth (Millard *et al.*, 2001; Salifu & Timmer, 2003; Millard & Grelet, 2010). Nitrogen storage can occur not only through increasing shoot N concentration but also through increasing the amount of shoot tissues, such as foliage. In our experiment, fertilization increased shoot growth 40% more in females than in males. Similar to our findings, other studies have detected higher branch and leaf growth on female plants (Nicotra, 1999b; Nicotra *et al.*, 2003). Moreover, Sala (2006) suggested that leaves are nutrient stores for species producing exceptionally large crops during masting years. Thus, an increase in shoot growth might be a strategy used by females to devote resources to photosynthetic structures which can be used to support future reproduction. Male trees, by contrast, seem to modulate their vegetative growth and reproduction according to current resource availability. Nicotra (1999a,b) found a similar pattern for *Siparuna grandiflora*, with females showing delayed reproductive costs as

well as physiological compensation mechanisms to compensate for such costs. This suggests that *J. thurifera* females may have a long-term specific physiological strategy to cope with very high reproductive investments during masting years that are unlikely to be paid for by short-term compensation mechanisms (Nicotra, 1999a,b; Nicotra *et al.*, 2003; Dudley & Galen, 2007). This hypothesis is supported by a reduction in female tree ring growth in the long term (Montesinos *et al.*, 2006).

In conclusion, the sexes in *J. thurifera* showed functional differences and responded differently to changes in nutrient availability, indicating different strategies to cope with nutrient surplus, particularly a surplus of N. Different reproductive investments by each sex are expected to select for different resource acquisition traits (Dawson & Ehleringer, 1993; Delph & Meagher, 1995; Geber *et al.*, 1999), particularly in resource-limiting environments where sexual dimorphism is expected to be larger (Lloyd & Webb, 1977; Gehring & Linhart, 1993; Geber *et al.*, 1999). Dioecy has been proposed to have evolved from monoecy (Charlesworth & Charlesworth, 1978; Lloyd, 1980; Webb, 1999; Renner & Won, 2001; Dorken *et al.*, 2002) either as a consequence of sexual specialization or to avoid inbreeding (Gleiser & Verdú, 2005). Among the European junipers, dioecy is the common breeding system and seems to be evolutionarily derived from monoecy (Jordano, 1991). The few monoecious junipers are known to be functionally subdioecious, indicating that the functional separation of the sexes is a common feature in the genus. Although *J. thurifera* is strictly dioecious, with just a few anecdotal cases of limited monoecy, our study supports the theoretical expectation that dioecy might have been favoured by selection in *J. thurifera* to maximize the ability to respond to the different physiological demands of each sexual function.

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References

- Bertaudière V, Montes N, Badri W, Gauquelin T. 2001. Multicaulis structure of *Juniperus thurifera*: adaptation to a severe environment? *Comptes Rendus De L'Académie Des Sciences Serie III Sciences De La Vie* 324: 627–634.
- Bertaudière V, Montes N, Gauquelin T, Edouard JL. 1999. Dendroecology of thuriferous juniper (*Juniperus thurifera* L.): example from a French Pyrenean site at Rie mountain. *Annals of Forest Science* 56: 685–697.
- Ceballos L, Ruiz de la Torre J. 1979. *Árboles y arbustos de la España peninsular*. Madrid, Spain: E.T.S.I. de Montes.
- Charlesworth D, Charlesworth B. 1978. Population-genetics of partial male-sterility and evolution of monoecy and dioecy. *Heredity* 41: 137–153.
- Chen L, Zhang S, Zhao H, Korpeläinen H, Li C. 2010. Sex-related adaptive responses to interaction of drought and salinity in *Populus vunnanensis*. *Plant, Cell & Environment* 33: 1767–1778.
- Correia O, Diaz Barradas MC. 2000. Ecophysiological differences between male and female plants of *Pistacia lentiscus* L. *Plant Ecology* 149: 131–142.
- Dawson TE, Ehleringer JR. 1993. Gender-specific physiology, carbon isotope discrimination, and habitat distribution in Boxelder, *Acer negundo*. *Ecology* 74: 798–815.
- Dawson TE, Ward JK, Ehleringer JR. 2004. Temporal scaling of physiological responses from gas exchange to tree rings: a gender-specific study of *Acer negundo* (Boxelder) growing under different conditions. *Functional Ecology* 18: 212–222.
- Delph LF. 1990. Sex-differential resource-allocation patterns in the subdioecious shrub *Hebe subalpina*. *Ecology* 71: 1342–1351.
- Delph LF. 1999. Sexual dimorphism in life history. In: Geber MA, Dawson TE, Delph LF, eds. *Gender and sexual dimorphism in flowering plants*. Berlin, Germany: Springer-Verlag, 149–173.
- Delph LF, Meagher TR. 1995. Sexual dimorphism masks life-history trade-offs in the dioecious plant *Silene latifolia*. *Ecology* 76: 775–785.
- Di Castri F. 1981. Mediterranean-type shrublands of the world. In: Di Castri F, Goodall DW, Specht RL, eds. *Mediterranean-type shrublands*. Amsterdam, the Netherlands: Elsevier, 1–52.
- Dorken ME, Friedman J, Barrett SCH. 2002. The evolution and maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). *Evolution* 56: 31–41.
- Dudley LS, Galen C. 2007. Stage-dependent patterns of drought tolerance and gas exchange vary between sexes in the alpine willow, *Salix glauca*. *Oecologia* 153: 1–9.
- Eckhart VM, Chapin FS III. 1997. Nutrient sensitivity of the cost of male function in gynodioecious *Phacelia linearis*. *American Journal of Botany* 84: 1092–1098.
- Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40: 503–537.
- Field C, Mooney HA. 1983. The photosynthesis-nitrogen relationship in wild plants. In: Givnish TJ, ed. *On the economy of plant form and function*. Cambridge, UK: Cambridge University Press, 25–56.
- Geber MA, Dawson TE, Delph LF. 1999. *Gender and sexual dimorphism in flowering plants*. Berlin, Germany: Springer.
- Gehring JL, Linhart YB. 1993. Sexual dimorphisms and response to low resources in the dioecious plant *Silene latifolia* (Caryophyllaceae). *International Journal of Plant Sciences* 154: 152–162.
- Gleiser G, Verdú M. 2005. Repeated evolution of dioecy from androdioecy in *Acer*. *New Phytologist* 165: 633–640.
- Havens K, Preston K, Richardson C, Delph LF. 1995. Nutrients affect allocation to male and female function in *Abutilon theophrasti* (Malvaceae). *American Journal of Botany* 82: 726–733.
- He WM, Zhang XS, Dong M. 2003. Gas exchange, leaf structure, and hydraulic features in relation to sex, shoot form, and leaf form in an evergreen shrub *Sabina vulgaris* in the semi-arid Mu Us Sandland in China. *Photosynthetica* 41: 105–109.
- Herrera CM, Jordano P, Guitián J, Traveset A. 1998. Annual variability in seed production by woody plants and the masting concept: reassessment of principles and relationship to pollination and seed dispersal. *American Naturalist* 152: 576–594.
- Hesse E, Pannell JR. 2011. Sexual dimorphism in a dioecious population of the wind-pollinated herb *Mercurialis annua*: the interactive effects of resource availability and competition. *Annals of Botany* 106: 1039–1045.
- Hill PW, Handley LL, Raven JA. 1996. *Juniperus communis* L. ssp. *communis* at Balnaguard, Scotland: foliar carbon discrimination ($d^{13}C$) and $15-N$ natural abundance ($d^{15}N$) suggest gender-linked differences in water and N use. *Botanical Journal of Scotland* 48: 209–224.
- Hubick KT, Farquhar GD, Shorter R. 1986. Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. *Australian Journal of Plant Physiology* 13: 803–816.

- Jordano P. 1991. Gender variation and expression of monoecy in *Juniperus phoenicea* (L.) (Cupressaceae). *Botanical Gazette* 152: 476–485.
- Karlsson PS. 1994. Photosynthetic capacity and photosynthetic nutrient-use efficiency of *Rhododendron lapponicum* leaves as related to leaf nutrient status, leaf age and branch reproductive status. *Functional Ecology* 8: 694–700.
- Kelly D, Sork VL. 2002. Mast seeding in perennial plants: why, how, where? *Annual Review of Ecology and Systematics* 33: 427–447.
- Lambers H, Poorter H. 1992. Inherent variations in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research* 23: 187–261.
- Lasanta T. 1996. El proceso de marginación de tierras en España. In: Lasanta T, García-Ruiz JM, eds. *Erosión y recuperación de tierras en áreas marginales*. Logroño, Spain: Instituto de Estudios Riojanos & Sociedad Española de Geomorfología, 7–32.
- Livingston NJ, Guy RD, Sun ZJ, Ethier GJ. 1999. The effects of nitrogen stress on the stable carbon isotope composition, productivity and water use efficiency of white spruce (*Picea glauca* (Moench) Voss) seedlings. *Plant, Cell & Environment* 22: 281–289.
- Lloyd DG. 1980. The distributions of gender in four angiosperm species illustrating two evolutionary pathways to dioecy. *Evolution* 34: 123–134.
- Lloyd DG, Webb CJ. 1977. Secondary sex characters in plants. *Botanical Review* 43: 177–216.
- Marschner H. 1986. *Mineral nutrition of higher plants*. London, UK: Academic Press.
- Martínez-Vilalta JH, Cochard M, Mencuccini F, Sterck A, Herrero JFJ, Korhonen P, Llorens E, Nikinmaa A, Nolé R, Poyatos F *et al.* 2009. Hydraulic adjustment of Scots pine across Europe. *New Phytologist* 184: 353–364.
- Millard P, Grelet GA. 2010. Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. *Tree Physiology* 30: 1083–1095.
- Millard P, Hester AJ, Wendler R, Baillie G. 2001. Interspecific defoliation responses of trees depend on sites of winter nitrogen storage. *Functional Ecology* 15: 535–543.
- Montès N, Bertaudière V, Badri W, Zaoui ELH, Gauquelin T. 2002. Biomass and nutrient content of a semi-arid mountain ecosystem: the *Juniperus thurifera* L. woodland of Azzaden Valley (Morocco). *Forest Ecology and Management* 166: 35–43.
- Montesinos D. 2007. *Resource availability and reproductive efficacy of the dioecious tree Juniperus thurifera*. Dissertation thesis, Universitat de València, Valencia, Spain.
- Montesinos D, De Luis M, Verdú M, Raventós J, García-Fayos P. 2006. When, how and how much: gender-specific resource use strategies in the dioecious tree *Juniperus thurifera*. *Annals of Botany* 98: 885–889.
- Montesinos D, García-Fayos P, Verdú M. 2010. Relictual distribution reaches the top: elevation constrains fertility and leaf longevity in *Juniperus thurifera*. *Acta Oecologica* 36: 120–125.
- Nicotra AB. 1999a. Reproductive allocation and the long-term costs of reproduction in *Siparuna grandiflora*, a dioecious neotropical shrub. *Journal of Ecology* 87: 138–149.
- Nicotra AB. 1999b. Sexually dimorphic growth in the dioecious tropical shrub, *Siparuna grandiflora*. *Functional Ecology* 13: 322–331.
- Nicotra AB, Chazdon RL, Montgomery RA. 2003. Sexes show contrasting patterns of leaf and crown carbon gain in a dioecious rainforest shrub. *American Journal of Botany* 90: 347–355.
- Obeso JR. 2002. The costs of reproduction in plants. *New Phytologist* 155: 321–348.
- Padilla FM, Pugnaire FI. 2007. Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. *Functional Ecology* 21: 495.
- Reich PB, Kloeppel DS, Ellsworth DS, Walters MB. 1995. Different photosynthesis-nitrogen relations in deciduous hardwood and evergreen coniferous tree species. *Oecologia* 104: 24–30.
- Renner S, Won H. 2001. Repeated evolution of dioecy from monoecy in Siparunaceae (Laurales). *Systematic Biology* 50: 700–712.
- Retuerto R, Fernandez Lema B, Rodríguez Roiloa S, Obeso JR. 2000. Gender, light and water effects in carbon isotope discrimination, and growth rates in the dioecious tree *Ilex aquifolium*. *Functional Ecology* 14: 529–537.
- Rodà F, Mayor X, Sabatés S, Diego V. 1999. Water and nutrient limitations to primary production. In: Rodà F, Retana J, Gracia C, Bellot J, eds. *Ecology of Mediterranean evergreen oak forests*. Berlin, Germany: Springer, 183–194.
- Rodrigo C. 1999. *Puebla de San Miguel*. Valencia, Spain: Ayuntamiento de Puebla de San Miguel.
- Rozas V, DeSoto L, Olano JM. 2009. Sex-specific, age dependent sensitivity of tree-ring growth to climate in the dioecious tree *Juniperus thurifera*. *New Phytologist* 182: 687–697.
- Sala A. 2006. Hydraulic compensation in northern Rocky Mountain conifers: does successional position and life history matter? *Oecologia* 149: 1–11.
- Salifu KF, Timmer VR. 2003. Nitrogen retranslocation response of young *Picea mariana* to nitrogen-15 supply. *Soil Science Society of America Journal* 67: 309–317.
- Saulnier TP, Reekie EG. 1995. Effect of reproduction on nitrogen allocation and carbon gain in *Oenothera biennis*. *Journal of Ecology* 83: 23–29.
- SPSS. 2005. *Linear mixed-effects modeling in SPSS: an introduction to the MIXED procedure*. Chicago, IL, USA: SPSS Inc.
- Stearns SC. 1989. Trade-offs in life-history evolution. *Functional Ecology* 3: 259–268.
- Turner NC. 1986. Adaptation to water deficits: a changing perspective. *Plant Physiology* 13: 175–190.
- Verdú M, Villar-Salvador P, García-Fayos P. 2004. Gender effects on the post-facilitation performance of two dioecious *Juniperus* species. *Functional Ecology* 18: 87–93.
- Wang X, Curtis P. 2001. Gender-specific responses of *Populus tremuloides* to atmospheric CO₂ enrichment. *New Phytologist* 150: 675–684.
- Webb CJ. 1999. Empirical studies: evolution and maintenance of dimorphic breeding systems. In: Geber MA, Dawson TE, Delph LE, eds. *Gender and sexual dimorphism in flowering plants*. New York, NY, USA: Springer, 61–95.
- Wheelwright NT, Logan BA. 2004. Previous-year reproduction reduces photosynthetic capacity and slows lifetime growth in females of a neotropical tree. *Proceedings of the National Academy of Sciences, USA* 101: 8051–8055.
- Xu X, Yang F, Xiao X, Zhang S, Korpelainen H, Li C. 2008. Sex-specific responses of *Populus cathayana* to drought and elevated temperatures. *Plant, Cell & Environment* 31: 850–860.
- Zhang J, Marshall JD, Jaquish C. 1993. Genetic differentiation in carbon isotope discrimination and gas exchange in *Pseudotsuga menziesii*. *Oecologia* 93: 80–87.
- Zhang JW, Marshall JD. 1995. Variation in carbon isotope discrimination and photosynthetic gas exchange among populations of *Pseudotsuga menziesii* and *Pinus ponderosa* in different environments. *Functional Ecology* 9: 402–412.
- Zhao H, Xu X, Zhang Y, Korpelainen H, Chunyang L. 2011. Nitrogen deposition limits photosynthetic response to elevated CO₂ differentially in a dioecious species. *Oecologia* 165: 41–54.

Supporting Information

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

Table S1 *F* values and significance level obtained from a linear mixed model of the effects of sex, fertirrigation, year, month and time (morning or midday) and their interaction on water potential (ψ), net photosynthesis rate (*A*) and stomatal conductance (*g*)

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