

Gender effects on the post-facilitation performance of two dioecious *Juniperus* species

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Summary

1. Plant facilitation usually changes to competition as plants age. In dioecious plants, females should be affected more negatively than males by stressful conditions because of the greater costs of female reproduction.
2. We investigated the gender effects on the post-facilitation performance of adult plants of two dioecious *Juniperus* species from the high mountains of eastern Spain: *J. sabina* L. acts as a nurse plant for *J. communis* L. We compared physiological (water potential, carbon isotope discrimination and nitrogen concentration), vegetative (shoot growth) and reproductive (number of male flowers, and number of fruits and seeds) characters of associated and non-associated plants of both species, to test the hypothesis that this association represents a more stressful condition for females than for males because of the greater costs of female reproduction.
3. Despite their close phylogenetic relatedness, both species showed a distinct performance pattern after the facilitation phase. Association with the nurse plant reduced the growth and reproductive capacity of both genders in *J. communis*, the facilitated species. In contrast, the association with *J. communis* did not affect the fitness of the nurse plant, *J. sabina*, although in accordance with our hypothesis a gender effect was found on several physiological parameters. Thus *J. sabina*-associated females had a more negative water potential and carbon isotope discrimination than the associated males, but there were no differences between genders when growing in isolation.
4. The consequences of the post-facilitation interaction between the two long-lived woody *Juniperus* species are asymmetrical: harmful for the facilitated species, but harmless for the nurse.
5. Gender had also asymmetrical consequences on some functional traits of the nurse – but not the facilitated – species.

Key-words: carbon isotope discrimination, competition, juniper, Mediterranean mountains, nurse plant

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Introduction

The establishment and growth of seedlings can be facilitated in microhabitats formed under the canopy of adult plants of the same or other species (Franco & Nobel 1989; Callaway 1995; Pugnaire *et al.* 1996a). Canopies tend to create fertile microhabitats where extreme environmental conditions are buffered, and provide protection from grazing (Verdú & García-Fayos 1996; Rousset & Lepart 2000). Establishment is promoted because facilitated plants can attain a superior physiological performance than non-associated individuals.

Facilitation studies in plants have often emphasized the positive effects of nurse plants on the facilitated plants, without considering that facilitation is a bi-directional process between nurse and facilitated plant (Holzapfel & Mahall 1999). Facilitative interactions are encounters between individuals that benefit at least one of the participants and cause harm to neither (Stachowicz 2001; Bruno *et al.* 2003). Therefore the influence of the facilitated plants on their nurses should also be measured to ensure no negative effects appear in the interaction. The balance between positive (facilitation) and negative (competition) forces may change temporally during the life of nurse and facilitated plant (Sans *et al.* 1998; Rousset & Lepart 2000; Tielbörger & Kadmon 2000). These temporal changes have rarely been detected because most studies on facilitation have

focused on the early growth stages of the facilitated species, or on species with short life spans (Callaway 1995; Callaway & Pugnaire 1999). The few studies that have evaluated the interaction at later life stages in long-lived facilitated plants have shown that facilitation gradually becomes competition, the facilitated plant increasing its performance to the detriment of the nurse species (McAuliffe 1984; Valiente-Banuet *et al.* 1991; Flores-Martínez *et al.* 1994).

Facilitation studies usually measure plant survival and vegetative growth as the only fitness components, neglecting reproduction (Tielbörger & Kadmon 2000). Holzapfel & Mahall (1999) reported that facilitated annual species induced a shift towards a greater proportion of male flowers in nurse shrubs of *Ambrosia dumosa*. Stress usually favours maleness over femaleness in monoecious species because of the higher costs of female reproduction (Willson 1983 and references therein). In dioecious species, gender-specific differences in growth, survival, reproductive output, resource allocation and physiology have been described (Dawson & Geber 1999; Delph 1999; Obeso 2002). However, some of these differences might depend on the environment in which both genders develop (Retuerto *et al.* 2000), tending to be enhanced in stressful environments (Dawson & Bliss 1989; Dawson & Ehleringer 1993). Therefore in dioecious species the performance of female plants in stressful habitats might be different from that of males (Cox 1981; Bierzychudek & Eckhart 1988). If the interaction between species changes from facilitation to competition, it might be expected that the genders will perform differently, and that gender differences in functional traits should be taken into account when studying the post-facilitation performance of adult plants.

In the high mountains of eastern Spain, *Juniperus sabina* acts as a nurse plant for *Juniperus communis* (Verdú & García-Fayos 2003). Both species are dioecious, so they provide an appropriate ecological model to test for gender effects on the post-facilitation performance. This study aimed to test the hypothesis that this association is more stressful for females than for males in terms of physiological, vegetative and reproductive traits.

Materials and methods

NATURAL HISTORY OF THE SYSTEM

The study was carried out in Puebla de San Miguel (Valencia, East Spain) (40°1' N, 1°12' W) at 1640 m a.s.l. All measurements were taken in individuals from a 5 ha stand on a south-west oriented slope with a 10° angle. The soils are limestones <30 cm deep, although cracks in the rock allow roots to go deeper than this. Gymnosperms (*Juniperus sabina*, *J. communis*, *J. thurifera*, *Pinus sylvestris* and *P. nigra* ssp. *salzmannii*) are the dominant species at the study site. A discontinuous herb and subshrub layer is also present, composed of

Helleborus foetidus, *Helianthemum canum*, *Thymus godayanus*, *Euphorbia nicaeensis*, *Koeleria vallesiana* and *Festuca hystrix* (nomenclature of all taxa follows Mateo & Crespo 1998).

The climate is Mediterranean, with cold winters (mean temperature in January, 2.5 °C) and warm, dry summers (mean temperature in July, 21 °C). Mean annual precipitation averages 600 mm.

Leaf type, growth form and shape differ between the *Juniperus* species. *Juniperus sabina* has scale-like leaves, and a prostrate and creeping growth form which develops in a centrifugal pattern to form an elliptical shrub of up to 20 m diameter. *Juniperus communis* ssp. *hemisphaerica* has needle-like leaves and grows as an erect shrub up to 2.5 m tall. Both species are dioecious and have fleshy fruits that ripen at the same time. Birds of the genus *Turdus* are the main seed dispersers for both species (Jordano 1993; García 1998; Verdú & García-Fayos 2003).

The association between the species is mediated by birds dispersing the seeds of *J. communis* within the canopy of the nurse plant, *J. sabina* (Verdú & García-Fayos 2003). Thus *J. communis* plants emerging from inside the canopy of *J. sabina* shrubs were considered as associated individuals. Similarly, all the *J. sabina* plants with *J. communis* shrubs emerging from inside their canopies were considered as associated individuals. Eight individuals of *J. sabina* in each of the following four combinations (treatments) were selected: males associated with *J. communis*; females associated with *J. communis*; non-associated males; non-associated females. An equivalent design was made for *J. communis*. Small and large individuals of both species were excluded. The size of *J. sabina* shrubs was estimated as the product of two perpendicular diameters, whereas the size of *J. communis* shrubs was estimated as the product of two perpendicular diameters and their height. The sizes (mean \pm SE) of the associated and non-associated females and males in *J. sabina* were (in m²) 45.6 \pm 5.71, 39.8 \pm 3.20, 39.8 \pm 6.02 and 44.6 \pm 4.65, respectively; in *J. communis* they were (in m³) 8.0 \pm 2.17, 8.7 \pm 2.77, 9.4 \pm 2.82 and 10.4 \pm 1.72, respectively. In each species there were no significant differences among groups ($P > 0.05$).

VEGETATIVE GROWTH AND REPRODUCTIVE OUTPUT

Vegetative growth was measured on July 1999 and July 2000 as the dry weight of 10 shoots from the current year's growth. Shoots from the current year can be identified because the stem is not lignified and, in the case of *J. sabina*, because they grow more vigorously on the periphery of the shrub. Current-year shoots were cut around the whole plant to avoid any effect of orientation, and were oven-dried at 60 °C for 48 h. Reproductive output in females of both species was estimated in October 1999 by (1) counting the number of fruits of the whole plant, and (2) counting the

number of seeds and estimating their viability in 50 fruits per plant. Seed viability was confirmed by splitting the seeds to check if the embryo was aborted or viable. Reproductive output in males was estimated by counting the number of flowers from 10 branches in March 2000.

PHYSIOLOGICAL ATTRIBUTES

Oven-dried shoots collected for the 1999 growth measurements were finely ground with a mill. A 1 g subsample was used for total nitrogen determination following the standard Kjeldahl method. A second 20 mg subsample was analysed in the Laboratorio de Isótopos Estables (Universidad Autónoma de Madrid, Spain). 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-Isochrom, Manchester, UK) with a measurement error of 0.1‰. The isotopic values are expressed in delta notation and calculated as:

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

where R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ ratios of the sample and the Pee Dee Belemnite carbonate standard, respectively.

Shoot water potential (Ψ) measurements were carried out on a typical cloudless summer day at the end of July 1999. Predawn and midday water potentials were determined with a Scholander chamber on three to nine individuals per treatment and species, using sun-exposed shoots excised from the southern sides of the plants.

DATA ANALYSES

Individual differences on the measured parameters were analysed by fitting a factorial ANOVA with

association and gender as fixed factors (except for reproductive output, in which each gender was analysed separately by means of t -tests). Plant size was included as a covariate when it was correlated with the dependent variable, as in the measures of carbon isotope discrimination and shoot growth in 2000 for *J. sabina*. In ANCOVA analyses, slope homogeneity was checked.

The Ψ determinations were analysed with a two-way repeated-measures ANOVA, the levels of the repeated factor being the predawn and midday water potential. Two orthogonal contrasts were constructed, when the interaction term between gender and association was statistically significant, to compare the means of (1) associated females vs associated males, and (2) non-associated males vs non-associated females. Normality and homogeneity of variances was checked and variables were transformed as needed.

Results

VEGETATIVE GROWTH AND REPRODUCTIVE OUTPUT

Growth in 1999 and 2000 of the nurse plant, *J. sabina*, was similar between genders and was unaffected by the association with *J. communis* or by the interaction gender \times association (Table 1; Fig. 1). In contrast, *J. communis* individuals growing in association with *J. sabina* grew less than the non-associated plants during both years, irrespective of gender.

The reproductive parameters of the female nurse plants (*J. sabina*) were not affected by the association with *J. communis*. Fruit crop (Fig. 2; $t = -0.22$, $df = 14$, $P > 0.05$), the number of seeds per fruit (2.23 ± 0.10 vs 2.33 ± 0.11 , $t = -0.70$, $df = 15$, $P > 0.05$), or the percentage of viable seeds (54.1 ± 6.9 vs 53.1 ± 2.6 , $t = 0.15$, $df = 10.3$, $P > 0.05$) were similar in non-associated and associated nurse plants. Conversely, the associated *J. communis* female plants experienced a 2.5-fold reduction in their fruit crop in relation to the

Table 1. Results of AN(C)OVA (F values) for effects of gender, association and their interaction on shoot growth (log-transformed), carbon isotope ratios ($\delta^{13}\text{C}$), shoot nitrogen concentration (N) and water potential (Ψ). Plant size was used as a covariate in the model when significant

	Shoot growth		$\delta^{13}\text{C}$	N	Ψ
	1999	2000			
Nurse plant, <i>Juniperus sabina</i>					
Gender	0.28	0.04	1.57	0.87	9.7**
Association	3.79	2.69	0.41	3.00	5.1*
Gender \times association	3.17	1.00	8.05**	1.50	5.7*
Plant size (covariate)	–	5.19*	4.05*	–	–
Facilitated plant, <i>Juniperus communis</i>					
Gender	0.91	1.47	0.41	0.08	2.87
Association	6.04*	15.1***	0.42	0.23	0.01
Gender \times association	1.38	1.00	1.45	0.27	1.06
Plant size (covariate)	–	–	–	–	–

*, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P < 0.001$.

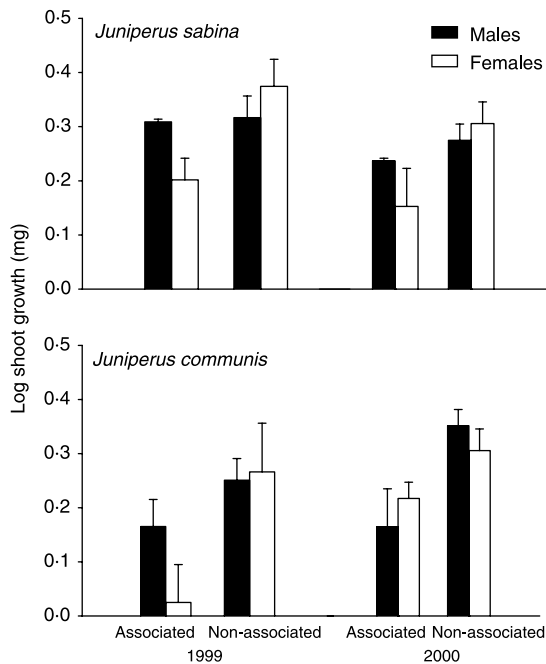


Fig. 1. Current year shoot growth (mean \pm 1 SE) measured in the 1999 and 2000 growing seasons in males and females of the nurse plant, *Juniperus sabina* and the facilitated plant, *Juniperus communis*, living either non-associated or associated with the other species.

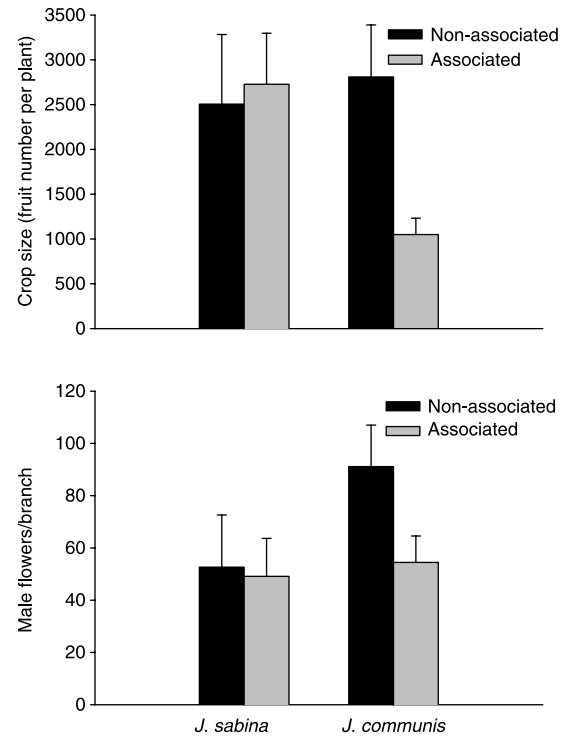


Fig. 2. Crop size (number of fruits per plant) and number of male flowers per branch (mean \pm 1 SE) in *Juniperus sabina* and *Juniperus communis* living either isolated or in association with the other species.

non-associated plants (Fig. 2; $t = 2.88$, $df = 14$, $P = 0.006$), although there were no differences in the number of seeds per fruit (2.70 ± 0.05 vs 2.69 ± 0.14 , $t = 0.15$, $df = 15$, $P > 0.05$), or the percentage of viable seeds (51.3 ± 8.8 vs 49.7 ± 5.5 , $t = 0.15$, $df = 14$, $P > 0.05$). The same trend was observed in male reproductive parameters. The mean number of male flowers per branch was reduced by 40% in the facilitated plants ($t = 1.94$, $df = 18$, $P = 0.03$) but was not affected in the nurse plants ($t = 0.14$, $df = 18$, $P > 0.05$) as a consequence of the association (Fig. 2).

PHYSIOLOGICAL ATTRIBUTES

Intraspecific differences in shoot $\delta^{13}\text{C}$ between *J. sabina* and *J. communis* were 1.8 and 2.4‰, respectively. In *J. sabina* $\delta^{13}\text{C}$ a highly significant interaction was observed between gender and association with *J. communis* (Table 1). Thus associated females had a significantly more negative $\delta^{13}\text{C}$ than associated males, but no significant difference between the genders was observed in non-associated individuals (Table 2). Gender or association with *J. sabina* did not affect $\delta^{13}\text{C}$ variation in *J. communis*.

In both species, shoot N concentration did not differ either between genders or between associated and non-associated individuals. Similarly, no significant effect of N on the interaction between gender and association was observed.

In both species, Ψ changed significantly during the day ($P < 0.001$), but similarly between females and

males or between associated and non-associated plants (non-significant interactions between within- and between-subjects factors). In *J. sabina* a significant interaction was observed (Table 1), associated females having a more negative Ψ than associated males, and there were no differences between genders when isolated (Table 2).

Discussion

Despite their close phylogenetic relatedness, the response of the two juniper species was distinct after the facilitation phase. Association with the nurse plant reduced the growth and reproductive capacity of both genders in *J. communis*, the facilitated species. In contrast, the association with *J. communis* did not affect the fitness of the nurse plant, *J. sabina*, although in accordance with our hypothesis a gender effect was found on several physiological parameters.

PERFORMANCE OF THE NURSE PLANT

Associated individuals of *J. sabina* did not show any variation in growth or reproductive output compared with isolated individuals. These results contrast with those found for other species, where reductions in growth and reproductive output in nurse plants were documented for *Mimosa luisana* (Flores-Martínez *et al.* 1994) and *Prosopis glandulosa* (Barnes & Archer 1999) by the effect of facilitated woody species, and in *A. dumosa* by the effect of annual herbs (Holzapfel &

Table 2. Carbon isotope ratios ($\delta^{13}\text{C}$), shoot nitrogen concentration (N) and water potential (Ψ) (mean \pm 1 SE) of associated and non-associated male and female individuals of nurse and facilitated plants

	Nurse plant, <i>Juniperus sabina</i>				Facilitated plant, <i>Juniperus communis</i>			
	Associated		Non-associated		Associated		Non-associated	
	Male	Female	Male	Female	Male	Female	Male	Female
$\delta^{13}\text{C}$ (‰)	-23.1 \pm 0.1	-23.7 \pm 0.1*	-23.4 \pm 0.1	-23.2 \pm 0.1	-23.8 \pm 0.2	-23.7 \pm 0.2	-23.7 \pm 0.2	-24.1 \pm 0.3
N (mg g ⁻¹)	13.1 \pm 0.40	12.2 \pm 0.48	11.9 \pm 0.34	12.0 \pm 0.41	11.5 \pm 0.24	11.2 \pm 0.26	11.5 \pm 0.34	11.5 \pm 0.35
Ψ (MPa)	-1.06 \pm 0.03	-1.23 \pm 0.04*	-1.06 \pm 0.03	-1.08 \pm 0.02	-0.97 \pm 0.05	-0.99 \pm 0.04	-0.92 \pm 0.03	-1.03 \pm 0.03

* $P < 0.05$. Asterisks indicate significant differences of the two orthogonal contrasts comparing associated males vs associated females and non-associated males vs non-associated females.

Mahall 1999). Our results also disagree with those of Pugnaire *et al.* (1996b), who found that *Retama sphaerocarpa* grew and reproduced more when associated with the understorey perennial herb *Marrubium vulgare*.

However, the post-facilitative patterns cannot be interpreted so straightforwardly in the case of dioecious species, where females are expected to be more susceptible to stress than males. According to this hypothesis, we found a physiological differentiation among genders of *J. sabina* that appears when associated with the facilitated plants. Thus *J. sabina* females, but not males, had a more negative $\delta^{13}\text{C}$ and Ψ when they grew with *J. communis*, but these differences were not apparent in female and male individuals when isolated. These results agree with the conclusions reached by Retuerto *et al.* (2000), who observed that functional differences between genders in *Ilex aquifolium* depended on the environmental context in which both genders grew. Water potential of nurse plants of *R. sphaerocarpa* were less negative when associated with their facilitated plants, *M. vulgare* (Pugnaire *et al.* 1996b), but both positive and negative effects can occur depending on the season (Barnes & Archer 1999; Holzapfel & Mahall 1999).

Carbon isotopic discrimination is a long-term estimate of internal CO_2 concentration and a proxy for potential water-use efficiency (WUE) in C_3 species (Hubick *et al.* 1986; Farquhar *et al.* 1989; Zhang & Marshall 1995). More negative $\delta^{13}\text{C}$ values are usually associated with smaller WUE (Hubick *et al.* 1986; Zhang & Marshall 1993). The more negative $\delta^{13}\text{C}$ in the associated *J. sabina* females with respect to the associated males therefore indicates that those individuals probably had a smaller WUE. Similar differences between genders in $\delta^{13}\text{C}$ have also been reported in *Acer negundo* (Dawson & Ehleringer 1993) and in *I. aquifolium* (Retuerto *et al.* 2000). In both species, females had a more negative $\delta^{13}\text{C}$ than males when grown under adverse conditions, but no difference existed under favourable conditions.

A more negative $\delta^{13}\text{C}$ could be attributed to a smaller CO_2 assimilation capacity of the foliage if no stomatal conductance changes occur. However, differences in carboxylation capacity are unlikely to have been the cause of the more negative $\delta^{13}\text{C}$ in the females in our

study, as foliage N concentration, which is an estimate of photosynthetic capacity (Field & Mooney 1986), did not differ among treatments. A second possible cause of the more negative $\delta^{13}\text{C}$ in associated *J. sabina* females is the different stomatal sensitivity to environmental conditions and tissue water potential in both genders. In *A. negundo*, a more negative $\delta^{13}\text{C}$ and smaller WUE of females compared with males was explained by their less sensitive stomatal closure in response to changes in water vapour pressure deficit and leaf water potential (Dawson & Ehleringer 1993). The more negative water potential of the associated *J. sabina* females, in comparison with plants in the other treatments, suggests that they maintain a greater transpiration rate at midday, probably because of a greater stomatal conductance. Higher stomatal conductance can increase CO_2 assimilation rate.

Plant growth under water-limited conditions is frequently correlated with WUE and $\delta^{13}\text{C}$ (Ehleringer 1993; Wright *et al.* 1993). The sign of the relationship between plant growth and $\delta^{13}\text{C}$ might vary depending on where the plants grow and on the scale of the study (Condon & Richards 1993; Johnson *et al.* 1993; Livingston & Spittlehouse 1993; Flanagan & Johnsen 1995; Retuerto *et al.* 2000). In *J. sabina*, associated females had more negative $\delta^{13}\text{C}$ than associated males, but similar growth. In *A. negundo*, males and females had similar growth in dry habitats despite the lower WUE of females (Dawson & Ehleringer 1993). The potential growth advantages of a lower WUE, as inferred by the $\delta^{13}\text{C}$ results, might have not been realized in associated *J. sabina* females because they could have been offset by the negative effects of competition with *J. communis* and the reproductive effort associated with fruit formation.

PERFORMANCE OF THE FACILITATED PLANT

The post-facilitation effect was detrimental to *J. communis* because the growth and reproduction capacity of adults of both genders was reduced when growing associated with the nurse plant, which remained unaffected. These results contrast with those found in other studies, in which bidirectional effects between nurse

and facilitated plants were found. For example, growth of facilitated plants was increased in the case of *M. vulgare* (Pugnaire *et al.* 1996b) and of the annual herbs living under *A. dumosa* shrubs (Holzapfel & Mahall 1999), but it remained unaffected in the shrubs nursed by *P. glandulosa* (Barnes & Archer 1999).

Juniperus communis individuals associated with *J. sabina* reduce their growth and reproductive output, indicating that after the initial facilitation process, there is a competition stage in which the performance of the facilitated species is reduced with no effect on the nurse species. Growth reduction in associated *J. communis* individuals was not related to a lower shoot N concentration, as no differences existed among associated and non-associated individuals, contrasting again with the results of previous studies where leaf N concentration increased in facilitated plants (Pugnaire *et al.* 1996b; Barnes & Archer 1999).

Stress induced by competition for soil water could explain some of the performance differences (Callaway *et al.* 1996; Collet *et al.* 1996). However, the lack of water potential differences measured in summer, as well as differences in $\delta^{13}\text{C}$, indicates that water stress did not limit growth or reproductive output in the associated *J. communis* individuals. Similar results were reported by Collet *et al.* (1996), who observed that, when water was not a limiting factor, no differences in $\delta^{13}\text{C}$ or nutritional status existed among competing and non-competing seedlings of *Quercus petraea*.

The functional attributes studied here do not provide any clear evidence for the physiological mechanisms of the differences in growth and reproduction of associated vs non-associated *J. communis* plants. Further research on other features such as root competition, different biomass allocation patterns, or even allelopathic inhibition could help unravel this question.

Unlike the nurse species, no gender effect was found in *J. communis* because females and males did not differ in their growth or physiological traits. This result agrees with those reported by Marion & Houle (1996), who found no growth differences between genders in *J. communis* var. *depressa* either under mesic or stressful conditions in subarctic dunes. In contrast, Hill *et al.* (1996) found gender-linked differences in water use in *J. communis* from drier sites.

In summary, the consequences of the post-facilitation interaction between the two long-lived woody *Juniperus* species are asymmetrical: harmful for the facilitated species, harmless for the nurse species. Gender also had asymmetrical consequences for some functional traits of the nurse – but not the facilitated – species.

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