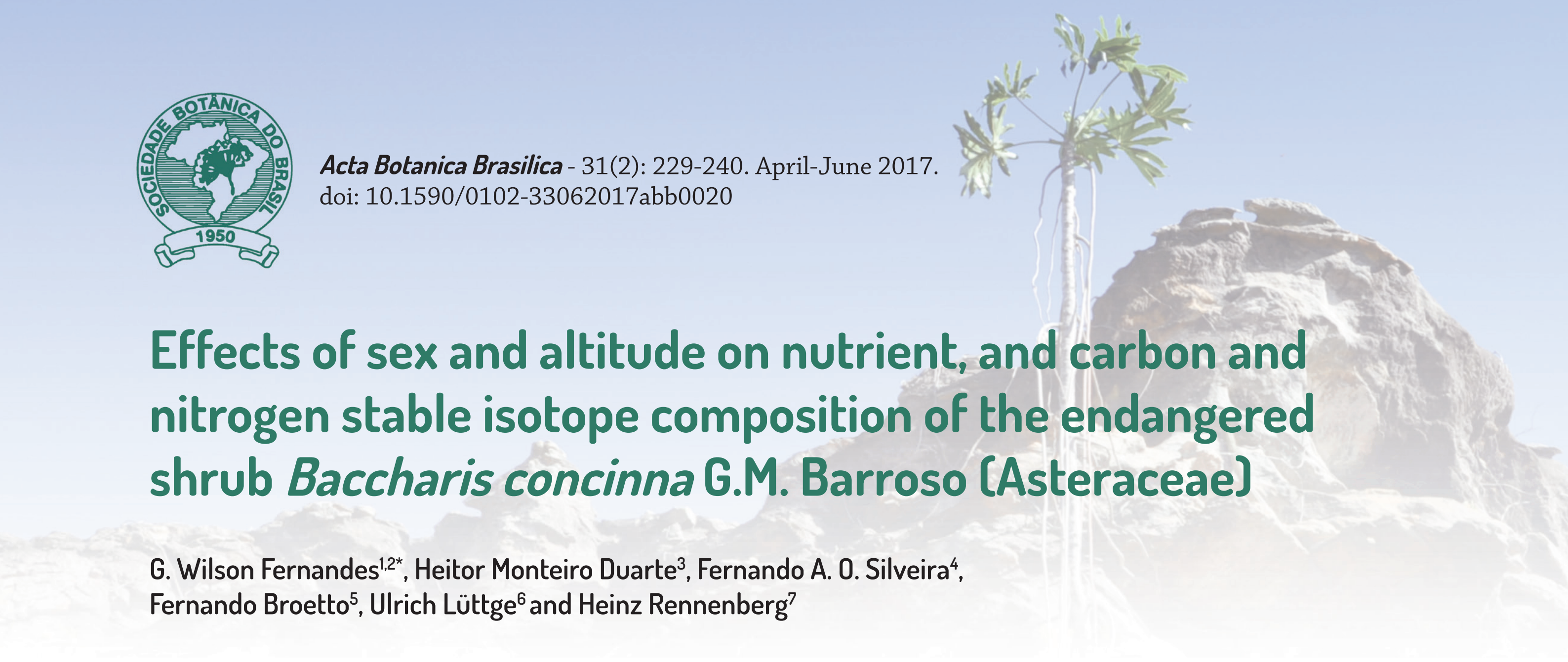

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Effects of sex and altitude on nutrient, and carbon and nitrogen stable isotope composition of the endangered shrub *Baccharis concinna* G.M. Barroso (Asteraceae)

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ABSTRACT

Previous ecological studies of dioecious plant species have found that female plants preferentially occur at lower altitudes where there are typically better nutritional conditions, while male plants often occur in less favorable sites. We compared the ecophysiological performance of male and female plants in three populations of the dioecious *Baccharis concinna*, an endemic species of rupestrian grasslands of Serra do Cipó, in southeastern Brazil. We hypothesized that physiological differences between the sexes would explain the distribution patterns of the populations. Analyses of the tissue content of phosphorus (P), calcium (Ca), potassium (K) and sodium (Na), and carbon and nitrogen stable isotopes, were used to assess nutritional status and water use efficiency (WUE) in plant leaves, stems and roots of male and female plants in three populations located along an elevational gradient. Differences among populations were related to decreased nutrient levels and WUE at higher elevations, but an effect of sex was found only for %C, with male plants having slightly higher values. In conclusion, the sex ratios in the studied populations of *B. concinna* could not be attributed to differences in nutrient acquisition and WUE.

Keywords: Cerrado, *campos rupestres*, dioecy, plant sex, resource allocation, rupestrian grasslands, Serra do Cipó

Introduction

Many aspects of the natural history, ecology, and physiology of dioecious plants have been studied in an attempt to unravel the evolutionary processes and mechanisms involved in the differential distribution of the sexes among habitats of differing quality (Lloyd 1973; 1974; Freeman *et al*. 1976; Varga & Kytöviita 2011; Castilla *et al*. 2012). Females have been reported to be relatively more abundant in habitats at lower elevations with higher humidity, lower salinity and less light exposure, and also in populations of low-density. Males in contrast, exhibit the opposite trend relative to these conditions (e.g., Pavón & Ramírez 2008; but see Vega-Fruits *et al*. 2013; Riba-Hernandez *et al*. 2014). The spatial segregation of males and females of dioecious plants among habitats has been mostly attributed to sex-dependent differential resource requirements (e.g., Cox 1981; Meagher 1984), in which female plants allocate more resources to reproduction, and preferentially occupy relatively more favorable habitats (e.g., Bierzychudek & Eckhart 1988; Krischik & Denno 1990; Herms & Mattson 1992).

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Analyses of $\delta^{13}C$ and $\delta^{15}N$ stable isotope composition have been successfully used to assess differences in carbon metabolism, water use and the magnitude of different N sources available to plants both at ecosystem (Pate & Arthur 1998; Ehleringer *et al*. 2000), and individual levels (Robinson *et al*. 2000). $\delta^{13}C$-ratios in organic material provide an estimate of the extent to which different components of gas exchange affect productivity (Farquhar *et al*. 1989a), as well as screen C_3 genotypes for potential water-use efficiency (Ehleringer *et al*. 1993). $\delta^{15}N$-ratios, on the other hand, provide a quantitative estimate of the balance between nitrogen inputs and losses from soil, and species-specific patterns of nitrogen use (Evans & Ehleringer 1993). Environmental stresses, such as drought, modify $\delta^{13}C$ in a largely predictable way (Guy *et al*. 1998), which is another useful tool for assessing the physiological mechanisms that influence the differential distribution of male and female dioecious plants.

The dioecious shrub *Baccharis concinna* (Asteraceae) is a threatened, endemic and locally rare species found in patches of rupestrian grasslands of the Serra do Cipó in southeastern Brazil (Gomes *et al*. 2004; Marques & Fernandes 2016). A higher proportion of female plants has been observed at lower elevations, while male plants are at higher relative proportions at higher elevations (Marques *et al*. 2002). These authors have reported that soils at higher elevations were more nutrient-impoverished than those at lower elevations. They also demonstrated that there is a trend towards the aggregation of plants of the same sex within habitats, but that the distribution of the sexes within habitats was random, perhaps owing to nutrient homogeneity of the soils within habitats as well as the absence of antagonism between the sexes. Therefore, this scenario presents an interesting opportunity to investigate the nutritional content of plants along an elevational gradient in order to make inferences about the functional responses of sex along an environmental gradient.

Here, we examined dried leaf, stem, and root samples from male and female plants from three populations of *B. concinna* along an elevational gradient. Both $\delta^{13}C$ and $\delta^{15}N$ ratios were determined, aiming to assess C and N acquisition and water-use efficiency, and levels of C, N, C/N, P, Ca, K and Na were determined in order to assess the nutritional content of the samples analyzed. We hypothesize a strong sex effect, with female plants exhibiting a greater accumulation of nutrients, higher C and N acquisition and higher water-use efficiency than male plants. We also expect physiological differences along the elevational gradient because resource availability decreases with increasing elevation.

Materials and methods

Species, population sites and sample collection

The dioecious shrub *Baccharis concinna* G.M. Barroso (Asteraceae) is narrowly distributed in the Espinhaço Mountain Range in southeastern Brazil (Barroso 1976). The plant is an ericoid shrub reaching between 0.5 and 1.7 meters in height, and is endemic to the rupestrian grasslands of Serra do Cipó and Diamantina. Despite its local rarity, it supports one of the richest fauna of galling insects in rupestrian grasslands (Fernandes *et al*. 1996; 2014a; Marques & Fernandes 2016).

Rupestrian grasslands vegetation is a physiognomic formation of the Cerrado biome known for its high daily variation in temperature and humidity, and intense solar irradiance (Fernandes *et al*. 2014b; Fernandes 2016a; Silveira *et al*. 2016). The soils are shallow, sandy, rocky (quartzite-derived) and with variable water retention capacity and organic matter content (Negreiros *et al*. 2009; Oliveira *et al*. 2015). In tropical mountains, soil nutrient concentration generally decreases with increasing elevation (Harrison *et al*. 1991; Morecroft *et al*. 1992). Aluminum concentration also exhibits the same negative relationship with elevation (Marques *et al*. 2002), and has an important influence on nitrogen availability in the soil (Goodland & Pollard 1972). The climate of Serra do Cipó has four distinct seasons: a rainy season from November to January, a post-rainy season from February to April, a dry season from May to September, and a post-dry transition in October. Mean annual precipitation is around 1350 mm, low temperatures can be below 8 °C in the winter while high temperatures may rise above 35 °C in the summer (Madeira & Fernandes 1999)

We studied three large patches (hereafter assumed to be distinct populations) of *B. concinna* at different elevations. The first population was located at km 101 of highway MG-010 at 900 m a.s.l. (19°18'S, 43°36'W), hereafter named *Population 101*. This population is in an area undergoing natural regeneration and contains numerous other shrub, grass and tree species. The second population was located at km 107 of highway MG-010 on privately owned property (Reserva Vellozia) with a relatively flat slope. This population is in a degraded area of rocky outcrop terrain at 1,150 m a.s.l. (19°17'S, 43°35'W) that is undergoing natural regeneration; hereafter named *Population 107*. The third population was in an eroded area outside Parque Nacional da Serra do Cipó at 1,500 m a.s.l. (km 123 highway MG-010, 19°14'S, 43°30'W); hereafter named *Population 123*. At each population, we randomly selected five male and five female individual plants and removed them in their entirety from the soil, taking care to completely remove the root system. Because $\delta^{13}C$ signatures may vary considerably between individuals in shaded and non-shaded habitats (see Damesin *et al*. 1997; Pate & Arthur 1998), samples were taken only from individuals exposed to full sunlight. Plants were then placed into plastic bags, deposited in an icebox and taken to the laboratory where they were separated into roots, stems, and leaves, and dried in an oven at 50 °C for 72 h. The plants were then powdered in a mortar for analyses.

Nutrient analyses

To determine the concentration of sodium, calcium, and potassium in the tissues of *B. concinna*, 50 mg of dry powdered plant material was extracted with 5 ml distilled water for 30 min and centrifuged at 4000 x for 10 min. The supernatant was used to measure ion content by flame photometry (Eppendorf, Hamburg, Germany). To determine total phosphate content, 50 mg of dry powdered plant material was digested by boiling five times with 1 ml H_2SO_4 (95 %), HCl (70 %) and 3 droplets of H_2O_2 (30 %). Phosphate was determined colorimetrically according to Strickland & Parsons (1965).

Stable isotope analyses

Isotope ratio mass spectrometry (IRMS) was used to determine $\delta^{15}N$, $\delta^{13}C$, nitrogen and carbon content. Aliquots of dried plant material were weighed, placed in tin capsules (CE instruments, Milan, Italy) and injected into an elemental analyzer (EA) NC 2500 (CE instruments, Milan, Italy). Samples were combusted and oxidized at 1000 °C in an oxidation oven (HE 46820999 Hekatech, Wegberg, Germany). From the resulting gases (H_2O, CO_2, N_2, NO_x), nitrogen-oxides were reduced at 750 °C in a copper reduction oven (HE 46820899 Hekatech, Wegberg, Germany) to final N_2. H_2O was fixed with magnesium perchlorate. N_2 and CO_2 in the helium carrier gas flow were separated in a gas chromatographic column. Flow was split and N_2, which was diluted first from the column, was conducted without dilution into the Con Flow II interface (Finnigan MAT GmbH, Bremen, Germany), which connected the EA with the Delta^{plus} isotope ratio mass spectrometer (Finnigan MAT GmbH, Bremen, Germany). CO_2 was diluted with helium when passing through the interface. In IRMS, N_2 molecules (mass 28: 14N-14N, mass 29: 14N-15N, and mass 30: 15N-15N) and CO_2 molecules (mass 44: $^{12}C\text{-}^{16}O_2$, mass 45: $^{13}C\text{-}^{16}O_2$, $^{12}C\text{-}^{17}O\text{-}^{16}O$, $^{12}C\text{-}^{16}O\text{-}^{17}O$, and mass 46: $^{12}C\text{-}^{18}O\text{-}^{16}O$, $^{12}C\text{-}^{16}O\text{-}^{18}O$, $^{13}C\text{-}^{17}O\text{-}^{16}O$, $^{13}C\text{-}^{16}O\text{-}^{17}O$, $^{12}C\text{-}^{17}O_2$, $^{14}C\text{-}^{16}O_2$) were fractionated and detected by mass. Results were corrected with standard L-glutamate samples, calibrated against Pee Dee Be (CO_2) and N_2 in air (N_2), which were measured. The d^{13}C values were calculated as:

$$\delta^{13}C\ (‰) = (R_{Sample}/R_{PDB} - 1) \times 1000$$

where R_{Sample} and R_{PDB} are $^{13}C/^{12}C$ ratios of the sample and the standard Pee Dee belemnite (PDB), respectively.

The $\delta^{15}N$ values were calculated as:

$$\delta^{15}N\ (‰) = (R_{Sample}/RN_{2\ \text{in air}} - 1) \times 1000$$

where R_{Sample} and $RN_{2\ \text{in air}}$ are $^{15}N/^{14}N$ ratios of the sample and N_2 in air, respectively.

Total N content and total C content of the samples were calculated as sum of the single isotopes and expressed as proportion (%) of sample mass. From the measurements of carbon isotope ratio ($\delta^{13}C$), ^{13}C discrimination ($\Delta^{13}C$) was calculated as follows:

$$\Delta = (\delta_a - \delta_p)/(1000 + \delta_p) \times 1000(‰)$$

where δ_a and δ_p (in ‰) are carbon isotope ratios of the ambient air (assumed here to be - 8 ‰) and dried plant material, respectively (Farquhar *et al*. 1989a; b; Broadmeadow *et al*. 1992).

Statistical analyses

For statistical analyses of ion, nutrient, and isotope concentrations in tissues we used two-way analyses of variance, which allowed us to compare the effects of the independent factors of plant sex and population, plus their interaction. Analyses were done separately for each plant organ. When significant differences were detected we performed post-hoc tests using the Tukey HSD test (Zar 1996). For all analyses, we assessed the statistical assumptions and considered an α value of ≤ 0.05 as significant.

Results

Tables 1, 2 and 3 show the effects of the variables plant sex and population, and their interaction, for all measured parameters in leaf, stem and root tissue, respectively. The subsequent figures illustrate the differences found whenever there were significant effects of plant sex, population, or their interaction, on sex differences within populations.

The effect of plant sex

The effect of plant sex was restricted to the leaves of *B. concinna*, and only for %C (Tab. 1), with the average value for males of all three populations being higher than the average for female plants (male = 47.4±4.1, female = 42.9±5.4, Fig. 1). No other significant difference was observed within populations. All the other parameters from stable isotope analysis ($\delta^{13}C$, $\Delta^{13}C$, $\delta^{15}N$ and C/N), as well as tissue concentration of P, K^+, Na^+ and Ca^{++}, varied independently of plant sex.

The effect of population, and its interactions with plant sex

The variable population had a much clearer effect on a variety of parameters and appeared to be an important factor to the physiological behavior of *B. concinna*. Among the parameters of the isotope analysis, $\delta^{13}C$-values exhibited the clearest effect of population, which was observed across all studied organs (Tabs. 1- 3). *Population 123* clearly possessed a

Table 1. Summary of the two-way ANOVA of foliar tissue parameters from C and N stable isotope analysis (%C, %N, C/N, $\delta^{13}C$, $\Delta^{13}C$ and $\delta^{15}N$) and from concentrations of P and the ions K^+, Na^+, Ca^{++}, where the effect of plant sex, population and their interaction are given. Values in bold are statistically significant.

Main Effect	Parameter	Mean Sqr. Effect	Mean. Sqr. Error	*F*	*p*
plant sex	**%C**	**157.5**	**157.45**	**9.195**	**0.006**
df (2,24)	%N	0.0624	0.06238	1.557	0.224
	C/N	33.0	32.7	0.196	0.662
	$\delta^{13}C$	1.028	1.028	1.694	0.205
	$\Delta^{13}C$	1.083	1.083	1.604	0.217
	$\delta^{15}N$	7.48	7.482	0.719	0.405
	P	22359	22359	0.302	0.588
	K^+	6729	6729	3.082	0.092
	Na^+	0.010	0.012	0.002	0.988
	Ca^{++}	128.5	128.55	1.399	0.248
population site	**%C**	**205.1**	**102.57**	**5.990**	**0.008**
df (2,24)	%N	0.2532	0.1266	3.159	0.060
	C/N	**1707**	**854**	**5.112**	**0.014**
	$\delta^{13}C$	**25.154**	**12.577**	**20.723**	**<0.001**
	$\Delta^{13}C$	28.061	14.030	20.781	**<0.001**
	$\delta^{15}N$	8.75	4.376	0.420	0.662
	P	**1300235**	**650117**	**8.770**	**0.001**
	K^+	1797	899	0.412	0.667
	Na^+	311.53	155.76	3.109	0.063
	Ca^{++}	298.5	149.23	1.624	0.218
Interaction	%C	20.8	10.39	0.607	0.553
plant sex vs.	%N	0.2291	0.1146	2.859	0.077
population site	C/N	1125	562.6	3.369	0.051
df (2,24)	$\delta^{13}C$	0.445	0.223	0.367	0.697
	$\Delta^{13}C$	0.486	0.243	0.360	0.701
	$\delta^{15}N$	2.36	1.178	0.113	0.893
	P	391814	195907	2.643	0.092
	K^+	**24059**	**12030**	**5.510**	**0.011**
	Na^+	8.38	4.188	0.0836	0.920
	Ca^{++}	464.8	232.40	2.530	0.101

less negative isotope ratio (in foliar, stem and root tissue) while *Population 101* and *Population 107* had either more negative or intermediately negative values (stems and roots) (Fig. 2). A similar pattern was also observed with ^{13}C discrimination ($\Delta^{13}C$) in leaf and stem tissue (Tabs. 1, 2; Figs. 2, 3).

The %C and C/N ratio experienced an effect from the variable population only in foliar tissue (Tab. 1 and Fig. 1). The population average %C was similar in *Population 101* and *Population 123*, while it was lower in *Population 107* than *Population 123* at higher elevations. The C/N ratio was also lower in *Population 107*, but higher in *Population 123* and *Population 101*, which did not differ significantly.

With regard to P concentration (Fig. 4), the effect of population was found in foliar and root tissues, with the overall plant average of *Population 107* having the highest values. For root tissue, *Population 123* had the lowest average value. *Population 101* had intermediate values, being similar to *Population 123* with regard to leaves, and similar to *Population 107* with regard to roots.

Among the ions analyzed, the effect of population on Ca^{++} concentration was evident in root and stem tissue, with lower values for *Population 123* at the highest elevation (Fig. 5), while effects on K^+ concentration were restricted to stem tissue (Fig. 6), where a decreasing gradient was found with increasing elevation from *Population 101* to *Population 123*.

Significant interaction between plant sex and population was rare among the parameters studied, and restricted to P concentration in stem and root tissue (Fig. 4), and K^+ concentration in leaf tissue (Fig. 6). Stem P concentration in female plants was significantly higher than that in male plants in *Population 101*. However, the inverse pattern was observed in *Population 107*, and no difference was observed in *Population 123*. In roots, significant differences between sexes regarding P concentration exhibited an inverse pattern to that of stems: female plants had lower P-values in *Population 101* and higher values in *Population 107*, while *Population 123* exhibited no difference.

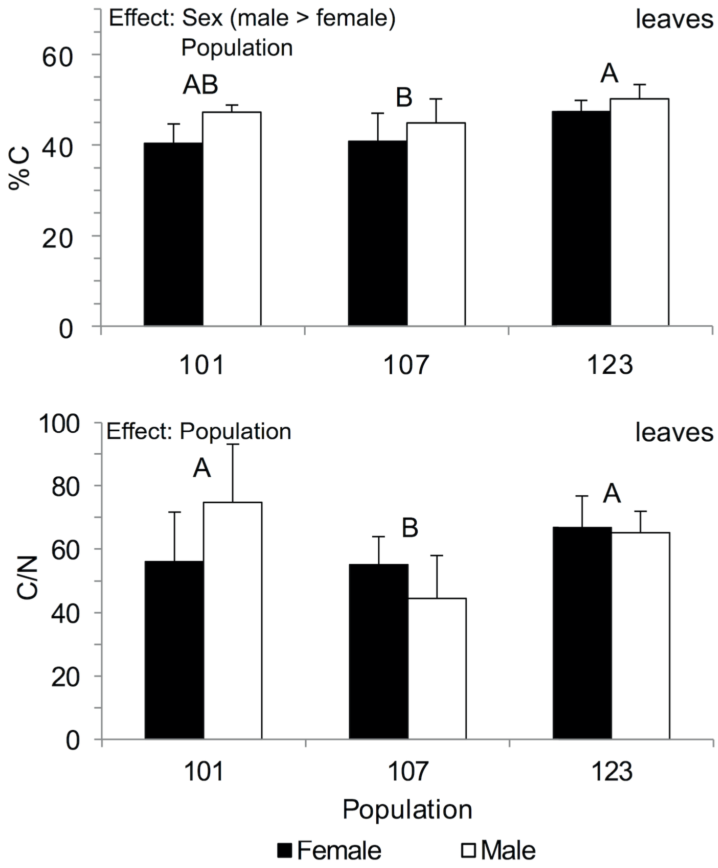

Figure 1. % C and C/N ratio of leaves of male and female plants of *B. concinna* from three different populations. Different capital letters denote significant differences among population means ($p<0.05$, Tukey HSD).

Lastly, K^+ concentration only differed between the sexes in leaves of *Population 101*, where male plants had greater values than female plants. The other two populations did not exhibit any clear differences. Overall, the patterns observed for P and K^+ concentrations regarding sex were irregular, with no absolute sex effect being observed in all cases.

Discussion

Male and female plants of dioecious species may differ in traits related to plant phenology and defense against herbivory, as well as in physiological traits such as photosynthetic performance and water use (Dawson & Geber 1999; Rowland & Johnson 2001; Cornelissen & Stiling 2005; Vega-Fruits *et al*. 2013). Male and female plants of many species have adapted to different ecological niches (Shine 1989; Obeso 2002; Vega-Fruits *et al*. 2013), and many authors have argued that female individuals are more common in habitats of better quality (e.g., Freeman *et al*. 1976; Cox 1981; Wef & Berg 1995; see review by Vega-Fruits *et al*. 2013). Marques *et al*. (2002) corroborated the hypothesis of sex segregation between habitats in an investigation of *B. concinna*, by finding a greater proportion of males at higher elevations and greater proportion of females at lower elevations. In the present study, we found minor differences in the physiological behavior of male and female plants of *B. concinna*, and argue that the unbalanced distribution of the sexes could have been influenced by soil quality, since the dominance of female plants at lower elevations coincided with greater soil fertility, in terms of P/N and Ca/Al ratios, at lower elevations (Marques *et al*. 2002).

Plant communities of rupestrian grasslands exist on extremely nutrient-impoverished soils (Oliveira *et al*. 2015; Fernandes 2016a; b). These soils possess exceptionally low levels of P, which is an essential element for seed production (Fujita *et al*. 2014). Therefore, we anticipated to find pronounced differences in resource acquisition strategies between male and female plants. However, we found only minor differences in tissue nutrient accumulation between male and female plants, with the most significant differences occurring between populations at different elevations. Plants of *B. concinna* at higher elevations had the lowest nutrient concentrations, irrespective of their sex. At the study site, soil fertility decreased with elevation, and so individuals of *B. concinna* at higher elevations experienced

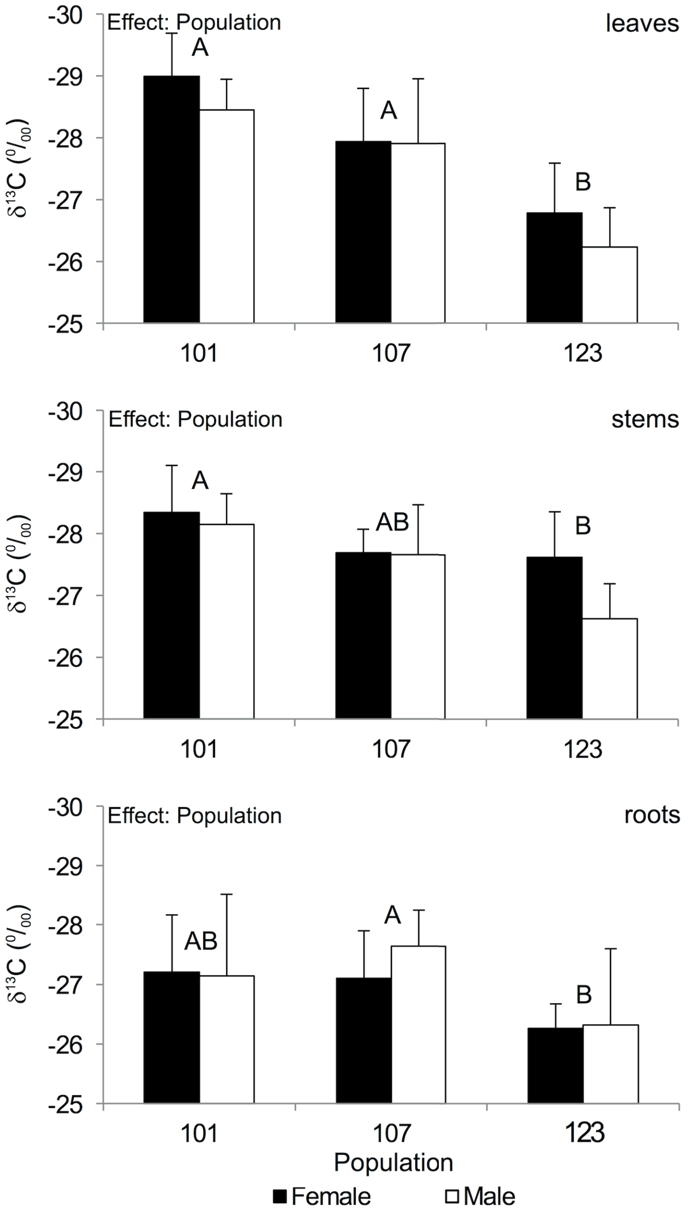

Figure 2. $\delta^{13}C$ values of leaves, stems and roots of male and female plants of *B. concinna* from three different populations. Different capital letters denote significant differences among population means ($p<0.05$, Tukey HSD).

the most extreme conditions of resource scarcity (Fernandes *et al*. 2007). Therefore, sexual reproduction in populations at the highest elevations can be severely constrained by resource limitation.

Values of $\Delta^{13}C$ are known to be correlated with long-term average stomatal opening (g_{H_2O}), and show a negative correlation with plant overall water-use-efficiency (WUE). Thus, this parameter is a good indicator of the overall WUE throughout the lifetime of a plant (Farquhar *et al*. 1989 a; b; Broadmeadow *et al*. 1992; Ehleringer *et al*. 1993; Guehl *et al*. 2004). This made it possible to infer that the population at the highest elevation had a higher WUE than other populations.

We had postulated that the differential occupation of habitats by male and female plants would result in different concentrations of ions in plant organs. Our study showed differences in performance among populations of *B. concinna* in traits related to water use efficiency ($\Delta^{13}C$) and nutrient acquisition (%C, C/N, P, $Ca^{++}$and K^{+}). Among them, a minor effect of sex was suggested by the interaction of sex and population with regard to P and K^{+} concentrations, although the patterns were not enough to establish a clear difference in behavior between male and female plants. Foliar %C, however, was always higher in male plants independent of intra-population variation. This result could indicate that male plants of *B. concinna* are more efficient at fixing carbon than female plants, although the latter are more

Table 2. Summary of the two-way ANOVA of stem tissue parameters from C and N stable isotope analysis (%C, %N, C/N, $\delta^{13}C$, $\Delta^{13}C$ and $\delta^{15}N$) and from concentrations of P and the ions K^+, Na^+, Ca^{++}, where the effect of plant sex, population and their interaction are given. Values in bold are statistically significant.

Main Effect	Parameter	Mean Sqr. Effect	Mean. Sqr. Error	*F*	*p*
plant sex	%C	34.4	34.3	0.525	0.476
df (1,24)	%N	0.007550	0.007553	1.438	0.242
	C/N	43	43	0.018	0.895
	$\delta^{13}C$	1.245	1.2448	3.013	0.095
	$\Delta^{13}C$	1.408	1.408	3.118	0.090
	$\delta^{15}N$	18.9	18.94	0.181	0.675
	P	340	340	0.023	0.881
	K^+	25	25	0.042	0.840
	Na^+	17.8	17.787	0.707	0.409
	Ca^{++}	0.94	0.94	0.090	0.767
population site	%C	152.0	75.99	1.160	0.331
df (2,24)	%N	0.0114	0.0057	1.087	0.353
	C/N	6334	3167	1.319	0.286
	$\delta^{13}C$	**6.254**	**3.1270**	**7.569**	**0.003**
	$\Delta^{13}C$	**6.614**	**3.307**	**7.322**	**0.003**
	$\delta^{15}N$	350.6	175.29	1.672	0.209
	P	59030	29515	1.993	0.158
	K^+	**10051**	**5025**	**8.258**	**0.002**
	Na^+	33.5	16.729	0.665	0.523
	Ca^{++}	**189.51**	**94.76**	**9.065**	**0.001**
Interaction	%C	33.3	16.6	0.254	0.778
plant sex vs.	%N	0.01131	0.006	1.076	0.357
population site	C/N	7831	3916	1.631	0.217
df (2,24)	$\delta^{13}C$	1.333	0.666	1.613	0.220
	$\Delta^{13}C$	1.421	0.710	1.573	0.228
	$\delta^{15}N$	70.4	35.2	0.336	0.718
	P	**555902**	**277951**	**18.766**	**<0.001**
	K^+	2731	1365	2.243	0.128
	Na^+	2.8	1.396	0.056	0.946
	Ca^{++}	1.03	0.52	0.049	0.952

Table 3. Summary of the two-way ANOVA of root tissue parameters from C and N stable isotope analysis (%C, %N, C/N, $\delta^{13}C$, $\Delta^{13}C$ and $\delta^{15}N$) and from concentrations of P and the ions K^+, Na^+, Ca^{++}, where the effect of plant sex, population and their interaction are given. Values in bold are statistically significant.

Main Effect	Parameter	Mean Sqr. Effect	Mean. Sqr. Error	*F*	*p*
plant sex	%C	9.00	8.63	0.050	0.824
df (1,24)	%N	0.0083	0.0083	0.626	0.437
	C/N	670	670	0.068	0.797
	$\delta^{13}C$	0.245	0.245	0.262	0.613
	$\Delta^{13}C$	0.225	0.225	0.217	0.646
	$\delta^{15}N$	8.10	8.06	0.309	0.584
	P	0.0831	0.0831	1.630	0.214
	K^+	143.0	143.4	0.156	0.696
	Na^+	23.9	23.9	1.100	0.305
	Ca^{++}	3.07	3.07	0.510	0.482
population site	%C	226	113.19	0.661	0.525
df (2,24)	%N	0.0281	0.0140	1.061	0.362
	C/N	25538	12769	1.294	0.293
	$\delta^{13}C$	**6.612**	**3.306**	**3.534**	**0.045**
	$\Delta^{13}C$	7.001	3.500	3.365	0.051

Table 3. Cont.

Main Effect	Parameter	Mean Sqr. Effect	Mean. Sqr. Error	*F*	*p*
	$\delta^{15}N$	35.4	17.711	0.678	0.517
	P	**0.7850**	**0.3925**	**7.696**	**0.003**
	K^+	1341	670.6	0.731	0.492
	Na^+	34.1	17.1	0.784	0.468
	Ca^{++}	**122.49**	**61.24**	**10.162**	**0.001**
Interaction plant sex vs. population site df (2,24)	%C	201	101	0.588	0.563
	%N	0.0262	0.0131	0.990	0.386
	C/N	11013	5507	0.558	0.580
	$\delta^{13}C$	0.505	0.253	0.270	0.766
	$\Delta^{13}C$	0.645	0.322	0.310	0.736
	$\delta^{15}N$	17.70	8.86	0.339	0.716
	P	**1212652**	**606326**	**11.889**	**<0.001**
	K^+	1136	568	0.619	0.547
	Na^+	18.3	9.2	0.421	0.661
	Ca^{++}	4.24	2.12	0.352	0.707

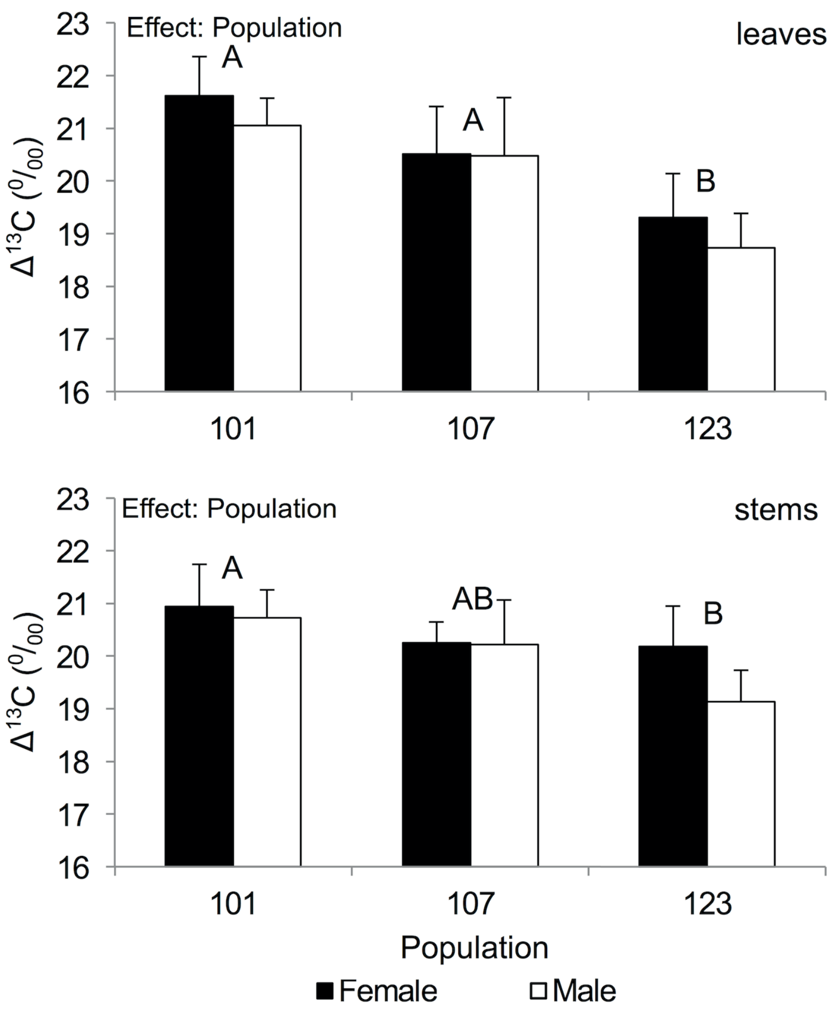

Figure 3. $\delta^{13}C$ values of stems and roots of male and female plants of *B. concinna* from three different populations. Different capital letters denote significant differences among population means ($p<0.05$, Tukey HSD).

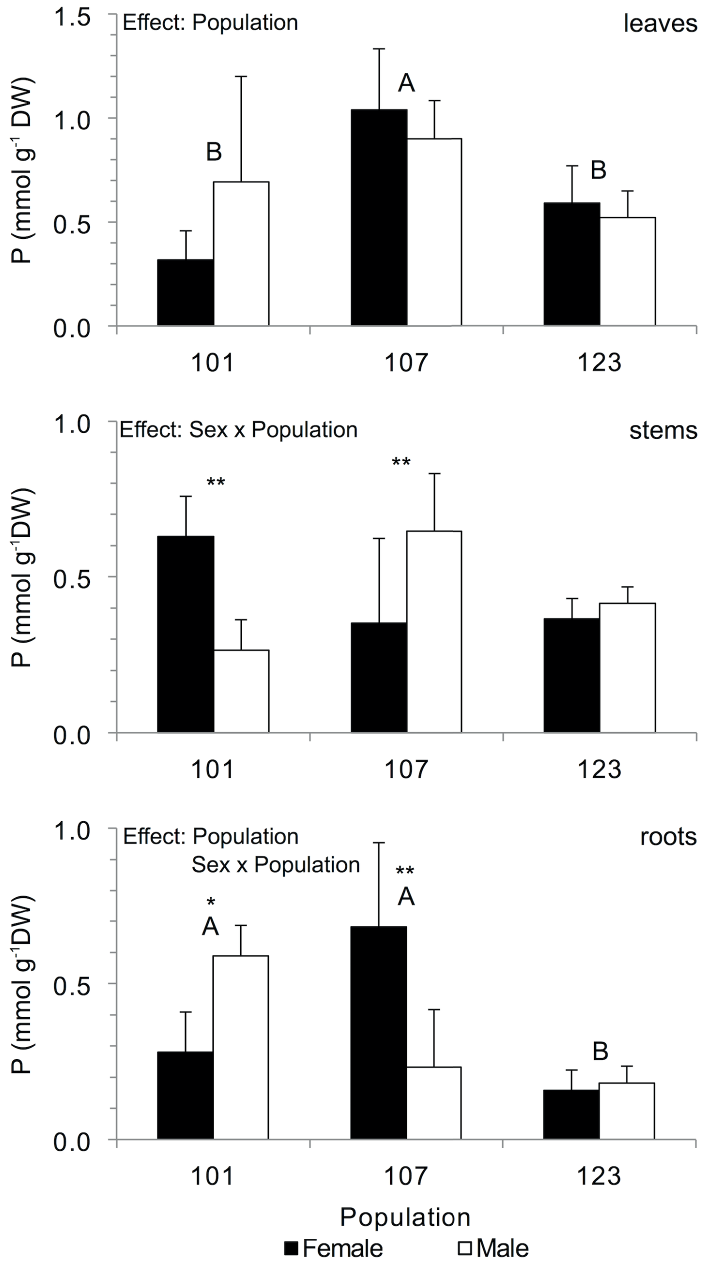

Figure 4. Phosphorous concentration in leaves, stems and roots of male and female plants of *B. concinna* from three different populations. Different letters denote significant differences among population means ($p<0.05$, Tukey HSD); ** indicates significant differences between sexes ($p<0.01$, Tukey HSD).

abundant at more nutritional sites (Marques *et al*. 2002). Nevertheless, this difference was very subtle since no significant differences were found within populations, and only in pooled averages from all sites. Zhao *et al*. (2012) showed that adult and fully expanded leaves of male plants of the riparian species *Populus cathayana* exhibited improved photosynthesis-dependent traits related to source and sink differences compared to female plants. Conversely, Liebig *et al*. (2001) found that sex expresion has no important general consequences for the physiology of the photosynthetic light reactions of *Clusia hilariana,* a dioecious and obligate CAM plant that grows on oligotrophic sandy coastal planes (*restingas*) in Brazil

In conclusion, our study found minor differences in tissue nutrient content between male and female plants of *Baccharis concinna*, although a consistent decrease in nutrient content was detected with increasing elevation. It remains unclear whether the higher %C in males of *B. concinna* are a result of resource partitioning between male and female plants, and studies should be conducted to

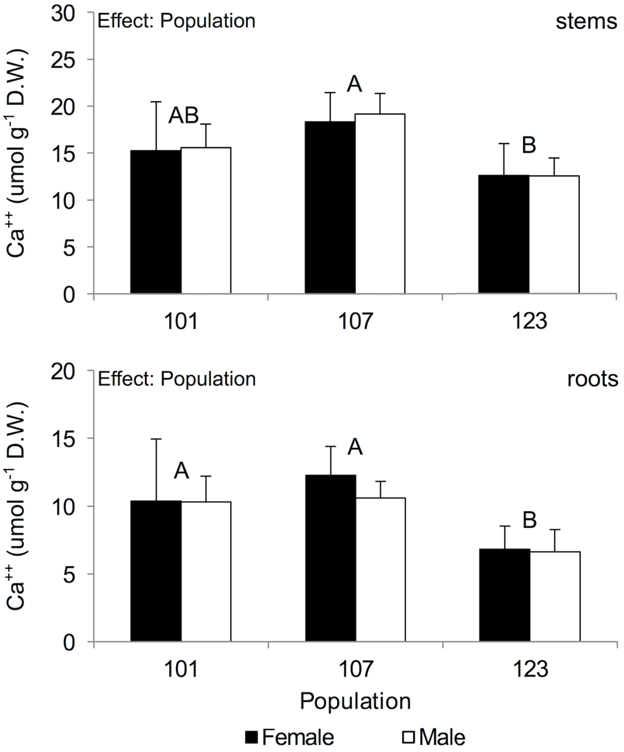

Figure 5. Ca^{++} concentration in stems and roots of male and female plants of *B. concinna* from three different populations. Different letters denote significant differences among population means ($p<0.05$, Tukey HSD).

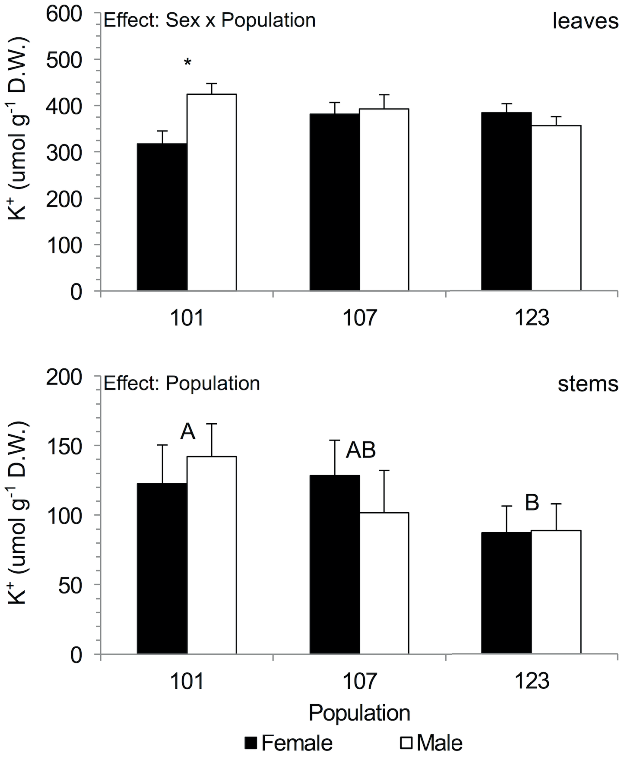

Figure 6. K^{+} concentration in leaves and stems of male and female plants of *B. concinna* from three different populations. Different letters denote significant differences among population means ($p<0.05$, Tukey HSD); * indicates significant differences between sexes ($p<0.05$, Tukey HSD)

attempt to explain this finding. Such studies will serve to advance our understanding of the ecology of dioecious plants, and improve our ability to predict the response of such plants to changing climate conditions in mountains.

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