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Permalink

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Journal

Oecologia, 175(3)

ISSN

0029-8549

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Publication Date

2014-07-01

DOI

10.1007/s00442-014-2947-1

Peer reviewed

Heavy browsing affects the hydraulic capacity of *Ceanothus rigidus* (Rhamnaceae)

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Received: 24 July 2013 / Accepted: 15 April 2014 / Published online: 10 May 2014
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Abstract Defoliation by herbivores can reduce carbon assimilation, change plant water relations, and even shift the biotic structure of plant communities. In this study, we took advantage of a long-term deer exclosure experiment to examine the consequences of persistent deer herbivory on plant water relations and the xylem structure–function relationships in *Ceanothus rigidus*, a maritime chaparral shrub in coastal California. Browsed plants had thicker stems with many intertwined short distal twigs, and significantly higher sapwood-to-leaf area ratios than their non-browsed counterparts. Leaf area-specific hydraulic conductivity was similar in both browsed and non-browsed plants, but xylem area-specific conductivity was significantly lower in the browsed plants. Vessel diameters were equivalent in both plant groups, but the number of vessels on a transverse area basis was nearly 40 % lower in the browsed plants, accounting for their

lower transport efficiency. Mid-day in situ water potentials and losses of hydraulic conductivity due to embolism were similar in both groups of plants but stomatal conductance was higher in the browsed shrubs in the early part of the growing season. We discuss our findings in the context of whole-plant ecophysiology, and explore the consequences of herbivory on hormonal signals, wood anatomy, and xylem function.

Keywords Embolism · Xylem anatomy · Stomatal conductance · Herbivory · Water transport

Introduction

Herbivory can affect plants at all life stages, potentially influencing other biotic interactions that may shape community structure and vegetation heterogeneity at many scales. For example, herbivores are often the intermediaries that affect how vegetation recovers from disturbances such as fire, and herbivores may shape ecosystem structure in ways that deviate from simple climate-based predictions (Minnich 1982; Tyler 1995; 1996; Bond 2005; Waldram et al. 2008). Fossil evidence indicates that herbivores were present throughout deep time (Wilf et al. 2001), so plants would have needed to adapt to defoliation by insects and vertebrates as soon as they appeared on land. As such, both browsers and grazers may have spurred the evolution of traits that minimize the effects of herbivory; such traits include signaling by the release of secondary compounds (Wu and Baldwin 2009), production and up-regulation of defensive chemicals (Endara and Coley 2011), increased modularity of the shoot (Haukioja et al. 1991), and the evolution of basal meristems and faster growth rates in grasses (Stowe et al. 2000; Turley et al. 2013). Altogether, there is good evidence that herbivores have had profound effects on

Communicated by Susanne Schwinning.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-014-2947-1) contains supplementary material, which is available to authorized users.

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the structure and function of land plants (Fox 1981; Strauss and Irwin 2004; Wise and Rausher 2013).

Consumers may impact any or all portions of a plant, shifting resource allocation away from storage, new growth, and reproductive structures to increased physical and chemical defenses and possibly compensatory responses. Compensatory responses appear to be temporary, but examples include increased growth of new stems or leaves as well as higher rates of photosynthesis in newly developed foliage (Thomson et al. 2003; Johnston et al. 2007; Gálvez and Tyree 2009; Ayres et al. 2004 but see Belsky et al. 1993). Depending on the magnitude, persistence, and nature of the defoliation events, subsequent plant architecture may vary from appearing relatively unchanged, to being irreversibly altered by the presence of either copious new sprouts as in the case of willows (Johnston et al. 2007) or the production of stunted shoots for which re-growth is impossible due to persistent browsing pressure (Huntsinger 1996; Cooper et al. 2003; Knight et al. 2009; Ramirez et al. 2012). Indeed, deer often nibble away at juvenile foliage at the base of a tree throughout the growing season, giving rise to such stubby branches. Our study addresses whether such long-term herbivory affects water transport and xylem anatomy in *Ceanothus rigidus*, an evergreen shrub common to the maritime chaparral of central California.

Herbivory can shift plant physiology by altering root-to-shoot ratios, whereby greater root mass becomes available to support hydration and nutrient demands of the defoliated shoot. Consequently, during recovery, browsed plants often exhibit increased shoot water potentials, higher rates of stomatal conductance and photosynthesis and thus increased growth rates relative to individuals that have not been browsed (Thomson et al. 2003; Johnston et al. 2007; Gálvez and Tyree 2009; Ayres et al. 2004; Ramirez et al. 2012). Similarly, when over 50 % of foliage was removed due to simulated and observed herbivory in *Eucalyptus blakelyi* and *Pinus taeda*, respectively, sap flow was reduced in both species by up to 50 % due to reduced leaf-level demand for water (Cunningham et al. 2009; Pataki et al. 1998). Taken together, these studies suggest that hydraulic co-ordination between transpiration and xylem-mediated supply can be a dynamic process, responding rapidly to shifts in canopy demand.

Since the effects of long-term herbivory on xylem anatomy and hydraulic function have not been studied, we examined this in *Ceanothus rigidus* shrubs that were part of a long-term herbivore exclosure experiment in maritime chaparral along the central California coast. These shrubs were either protected from herbivores by fencing, or left unprotected and thus consumed, primarily by deer. Browsed *C. rigidus* individuals have thick, intertwined and clustered distal stems on which masses of short shoots form a deformed, topiary-like canopy (Fig. 1a–c, e, f). We

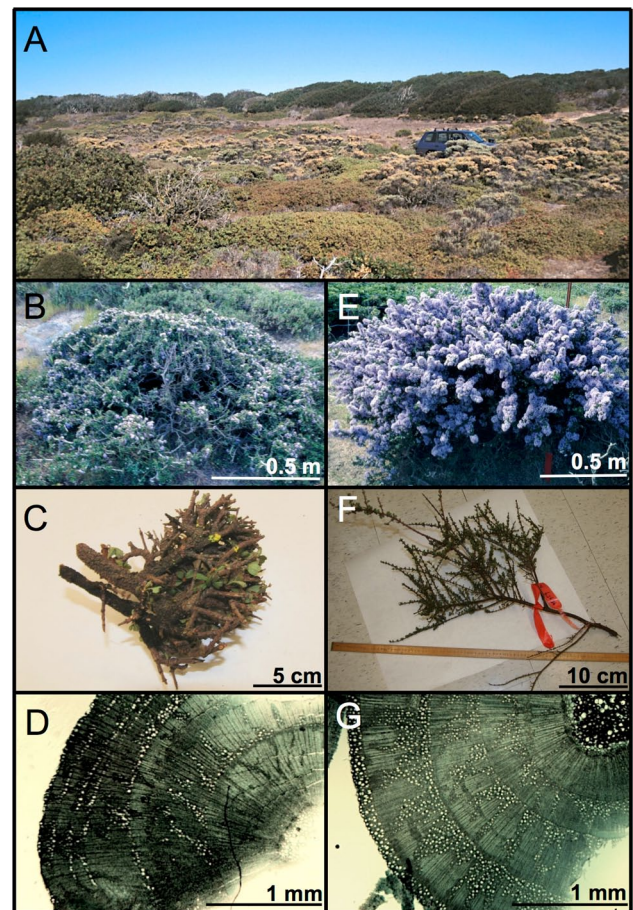


Fig. 1 The morphology and anatomy of browsed (**b–d**) and non-browsed (**e–g**) *Ceanothus rigidus*. The maritime chaparral vegetation growing on the sandy soil of Fort Ord Natural Reserve is pictured in **a**. Note the browsed *C. rigidus* shrubs in the foreground. **b**, **e** Browsed and non-browsed shrubs, respectively, during flowering. Browsed plants exhibit shorter, thicker stems with intertwined clumps of distal twigs (**c**) as compared to the non-browsed *C. rigidus* stems (**f**). Cross sections of browsed (**d**) and non-browsed (**g**) *C. rigidus* stems showing differences in vessel distribution as well as the thickness of the outer growth rings

compared the physiological performance of browsed and non-browsed *C. rigidus* individuals by characterizing the differences in shoot architecture, hydraulic performance, wood anatomy, and in situ measurements of water potential and stomatal conductance. We predicted that browsed plants would exhibit less mid-day water stress but expected no significant differences in xylem function because xylem-mediated supply of water scales with leaf-level demand (Santiago et al. 2004). However, the results show that continual browsing conspicuously alters xylem anatomy and hydraulic function, with no effect on leaf water potentials. Hence, we consider the effects of leaf removal on water transport by way of hormonal signals, and discuss the broader implications of herbivory on plant structure and overall water relations.

Methods

Site description and sample collection

Maritime chaparral is the dominant habitat on the Fort Ord Natural Reserve (FONR), a 242-ha reserve that is part of the University of California Natural Reserve System, and lies ~2 km inland from Monterey Bay at an elevation of 21–58 m. Fort Ord has a Mediterranean-type climate, with late autumn and winter rains and dry (though cool and foggy) summers. The soils are aeolian dunes, 10⁴–10⁵ years old (Dupre 1990), composed mainly of quartz sands with low levels of most nutrients at 0–10 cm depth: e.g., total $n = 0.084\%$; $p = 21.8$ ppm; and organic matter = 1.89 % (Fox et al. 1998). The site is at least 70 years post-fire. The manzanitas, *Arctostaphylos pumila* (Nutt.) and *A. tomentosa* (Pursh) Lindley ssp. *tomentosa*, are the dominant shrubs in the maritime chaparral on the reserve, while *Ceanothus rigidus* (Nutt.) Hoover and *Ericameria ericoides* (Less.) Jepson are distributed patchily amongst them. Several other shrubs or young trees occur much less commonly, particularly *Quercus agrifolia* Née, *Fragula* (syn. *Rhamnus*) *californica* (Eschsch.) and *Acmispon glaber* var. *glaber* (Vogel) (syn. *Lotus scoparius*). Only the *C. rigidus*, *Q. agrifolia* and *A. glaber* are browsed heavily.

Experimental design

The experiment began in August 1996, in two areas of FONR that are ~1 km apart, with the goal of assessing how shrub and habitat dynamics responded to deer and rabbit browsing, particularly on *C. rigidus*, since it is heavily browsed, sufficiently abundant and evenly distributed to allow for experimental treatments, and easily enclosed in a fence. The experiment had three treatments arranged in a randomized block design, and replicated four times in each area (24 plots total). Treatments were control plots with natural levels of browsing by deer and rabbits, and plots that excluded these herbivores. Each plot is 9 m² and initially contained at least one shrub of *C. rigidus*, *A. pumila* or *A. tomentosa*, and *E. ericoides*, and some “open space” between shrubs that is habitat for herbaceous annuals; other woody plants were in some plots as well. Plots were grouped by proximity into blocks, with treatments assigned randomly within each block. Deer were excluded by 2-m-high cages, with 20 cm × 10-cm mesh. Rabbits were excluded by 1.5-cm mesh on the lower 1 m of half of the deer exclosures. Because many *C. rigidus* shrubs in control plots died during this experiment, we randomly selected other individuals growing near the deer exclosures for the data in this paper. In addition, since rabbit effects were minimal, we consider only deer exclosures (no deer

browsing, but ± rabbits) and controls (natural browsing) for this paper.

Photosynthesis measurements

It was difficult to measure gas-exchange rates in situ with a standard portable gas-exchange instrument because the deformed twig architecture interfered with the use of the leaf cuvette, and because the space inside the fence enclosures was limited. Hence, we collected plant material in the field, and transported it back to the laboratory for gas-exchange and other measures (see below). Plant segments were collected in April and May 2011 under fog or low cloud with temperatures between 11–14 °C. Leafy stems ranging from 30–60 cm in length and 4–10 mm in diameter were clipped in the field, quickly re-cut 5–10 cm under water, trimmed with a razor blade, covered with a plastic bag, and transported in a water-filled container to the lab where they were left overnight to re-hydrate to water potentials between −0.1 to −0.5 MPa as measured by a Scholander-style pressure chamber (PMS Instruments, Corvallis, OR, USA). We rehydrated the plant material to standardize leaf water potential, and thus compare intrinsic rates of gas exchange. Wounding-induced changes in gas-exchange rates were not observed over the 24-h time frame during which the measurements were made. We assumed that plant material collected earlier in the growing season would be free of the confounding effects of drought-induced air entry into the xylem (embolism; Hacke et al. 2000; Jacobsen et al. 2007). However, Wheeler et al. (2013) recently showed that clipping plant segments in the field while the xylem is under tension may inflate embolism, an artefact of significant concern. Given that material was collected early in the growing season, at low temperature and under cloud cover, and then re-cut again, we suspect that these effects were minimal, although they cannot be ruled out.

Gas-exchange measurements were made the following day in an outdoor area at the UCSC greenhouses, under ambient conditions (12–15 °C, overcast) using a Li-Cor LI-6400 XT photosynthesis instrument (Li-Cor Biosciences Inc., Lincoln, NE). We used a 2 × 3-cm cuvette fit with a standard mounted external LED light source with the flow rate set to 400 ml min^{−1}, the CO₂ mixer at 400 μmol mol^{−1} with vapor pressure deficit ranging from 1.1 to 1.5 kPa. Leaf temperature was maintained at 20 °C and measurements were obtained at saturated light levels (2,000 μmol quanta m^{−2} s^{−1}, which were increased in increments of 200–500 every 5–7 min starting at ambient light (150–300 μmol m^{−2} s^{−1}). For each stem segment, the most accessible short shoots were carefully inserted inside the cuvette, taking care to seal any leaks with Poster Tack plasticine (Elmer's, Columbus, OH, USA). Following each

measurement, leaves were scanned (Epson 4200X Printer/Scanner, Epson Corp., USA) and the projected leaf area was measured using ImageJ software (Rasband 2012). These leaf areas were used to re-calculate the true photosynthetic rates per unit leaf area.

Hydraulic measurements

Hydraulic measurements were performed on the same plant material on which gas-exchange measurements were made in order to reduce the effect of foliage removal on other concurrent studies at FONR. Stems were re-cut under water, with the cut ends smoothed using a sharp razor blade. It is customary to use segments longer than the longest vessel, and we attempted to find the longest possible segments but could find no usable stems from the browsed plants that were longer than 110 mm. Consequently, we chose similarly sized segments from the non-browsed plants with lengths for both treatments ranging from 70 to 120 mm. Segment length effects on conductivity are negligible (Sperry et al. 2005) and the vast majority of *C. rigidus* vessels are less than 100 mm in length (Jacobsen et al. 2007, 2012).

Hydraulic conductivity was measured using the standard gravimetric method (Pratt et al. 2012; Hacke et al. 2000) whereby stems were mounted on a tubing apparatus connected to a four-digit balance, and the flow rate was measured using a 20-mM KCl solution. To increase precision, background flow rates were measured under zero pressure before and after each gravimetric pressure measurement, then averaged and subtracted from the gravimetric flow measurement (~6–8 kPa). Hydraulic conductivity (K) was computed as the flow rate for a given pressure, multiplied by stem length. Native K (K_{native}) was measured first, followed by an overnight degassing treatment to remove any air embolisms, after which K was re-measured (K_{max}). This was accomplished by submerging stems in filtered water (0.2 μm filter, Nanopure, Barnstead International) and then connecting the sealed vessel to a standard in-lab vacuum system, for 12 h. Our tests show that K_{max} is reached after 7 h with no changes to K with further degassing. The % loss of conductivity (PLC) was computed as $100 \times (1 - K_{\text{native}}/K_{\text{max}})$. K_{max} was further standardized for functional xylem area (K_s), which was identified by perfusing the distal portions of stems with basic fuchsin, and measured with ImageJ. Hand-made, transverse sections of the stems were mounted in glycerin, and the red-stained xylem tissue was photographed at 20 $\times$ with a digital compound microscope (Motic BA210, Richmond, BC, Canada). Since the plant material was re-hydrated overnight, we cannot rule out the possibility that some embolism repair may have occurred, thereby reducing native PLC. To standardize K_{max} by distal leaf area (K_{leaf}), the leaves were scanned, and the projected area was measured with ImageJ

software. The Huber ratio was computed by dividing the functional xylem area by the distal leaf area (Tyree and Zimmermann 2002).

Xylem anatomy and wood density

Wood density was measured according to the methods of Hacke et al. (2000) whereby 1 to 1.5-cm-long samples were cut from the xylem, taking care to remove the bark and pith. The fresh volume was obtained by immersing the samples in a water-filled vessel on a balance, and converting the displacement weight (g) to volume (cm^3) by dividing by 0.998 g cm^{-3} , which is the density of water at 20 °C. Each sample was immersed at least 5 $\times$ to generate an average displacement weight. The xylem dry weight was obtained after 3 days at 65 °C, and wood density was computed as the ratio of dry weight to fresh volume.

Conduit anatomy was determined on stems used for K measurements. Hand-sections were made on the middle portion of the stem segment, stained with toluidine blue and mounted in glycerol. Three radial sectors corresponding to the outer, transporting growth rings were photographed under 200 $\times$. Conduit size and density were determined by manually measuring conduit dimensions with ImageJ software, and by counting the number of conduits for a given xylem area, respectively. Growth rings were difficult to distinguish in the browsed plants and could not be reliably measured (Fig. 1d).

Mid-day water potential and stomatal conductance

Field measurements of mid-day water potential and stomatal conductance were first made in August 2011 when the plants would have experienced high levels of seasonal drought stress, and differences between the treatments would be most apparent. Whenever possible, we attempted to collect data on clear days but fog or low cloud cover were persistent at Fort Ord such that mid-day temperatures rarely peaked above 18 °C, with PAR levels typically less than $700 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Subsequently, in October 2013, several hot, cloud-free days with air temperatures above 32 °C afforded us the opportunity to collect additional late-season data. Stomatal conductance was obtained with a hand-held porometer (Decagon Devices, Pullman, WA, USA) taking care to cover the sensor completely by the leaf. The shoot from which the leaf was sampled was clipped, placed immediately in a plastic bag, and the water potential measured using the pressure chamber as described above. The 2011 measurements were made following a year of slightly above average rainfall (17.4 vs. 15.95 cm), while the 2013 measurements followed 2 years with <70 % of average rainfall.

Statistics

Multiple measurements on each individual shrub were averaged, such that each datum represents a single individual unless indicated otherwise. Our sample size varied from 6–9 individual browsed plants and 5–8 non-browsed (caged) plants. We used SAS 9.3 software (SAS Institute, Cary, NC, USA) for all control-treatment comparisons. We tested all variables for normality, using Proc Univariate on residuals from ANOVAs (Proc GLM), and transformed the variables as appropriate. The few variables that could not be normalized were analyzed using a two-sample Wilcoxon test in Proc Npar1way. Findings were considered statistically significant when $p < 0.05$. Regression analyses were performed using the R statistical environment (R Core Team 2012).

Results

The Huber ratio of sapwood to distal leaf area in browsed *C. rigidus* was 57 % greater than in the non-browsed shrubs (Fig. 2a; $p = 0.004$). Although the stems of browsed plants were generally thicker than those of non-browsed *C. rigidus* (Figs. 1c, f, 2b, $p = 0.03$), the functional xylem area was not statistically different ($p = 0.12$). Specific leaf areas (SLA) of the browsed plants were marginally (though not significantly) higher at $4.55 \pm 0.59 \text{ mm}^2 \text{ mg}^{-1}$ (mean $\pm$ SD) relative to the non-browsed shrubs ($4.05 \pm 0.70 \text{ mm}^2 \text{ mg}^{-1}$, Supplemental Figure 1; $p = 0.07$).

The leaf-specific conductivity was the same in the browsed and non-browsed shrubs averaging 3.93 ± 1.55 ($\times 10^{-4}$) $\text{kg m}^{-1} \text{ MPa}^{-1} \text{ s}^{-1}$ (Fig. 3a). By contrast, K_s in the browsed plants was 53 % lower than in protected plants (0.59 ± 0.25 vs. $1.11 \pm 0.48 \text{ kg m}^{-1} \text{ MPa}^{-1} \text{ s}^{-1}$, respectively; $p = 0.02$; Fig. 3b). Vessel size was equivalent in both treatments (17.70 ± 2.19 and $17.00 \pm 1.66 \mu\text{m}$, respectively) but the number of conduits per area, that is vessel density, was significantly lower in the browsed plants ($263.38 \pm 61.88 \text{ mm}^{-2}$) than in those that were fenced in ($409.77 \pm 61.07 \text{ mm}^{-2}$; $p = 0.01$; Fig. 4, Supplemental Figure 2). Figure 4 shows that vessel density explained 46 % of the variation in K_s with higher K_s in response to greater vessel number per area. Wood density was the same in browsed and non-browsed stems with values of 0.705 ± 0.021 and $0.719 \pm 0.045 \text{ g cm}^{-3}$, respectively.

Browsing had no measurable effect on xylem embolism, with mean values of the % loss of hydraulic conductivity in the non-browsed and browsed plants at 56.1 ± 12.4 and 46.5 ± 24.3 %, respectively ($p = 0.12$; Fig. 5). The native PLC values in the browsed plants were more variable than in the non-browsed shrubs, but interestingly, three-quarters of the sampled stems had PLC values below 55 %.

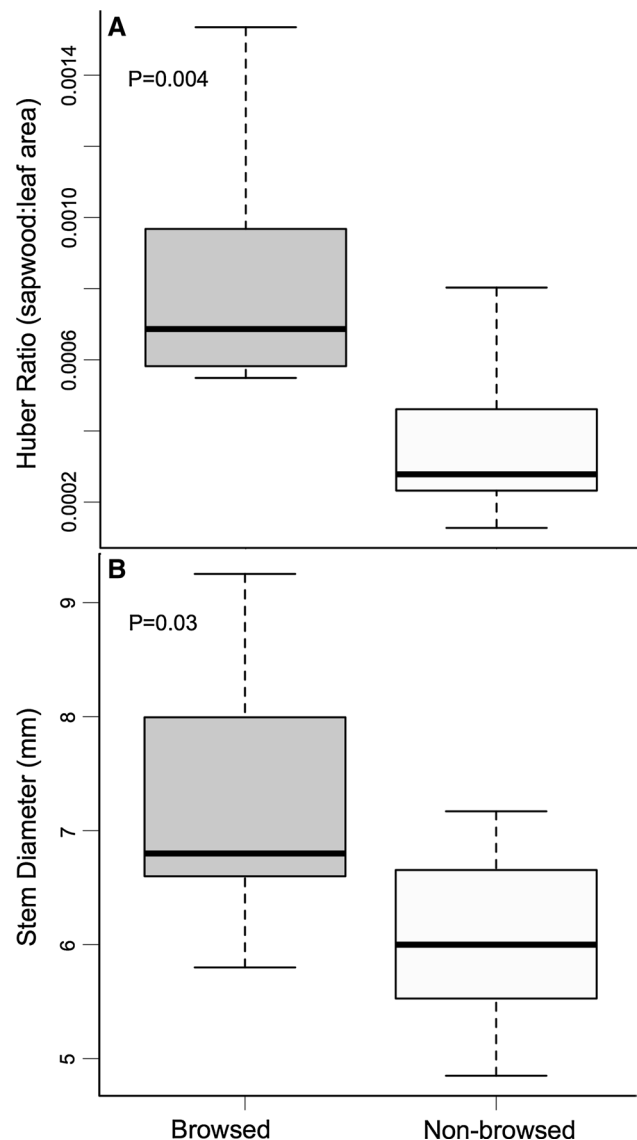


Fig. 2 Box-and-whisker plots of the sapwood-to-leaf area ratio (a) and stem diameter (b) in browsed and non-browsed *C. rigidus* stems. The box indicates the mid 50 % of the data distribution where the thickened line is the median. The whiskers represent the upper and lower quartiles

Mid-day water potentials were similar in the browsed and non-browsed plants during both August 2011 and October 2013 ($p = 0.49$; Fig. 6). It is notable that water potentials were 2–3-fold lower in October 2013 (as opposed to the data collected in the cooler, foggy days of August 2011) due to the prolonged drought, with several non-browsed plants dropping to less than -9 MPa (Fig. 6, Supplemental Figure 3). In the field, browsed plants had higher stomatal conductance ($130.08 \pm 42.83 \text{ mmol m}^{-2} \text{ s}^{-1}$) than non-browsed shrubs in August 2011 ($81.64 \pm 30.27 \text{ mmol m}^{-2} \text{ s}^{-1}$; Fig. 6; Supplemental Figure 3; $p = 0.012$) but these differences disappeared in October when water potentials

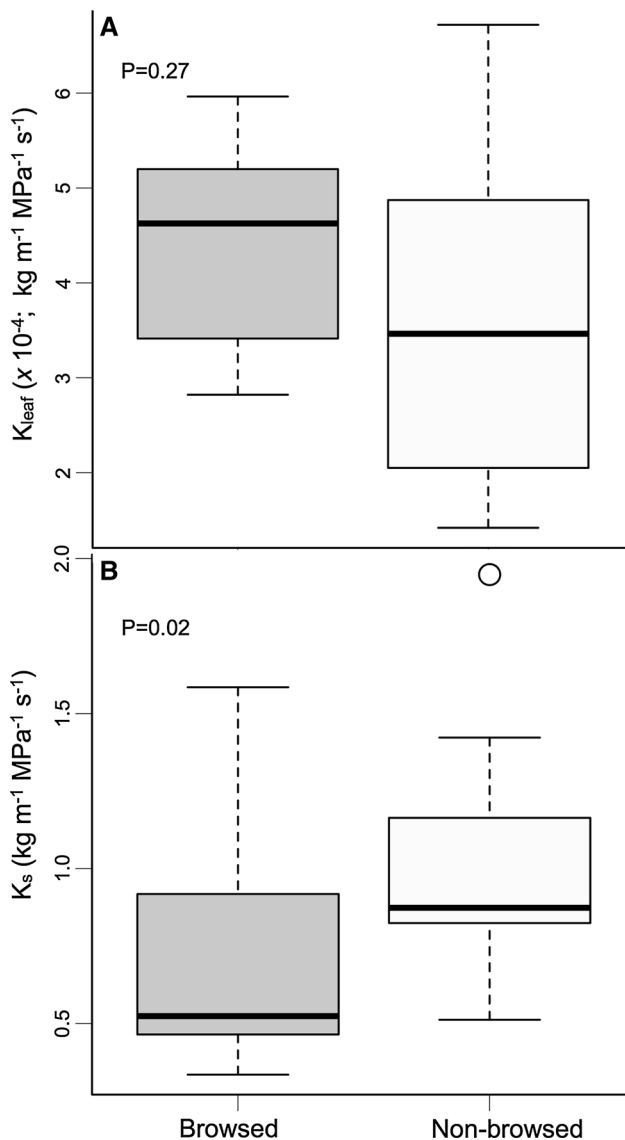


Fig. 3 The leaf-specific conductivity (a) and xylem-area specific conductivity (b) of stem segments belonging to browsed and non-browsed *C. rigidus*

in the majority of the sampled plants dropped to -6 MPa or lower ($p = 0.49$). Lastly, photosynthesis and stomatal conductance on re-hydrated samples were nearly identical in both plant groups, consistent with previous findings that leaf nitrogen levels (1.5 %) were not affected by browsing (Fox, unpublished data; Supplemental Figure 4).

Discussion

Plants have evolved a suite of defensive and compensatory strategies to minimize leaf loss to herbivores that may reduce plant growth rates and fitness and alter plant architecture over long periods of time (Fig. 1a–c; Fox 1981; Archibald

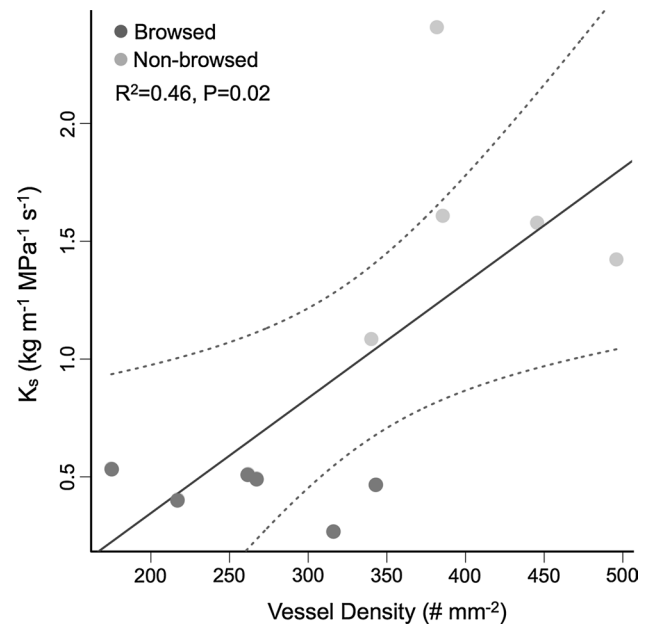


Fig. 4 The relationship between stem vessel density and xylem area-specific conductivity in browsed and non-browsed shrubs. Dotted lines represent 95 % confidence intervals

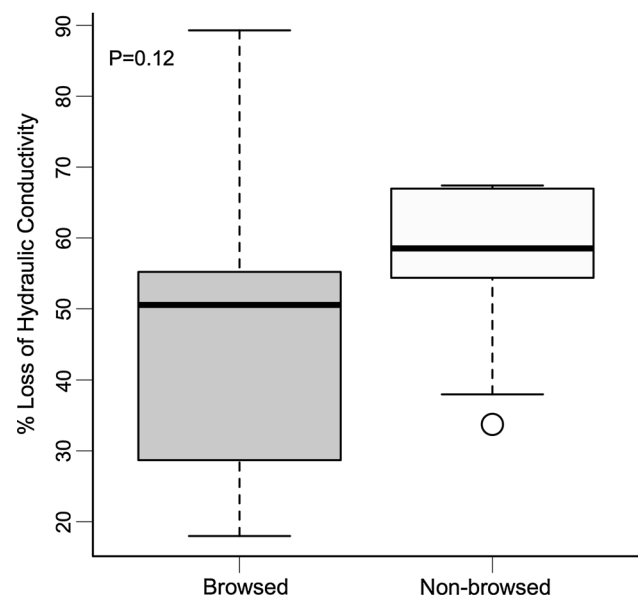


Fig. 5 Boxplots showing the native % loss of hydraulic conductivity in stems of browsed and non-browsed *C. rigidus*

and Bond 2003; Strauss and Irwin 2004; Moncrieff et al. 2011; Wise and Rausher 2013). Compensatory responses such as higher photosynthesis rates (Thomson et al. 2003; Ayres et al. 2004; Johnston et al. 2007) and higher sap flow may facilitate vegetation recovery (Pataki et al. 1998; Cunningham et al. 2009), but whether they persist over multiple growing seasons is unknown. We observed no difference in

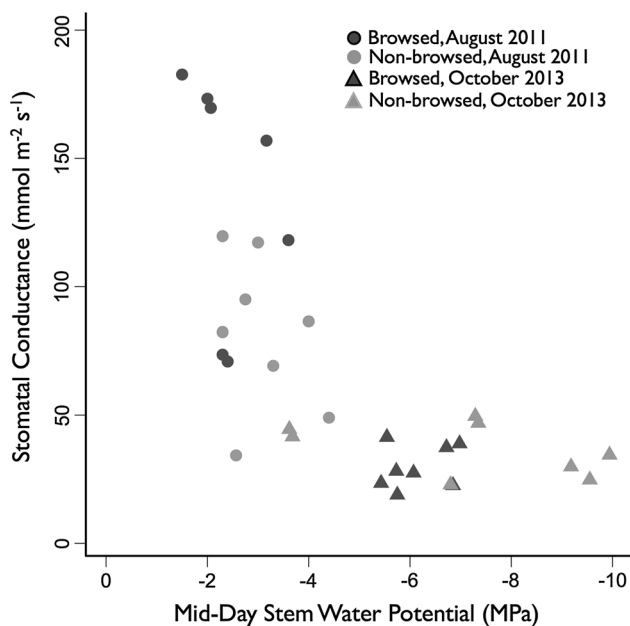


Fig. 6 The relationship between in situ mid-day water potential and leaf stomatal conductance in browsed and non-browsed *C. rigidus* plants in August 2011 and October 2013. Each point in the August 2011 data represents an average of at least two water potential and three to five conductance measures per plant. Three browsed and three non-browsed plants were sampled in October 2013 with each datum corresponding to an individual conductance and an associated water potential measurement. Each plant was sampled at least twice

photosynthesis rates between rehydrated browsed and non-browsed *C. rigidus* foliage, but in situ photosynthesis rates may have been greater in browsed *C. rigidus* during August 2011 when these plants had higher stomatal conductance than their protected counterparts. It may be that compensatory responses are nuanced, and more or less dependent on how the plants are sampled.

Chaparral shrubs evolved several post-fire recovery strategies including sprouting (Ramirez et al. 2012; Pratt et al. 2007), but as a non-sprouter, *C. rigidus* allocates few resources to the root crown and quiescent buds, and instead produces more copious quantities of seeds that lie dormant in seed banks (Keeley 1991; Deveny and Fox 2006). Over time at Fort Ord, unbrowsed *C. rigidus* produced $\sim 35\times$ more seeds than their browsed counterparts, though this varies markedly in different years (Deveny and Fox 2006; compare flower abundance in Fig. 1b, e); persistent herbivory weakens this important compensatory response to disturbance. Deer browsing significantly slows immediate post-fire re-growth in *Ceanothus* and other plants in the chaparral (Minnich 1982; Quinn 1992, 1994; Mills 1983, 1986; Davis 1967; Tyler 1995, 1996; Ramirez 2012), but the studies at Fort Ord are the first to show long-term impacts of browsing (at least 70 years post fire; Fox unpublished and Coale et al. 2011). Taken together, reduced *Ceanothus* biomass, as well

as delayed recovery after fires, may be attributed to fewer seeds as well as some persistent physiological constraints due to prolonged, intense browsing.

Our study addresses physiological adjustments that balance investment in xylem tissue with leaf-level water loss in continually browsed plants. In contrast to the plants protected from herbivores in our experiment, sapwood-to-leaf area ratios of browsed shrubs were over 50 % higher, so all else being equal, we expected similar or slightly higher leaf-specific conductivity in browsed than non-browsed plants. However, K_{leaf} is similar in both groups of shrubs, consistent with lower xylem-specific conductivity in the browsed plants. Further, the lower K_s can be attributed to a nearly 40 % reduction in vessel density, implying a dynamic connection between leaf area and xylem function. Although we could not reliably document growth ring width in stems on heavily browsed plants because growth was severely constricted, we did observe narrower, compressed and incompletely formed rings. These observations support previous work at the Fort Ord site showing poorly developed rings on main stems of heavily browsed *C. rigidus*, compared to plants sustaining less browsing in other parts of Fort Ord (Coale et al. 2011). Narrower rings in response to browsing also occur in other species (e.g., *Betula pubescens*, Speed et al. 2011).

How continual defoliation affected root-to-shoot ratios in *C. rigidus* is unknown, but one might expect some degree of scaling whereby these ratios are similar in the browsed and non-browsed plants, assuming that both groups are of similar age. Given that water potentials in browsed and non-browsed plants were not significantly different, it seems unlikely that root-to-shoot ratios varied between these two groups. Indeed, reduced root biomass has been reported in artificially defoliated seedlings of *Fagus* and *Abies* grown in vermiculite, suggesting that co-variation in above- and below-ground allocation may be a common response (Ayres et al. 2004). The below-ground response to herbivory in the Fort Ord system is much less tractable due to on-going research, as well as varied topography and patchy vegetation patterns, both of which could affect root distribution and depth.

Herbivory has important ramifications for plant hydraulic function under both natural and experimental conditions. The compensatory responses that favor the development of vigorous new shoots with increased rates of gas exchange, faster growth, and larger leaves (Ayres et al. 2004; Johnston et al. 2007; Gálvez and Tyree 2009; Turley et al. 2013) may be due to favorable root-to-shoot ratios that improve leaf water status (Pataki et al. 1998; Johnston et al. 2007; Gálvez and Tyree 2009). However, rapid stem growth combined with faster leaf production may eventually predispose the shoots to hydraulic failure due to a combination of younger, possibly weaker wood and a high

leaf-level demand for water (Gálvez and Tyree 2009). We did not observe significantly different degrees of embolism in our study, probably because many species of Rhamnaceae are highly drought resistant to begin with (Jacobsen et al. 2007).

This study presented us with unique challenges to evaluating hydraulic function that were particular to heavily browsed *C. rigidus* plants. The first such hurdle was to find stems that were sufficiently long and straight to fit either in the centrifuge (Alder et al. 1997) or the air-injection sleeve (PMS Instruments, Corvallis, OR, USA; Sperry and Saliendra 1994) for determining vulnerability to embolism. Since we could not locate appropriate material in the browsed plants, we only measured native percent loss of conductivity for both groups. Secondly, using such short segments can in theory inflate estimates of K due to removal of vessel endwalls, but our K_s values are on par, if not slightly lower than the K_s reported in longer stems segments of *Ceanothus* (e.g., in the closely related *C. cuneatus* in Pratt et al. 2007) and other members of the Rhamnaceae (Pratt et al. 2007; Jacobsen et al. 2007; see also Sperry et al. 2005). Further, our native embolism values (Fig. 5) may be higher than what we would expect in spring, probably because measurements of maximum conductivity did not exclude vessels produced in earlier years that are typically more vulnerable to embolism, and thus dysfunctional in the current year (Hacke et al. 2000; Pratt et al. 2007; Jacobsen et al. 2007). In retrospect, a better approach would have been to disable this population of weak conduits by axial pressurization of the stems at 0.25–0.5 MPa following degassing, and thus to consider the resulting, adjusted conductivity as the $K_{\max}$. Despite these challenges, we captured important trends and physiological responses that may shed light on future studies of the effects of herbivory on woody plants.

The key finding of our study is that persistent defoliation reduces stem water transport in a manner that scales with distal leaf area. Specifically, the continual removal of leaves and apices results in xylem with a lower vessel density. We suspect that the connection between leaf and xylem function in *C. rigidus* is mediated by cambial levels of indole-3-acetic acid (auxin), a hormone that is synthesized in new leaves and apical meristems. Auxin moves basipetally to the vascular cambium, where it stimulates mitosis in the cambial zone (Uggla et al. 1996) so reductions in auxin gradients limit cell division in the cambium. Lower auxin levels have been shown to reduce vessel densities in *Phaseolus* (Aloni and Zimmermann 1983), so persistent browsing in *C. rigidus* could have similar effects via auxin-mediated shifts in cambial activity. Given the higher fiber fraction and thicker stems in the browsed plants, it appears that fibers replaced the wood volume that would otherwise belong to vessels. Fiber differentiation responds to both auxin and gibberellin (Aloni 1987), thus browsing may have tipped the balance

of these two hormones. Furthermore, high concentrations of auxin are associated with suppressed lateral branch formation (Taiz and Zeiger 2010), so the potentially lower levels of auxin in browsed *C. rigidus* release the stems from apical dominance giving rise to the intertwined small twigs on the distal portions of the main stems (Fig. 1c). We might expect normal xylem formation in more sporadically browsed plants with the opportunity to re-grow.

Few studies have explicitly tested the effects of herbivory on plant water relations, but given that most plants experience some defoliation by herbivory or disturbance, both sporadic and continual defoliation may have important implications for community water balance and large-scale plant allometry. Ecosystems with high and persistent herbivory or animal disturbance might have lower leaf area indices, which could reduce vegetation demand for water and thus impact soil hydration. Large mammalian herbivores are often ecosystem engineers that trample and consume vegetation. While predators may keep herbivore numbers in check, as is the case with wolves and elk in Yellowstone National Park (Johnston et al. 2007), tipping the balance of such predator–prey interactions may ultimately have indirect effects on local hydrology. Secondly, plant allometry is also altered by herbivores, shifting tree stem diameter versus height-scaling exponents away from theoretical predictions of 2/3 depending on how the plant regrows (Archibald and Bond 2003; Moncrieff et al. 2011). This implies that some of the deviations from the idealized scaling laws reported in the literature on plants (see Coomes 2006) may be due to consumer activity that alters plant hormone patterns, and affects physiology and structure in ways that have previously not been accounted for.

Our work with *C. rigidus* shows that biotic interactions such as herbivory may cause observable shifts in plant allometry and hydraulic plant structure–function relationships. Hence, the effects of herbivores on plant performance should be considered in future studies of ecological plant physiology.

Acknowledgments We thank the U.C. Santa Cruz Natural Reserves office for support and access to the Fort Ord Natural Reserve. We also appreciate A. Jacobsen's advice regarding vessel length in *Ceanothus*, as well as the helpful comments on the manuscript from Susan Schwinning and two anonymous reviewers. The study was supported in part by UCSC Start-Up Funds to JP.

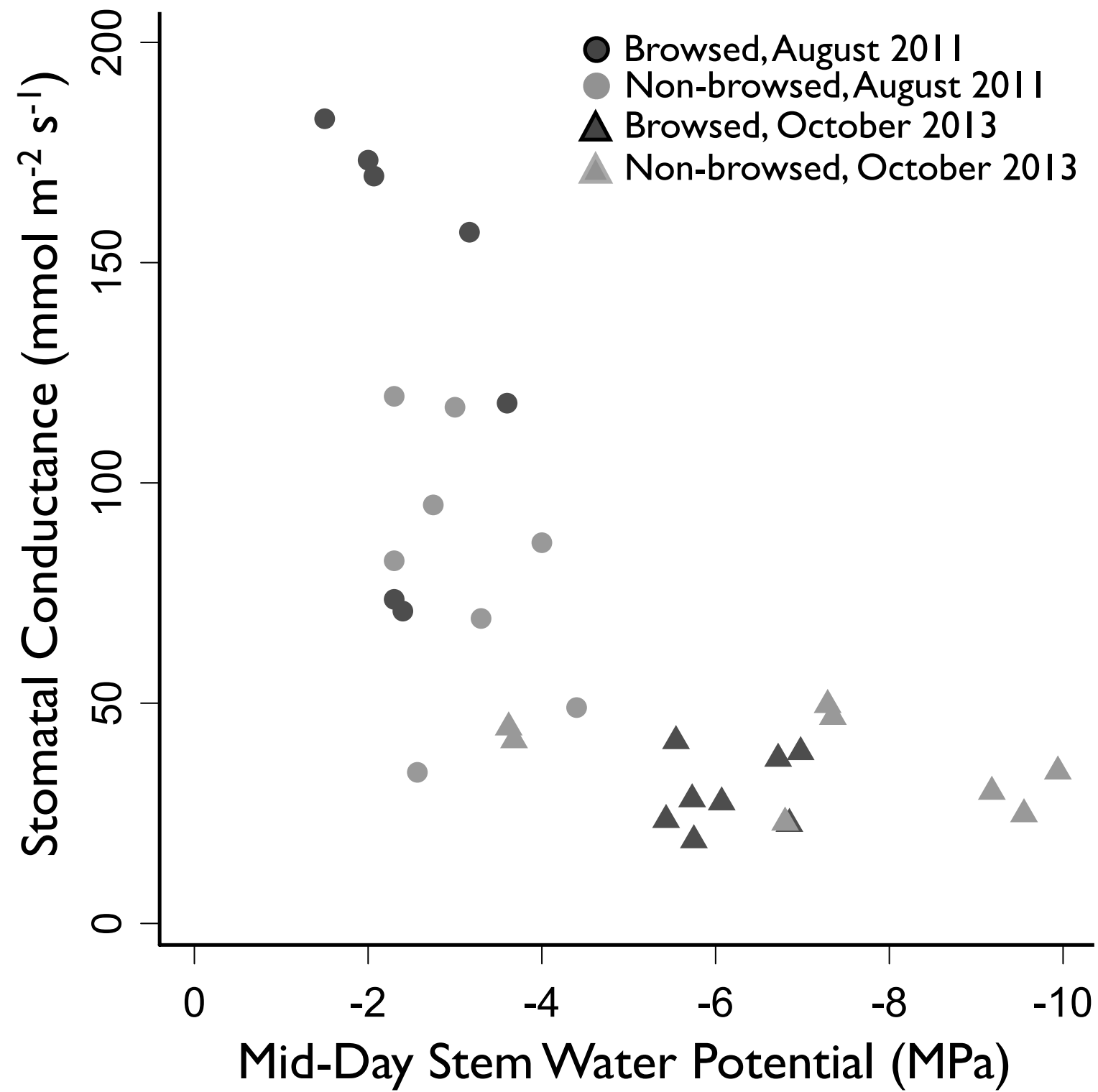
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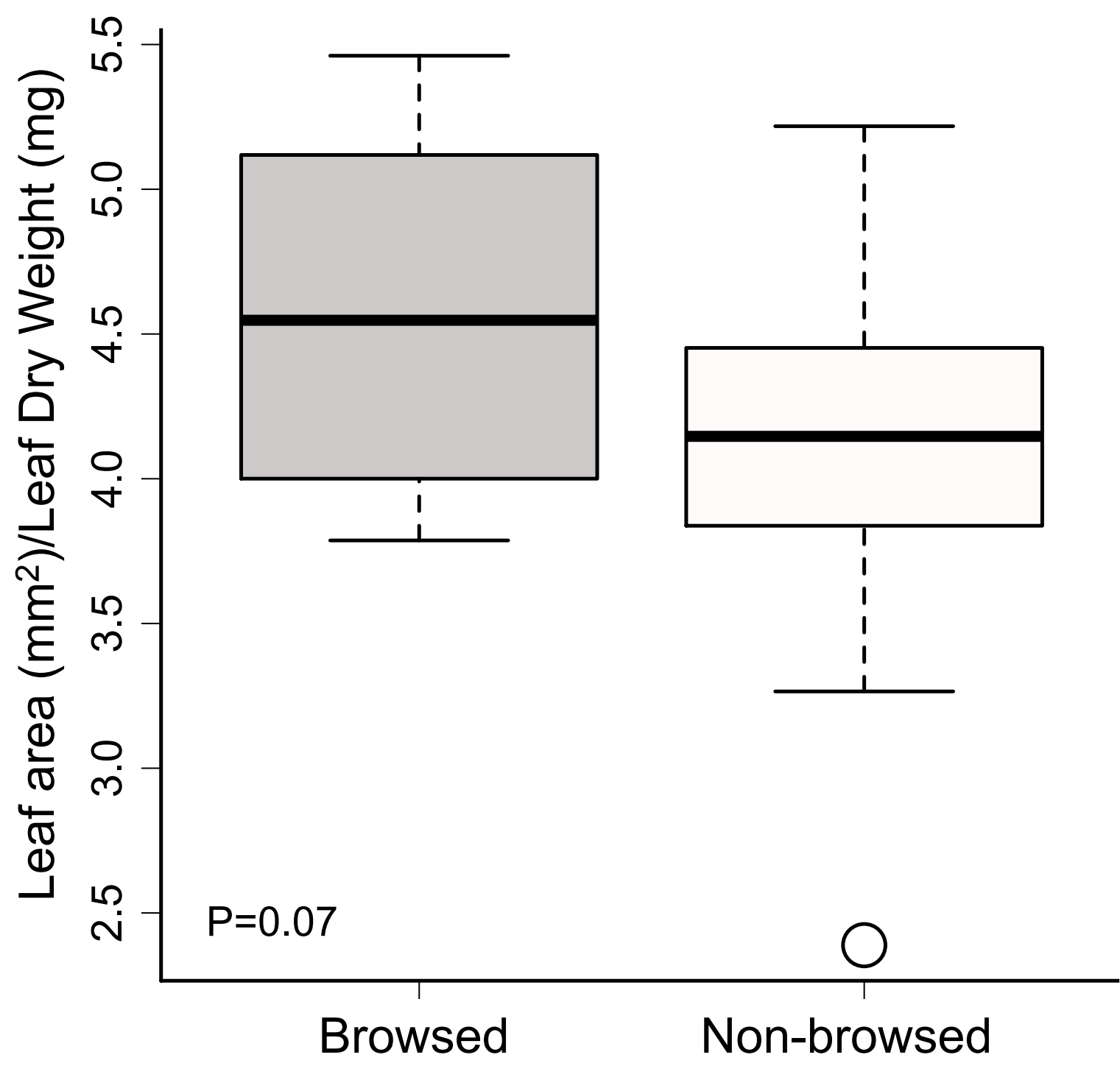
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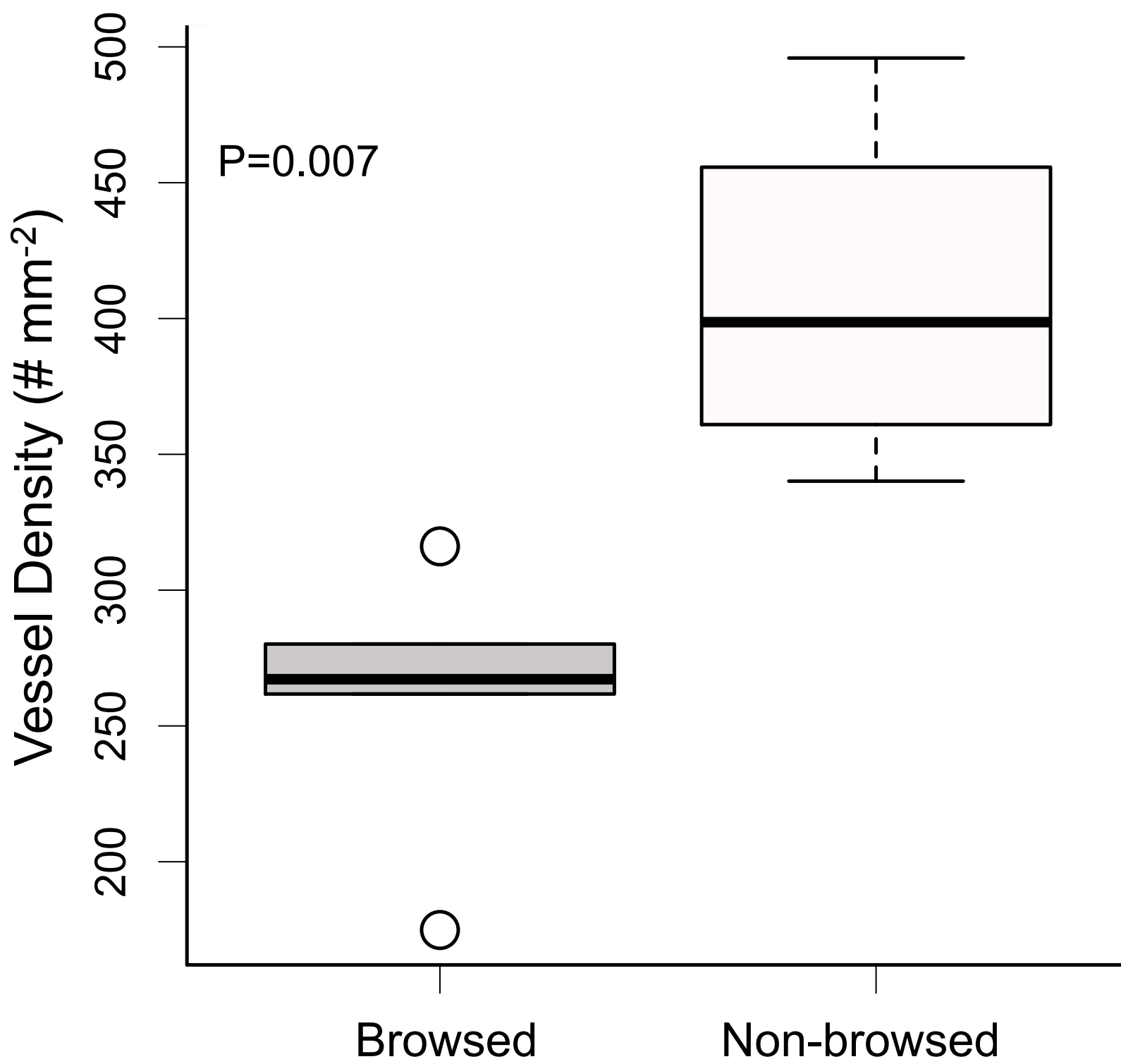
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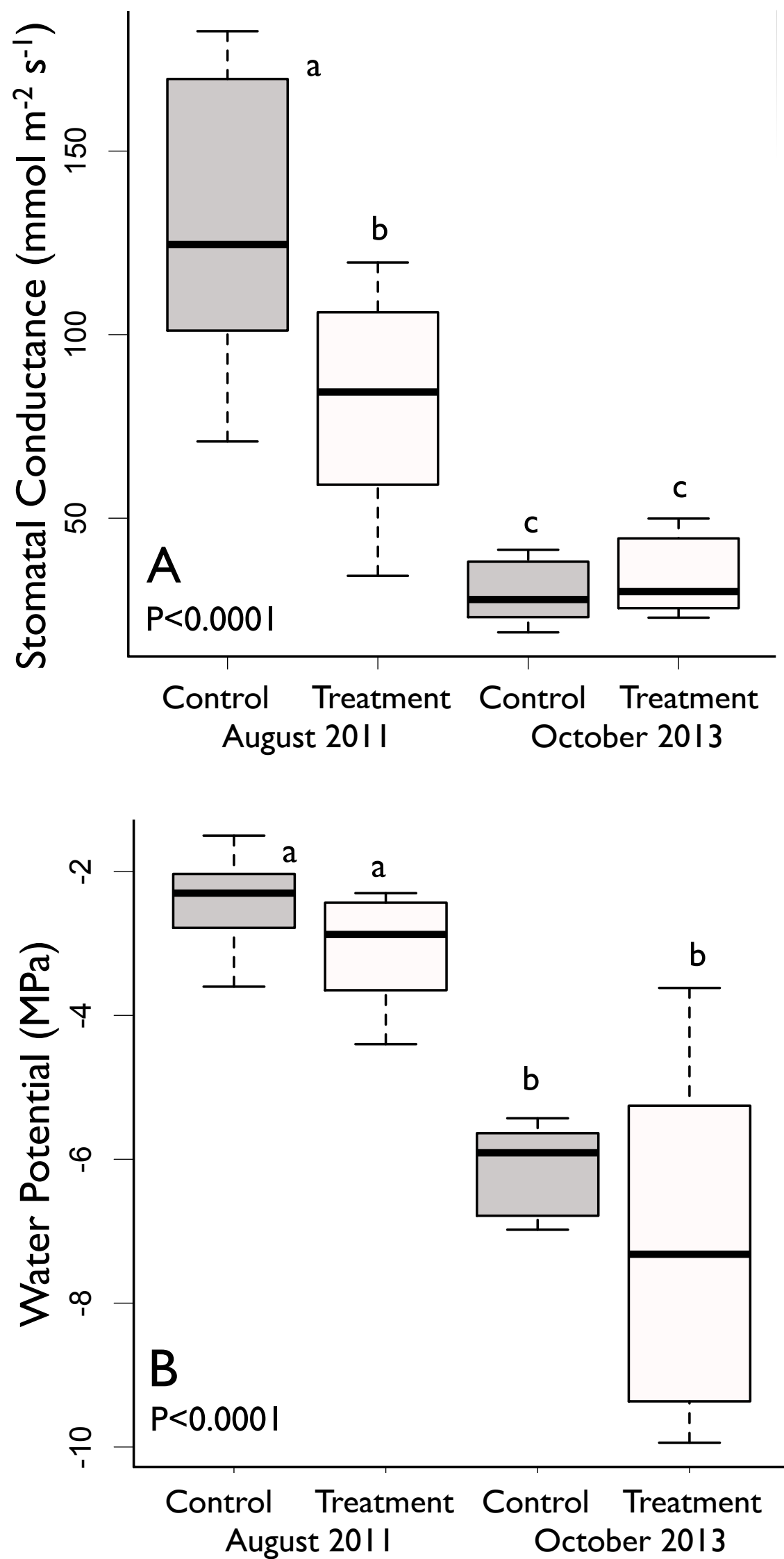




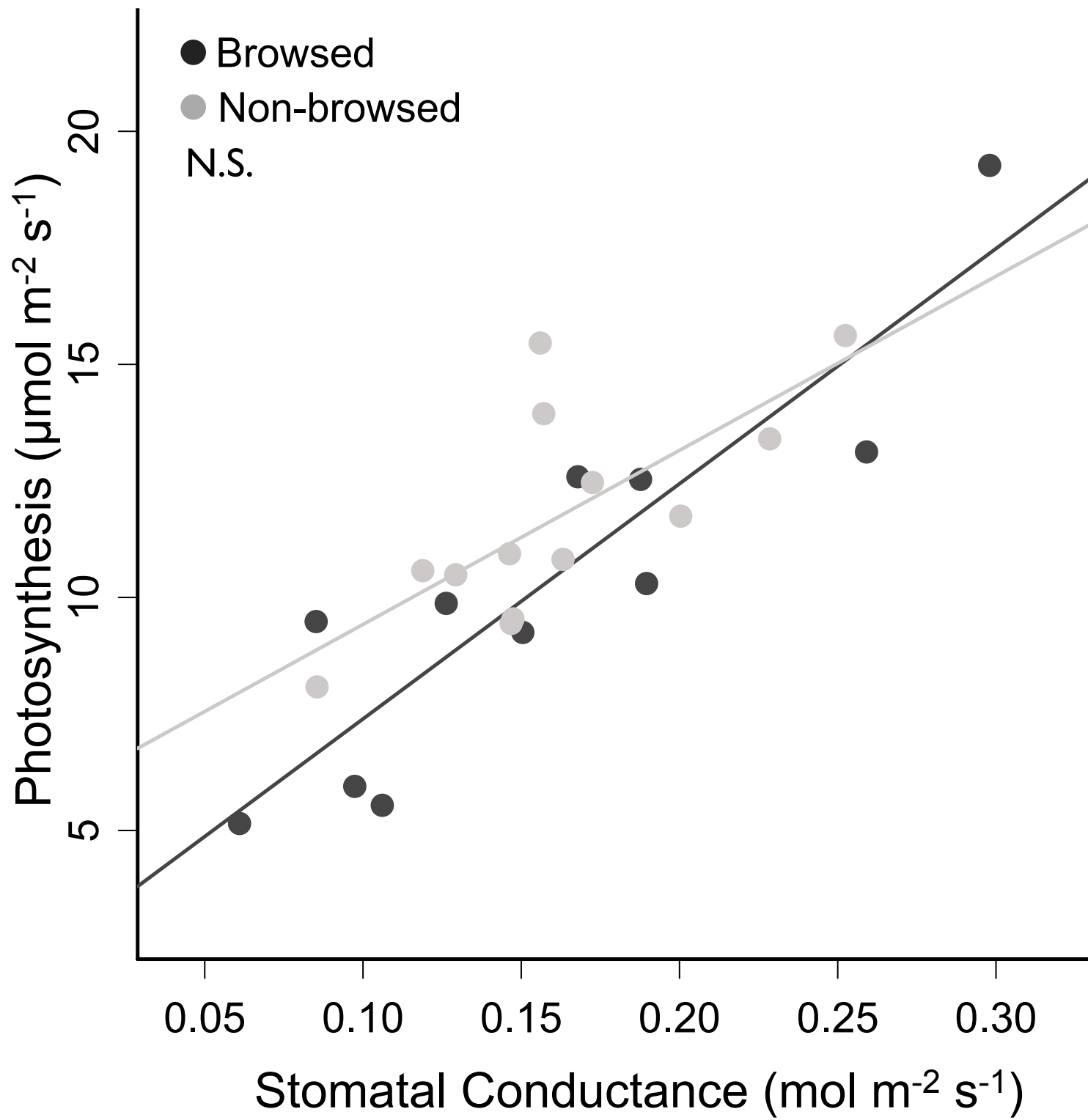
Supplemental Figure 1 Boxplots of the leaf area to leaf dry weight ratio in browsed and non-browsed *C. rigidus* shrubs.



Supplemental Figure 2 Boxplots of the vessel density in the stems of browsed and non-browsed *C. rigidus* plants.



Supplemental Figure 3 Boxplots of leaf stomatal conductance (A) and water potential (B) in browsed and non-browsed *C. rigidus* plants.



Supplemental Figure 3 The relationship between stomatal conductance and photosynthesis as measured on hydrated browsed and non-browsed *C. rigidus* leaves using an LI-6400 gas-exchange instrument.

Heavy browsing affects the hydraulic capacity of *Ceanothus rigidus* (Rhamnaceae)

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Received: 24 July 2013 / Accepted: 15 April 2014 / Published online: 10 May 2014
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Abstract Defoliation by herbivores can reduce carbon assimilation, change plant water relations, and even shift the biotic structure of plant communities. In this study, we took advantage of a long-term deer exclosure experiment to examine the consequences of persistent deer herbivory on plant water relations and the xylem structure–function relationships in *Ceanothus rigidus*, a maritime chaparral shrub in coastal California. Browsed plants had thicker stems with many intertwined short distal twigs, and significantly higher sapwood-to-leaf area ratios than their non-browsed counterparts. Leaf area-specific hydraulic conductivity was similar in both browsed and non-browsed plants, but xylem area-specific conductivity was significantly lower in the browsed plants. Vessel diameters were equivalent in both plant groups, but the number of vessels on a transverse area basis was nearly 40 % lower in the browsed plants, accounting for their

lower transport efficiency. Mid-day in situ water potentials and losses of hydraulic conductivity due to embolism were similar in both groups of plants but stomatal conductance was higher in the browsed shrubs in the early part of the growing season. We discuss our findings in the context of whole-plant ecophysiology, and explore the consequences of herbivory on hormonal signals, wood anatomy, and xylem function.

Keywords Embolism · Xylem anatomy · Stomatal conductance · Herbivory · Water transport

Introduction

Herbivory can affect plants at all life stages, potentially influencing other biotic interactions that may shape community structure and vegetation heterogeneity at many scales. For example, herbivores are often the intermediaries that affect how vegetation recovers from disturbances such as fire, and herbivores may shape ecosystem structure in ways that deviate from simple climate-based predictions (Minnich 1982; Tyler 1995; 1996; Bond 2005; Waldram et al. 2008). Fossil evidence indicates that herbivores were present throughout deep time (Wilf et al. 2001), so plants would have needed to adapt to defoliation by insects and vertebrates as soon as they appeared on land. As such, both browsers and grazers may have spurred the evolution of traits that minimize the effects of herbivory; such traits include signaling by the release of secondary compounds (Wu and Baldwin 2009), production and up-regulation of defensive chemicals (Endara and Coley 2011), increased modularity of the shoot (Haukioja et al. 1991), and the evolution of basal meristems and faster growth rates in grasses (Stowe et al. 2000; Turley et al. 2013). Altogether, there is good evidence that herbivores have had profound effects on

Communicated by Susanne Schwinning.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-014-2947-1) contains supplementary material, which is available to authorized users.

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the structure and function of land plants (Fox 1981; Strauss and Irwin 2004; Wise and Rausher 2013).

Consumers may impact any or all portions of a plant, shifting resource allocation away from storage, new growth, and reproductive structures to increased physical and chemical defenses and possibly compensatory responses. Compensatory responses appear to be temporary, but examples include increased growth of new stems or leaves as well as higher rates of photosynthesis in newly developed foliage (Thomson et al. 2003; Johnston et al. 2007; Gálvez and Tyree 2009; Ayres et al. 2004 but see Belsky et al. 1993). Depending on the magnitude, persistence, and nature of the defoliation events, subsequent plant architecture may vary from appearing relatively unchanged, to being irreversibly altered by the presence of either copious new sprouts as in the case of willows (Johnston et al. 2007) or the production of stunted shoots for which re-growth is impossible due to persistent browsing pressure (Huntsinger 1996; Cooper et al. 2003; Knight et al. 2009; Ramirez et al. 2012). Indeed, deer often nibble away at juvenile foliage at the base of a tree throughout the growing season, giving rise to such stubby branches. Our study addresses whether such long-term herbivory affects water transport and xylem anatomy in *Ceanothus rigidus*, an evergreen shrub common to the maritime chaparral of central California.

Herbivory can shift plant physiology by altering root-to-shoot ratios, whereby greater root mass becomes available to support hydration and nutrient demands of the defoliated shoot. Consequently, during recovery, browsed plants often exhibit increased shoot water potentials, higher rates of stomatal conductance and photosynthesis and thus increased growth rates relative to individuals that have not been browsed (Thomson et al. 2003; Johnston et al. 2007; Gálvez and Tyree 2009; Ayres et al. 2004; Ramirez et al. 2012). Similarly, when over 50 % of foliage was removed due to simulated and observed herbivory in *Eucalyptus blakelyi* and *Pinus taeda*, respectively, sap flow was reduced in both species by up to 50 % due to reduced leaf-level demand for water (Cunningham et al. 2009; Pataki et al. 1998). Taken together, these studies suggest that hydraulic co-ordination between transpiration and xylem-mediated supply can be a dynamic process, responding rapidly to shifts in canopy demand.

Since the effects of long-term herbivory on xylem anatomy and hydraulic function have not been studied, we examined this in *Ceanothus rigidus* shrubs that were part of a long-term herbivore exclosure experiment in maritime chaparral along the central California coast. These shrubs were either protected from herbivores by fencing, or left unprotected and thus consumed, primarily by deer. Browsed *C. rigidus* individuals have thick, intertwined and clustered distal stems on which masses of short shoots form a deformed, topiary-like canopy (Fig. 1a–c, e, f). We

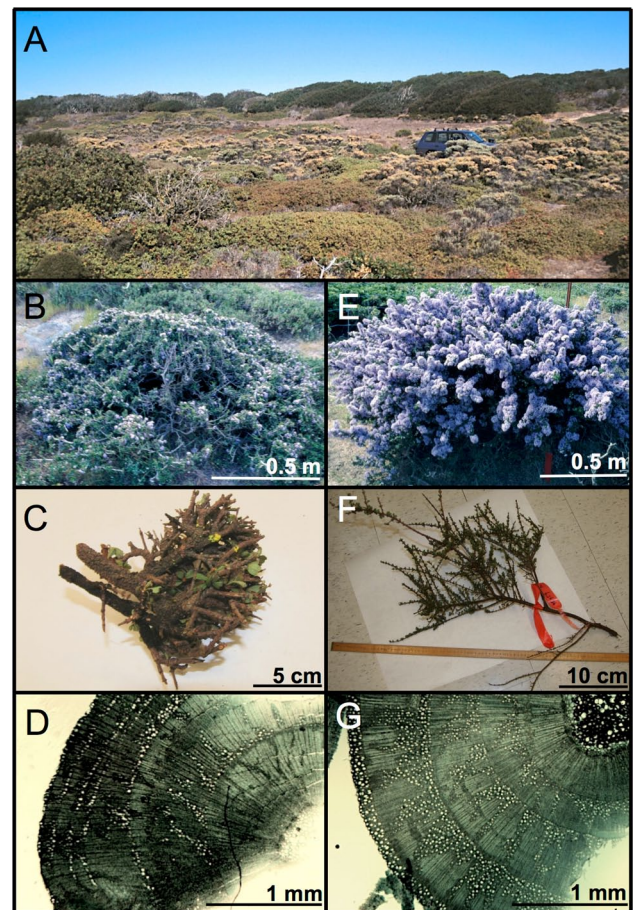


Fig. 1 The morphology and anatomy of browsed (**b–d**) and non-browsed (**e–g**) *Ceanothus rigidus*. The maritime chaparral vegetation growing on the sandy soil of Fort Ord Natural Reserve is pictured in **a**. Note the browsed *C. rigidus* shrubs in the foreground. **b**, **e** Browsed and non-browsed shrubs, respectively, during flowering. Browsed plants exhibit shorter, thicker stems with intertwined clumps of distal twigs (**c**) as compared to the non-browsed *C. rigidus* stems (**f**). Cross sections of browsed (**d**) and non-browsed (**g**) *C. rigidus* stems showing differences in vessel distribution as well as the thickness of the outer growth rings

compared the physiological performance of browsed and non-browsed *C. rigidus* individuals by characterizing the differences in shoot architecture, hydraulic performance, wood anatomy, and in situ measurements of water potential and stomatal conductance. We predicted that browsed plants would exhibit less mid-day water stress but expected no significant differences in xylem function because xylem-mediated supply of water scales with leaf-level demand (Santiago et al. 2004). However, the results show that continual browsing conspicuously alters xylem anatomy and hydraulic function, with no effect on leaf water potentials. Hence, we consider the effects of leaf removal on water transport by way of hormonal signals, and discuss the broader implications of herbivory on plant structure and overall water relations.

Methods

Site description and sample collection

Maritime chaparral is the dominant habitat on the Fort Ord Natural Reserve (FONR), a 242-ha reserve that is part of the University of California Natural Reserve System, and lies ~2 km inland from Monterey Bay at an elevation of 21–58 m. Fort Ord has a Mediterranean-type climate, with late autumn and winter rains and dry (though cool and foggy) summers. The soils are aeolian dunes, 10⁴–10⁵ years old (Dupre 1990), composed mainly of quartz sands with low levels of most nutrients at 0–10 cm depth: e.g., total $n = 0.084\%$; $p = 21.8$ ppm; and organic matter = 1.89 % (Fox et al. 1998). The site is at least 70 years post-fire. The manzanitas, *Arctostaphylos pumila* (Nutt.) and *A. tomentosa* (Pursh) Lindley ssp. *tomentosa*, are the dominant shrubs in the maritime chaparral on the reserve, while *Ceanothus rigidus* (Nutt.) Hoover and *Ericameria ericoides* (Less.) Jepson are distributed patchily amongst them. Several other shrubs or young trees occur much less commonly, particularly *Quercus agrifolia* Née, *Fragula* (syn. *Rhamnus*) *californica* (Eschsch.) and *Acmispon glaber* var. *glaber* (Vogel) (syn. *Lotus scoparius*). Only the *C. rigidus*, *Q. agrifolia* and *A. glaber* are browsed heavily.

Experimental design

The experiment began in August 1996, in two areas of FONR that are ~1 km apart, with the goal of assessing how shrub and habitat dynamics responded to deer and rabbit browsing, particularly on *C. rigidus*, since it is heavily browsed, sufficiently abundant and evenly distributed to allow for experimental treatments, and easily enclosed in a fence. The experiment had three treatments arranged in a randomized block design, and replicated four times in each area (24 plots total). Treatments were control plots with natural levels of browsing by deer and rabbits, and plots that excluded these herbivores. Each plot is 9 m² and initially contained at least one shrub of *C. rigidus*, *A. pumila* or *A. tomentosa*, and *E. ericoides*, and some “open space” between shrubs that is habitat for herbaceous annuals; other woody plants were in some plots as well. Plots were grouped by proximity into blocks, with treatments assigned randomly within each block. Deer were excluded by 2-m-high cages, with 20 cm × 10-cm mesh. Rabbits were excluded by 1.5-cm mesh on the lower 1 m of half of the deer exclosures. Because many *C. rigidus* shrubs in control plots died during this experiment, we randomly selected other individuals growing near the deer exclosures for the data in this paper. In addition, since rabbit effects were minimal, we consider only deer exclosures (no deer

browsing, but ± rabbits) and controls (natural browsing) for this paper.

Photosynthesis measurements

It was difficult to measure gas-exchange rates in situ with a standard portable gas-exchange instrument because the deformed twig architecture interfered with the use of the leaf cuvette, and because the space inside the fence enclosures was limited. Hence, we collected plant material in the field, and transported it back to the laboratory for gas-exchange and other measures (see below). Plant segments were collected in April and May 2011 under fog or low cloud with temperatures between 11–14 °C. Leafy stems ranging from 30–60 cm in length and 4–10 mm in diameter were clipped in the field, quickly re-cut 5–10 cm under water, trimmed with a razor blade, covered with a plastic bag, and transported in a water-filled container to the lab where they were left overnight to re-hydrate to water potentials between −0.1 to −0.5 MPa as measured by a Scholander-style pressure chamber (PMS Instruments, Corvallis, OR, USA). We rehydrated the plant material to standardize leaf water potential, and thus compare intrinsic rates of gas exchange. Wounding-induced changes in gas-exchange rates were not observed over the 24-h time frame during which the measurements were made. We assumed that plant material collected earlier in the growing season would be free of the confounding effects of drought-induced air entry into the xylem (embolism; Hacke et al. 2000; Jacobsen et al. 2007). However, Wheeler et al. (2013) recently showed that clipping plant segments in the field while the xylem is under tension may inflate embolism, an artefact of significant concern. Given that material was collected early in the growing season, at low temperature and under cloud cover, and then re-cut again, we suspect that these effects were minimal, although they cannot be ruled out.

Gas-exchange measurements were made the following day in an outdoor area at the UCSC greenhouses, under ambient conditions (12–15 °C, overcast) using a Li-Cor LI-6400 XT photosynthesis instrument (Li-Cor Biosciences Inc., Lincoln, NE). We used a 2 × 3-cm cuvette fit with a standard mounted external LED light source with the flow rate set to 400 ml min^{−1}, the CO₂ mixer at 400 μmol mol^{−1} with vapor pressure deficit ranging from 1.1 to 1.5 kPa. Leaf temperature was maintained at 20 °C and measurements were obtained at saturated light levels (2,000 μmol quanta m^{−2} s^{−1}, which were increased in increments of 200–500 every 5–7 min starting at ambient light (150–300 μmol m^{−2} s^{−1}). For each stem segment, the most accessible short shoots were carefully inserted inside the cuvette, taking care to seal any leaks with Poster Tack plasticine (Elmer's, Columbus, OH, USA). Following each

measurement, leaves were scanned (Epson 4200X Printer/Scanner, Epson Corp., USA) and the projected leaf area was measured using ImageJ software (Rasband 2012). These leaf areas were used to re-calculate the true photosynthetic rates per unit leaf area.

Hydraulic measurements

Hydraulic measurements were performed on the same plant material on which gas-exchange measurements were made in order to reduce the effect of foliage removal on other concurrent studies at FONR. Stems were re-cut under water, with the cut ends smoothed using a sharp razor blade. It is customary to use segments longer than the longest vessel, and we attempted to find the longest possible segments but could find no usable stems from the browsed plants that were longer than 110 mm. Consequently, we chose similarly sized segments from the non-browsed plants with lengths for both treatments ranging from 70 to 120 mm. Segment length effects on conductivity are negligible (Sperry et al. 2005) and the vast majority of *C. rigidus* vessels are less than 100 mm in length (Jacobsen et al. 2007, 2012).

Hydraulic conductivity was measured using the standard gravimetric method (Pratt et al. 2012; Hacke et al. 2000) whereby stems were mounted on a tubing apparatus connected to a four-digit balance, and the flow rate was measured using a 20-mM KCl solution. To increase precision, background flow rates were measured under zero pressure before and after each gravimetric pressure measurement, then averaged and subtracted from the gravimetric flow measurement (~6–8 kPa). Hydraulic conductivity (K) was computed as the flow rate for a given pressure, multiplied by stem length. Native K (K_{native}) was measured first, followed by an overnight degassing treatment to remove any air embolisms, after which K was re-measured (K_{max}). This was accomplished by submerging stems in filtered water (0.2 μm filter, Nanopure, Barnstead International) and then connecting the sealed vessel to a standard in-lab vacuum system, for 12 h. Our tests show that K_{max} is reached after 7 h with no changes to K with further degassing. The % loss of conductivity (PLC) was computed as $100 \times (1 - K_{\text{native}}/K_{\text{max}})$. K_{max} was further standardized for functional xylem area (K_s), which was identified by perfusing the distal portions of stems with basic fuchsin, and measured with ImageJ. Hand-made, transverse sections of the stems were mounted in glycerin, and the red-stained xylem tissue was photographed at 20 $\times$ with a digital compound microscope (Motic BA210, Richmond, BC, Canada). Since the plant material was re-hydrated overnight, we cannot rule out the possibility that some embolism repair may have occurred, thereby reducing native PLC. To standardize K_{max} by distal leaf area (K_{leaf}), the leaves were scanned, and the projected area was measured with ImageJ

software. The Huber ratio was computed by dividing the functional xylem area by the distal leaf area (Tyree and Zimmermann 2002).

Xylem anatomy and wood density

Wood density was measured according to the methods of Hacke et al. (2000) whereby 1 to 1.5-cm-long samples were cut from the xylem, taking care to remove the bark and pith. The fresh volume was obtained by immersing the samples in a water-filled vessel on a balance, and converting the displacement weight (g) to volume (cm^3) by dividing by 0.998 g cm^{-3} , which is the density of water at 20 °C. Each sample was immersed at least 5 $\times$ to generate an average displacement weight. The xylem dry weight was obtained after 3 days at 65 °C, and wood density was computed as the ratio of dry weight to fresh volume.

Conduit anatomy was determined on stems used for K measurements. Hand-sections were made on the middle portion of the stem segment, stained with toluidine blue and mounted in glycerol. Three radial sectors corresponding to the outer, transporting growth rings were photographed under 200 $\times$. Conduit size and density were determined by manually measuring conduit dimensions with ImageJ software, and by counting the number of conduits for a given xylem area, respectively. Growth rings were difficult to distinguish in the browsed plants and could not be reliably measured (Fig. 1d).

Mid-day water potential and stomatal conductance

Field measurements of mid-day water potential and stomatal conductance were first made in August 2011 when the plants would have experienced high levels of seasonal drought stress, and differences between the treatments would be most apparent. Whenever possible, we attempted to collect data on clear days but fog or low cloud cover were persistent at Fort Ord such that mid-day temperatures rarely peaked above 18 °C, with PAR levels typically less than $700 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Subsequently, in October 2013, several hot, cloud-free days with air temperatures above 32 °C afforded us the opportunity to collect additional late-season data. Stomatal conductance was obtained with a hand-held porometer (Decagon Devices, Pullman, WA, USA) taking care to cover the sensor completely by the leaf. The shoot from which the leaf was sampled was clipped, placed immediately in a plastic bag, and the water potential measured using the pressure chamber as described above. The 2011 measurements were made following a year of slightly above average rainfall (17.4 vs. 15.95 cm), while the 2013 measurements followed 2 years with <70 % of average rainfall.

Statistics

Multiple measurements on each individual shrub were averaged, such that each datum represents a single individual unless indicated otherwise. Our sample size varied from 6–9 individual browsed plants and 5–8 non-browsed (caged) plants. We used SAS 9.3 software (SAS Institute, Cary, NC, USA) for all control-treatment comparisons. We tested all variables for normality, using Proc Univariate on residuals from ANOVAs (Proc GLM), and transformed the variables as appropriate. The few variables that could not be normalized were analyzed using a two-sample Wilcoxon test in Proc Npar1way. Findings were considered statistically significant when $p < 0.05$. Regression analyses were performed using the R statistical environment (R Core Team 2012).

Results

The Huber ratio of sapwood to distal leaf area in browsed *C. rigidus* was 57 % greater than in the non-browsed shrubs (Fig. 2a; $p = 0.004$). Although the stems of browsed plants were generally thicker than those of non-browsed *C. rigidus* (Figs. 1c, f, 2b, $p = 0.03$), the functional xylem area was not statistically different ($p = 0.12$). Specific leaf areas (SLA) of the browsed plants were marginally (though not significantly) higher at $4.55 \pm 0.59 \text{ mm}^2 \text{ mg}^{-1}$ (mean $\pm$ SD) relative to the non-browsed shrubs ($4.05 \pm 0.70 \text{ mm}^2 \text{ mg}^{-1}$, Supplemental Figure 1; $p = 0.07$).

The leaf-specific conductivity was the same in the browsed and non-browsed shrubs averaging 3.93 ± 1.55 ($\times 10^{-4}$) $\text{kg m}^{-1} \text{ MPa}^{-1} \text{ s}^{-1}$ (Fig. 3a). By contrast, K_s in the browsed plants was 53 % lower than in protected plants (0.59 ± 0.25 vs. $1.11 \pm 0.48 \text{ kg m}^{-1} \text{ MPa}^{-1} \text{ s}^{-1}$, respectively; $p = 0.02$; Fig. 3b). Vessel size was equivalent in both treatments (17.70 ± 2.19 and $17.00 \pm 1.66 \mu\text{m}$, respectively) but the number of conduits per area, that is vessel density, was significantly lower in the browsed plants ($263.38 \pm 61.88 \text{ mm}^{-2}$) than in those that were fenced in ($409.77 \pm 61.07 \text{ mm}^{-2}$; $p = 0.01$; Fig. 4, Supplemental Figure 2). Figure 4 shows that vessel density explained 46 % of the variation in K_s with higher K_s in response to greater vessel number per area. Wood density was the same in browsed and non-browsed stems with values of 0.705 ± 0.021 and $0.719 \pm 0.045 \text{ g cm}^{-3}$, respectively.

Browsing had no measurable effect on xylem embolism, with mean values of the % loss of hydraulic conductivity in the non-browsed and browsed plants at 56.1 ± 12.4 and 46.5 ± 24.3 %, respectively ($p = 0.12$; Fig. 5). The native PLC values in the browsed plants were more variable than in the non-browsed shrubs, but interestingly, three-quarters of the sampled stems had PLC values below 55 %.

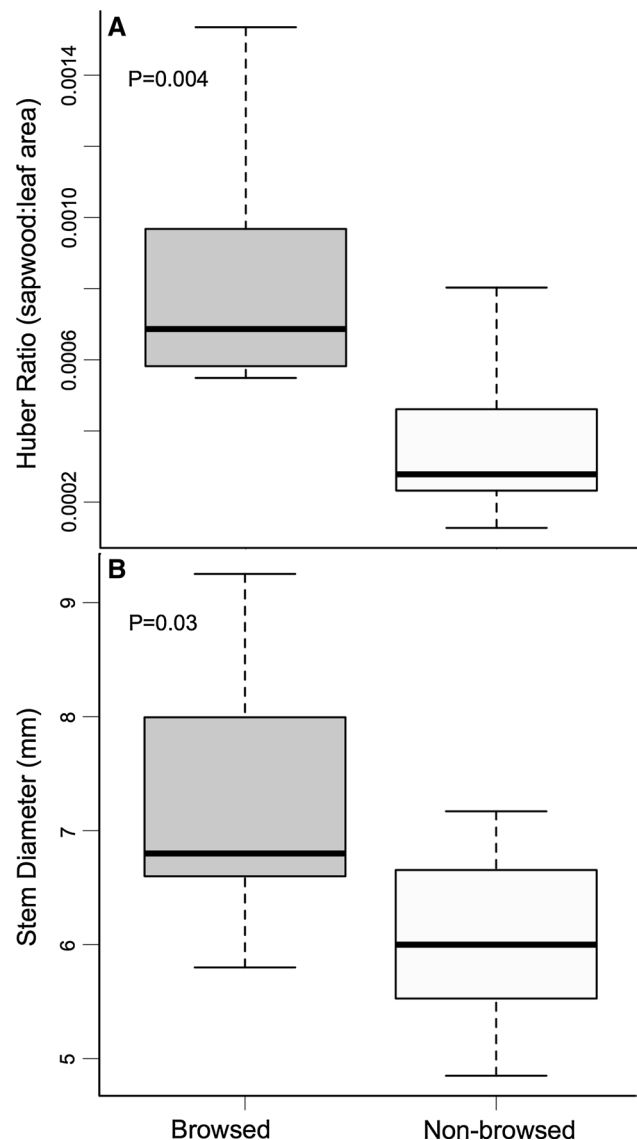


Fig. 2 Box-and-whisker plots of the sapwood-to-leaf area ratio (a) and stem diameter (b) in browsed and non-browsed *C. rigidus* stems. The box indicates the mid 50 % of the data distribution where the thickened line is the median. The whiskers represent the upper and lower quartiles

Mid-day water potentials were similar in the browsed and non-browsed plants during both August 2011 and October 2013 ($p = 0.49$; Fig. 6). It is notable that water potentials were 2–3-fold lower in October 2013 (as opposed to the data collected in the cooler, foggy days of August 2011) due to the prolonged drought, with several non-browsed plants dropping to less than -9 MPa (Fig. 6, Supplemental Figure 3). In the field, browsed plants had higher stomatal conductance ($130.08 \pm 42.83 \text{ mmol m}^{-2} \text{ s}^{-1}$) than non-browsed shrubs in August 2011 ($81.64 \pm 30.27 \text{ mmol m}^{-2} \text{ s}^{-1}$; Fig. 6; Supplemental Figure 3; $p = 0.012$) but these differences disappeared in October when water potentials

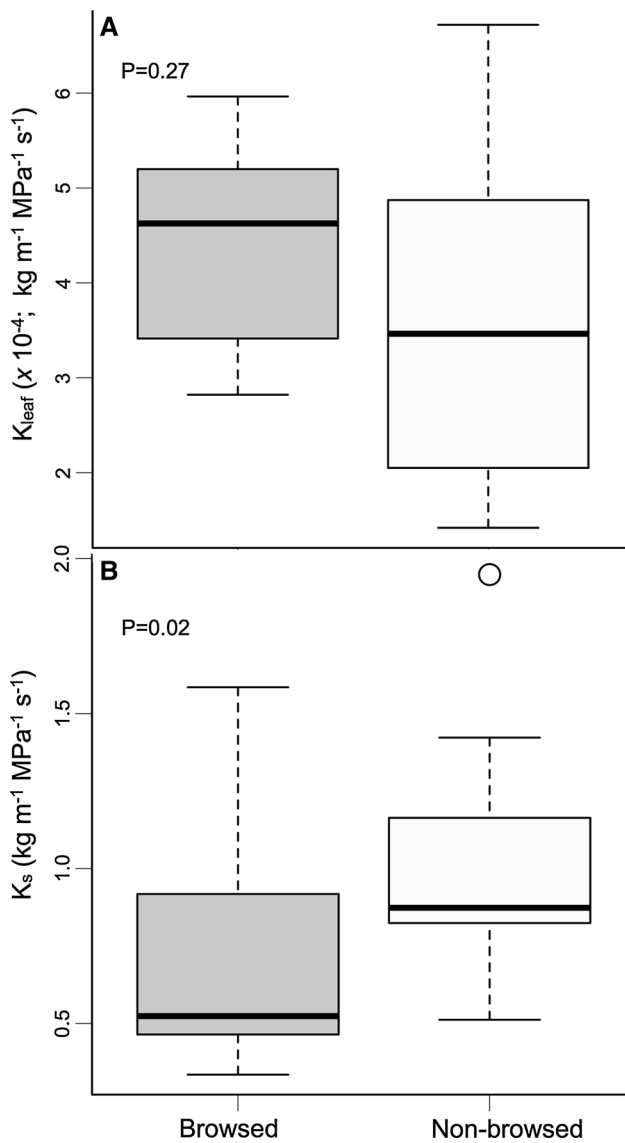


Fig. 3 The leaf-specific conductivity (a) and xylem-area specific conductivity (b) of stem segments belonging to browsed and non-browsed *C. rigidus*

in the majority of the sampled plants dropped to -6 MPa or lower ($p = 0.49$). Lastly, photosynthesis and stomatal conductance on re-hydrated samples were nearly identical in both plant groups, consistent with previous findings that leaf nitrogen levels (1.5 %) were not affected by browsing (Fox, unpublished data; Supplemental Figure 4).

Discussion

Plants have evolved a suite of defensive and compensatory strategies to minimize leaf loss to herbivores that may reduce plant growth rates and fitness and alter plant architecture over long periods of time (Fig. 1a–c; Fox 1981; Archibald

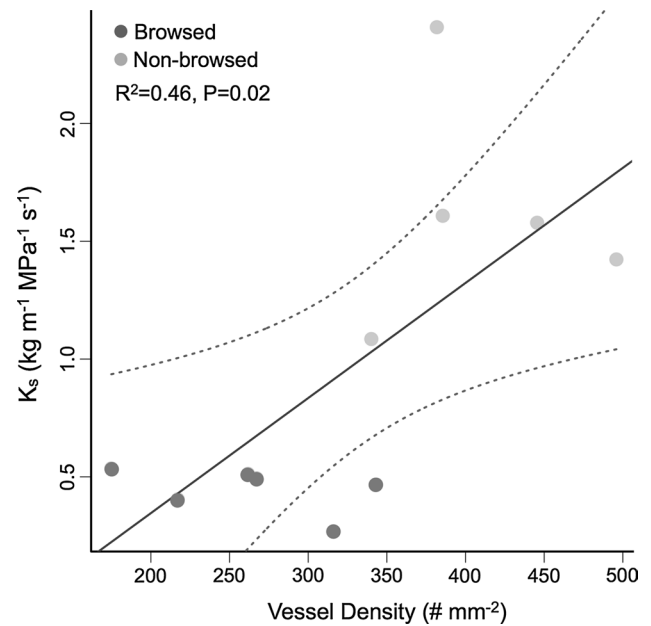


Fig. 4 The relationship between stem vessel density and xylem area-specific conductivity in browsed and non-browsed shrubs. Dotted lines represent 95 % confidence intervals

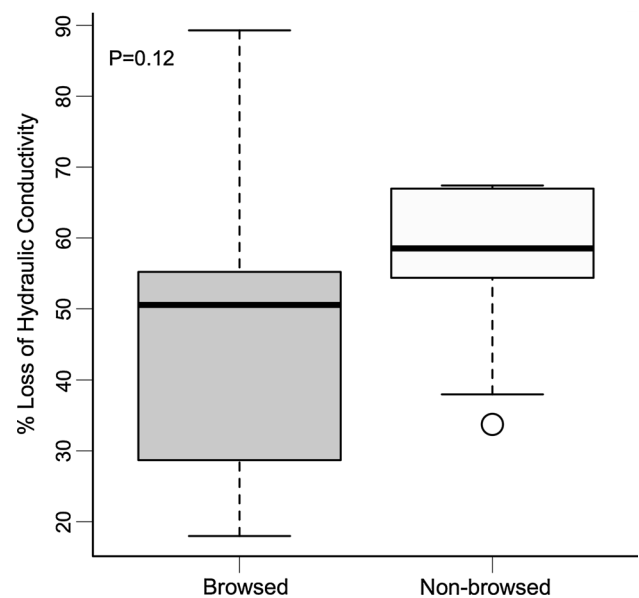


Fig. 5 Boxplots showing the native % loss of hydraulic conductivity in stems of browsed and non-browsed *C. rigidus*

and Bond 2003; Strauss and Irwin 2004; Moncrieff et al. 2011; Wise and Rausher 2013). Compensatory responses such as higher photosynthesis rates (Thomson et al. 2003; Ayres et al. 2004; Johnston et al. 2007) and higher sap flow may facilitate vegetation recovery (Pataki et al. 1998; Cunningham et al. 2009), but whether they persist over multiple growing seasons is unknown. We observed no difference in

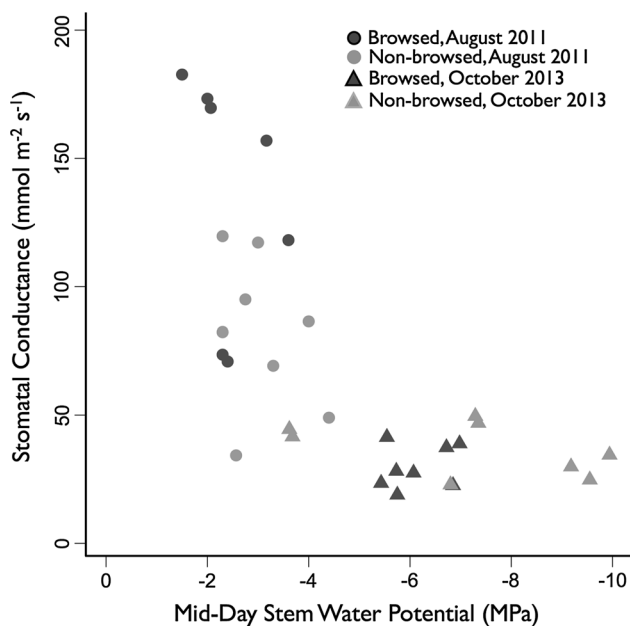


Fig. 6 The relationship between in situ mid-day water potential and leaf stomatal conductance in browsed and non-browsed *C. rigidus* plants in August 2011 and October 2013. Each point in the August 2011 data represents an average of at least two water potential and three to five conductance measures per plant. Three browsed and three non-browsed plants were sampled in October 2013 with each datum corresponding to an individual conductance and an associated water potential measurement. Each plant was sampled at least twice

photosynthesis rates between rehydrated browsed and non-browsed *C. rigidus* foliage, but in situ photosynthesis rates may have been greater in browsed *C. rigidus* during August 2011 when these plants had higher stomatal conductance than their protected counterparts. It may be that compensatory responses are nuanced, and more or less dependent on how the plants are sampled.

Chaparral shrubs evolved several post-fire recovery strategies including sprouting (Ramirez et al. 2012; Pratt et al. 2007), but as a non-sprouter, *C. rigidus* allocates few resources to the root crown and quiescent buds, and instead produces more copious quantities of seeds that lie dormant in seed banks (Keeley 1991; Deveny and Fox 2006). Over time at Fort Ord, unbrowsed *C. rigidus* produced $\sim 35\times$ more seeds than their browsed counterparts, though this varies markedly in different years (Deveny and Fox 2006; compare flower abundance in Fig. 1b, e); persistent herbivory weakens this important compensatory response to disturbance. Deer browsing significantly slows immediate post-fire re-growth in *Ceanothus* and other plants in the chaparral (Minnich 1982; Quinn 1992, 1994; Mills 1983, 1986; Davis 1967; Tyler 1995, 1996; Ramirez 2012), but the studies at Fort Ord are the first to show long-term impacts of browsing (at least 70 years post fire; Fox unpublished and Coale et al. 2011). Taken together, reduced *Ceanothus* biomass, as well

as delayed recovery after fires, may be attributed to fewer seeds as well as some persistent physiological constraints due to prolonged, intense browsing.

Our study addresses physiological adjustments that balance investment in xylem tissue with leaf-level water loss in continually browsed plants. In contrast to the plants protected from herbivores in our experiment, sapwood-to-leaf area ratios of browsed shrubs were over 50 % higher, so all else being equal, we expected similar or slightly higher leaf-specific conductivity in browsed than non-browsed plants. However, K_{leaf} is similar in both groups of shrubs, consistent with lower xylem-specific conductivity in the browsed plants. Further, the lower K_s can be attributed to a nearly 40 % reduction in vessel density, implying a dynamic connection between leaf area and xylem function. Although we could not reliably document growth ring width in stems on heavily browsed plants because growth was severely constricted, we did observe narrower, compressed and incompletely formed rings. These observations support previous work at the Fort Ord site showing poorly developed rings on main stems of heavily browsed *C. rigidus*, compared to plants sustaining less browsing in other parts of Fort Ord (Coale et al. 2011). Narrower rings in response to browsing also occur in other species (e.g., *Betula pubescens*, Speed et al. 2011).

How continual defoliation affected root-to-shoot ratios in *C. rigidus* is unknown, but one might expect some degree of scaling whereby these ratios are similar in the browsed and non-browsed plants, assuming that both groups are of similar age. Given that water potentials in browsed and non-browsed plants were not significantly different, it seems unlikely that root-to-shoot ratios varied between these two groups. Indeed, reduced root biomass has been reported in artificially defoliated seedlings of *Fagus* and *Abies* grown in vermiculite, suggesting that co-variation in above- and below-ground allocation may be a common response (Ayres et al. 2004). The below-ground response to herbivory in the Fort Ord system is much less tractable due to on-going research, as well as varied topography and patchy vegetation patterns, both of which could affect root distribution and depth.

Herbivory has important ramifications for plant hydraulic function under both natural and experimental conditions. The compensatory responses that favor the development of vigorous new shoots with increased rates of gas exchange, faster growth, and larger leaves (Ayres et al. 2004; Johnston et al. 2007; Gálvez and Tyree 2009; Turley et al. 2013) may be due to favorable root-to-shoot ratios that improve leaf water status (Pataki et al. 1998; Johnston et al. 2007; Gálvez and Tyree 2009). However, rapid stem growth combined with faster leaf production may eventually predispose the shoots to hydraulic failure due to a combination of younger, possibly weaker wood and a high

leaf-level demand for water (Gálvez and Tyree 2009). We did not observe significantly different degrees of embolism in our study, probably because many species of Rhamnaceae are highly drought resistant to begin with (Jacobsen et al. 2007).

This study presented us with unique challenges to evaluating hydraulic function that were particular to heavily browsed *C. rigidus* plants. The first such hurdle was to find stems that were sufficiently long and straight to fit either in the centrifuge (Alder et al. 1997) or the air-injection sleeve (PMS Instruments, Corvallis, OR, USA; Sperry and Saliendra 1994) for determining vulnerability to embolism. Since we could not locate appropriate material in the browsed plants, we only measured native percent loss of conductivity for both groups. Secondly, using such short segments can in theory inflate estimates of K due to removal of vessel endwalls, but our K_s values are on par, if not slightly lower than the K_s reported in longer stems segments of *Ceanothus* (e.g., in the closely related *C. cuneatus* in Pratt et al. 2007) and other members of the Rhamnaceae (Pratt et al. 2007; Jacobsen et al. 2007; see also Sperry et al. 2005). Further, our native embolism values (Fig. 5) may be higher than what we would expect in spring, probably because measurements of maximum conductivity did not exclude vessels produced in earlier years that are typically more vulnerable to embolism, and thus dysfunctional in the current year (Hacke et al. 2000; Pratt et al. 2007; Jacobsen et al. 2007). In retrospect, a better approach would have been to disable this population of weak conduits by axial pressurization of the stems at 0.25–0.5 MPa following degassing, and thus to consider the resulting, adjusted conductivity as the $K_{\max}$. Despite these challenges, we captured important trends and physiological responses that may shed light on future studies of the effects of herbivory on woody plants.

The key finding of our study is that persistent defoliation reduces stem water transport in a manner that scales with distal leaf area. Specifically, the continual removal of leaves and apices results in xylem with a lower vessel density. We suspect that the connection between leaf and xylem function in *C. rigidus* is mediated by cambial levels of indole-3-acetic acid (auxin), a hormone that is synthesized in new leaves and apical meristems. Auxin moves basipetally to the vascular cambium, where it stimulates mitosis in the cambial zone (Uggla et al. 1996) so reductions in auxin gradients limit cell division in the cambium. Lower auxin levels have been shown to reduce vessel densities in *Phaseolus* (Aloni and Zimmermann 1983), so persistent browsing in *C. rigidus* could have similar effects via auxin-mediated shifts in cambial activity. Given the higher fiber fraction and thicker stems in the browsed plants, it appears that fibers replaced the wood volume that would otherwise belong to vessels. Fiber differentiation responds to both auxin and gibberellin (Aloni 1987), thus browsing may have tipped the balance

of these two hormones. Furthermore, high concentrations of auxin are associated with suppressed lateral branch formation (Taiz and Zeiger 2010), so the potentially lower levels of auxin in browsed *C. rigidus* release the stems from apical dominance giving rise to the intertwined small twigs on the distal portions of the main stems (Fig. 1c). We might expect normal xylem formation in more sporadically browsed plants with the opportunity to re-grow.

Few studies have explicitly tested the effects of herbivory on plant water relations, but given that most plants experience some defoliation by herbivory or disturbance, both sporadic and continual defoliation may have important implications for community water balance and large-scale plant allometry. Ecosystems with high and persistent herbivory or animal disturbance might have lower leaf area indices, which could reduce vegetation demand for water and thus impact soil hydration. Large mammalian herbivores are often ecosystem engineers that trample and consume vegetation. While predators may keep herbivore numbers in check, as is the case with wolves and elk in Yellowstone National Park (Johnston et al. 2007), tipping the balance of such predator–prey interactions may ultimately have indirect effects on local hydrology. Secondly, plant allometry is also altered by herbivores, shifting tree stem diameter versus height-scaling exponents away from theoretical predictions of 2/3 depending on how the plant regrows (Archibald and Bond 2003; Moncrieff et al. 2011). This implies that some of the deviations from the idealized scaling laws reported in the literature on plants (see Coomes 2006) may be due to consumer activity that alters plant hormone patterns, and affects physiology and structure in ways that have previously not been accounted for.

Our work with *C. rigidus* shows that biotic interactions such as herbivory may cause observable shifts in plant allometry and hydraulic plant structure–function relationships. Hence, the effects of herbivores on plant performance should be considered in future studies of ecological plant physiology.

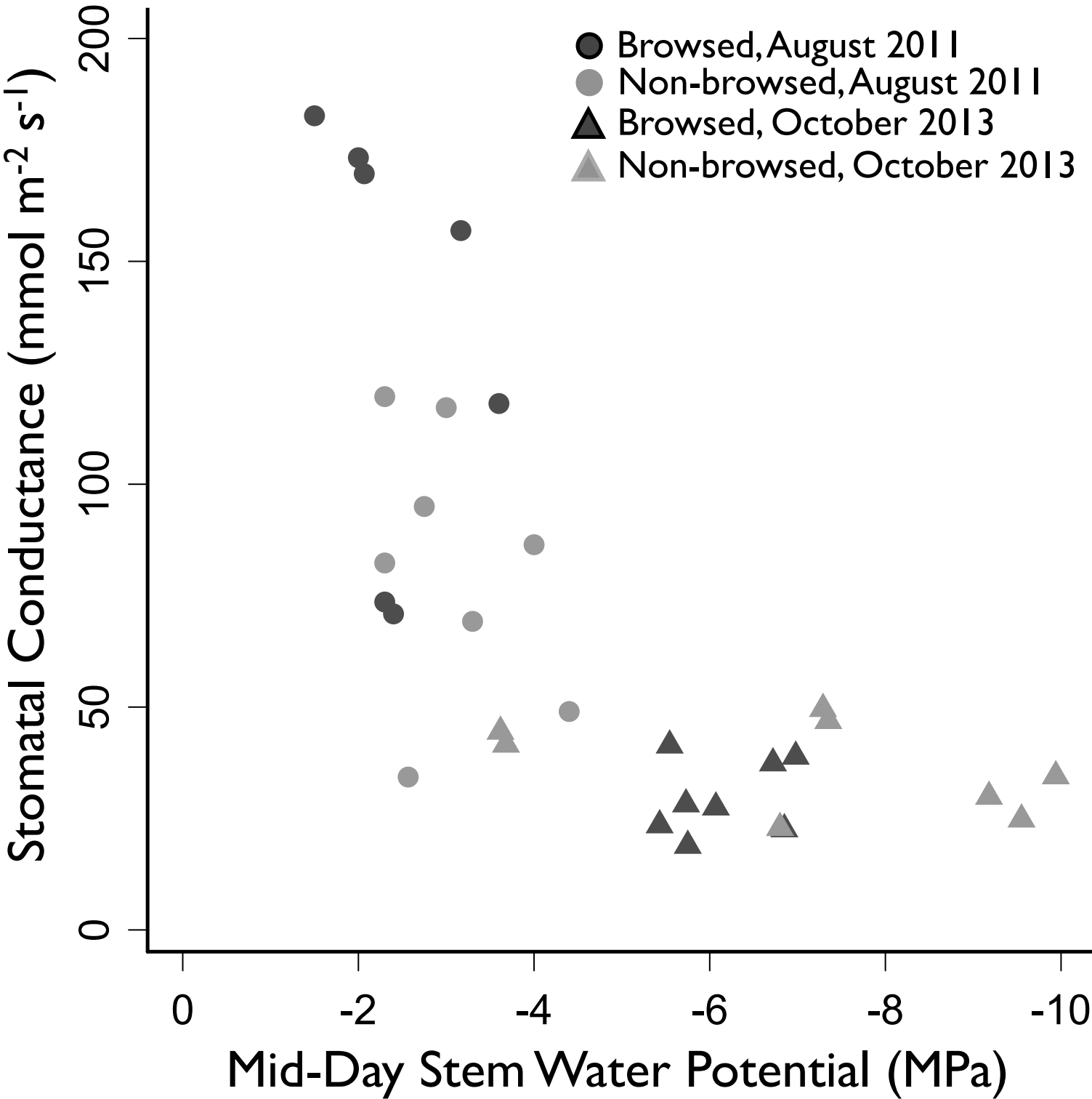
Acknowledgments We thank the U.C. Santa Cruz Natural Reserves office for support and access to the Fort Ord Natural Reserve. We also appreciate A. Jacobsen's advice regarding vessel length in *Ceanothus*, as well as the helpful comments on the manuscript from Susan Schwinning and two anonymous reviewers. The study was supported in part by UCSC Start-Up Funds to JP.

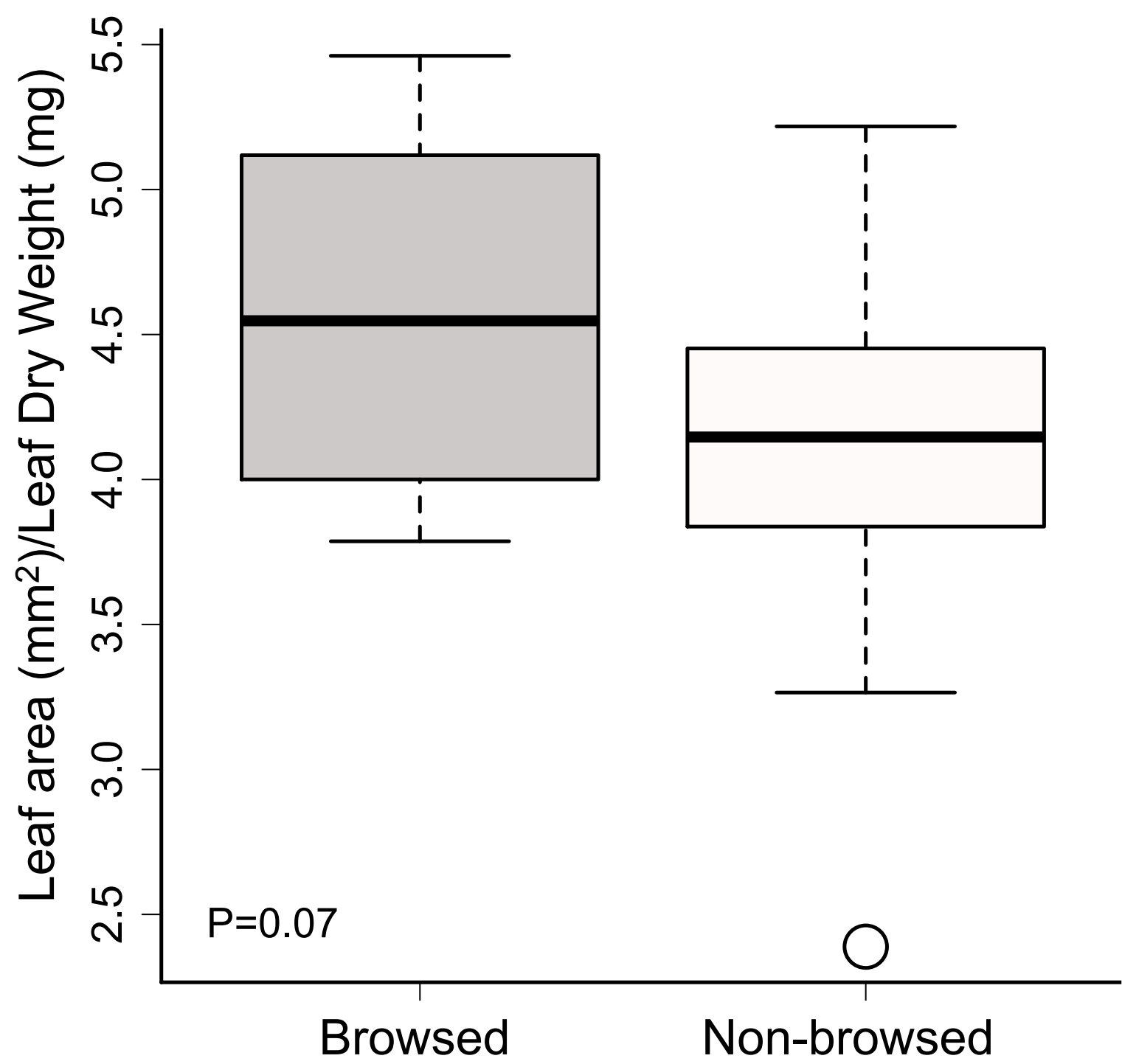
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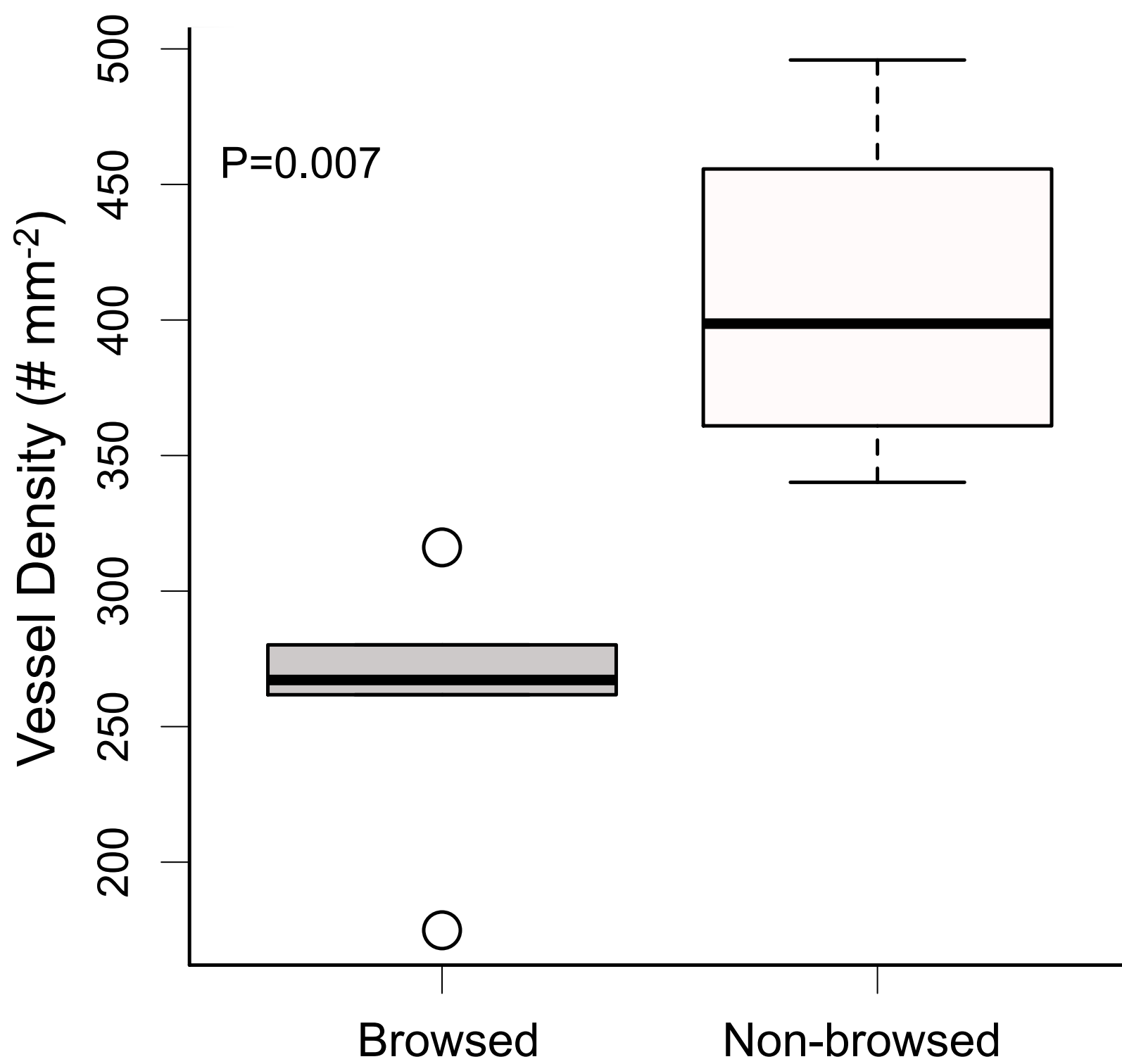
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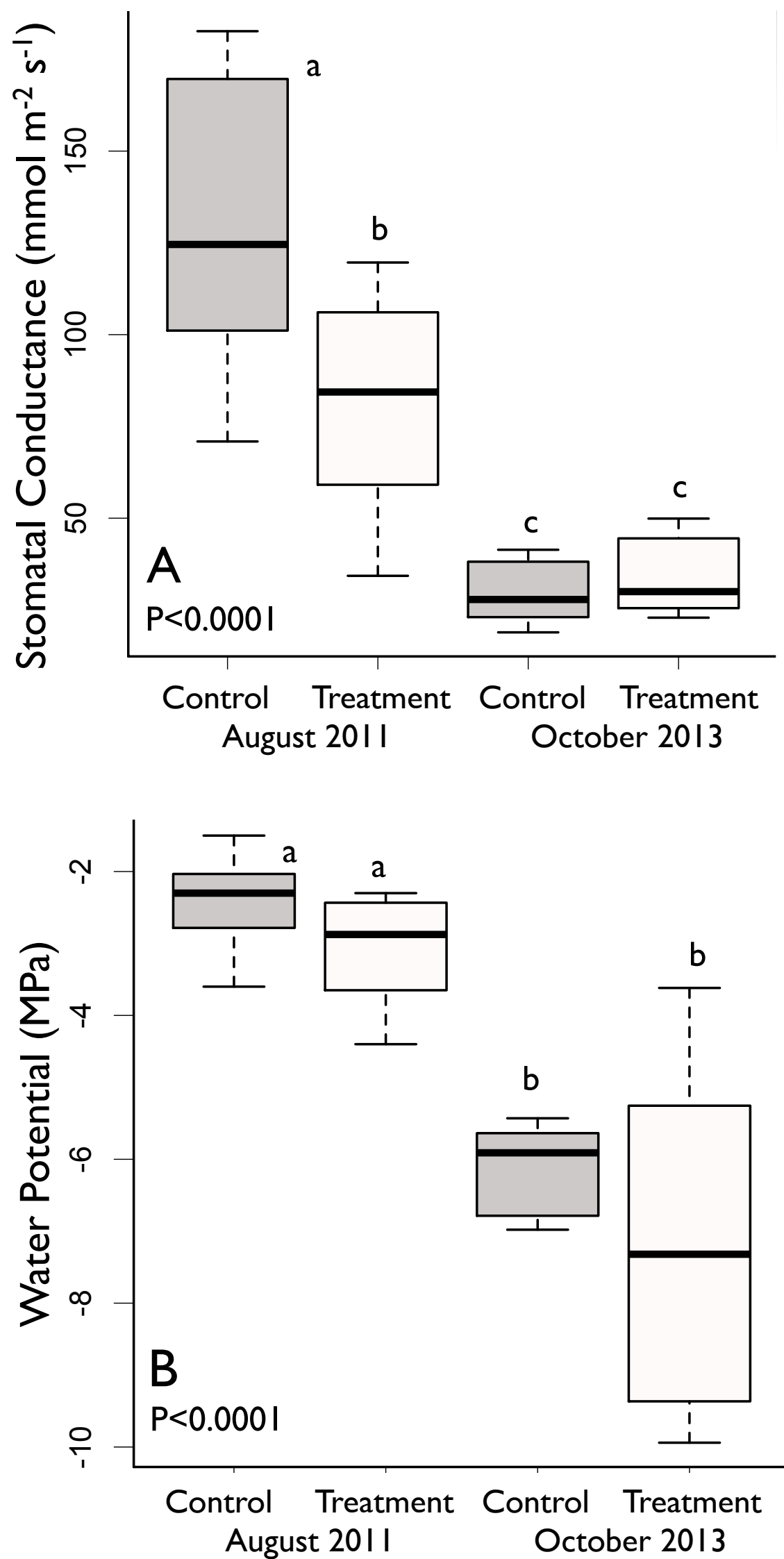




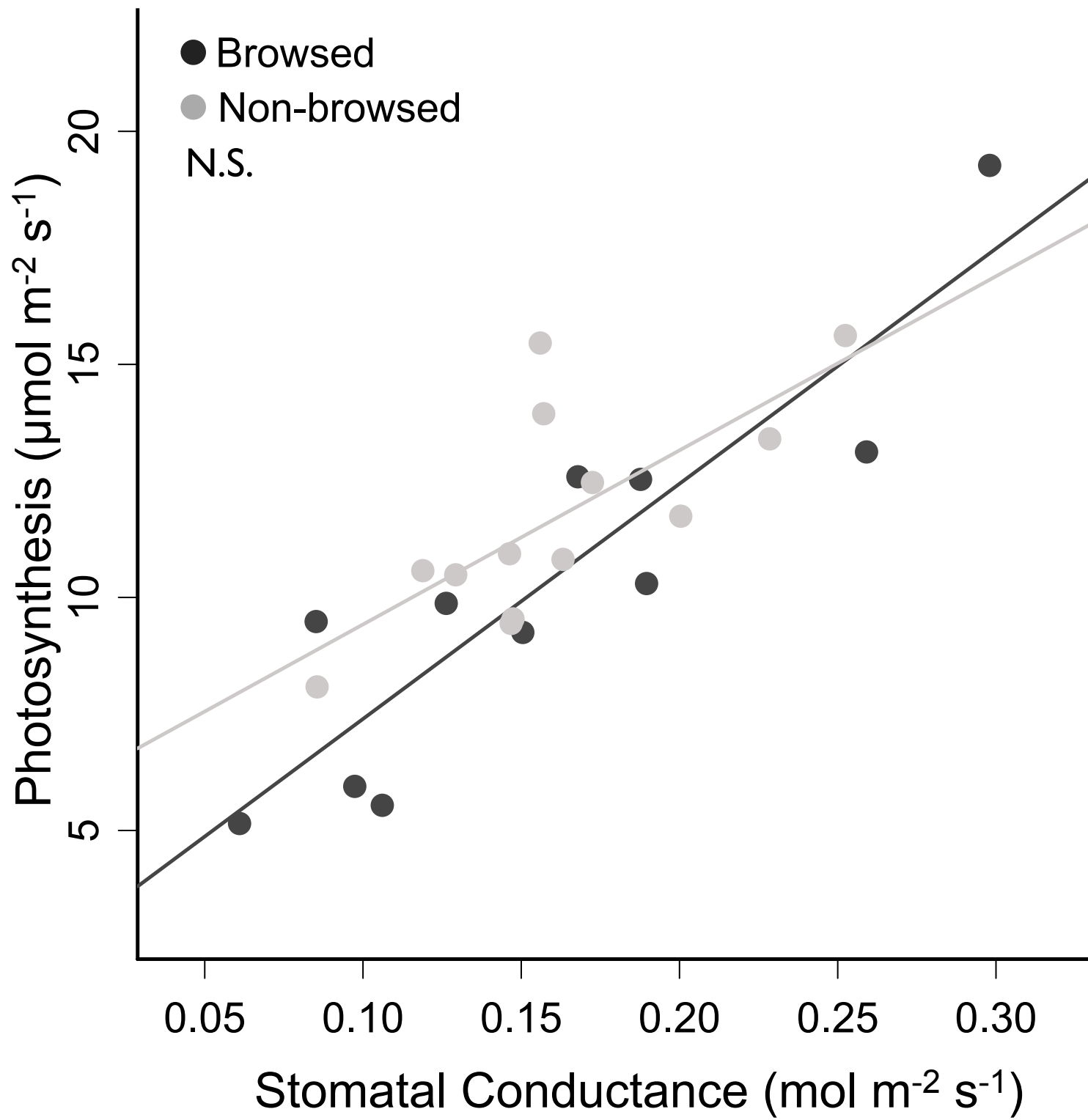
Supplemental Figure 1 Boxplots of the leaf area to leaf dry weight ratio in browsed and non-browsed *C. rigidus* shrubs.



Supplemental Figure 2 Boxplots of the vessel density in the stems of browsed and non-browsed *C. rigidus* plants.



Supplemental Figure 3 Boxplots of leaf stomatal conductance (A) and water potential (B) in browsed and non-browsed *C. rigidus* plants.



Supplemental Figure 3 The relationship between stomatal conductance and photosynthesis as measured on hydrated browsed and non-browsed *C. rigidus* leaves using an LI-6400 gas-exchange instrument.