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**VARIABILITY IN AMOUNT AND FREQUENCY OF WATER SUPPLY
AFFECTS ROOTS BUT NOT GROWTH OF ARID SHRUBS**

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Running title: *Heterogeneous water supply and plant responses*

1 Key-words: arid environments; drought, functional traits; Mediterranean shrubs; pulsed
2 events; relative growth rate; specific root length; water heterogeneity.

3
4 ABSTRACT

5 Rainfall and soil moisture variability have a strong effect on plant survival and
6 seed germination in arid environments, yet very little is known about the effects on
7 roots and growth of woody seedlings. Here we focused on the effects of variability in
8 both amount and frequency of water supply on juvenile root and leaf functional traits
9 and growth of seven Mediterranean shrub species occurring in arid SE Spain, *Anthyllis*
10 *cytisoides*, *Atriplex halimus*, *Ephedra fragilis*, *Genista umbellata*, *Lycium intricatum*,
11 *Retama sphaerocarpa* and *Salsola oppositifolia*. In a 14-month greenhouse experiment
12 we manipulated water supply expecting that reduced water amount and pulses of
13 watering of different magnitude affected functional traits and seedling growth, even if
14 the amount of water provided was the same. Different watering patterns altered soil
15 drying dynamics, with reduced supply of water amount and frequent watering becoming
16 the driest treatment. We found that roots of all species responded to alterations in water
17 supply by changing biomass allocation patterns (i.e., higher root-to-shoot mass [R:S]
18 ratio in droughted plants), and by altering fine roots diameter, measured in terms of
19 specific root length (SRL). Indeed, differences in growth rate among species were
20 significantly linked to fine roots diameter and biomass allocation, which relates to
21 uptake capacity of roots. However, relative growth rate (RGR) and leaf traits such as
22 specific leaf area (SLA) were insensitive, likely because prolonged droughts over longer
23 periods of time seem necessary to constraint growth in all these arid shrubs.

1 INTRODUCTION

2 Understanding how plant species deal with soil resource availability is a central
3 theme of plant ecological research (Chapin 1991; Lambers et al. 1998). Soil resources
4 required for plant growth are highly heterogeneous at a wide variety of scales both in
5 time and space. Nutrients (Schlesinger and Pilmanis 1998; Gallardo 2003) and water
6 (Burgess et al. 1998; Cantón et al. 2004) are not evenly distributed in space in natural
7 soils, and their temporal availability is not regular (Austin et al. 2004; Reynolds et al.
8 2004). Resource heterogeneity can impact individual plants in terms of survival,
9 growth, fitness, and biotic interactions (Hutchings and Kroon 1994; Cahill and Casper
10 1999; Poorter and Lager 2000; Hodge 2004; Padilla et al. 2007; Maestre and Reynolds
11 2007), and therefore can affect population dynamics.

12 In arid environments water availability is highly pulsed, and discrete rainfall
13 events interspersed with drought periods are important components of the annual water
14 supply (Noy-Meir 1985). Vegetation not only responds to rainfall amount (Noy-Meir
15 1985; Reynolds et al. 2004), but also to variations in time (Sala and Lauenroth 1982;
16 Turner & Randall 1989; Lázaro et al. 2001) in such a way that relatively small changes
17 in rainfall frequency might have strong effects on some species, particularly seedlings
18 of annuals (Novoplansky and Goldberg 2001; Sher et al. 2004). Since seedlings and
19 juveniles are more sensitive to dehydration than seeds or adults (Evans and Etherington
20 1991), variations in amount and frequency of water supply (i.e., greater, less frequent
21 events followed by longer drought periods) is bound to affect plants in different ways
22 (Easterling et al. 2000; Weltzin et al. 2003; Sher et al. 2004). However, the magnitude
23 of the response depends on species identity and habitat, and research has shown that
24 species from dry habitats tend to exhibit smaller responses than those from more mesic

1 environments (Novoplansky and Goldberg 2001; Volis et al. 2001; Sher et al. 2004)
2 presumably due to physiological constraints preventing them from responding to peaks
3 of resources (Sher et al. 2004; Chapin 1991). Growing attention has been paid recently
4 to the relationship between water inputs, species responses and ecosystems dynamics
5 (Reynolds et al. 2004; Schwinning et al. 2004; Heisler and Weltzin 2006) although most
6 research conducted in greenhouses focused on annual and grassland species
7 (Novoplansky and Goldberg 2001; Sher et al. 2004; Maestre and Reynolds 2007).
8 However, woody species could show different behavior and differ in their responses
9 from annuals, yet to our knowledge very little is known about the effects of
10 heterogeneity in water supply on shrub seedlings. Addressing these effects is not only
11 important for a better understanding of seedling strategies, but also to provide insights
12 into how rainfall variability and climate change could affect ecosystems.

13 In this paper we focus on the effects of variation in amount and temporal supply
14 of water on seven shrub species from arid SE Spain. In this area, among the driest in
15 Europe with less than 250 mm year⁻¹ (Capel-Molina 2000), rainfall timing and amount
16 greatly influence germination and seedling establishment (Pugnaire & Lázaro 2000;
17 Lázaro 2004; Pugnaire et al. 2006; Padilla and Pugnaire 2007), but there is little
18 information about the effects of heterogeneity in water input on shrub performance. In a
19 greenhouse experiment we modified water supply and analyzed the effects on plant
20 functional traits -as surrogates of performance (Violle et al. 2007), and growth,
21 expecting that pulses of water of different magnitude have different effects on plants,
22 even if the amount of water provided was kept constant (Knapp et al. 2002; Reynolds et
23 al. 2004). Recent results highlighted the plasticity of root growth in very young
24 seedlings of Mediterranean species (Padilla et al. 2007), and according to this evidence

1 we first hypothesized that roots would be very responsive to drying soil caused by
2 heterogeneity in watering. Secondly, we hypothesized that seedling growth and leaf
3 traits would be affected by water supply so that seedlings subjected to larger water
4 supplies would show greater growth rates.

5

6 MATERIALS AND METHODS

7 *Species*

8 We selected seven native shrub species occurring in Mediterranean arid
9 shrublands in the Tabernas basin (Almería, SE Spain, 37°08' N, 2°22' W, 490 m
10 elevation). This area is characterized by mild temperatures (17.8 °C average annual
11 temperature), and low and variable rainfall (235 mm annual rainfall, 1967-1997 period,
12 Confederación Hidrográfica del Sur), with a markedly dry season from June to
13 September (Lázaro et al. 2001). Species differed widely attending to leaf habit (nearly
14 leafless shrubs with photosynthetic stems, drought-deciduous shrubs and evergreen
15 species), drought tolerance based on minimum xylem pre-dawn water potential (Ψ_{pd})
16 recorded in the field and photosynthetic pathway (Table 1). While *Anthyllis cytisoides*
17 L., a small, drought-deciduous shrub (Haase et al. 2000), *Lycium intricatum* Boiss.,
18 *Atriplex halimus* L., *Salsola oppositifolia* Desf. (the two latter C₄ xero-halophyte
19 shrubs; Pyankov et al. 2001; Martínez et al. 2004), *Ephedra fragilis* Desf., and the
20 shallow-rooted *Genista umbellata* (L'Hér.) Dum. Cours., stand low water potentials
21 ($\Psi_{pd} < -5$ MPa; Lansac et al. 1994; Pugnaire et al. 2004), *Retama sphaerocarpa* (L.)
22 Boiss., a deep-rooted species, shows a more drought-sensitive behavior revealed by less
23 negative Ψ_{pd} (~ -1.5 MPa; Haase et al. 1999).

24

1 *Experimental design*

2 Seeds of the seven species were separately sown in germination trays containing
3 grade III vermiculite (Verlite®, Vermiculita y Derivados SL, Gijón, Spain) in
4 laboratory at room temperature and day light on 22 March 2005. Seeds from the
5 Tabernas basin were collected manually or provided by local nurseries. All seeds
6 germinated within three weeks, and very young seedlings were carefully transferred to
7 pots on 14 April, once cotyledons had fully emerged from seed coats. Six randomly
8 selected seedlings of every species were harvested before transplanting. At transplant,
9 one seedling was planted in each pot and tap water was provided daily. Pots of 300 mL
10 in volume contained vermiculite as above (bulk density $90 \text{ Kg m}^3 \pm 15 \text{ Kg m}^3$) and were
11 4.5 cm in diameter and 18 cm deep (Forest Pot 300®). We selected vermiculite because
12 of its relatively infertility, lower compaction and greater oxygenation than other
13 growing media. A nutrient solution (2 mL/L water) of a 4-5-6 NPK fertilizer (KB, Scott
14 France, Lyon, France) was added weekly for one month, and seedlings that died during
15 this period were replaced.

16 Pots were arranged in a factorial design with two factors (amount of water and
17 watering frequency) on 16 May. Watering treatments were established according to
18 climate change forecasts for the western Mediterranean Basin, consisting in a reduction
19 of annual rainfall of ~30% with a trend towards extended drought periods (IPCC 2001;
20 Sánchez-Rodrigo 2002). Although potted experiments deviate from natural conditions
21 in the field, we would rather to be consistent with these predictions and not apply
22 stronger yet arbitrary reductions. Amount of watering included a ‘control’ and a
23 ‘reduced’ level consisting of 30% less than the control, and frequency comprised a
24 ‘normal’ level (four watering events per week) and ‘half’ the number of events (two per

1 week). Since we focused on growth rather than survival, we considered that two
2 watering events per week were necessary to keep seedlings alive on the course of the
3 experiment. The amount and frequency factors were fully crossed in all species, and
4 seedlings subjected to ‘normal’ frequency were watered four times a week, either with
5 20 mL (‘control’) or 14 mL (‘reduced’) each, whereas those subjected to ‘half’
6 frequency were watered twice a week, either with 40 or 28 mL. Plants grew in a
7 greenhouse at the Estación Experimental de Zonas Áridas (CSIC, Almería) without
8 supplemental irradiance, temperature and further fertilization, and were kept for 14
9 months. Pot position was re-arranged at random every two weeks.

10

11 *Measurements and plant harvests*

12 To estimate the effect of altered watering on vermiculite moisture, we calculated
13 the gravimetric water content (%) corresponding to each treatment by weighing pots
14 before and after each watering during a two-week period before the final harvest. At the
15 end of the monitoring period pots were dried at 105 °C for 48 hours, emptied out and
16 weighed. The gravimetric water content (WC) was calculated following:

$$17 \quad WC \text{ (\%)} = \frac{(W_{wet} - W_{dry})}{(W_{dry} - W_{pot})} \times 100 \quad (1)$$

18 where W_{wet} was pot weight before and after watering, W_{dry} pot weight after drying, and
19 W_{pot} pot weight. Measurements were done in five unplanted pots per treatment because
20 it is a destructive method. Water potential corresponding to each moisture value was
21 estimated from vermiculite water retention curve (M.J. Steinbauer, *unpublished*),
22 obtained with a dewpoint potential meter (WP4-T, Decagon Devices, Inc., Pullman,
23 WA, USA).

1 Before plant harvest in June 2006, 5 to 15 leaves from the same aspect of each
2 plant, or 5 to 10 stem segments 5 cm long of leafless shrubs, were excised, scanned with
3 a portable scanner (Epson GT7000, Seiko Epson Corp., Nagano, Japan) at 300 dpi, and
4 the projected area measured with appropriate software (Midebmp v.4.2, R. Ordiales-
5 Plaza, 2000) to calculate leaf area (LA). LA of cylindrical leaves and stems were
6 corrected by $\pi/2$. Due to the small leaf size, leaves of each plant were scanned and
7 weighed together after drying at 72°C for > 48 hours, and averaged. Specific leaf area
8 (SLA, $\text{m}^2 \text{kg}^{-1}$) was computed as the ratio between leaf area and mass. *Lycium* leaves
9 were not measured because of their small size. At harvest, plants were clipped at ground
10 level and shoots were immediately labeled and stored in paper bags, dried and weighed.
11 Pots were emptied out into water and vermiculite attached to roots was removed by
12 brushing gently. Roots were then labeled, placed into wet paper towels and kept cool in
13 zip bags in a refrigerator before processing. To calculate specific root length (SRL, cm
14 g^{-1}), 5 to 10 fresh root segments 5 cm long of each plant were excised and digitalized.
15 Segment length was measured from digitalized traces using the macro RootMeasure
16 v.1.80 (Kimura and Yamasaki 2003) implemented on the software Scion Image Beta v.
17 4.02 (Scion Corp., Maryland, USA). Segment dry mass and root mass were obtained as
18 with leaves. Root-to-shoot mass (R:S) ratio for each plant was calculated from above
19 and belowground masses. Relative growth rate on plant mass (RGR, $\text{mg g}^{-1} \text{day}^{-1}$)
20 during the monitoring period was calculated from data at harvest (W_2) and transplant
21 (W_1) following:

$$22 \quad RGR = \frac{(\log W_2 - \log W_1)}{(t_2 - t_1)} \quad (2)$$

23 where $t_2 - t_1$ was 425 days, using the Hunt et al. (2002) spreadsheet tool.

24

1 *Statistics*

2 Vermiculite drying dynamics was analyzed using ANCOVA on daily water
3 content with time as covariate. Differences among treatments were considered
4 significant when the treatment x time interactions was significant at $P < 0.05$. We tested
5 differences in vermiculite water content at the end of the monitoring period through
6 factorial ANOVA followed by Tukey *post hoc* tests. This gives an estimate of the
7 lowest soil moisture plants dealt with.

8 Plant data were analyzed as a non-balanced nested factorial ANOVA with three
9 factors, species, water amount and frequency of watering. Since the ‘half’ level of the
10 frequency factor was lacking in *Atriplex* because most replicates died by summer, we
11 nested this factor within species. We ran independent ANOVA for each variable
12 followed by Tukey tests when significant differences at $P < 0.05$ were detected.
13 Heteroscedastic variables were transformed to meet ANOVA assumptions. Since plant
14 mass was unaffected by watering patterns, differences in RGR among species were
15 detected by one-way ANOVA using each treatment as a replicate ($n = 4$). Simple linear
16 regressions were performed to test correlation strength between variables, using
17 adjusted R^2 to correct for the degrees of freedom.

18 All tests were conducted with Statistica v.6.0 (Statsoft Inc, Tulsa, OK, USA) and
19 data are presented as means \pm one standard error. Because of differing mortality at
20 transplant, the final sample size of each combination ranged 6-14.

21

22 RESULTS

23 Watering treatments led to differences in vermiculite drying dynamics
24 (ANCOVA $_{\text{treatment} \times \text{time}} F_{3,312} = 4.135, P < 0.01$, Figure 1). Vermiculite moisture greatly

1 fluctuated with time, but in general it was lower in pots supplied with reduced water
2 amount (ANOVA_{amount} $F_{1,16}=80.580$, $P<0.001$) and normal frequency of watering
3 (ANOVA_{frequency} $F_{1,16} = 52.869$, $P<0.001$). Considering the lowest vermiculite moisture
4 registered, our treatments created a gradient that ranged from $24\pm 2\%$ (≈ 0 MPa) in the
5 control amount-half events combination, to $12\pm 0.7\%$ (-1.2 ± 0.4 MPa) for the control-
6 normal frequency, to $11\pm 1\%$ (-1.7 ± 0.2 MPa) for the reduced amount-half events, and to
7 $2\pm 1\%$ (< -12 MPa) for the reduced amount-normal frequency, which entailed reductions
8 in water moisture of 50, 54 and 92%, respectively. However, despite these reductions, it
9 is worth noting that only one watering treatment imposed severe levels of water deficit,
10 albeit only for short periods of time.

11 Water supply treatments affected the root traits R:S ratio and SRL (Table 2).
12 Plants subjected to reduced water amount allocated proportionally more biomass to
13 roots (i.e., higher R:S ratio, ANOVA_{amount} $F_{1,204} = 4.934$, $P<0.03$, Figure 2A) but no
14 consistent differences were found in species responsiveness (ANOVA_{species x water} $F_{6,204}$
15 $= 2.084$, $P>0.05$). Frequency of water supply had no effect on biomass allocation
16 patterns in any species (ANOVA_{frequency} $F_{6,204} = 1.125$, $P>0.3$), whereas it did affect
17 SRL ($P<0.05$), interacting with the amount of water provided (ANOVA_{amount x frequency}
18 $F_{6,204} = 2.363$, $P<0.04$). This is, regular watering at reduced water amount (thus our
19 driest treatment) increased SRL, meaning roots became thinner, while there was no
20 effect of frequency on SLR at higher watering level (Figure 2B).

21 Neither amount nor frequency of water supply consistently affected plant, shoot
22 and root mass at harvest in any species ($P>0.07$). Leaf traits such as LA and SLA did
23 not differ among watering treatments ($P>0.1$, Figure 3), and drought-deciduous shrubs
24 did not shed leaves throughout the monitoring period.

1 When comparing among species, we found significant differences in plant mass
2 and growth rate (ANOVA_{RGR} $F_{6,19} = 268.02$, $P < 0.001$). The highest RGR was achieved
3 by *Atriplex*, followed by *Anthyllis* and *Lycium*, while *Retama* and *Salsola* showed
4 distinctly lower growth rates (Table 3). We also detected differences in biomass
5 allocation (Figure 3), with the R:S ratio being especially high in *Anthyllis* (2.59 ± 0.16),
6 and well above 1 in *Retama* (1.67 ± 0.04) and *Genista* (1.17 ± 0.07). In contrast, *Salsola*
7 allocated proportionally the least to roots (0.81 ± 0.04). R:S ratio of summer-deciduous
8 species showed a great variability, ranging from the largest value in *Anthyllis* to one of
9 the lowest in *Lycium*. Nevertheless, the lack of clear links between leaf habit and
10 biomass allocation does not rule out their existence, which have been revealed by
11 Antúnez et al. (2001) in other Mediterranean species, but may rather reflect the small
12 number of replicates within each functional group. As for leaf traits, SLA showed
13 considerable contrast among species ($P < 0.001$), with *Anthyllis* and *Atriplex* having the
14 highest SLA, which differed from other species, notably from the species with
15 photosynthetic stems *Ephedra* and *Retama*. As for root traits, *Salsola* showed the lowest
16 SRL ($2210 \pm 157 \text{ cm g}^{-1}$), and *Lycium* and *Atriplex* the largest ($\sim 5100 \pm 330 \text{ cm g}^{-1}$).

17 We found a positive relationship between seedling growth rate (RGR) and
18 specific root length (SRL, $R^2 = 0.50$, $P < 0.001$). RGR was also positively related to a
19 lesser extent to specific leaf area ($R^2 = 0.29$, $P < 0.01$), leaf area ($R^2 = 0.23$, $P < 0.02$), and
20 root-to-shoot mass ratio ($R^2 = 0.12$, $P < 0.05$, Figure 4). In agreement with published data
21 (Wright and Westoby 1999; Antúnez et al. 2001), we found that summer-deciduous
22 species, *Anthyllis* and *Lycium*, had in general greater SLA and SRL, and consequently
23 faster RGR than evergreen species. Differences in growth rate among species were
24 linked to differences in traits that maximize uptake capacity of roots and leaves such as

1 SRL and SLA (Garnier 1991; Cornelissen et al. 1996; Reich et al. 1998; Comas and
2 Eissenstat 2004), rather than to differences in biomass allocation to roots.

3

4 DISCUSSION

5 By modifying water supply patterns we caused a strong alteration of substrate
6 drying dynamics, as well as large decreases in vermiculite moisture and water potential,
7 especially in the reduced water amount and frequent (normal) watering treatment, which
8 was the only watering treatment that imposed severe levels of water deficit, albeit only
9 for short periods of time. Less frequent (half) watering events resulted the moistest,
10 perhaps due to the effect of large water pulses on vermiculite water holding capacity.

11 Our first hypothesis that roots would be very responsive to modified soil drying
12 dynamics holds since functional traits related to water acquisition changed in droughted
13 roots, regardless of the species. Despite the wide variation in species leaf habit
14 (evergreen, drought-deciduous, and leafless shrubs), photosynthetic pathway (C_3 and
15 xero-halophyte C_4 shrubs) and drought tolerance (tolerants and avoiders), juveniles of
16 all species dealt with heterogeneous water supply by changing biomass allocation
17 patterns and root diameter, which agrees with previous studies (Padilla et al. 2007). Our
18 driest watering (i.e., reduced water amount at regular intervals), which was the only
19 treatment that imposed severe water deficit, resulted in higher SRL, while there was no
20 effect on SLR at moister watering levels. Likely, this was due to reduced average root
21 diameter (i.e., thinner roots) as we quantified SRL from root segments, instead of using
22 the whole root system. Nevertheless, we cannot rule out that severe water deficit may
23 also have enlarged the overall proportion of fine roots.

24

1 Biomass allocation to roots relative to shoots (i.e., R:S ratio) and root diameter
2 (measured in terms of SRL) are believed to be involved in water and nutrient uptake
3 rates (Chapin et al. 1987; Eissenstat 1992; Lambers et al. 1998; Cornelissen et al. 2003);
4 thus, the larger R:S ratio and thinner roots reported in plants subjected to severe water
5 stress could be interpreted as a strategy to maximize absorptive root surfaces (Reich et
6 al.1998; Wright and Westoby 1999; Fernández and Reynolds 2000).

7 It was expected growth and leaf traits to be affected in the driest watering
8 pattern, but no significant effects on these variables were observed. Water limitation
9 selects for smaller leaves and lower SLA (Cornelissen et al. 2003; Wright et al. 2006);
10 however, we did not detect leaf adjustments in response to lower soil moisture and,
11 because of the tight correlation between SLA and growth rate (Cornelissen et al. 1996;
12 Wright and Westoby 1999), we likely did not find differences in biomass or relative
13 growth rate (RGR). These results contrast with reports conducted under controlled
14 conditions. Fernández and Reynolds (2000) found that plant mass and SLA of eight
15 perennial C₄ desert grasses were markedly reduced by severe drought. In other
16 Mediterranean perennial species, soil water deficits also decreased SLA and growth rate
17 (Sack and Grubb 2002; Galmés et al. 2005; Sánchez-Gómez et al. 2006).

18 In our driest treatment, water content reached as low as 2% (< -12 MPa), but
19 roots faced such dry soil for few days, being in fairly moister vermiculite (> -1.7 MPa)
20 the remaining time. It is thus reasonable to think that watering every three days
21 maximized water uptake at moisture peaks, making less relevant for plant growth the
22 inter-pulse dry period. So, although the severe habitats where our species inhabit could
23 have led to plant adaptation to very variable water inputs, stronger and more prolonged
24 water stress periods than that we applied seems to be needed to constraint plant growth.

1 However, roots at the juvenile stage seem to be very sensitive to soil water deficits,
2 presumably responding to compensate to some extent for fluctuating soil water
3 availability.

4

5 ACKNOWLEDGEMENTS

6 We thank an anonymous referee for comments on an earlier draft, and vivero de
7 Rodalquilar (Junta de Andalucía) for seed donation. The Spanish Ministry of Education
8 and Science funded this work (grant CGL2004-00090/CLI). FMP was supported by an
9 I3P-predocctoral fellowship (CSIC-European Social Fund).

10

11 REFERENCES

- 12 Antúnez I, Retamosa EC, Villar R (2001) Relative growth rate in
13 phylogenetically related deciduous and evergreen woody species. *Oecologia*
14 128:172-180.
- 15 Austin AT, Yahdjian L, Stark JM et al. (2004) Water pulses and biogeochemical cycles
16 in arid and semiarid ecosystems. *Oecologia* 141:221-235.
- 17 Burgess SSO, Adams MA, Turner MC et al. (1998) The redistribution of soil water by
18 tree root systems. *Oecologia* 115:306-311.
- 19 Cahill J R, Casper B B (1999) Growth consequences of soil nutrient heterogeneity for
20 two old-field herbs, *Ambrosia artemisiifolia* and *Phytolacca americana*, grown
21 individually and in combination. *Ann Bot* 83:471-478.
- 22 Cantón Y, Solé-Benet A, Domingo F (2004) Temporal and spatial pattern of soil
23 moisture in semiarid badlands of SE Spain. *J Hydrol* 285:199-214.
- 24 Capel-Molina JJ (2000) *El clima de la Península Ibérica*. Ariel Geografía, Barcelona.

- 1 Chapin FS, Bloom AJ, Field CB et al. (1987) Plant responses to multiple environmental
2 factors. *BioScience* 37:49-57.
- 3 Chapin FS (1991) Integrated responses of plants to stress. *BioScience* 41:29-36.
- 4 Comas LH, Eissenstat DM (2004) Linking fine root traits to maximum potential growth
5 rate among 11 mature temperate tree species. *Funct Ecol* 18:388-397.
- 6 Cornelissen JHC, Diez PC, Hunt R (1996) Seedling growth, allocation and leaf
7 attributes in a wide range of woody plant species and types. *J Ecol* 84:755-765.
- 8 Cornelissen JHC, Lavorel S, Garnier E et al. (2003) A handbook of protocols for
9 standardised and easy measurements of plant functional traits worldwide. *Aust J*
10 *Bot* 51:335-380.
- 11 Easterling DR, Meehl GA, Parmesan C et al. (2000) Climate extremes: Observations,
12 modeling, and impacts. *Science* 289:2068-2074.
- 13 Eissenstat DM (1992) Costs and benefits of constructing roots of small diameter. *J Plant*
14 *Nutr* 15:763-782.
- 15 Evans CE, Etherington JR (1991) The effect of soil water potential on seedling growth
16 of some British plants. *New Phytol* 118:571-579.
- 17 Fernández RJ, Reynolds JF (2000) Potential growth and drought tolerance of eight
18 desert grasses: lack of a trade-off? *Oecologia* 123:90-98.
- 19 Gallardo A (2003) Spatial variability of soil properties in a floodplain forest in
20 northwest Spain. *Ecosystems* 6:564-576.
- 21 Galmés J, Cifre J, Medrano H et al. (2005) Modulation of relative growth rate and its
22 components by water stress in Mediterranean species with different growth
23 forms. *Oecologia* 145:21-31.

- 1 Garnier E (1991) Resource capture, biomass allocation and growth herbaceous plants.
2 Trends Ecol Evol 6:126-131.
- 3 Haase P, Pugnaire FI, Clark SC et al. (1999) Diurnal and seasonal changes in cladode
4 photosynthetic rate in relation to canopy age structure in the leguminous shrub
5 *Retama sphaerocarpa*. Funct Ecol 13:640-649.
- 6 Haase P, Pugnaire FI, Clark SC et al. (2000) Photosynthetic rate and canopy
7 development in the drought-deciduous shrub *Anthyllis cytisoides* L. J Arid
8 Environ 46:79-91.
- 9 Heisler JL, Weltzin JF (2006) Variability matters: towards a perspective on the
10 influence of precipitation on terrestrial ecosystems. New Phytol 172:189-192.
- 11 Hodge A (2004) The plastic plant: root responses to heterogeneous supplies of nutrients.
12 New Phytol 162:9-24.
- 13 Hunt R, Causton DR, Shipley B et al. (2002) A modern tool for classical plant growth
14 analysis. Ann Bot 90:485-488.
- 15 Hutchings M J, de Kroon H (1994) Foraging in plants - the role of morphological
16 plasticity in resource acquisition. Adv Ecol Res 25:159-238.
- 17 IPCC (2001) Third assessment report of the intergovernmental panel on climate change.
18 Cambridge University Press, New York.
- 19 Kimura K, Yamasaki S (2003) Accurate root length and diameter measurement using
20 NIH Image: use of Pythagorean distance for diameter estimation. Plant Soil
21 254:305-315.
- 22 Knapp AK, Fay PA, Blair JM et al. (2002) Rainfall variability, carbon cycling, and
23 plant species diversity in a mesic grassland. Science 298:2202-2205.

- 1 Lambers H, Chapin FS, Pons TL (1998) Growth and allocation. In: Plant Physiological
2 Ecology. Springer-Verlag, New York, pp299-351.
- 3 Lansac AR, Zaballos JP, Martin A (1994) Seasonal water potential changes and proline
4 accumulation in Mediterranean shrubland species. Plant Ecol 113:141-154.
- 5 Lázaro R, Rodrigo FS, Gutiérrez L et al. (2001) Analysis of a 30-year rainfall record
6 (1967-1997) in semi-arid SE Spain for implications on vegetation. J Arid
7 Environ 48:373-395.
- 8 Lázaro R (2004) Implications of precipitation on vegetation of water-limited lands. In:
9 Recent Research Development in Environmental Biology. Research Signpost,
10 Kerala, India, pp553–591.
- 11 Maestre FT, Reynolds JF (2007) Amount of pattern? Grassland responses to the
12 heterogeneity and availability of two key resources. Ecology 88:501-511.
- 13 Martínez JP, Lutts S, Schanck A et al. (2004) Is osmotic adjustment required for water
14 stress resistance in the Mediterranean shrub *Atriplex halimus* L.? J Plant Physiol
15 161:1041-1051.
- 16 Novoplansky A, Goldberg DE (2001) Effects of water pulsing on individual
17 performance and competitive hierarchies in plants. J Veg Sci 12:199-208.
- 18 Noy-Meir I (1985) Desert ecosystem structure and function. In: Evenari M, Noy-Meir I,
19 Goodall DW (eds) Ecosystem of the world. Hot deserts and arid shrublands.
20 Elsevier, Amsterdam, pp93-103.
- 21 Padilla FM, Pugnaire FI (2007) Rooting depth and soil moisture control Mediterranean
22 woody seedling survival during drought. Funct Ecol 21:489-495.
- 23 Padilla FM, Miranda JD, Pugnaire FI (2007) Early root growth plasticity in seedlings of
24 three Mediterranean woody species. Plant Soil 296:103-113.

- 1 Poorter H, Nagel O (2000) The role of biomass allocation in the growth response of
2 plants to different levels of light, CO₂, nutrients and water: a quantitative review.
3 *Aust J Plant Physiol* 27:595-607.
- 4 Pugnaire FI, Lázaro R (2000) Seed bank understorey species composition in a semi-arid
5 environment: the effect of scrub age and rainfall. *Ann Bot* 86:807-813.
- 6 Pugnaire FI, Armas C, Valladares F (2004) Soil as a mediator in plant-plant interactions
7 in a semi-arid community. *J Veg Sci* 15:85-92.
- 8 Pugnaire FI, Luque MT, Armas C et al. (2006) Colonization processes in semi-arid
9 Mediterranean old-fields. *J Arid Environ* 65:591-603.
- 10 Pyankov V, Ziegler H, Kuz'min A et al. (2001) Origin and evolution of C-4
11 photosynthesis in the tribe Salsoleae (Chenopodiaceae) based on anatomical and
12 biochemical types in leaves and cotyledons. *Plant Syst Evol* 230:43-74.
- 13 Reich PB, Tjoelker MG, Walters MB et al. (1998) Close association of RGR, leaf and
14 root morphology, seed mass and shade tolerance in seedlings of nine boreal tree
15 species grown in high and low light. *Funct Ecol* 12:327-338.
- 16 Reynolds JF, Kemp PR, Ogle K et al. (2004) Modifying the 'pulse-reserve' paradigm
17 for deserts of North America: precipitation pulses, soil water, and plant
18 responses. *Oecologia* 141:194-210.
- 19 Reynolds JF, Virginia RA, Kemp PR et al. (1999) Impact of drought on desert shrubs:
20 effects of seasonality and degree of resource island development. *Ecol Monogr*
21 69:69-106.
- 22 Sack L, Grubb PJ (2002) The combined impacts of deep shade and drought on the
23 growth and biomass allocation of shade-tolerant woody seedlings. *Oecologia*
24 131:175-185.

- 1 Sala OE, Lauenroth WK (1982) Small rainfall events – An ecological role in semi-arid
2 regions. *Oecologia* 53:301-304.
- 3 Sánchez-Gómez D, Valladares F, Zavala MA (2006) Performance of seedlings of
4 Mediterranean woody species under experimental gradients of irradiance and
5 water availability: trade-offs and evidence for niche differentiation. *New Phytol*
6 170:795-806.
- 7 Sánchez-Rodrigo F (2002) Cambio climático y extremos pluviométricos. In: Contreras
8 S, Piquer M, Cabello J (eds) *Agricultura, Agua y Sostenibilidad en la provincia*
9 *de Almería*. Junta de Andalucía, Almería, pp283-299.
- 10 Schlesinger WH, Pilmanis AM (1998) Plant-soil interactions in deserts.
11 *Biogeochemistry* 42:169-187.
- 12 Schwinning S, Sala OE, Loik ME et al. (2004) Thresholds, memory, and seasonality:
13 understanding pulse dynamics in arid/semi-arid ecosystems. *Oecologia* 141:191-
14 193.
- 15 Sher AA, Goldberg DE, Novoplansky A (2004) The effect of mean and variance in
16 resource supply on survival of annuals from Mediterranean and desert
17 environments. *Oecologia* 141:353-362.
- 18 Turner FB, Randall DC (1989) Net production by shrubs and winter annuals in
19 Southern Nevada. *J Arid Environ* 17:23-36.
- 20
- 21 Violle C, Navas ML, Vile D et al. (2007) Let the concept of trait be functional! *Oikos*
22 116:882-892.
- 23 Volis S, Mendlinger S, Ward D (2001) Differentiation in populations of *Hordeum*
24 *spontaneum* Koch along a gradient of environmental productivity and

- 1 predictability: plasticity in response to water and nutrient stress. *Biol J Linn Soc*
2 75:301-312.
- 3 Weltzin JF, Loik ME, Schwinning S et al. (2003) Assessing the response of terrestrial
4 ecosystems to potential changes in precipitation. *BioScience* 53:941-952.
- 5 Wright IJ, Westoby M (1999) Differences in seedling growth behaviour among species:
6 trait correlations across species, and trait shifts along nutrient compared to
7 rainfall gradients. *J Ecol* 87:85-97.
- 8 Wright IJ, Reich PB, Westoby M et al. (2004) The worldwide leaf economics spectrum.
9 *Nature* 428:821-827.
- 10

1 TABLES

2 **Table 1.** Main plant traits

Species	Family	Leaf habit	Drought strategy	Photosynthetic pathway
<i>Ephedra fragilis</i>	Ephedraceae	Leafless	Tolerant	C ₃ , photosynthetic stems
<i>Genista umbellata</i>	Leguminosae	Leafless	Tolerant	C ₃ , photosynthetic stems
<i>Retama sphaerocarpa</i>	Leguminosae	Leafless	Avoider	C ₃ , photosynthetic stems
<i>Anthyllis cytisoides</i>	Leguminosae	Deciduous	Tolerant	C ₃ , leaves
<i>Lycium intricatum</i>	Solanaceae	Deciduous	Tolerant	C ₃ , succulent leaves
<i>Atriplex halimus</i>	Chenopodiaceae	Evergreen	Tolerant	C ₄ , leaves
<i>Salsola oppositifolia</i>	Chenopodiaceae	Evergreen	Tolerant	C ₄ , succulent leaves

3

1 **Table 2.** P-values of nested factorial-ANOVA at harvest on plant, shoot and root mass,
 2 root-to-shoot mass (R:S) ratio, leaf area, specific leaf area (SLA), and specific root
 3 length (SRL). Frequency factor was nested within species. Significant effects are shown
 4 by bold at P<0.05.

	Effect				
	Species (S)	Quantity (Q)	Frequency (F(S))	S x Q	Q x F(S)
Plant mass	< 0.001	0.907	0.314	0.072	0.687
Shoot mass	< 0.001	0.633	0.470	0.091	0.787
Root mass	< 0.001	0.618	0.078	0.178	0.529
R:S ratio	< 0.001	0.027	0.349	0.057	0.627
Leaf area	< 0.001	0.895	0.434	0.329	0.914
SLA	< 0.001	0.793	0.103	0.193	0.482
SRL	< 0.001	0.589	0.048	0.929	0.031

5

1 **Table 3.** Relative growth rate (RGR, mg g⁻¹ week⁻¹) for each species x combination and
 2 average (\pm SE). Control and reduced refer to water amount, and normal and half to
 3 frequency of watering. Significant differences among species are indicated at P<0.05 by
 4 differing superscript letters (ANOVA after Tukey test).

5

Species	Control		Reduced		Average
	Normal	Half	Normal	Half	
<i>Anthyllis</i>	100.9 \pm 14.9	97.0 \pm 15.4	101.8 \pm 14.8	98.2 \pm 13.3	99.5 \pm 14.6 ^a
<i>Atriplex</i>	113.2 \pm 7.0	-	107.5 \pm 8.7	-	110.4 \pm 7.9 ^b
<i>Ephedra</i>	76.8 \pm 6.7	73.1 \pm 13.0	73.7 \pm 9.6	77.9 \pm 7.5	75.4 \pm 9.2 ^c
<i>Genista</i>	67.5 \pm 14.1	65.7 \pm 21.3	75.2 \pm 14.1	73.0 \pm 14.1	70.4 \pm 15.9 ^c
<i>Lycium</i>	90.7 \pm 7.9	84.8 \pm 9.7	85.9 \pm 9.2	83.7 \pm 10.4	86.3 \pm 9.3 ^d
<i>Retama</i>	57.6 \pm 7.6	61.3 \pm 6.5	56.8 \pm 9.4	57.5 \pm 7.4	58.3 \pm 7.7 ^e
<i>Salsola</i>	32.2 \pm 4.8	31.3 \pm 4.8	35.8 \pm 7.3	33.2 \pm 5.4	33.1 \pm 5.6 ^f

6

7

1 FIGURE LEGENDS

2

3 Figure 1. Mean gravimetric water content (%) and estimated water potential (MPa) of
4 vermiculite, recorded in five unplanted pots for every combination during a 16-day
5 watering cycle before the final harvest. Normal and half events refer to frequency of
6 watering. Crosses show vermiculite water potentials below -12 MPa.

7

8 Figure 2. (A) Effect of water amount on root-to-shoot (R:S) mass ratio for each species.
9 Ant, *Anthyllis cytisoides*; Atr, *Atriplex halimus*; Eph, *Ephedra fragilis*; Gen, *Genista*
10 *umbellata*; Lyc, *Lycium intricatum*; Ret, *Retama sphaerocarpa*; Sal, *Salsola*
11 *oppositifolia*. (B) Effect of water amount and water frequency on specific root length
12 pooling over species. P-values show significance of main factors and interactions after
13 ANOVA. Values are means \pm 1 SE.

14

15 Figure 3. Plant, root and shoot mass, R: S ratio, leaf area (LA), specific leaf area (SLA),
16 and specific root length (SRL) for each species x combination at harvest. Control and
17 reduced refer to water amount, and normal and half to frequency of watering. Different
18 letters show significant differences among species after Tukey's *post hoc* test
19 (ANOVA_{species} for all species P<0.001). Species abbreviations as in Fig. 2.

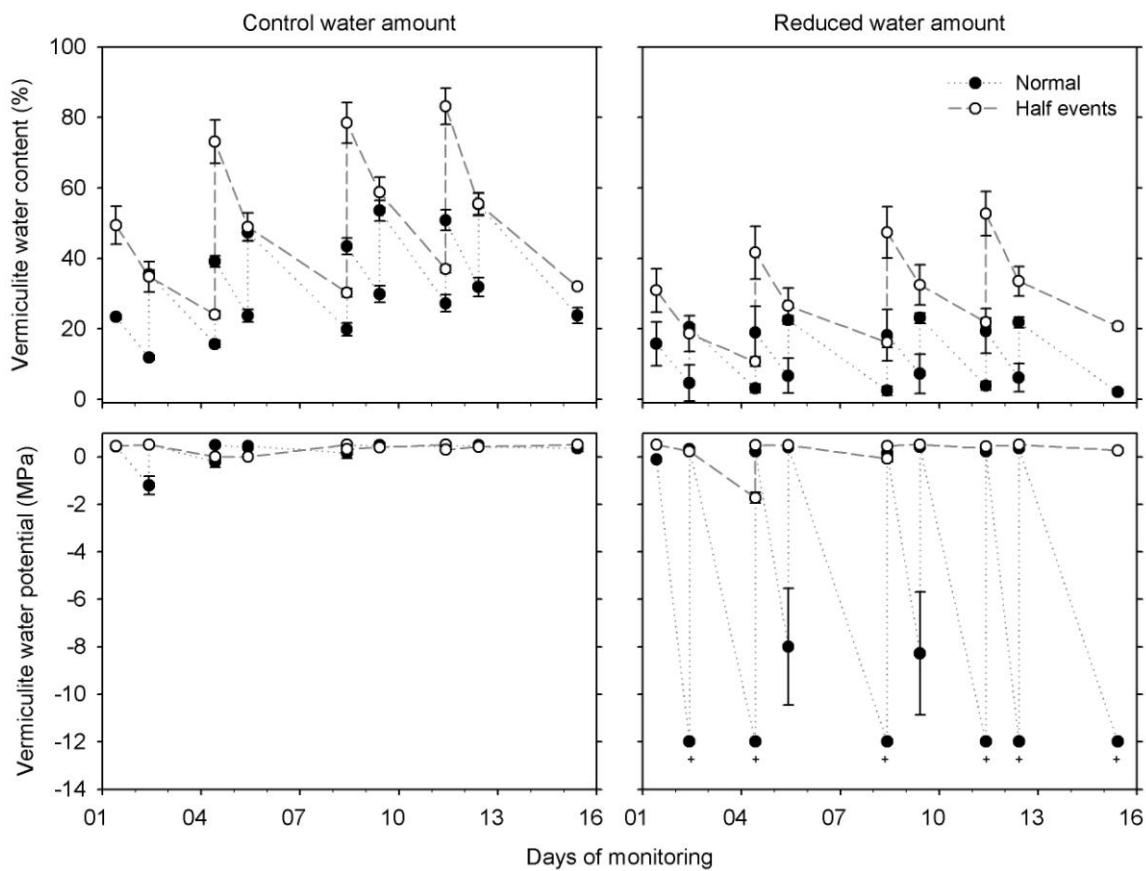
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21 Figure 4. Relationships between relative growth rate (RGR) and, leaf area (LA), specific
22 leaf area (SLA), specific root length (SRL), and root:shoot (R:S) ratio. Each point
23 represents mean value for each treatment.

24

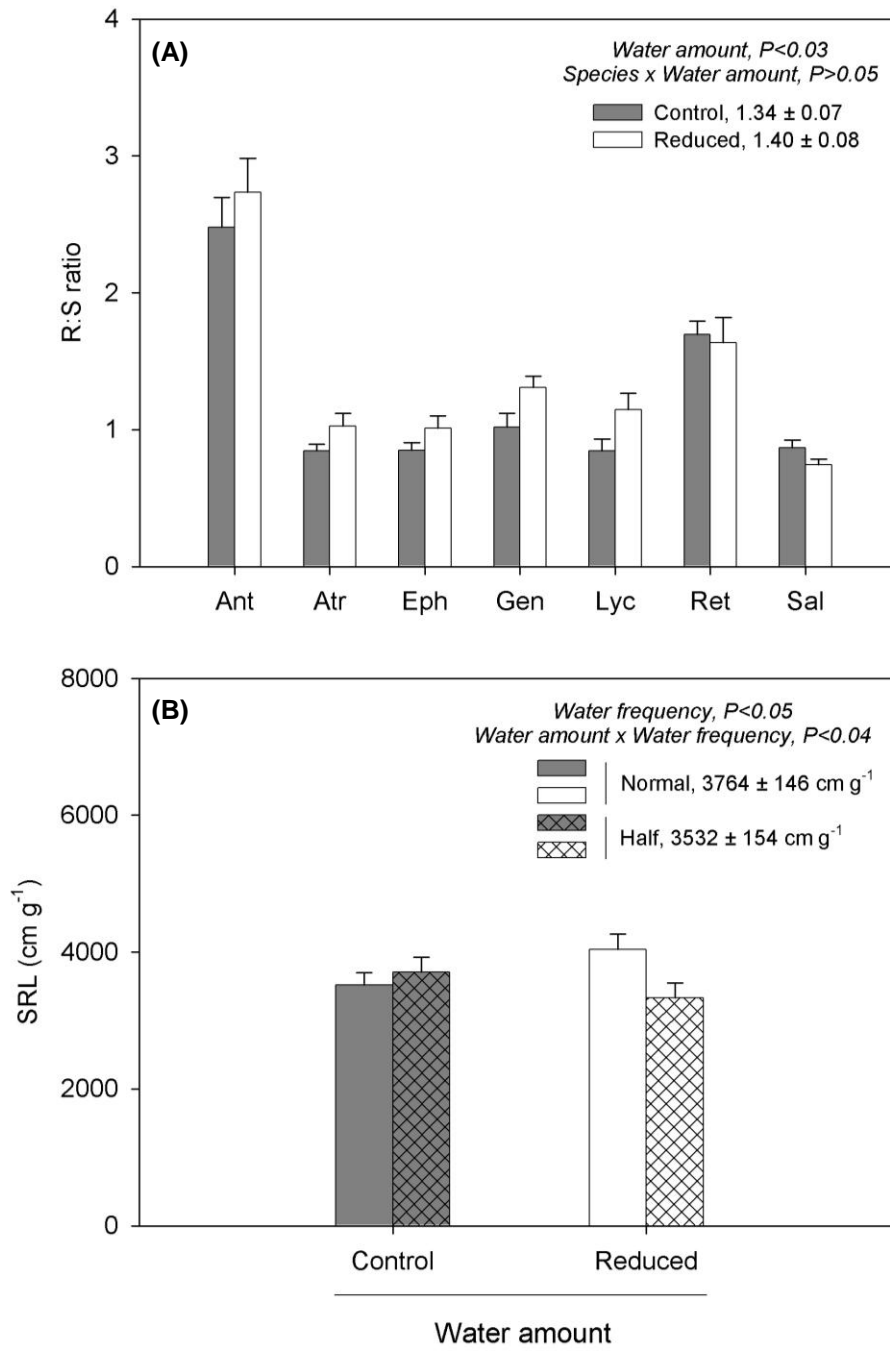
1 FIGURES

2 Figure 1



3

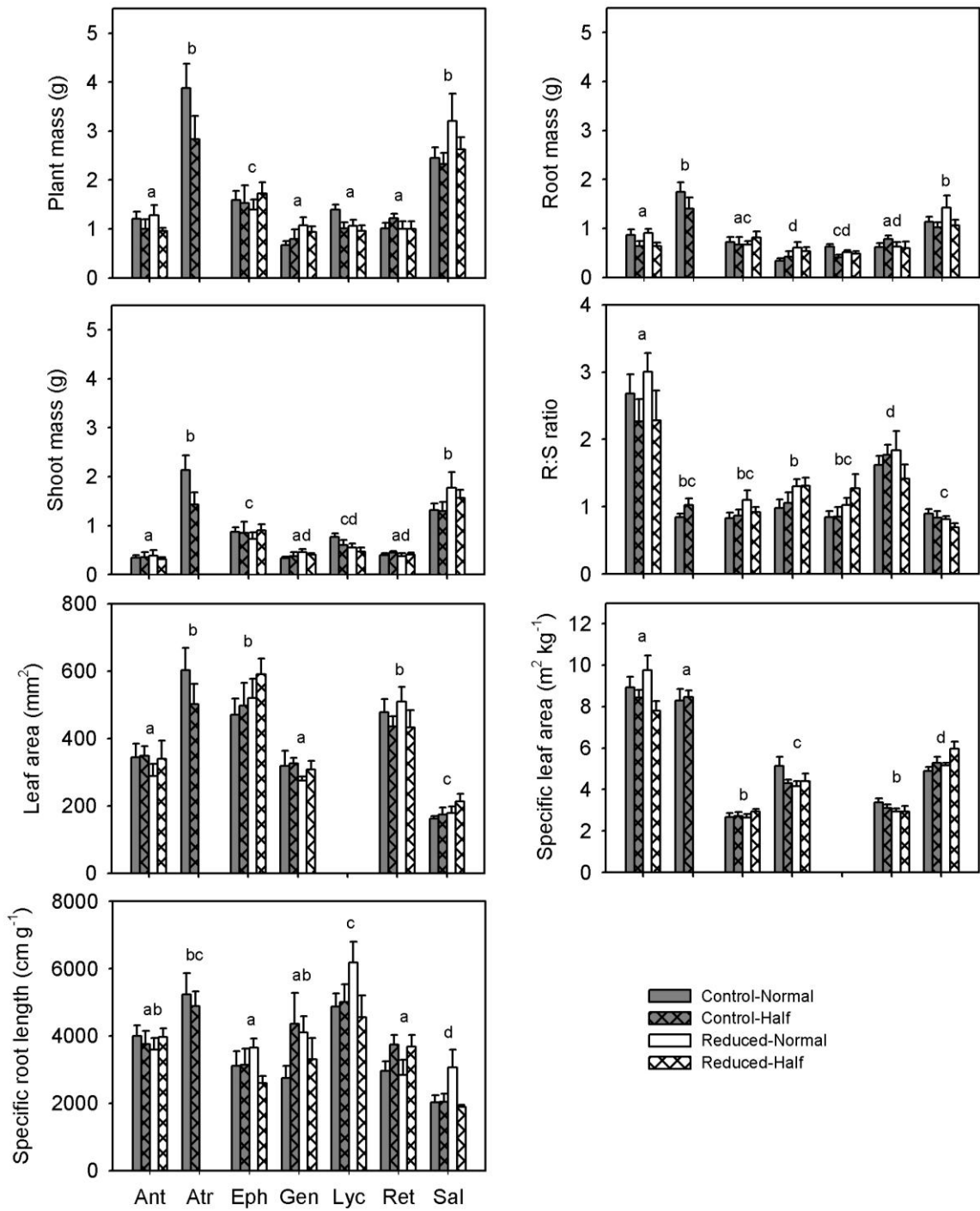
4 Figure 2



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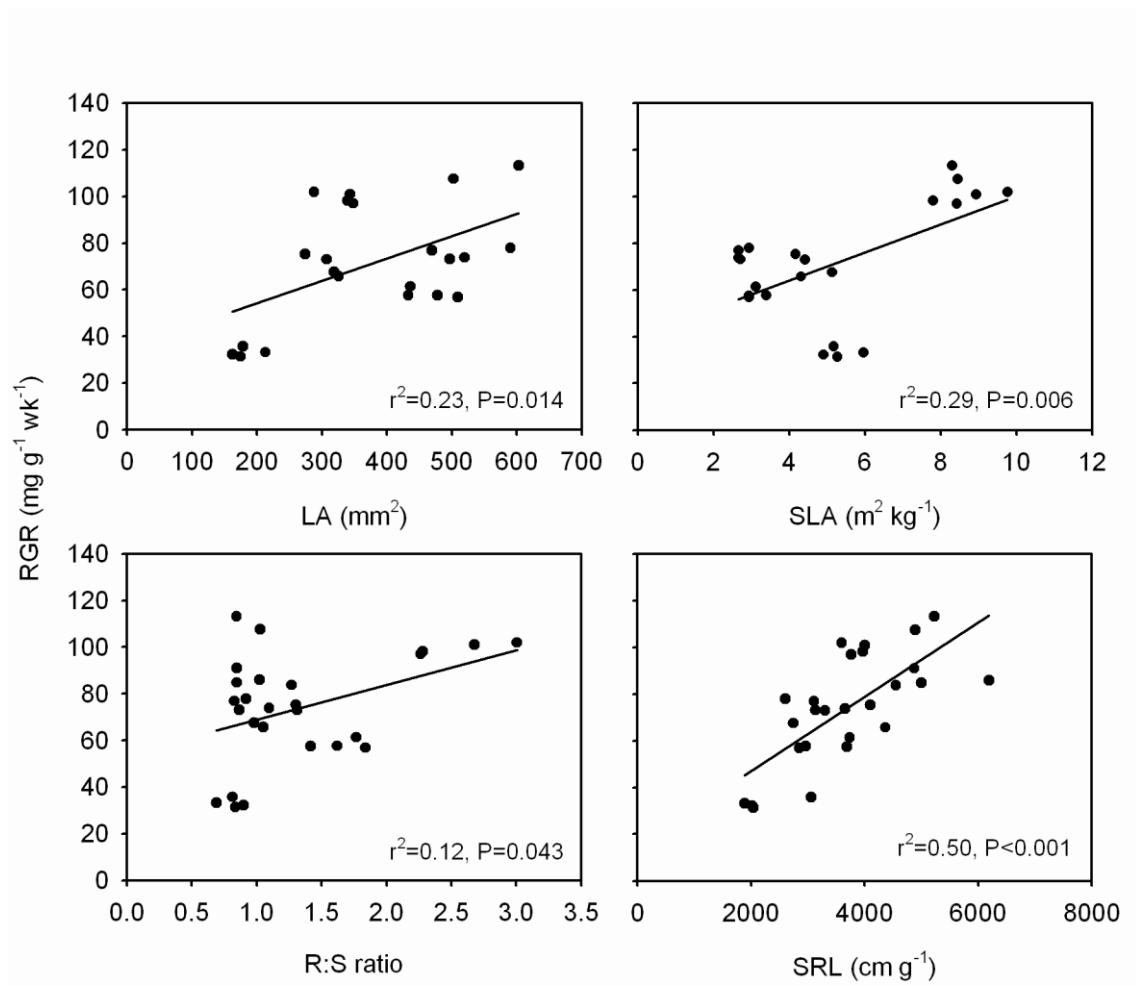
7 Figure 3



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10 Figure 4



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