

# Hydrotropism in lateral but not in pivotal roots of desert plant species under simulated natural conditions

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## Abstract

**Aims** Root hydrotropism has been widely studied in seedling radicles through artificial experiments that reduce the influence of gravity and soil. In this work we aimed to study hydrotropism of primary lateral and pivotal roots in developed root systems of desert plants under simulated natural conditions.

**Methods** We grew *Bulnesia retama* Griseb. (non phreatophyte), *Prosopis flexuosa* DC. (facultative phreatophyte) and *Prosopis alpataco* Phil.(obligate phreatophyte) seedlings in observation boxes with sand. Lateral and pivotal roots were stimulated by lateral water gradients and hydrotropic responses, root proliferation and root : shoot ratios were measured.

**Results** We found that  $65 \pm 15$  % of lateral roots that grew in response to water gradients in *B. retama*,  $84 \pm 8$  % in *P. flexuosa* and  $88 \pm 8$  % in *P. alpataco* displayed hydrotropism. Conversely, pivotal roots did not show hydrotropic growth. This was accompanied by root proliferation inside water patches, and biomass partitioning to shoot growth.

**Conclusions** Our results provide evidence that root hydrotropism is a relevant response that could occur in nature. Lateral and pivotal roots manifest different hydrotropic responses under the conditions assayed. The combination of hydrotropism and precise root proliferation can shape root architecture, leading to optimum water patch exploration.

**Keywords** *Bulnesia retama* · Hydrotropism · *Prosopis alpataco* · *Prosopis flexuosa* · Root proliferation · R : S ratio

## Abbreviations

R : S ratio	Root : shoot ratio
GSA	Gravitropic set point angle
$\psi_s$	Soil water potential
d.f	Degrees of freedom

## Introduction

Roots are plastic organs that respond to a variety of below and above ground signals that modulate root system architecture (Hodge 2010; Sassi et al. 2012). Belowground, patches of water and nutrients stimulate local root proliferation, which enhances the efficiency of resource acquisition (Hodge 2003, 2010). Local root proliferation involves perception of the external stimulus, transduction of the incoming signal and interaction with endogenous signals that result in promotion of lateral root emergence and growth via changes in hormone levels such as auxins, cytokinins, and ABA, that

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promote or inhibit both processes (Malamy 2005; Nibau et al. 2008; Kiba et al. 2011; Bouguyon et al. 2012). The direction of growth of pivotal and lateral roots is guided by internal and external cues. The ubiquitous external signal that modulates root position is gravity (Blancaflor and Masson 2003; Strohm et al. 2012). Roots respond to gravity by gravitropism, and grow at a certain angle to the gravity vector, called gravitropic set point angle (GSA; Mullen and Hangarter 2003). GSA varies among root types and with root length; some roots grow downward as pivotal roots and lateral sinker roots, and others grow obliquely or shallowly, as lateral roots of first or higher orders (Danjon and Reubens 2008; Guevara et al. 2010). GSA is altered when roots are faced with tropistic stimuli that trigger directional changes in growth: water, light or temperature gradients that induce hydro, photo and thermotropism respectively (Fortin and Poff 1990; Kutschera and Briggs 2012; Cassab et al. 2013), or solid objects that induce thigmotropism (Massa and Gilroy 2003). Tropistic growth responses involve perception of the signal in the root cap, and its transduction to a basal elongation zone, where different levels of auxins between the stimulated and non stimulated sides of the root produce bending towards or against the direction of the stimulus (Takahashi et al. 2009; Strohm et al. 2012).

Water in the soil is heterogeneously distributed both horizontally and vertically. Roots are able to respond to and exploit the water patches they encounter in the soil with increased local root proliferation and absorption capacity in the wettest portion of the root system. This allows plants to shift water uptake from drier to wetter soil areas at the same time as roots in the drier zone send signals to shoot, inducing a conservative use of water (Stoll et al. 2000; Wang et al. 2005; Hodge 2010). Hypothetically, roots can also grow towards increasing moisture gradients in the soil by positive hydrotropism, which could contribute to precise exploitation of water patches and drought avoidance. However, this has been demonstrated principally in artificial experimental systems designed to reduce root responses that mask hydrotropism, such as gravitropism and thigmotropism, by clinorotation, use of reduced or non graviresponsive genotypes, and substitution of soil with agar or air (Kiss 2007; Kobayashi et al. 2007; Takahashi et al. 2009; Moriwaki et al. 2012). Following this reasoning, a variety of experimental settings were developed, which share some relevant features that can bias our understanding of hydrotropism: water potential

gradients are often steeper than those reported in soil, roots are usually grown under light, and pivotal down-growing radicles are mostly assessed, with little representation of lateral roots (Takahashi et al. 2009; Iwata et al. 2012). These studies allowed fundamental and significant advances in our understanding of the mechanism of hydrotropism and its interaction with gravitropism at the cellular, molecular and genetic level. However, they told little about how hydrotropism operates in the real world, where pivotal and lateral roots with different GSA are surrounded by soil in the dark, confronted with dynamic water gradients, and constantly stimulated by gravity.

The first demonstration of positive root hydrotropism and its contribution to drought avoidance in a commercially prepared soil substrate and under gravity was recently published by Iwata et al. (2013) for *Arabidopsis thaliana*. They found that lateral roots grew towards soil moisture gradients affecting root system architecture and enhancing drought tolerance. Previously, Cole and Mahall (2006) had found no evidence of root hydrotropism in xerophyte dune shrubs from a water-limited habitat, in sand-filled boxes under simulated natural conditions. Tsutsumi et al. (2003) demonstrated that when gravity was ruled out in the longitudinal axis of the root, hydrotropism could shape soybean root architecture in a mixture of sand and organic soil. Loomis and Ewan (1936) found no evidence of hydrotropic growth in 29 genera of crops in sandy loam soil, by testing root growth direction from a moist soil layer when faced with a drier soil layer. In the light of the scarce research work on root hydrotropism under natural conditions and its controversial results, our comprehension regarding the operation and relevance of this response in nature is limited.

In this work, we aimed to study how root hydrotropism develops in root systems faced with realistic and dynamic water patches in soil and under gravity. We studied this in desert plant species, in which positive responses to water are crucial for survival. We also wanted to know if the hydrotropic behavior of primary lateral roots and pivotal roots, with different GSA and relative position within the root system, differ in species that explore different water sources when adults. We hypothesize that the manifestation of hydrotropism under natural-simulated conditions varies with the type of root and its spatial location in the adult plant, and predict that upon stimulation by lateral water patches, roots that exploit surface water reservoirs will manifest stronger

hydrotropic growth towards water gradients than roots that are committed to grow several meters deep to reach phreatic water. To test this hypothesis, we selected three species from the Central Monte Desert (Argentina) that exploit different combinations of spatially segregated water reservoirs in the field in the adult stage (Jobbágy et al. 2011): the non-phreatophyte *Bulnesia retama* Griseb that uses rainfall water exclusively; the facultative phreatophyte *Prosopis flexuosa* DC, that uses phreatic water and rainfall water and the obligate phreatophyte *Prosopis alpataco* Phil., that uses phreatic water exclusively (Jobbágy et al. 2011; Giordano et al. 2011). We can infer that in the field, *B. retama* root system is relatively shallow and *P. alpataco* root system is relatively deep. We have more information about *P. flexuosa* and know that its root system is dichotomous and spreads both shallowly and deeply (Morelo 1958; Guevara et al. 2010; Giordano et al. 2011). If our hypothesis is true, we expect lateral roots of *B. retama* and *P. flexuosa* to be more hydrotropic than lateral roots of *P. alpataco*, pivotal root of *B. retama* to be more hydrotropic than pivotal root of *P. flexuosa* and *P. alpataco*, and lateral roots of *P. flexuosa* to be more hydrotropic than its pivotal root. We also analyzed root proliferation and biomass distribution between roots and shoot, to assess whole root system and whole plant responses to water patches.

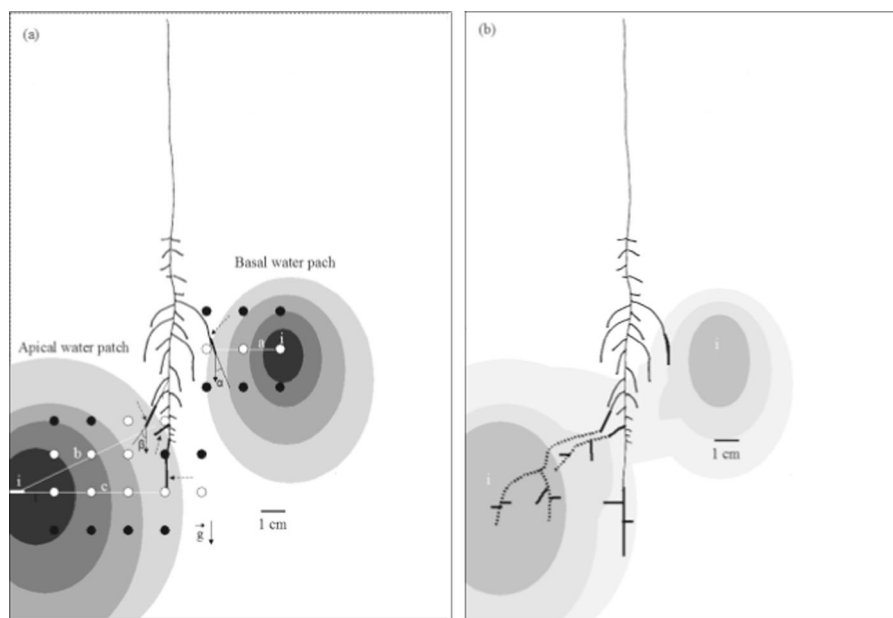
## Materials and methods

### Experimental setting

We grew seedlings of *Bulnesia retama* Griseb., *Prosopis flexuosa* DC. and *Prosopis alpataco* Phil. in observation boxes and followed root growth. Boxes were 0.2 m width  $\times$  0.65 m height  $\times$  0.03 m deep. The front 0.2  $\times$  0.65 m side was made of transparent acrylic, the rest of the structure of corrugated plastic board and wood. Boxes were coated with a black polyethylene sheet, polyethylene foam and aluminum film to insulate from light and air temperature. They were filled with a sandy substrate and placed in a greenhouse at IADIZA in Mendoza city (32°52' S; 68°49' W), at an angle of 45° from vertical to induce roots to grow toward the translucent side, so gravity was reduced by 30 % in the longitudinal axis of the boxes. Substrate chemical properties were: pH 7.6; CE 4.16 dS m<sup>-1</sup>; 150 mg kg<sup>-1</sup> of total N; 5.1 mg kg<sup>-1</sup> of P-H<sub>2</sub>CO<sub>3</sub> 1:10; 119 mg kg<sup>-1</sup> K

int Ac-NH<sub>4</sub> pH 7 [Soil, Water and Plant Material Laboratory of Instituto Nacional de Tecnología Agropecuaria (INTA) Mendoza, Argentina]. Three seeds per species [collected from Telteca Natural Reserve (32° 20' S; 68° 00' W)] were sown in the boxes and watered from top with 150 ml of a fungicide solution of N-(trichloromethyltio) cyclohex-4-ene-1,2dicarboximide 0.13 g l<sup>-1</sup> (Orthocide 80 WP, Arysta LifeScience, Tokyo, Japan). This initial irrigation generated a wet soil profile of ~20 cm. Once emerged, one seedling was left per box, so in some boxes seedlings were centered and in others they were displaced laterally. Under these well-water conditions, seedlings developed three true leaves and a root system with a pivotal root and first-order lateral roots, and stopped growing once soil dried. After 35 days of the single initial irrigation, and immediately before water patch application, we measured pre-dawn water potential at the shoot base with a pressure chamber (Bio Control Model 12, Buenos Aires, Argentina) using Scholander et al.'s (1965) method, in seedlings grown in replicated boxes that were sacrificed for the measurement. We then injected water in two patches per box in half the boxes (Treated), and used the other half as control without water patches (Control). Water patches were applied on opposite sides of the same root system: one near the root system base, called *basal*, and one near the pivotal root apex, called *apical*, to stimulate pivotal and first-order lateral roots, referred to as 'target roots' (Fig. 1a). Water was injected with a syringe (3 to 7 ml depending on seedling position in the box) through the corrugated plastic board from lateral or back sides. The experiment ended once seedlings displayed root growth in response to water patches, and stopped growing once water moved into the unsaturated soil matrix or was absorbed by seedlings.

The experiment was arranged in 8 complete blocks, and the combination of the three species, *B. retama*, *P. flexuosa* and *P. alpataco*, growing in Treated and Control boxes, was assigned randomly within each block. One block had to be discarded because all plants died prematurely due to vicinity of a heating device that malfunctioned during the experiment, and in another block *P. flexuosa* seedlings were severely attacked by fungi. So there remained for analysis: 7 blocks for *B. retama* and *P. alpataco*, and 6 blocks for *P. flexuosa*. The experiment started on 21st July 2012 and ended on 31st October 2012. Mean daily air temperature inside the greenhouse was 22 °C, and relative humidity 32 % (measured with HOBO Pro Series data



**Fig. 1** Schematic representation of apical and basal water patches and root responses. Water patches are drawn in a grey intensity scale; darker shades are higher soil moisture contents near the water injection point, indicated by *i*. **a** Soil moisture gradients at the moment of sampling, previous to root responses. Soil samples were extracted at equidistant points indicated by *white* and *black* circles. *White* circles were selected to quantify soil moisture gradients inside each water patch along different spatial trajectories

from the position of ‘target root’ tips (indicated by *dashed arrows*) towards the point of water injection. *Line a* represents a *basal gradient for lateral roots*; *line b* represents an *apical gradient for lateral roots*; and *line c* represents an *apical gradient for pivotal root*.  $\alpha$  and  $\beta$  indicate lateral root GSA; pivotal root GSA is 0 as it coincides with the gravity vector indicated by *g*. **b** An example of typical root responses. *Dotted roots* are the roots that resumed growth after water patch application

loggers, Onset Computer Corporation, Bourne, MA, USA). Mean midday solar photosynthetic photon fluence rate inside the greenhouse during the experiment was  $\sim 1080 \mu\text{mol m}^{-2} \text{s}^{-1}$  over the waveband 400–700 nm. It was estimated from Luján de Cuyo ( $33^\circ 00' \text{S}$ ;  $68^\circ 51' \text{W}$ ) weather data from INTA-Mendoza, and greenhouse transmittance to photosynthetic active radiation (PAR) measured with a radiometer (Li-Cor LI-185B, Lincoln, Nebraska, USA).

#### Measurement of soil moisture gradients

Injected water moved from the point of injection into the unsaturated soil forming visible circular patches (Fig. 1a). Two days after treatment application (when the edges of the water patches approached target root tips), we extracted soil samples at equidistant points inside each water patch (Fig. 1a) from Treated and Control replicated boxes (as the measurement was destructive), to determine whether the treatment generated soil moisture gradients. Soil was taken with 0.15 ml stainless steel tubes from the back side of the boxes after

drilling holes at 1.5-cm distant points within each water patch (Fig. 1a). We then measured soil moisture gravimetrically after oven-drying soil at  $100^\circ \text{C}$  for 2 days. To relate soil moisture (% w/w) with soil water potential ( $\psi_s$ ), which indicates water availability for the plant, we constructed the moisture retention curve of the substrate. For this, we measured pre-dawn water potential of *P. flexuosa* seedlings growing in 180 ml pots, which had been covered 12 hs previous to the measurement and equilibrated at different soil moisture contents; pre-dawn water potential was considered to represent  $\psi_s$ . To quantify soil moisture and  $\psi_s$  change with distance from target root tips position towards the point of water injection we selected soil samples that corresponded to points along or near the radius of the water patch, or the equivalent area in the Control boxes, that coincided with this trajectory (Fig. 1a). When more than one lateral root grew, we started from an imaginary point situated in the middle of their root tips to define the trajectory (see line *b* in Fig. 1a). If none of the lateral roots that were near a water patch grew (as occurred in most basal water patches), we likewise quantified the moisture gradient

along the radius of the water patch that intercepted the nearest lateral root (see line a in Fig. 1a). The three different soil moisture gradients most probably perceived by ‘target roots’ were called: *basal moisture gradient for lateral roots*, *apical moisture gradient for lateral roots* and *apical moisture gradient for pivotal root* (Fig. 1a).

### Root measurements

Root growth was followed by tracing root segments with markers of different colors on acetate films fixed to the translucent front side of the boxes. *Root system morphology*: In the root systems formed during initial growing conditions before water patch application, we measured length of pivotal and lateral roots, number of lateral roots, GSA of pivotal and lateral roots every 5 mm, and drew representative root systems based on averaged data. To describe the position of lateral roots with respect to gravity we generated a function of GSA vs. root length for each species. Each pair of  $x$ :  $y$  values (lateral root length in cm and its GSA in degrees) came from an average value per seedling per block. *Lateral root hydrotropism*: We registered the number of lateral roots whose main axis grew penetrating water patches and, among them, the ones that grew hydrotropically. As lateral roots did not grow vertically but obliquely, and their GSA changed with root length, we compared the lateral root GSA observed after water patch application with the expected GSA. For this, we fitted a cubic spline curve of the GSA vs. length function of each growing lateral root, and compared it with the GSA vs. length function of lateral roots characteristic of the species. We considered that lateral roots grew hydrotropically if their GSA vs. length function deviated from the 95 % prediction bands of their species’ function, in direction toward the moisture gradient (positive values of GSA). Ninety five % prediction bands are expected to enclose 95 % of future data points and include both the uncertainty in the true position of the curve (enclosed by the confidence bands), and also account for scatter of data around the curve (GraphPad Prism v. 5.0, 2007, User’s Guide, GraphPad Software Inc). This method is very conservative as we did not consider hydrotropic roots that increased their GSA but were enclosed within the prediction bands. We registered the number of growing roots and the number of

hydrotropic roots at each water patch, calculated a ratio between both variables per seedling (adding up observations of both water patches) as an index of lateral root hydrotropism, and expressed the ratio as a percentage of lateral root hydrotropism. *Pivotal root hydrotropism*: We recorded the GSA of pivotal roots before and after the application of water patches, and compared both. Roots were considered hydrotropic if both values of GSA differed. We defined positive values of GSA when roots deviated from the vertical (GSA = 0) towards the apical water gradient, and negative values of GSA when roots deviated towards the opposite side. *Root proliferation*: We measured root segment lengths in an observation area of 600 cm<sup>2</sup>, and added up segments that grew after water patch application inside and outside the water patch area. We divided each recording by a common root growth area of 600 cm<sup>2</sup> and expressed root proliferation as cm of root per cm<sup>2</sup> of observation area.

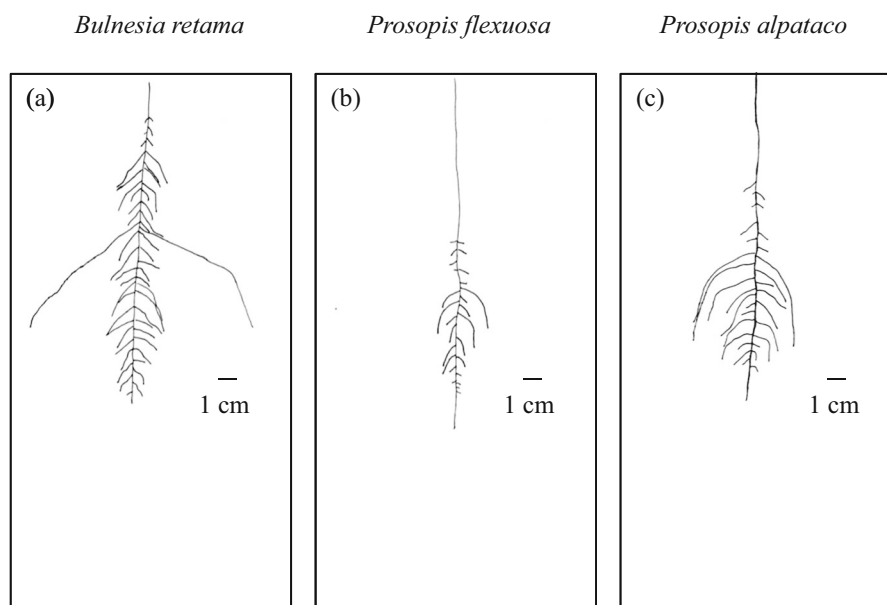
### Biomass distribution between root and shoot

We harvested seedlings from Treated and Control boxes, separated shoots from roots, and recovered roots from substrate by careful sieving. Harvested material was dried at 60 °C for 2 days and weighted to calculate total, shoot and root dry biomass and the root : shoot ratio (R : S).

### Statistical analysis

*Root systems* We compared species means of pivotal root length and number of lateral roots with two-way ANOVA, with species as random factor and block as fixed factor. GSA vs. length functions of lateral roots for each species were fit by non-linear regression to a one-phase exponential decay function with the general equation:  $y = span e^{-kx} + plateau$ . Mean comparison between species of GSA at lateral root emergence [ $y_{(x0)} = span + plateau$ ], change in GSA with length ( $k$ ) and in GSA at lateral root tip ( $plateau$ ) was made by one-way ANOVA.

*Water retention curve*  $\psi_s$  vs. % soil moisture function was fit by non-linear regression to a one-phase exponential decay function.



**Fig. 2** Bi-dimensional root systems morphologies of 35 days-old seedlings of *Bulnesia retama*, *Prosopis flexuosa* and *Prosopis alataco* under initial irrigation conditions

**Root hydrotropism** GSA vs. length functions of individual lateral roots was traced by fitting cubic spline curves to original data. We compared species pre-dawn water potential previous to water patch application, and percentage of hydrotropic response of lateral roots per seedling to water patches by two-way ANOVA. GSA of pivotal roots before and after water patch application within each species was compared with bilateral paired *t*-test.

**Root proliferation** Root proliferation inside and outside water patches within each species was compared with bilateral paired *t*-test.

**Biomass partitioning** Mean comparison of total dry biomass and R : S ratios was made by three-way ANOVA, with species and treatment as random factors, and block as fixed factor.

One, two and three-way ANOVAs, paired and unpaired *t*-tests were done with Infostat (v. 2011, Grupo InfoStat, Facultad de Ciencias Agrarias, Universidad Nacional de Córdoba, Argentina). Normal distribution of errors and homocedasticity for ANOVA were tested with Shapiro-Wilks test modified by Mahibbur and Govindarajulu (1997), Levene test, and graphics of observed vs. predicted residue values with Infostat.

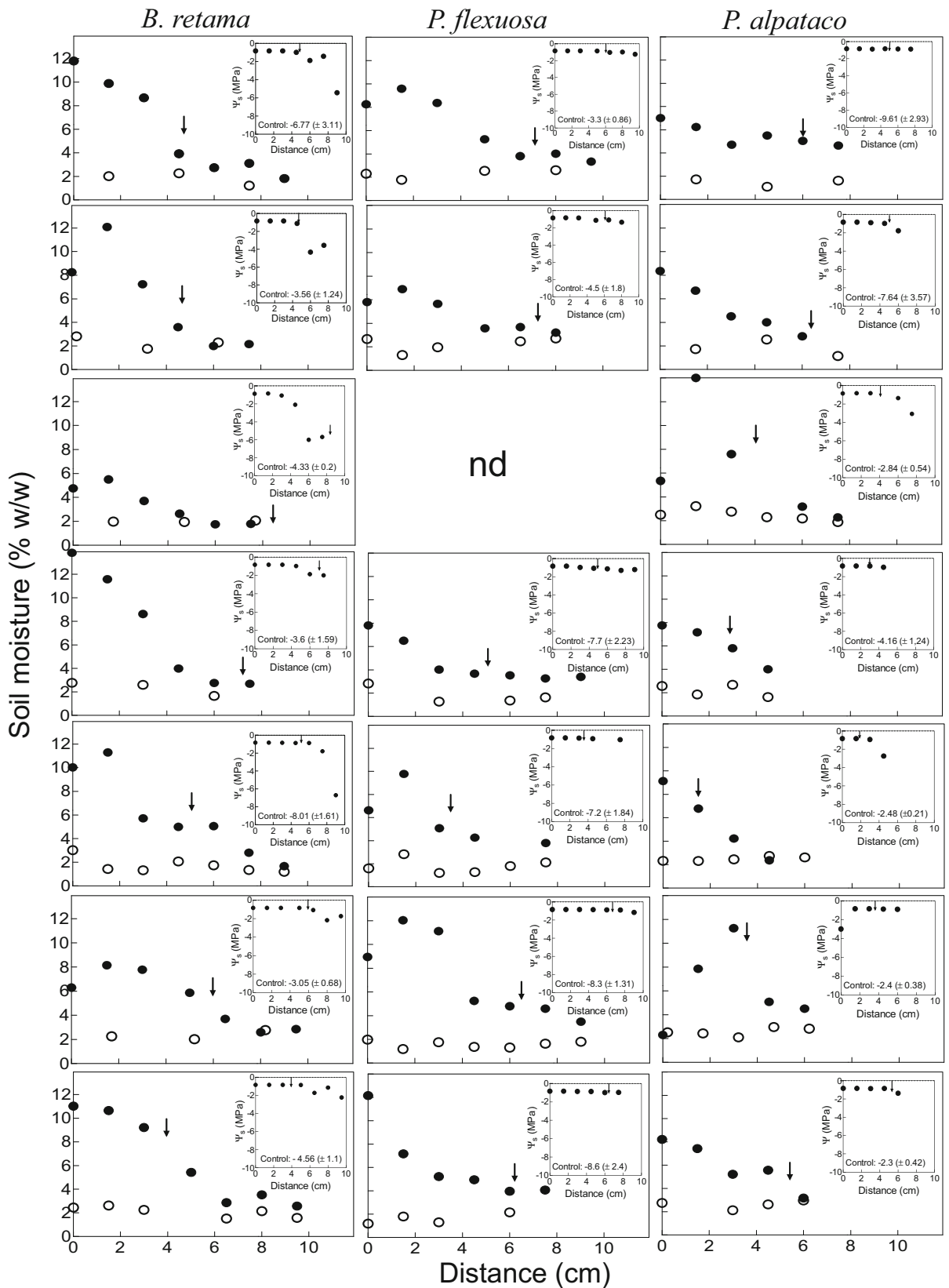
Non-linear regressions and cubic spline curves were done with GraphPad Prism (v. 5.0, GraphPad Software Inc.).

## Results

### Root systems

*B. retama*, *P. flexuosa* and *P. alataco* 35 days-old seedlings developed root systems with different morphologies (Fig. 2). Just before water patch application, pivotal roots had similar lengths (species effect  $P=0.61$ ;  $N=20$ ; d. f. = 11):  $20.36 \pm 0.55$  cm in *B. retama*,  $20.98 \pm 0.59$  cm in *P. flexuosa*, and  $21.09 \pm 0.55$  cm in *P. alataco* seedlings. The number of first-order lateral roots differed among species (species effect  $P=0.01$ ;  $N=20$ ; d. f. = 11), with the highest number displayed by *B. retama* ( $35.71 \pm$

**Fig. 3** Basal moisture gradients for lateral roots, represented in percent soil moisture ( $w/w$ ) content and  $\psi_s$  (insets) vs. distance in spatial trajectories that go from points of water injection (Distance = 0) to position of target root tips (indicated by arrows). Closed circles are data from Treated boxes, open circles are data from Control boxes. Graphics are organized by species (*Bulnesia retama*, *Prosopis flexuosa* and *Prosopis alataco*) in columns, and by blocks (A to G) in rows



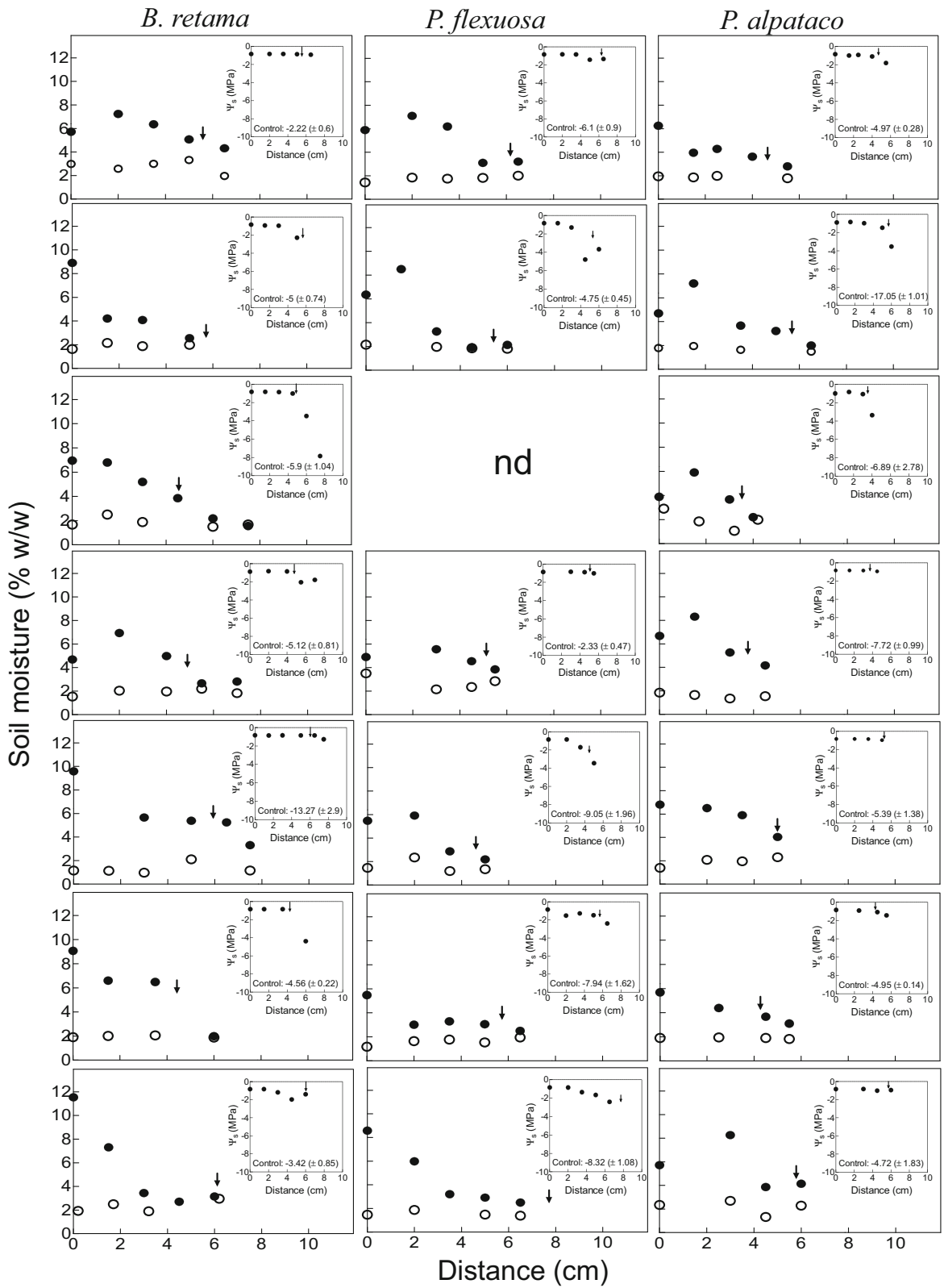


Fig. 4 Apical moisture gradients for lateral roots. References and graphics organized as in Fig. 3



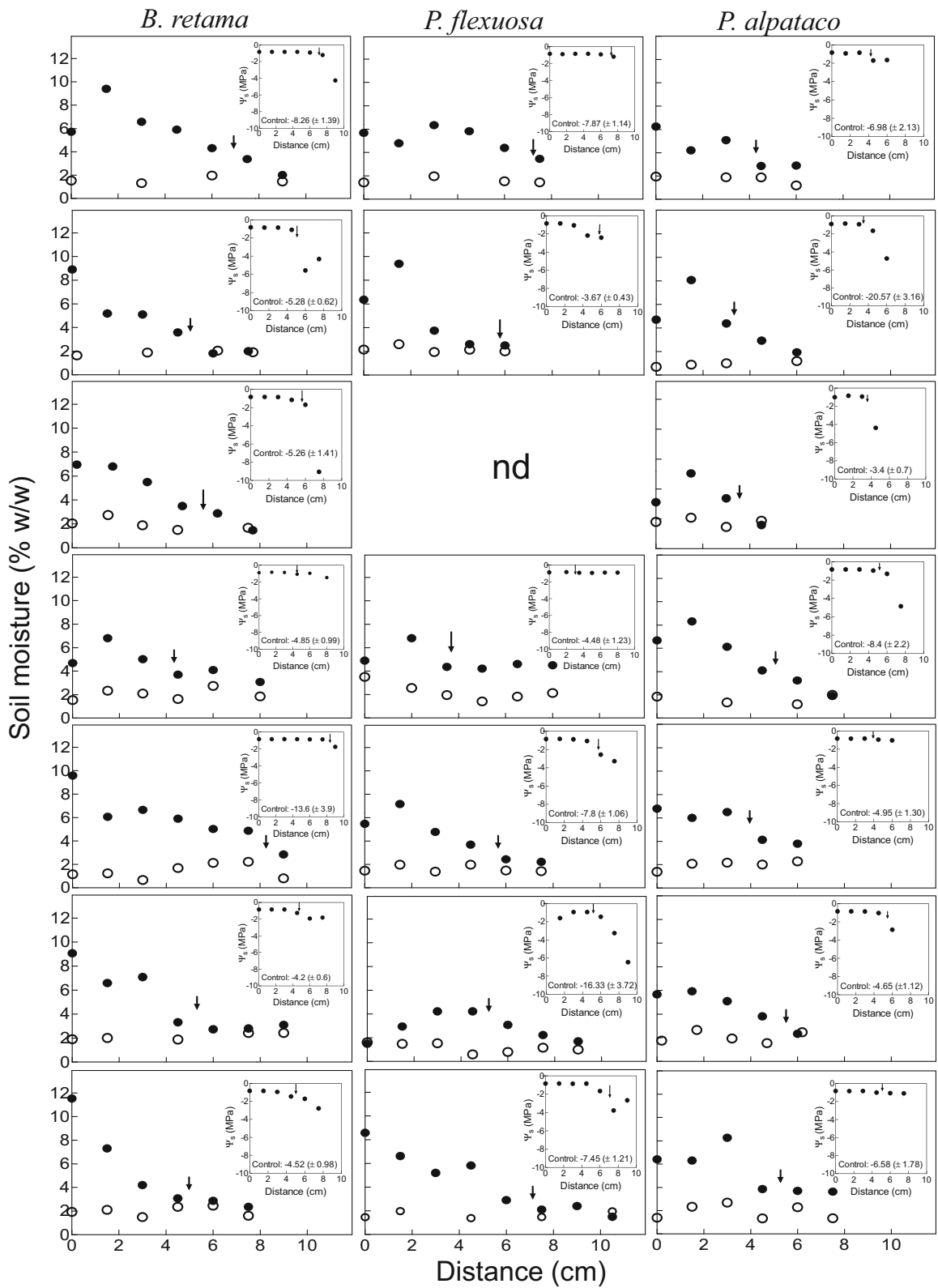


Fig. 5 Apical moisture gradients for pivotal roots. References and graphics organized as in Figs. 3 and 4

**Table 1** Lateral root responses to water patches

Species	Block	Lateral root responses				Hydrotropic ratio per seedling <sup>1</sup>	Percentage of hydrotropic roots per seedling
		In basal water patches		In apical water patches			
		No. of growing roots	No. of hydrotropic roots	No. of growing roots	No. of hydrotropic roots		
<i>B. retama</i>	A	0	0	1	1	1/1	100
	B	0	0	2	2	2/2	100
	C	0	0	3	3	3/3	100
	D	0	0	2	1	1/2	50
	E	0	0	2	0	0/2	0
	F	2	2	2	1	3/4	75
	G	0	0	3	1	1/3	33
<i>P. flexuosa</i>	A	1	1	1	1	2/2	100
	B	0	0	5	4	4/5	80
	C	Nd.					
	D	0	0	2	1	1/2	50
	E	0	0	4	3	3/4	75
	F	1	1	1	1	2/2	100
	G	0	0	1	1	1/1	100
<i>P. alpataco</i>	A	1	1	2	2	3/3	100
	B	2	2	0	0	2/2	100
	C	0	0	1	1	1/1	100
	D	2	2	1	1	3/3	100
	E	1	1	1	0	1/2	50
	F	1	1	2	1	2/3	66
	G	0	0	1	1	1/1	100

Nd Not determined

<sup>1</sup> No. of hydrotropic roots / No. of growing roots per seedling, adding up root responses observed in basal and apical water patches

4.13), the lowest by *P. flexuosa* (19.5±4.46) and an intermediate number of lateral roots developed by *P. alpataco* (30.71±4.13).

Position of lateral roots with respect to gravity was described by the following GSA vs. length functions for each species:

*B. retama*:

$$\text{GSA} (^{\circ}) = 50.57 \pm 12.44 e^{-1.035 \pm 0.316 \text{ Length (cm)}} + 30.30 \pm 1.532R^2 = 0.24; N = 167; \text{d.f.} = 164$$

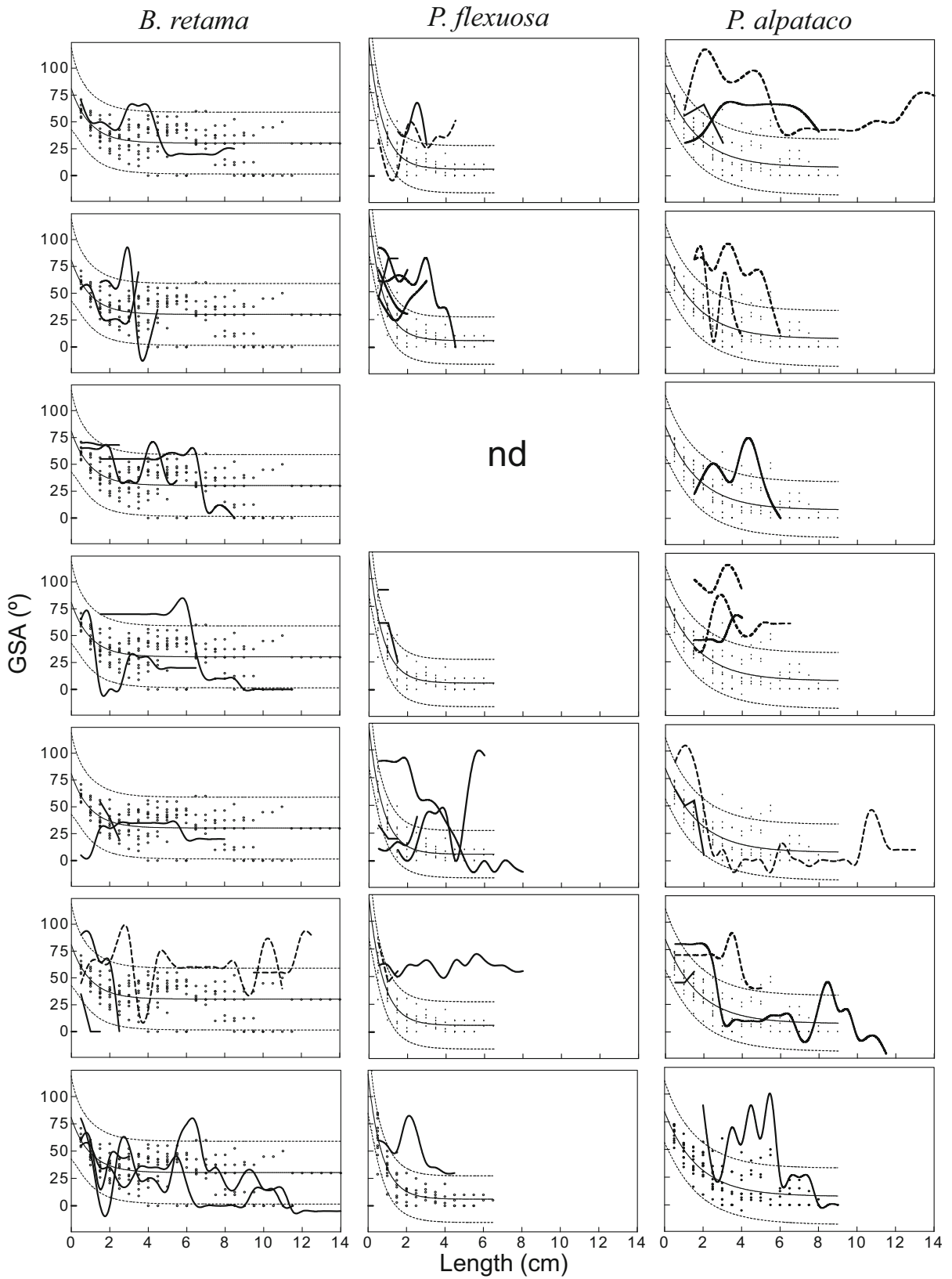
*P. flexuosa*:

$$\text{GSA} (^{\circ}) = 111.4 \pm 15.51 e^{-1.373 \pm 0.2437 \text{ Length (cm)}} + 6.95 \pm 2.439R^2 = 0.79; N = 71; \text{d.f.} = 68$$

*P. alpataco*:

$$\text{GSA} (^{\circ}) = 76.56 \pm 5.616 e^{-0.5919 \pm 0.096 \text{ Length (cm)}} + 7.67 \pm 2.817R^2 = 0.67; N = 130; \text{d.f.} = 127$$

**Fig. 6** GSA vs. length functions of lateral roots that grew in response to water patches. Graphics are organized by species (*Bulnesia retama*, *Prosopis flexuosa* and *Prosopis alpataco*) in columns, and by blocks (A to G) in rows. *Narrow lines* represent GSA vs. length functions of lateral roots characteristic of each species (repeated in all graphics in the species' column); *dotted lines* are the bands of prediction, and dots are the original data. *Bold lines* indicate the trajectory of growing roots (adjusted by spline curves): the *dashed ones* represent roots that grew in basal water patches and the *continuous ones* represent roots that grew in apical water patches



At their emergence (Length = 0), lateral roots tended to be more horizontal in *P. flexuosa* (GSA =  $117.35 \pm 17.9^\circ$ ) than in *B. retama* (GSA =  $80.87 \pm 14^\circ$ ) or *P. alpataco* (GSA =  $84.23 \pm 8.4^\circ$ ; Fig. 2). This was visually evident (Fig. 2), although mean GSAs at lateral root emergence did not differ statistically (species effect  $P=0.12$ ;  $N=34$ ; d. f. = 16). *P. flexuosa* lateral roots decreased the most their GSA per unit length and *P. alpataco* the least, with intermediate values in *B. retama* (see  $k$  values of the functions, species effect  $P=0.07$ ;  $N=34$ ; d. f. = 31), and at their tips, roots of *B. retama* were more oblique than roots of *Prosopis spp.* that were more vertical (see  $plateau$  values of the functions, species effect  $P<0.0001$ ;  $N=34$ ; d. f. = 31).

### Soil moisture gradients

Soil moisture gradients developed in water patches in Treated boxes, along the three trajectories that we defined as *basal moisture gradients for lateral roots*, *apical moisture gradients for lateral roots* and *apical moisture gradients for pivotal roots* (Figs. 3, 4 and 5). Variations in soil moisture (% w/w) caused variations in  $\psi_s$  when soil had lost around 70 % of the water it could hold at field capacity, which was 14 % w/w [see water retention curve of the substrate in Online Resource, Fig. S1]. So, plants started to perceive a decrease in water availability when soil had  $\leq 4$  % w/w moisture content: this decline was subtle between 4 and 2 % w/w of soil moisture and abrupt when soil moisture was less than 2 % w/w [Fig. S1, and Figs. 3, 4 and 5]. At the moment of soil sampling, some gradients of  $\psi_s$  were steeper than others, however, all of them were expected to change continuously and get steeper with time as soil pores became depleted of water that flowed into the unsaturated soil matrix surrounding water patches, with  $1.92 \pm 0.06$  % w/w soil moisture and  $\psi_s -6.26 \pm 0.48$  MPa on average (measured in Control boxes).

### Lateral root hydrotropism

Following 35 days of initial irrigation and immediately before the offer of water in patches, seedlings were under water stress: *B. retama* was at  $-5.6 \pm 0.3$  MPa, *P. flexuosa* was at  $-5.2 \pm 0.5$  MPa and *P. alpataco* was at  $-5.0 \pm 0.6$  MPa of pre-dawn shoot water potential (species effect  $P=0.66$ ;  $N=20$ ; d. f. = 11). After water patches application, stimulated lateral roots resumed growth in all seedlings (Table 1).

GSA vs. length curves of these growing lateral roots were compared to the corresponding species' GSA vs. length functions to define which roots grew hydrotropically (Fig. 6), and calculate the hydrotropic ratio per seedling and the percentage of hydrotropic roots (Table 1). Average percentages of hydrotropic roots per seedling were  $65 \pm 15$  % for *B. retama*,  $84 \pm 8$  % for *P. flexuosa* and  $88 \pm 8$  % for *P. alpataco* (Table 1), with no significant differences among species (species effect  $P=0.17$ ;  $N=20$ ; d. f. = 11). So, among lateral roots that grew in response to the presence of a water patch, the majority of them grew with GSAs that could not be explained by their expected response to gravity, and grew at higher GSAs that coincided with the direction of increasing soil moisture gradients.

### Pivotal root hydrotropism

Downward growing pivotal roots recovered growth after water patch application, once first- and second-order lateral roots had colonized them. Pivotal roots did not change their GSA significantly after the offer of water in patches (Table 2), so they did not demonstrate hydrotropic behavior in neither of the species studied.

### Root proliferation

Following the offer of water in patches, *P. flexuosa* and *P. alpataco* root main axis re-growth and new branching occurred nearly exclusively inside these patches, while *B. retama* presented 4 % of root proliferation also in dry soil (Table 3).

**Table 2** GSA of pivotal roots before and after water patch application

	GSA ( $^\circ$ )		
	<i>B. retama</i>	<i>P. flexuosa</i>	<i>P. alpataco</i>
Before treatment	4.71 ( $\pm 2.89$ )	2.33 ( $\pm 2.12$ )	4 ( $\pm 3.82$ )
After treatment	9.14 ( $\pm 3.23$ )	5.67 ( $\pm 3.28$ )	0 ( $\pm 0$ )
<i>P</i> values	0.16	0.31	0.34
N; d. f.	7; 6	6; 5	7; 6

*P* values are from bilateral paired *t*-test

**Table 3** Root proliferation inside and outside water patches

	Root proliferation (cm cm <sup>-2</sup> )		
	<i>B. retama</i>	<i>P. flexuosa</i>	<i>P. alpataco</i>
Inside water patches	0.49 (±0.13)	0.27 (±0.10)	0.24 (±0.02)
Outside water patches	0.02 (±0.01)	0 (±0)	1.44 e <sup>-6</sup> (±1.40 e <sup>-6</sup> )
<i>P</i> values	0.008	<0.0001	0.046
<i>N</i> ; d. f.	14; 12	12; 10	14; 12

*P* values are from bilateral paired *t*-test

### Biomass partitioning between roots and shoot

All three species augmented their total biomass (shoot + root) in response to water offered in patches after the stress period following initial irrigation (Table 4). So, water patches were effectively used to increase biomass. The way total biomass was partitioned between shoot and roots in response to treatment was different for each species. Under initial conditions of growth with non-limiting availability of water, *B. retama* invested more biomass in roots (R : S > 1) than did *Prosopis spp.*, which favored biomass allocation to shoot (R : S ratios < 1; Table 4). When water became available in patches, *B. retama* favored shoot growth reducing significantly its R : S ratio, while *Prosopis spp.* maintained a biomass distribution between both organs that did not differ significantly from values in initial conditions of growth (Table 4). In the three species the R : S ratio after water patch availability at early seedling stage converged to an average value of 0.74, so shoot received around a quarter more biomass than roots when water resource was transiently available in patches.

### Discussion

Lateral roots of all three species changed their GSA dictated by gravitropism under optimal irrigation, in the presence of water patches after a period of water stress, and grew towards increasing moisture gradients in the soil. In the three species, over 60 to 90 % of lateral roots that resumed their growth stimulated by the presence of water did it hydrotropically. These results indicate that hydrotropism is a root response likely to occur under the influence of gravity in a sandy substrate, induced by the intensity and dynamics of water gradients naturally developed in localized water patches. The fact that the major body of research work that

demonstrated root hydrotropism was done with minimum substrate interference (in agar or air) and with null or reduced gravity stimulus (using agravitropic mutants, in clinostats or space flights), while the majority of research work done with both stimuli failed to observe root hydrotropism, led to question the relevance of this response in nature (Loomis and Ewan 1936; Cole and Mahall 2006; Takahashi et al. 2009; Cassab et al. 2013). This work contributes evidence that root hydrotropism could manifest itself under natural conditions, enhancing plant chances to exploit transient water availability. This is one of the two works that demonstrated this, being preceded by Iwata et al. (2013) who reported hydrotropism in lateral roots of *A. thaliana* in soil under gravity. In contrast, Cole and Mahall (2006) failed to observe root hydrotropism in experimental conditions similar to ours, in two xerophytes shrubs (*Artemisia californica* and *Eriogonum parvifolium*) growing in a sandy substrate, with dynamic water patches generated in a matrix of dry soil. These discrepancies indicate there is a need to explore different substrates, species and experimental systems to understand how root hydrotropism expresses itself with gravitropism, to gain comprehension about its role and function in plant responses to water availability.

We found that primary lateral roots manifested hydrotropic growth while pivotal roots did not, in the three species tested. Primary lateral roots acted as water ‘detectors’, contributed to exploration of transient water patches and, consequently, modeled root architecture in relation to water location. Pivotal roots grew downward independently of water stimuli, probably favoring seedling anchorage in the soil. So, in 35 days-old seedlings, root hydrotropism was more associated to root type than to differences in their spatial fate (shallow or deep) in adult plants. It is possible that the stronger hydrotropic response of lateral roots than of pivotal roots was associated to their lower gravitropism,

**Table 4** Total dry biomass and root: shoot ratio (R : S) in response to water patch application

	<i>B. retama</i>		<i>P. flexuosa</i>		<i>P. alpataco</i>		<i>P</i> values			
	Control	Treated	Control	Treated	Control	Treated	Species effect	Block effect	Treatment effect	Species* treatment
	Total dry mass (mg)	35.86 ( $\pm 3.69$ )	59.14 ( $\pm 8.49$ )	34.33 ( $\pm 3.98$ )	49.15 ( $\pm 5.58$ )	23.33 ( $\pm 2.5$ )	47.61 ( $\pm 5.48$ )	0.076	0.28	0.0001
R : S	1.35 ( $\pm 0.27$ )	0.73 ( $\pm 0.39$ )	0.53 ( $\pm 0.14$ )	0.75 ( $\pm 0.13$ )	0.83 ( $\pm 0.14$ )	0.74 ( $\pm 0.08$ )	0.02	0.08	0.13	0.02

Average values  $\pm$  s. e. m. from Treated (with water patches) and Control (without water patches) boxes are reported. *P* values are from three-way ANOVA. N: 40; d. f.: 28

evidenced by their higher GSA. In *A. thaliana* it was demonstrated that lateral roots acquired gravitropism later than the pivotal root (Guyomarc'h et al. 2012), and in soybean, both pivotal and lateral roots grew hydrotropically towards the direction of water when gravity was excluded artificially (Tsutsumi et al. 2003). However, both types of roots might differ not only in their interaction between hydrotropism and gravitropism, but also in their hydrotropic capacity (e.g. water gradient sensing, downstream growth responses), which has been unexplored and is unknown at present.

Precise local root proliferation inside water patches accompanied lateral root hydrotropism in all three species. This response was very specific, as nearly no root proliferation was observed in dry soil. *B. retama* was the only species that showed 4 % of total new root proliferation outside water patches.

*B. retama*, *P. flexuosa* and *P. alpataco* took advantage of transient water availability in patches after a period of drought, increasing whole seedling biomass. The way total biomass was distributed between above and underground organs during initial optimal watering conditions and during patchy water availability after drought, differed among species. Initially, *B. retama* invested more biomass in roots than *Prosopis spp.* that invested more in shoots. After a drought period, *B. retama* used water for shoot growth, as *Prosopis spp.* did.

Integrating root morphologies, root responses to water and biomass partitioning patterns, we were able to outline some differences between the non-phreatophyte *B. retama* and the phreatophytes *Prosopis spp.* that can influence their ability to use transient water patches, common in their natural habitat where rainfall events are scarce, discrete and unpredictable (Giordano et al. 2011). Following an initial pulse of water that induced seedling emergence from seed, *B. retama* invested more biomass in roots and produced more primary lateral roots than *Prosopis spp.*, enabling *B. retama* to count on a higher probability to encounter and exploit future water patches than *Prosopis spp.* Moreover, its lower precision in root proliferation after additional pulses of water enhanced soil exploration in different directions, augmenting the likelihood to encounter future water patches in a broader three-dimensional spatial range. These responses, that rely more in biomass allocation patterns and root proliferation precision than in hydrotropism, might be particularly advantageous for a non phreatophyte species that uses solely rainfall water

throughout its life. On the other hand, the phreatophytes invested more in shoot development, and developed lesser primary lateral roots than *B. retama*, but were more precise in water patch exploration.

From a technological point of view, the results presented in this work give evidence that it is possible to modify root architecture of desert plant seedlings grown in pots by regulating the place and amount of watering. Modulating root architecture might be valuable for cultivation of species for re-vegetation of degraded arid ecosystems (a current global issue that comprises 20 % of drylands; Reynolds et al. 2007; Cowie et al. 2011) where the successful access and exploration of water reservoirs is crucial for plant survival.

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## References

- Blancaflor EB, Masson PH (2003) Plant gravitropism. Unraveling the ups and downs of a complex process. *Plant Physiol* 133:1677–1690
- Bouguyon E, Gojon A, Nacry P (2012) Nitrate sensing and signaling in plants. *Semin Cell Dev Biol* 23:648–654
- Cassab GI, Eapen D, Campos ME (2013) Root hydrotropism: an update. *Am J Bot* 100:14–24
- Cole ES, Mahall BE (2006) A test for hydrotropic behavior by roots of two coastal dune shrubs. *New Phytol* 172:358–368
- Cowie AL, Penman TD, Gorissen L, Winslow MD, Lehmann J, Tyrrell TD, Twomlow S, Wilkes A, Lal R, Jones JW, Paulsch A, Kellner K, Akhtar-Schuster M (2011) Towards sustainable land management in the drylands: scientific connections in monitoring and assessing dryland degradation, climate change and biodiversity. *Land Degrad Dev* 22:248–260
- Danjon F, Reubens B (2008) Assessing and analyzing 3D architecture of woody root systems, a review of methods and applications in tree and soil stability, resource acquisition and allocation. *Plant Soil* 303:1–34
- Fortin MCA, Poff KL (1990) Temperature sensing by primary roots of maize. *Plant Physiol* 94:367–369
- Giordano CV, Guevara A, Boccalandro HE, Sartor C, Villagra PE (2011) Water status, drought responses, and growth of *Prosopis flexuosa* trees with different access to the water table in a warm South American desert. *Plant Ecol* 212:1123–1134
- Guevara A, Giordano CV, Aranibar J, Quiroga M, Villagra PE (2010) Phenotypic plasticity of the coarse root system of *Prosopis flexuosa*, a phreatophyte tree, in the Monte Desert (Argentina). *Plant Soil* 330:447–464
- Guyomarc'h S, Lérans S, Auzon-Cape M, Perrine-Walker FML, Laplaze L (2012) Early development and gravitropic response of lateral roots in *Arabidopsis thaliana*. *Phil Trans R Soc B* 367:1509–1516
- Hodge A (2003) The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytol* 162:9–24
- Hodge A (2010) Roots: the acquisition of water and nutrients from the heterogeneous soil environment. *Prog Bot* 71:307–337
- Iwata S, Miyazawa Y, Takahashi H (2012) MIZU-KUSSE1 plays an essential role in the hydrotropism of lateral roots in *Arabidopsis thaliana*. *Environ Exp Bot* 75:167–172
- Iwata S, Miyazawa Y, Fujii N, Takahashi H (2013) MIZ1-regulated hydrotropism functions in the growth and survival of *Arabidopsis thaliana* under natural conditions. *Ann Bot* 112:103–114
- Jobbágy EG, Noyes MD, Villagra PE, Jackson RB (2011) Water subsidies from mountains to deserts: their role in sustaining groundwater-fed oases in a sandy landscape. *Ecol Appl* 21:678–694
- Kiba T, Kudo T, Kojima M, Sakakibara H (2011) Hormonal control of nitrogen acquisition: roles of auxin, abscisic acid, and cytokinin. *J Exp Bot* 62:1399–1409
- Kiss JZ (2007) Where's the water? Hydrotropism in plants. *Proc Natl Acad Sci* 104:4247–4248
- Kobayashi A, Takahashi A, Kakimoto Y, Miyazawa Y, Fujii N, Higashitani A, Takahashi H (2007) A gene essential for hydrotropism in roots. *Proc Natl Acad Sci* 104:4724–4729
- Kutschera U, Briggs WR (2012) Root phototropism: from dogma to the mechanism of blue light perception. *Planta* 235:443–452
- Loomis WE, Ewan LM (1936) Hydrotropic responses of roots in soil. *Bot Gaz* 97:728–743
- Mahibbur RM, Givbardarajulu Z (1997) A modification of the test of Shapiro and Wilk for normality. *J Appl Stat* 24:219–235
- Malamy JE (2005) Intrinsic and environmental response pathways that regulate root system architecture. *Plant Cell Environ* 28:67–77
- Massa GD, Gilroy S (2003) Touch modulates gravity sensing to regulate the growth of primary roots of *Arabidopsis thaliana*. *Plant J* 33:435–445
- Morello J (1958) La Provincia Fitogeográfica del Monte. *Opera Lilloana* 2:1–155
- Moriwaki T, Miyazawa Y, Fujii N, Takahashi H (2012) Light and abscisic acid signalling are integrated by MIZ1 gene expression and regulate hydrotropic response in roots of *Arabidopsis thaliana*. *Plant Cell Environ* 35:1359–1368
- Mullen JL, Hangarter RP (2003) Genetic analysis of the gravitropic set-point angle in lateral roots of *Arabidopsis*. *Adv Space Res* 31:2229–2236
- Nibau C, Gibbs DJ, Coates JC (2008) Branching out in new directions: the control of root architecture by lateral root formation. *New Phytol* 179:595–614
- Reynolds JF, Stafford Smith DM, Lamborn EF, Turner BL II, Mortimore M, Batterbury SPJ, Dowling TE, Dowlatabadi H, Fernández RJ, Herrick JE, Huber-Sannwald E, Jiang H, Leemans R, Lynham T, Maestre FT, Ayarza M, Walker B (2007) Global desertification: building a science for dryland development. *Science* 316:847–851

- Sassi M, Lu Y, Zhang Y, Wang J, Dhonukshe P, Blilou I, Dai M, Li J, Gong X, Jaillais Y, Yu X, Traas J, Ruberti I, Wang H, Scheres B, Vernoux T, Xu J (2012) COP1 mediates the coordination of root and shoot growth by light through modulation of PIN1- and PIN2-dependent auxin transport in *Arabidopsis*. *Development* 139:3402–3412
- Scholander PF, Hammel HT, Hemingsen EA, Bradstreet ED (1965) Sap pressure in vascular plants. *Science* 148: 339–346
- Stoll M, Loveys B, Dry P (2000) Hormonal changes induced by partial root zone drying of irrigated grapevine. *J Exp Bot* 51: 1627–1634
- Strohm AK, Baldwin KL, Masson PH (2012) Molecular mechanisms of root gravity sensing and signal transduction. *WIREs Dev Biol* 1:276–285
- Takahashi H, Miyazawa Y, Fujii N (2009) Hormonal interactions during root tropic growth: hydrotropism versus gravitropism. *Plant Mol Biol* 69:489–502
- Tsutsumi D, Kosugi K, Mizuyama T (2003) Effect of hydrotropism on root system development in soybean (*Glycine max*): growth experiments and a model simulation. *J Plant Growth Regul* 21:441–458
- Wang L, de Kroon H, Bögemann GM, Smits AJM (2005) Partial root drying effects on biomass production in *Brassica napus* and the significance of root responses. *Plant Soil* 276:313–326