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Volume 170 · Number 4 · December 2012

Oecologia

Springer

Springer

PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

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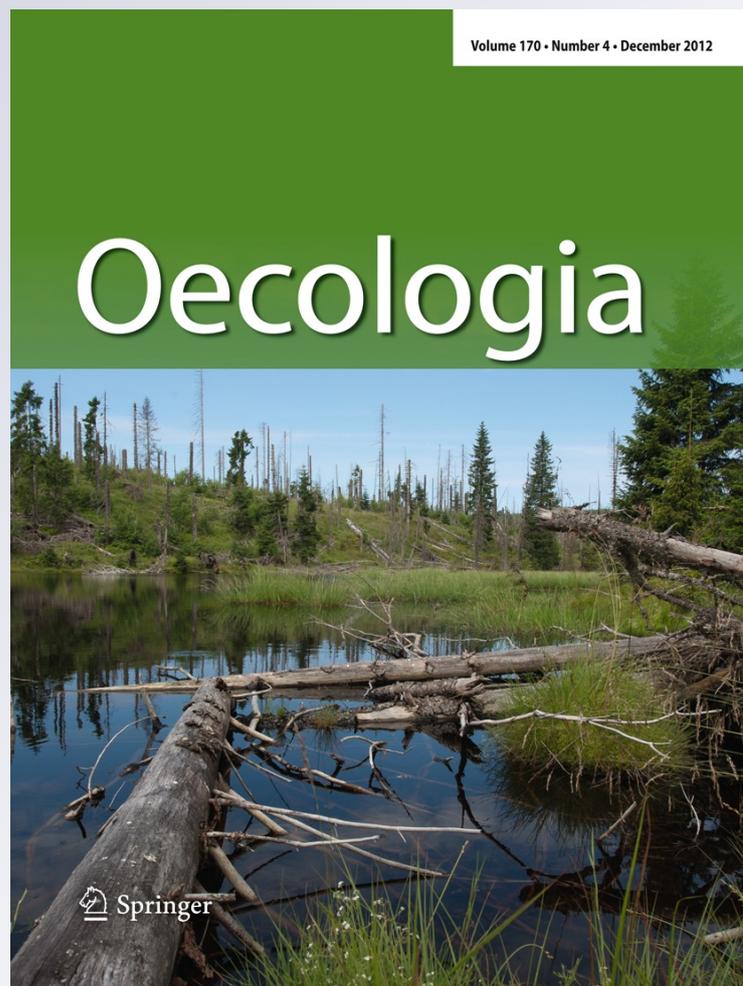
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Abstract Plants respond to low nutrient availability by modifying root morphology and root system topology. Root responses to nitrogen (N) and phosphorus (P) limitation may affect plant capacity to withstand water stress. But studies on the effect of nutrient availability on plant ability to uptake and transport water are scarce. In this study, we assess the effect of nitrogen and phosphorus limitation on root morphology and root system topology in *Pistacia lentiscus* L seedlings, a common Mediterranean shrub, and relate these changes to hydraulic conductivity of the whole root system. Nitrogen and phosphorus deprivation had no effect on root biomass, but root systems were more branched in nutrient limited seedlings. Total root length was higher in seedlings subjected to phosphorus deprivation. Root hydraulic conductance decreased in nutrient-deprived seedlings, and was related to the number of root junctions but not to other architectural traits. Our study shows that changes in nutrient availability affect seedling water use by modifying root architecture. Changes in nutrient availability should be taken into account when evaluating seedling response to drought.

Communicated by Marilyn Ball.

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Introduction

Nutrient limitation of plant productivity is widespread in agricultural soils. In 2000, nitrogen- and phosphorus-depleted soils covered 59 and 85 % of the harvested area worldwide, respectively (Tan et al. 2005). The extent of nutrient-depleted areas has increased in the last decades, threatening agricultural production and increasing the magnitude of food-insecure human populations (Lal 2009). Natural ecosystems also show limited availability of nutrients, particularly nitrogen and phosphorus (Lynch and Deikman 1998; Vitousek et al. 2010).

Nutrient limitation is often accompanied by limitation of other resources, particularly water (Field et al. 1992; Vitousek et al. 2010). Multiple resource limitation probably represents the normal situation in terrestrial ecosystems. However, understanding multiple resource limitation and morpho-physiological responses to be faced has challenged researchers for decades (Bloom et al. 1985; Vitousek et al. 2010). Responses to low availability of a given resource may have synergistic, antagonistic or neutral effects on plant ability to access other resources. For example, deep rooted genotypes may be favoured in water-limited environments, whereas shallow rooting may be advantageous for plants living in P-limited areas (Ho et al. 2005; Zhu et al. 2011).

In terrestrial ecosystems, plants have developed mechanisms to maximize the efficiency of nutrient and water uptake (Wu et al. 2005), including changes in root system topology (Fitter et al. 1991). Root system topology can be defined as the spatial configuration of the root system (Lynch 1995). In terms of topology, there is a wide range of root systems, including the herringbone type, where branching is restricted to the main axis, and the dichotomous type, in which each lateral root bifurcates (Eissenstat

1997). Topological modeling has been successfully used to explore the effects of branching patterns on resource uptake efficiency (Fitter 1991; Simunek and Hopmans 2009). Their results emphasize the importance of root topology and spatial deployment of fine roots, and their potential implications on acquisition of soil resources. To our knowledge, however, no model has explicitly incorporated the effect of changes in root topology on water transport.

Root system architecture may affect water and nutrient acquisition, carbon metabolism and resistance to environmental stresses. Root systems are commonly branched, and water fluxes between individual root segments (i.e., links) involve flows through root junctions. Changes in root hydraulic conductance (K_R) may occur in root junctions as a result of the decrease in vessel and tracheid diameter, the presence of vessel ends and the abundance of pit membranes (Zimmermann 1983; Schulte 2006). The hydraulic significance of root junctions and their role in the redistribution of water within the soil is not well understood. According to Zimmermann (1983), hydraulic limitation and embolism in the junctions may contribute to partition the root system and sacrifice lateral segments during periods of high water stress (Zimmermann 1983). Boundary pit membranes at root junctions can be efficient filters for microbes and particles entering the root system from damaged and senescent lateral roots (Shane et al. 2000). Previous works on xylem junctions aboveground suggest that they act as hydraulic constrictions or bottlenecks for water transport (Cruiziat et al. 2002). Since root junctions affect water flow, root systems differing in the number of root junctions, as root systems of contrasted topology, may also differ in their ability to transport water and their vulnerability to cavitation (Sperry and Ikeda 1997).

Plants respond to nutrient limitation by modifying branching patterns and root system architecture (Al-Ghazi et al. 2003; Lynch and Ho 2005). Phosphorus deficiency promotes the development, and increases the length and density of lateral roots and root hairs (Ma et al. 2001; Williamson et al. 2001; Bucio et al. 2002; Wu et al. 2005), although there are exceptions to this (Lynch 2011). Phosphorus deficiency also promotes shallower root angles and lateral branching, increased basal root whorl number, and the formation of adventitious roots and aerenchyma (Lynch 2011), whereas nitrogen deficiency promotes the increase in total and specific root length, steeper root growth angles, (Trubat et al. 2006; Sorgonà et al. 2007). Conversely, high NO_3^- or NH_4^+ availability promotes lateral root branching by triggering the production of lateral root primordia and higher order root branching (Jones and Ljung 2012). Plants respond to water limitation by inhibiting lateral branching (Malamy 2005), thus forming herringbone-like root systems (Berntson 1994), and promoting deep rooting (Ho et al. 2005; Zhu et al. 2011). In drylands, plants often produce roots with long links and high specific root length (Nicotra et al. 2002; Tjolker et al. 2005).

Anatomical and architectural changes resulting from contrasted changes in nutrient availability may affect root system ability to transport water. Several studies have shown that N and P deficiency reduce hydraulic conductance of entire plants (Radin and Matthews 1989; Radin and Ackerson 1981; Trubat et al. 2006). The decline in hydraulic conductance may result from various anatomical and morphological changes (Steudle 2000). Our understanding of the relationship between changes in root system topology in nutrient deficient plants and water acquisition and transport has been emphasized.

In this study we assess the effect of different nutrient levels on root morphology and architecture of *Pistacia lentiscus* L. seedlings. Then, we explore the relationship between these changes and root system ability to transport water. Finally, we assess the importance of the various components of root architecture in determining hydraulic conductance of the whole root system. *Pistacia lentiscus* is a common resprouting species in the Mediterranean basin, where it has been extensively used to restore degraded areas (Tomaselli 1981). We selected *Pistacia lentiscus* because (1) it has a strong impact on community composition and ecosystem function, (2) it thrives in areas subjected to seasonal drought, thanks to a fast growing tap root, (3) it grows on alkaline soils, where P availability is low, and (4) it is highly plastic (Caravaca et al. 2002; Maestre et al. 2004; Valdecantos et al. 2006; Trubat et al. 2008, 2011).

Materials and methods

Seedling production

Pistacia lentiscus seeds from local provenance (SE Spain Mountain Ranges, Forest Seed Bank, Generalitat Valenciana) were sown in March 2002 in $5 \times 5 \times 17$ cm polyethylene plugs filled with quartz sand. Plants were kept in a greenhouse from February to August 2002 and watered on alternate days with 40 mL of a modified Hoagland's solution containing either 150 mg N L^{-1} [as $Ca(NO_3)_2$ and KNO_3], 80 mg P L^{-1} (as KH_2PO_4) and 100 mg K L^{-1} (control, C), or identical solutions without nitrogen (nitrogen deficient treatment, ND) or phosphorus (phosphorus deficient treatment, PD). In ND and PD seedlings, osmolarity of the nutrient solution was adjusted with KCl (Radin 1984). Each treatment was replicated 20 times.

Root hydraulic conductance

On August 2002, we selected five well-watered seedlings per treatment randomly, and cut their stem 5 cm above the

root collar. Intact root plugs were placed in a pressure bomb with the excised stems protruding from the chamber (Scholander et al. 1965). Hydraulic conductance was measured as described by Nardini et al. (1998). Pressure in the chamber was gradually increased at a rate of 0.07 MPa min^{-1} up to 0.69 MPa. Flow was then measured at this pressure after 30 min equilibration to constant flow. Flow measurements were made every 2 min over a period of 10 min ($n = 5$ measures per pressure level) by placing a pre-weighted capsule with a sponge in contact with the sectioned stem and determining the increase in weight on a digital balance. Pressure was then released in steps of 0.17 MPa at a rate of 0.07 MPa min^{-1}, and the same procedure was followed to measure sap flow at each pressure level tested (i.e., 0.69, 0.52, 0.34 and 0.17 MPa). Flow was plotted against pressure, and K_R was calculated as the slope of the linear regression between both variables (Fiscus 1975). Finally, we calculated root specific hydraulic conductance (K_{RR}) as the ratio between K_R and root surface area (see below).

Morphological traits

Morphological traits were measured in August 2002 on 20 seedlings per treatment, including seedlings used for root hydraulic conductance measurements. Root surface area, total root length and number of root tips were measured after washing and scanning the root systems (8-bit gray scale image, resolution 300 dpi), and analyzing the image by means of specific image analysis software (WinRhizo, Regent Instruments Inc., Québec, Canada). Leaf area was measured by using the same procedure. Finally all biomass fractions were dried at 65 °C for 48 h to constant weight. Biomass allocation below ground was evaluated by calculating the root weight ratio (RWR) as the ratio between root dry weight and total plant dry weight (Hunt 1978). We calculated specific root length (SRL), as the ratio between root length and root dry weight. Finally, we calculated specific leaf area (SLA) as the ratio between leaf area and foliar dry weight.

Root system topology

The topology of root systems on a two dimensional plane can be described by using three variables, magnitude (μ), total exterior path length (P_e) and altitude (a). Magnitude is the number of root tips in the whole root system. Total exterior path length is calculated as the sum of all links counted from each of the root tips back to the base of the root system. Altitude is the single longest individual path from a root tip back to the base link. We counted the number of root tips and calculated the topological indices for three lateral roots from each seedling. Then, we

estimated expected altitude ($E(a)$) and expected total exterior path length ($E(P_e)$), that is the value of a and P_e for a randomly branched root system of a given μ, following the method described in Werner and Smart (1973), modified by Berntson (1994). Increases in the ratio $a/E(a)$ and $P_e/E(P_e)$ indicate more herringbone-like root systems whereas decreases in these ratios correspond to dichotomous branching. Links were further categorised as external–internal links (EI), external–external links (EE), and internal–internal links (II; Fitter and Stickland 1991), where external links end in a root tip, and external–external links differ from external–internal links in that the former are connected to external links.

$$P_e = 1.63\mu^{1.52} \quad a = 1.98\mu^{0.59}$$
$$E(P_e) = 2.17\mu^{1.42} \quad E(a) = 2.65\mu^{0.50}$$

Calculation of topological indices:

- Altitude slope: the slope of the regression of $\log_{10}a$ on $\log_{10}a\ \mu$.
- Pathlength slope: the slope of the regression of $\log_{10}P_e$ on $\log_{10}a\ \mu$.
- Altitude ratio $a/E(a)$ and $P_e/E(P_e)$.

Nutrient status of leaves

Dry samples of leaves were fragmented and digested in a heating block at 250 °C with a mixture of H_2SO_4 and H_2O_2 (1:1, v/v). We determined total N concentration by using semi-micro Kjeldahl distillation (Tecator Kjeltec Auto 1030 Analyzer, Hogana, Sweden), and P and K concentration by ICP spectrometry (Perkin Elmer Optima 3000, Perkin Elmer Corp., Norwalk, CT, USA).

Statistical analyses

We used analysis of variance (ANOVA) to evaluate the significance of the effect of nutrient deficiency on morphological and physiological variables. Tukey's HSD test was used to compare treatment means when ANOVA showed significant ($P < 0.05$) treatment effects. Statistical analysis was performed by using SPSS 10.6 statistical package (SPSS Inc., Chicago, USA). The proportion of variance in K_{RR} and K_R explained independently and jointly by topological variables was quantified using hierarchical partitioning (HP) of negative log-likelihoods methods (Mac Nally 2000; R 2.11.1, the R Foundation for Statistical Computing). This method allows identification of variables whose independent correlation with dependent variables is strong, in contrast to variables with little independent effect. The latter may be highly correlated with the dependent variable as a result of joint covariation
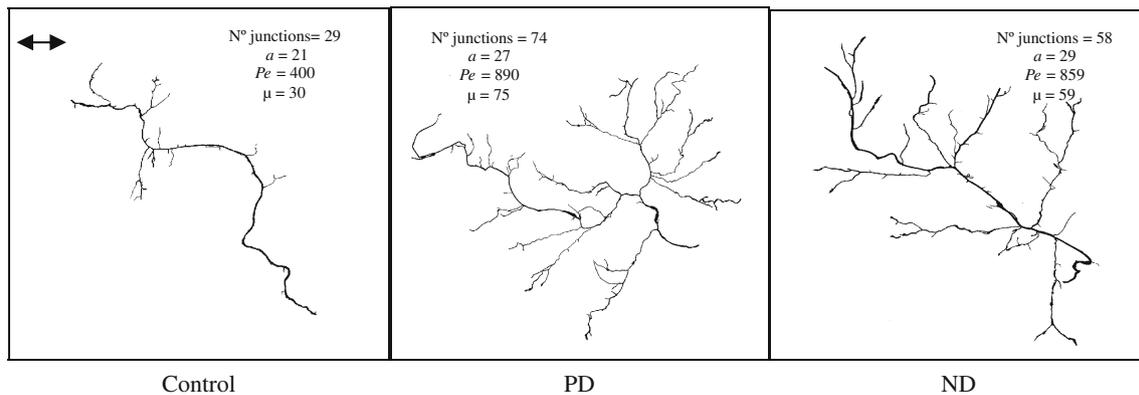
Fig. 1 Lateral roots of 6-month-old *Pistacia lentiscus* seedlings receiving a complete nutrient solution (control) or nutrient solutions with no N (ND) and no P (PD). The number of junctions, altitude (a), total exterior path length (P_e), and magnitude (μ) are given for each root. The *inset double arrow* corresponds to 2 cm for the three images

with other independent variables. The output of an HP analysis is a list of predictor variables and their independent (I) and joint (J) influences on the response variable. Variables that independently explained a larger proportion of variance than would be expected by chance were identified by comparing their observed value of independent contribution to variance (I) to a population of "I" values from 1,000 randomizations of the data matrix. Results of HP analyses for each variable were expressed as Z scores ([observed − mean {randomizations}]/SD {randomizations}), and the statistical significance based on the upper 95 % confidence limit ($Z \geq 1.65$). Thus hierarchical partitioning does not produce any kind of predictive model. Rather it allows identification of the predictors that explain most variance independently of the others, helping to overcome the problems presented by multi-collinearity. Hierarchical partitioning will be used here to assess the amounts of variance attributable to each predictor variable (Figs. 1, 2)

Results

The reduction in N and P availability had a strong effect on seedling nutritional status. The absence of N in the nutrient solution reduced foliar N concentration by 42 %, and increased foliar K concentration, which almost duplicated the concentration found in control and PD seedlings. P deficient reduced foliar P concentration to values below 1 mg g^{-1} (Table 1).

Seedling morphology was strongly affected by low levels of N and P (Table 2). Leaf area was significantly reduced by both treatments. ND seedlings produced 20 % less shoot dry weight than control seedlings. There was no effect of P deprivation on above ground biomass
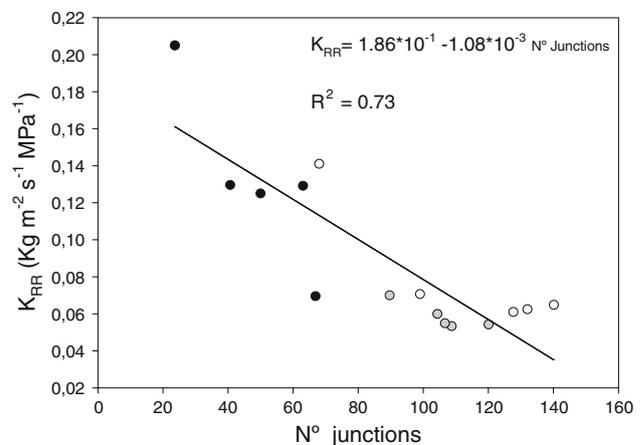

Fig. 2 Relationship between specific root hydraulic conductance (K_{RR}) and number of junctions of *Pistacia lentiscus* seedlings receiving complete nutrient solution (black symbols), a nutrient solution with no N (*grey symbols*), and nutrient solution with no P (*open symbols*)

accumulation. The decrease in above ground biomass in ND seedlings was not paralleled by a decrease in below ground biomass, and thus, ND seedlings had higher RWR than control seedlings. Phosphorus deprivation decreased SLA compared to control and ND seedlings. Phosphorus deprivation had a positive effect on root area and root length, and a marginally significant effect on root biomass (Table 2). Root tissue density was higher in ND seedlings compared to control seedlings, and showed no significant response to P deprivation.

Root hydraulic conductance was affected by the treatments. Pressure gradient and water flow rate of whole root systems were highly correlated in all seedlings ($r > 0.97$, $N = 12$, $p < 0.001$). Root specific hydraulic conductance and leaf specific hydraulic conductance were substantially

Table 1 Foliar nutrient concentration in 6-month-old *Pistacia lentiscus* seedlings receiving a complete nutrient solution (control), and nutrient solution with no N (ND) and no P (NP)

	Control	ND	PD	*F*	*P*
N (mg g^{-1})	27.1^a ± 1.70	15.6^b ± 0.90	22.5^a ± 1.40	1.67	<0.001
P (mg g^{-1})	5.1^a ± 1.10	5.9^a ± 0.14	0.8^b ± 0.20	10.83	0.002
K (mg g^{-1})	12.9^a ± 1.20	20.6^b ± 0.90	10.3^a ± 0.50	29.66	<0.001

Means followed by the same letter for a given nutrient are not significantly different (Tukey's HSD test, $p < 0.05$). Data are mean ± SE ($n = 5$ seedlings)

Table 2 Morphological characteristics of *Pistacia lentiscus* seedlings grown for 6 months with complete nutrient solution (control), and nutrient solutions with no N (ND) and no P (PD)

	Control	ND	PD	*F*	*P*
Root dry weight (g)	0.19 ± 0.01	0.21 ± 0.03	0.15 ± 0.05	2.89	0.094
Shoot dry weight (g)	0.37^a ± 0.01	0.26^b ± 0.01	0.35^a ± 0.01	1.39	0.028
RWR	0.31^a ± 0.03	0.42^b ± 0.09	0.29^a ± 0.03	15.95	<0.001
Leaf area (cm^2)	32.6^a ± 2.5	25.6^b ± 1.3	25.7^b ± 3.1	4.44	0.007
Root surface area (cm^2)	59.0^a ± 4.7	61.1^a ± 5.8	83.2^b ± 3.1	2.55	0.020
SRL (cm g^{-1})	2,667^a ± 508	3,062^a ± 424	5,207^b ± 523	48.69	<0.001
SLA (cm^2 g^{-1})	93.4^a ± 8.4	98.3^a ± 5.2	70.4^b ± 3.1	1.01	0.046
Root tissue density (g cm^{-3})	0.016^a ± 0.01	0.023^b ± 0.01	0.019^{ab} ± 0.01	4.37	0.024
Root length (cm)	512^a ± 21	635^a ± 30	785^b ± 67	3.14	0.04

Results of the ANOVA to test the significance of nutrient deficiencies are also shown. Data are means ± SE ($n = 20$). Different letters within each row indicate significant differences (Tukey's HSD test, $p < 0.05$)

Table 3 Root hydraulic conductance (K_R) and specific root hydraulic conductance (K_{RR}) of *Pistacia lentiscus* seedlings receiving a complete nutrient solution (control) or nutrient solutions with no N (ND) and no P (PD)

	Control	PD	ND	*F*	*P*
K_R (Kg s^{-1} MPa^{-1} 10^{-8})	7.23^a ± 1.41	4.64^b ± 1.09	4.78^b ± 1.21	6.84	0.001
K_{RR} (Kg m^{-2} s^{-1} MPa^{-1})	0.13^a ± 0.04	0.08^b ± 0.03	0.06^b ± 0.01	5.93	0.016

Data are means ± SE ($n = 5$ seedlings). Different letters indicate significant differences (Tukey's HSD test, $p < 0.05$)

reduced by N and P deficiency (e.g., K_{RR} in ND seedlings was 64 % of the K_{RR} observed in control seedlings; Table 3).

The number of junctions was higher in ND and PD seedlings than in control seedlings (Table 4). The topology of lateral roots was significantly affected by nutrient deficiency. Root system altitude ($a/E(a)$) and total exterior pathlength ($P_e/E(P_e)$) were lower in ND and PD seedlings, reflecting a trend towards more branched dichotomous systems (Table 4). Phosphorus deficiency resulted in a significant increase in the average length of external links (EE and EI).

Hierarchical partitioning analysis showed that the number of junctions was the main variable explaining the observed variability in root specific hydraulic conductance (Table 5). The contribution of the topological indices to the variability of hydraulic conductance was not statistically significant (Fig. 3).

Discussion

Carbon allocation patterns depend on above ground and below ground resources limiting plant growth, its distribution and mobility (Bloom et al. 1985; Poorter and Nagel 2000). In areas with seasonal rainfall, water is a "deep resource" that can be accessed by extended vertical growth of the primary root (i.e., herringbone structures where lateral roots are restricted to the main axis). In contrast, N and P are "shallow resources" whose availability is higher in the topsoil, and they are more readily acquired by highly branched roots (e.g., dichotomous systems) growing close to the soil surface (Lynch and Brown 2001; Ho et al. 2005). When low mobility resources, such as P, are limiting, plants respond by decreasing root system altitude and total exterior path length, root growth angle and increasing root system magnitude and length, average link length, SRL,

Table 4 Magnitud$e$ (μ), topological indices ($a/E(a)$, $Pe/E(P_e)$), number of junctions and average length of external–external (EE), external–internal (EI) and internal–internal (II) links of secondary roots of *Pistacia lentiscus* seedlings receiving a complete nutrient solution (control), a nutrient solution with no N (ND), and a nutrient solution with no P (PD)

	Control	ND	PD	F	P
μ (number)	$29^a \pm 4$	$53^b \pm 5$	$54^b \pm 6$	8.55	0.001
$a/E(a)$	$1.59^a \pm 0.08$	$1.17^b \pm 0.1$	$1.08^b \pm 0.07$	9.35	<0.001
$P_e/E(P_e)$	$1.34^a \pm 0.13$	$1.01^b \pm 0.1$	$0.94^b \pm 0.07$	5.27	0.014
Number of junctions	$28^a \pm 7.0$	$52^b \pm 8.0$	$53^b \pm 10.3$	36.13	<0.001
EE (cm)	$0.56^a \pm 0.07$	$0.57^a \pm 0.05$	$0.64^b \pm 0.07$	32.50	<0.001
EI (cm)	$0.55^a \pm 0.05$	$0.50^a \pm 0.05$	$0.63^b \pm 0.04$	2.89	0.004
II (cm)	0.42 ± 0.03	0.45 ± 0.03	0.47 ± 0.02	0.58	0.560

Data are means $\pm$ SE ($n = 15$ seedlings). Means followed by the same letter for a given nutrient are not significantly different (Tukey's HSD test, $p < 0.05$)

Table 5 Results of the hierarchical partitioning of root specific hydraulic conductance (K_{RR})

Variable	J	Z
μ	0.043	0.56
a	0.458	0.61
P_e	0.256	−0.18
$a/E(a)$	−0.314	0.53
$P_e/E(e)$	0.224	−0.15
Number of junctions	0.395	2.02*
Root surface area (cm^2)	0.295	0.22
Root dry weight (g)	−0.003	−0.44
Root length (cm)	0.381	0.17

Given are the variance explained by individual variables (I), the variance explained together with other variables (J) and the Z score from the randomization procedure. The asterisk identifies a significant difference based on the upper 0.95 confidence limit ($Z \geq 1.65$)
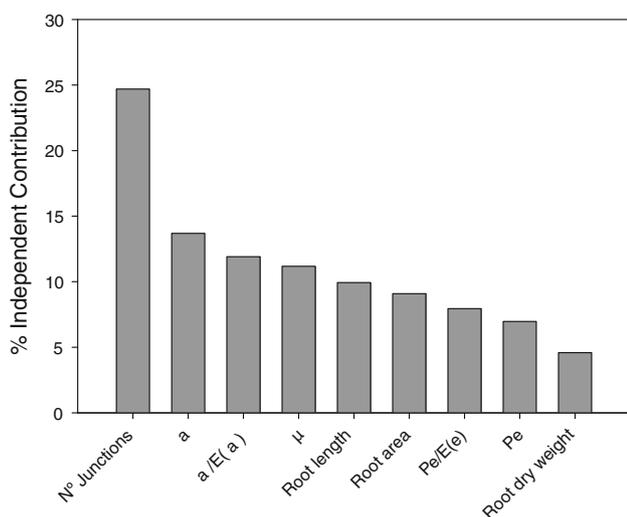

Fig. 3 Percentage-distribution of independent effects of root architectural traits on root specific hydraulic conductance (K_{RR}) calculated from the hierarchical partitioning analysis

basal root whorl number, lateral root dispersion, the number of root hairs and the amount of aerenchyma (Lynch and Brown 2001; Lynch 2011). Our results partly support these observations, as we found that *P. lentiscus* seedlings responded to P deprivation by increasing lateral root growth and lateral root density (Williamson et al. 2001). We may note, however, that various exceptions to these rules have been observed in dicots and grasses (Fitter and Stickland 1991; Taub and Goldberg 1996). Herringbone-like root systems can be more effective at acquiring deep soil resources than dichotomous systems, but the relationship between root topology and competition is poorly understood (Fitter and Stickland 1991; Lynch 2011).

RWR in N-deficient seedlings increased and below ground biomass was maintained despite the likely decrease in C fixation rate. Since nutrient deficiency reduces leaf growth and leaf demand for carbohydrates, more carbohydrates may be available for root growth (Clarkson et al. 2000). In contrast, P deficiency did not affect biomass accumulation or allocation patterns, although it favoured a decrease in foliar surface and changes in root morphology.

The increase in SRL in P deficient plants was not paralleled by a decrease in root tissue density but rather by a decrease in average root diameter. This decrease was accompanied by an increase in average link length of external links. These changes have important implications for the functioning of the root system. On the one hand, lateral roots are more efficient in capturing soil resources because of their higher root surface area, and may increase nutrient uptake per unit of carbon invested in root construction and maintenance (Robinson 1996). In this way, P deprivation probably increased seedling efficiency in P uptake. On the other hand, fine root diameter is directly related to fine root longevity (Eissenstat et al. 2000), and thus increases in the efficiency of soil exploration in P-deficient plants were probably achieved at the expense of a higher root turnover.

Root response to phosphorus limitation is different than for mobile resources such as nitrate and water (Fitter et al. 2002; Ho et al. 2005). Nitrate diffusion is three to four orders of magnitude faster than that of phosphate (Tinker and Nye 2000), and a low root density is sufficient to capture nitrate in a large volume of soil (Linkohr et al. 2002). Response to N deprivation in *P. lentiscus* seedlings was somewhat unexpected, as N-deficient seedlings showed no changes in root length, root surface area, average link length and SRL, but increases in the number of junctions. In addition, N-deficient seedlings changed their topology towards a dichotomous-like root system.

Several studies have found a decrease in root hydraulic conductance in nutrient deficient plants (Radin and Eidenbock 1984; Syvertsen and Graham 1985; Radin and Matthews 1989; Trubat et al. 2006). In our study, plants deprived of N and P showed lower capacity for water transport per unit of root surface area than control plants. Nutrient-deprived plants also showed higher SRL, which is commonly associated with smaller xylem vessel diameter and high resistance to water flow (Castro-Díez et al. 1998). The reduction in the ability for transporting water may affect the water balance of nutrient deficient plants (Clearwater and Meinzer 2001), and may limit growth (Hsiao 1973). Low hydraulic conductance hampers an efficient transport of water and nutrients above ground, promoting the development of high water potential gradients. Limitations to water transport due to reductions in hydraulic conductance may enhance a conservative water use under moderate water stress (Sperry 2003). On the other hand, narrow xylem vessels may be less prone to cavitation and embolism in response to low water availability (Pockman and Sperry 2000). A reduction in hydraulic conductance has been associated with lower risk of xylem cavitation (Martínez-Vilalta et al. 2002; Vilagrosa et al. 2003), and higher tolerance to drought (Trubat et al. 2006; Chirino et al. 2008).

Water flow from soil to leaves depends on hydraulic conductance, root system architecture and their interaction (Doussan et al. 1998). The weak relationship found between K_{RR} and μ is puzzling. Root apices are commonly thinner and less lignified and suberized, and decreases in nutrient uptake capacity with aging can be very rapid (Wells and Eissenstat 2003). Thus, we expected that a higher proportion of terminal links (EE links and root system magnitude) would promote water flow, but this was not the case. Other studies have shown that old roots can be highly efficient in absorbing water (Kramer and Bullock 1966; MacFall et al. 1991; North and Baker 2007), and they play a crucial role in capturing water from small infrequent rainfall events in deserts (North and Baker 2007). Indeed, the extent of lignification and suberization cannot be estimated from visual observations (McCrady

and Comerford 1998), and may depend on soil conditions, and not only on root age (North and Nobel 2000). Changes in root structure are directly associated with the hydraulic root resistance and the pathways used for water movement in the root, both for axial transport in vessels and for the radial movement across the root. Various studies have shown that axial resistance is substantially smaller than radial resistance (Rowse and Goodman 1981). Radial hydraulic resistance has been mostly attributed to the endodermis and exodermis, where hydrophobic deposits block the transport of water and ions in the cell walls (Passioura 1988). The increase in SRL in nutrient-deprived plants may have reduced radial resistance to water flow, and contributed to increase hydraulic conductance (Eissenstat 1997). In our study, other factors may have compensated for the positive effect of increased SRL on water transport capacity, as nutrient deprivation resulted in lower hydraulic conductance.

In contrast, the significant correlation found between K_{RR} and K_R, on one hand, and the number of root junctions, on the other, suggests that resistance to water flow is more heavily dependent on the number of links (and their connections), than the way they are arranged. The effect of root junctions on hydraulic conductance is still under discussion. Junctions may represent constrictions to water flow and contribute to protect the main root during soil drying (North et al. 1992). However, water paths in root junctions are complex (Shane et al. 2000) and water flow may not follow simple resistance analogue models (Schulte and Brooks 2003). Recently developed 3-D models of water flow in plant stems, where conduits show a varied degree of interconnection (Loepfe et al. 2007), will probably shed light on the hydraulic significance of junctions. At the leaf level, junctions are sites of high hydraulic resistance and embolism during drought (Chave et al. 2002). Some studies argue that the highest resistance in the hydraulic pathway occurs at the ends of vessels or tracheids, as water flows from one conduit element into the next via perforation in the conduit ends walls (Chave et al. 2002; Zimmermann 1978). It has been suggested that the increase in resistance to water flow due to branch junctions is small in seedlings compared with adult trees (Tyree and Ewers 1991). If this were the case, we would expect that the effect of nutrient limitation on hydraulic conductance mediated by changes in root architecture would increase with time.

Plant response to limited N and P availability may have strong implications for plant capacity to face drought. On the one hand, limitation by N and, particularly P, promotes root density by increasing biomass allocation below ground and promoting root branching. This strategy may confer additional advantages in environments subjected to small rainfall events, where water remains in the topsoil layers,

but not in areas subjected to seasonal rains, where deep rooting is crucial to withstand long periods of drought (Padilla and Pugnaire 2007). On the other hand, lower water transport capacity resulting from increased branching in nutrient-limited plants may promote plant ability to withstand drought but may compromise its capacity to grow when water is not limiting.

Conclusion

Nitrogen and phosphorus deprivation strongly affected root system morphology and architecture of *Pistacia lentiscus* seedlings, and decreased hydraulic conductance of the whole root system. Specific hydraulic conductance was significantly related to the number of root junctions but not to other architectural traits of the root system, emphasizing the importance of root branching as a determinant of root function. Our results show that N and P limitations may have a strong impact on seedling water use and seedling ability to withstand drought.

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