ORIGINAL ARTICLE

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Plant morphology and root hydraulics are altered by nutrient deficiency in *Pistacia lentiscus* (L.)

Abstract The plants in arid and semiarid areas are often limited by water and nutrients. Morpho-functional adjustments to improve nutrient capture may have important implications on plant water balance, and on plant capacity to withstand drought. Several studies have shown that N and P deficiencies may decrease plant hydraulic conductance. Surprisingly, studies on the implications of nutrient limitations on water use in xerophytes are scarce. We have evaluated the effects of strong reductions in nitrogen and phosphorus availability on morphological traits and hydraulic conductance in seedlings of a common Mediterranean shrub, *Pistacia lentiscus* L.. Nitrogen deficiency resulted in a decrease in aboveground biomass accumulation, but it did not affect belowground biomass accumulation or root morphology. Phosphorus-deficient plants showed a decrease in leaf area, but no changes in aboveground biomass. Root length, root surface area, and specific root length were higher in phosphorus-deficient plants than in control plants. Nitrogen and phosphorus deficiency reduced both root hydraulic conductance and root hydraulic conductance scaled by total root surface area. On the other hand, nutrient limitations did not significantly affect root conductance per unit of foliar surface area. Thus, adaptation to low nutrient availability did not affect seedling capacity for maintaining water supply to leaves. The implications for drought resistance and survival during seedling establishment in semi-arid environments are discussed.

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Introduction

There is a vast amount of information on plant strategies to cope with limiting resources. However, in natural environments, limitation by a single resource is uncommon, and plants must simultaneously optimize the use of multiple resources (Schulze et al. 1991). Xeric environments are characterized by excessive radiation, high-evaporative demand, and low-water availability. Under these conditions, plants have evolved morpho-functional traits to enhance water absorption and transport (Levitt 1980; Larcher 1995). Species respond to water deficit by developing avoidance mechanisms based on stomatal control, reductions in leaf area, leaf size and specific leaf area (Waring et al. 1985), and changes in root hydraulic conductance, which can be considered as a complementary mechanisms for regulating transpiration (Blanco et al. 2002). In drylands, soil fertility is frequently low, due to low rates of nitrogen fixation, organic matter inputs and mineralisation rates, and high rates of phosphorus immobilization (Henkin et al. 1998; Vallejo et al. 1998). Disturbances such as recurrent wildfires, and historical land uses such as low-input agriculture and fiber cropping may have further decreased soil fertility in these areas (Albaladejo et al. 1998). Low-moisture levels can substantially reduce soil nutrient availability by decreasing nutrient diffusion and mass flow (Kramer 1988; Passioura 1988), and nitrification rate (Killham 1995), and by promoting nutrient losses at leaf level (Heckathorn et al. 1997; De Lucia et al. 1998). Thus, it is not surprising that dryland plants frequently show low nutrient levels (Osonubi et al. 1988) and may respond to fertilization (Hamilton et al. 1998).

A negative relationship between water-use efficiency (WUE) and nutrient use efficiency has been observed (Ewers et al. 2000), suggesting that plants cannot fully use nutrients for growth when water is limiting. Increasing N

investment in leaves may not result in a significant increase in photosynthesis when plants are under strong water limitation. In contrast, at constant water loss, increases in tissue N may increase WUE (Field and Mooney 1982). Also, at a constant N concentration, decreases in stomatal conductance may increase WUE but decrease overall photosynthesis, and N use efficiency (Nielsen and Orcutt 1996). Some plant strategies to cope with drought, such as increased biomass allocation belowground, increased WUE or changes in root morphology (Ingestad and Ågren 1991; Jackson et al. 2000) have been described for nutrient-limited plants (Forde and Lorenzo 2001). However, changes in root architecture in drought-stressed plants may reduce plant ability to capture relatively immobile nutrients such as phosphorus (Fitter et al. 1991; Huang and Nobel 1994). Deep rooting, a commonly reported strategy to withstand water limitation, may also promote the exploration of soils horizons that are commonly poor in organic matter and nutrients (Canadell and Zedler 1995).

The limited N and P availability may affect the growth rate and morphology of roots and root hairs. As P deficiency can have a profound effect on root system morphology and architecture (Williamson et al. 2001; Búcio et al. 2002), it may alter plant capacity for water transport, as observed in intact plants and excised roots (Radin and Matthews 1989). Under high-evaporative demand, reductions in the water transport capacity may promote drought stress above ground and increase the risk of hydraulic conductance loss due to the xylem cavitation (Sperry 2000). In xeric environments plants have evolved morphological traits to optimize water absorption and transport, maximizing transport efficiency and avoiding the risk of xylem failure (Martínez-Vilalta et al. 2002).

Despite its importance, little information exists on the effects of nutrient limitation on the water balance of woody plants and, more specifically, on the capacity of the root system to transport water. In semi-arid ecosystems seedling survival is strongly coupled to soil water availability (Cortina et al. 2004). Therefore, changes in root capacity for water transport should affect survival and growth under field conditions. The objective of this work is to evaluate the effect of nitrogen and phosphorus deficiency on the morphology, biomass allocation and root hydraulic conductance of a common Mediterranean shrub (mastic tree, *Pistacia lentiscus* L.). *P. lentiscus* L. is a widely distributed sprouting species in the Mediterranean (Le Houérou 1981; Naveh 1989) and exhibits a high degree of plasticity in response to drought (Vilagrosa et al. 2003). Previous studies have shown that short-term survival of the Mediterranean shrubs, such as *P. lentiscus* L., under semiarid field conditions can be enhanced when N or P is removed from the nutrient solution during the nursery phase (Trubat et al. 2004).

Materials and methods

P. lentiscus L. seeds from a local provenance (E Spain Mountain Ranges) were sown in March 2002 in 305 cm^3 (5 cm×5 cm×17 cm) polyethylene plugs with quartz sand as the culture medium. The plants were kept in the open air and watered on alternate days with 40 mL per plant of a modified Hoagland's solution containing 150 mgL^{-1} nitrogen (as $Ca(NO_3)_2$ and KNO_3), 80 mg L^{-1} phosphorus (as KH_2PO_4) and 100 mg L^{-1} potassium (control seedlings, hereafter C), or with similar solutions containing either no N (Nitrogen-deficient seedlings, hereafter N^-) or no P (phosphorus-deficient, hereafter P^-). In N^- and P^- seedlings, osmolarity of the nutrient solution was adjusted with KCl (Radin 1984). Each treatment was replicated 20 times.

After 6 months, five randomly selected seedlings per treatment were removed from the field in the night before they were to be measured in the laboratory. There, after the stems were then cut 5 cm above the root collar and the root plugs were placed in a pressure bomb (Scholander et al. 1965) with the excised stems protruding from the chamber, hydraulic conductance was measured as described in Nardini et al. (1998). The pressure in the chamber was increased at a rate of about 0.07–0.69 MPa min^{-1}. The flow was then measured at this pressure after 30 min of equilibration. Flow measurements were made every 2 min over a period of 10 min (n=5 measures per pressure level) by placing a plastic capsule with a sponge in contact with the sectioned stem and determining the increase in weight on a digital balance. The pressure was then decreased 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 root hydraulic conductance (K_R) was estimated as the slope of the linear regression between both variables (Fiscus 1975). Hydraulic conductance of the whole root system is mainly a function of the root surface area (A) in contact with the soil (Nardini et al. 1998), and thus we estimated root specific hydraulic conductance (K_{RR}) as the ratio between root hydraulic conductance (K_R) and root surface area, and leaf specific hydraulic conductance (K_{RL}) as the ratio between root hydraulic conductance (K_r) and leaf area, according to Nardini et al. (2000).

Morphological traits were measured on 20 seedlings per treatment. Root length (cm) and root surface area (cm^2) were measured after washing out the sand, spreading the root system on a A4 size tray to minimize overlaps, scanning (on a professional scanner with transparency adapter; 8-bit grayscale image, resolution 300 dpi), and analyzing the image by means of specific image analysis software (WinRhizo, Régent Instruments Inc., Quebec Canada). Leaf area (cm^2) was measured by the same procedure. Finally, all biomass fractions were dried at 65°C to constant weight. Biomass allocation belowground was evaluated by calculating the root weight ratio (RWR, g g^{-1}) as the ratio between root dry weight and total dry weight (Hunt 1978). We calculated the specific root length (SRL, cm g^{-1}) as the ratio between root length and root dry weight. The specific leaf area (SLA, cm^2 g^{-1}) was calculated as the ratio between leaf area and foliar dry weight. We estimated root tissue density (RTD, g cm^{-3}) as the ratio between root dry weight and root volume.

Table 1 Foliar nutrient concentration in *P. lentiscus* L. seedlings receiving a complete nutrient solution, a nutrient solution with no N (N$^-$) or a no P (P$^-$)

	Control	N$^-$	P$^-$	F	P
N (mg g^{-1})	27.1±1.70 a	15.6±0.90 b	22.5±1.40 a	1.67	<0.001
P (mg g^{-1})	5.1±1.10 a	5.9±0.14 a	0.8±0.20 b	10.83	0.002
K (mg g^{-1})	12.9±1.20 a	20.6±0.9 b	10.4±0.50 a	29.66	<0.001

Data are means ± S.E. (n=5 plants). Means followed by the same letter are not significantly different at $P<0.05$

The dried leaves were ground in a ring mill 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).

We evaluated the effect of N and P deficiency on seedling morphology and root hydraulic conductance by one-way analysis of variance with one fixed factor (nutrients). When nutrients had a significant effect on any variable, we compared the means by using Tukey's b HSD test. Statistical analyses were performed by using the SPSS 10.6 statistical package (SPSS Inc., Chicago, USA).

Results

The reductions in N and P availability had a strong effect on foliar nutrient concentrations. The absence of N in the nutrient solution reduced foliar N concentration from 27.1 to 1.56 mg N g^{-1} and increased foliar K concentration from 12.9 to 20.6 mg K g^{-1}. P suppression resulted in an 84% decrease in foliar P concentration (Table 1).

Both the number of leaves per plant and the leaf area were reduced by N deficiency (Table 2). Specific leaf area was not affected by this treatment, and thus aboveground biomass was lower in N$^-$ plants. Leaf number and leaf area were also lower in P$^-$ plants than in control plants. However, specific leaf area decreased from 93.4 to 70.4 cm^2 g^{-1} in the absence of P, and aboveground biomass accumulation in P$^-$ plants did not differ from that of Control plants.

Belowground growth was not affected by N deficiency. Trends toward increasing root length, root surface area, root biomass, and specific root length were not statistically significant. Only root tissue density was higher in N$^-$ plants than in control plants. In contrast, root surface area

and, marginally, root length in P$^-$ seedlings increased as compared to control seedlings. Root biomass accumulation and root tissue density did not differ between control and P$^-$ seedlings. Thus, similar values of root biomass accumulation in control and P$^-$ seedlings resulted from higher specific root length in the latter. Biomass allocation belowground, as reflected by the RWR, was not affected by N or P deficiency.

Sap flow increased linearly with pressure, generating correlation coefficients higher than 0.97 for all plants (Fig. 1). Sap flow was similar for all treatments at low pressure (0.2 MPa), but it was substantially higher in control seedlings than in N$^-$ or P$^-$ seedlings at higher pressure. Accordingly, root hydraulic conductance (K_R) was reduced by N and P deficiency ($F=6.84$; $P<0.01$). K_{RR} was also lower in N$^-$ and P$^-$ seedlings than in the control plants ($F=5.93$; $P<0.016$) (Fig. 2). In contrast K_{RL} was not affected by N and P deficiency (Fig. 3).

Discussion

We found a strong effect of N and P limitation on foliar nutrient concentrations. N concentration in N$^-$ seedlings was close to levels that have been commonly associated with N deficiency (Grundon et al. 1997; Connor and Fereres 2005). Foliar P concentration was also close to critical levels in P$^-$ seedlings. Potassium concentration strongly increased in N$^-$ seedlings, suggesting luxury consumption (Marschner 1986).

Aboveground biomass accumulation was reduced by low N availability, an effect that has been widely described in the literature (Ingestad and Ågren 1991; Rubio et al. 2003). N deficiency resulted in a decrease in the number of leaves and the whole plant leaf area, with no changes in specific leaf weight and average leaf area. P deficiency also lowered the number of leaves and whole plant leaf area. But

Table 2 Morphological traits of *P. lentiscus* L. seedlings growing under contrasted nutritional regimes

N$^-$ and P$^-$ plants received no N or P additions, respectively, throughout the period of study. Data are means ± S.E (n=20). Different letters in each row indicate significant differences at $P<0.05$
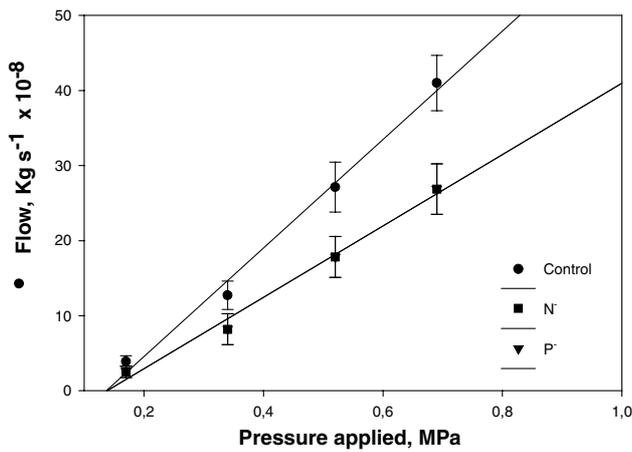
	Control	N$^-$	P$^-$	F	p
Root dry weight (g)	0.19±0.01	0.21±0.03	0.15±0.05	2.89	0.094
Shoot dry weight (g)	0.37±0.008 a	0.26±0.01 b	0.35±0.01 a	1.39	0.028
Leaf area (cm^2)	32.6±2.5 a	25.6±1.3 b	25.7±3.1 b	4.46	0.007
Root surface area (cm^2)	59.0±4.7 a	61.1±5.8 a	83.2±3.1 b	2.55	0.020
Specific root length (cm g^{-1})	2667±508 a	3062±424 a	5207±523 b	48.69	<0.001
Specific leaf area (cm^2 g^{-1})	93.4±8.4 a	98.3±5.2 a	70.4±3.1 b	1.01	0.046
N° Leaves	16.0±0.9 a	12.8±0.7 b	13±0.5 b	6.27	0.006
Root tissue density (g c^{-3})	0.016±0.01 a	0.023±0.01 b	0.019±0.01 ab	4.37	0.024
Root length (cm)	512±21.3 a	635±30.2 a	785±67.16 b	3.14	0.08
Root weight ratio (g g^{-1})	0.32±0.02 a	0.30±0.02 a	0.38±0.02 a	4.014	0.046

Fig. 1 Changes in water flow with pressure in root systems of *P. lentiscus* L. seedlings subjected to contrasted nutritional regimes. *Bars* correspond to standard errors of *n*=5 plants per treatment. The slopes of the linear relationships between flow and pressure were used to estimate hydraulic conductance
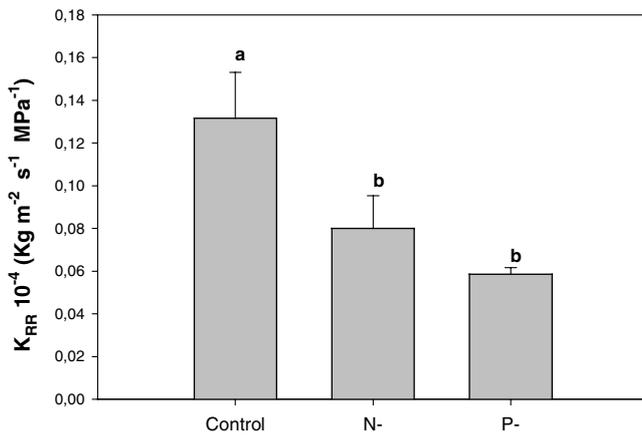

Fig. 2 Root hydraulic conductance scaled by total root surface area (K_{RR}) of *P. lentiscus* L. seedlings receiving a complete nutrient solution (control) or nutrient solutions with no N (N^-) or no P (P^-). Data are means ± S.E (*n*=5 plants). Different letters indicate significant differences at $P<0.05$, (F=593; P=0.016)
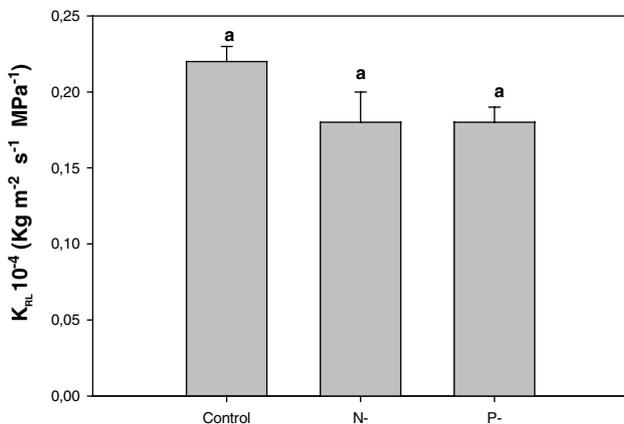

Fig. 3 Root hydraulic conductance scaled by total leaf surface area (K_{RL}) of *P. lentiscus* L. seedlings receiving complete nutrient solutions, or nutrient solutions with no N (N^-) or no P (P^-). Data are means ± S.E (*n*=5 plants). Different letters indicate significant differences at $P<0.05$, (F=1.26; P=0.31)

this was not reflected in a decrease in aboveground biomass. The reductions in leaf area in P-deficient plants have been observed elsewhere (Radin and Boyer 1982; Ewers et al. 2000; Yates et al. 2002). The decrease in leaf area may result in a decrease in the whole plant transpiration rates, which would enhance a drought-avoiding strategy (Levitt 1980; Nielsen and Orcutt 1996). Moreover, the proportion of root area versus leaf area increased from 1.8 in control seedlings to 2.4 and 3.2 in N^- and P^- seedlings, respectively. Thus, nutrient-deficient plants maintained a lower transpiring surface per unit of absorbing surface, an adjustment that may favour seedling resistance to water stress.

Nutrient deficiency promoted strong modifications in root system morphology, particularly in P^- seedlings. Specific root length and root surface area increased in P deficient seedlings. Several studies suggest that P deficiency affects root elongation through changes in H^+ excretion and subsequent effects on cell wall loosening (Anuradha and Narayanan 1991). The observed increase in the root surface area resulting from longer and denser fine root may be important in the acquisition of immobile phosphorus (Bielenberg et al. 2001). Higher SRL has been related to increases in exploitation efficiency (Fitter 1991), which would favor uptake efficiency (Comas and Eissenstat 2002). On the other hand, changes in SRL may affect plant capacity to capture and transport water. It has been suggested that higher SRL could favor higher hydraulic conductance (Eissenstat 1991). This does not seem to be the case in *P. lentiscus* L. seedlings in the present experiment. Other studies on *P. lentiscus* L. and other Mediterranean woody species have found increases in SRL in response to water limitation (Fonseca 1999), suggesting that modifications in root morphology due to low P availability, as observed in the present study, could have a positive net effect on water use.

The weak response of biomass allocation patterns to nutrient limitation was somewhat unexpected. Many studies have reported an increase in biomass allocation belowground under limiting nutrient availability (Ingestad and Ågren 1991). In contrast, our results suggest that root morphology was more sensitive than biomass allocation to P deficiency. Increased resource allocation belowground and reductions in leaf area are common strategies to cope with drought (Levitt 1980; Lloret et al. 1999).

However, other studies have found no changes in either the root weight ratio or the root-to-shoot ratio in *P. lentiscus* L. seedlings subjected to mild water stress (Fonseca 1999; Green et al. 2005). Mediterranean drought-resistant species may have acquired genetically determined characters that influence allocation patterns. In fact, several studies have shown that Mediterranean species have low phenotypic plasticity in comparison with species from humid climates (Valladares et al. 2000; Valladares et al. 2002).

Several works have reported a decreased water-transport capacity in nutrient-deficient plants (Radin and Eidenbock 1986; Syvertsen and Graham 1985; Radin and Matthews 1989). In the present study, plants subjected to N and P deficiency showed lower K_{RR} than control plants. These values represented a 27% reduction in the water transport

capacity as compared to control plants on a root surface area basis.

The reduction in the capacity for transporting water in nutrient-deficient plants may have important implications on the water balance of plants (Reinbott and Blevins 1999; Clearwater and Meinzer 2001). Reductions in K_{RR} could result either from decreased hydraulic conductance of the cells in the radial flow pathway or from changes in the hydraulic architecture of the whole root system (Ewers et al. 2000). Moreover, reductions in root hydraulic conductance could be the result of an increased SRL or a decreased conduit diameter (Linton et al. 1998). The parallel decreases in the leaf area and the root hydraulic conductance are consistent with the findings of Radin and Eidenbock (1984). They noted that differences in hydraulic conductance due to low phosphorus supply clearly preceded any effects on leaf area development, and they concluded that hydraulic conductance limited leaf expansion by restricting water transport. A major consequence of parallel decreases in root hydraulic conductance and leaf area was that $K_{RL,}$ a measure of root system capacity for water supply to leaves, was similar in both nutrient-deficient plants and control plants.

Several works have analyzed the relationship between changes in plant hydraulic conductance, and stomatal conductance and transpiration (Nardini et al. 2000; Sperry et al. 2002). High-hydraulic conductance may be advantageous because it facilitates efficient water and nutrient transport to leaves. However, under moderate water availability, limitations to water transport due to reductions in hydraulic conductance may enhance a conservative water use (Hubbard et al. 2001; Sperry 2003). In the present work, root hydraulic conductance scaled by leaf area (K_{RL}) showed the same transport capacity in all treatments. According to Darcy's law (Sperry 2000), the gradient of water potential from soil to leaves should be similar in both control and nutrient-deficient plants for a given evaporative flux, due to similar K_{RL}. The equivalent K_{RL} values show clearly that under a given evaporative flux, pressure gradients at the root level will be the same for all experimental groups.

In conclusion, N deficiency resulted in changes in aboveground biomass accumulation, but it had little effect on belowground morphology or biomass allocation. In contrast, P deficiency resulted in changes in both aboveground and belowground morphology, but not in root and shoot biomass accumulation. Both N and P deficiency strongly reduced hydraulic conductance and root specific hydraulic conductance but showed no significant effect on leaf specific hydraulic conductance. Survival of *P. lentiscus* L. seedlings under semiarid field conditions can be higher in plants that have been grown in nutrient-deficient conditions (Trubat et al. 2004). Our results suggest that the contrasting changes in the morphology of N and P deficient seedling, in conjunction with the decreases in root hydraulic conductance described in the present study, may have a positive net effect on plant performance under field conditions.

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