Hydraulic properties of *Eucalyptus grandis* in response to nitrate and phosphate deficiency and sudden changes in their availability

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Abstract

Some herbaceous plant species have been shown to dynamically alter the hydraulic properties of their roots in response to sudden changes in the concentrations of mobile nutrients. These hydraulic adaptations effectively allow plants to 'chase' mobile nutrients across the rhizosphere. Trees, on the other hand, could mitigate effects of heterogeneous, dynamic soil environments with extensive root systems as such systems would effectively equalize nutrient availability. In addition, large dendritic root systems would reduce the effectiveness of rapid, localized, physiological hydraulic changes as these local changes might cancel each other at lower-order root junctions. Thus, the aim of this study was to determine if trees (*Eucalyptus grandis*) employ short-term (minutes to hours), physiological hydraulic changes or rely on long-term (days), growth-based hydraulic acclimations to enhance mobile nutrient uptake. We used two nutrients, nitrogen (N) and phosphorus (P), that are characterized by contrasting soil mobility: N being mobile and P immobile. Transpiration, whole-plant hydraulic resistance (liquid phase), and the hydraulic resistance of single roots of *E. grandis* plants grown in high and low N combined with high and low P availability were measured. In general, plants grown with high N availability had lower whole-plant hydraulic resistance than plants grown with low N availability. When N or P were in short supply, a sudden addition of N or P did not change either single-root or whole-plant hydraulic resistance at a given leaf water potential. However, addition of N reduced the transpiration rate, thus, enhancing plant water status, suggesting that *E. grandis* behavior prioritizes water conservation over N uptake in short-term. Prolonged exposure to low nutrient availability resulted in high overall hydraulic resistance further suggesting prioritization of water conservation over N gain.

Key words: conductance / conductivity / nitrogen / phosphorus / transpiration

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1 Introduction

Plants respond to environmental heterogeneity by phenotypic changes that contribute to enhancing resource acquisition (*De Kroon* et al., 2009). However, as the heterogeneity of resource changes in both space and time, plants may not rely solely on fixed morphological adaptations but also on developed physiological responses that facilitate resource uptake. The physiological responses that influence the kinetics of resource acquisition might be especially important in forest ecosystems, where many organisms compete for limited resources (*Rennenberg* et al., 2009). Major soil resources, namely water, nitrogen (N) and phosphorus (P), are intertwined in a complicated network of interactions in which the kinetics of water uptake and nutrient acquisition are linked at a physiological level (*Cramer* et al., 2009; *Chapman* et al., 2011) with the physics of resource movement in soil (*Marschner*, 1995; *Kreuzwieser* and *Gessler*, 2010).

The most common form of N, nitrate, is soluble and highly mobile in the soil matrix, while P, in the form of phosphate, is immobile mainly due to sorption processes. One implication of this variance is that plants cannot employ the same strategy for the acquisition of these two nutrients (*Marschner*, 1995; *Richardson* et al., 2009). For example, the transport of mobile nutrients can be enhanced by changes in transpiration rate. Since transpiration generates mass flow of water in soil towards the roots, it can consequently transfer mobile nutrients into the rhizosphere (*Cramer* et al., 2009). It has been shown that some plants with high nitrate demand are able to 'chase' mobile nitrate around the soil matrix through temporally and spatially controlling root hydraulic conductivity (*Gloser* et al., 2007, 2009; *Gorska* et al., 2008a). However, this strategy of rapidly changing root hydraulic properties might not be useful in the acquisition of immobile P (*Górska* et al., 2010), because increases in water uptake will have minimal effects on the transport of an immobile nutrient, whose diffusion and concentration in soil solution is low due to sorption processes (*Gregory*, 2006). Therefore, in the case of immo-

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bile nutrients, long-lasting, morphological or growth-related strategies or physiological adaptations are more useful. Some examples of such strategies include the production of a wide-spreading root system (*Jeschke* et al., 1997), re-translocation and re-utilization of nutrients (*Doerner*, 2008), synthesis of specific high-affinity transporters (*Smith* et al., 2003; *Kraiser* et al., 2011), association with mycorrhizal fungi (*Chen* et al., 2000), and higher internal nutrient-use efficiency (*Ares* and *Fownes*, 2000). Most of these strategies seemed to be independent of water transport. However, as these changes relief nutrient deficiencies, they indirectly enhance growth and effective root size and directly influence the water-uptake path, linking plant hydraulics with the uptake of immobile ions.

While most previous work, showing the hydraulic responses of plants to nutrient availability, has been done on species with high nutrient demands (*Gorska* et al., 2008a, 2008b; *Cramer* et al., 2009; *Matimati* et al., 2014), more recent work suggests that species characterized by lower uptake rates of nitrate do not employ strategies that involve rapid changes in root hydraulic resistance in response to sudden changes in nitrate availability (*Górska* et al., 2010). Thus, we ask if plants with low nutrient demand employ different hydraulic strategies for the acquisition of mobile and/or immobile nutrients in the short or long term. As a model species, we selected *Eucalyptus grandis*, a fast growing tree species, yet one that has evolved in infertile soils and is considered to have low nutrient requirements (*Grove* et al., 1996; *McLaughlin*, 1996). Although *E. grandis* is a fast growing tree, its relative growth rate (mass) at seedling stage is 0.05 d^{-1} (*Graciano* et al., 2009), lower than crop species that were analyzed in previous works that grow 0.20–0.35 d^{-1} for *Solanum lycopersicum* (tomato) and *Zea mays* (maize), respectively (*Cramer* et al., 2009; *Górska* et al., 2010). *Eucalyptus grandis* has also a low N demand because N uptake is around 1 µmol NO_3^- h^{-1} g^{-1} FW (data recalculated from *Graciano* et al., 2009), while N uptake of tomato and maize plants is around 3.3 and 4.1 µmol NO_3^- h^{-1} g FW $^{-1}$ (*Górska* et al., 2010). Despite its low nutrient demand, *E. grandis* develops a set of responses to improve acquisition of nutrients from soil or internal utilization of growth-limiting nutrients in its native environments, especially of P (*Atwell* et al., 2009). Thus, we expect that morphological and physiological changes that can improve nutrient acquisition of *E. grandis* are most likely determined by a search for P. The aim of this study was to analyze the short- (minutes to hours) and long-term (days) hydraulic properties of *E. grandis* seedlings to determine if these trees employ any hydraulic strategies to enhance nutrient movement to the rhizosphere.

2 Material and methods

2.1 Growth conditions

Eucalyptus grandis plants were grown in either perlite or hydroponic solution (plants used for determination of root hydraulic properties) for 2 months following germination. Four modified Hoagland solutions (pH 6.1) were used to water plants:

– High-N and high-P (HNHP) solution was composed of: 795 µM KNO_3, 603 µM $Ca(NO_3)_2$, 270 µM $MgSO_4$ and 190 µM KH_2PO_4, as well as micronutrients 40.5 µM Fe(III)-EDTA, 20 µM H_3BO_4, 2 µM $MnSO_4$, 0.085 µM $ZnSO_4$, 0.15 µM $CuSO_4$, and 0.25 µM Na_2MoO_4.
– Low-N and high-P (LNHP) solution was composed of: 79.5 µM KNO_3, 60.3 µM $Ca(NO_3)_2$, 270 µM $MgSO_4$, and 190 µM KH_2PO_4, 376 µM K_2SO_4, 543 µM $CaCl_2$, and the same micronutrient composition as HNHP.
– High N and low P (HNLP) solution was composed of: 795 µM KNO_3, 603 µM Ca $(NO_3)_2$, 270 µM $MgSO_4$, 19 µM KH_2PO_4, 86 µM K_2SO_4, and the same micronutrient composition as HNHP.
– Low-N and low-P (LNLP) solution was composed of: 79.5 µM KNO_3, 60.3 µM $Ca(NO_3)_2$, 270 µM $MgSO_4$, 19 µM KH_2PO_4, 795 µM K_2SO_4, 603 µM $CaCl_2$, and the same micronutrient composition as HNHP.

Solutions were replaced twice a week for hydroponically grown plants and effectively replaced twice a week *via* ample watering for plants grown in perlite.

2.2 Transpiration

The transpiration rate of perlite-grown plants was measured by weighing at half-hour intervals. Pots were tightly sealed in plastic bags to avoid evaporation from substrate surfaces. After 2 h (four consecutive measurements), a sudden increase in nutrient availability (nutrient shock) was introduced: 250 mL of each nutrient solution enriched to 3 mM NO_3^- or 3 mM PO_4^{-3} was applied to each plant. At the same time that nutrient shocks were applied, control plants were supplied with 250 mL of fresh nutrient solutions to account for the possibility of time-dependent changes in transpiration rate due to circadian rhythm and to determine if differences in transpiration were due only to the fact that a solution was added. The volume of nutrient solution applied was enough to entirely replace solution in the pots, so pots were left to drain for 5 min, then sealed again in the plastic bags and weighed. Four additional weight measurements were taken, again at half-hour intervals. Four plants fed with each nutrient solution were used for each shock treatment (nitrate, phosphate, or control). The transpiration rate of each treatment reported in Table 1 was calculated as the mean of the four measurements prior to the sudden application of the nutrient, considering together the plants used for sudden addition of nitrate, phosphate, and nutrient solution. The transpiration rate reported in Fig. 1 corresponds to the mean of the plants used for each sudden addition treatment, to avoid the between-subjects error. After measurements, leaf area and dry weight of the plants were determined.

2.3 Whole plant hydraulic resistance (liquid phase)

To determine whole-plant hydraulic resistance a root pressure chamber was used (*Stirzaker* and *Passioura*, 1996) with modifications described in *Gloser* et al. (2007). In brief, the root pressure chamber technique allowed us to monitor instantaneous changes in the hydraulic resistances of intact, transpiring plants. Resistance was calculated as the pressure

gradient from the root chamber to the shoot divided by the transpiration rate. An active feedback control system adjusted the pressure applied to roots such that the pressure in the shoot xylem was held constant. Pressure in the shoot xylem was monitored using a pressure transducer attached to the petiole of the leaf near the top of the plant. Plants were grown in perlite and watered twice a week with low-nitrate solution (LN) or high-nitrate solution (HN). Both treatments received high phosphate concentration (HP). Transpiration rates were determined from changes in the weight of the pressure chamber which was placed on an electronic balance ($\pm$ 0.1 g). Changes in mass due to changes in gas concentration within the root pressure chamber were taken into account when calculating the transpiration rate. Access ports allowed us to amend the solution while maintaining the pressure in the root chamber. Nitrate concentration was increased to 3 mM nitrate. After measurements, leaf area and dry weight of the plants were determined.

2.4 Single-root hydraulic conductance

The hydraulic conductance of single roots of plants grown in hydroponic solutions was determined by measuring the flow induced in response to an applied pressure gradient following the methods described in previous work (*Gloser* et al., 2007; *Gorska* et al., 2008a). Briefly, an excised root was fitted with a plastic tube filled with distilled water and connected to a beaker located on a digital balance ($\pm$ 0.01 mg), then sealed in a pressure chamber containing the hydroponic solution in which plants had been grown (HNHP or LNHP). The chamber was pressurized with air regulated by the use of a needle valve at 0.125 MPa. A continuous small leak through a submerged aeration stone was maintained to avoid anoxic condition (for details on the setup see *Gorska* et al., 2008a; 2008b). The flow generated by root pressure was overcome by 0.125 MPa, *i.e.*, there was a linear relationship between pressure and flow. Water flow across the root was automatically recorded by a computer at 2 min intervals. As the flow rate stabilized (10–20 min after a root was placed in the chamber), plants were allowed an additional 1 h of acclimation time before a 1 M KNO_3 solution was added to the chamber, such that a final concentration of 5 mM NO_3^- was achieved in the immersion solution. This nitrate solution was injected through the aeration system, so that the pressure in the chamber remained unchanged and the added solution mixed well with the root medium. After measurements, the length and diameter of the root were determined in order to express the root conductivity with respect to root longitude ($k_{h\ root}$) and also root cross section ($k_{s\ root}$).

2.5 Shoot resistance and stem conductivity

Both, the hydraulic resistance of shoots (main stem with branches and leaves; ShootR, MPa s g^{-1}) and the specific hydraulic conductivity (k_s) of main stems (without branches and leaves) were determined with the low-pressure steady-state flow meter (SSFM) method. This method uses the drop in pressure across a tube of known resistance, together with the pressure at the stem fitting, to measure the flow rate into a stem segment or branch with leaves (*Brodribb* and *Feild*,

2000; *Zwieniecki* et al., 2000). The measurements were performed using plants from the HNHP and LNHP treatments growing in perlite. A given plant was placed in a plastic bag, cut at the root collar under water, and immediately connected to a plastic tube. Then, the bag was removed and the plant was lit to stimulate transpiration. Hydraulic resistance of the shoots was measured when flow was stable. First, the resistance (R) of an entire shoot was measured. Then, after cutting off the portion of the stem with branches and leaves, the hydraulic conductivity of the stem (k_h) was measured, such that only the basal portion of the branch (50 mm in length) was used. Shoot R was multiplied by the plant's total leaf area (ShootR$_{LA}$) in order to take into account the effect of size on this variable. Specific hydraulic conductivity (k_s) and leaf specific hydraulic conductivity of the stem (k_l) were calculated from k_h divided by the cross section of the stem (k_s) or the total leaf area supported by the stem (k_l).

2.6 Statistical analysis

Data were analyzed with an Analysis of Variance (ANOVA) considering one factor with four levels, derived by the combination of the dose of N (LN or HN) and the dose of P (LP or HP). If a nutrient sudden addition was applied, timing specified as "before" or "after" the sudden addition was included as another main factor. The analysis was done considering the same batch of plants before and after to avoid the between-subjects error. Means were compared using Duncan analysis ($P < 5\%$).

3 Results

Plants grown with HNHP were bigger, with greater leaf area, higher specific leaf area, and a higher shoot : root ratio than plants grown with low N (LNHP and LNLP; Table 1). P had no effects on size and dry mass partitioning between shoots and roots if N was low (LNHP *vs*. LNLP), but had some positive effect on dry mass accumulation if N was high (HNHP *vs*. HNLP; Table 1).

LNLP plants had a lower transpiration rate than HNHP plants, while the other treatments had intermediate values (Table 1).
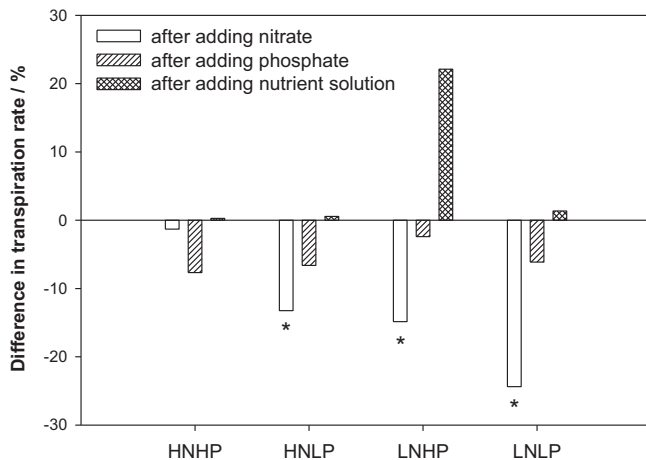
Table 1: Leaf area (LA), specific leaf area (SLA), total dry mass accumulation (DM), dry mass partitioning between shoot and roots (shoot : root), and transpiration rate (g m^{-2} h^{-1}) in *E. grandis* plants grown in perlite with high-N (HN), low-N (LN), high-P (HP), and low-P (LP) nutrient solutions. Different letters within columns indicate significant differences among nutrient treatments ($P < 5\%$).

	LA / m^2	SLA / m^2 g^{-1}	Total DM / g	Shoot : root	Transpiration rate / g m^{-2} h^{-1}
HNHP	0.064 c	0.036 b	2.76 b	7.10 b	185 b
HNLP	0.054 b	0.035 b	2.42 a	6.03 b	183 ab
LNHP	0.039 a	0.029 a	2.28 a	3.49 a	160 ab
LNLP	0.033 a	0.029 a	2.02 a	3.35 a	154 a

The sudden addition of nitrate had no effect on transpiration rate when plants were grown under HNHP conditions. However, there was a general reduction of transpiration rates in all other treatments in response to nitrate addition (Fig. 1). The sudden addition of concentrated P or standard nutrient solutions did not significantly change transpiration rates (Fig. 1). Although there were differences in leaf area between treatments, there was no correlation between plant leaf area and transpiration rate ($r^2 = 0.08$; $P = 53\%$; Fig. 2).

Whole-plant hydraulic resistance determined in the root pressure chamber was higher in LN than in HN plants (Fig. 3). However, whole-plant hydraulic resistance did not change in response to the sudden addition of nitrate (Fig. 3). Root-specific conductivity was similar in LN and HN plants ($k_{sroot} = 0.03$ and 0.04 kg MPa^{-1} s^{-1} m^{-1}, respectively; $P = 78\%$) for single roots. Root hydraulic conductivity did not change in response to the addition of nitrate of neither in HN nor LN plants (Fig. 4) for 100 min, although a small drop in conductance was observed between 100 and 200 min potentially reflecting a response to excision.
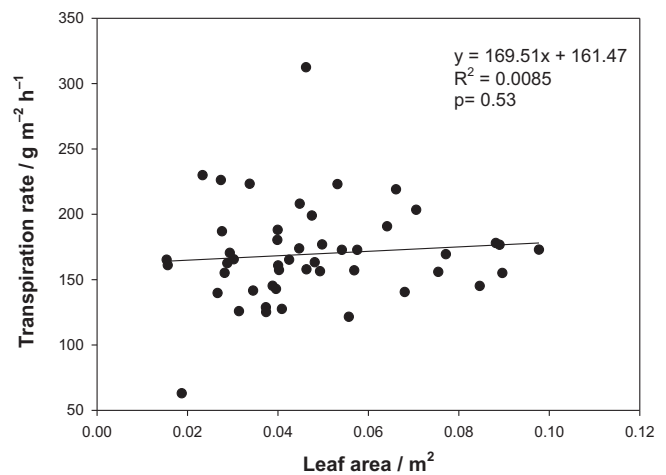
Similar to measurements at the whole-plant level, HN plants had lower shoot hydraulic resistance (Shoot R) than LN plants. However, stem-specific hydraulic conductivity (k_s) and
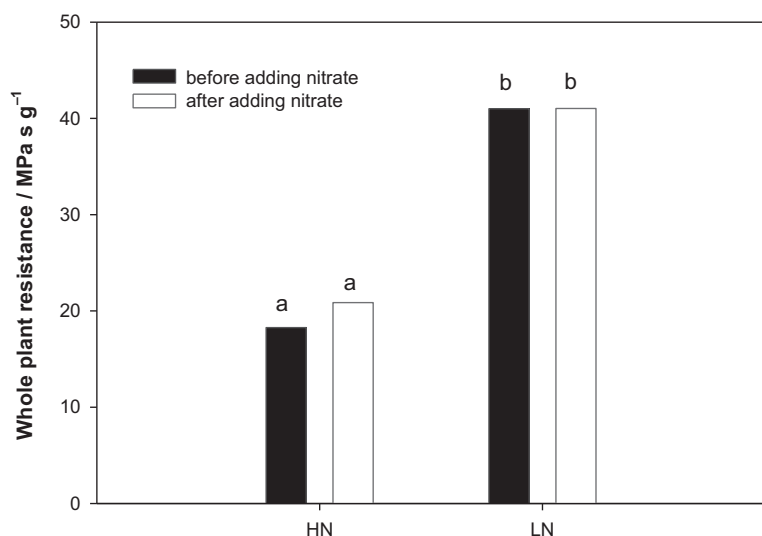

Figure 2: Relationship between transpiration rate (g m^{-2} h^{-1}) and plant leaf area (m^2) in *E. grandis* plants considering all growth conditions.

leaf-specific conductivity (k_l) were similar in both treatments (Table 2). Bigger plants (HN) had 60% lower shoot hydraulic resistance (Shoot R) than smaller plants (LN). However, when adjusted for leaf area specific resistance of the shoots of HN plants was bigger than of LN plants (Shoot R$_{LA}$; Table 2).
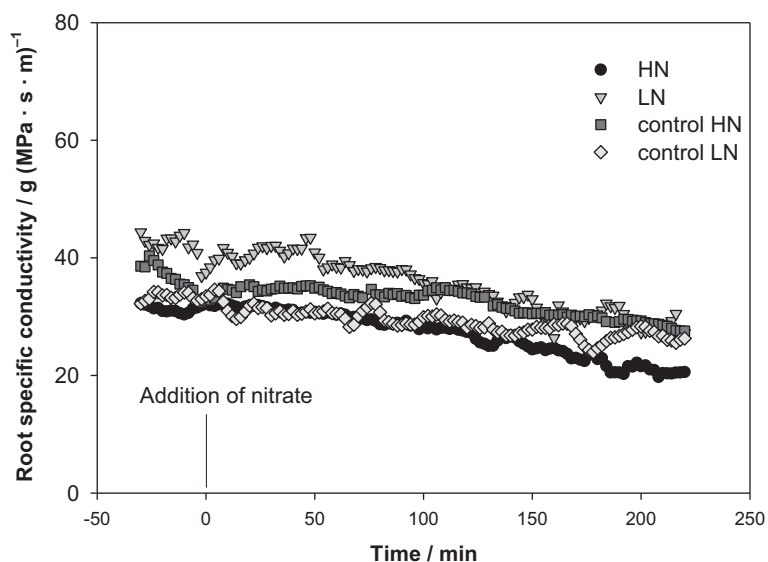
4 Discussion

We found some general changes demonstrating that *E. grandis* plants can alter their growth and hydraulic architecture in response to long-term low nutrient availability as well as in response to the sudden addition of nutrients. Hydraulic architecture is "the set of hydraulic characteristics of the conducting tissue of a plant which qualify and quantify the sap flux from roots to leaves" (*Cruiziat* et al., 2002). Often basic structural parameters (size, biomass, shoot : root ratio) are linked to plant hydraulics. In this study, the most pronounced change with potential influence on plant hydraulics was dry mass distribution between shoot and roots as a response to low N availability (Table 1). LN plants (with both high and low P availability) had lower shoot : root ratios than HN plants (Table 1). This is a well-known phenomenon and a widespread response of plants to N deficiency (*Kraiser* et al., 2011), promoting soil exploration for N. In the case of *E. grandis*, low N availability resulted in only a small drop of total plant DW and, thus, shift in shoot : root ratio resulted from larger root system composed mostly of fine roots promoting nutrient acquisition (*Comas* and *Eissenstat*, 2009). Such a

Figure 1: Difference in transpiration rate (%, respect to initial value) of *E. grandis* seedlings after they had been exposed to a sudden increase of N or P availability in the nutrient solution by the addition of 3 mM nitrate, 3 mM phosphate, or fresh nutrient solution according to treatment (control). Plants were grown in perlite with high (HN) or low (LN) nitrate concentration combined with high (HP) or low (LP) phosphate concentration. Asterisks indicate statistical differences in the transpiration rate before and after nutrient addition ($P < 5\%$).

Table 2: Stem conductivity normalized by sapwood area (k_s) or leaf area (k_l), shoot resistance (Shoot R) and shoot resistance normalized by plant leaf area (Shoot R$_{LA}$) of plants grown in perlite under low (LN) and high (HN) N availability. Different letters indicate statistical differences ($P < 5\%$).

	k_s / kg (s · Mpa · m)$^{-1}$		k_l / kg (s · Mpa · m)$^{-1}$		Shoot R / MPa s kg^{-1}		Shoot R$_{LA}$ / MPa s m^{-2} kg^{-1}	
LNHP	1.15	a	1.40 E^{-04}	a	10.96 E^{05}	b	17.07 E^{03}	b
HNHP	0.84	a	9.10 E^{-05}	a	6.40 E^{05}	a	33.45 E^{03}	a

Figure 3: Whole-plant hydraulic resistance (MPa s g^{-1}) of *E. grandis* plants grown in perlite with high N (HN) and low N (LN) nutrient solution, before and after the 3 mM sudden addition of nitrate. All plants were grown with high phosphate (HP). Different letters on the top of the columns indicate statistical differences between nutrient treatments ($P < 5\%$).

Figure 4: Specific root conductivity [g (MPa · s · m)$^{-1}$] in excised roots grown in hydroponics before and after the addition of 3 mM nitrate (time = 0). In control plants, no nitrate was added. Plants were grown in hydroponics with low (LN) or high (HN) nitrate availability, and all with high phosphate (HP) availability.

Long-term high N and P availability resulted in increase of plants' size and also higher transpiration rate per unit of leaf area (Table 1). As transpiration rates were independent of leaf area (Fig. 2) the higher transpiration rate of HNHP plants was not due to the increase in leaf area but was a consequence of the higher nutrient availability. Thus, HN plants were transpiring more water than LN plants because they had a larger leaf area and a higher transpiration rate (Table 1). On the contrary, a decrease in day-time stomatal conductance in young plants of *Eucalyptus tereticornis*, as P concentration in soil increased, was observed as a long-term acclimation, although in that case the response seemed to be due to anatomical changes and not directly related to P concentration in xylem sap (*De Dios* et al., 2013). Moreover, HN plants had lower overall resistance along the hydraulic pathway (Fig. 3) as shoot plant resistance was lower in HN plants than it was in LN plants (Table 2). However, on the basis of leaf area, specific shoot resistance of HN plants was bigger than LN plants (Table 2). Thus, low N availability resulted in impaired growth (Table 1) but specific resistance of the shoot was reduced facilitating water moment (Table 2). Stem hydraulic resistance of five savanna tree species decreased when N was applied, while no modification was observed if P was applied (*Bucci* et al., 2006). However, in subtropical tree species the addition of N and P reduced stem conductivity in only three of six species, while in the others there was no effect of nutrient enrichment in this trait (*Villagra* et al., 2013).

Consistent with lower hydraulic resistance, transpiration rate was higher in plants with high availability of N and P compared to plants with low availability of these nutrients (Table 1). This finding might be consistent with the idea that N availability affects transpiration such that if nitrate availability is high, transpiration is high, but if N availability is very low, transpiration is reduced to conserve water. The reduction in water consumption had probably low effects on carbon assimilation because as the plants were exposed to N starvation and had low photosynthetic capacity (low leaf area and/or low photosynthetic rate per unit leaf area). Thereafter, the long-term acclimation to low N availability is demonstrated by low shoot : root ratios, low water consumption, and high resistance to water movement.

larger root system could also play a role in reduction of overall root hydraulic resistance. On the other hand, P availability did not induce changes in dry mass partitioning, as LP and HP plants had the same shoot : root ratio (Table 1), suggesting that the plant strategy for acquisition/search of immobile nutrients is different from that for N or that P deficiency was not strong enough to induce changes in dry mass partitioning between shoot and root.

Although the long-term acclimation to HN implies higher rates of water use than under LN, plants that were growing either with low N or P responded to a sudden increase in nitrate concentration by decreasing transpiration rate (Fig. 1). As there was no response to the change of phosphate concentration (Fig. 1), the effect seems to be due to the change in nitrate availability and not to the decrease of water potential in the root environment caused by osmotic changes. The rapid change in transpiration rate following the addition of nitrate is

likely due to changes in stomatal conductance, as there were no changes in whole-plant resistance (Fig. 3) or root conductivity (Fig. 4). Thus, unlike in herbaceous species, such as cucumber, tomato, and maize (*Gorska* et al., 2008a), the root hydraulics of *E. grandis* were not affected in the short term by a sudden exposure to nitrate, but rather follow the behavior of plants with low nitrate needs (*Górska* et al., 2010). This finding further supports the idea that *E. grandis* does not rely on rapid hydraulic responses to changes in nutrient availability as the soil volume penetration of large trees might compensate for any small-scale changes in nutrient availability, effectively equalizing their access to nutrients. Thus, our results suggest that *E. grandis* plants most likely adapt to high nutrient concentrations (N) *via* changes in whole-plant hydraulic architecture.

Acknowledgments

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