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ORIGINAL ARTICLE

The spatial distribution of phosphate in the root system modulates N metabolism and growth in *Eucalyptus grandis* young plants

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Abstract

Key message **High P homogeneously applied in roots reduced growth and nitrate uptake. High P in a patch reduced N in tissues but did not reduce growth, because root hydraulic conductivity increased.**

Abstract Little is known about consequences of nutrient imbalances, i.e., when the increase of one nutrient's availability, in non-toxic concentration, reduces plant growth, because other nutrient availability is low. In a soil with low N (nitrogen) availability, high homogeneous P (phosphorus) availability reduced nitrate uptake and reduced growth of *Eucalyptus grandis* plants. However, if the same availability of P was applied only in a part of the root system (localized), no depressive effect was observed. In this work, it was analyzed which step in the early assimilation of N was affected by high homogeneous P and how localized P counteracted this depressive effect. Inorganic forms of N and protein in plant tissues as well as some plant hydraulic traits were analyzed in an experiment with *E. grandis* plants growing in perlite in a split-root system fed with low N. Control plants received low P. High P was applied in two spatial distributions: localized in one part of the root system (LP) or distributed homogeneously in both parts (HP). HP reduced growth, while LP had no depressive effect in growth. Both high P spatial distributions reduced concentration of nitrate in roots. Since concentration of nitrate in the xylem was similar in all treatments and nitrate in leaves was lower in high P than in control treatment, the reduction in root N was probably due to lower uptake. Nevertheless, plants growing with LP had no depressive effect in growth, because the decrease in N assimilation was counteracted by an increase of root hydraulic conductivity.

Keywords Nutrient interaction · Hydraulic conductivity · Mineral nutrition · Low nitrate · Localized phosphate · Homogeneous phosphate

Introduction

Plants require several mineral elements to complete their life cycles. It is well known that in certain environment, growth depends on the total availability of each nutrient as well as on the balance between them. If the concentration of one element is changed, interactions with other nutrients result in different responses in growth. Although this is a general state proved in many sites and species all around the word, little is known about the physiological causes of nutrient interaction (Kant et al. 2011). Nitrogen (N) and phosphorus (P) are two of the macronutrients that usually limit growth in natural and crop systems (Kuzyakov and Xu 2013). Both nutrients, in their most common form in soil solution (nitrate and phosphate), differ in their mobility in the soil matrix: nitrate is mobile, while phosphate moves slowly. Therefore, water movement from bulk soil to the rhizosphere can increase nitrate consumption, while it probably would have a little effect in phosphate uptake (Cramer et al. 2009). Nevertheless, if P is limiting growth, an increase in phosphate availability can increase nitrate demand to sustain the higher growth. In this case, the plant can develop different strategies to increase nitrate uptake,

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such as a wider root system to enlarge the surface contact between soil and roots (Hermans et al. 2006; Desnos 2008), higher root hydraulic conductivity to increase mass flow to the rhizosphere (Gorska et al. 2008a, 2010), or the synthesis of root nitrate transporters to increase the specific uptake rate (Bao et al. 2011).

If the availability of nutrients in the soil is not homogeneous, plants can sense the presence of nutrient-rich patches in the soil and they can respond to these patches by developing more roots in the rich area (López-Bucio et al. 2003; Hodge 2006), developing root in the rich area at the expense of growth outside the patch (Durieux et al. 1994; Gloser et al. 2008), or decreasing the shoot:root ratio (Hutchings and John 2004). Plants growing in agar exposed to a nitrate patch proliferated roots in the rich area, while plants exposed to a homogeneously high nitrate availability, decreased root proliferation (Zhang and Forde 2000). Root proliferation in the patch allows plants to efficiently take up the applied nutrient (Linkohr et al. 2002). On the other hand, fast root elongation in extremely poor nutrient hydroponic solution has been reported, which is a possible adaptation to explore more soil in poor environments (Walter et al. 2003; Hermans et al. 2006; Zhang et al. 2007).

Eucalyptus grandis Hill ex Maiden is a fast growing hardwood tree, native from Australia, and planted in more than 100 countries all around the world due to its high acclimatization to different environments. It is a tree model species to genomic and breeding researches, because its genome is nearly completely sequenced (Myburg et al. 2014). Although *E. grandis* evolved on fertile soils with relative low availability of phosphorus (Specht 1996; Tng et al. 2014), nowadays, it is planted on many type of soils and high yields are obtain even on soils with low availability of nutrients (Barros and Novais 1996; Dalla Tea and Marcó 1996; Herbert 1996). In most cases, productivity of eucalypts plantations depends on the application of fertilizers (Laclau et al. 2010). Fertilization increases the availability of certain nutrients in the soil, but it not always increases growth, because it produces physiological effects on the plants that are not completely understood.

In *E. grandis* plants growing with low N availability, a tenfold increase in the concentration of phosphate in soil solution had a depressive effect in growth and in N uptake (Graciano et al. 2009). Surprisingly, the depressive effect was reverted when the same amount of phosphate was held to the plant in a different spatial distribution: only part of the root system was fed with a very high phosphate solution, while the other part was fed with a very low concentration of phosphate. With this heterogeneous distribution of P, plants grew more and had normal N uptake, while plants fed with homogeneous concentration of P had reduced N uptake. These results suggest that the availability and spatial distribution of phosphate in the soil solution affect in some way the capacity of the plant to take up nitrate. This intercross between phosphorous distribution in the root environment and nitrogen metabolism was not studied in any plant species, and is very relevant to understand the effect of the placement of the fertilizers in tree growth. Broadcast application can increase fertilizer-root contact but can increase leaching if the root system is not wide enough (Alva et al. 2003); localized fertilization can increase the uptake rate of the nutrient applied, because the concentration of that nutrient in the soil is higher and this high concentration also diminishes immobilization by microorganisms (Smethurst et al. 2004). However, specific physiological responses can also explain the different effects of localized and broadcast fertilization.

Acquisition of nutrients depends on several factors, such as root architecture, root ion transporters, nutrient distribution in the plant and in the soil, and ion mobility in the soil by diffusion and mass flow (Chapman et al. 2012). In most soils, N is mainly taken up as nitrate. This anion can enter to the root through passive diffusion, but many nitrate transporters have been found in plants (Léran et al. 2014). Once inside the plant, nitrate can be reduced to nitrite in roots or leaves by the nitrate reductase (NR), and later, this form is reduced to ammonium by the nitrite reductase (NiR). Finally, the glutamine synthetase (GS) catalyzes the incorporation of ammonium to glutamine, the first form of organic N (Rennenberg et al. 2010). The diminish in nitrate uptake observed in *E. grandis* plants fed with a high homogeneous concentration of phosphate can be caused either by lower nitrate transporters activity or by down-regulation due to minor activity of some enzyme involved in nitrate assimilation route. Since there is a little information on the mechanisms that can explain the interaction of external P availability and nitrate uptake, our purpose was to analyze how nitrate uptake or assimilation was affected by the high homogeneous concentration of phosphate. We performed an experiment with *E. grandis* plants growing in a split-root system (hydroponics with perlite), in which we evaluated the inorganic forms of N in plant tissues and some hydraulic traits of the plants. The originality is that some steps in the assimilation of N are analyzed together with some traits of plant hydraulic architecture.

The aim of this work was to analyze which step in the early assimilation of N was affected by high homogeneous availability of P in the root system, and how high localized availability of P counteracted this depressive effect.

Materials and methods

Plant growing conditions

Eucalyptus grandis seeds were put to germinate in trays filled with perlite. Seeds were obtained from a clonal seed orchard of the INTA breeding program, Argentina (Marcó and White 2002). When plants were 2 months old, they were transplanted to a split-root system developed by Graciano et al. (2009). The root system was kept intact and manually placed in two 1-L black plastic pots: one pot contained the main root and lateral roots, while the other pot contained only lateral roots. The collar of the plant was above the division of the two pots. Plants grew in a natural lit greenhouse, in La Plata, Argentina (34°54′45.52″S, 57°55′51.11″W) during summer. During the experiment, inside the greenhouse, mean temperature was 24.7 ± 4.3 °C, relative humidity fluctuated along the day between 70 and 50 %, midday photosynthetic flux density on sunny days was around 1000 µmol photon m^{-2} s^{-1} (50 % of the radiation outside the greenhouse), and the photoperiod was around 14 h. The containers were filled with perlite, and plants were fed twice a week with nutrient solution. The excess of solution was allowed to drain through holes done in the bottom of the pots. Three solutions were prepared differing in the concentration of P: 0.08 mM (low), 0.8 mM (medium), and 1.52 mM (high). All the solutions had the same low concentration of N (0.75 mM) similar to that found in soil solution of non-fertilized soil used to plant *E. grandis* in Argentina (Graciano et al. 2009). Therefore, composition of the low P solution was: 0.08-mM KH_2PO_4, 6-mM KCl, 5-mM $CaCl_2$, 0.25-mM KNO_3, 0.25-mM $Ca(NO_3)_2$, 2-mM $MgSO_4$ and micronutrients: 5-µM B, 0.9-µM Mn, 0.8-µM Zn, 0.03-µM Cu, 0.01-µM Mo, and 0.9-µM Fe. The composition of medium P solution was: 0.80-mM KH_2PO_4, 5-mM KCl, and the same concentration of $CaCl_2$, KNO_3, $Ca(NO_3)_2$, $MgSO_4$, and micronutrients as low P solution. The concentrations in the high P solution were: 1.52-mM KH_2PO_4, 4-mM KCl, and the same concentration of $CaCl_2$, KNO_3, $Ca(NO_3)_2$, $MgSO_4$, and micronutrients as low and medium P solutions. Fe was added as ferric EDTA (Leggett and Frere 1971). Concentrations of N and P were chosen following the previous experiments, in which 0.8 mM of P was found to reduce N uptake (Graciano et al. 2009). The low concentration of P (C) corresponds to non-fertilized soil solution, while the other treatments correspond to the concentration found in soil solution when the P fertilizer is broadcasted around the plant (HP) or localized in a hole near the plant (LP). Nitrate was chosen as source of N, because it is the form that produces better growth in non-mycorrhizal seedlings (Turnbull et al. 1996) and it is the predominant inorganic form of N in this soil (unpublished data).

Each treatment received the same or different nutrient solution in each part of the root system (Fig. 1). C plants received low P solution in both pots. HP plants received medium P solution in both pots. LP plants received low P solution in the pot which contained the main root (principal root) and high P solution in the pot which contained only lateral roots (lateral root). In this way, HP and LP plants received the same amount of P but with a different distribution in the root system: HP had homogeneous availability of P, while LP received a localized application of P, mimicking a patch (Fig. 1). The assumption is that the only nutrients that are limiting growth in the C treatment are N and P, and only N in HP and LP treatments (Sands et al. 1992). It is also assume that the plant responds to the concentration of nutrients near the root system, and that ion and water movement in perlite is fast enough to compensate depletion of nutrients due to uptake.

Plants were fed twice a week with 100 mL of each nutrient solution during 2 months. Thereafter, six plants per treatment were used to perform hydraulic measurements and other four plants were used to take samples for biochemical determinations. After that, photographs of all the leaves of each plant were taken, roots of each pot were washed with tap water and manually divided in fine roots (diameter <2 mm) and coarse roots (diameter >2 mm).

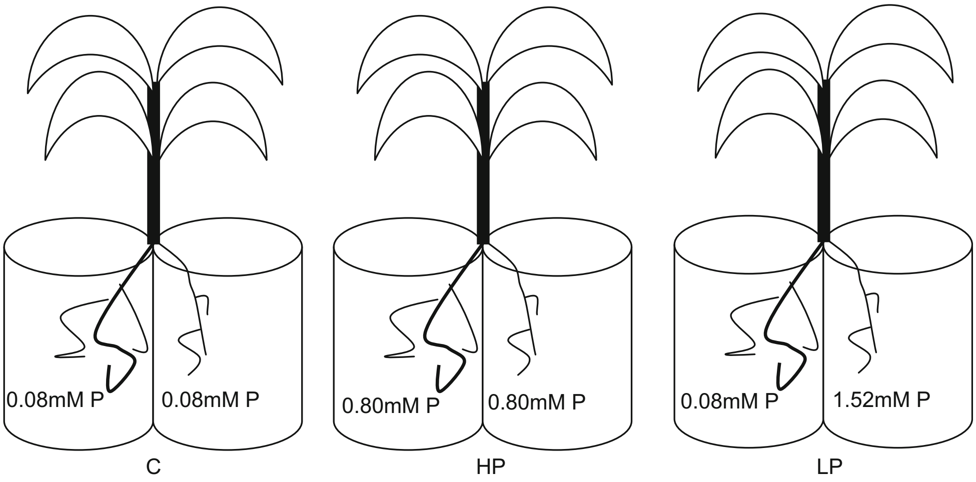

Fig. 1 Schematic design of the split-root experiment. *Numbers inside the pots* are the concentration of P applied in each pot. All treatments received the same low concentration of N (0.75 mM)

The classification of the roots was done manually immediately after washing the root system. Roots that had diameter near 2 mm were measured with a caliper. The tissues were dried in separate paper bags at 60 °C until constant mass, to determine growth as dry mass of leaves, stems, and principal and lateral fine and coarse roots. Leaf area was determined with digital photographs using the CMEIAS-IT v1.28 program (http://cme.msu.edu/cmeias/downloadarea.shtml).

Hydraulic measurements

The hydraulic conductance of shoots (main stem with branches and leaves), the specific hydraulic conductivity of main stems (basal segment without branches and leaves), and the hydraulic conductivity of segments of roots were determined with the pressure-drop hydraulic flow meter (Melcher et al. 2012). 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 shoot (Zwieniecki et al. 2000). 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. Measurements were taken when flow was stable. First, hydraulic conductance of the shoots (K_{shoot}, g MPa^{-1} s^{-1}) was measured. Then, after cutting off the portion of the stem with branches and leaves, the hydraulic conductivity of the stem (kh_{stem}, kg m MPa^{-1} s^{-1}) was measured, such that only the basal portion of the stem (5 cm in length) was used. Specific hydraulic conductivity (ks_{stem}, kg m^{-1} MPa^{-1} s^{-1}) and leaf-specific hydraulic conductivity of the stem (kl_{stem}, kg m^{-1} MPa^{-1} s^{-1}) were calculated from kh_{stem} divided by the cross section of the stem (ks_{stem}), or the total leaf area supported by the stem (kl_{stem}). Finally, the specific conductivity of 5 cm segments of fine roots (<2 mm of diameter) from lateral and principal pots were measured. The direction of the water flow was from the tip of the root to the collar of the plant. The conductivity was divided by the cross section of the root to calculate specific hydraulic conductivity of the root (ks_{root}; kg m^{-1} MPa^{-1} s^{-1}). Six plants per treatment were analyzed.

Biochemical determinations

Freshly cut leaf discs, xylem sap, and fresh fine roots (<2 mm of diameter) were stored at −80 °C to perform biochemical determinations. Shoots (stems and leaves) were put in a pressure chamber to obtain xylem sap. The basal end of the main stem was manually debarked and the shoot was fit in a pressure chamber. Low pressure (0.1–0.5 MPa) was applied during 2–5 min, time enough to collect 0.2 mL of xylem sap with a Hamilton syringe from the basal section of the stem. Four plants per treatment were analyzed for each determination.

Concentration of nitrate in leaves, roots, and xylem

To determine the concentration of NO_3^- in leaves, three leaf discs (0.5 cm diameter each) were homogenized with 1 mL of ultrapure water. Homogenates were heated for 10 min at 100 °C and, finally, were centrifuged at 10,000×*g* for 10 min at room temperature. Nitrate was determined by nitration of salicylic acid (Cataldo et al. 1975). KNO_3 was used as standard. To analyze the concentration of nitrate in roots, 0.3 g of roots were homogenized with 1 mL of ultrapure water and the same procedure as for the leaves was used. To analyzed nitrate in xylem sap, each sample was used directly to measure concentration of nitrate, with the same procedure used for leaves and roots.

Concentration of nitrite in leaves

Concentration of NO_2^- in leaves was analyzed using the same aqueous leaf extracts used to determine concentration of NO_3^-, as described above. Determination of nitrite was based on Zambelli spectrophotometric method. The reaction of the NO_2^- with Zambelli reagent (sulfanilic acid, hydrochloric acid medium, ammonium, and phenol) forms a yellow colored complex with peak absorption at 435 nm. $NaNO_2$ was used as standard.

Concentration of ammonium in leaves

Five leaf discs (0.5-cm diameter each) were homogenized with 0.4 mL of 0.3-mM H_2SO_4. Samples were centrifuged at 10,000×*g* for 10 min at 4 °C and ammonium was determined as Indophenol blue (Hung and Kao 2007). $(NH_4)_2SO_4$ was used as standard.

Concentration of proteins in leaves

Three leaf discs (1 cm diameter each) were homogenized in chilled buffer (50-mM Tris hydroxy-methylaminomethane–HCl, pH 8. 5-mM EDTA, 1-mM PMSF, 1-mM cysteine) and centrifuged at 10,000×*g* for 10 min at 4 °C. Proteins in the supernatant were determined according to Bradford (1976) with bovine serum albumin as standard. Concentration of proteins was expressed as concentration of N as protein by assuming that % N × 5.7 = % protein (Gooding 2007).

Concentration of phosphorous in leaves

Concentration of phosphorous in leaves was determined in samples of dry leaves. Three plants per treatment were analyzed. Samples were reduced to ashes in a muffle, then ashes were dissolved in HCl and phosphate was determined by the vanadomolybdophosphoric acid colorimetric method (Greenberg et al. 1985).

Content of nutrients

Content of nitrate, nitrite, ammonium, phosphorous, and protein in leaves was calculated as the product of the concentration of the sample of each plant with the dry mass of the leaves of the same plant. Content of nitrate in lateral and principal roots was calculated by multiplying concentration of nitrate of each compartment of each plant by the dry mass of fine roots (<2 mm of diameter) of each compartment of each plant.

Statistical analysis

Data were analyzed by one-way ANOVA, considering one factor with three levels: C, HP, and LP. If the effect of the factor was significant ($p < 0.05$), means were compared by the Tukey test ($p < 0.05$).

Results

The depressive effect of high homogeneous P in shoot dry mass and leaf area was corroborated

High homogeneous P availability (HP) decreased shoot growth, while high localized P availability (LP) did not change shoot dry mass comparing with low P availability (C) (Fig. 2). All treatments had similar root dry mass. LP had higher leaf area than HP, while specific leaf area was similar between all treatments (Table 1). Concentration of P in leaves was higher in HP and LP than in C plants (Table 1).

The availability of P altered the accumulation and partitioning of N to the organs

The concentration of nitrate in principal root was similar in all treatments, but it was higher in the lateral root of C plants than in HP plants (Fig. 3). Small changes in fine root dry mass counteracted the effect on changes in the concentration of nitrate; therefore, content of nitrate in lateral roots of all treatments was similar. Nevertheless, content of nitrate in C plants in principal root was higher than in both high P availability treatments (HP and LP) (Fig. 3). The stem xylem sap of all the treatments had similar concentration of nitrate (Fig. 4).

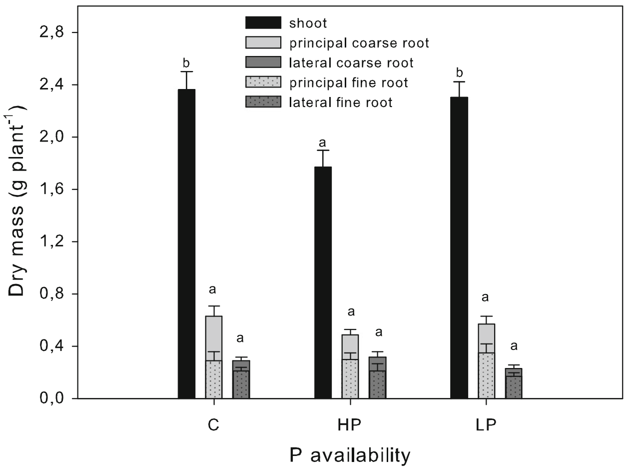

Fig. 2 Dry mass accumulation in shoot (DW_{Shoot}, in g) and roots (DW_{Root}, in g) in *E. grandis* plants growing in a split-root system with low P (C), high homogeneous (HP), or high localized (LP) availability of P. In LP plants, the high concentration of P was applied in the lateral root pot. *Different letters* indicate a significant difference ($p < 0.05$) between treatments for each variable

Plants with low availability of P (C) had higher concentration and content of nitrate in leaves than plants with high availability of P, irrespective of its distribution (HP or LP) (Fig. 5). Concentration and content of ammonium and nitrite in leaves were similar in all treatments (Fig. 5). Concentration of proteins in leaves of all treatments was similar, but content of proteins in leaves was lower in HP plants than in C plants, while LP plants has intermediate values (Fig. 6).

The localized availability of P increased root conductivity

The capacity of segments of the principal root to transport water (ks_{root}) was similar in all treatments, but ks_{root} of segments of lateral roots was higher in the LP treatment than in plants growing with high homogeneous P (HP) (Fig. 7). The lateral roots of HP treatment were those exposed to high concentration of P. The hydraulic conductivities of the stem (ks_{stem} and kl_{stem}) were similar in all treatments, as well as the hydraulic conductance of the shoot (stems plus leaves) (K_{shoot}) (Fig. 7).

Discussion

The depressive effect of high homogeneous P in shoot dry mass and leaf area was corroborated

Although in many forest systems, a depressive effect of fertilization in growth has been observed (Costa Muniz

Table 1 Plant leaf area (cm^2) (LA), specific leaf area ($cm^2\ g^{-1}$) (SLA), concentration of P ($mg\ g^{-1}$) (leaf [P]), and content of P ($mg\ plant^{-1}$) (leaf P content) in leaves of *E. grandis* plants growing in a split-root system with low P (C), high homogeneous (HP), or high localized (LP) availability of P

	LA (cm^2)	SLA ($cm^2\ g^{-1}$)	Leaf [P] ($mg\ g^{-1}$)	Leaf P content ($mg\ plant^{-1}$)
C	378ab	266a	1.20a	1.70a
HP	286a	313a	3.88b	3.54b
LP	398b	294a	2.86b	3.87b

Letters indicate a significant difference ($p < 0.05$) between treatments for each variable

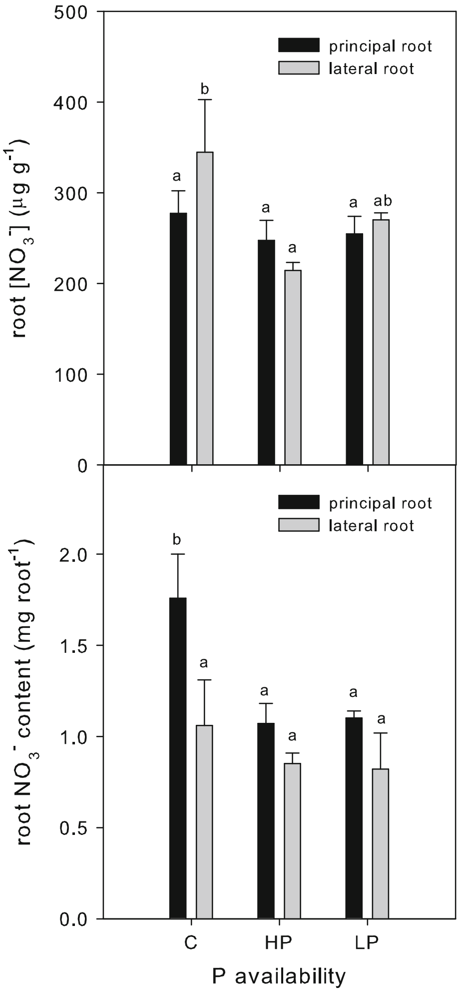

Fig. 3 Concentration (µg g^{-1}) and content (mg $root^{-1}$) of nitrate (NO_3^-) in roots of *E. grandis* plants growing in a split-root system with low concentration of P (C), high homogeneous (HP), or high localized (LP) availability of P. In LP plants, the high concentration of P was applied in the lateral root pot. *Different letters* indicate a significant difference ($p < 0.05$) between treatments for each variable

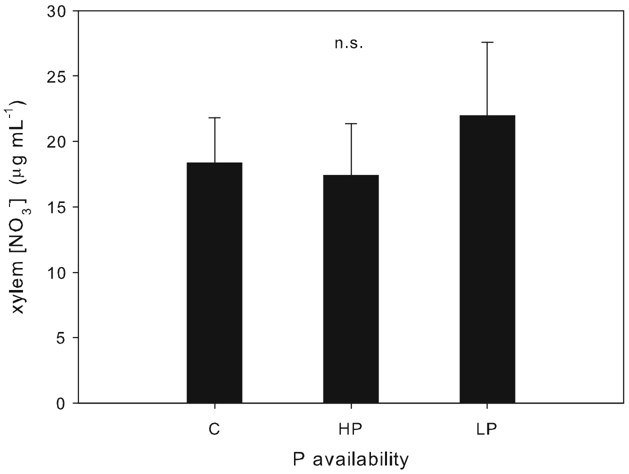

Fig. 4 Concentration of nitrate in stem xylem sap (µg mL^{-1}) in *E. grandis* plants growing in a split-root system with low concentration of P (C), high homogeneous (HP), or high localized (LP) availability of P. *n.s. not significant* differences between treatments ($p > 0.05$)

et al. 1975; Lopez-Zamora et al. 2001; Faustino et al. 2013), there are little functional explanations of the nutrient imbalances that finally generate growth reduction. The depressive effect in shoot growth of high concentration of P homogeneously applied in the root system (HP) in *E. grandis* plants growing with low N availability was corroborated (Fig. 2). It was also confirmed that the same availability of P applied only in a part of the root system (LP) has no depressive effect (Graciano et al. 2009). Moreover, LP treatment in the longer time will probably stimulate growth as leaf area was wider even than plants growing with low concentration of P (C) (Table 1). Probably, there were no changes in leaf morphology as was reflected in the same specific leaf area in all treatments (Table 1). As well, there were no changes in root dry mass between treatments in response to P availability. As in this experiment, nitrate availability was low, plants probably distributed growth to optimize N uptake, and they did not respond to the high concentration of P by increasing root growth in the lapse of the experiment, as it was observed in the previous trials (Graciano et al. 2009).

Higher concentration of P in HP and LP plants comparing with C (Table 1) is reflecting that plants took up more P as a consequence of the higher availability of this nutrient in the root environment. This result was found previously in plants growing in rhizotrons filled with sandy

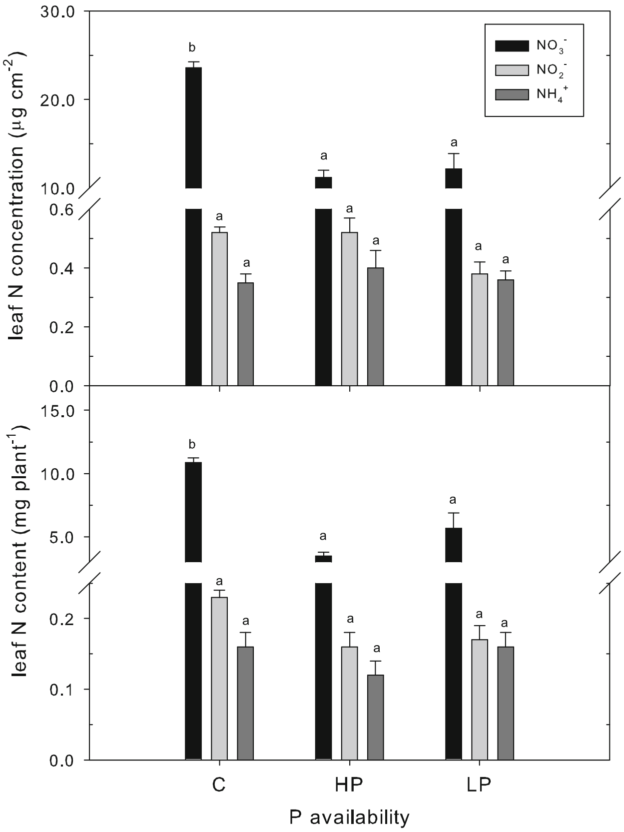

Fig. 5 Concentration (µg cm^{-2}) and content (mg) of nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+), in leaves of *E. grandis* plants growing in a split-root system with low concentration of P (C), high homogeneous (HP), or high localized (LP) availability of P. *Different letters* indicate a significant difference ($p < 0.05$) between treatments for each variable

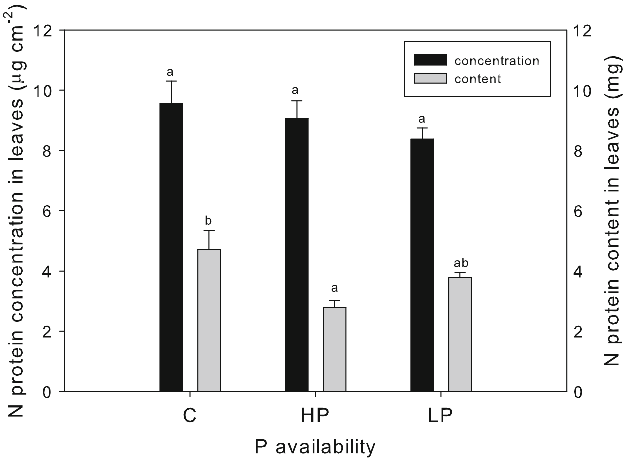

Fig. 6 Concentration (µg cm^{-2}) and content (mg) of N as protein in leaves of *E. grandis* plants growing in a split-root system with low availability of P (C), high homogeneous (HP) or high localized (LP) availability of P. *Different letters* indicate a significant difference ($p < 0.05$) between treatments for each variable

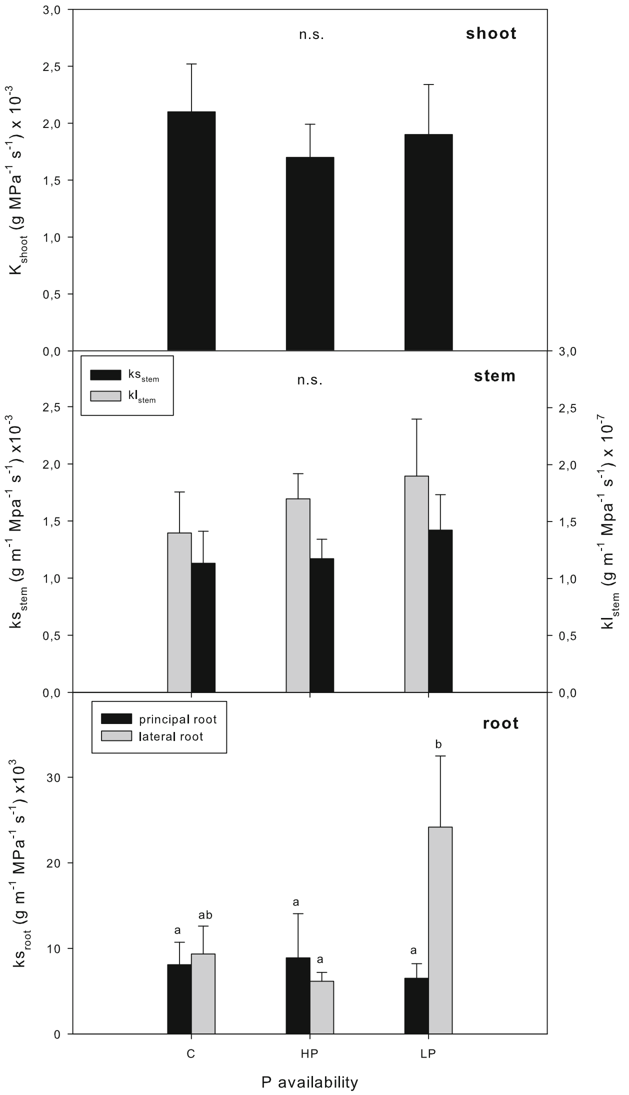

Fig. 7 Shoot hydraulic conductance (K_{shoot}; kg MPa^{-1} s^{-1}), stem specific hydraulic conductivity (ks_{stem}; kg MPa^{-1} s^{-1} m^{-1}), stem leaf-specific hydraulic conductivity (kl; kg MPa^{-1} s^{-1} m^{-1}), and root specific hydraulic conductivity (ks_{root}; kg MPa^{-1} s^{-1} m^{-1}) of fine root segments sampled from principal or lateral pots of *E. grandis* plants growing in a split-root system with low P (C), high homogeneous (HP) or high localized (LP) availability of P. In LP plants, the high concentration of P was applied in the lateral root pot. *Different letters* indicate a significant difference ($p < 0.05$) between treatments for each variable; *n.s.* not significant differences ($p > 0.05$)

soil (Graciano et al. 2009). HP and LP plants were not limited by P, because concentration of P in leaves was near the maximum found for *E. grandis* seedlings in aeroponics experiments (Kirschbaum and Tompkins 1990). The uptake of P was similar in both high P treatments; therefore, growth in HP was not limited by P, but it is limited by N metabolism.

The availability of P altered the accumulation and partitioning of N to the organs

HP plants had some alteration in their capacity of absorbing or assimilating N. This problem seems to be related with the homogeneous concentration of P and not with an imbalance between the concentrations of N and P in the substrate, because LP and HP plants had the same total availability of N and P, but LP grew similar to C plants, i.e., no depressive effect was observed. We found that some steps of the assimilation of N were altered in HP plants. For example, HP plants had lower concentration of nitrate in lateral roots than C plants, while LP plants had intermediate concentration (Fig. 3). Nevertheless, content of nitrate in lateral roots was similar in all treatments, because the small changes in root size counteracted changes in concentration (Figs. 2, 3). On the other hand, concentration of nitrate in principal roots was similar in all treatments, but content of nitrate was higher in C plants due to slightly larger root dry mass in plants of this treatment. A decrease in root concentration of nitrate can be due to lower uptake or higher assimilation rates or transport. In most trees, nitrate is assimilated in roots rather than shoots (Tang et al. 2012). Therefore, higher nitrate reductase activity in roots would reduce concentration of nitrate in roots (Adams and Attiwill 1982), but it should increase content of proteins in leaves. However, content of proteins was lower in HP plants (Fig. 6), indicating that the lower concentration of nitrate found in the roots was not due to a higher N assimilation. Higher transport should be reflected in higher concentration of nitrate in xylem sap; but this was not the case comparing HP with C plants (Fig. 4). Concentration of nitrate in the xylem of all treatments fell in the range found in other species (Guérin et al. 2007). Therefore, lower concentration of nitrate in HP roots probably indicates that uptake was reduced in this homogeneous P treatment. Nitrate enters to the plant by mass flow or by specific transporters. The concentration of nitrate in nutrient solution used in this experiment falls in the range of activity of high-affinity nitrate transporters (Gessler et al. 2004). It was demonstrated in *Arabidopsis thaliana* that high-affinity transporters respond not only to the specific ion that they transport but also to the concentration of other ions, indicating that control signals are affected by the general nutrition status of the plant (Bao et al. 2011). Phosphate can have an antagonist crosstalk with nitrate uptake, especially under low nitrate conditions (Kant et al. 2011), as in this experiment.

The localized availability of P increased root conductivity

As it was stated earlier, the lower concentration of nitrate in HP roots is not due to higher assimilation or transport of this anion to the leaves, because concentration of nitrate in the xylem was similar in all the treatments (Fig. 4). Furthermore, concentration and content of nitrate in leaves in HP and LP plants were lower than in C plants. Moreover, although the concentration of proteins in leaves was similar in all treatments, content of proteins in leaves was lower in HP plants (Fig. 6), demonstrating that the total assimilation of N is lower in HP plants. It seems that the concentration of proteins tends to be constant and, therefore, plants built the leaf area that can be supported with the N taken up.

Hence, a high availability of P diminishes nitrate uptake, but the effect is higher in the homogeneous treatment (HP) than in the localized treatment (LP). The possible explanation for the higher growth of LP plants respect to HP plants, although they have similar concentration of forms of N in roots and leaves, is that their roots had higher hydraulic conductivity when they were exposed to high concentration of P (Fig. 7). Higher hydraulic conductivity can increase the flow of water and nutrients to the rhizosphere, especially nitrate which is a mobile anion carried by mass flow. Therefore, although the concentration of nitrate measured in a certain moment in roots (Fig. 3), xylem (Fig. 4) and leaves (Fig. 5) were similar in LP and HP plants, the movement of nitrate into the root medium was faster in LP plants, and the uptake, integrated along the time, was consequentially higher, as it was reflected in higher leaf area and leaf content of proteins in LP than in HP plants (Table 1; Fig. 6).

Although root proliferation is a mechanism that allows plants to get more water in middle and long time, also short time responses to nutrient addition in hydraulic properties have been observed. Root flow rate increased 4 h after the addition of a high concentration of nitrate in plants growing in hydroponics with low availability of nitrate (Gloser et al. 2007; Gorska et al. 2008a). In that experiment, this effect was reversed upon return of the roots to a low nitrate solution. An important outcome of such a local response is that it will magnify the positive effect of the altered root resistance on total nitrate uptake by prioritizing water uptake from roots exposed to nutrient-rich patches (Gorska et al. 2008b). When the root system is exposed to heterogeneous conditions, roots inside and outside the nutrient-rich patch respond differently. The fact that this response was both localized and reversible supports the idea that plants can use this mechanism, probably mediated by aquaporin activity, to chase mobile patches of nitrate around the soil using only its ability to quick change of hydraulic root properties (Gorska et al. 2010). Genes that codify aquaporins of roots in *E. grandis* are overexpressed 6 h after the impose of water stress (Rodrigues et al. 2013), so aquaporin expression could also respond to changes in nutrient environment. Roots of *E. grandis* did not change their hydraulic conductivity in response to a sudden increase in N availability, but plants modify their hydraulic resistance in response to long-term reduction in the availability of N (Graciano et al. 2016). However, in those experiments, the availability of nutrients was homogeneous in all the root system. In our experiment, plants grew several days exposed to each concentration of P until root conductivity was measured, but the results demonstrate that P can also induce changes in root hydraulic conductivity, possibly associated to improve nitrate uptake. In this case, changes were probably related with anatomical modifications, although aquaporins could be involved. In any case, the increase in root hydraulic conductivity of the root exposed to high concentration of P in plants that have the most part of their roots exposed to low concentration of P (Fig. 2) reaffirms that plants can develop strategies to improve nitrate uptake by changing water uptake (Matimati et al. 2014; Graciano et al. 2016). In this case, a high root hydraulic conductivity counteracted the negative effect of high concentration of P in the root medium in nitrate uptake. Therefore, the intercross between P and N availability affects not only N metabolism, but also root hydraulic conductivity.

Relationship of the new evidences with the knowledge of nutrition of *E. grandis*

It is known that N and P are two nutrients that regulate *E. grandis* growth rate. The availability of N has a larger influence on dry mass partitioning to leaves than on leaf physiology, while availability of P impacts in leaf physiology (Kriedemann and Cromer 1996). Our results add the information that the distribution of P in the root system affects N uptake when the availability of N is limiting growth. The decrease in the concentration of N in individual leaves reduces the photosynthetic capacity, and therefore, growth is reduced (Kirschbaum and Tompkins 1990). Moreover, the distribution of P in the root system has an impact in plant hydraulic traits, i.e., a P-rich patch increases the hydraulic conductivity of the roots in contact with the high concentration of P. Therefore, the cross effect of availability of P with N and water uptake should be considered when deciding fertilization of *E. grandis* plantations.

The changes in hydraulic conductivity of the roots in response to the concentration of nutrients, give an explanation to the different nutrient uptake rate observed in *E. grandis* roots from different soil depth (Da Silva et al. 2011). Root specialization, i.e., different nutrient uptake potential in certain parts of the root system, can be reached by changing specific transporters activity or by changing mass flow from the soil to the roots through changes in root hydraulic conductivity. In addition, the increase in root hydraulic conductivity in nutrient-rich patches can give a possible explanation to the higher mortality in fertilized eucalypts under drought comparing with unfertilized plants (Stoneman et al. 1996; White et al. 2009), because higher hydraulic conductivity can be related with higher water consumption. If the availability of N is low, *E. grandis* adjust its hydraulic architecture to reduce water consumption, and although plants did not respond to a sudden increase of P in the whole root media (Graciano et al. 2016), they adjust root conductivity in P-rich patches. Therefore, *E. grandis* does not respond to sudden increases in nutrient availability, but it responds to longstanding changes in the root environment.

Conclusions

There is a clear intercross between phosphate availability in the roots and nitrate uptake. High concentration of P homogeneously applied in the root system (HP) has depressive effect in shoot growth, because nitrate uptake diminishes. However, the same availability of P applied localized in only a part of the root system (LP) has no depressive effect. The lower concentration of nitrate in HP roots is not due to higher assimilation or transport of this anion to the leaves, since the concentration of nitrate in the xylem was similar in all treatments and concentration and content of nitrate in leaves were lower in plants exposed to high concentration of P (HP and LP) than in plants fed with low concentration of P (C). Nevertheless, plants that received high P localized (LP) had no depressive effect in growth, because they had higher root hydraulic conductivity, and therefore, mass flow of nutrients to the rhizosphere was probably higher, enhancing N uptake. Finally, when nutrient interactions are analyzed, it is important to consider the concentration of each nutrient as well as their spatial distribution in the root system.

These results are relevant to understand the effect of different fertilizers placement in tree growth and to give a mechanistic explanation to nutrient interaction. They also highlight the relevance of the interaction between mineral nutrition and water consumption.

Author contribution statement LC designed and performed the biochemical determinations and wrote the manuscript, LIF perform the hydraulic measurements and took the samples for biochemical determinations, and CG designed the experiment, cultivate the plants, perform the biochemical and hydraulic measurements, and wrote the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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