



Tree Physiology 35, 1062–1074
doi:10.1093/treephys/tpv068



Research paper

Fertilization with urea, ammonium and nitrate produce different effects on growth, hydraulic traits and drought tolerance in *Pinus taeda* seedlings

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Received July 22, 2014; accepted June 29, 2015; published online July 31, 2015; handling Editor Guillermo Goldstein

Urea fertilization decreases *Pinus taeda* L. growth in clay soils of subtropical areas. The negative effect of urea is related to changes in some hydraulic traits, similar to those observed in plants growing under drought. The aims of this work were (i) to determine whether different sources of nitrogen applied as fertilizers produce similar changes in growth and hydraulic traits to those observed by urea fertilization and (ii) to analyze the impact of those changes in plant drought tolerance. Plants fertilized with urea, nitrate (NO_3^-) or ammonium (NH_4^+) were grown well watered or with reduced water supply. Urea and NO_3^- fertilization reduced plant growth and increased root hydraulic conductance scaled by root dry weight (DW). NH_4^+ fertilization did not reduce plant growth and increased shoot hydraulic conductance and stem hydraulic conductivity. We conclude that NO_3^- is the ion involved in the changes linked to the negative effect of urea fertilization on *P. taeda* growth. NH_4^+ fertilization does not change drought susceptibility and it produces changes in shoot hydraulic traits, therefore plants avoid the depressive effect of fertilization. Urea and NO_3^- fertilizers induce changes in DW and root hydraulic conductance and consequently plants are less affected by drought.

Keywords: allocation, dry weight, fertilizer, hydraulic conductance, hydraulic conductivity, nitrogen.

Introduction

Plant morphology and physiology are regulated by the environmental availability of water and nutrients. Shifting of dry weight partitioning to plant organs that acquire limiting resources is usual (De Kroon et al. 2009). If nutrient or water availabilities in the soil are low, plants would allocate relatively more carbon to their roots to enhance water or nutrient uptake. An inverse response occurs if the availability of a limiting resource increases, for example, by fertilization. In general, fertilized plants invest relatively less assimilates in roots and more in leaves (Sands and Mulligan 1990). The smaller root system reduces the volume of soil explored while the wider leaf area increases transpiring surface and water consumption. Therefore, changes in dry weight partitioning driven by fertilization can compromise plant fitness under drought and thus fertilization can diminish drought tolerance.

Changes in dry weight (DW) partitioning as a product of fertilization may be accompanied by physiological modifications at tissue or organ levels, altering the components of hydraulic architecture, i.e., the set of hydraulic characteristics of the plants' conducting tissues that qualify and quantify the water flow from the roots to the leaves (Cruziat et al. 2002). For example, fertilization can modify stomatal conductance (Guehl et al. 1995, Amponsah et al. 2004, Scholz et al. 2007), leaf water potential (Bucci et al. 2006, Samuelson et al. 2008) and the hydraulic conductance of roots (Radin and Eidenbock 1984, Gloser et al. 2007, Li et al. 2009) and stems (Harvey and van den Driessche 1997, Bucci et al. 2006). Changes in hydraulic conductive capacity can be related to the biophysical properties of the conductive system. These properties include xylem elements density and size, pit membrane characteristics and spatial

arrangement of conduits in the xylem tissue (Krasowski and Owens 1999, Yazaki et al. 2001, Kostianen et al. 2004, Choat et al. 2008, Sperry et al. 2008). Therefore, fertilization effects on patterns of carbon allocation and functional traits related to the hydraulic architecture may have consequences on water transport efficiency, drought tolerance and growth (Bucci et al. 2006, Goldstein et al. 2013).

Growth and productivity of *Pinus taeda* L., a commercially important forest tree in the USA and subtropical areas of South America, can be limited by nitrogen (N) availability (Allen et al. 1990, BassiriRad et al. 1997). Urea is nowadays the most widely used N fertilizer due to its low price and high N content (46%) (Jokela and Long 2012). Increases in growth due to urea fertilization have been extensively reported for *P. taeda* plantations in the USA, at different ages, in a wide variety of soils and with different application forms and doses (Albaugh et al. 1998, 2004, 2008, Allen and Albaugh 1999, Jokela et al. 2000, Nilsson and Allen 2003, Rahman et al. 2006, Fox et al. 2007). However, in subtropical areas of Northern Argentina and Southern Brazil decreases in growth related with urea fertilization have been observed (Costa Muniz et al. 1975, Faustino et al. 2011, 2012, Fernández et al. 1999, 2000a). The urea depressive effect was observed in different experimental situations, with different doses and placements of fertilizers, i.e., it is not the result of an operational problem like salinity or toxicity. The urea depressive effect during plantation establishment is associated with changes in some plant hydraulic traits, at different levels, that diminish the delivery of water to the needles compared with non-fertilized plants. The main changes observed were an increase in leaf area, a strong fall in needle midday water potential associated with stomatal closure and a reduction in the xylem hydraulic conductance at branch level (Faustino et al. 2013). The reduction in needle water potential, stomatal conductance and water supply to needles due to changes in stem hydraulic properties are also typical responses of plants subjected to drought (Maseda and Fernández 2006, McDowell et al. 2008, Faustino et al. 2012, 2013). Therefore, some changes produced by urea fertilization are similar to those produced by drought.

The negative effect of urea described above can be due to the urea itself or due to the ammonium (NH_4^+) and/or nitrate (NO_3^-) released to the soil shortly after the urea is applied. When urea is in contact with the soil it is rapidly hydrolyzed by the enzyme urease and releases NH_4^+ , which can remain in the soil exchange complex or can be converted to NO_3^- by microorganisms. NO_3^- and NH_4^+ are the predominant forms of inorganic N absorbed by roots (Guo et al. 2007), although some species, including conifers, can take up organic N forms (Näsholm et al. 2009). Fertilization with different N sources can produce diverse growth responses in some species (Seith et al. 1996, Claussen and Lenz 1999, Öhlund and Näsholm 2001, Smethurst et al. 2004) because each ion produces physiological and morphological

responses at different levels (cellular, tissues, organs). Often, these modifications involve changes in dry weight partitioning, root size and root architecture as a consequence of long-term availability of different N sources (Siemens et al. 2011, Siemens and Zwiazek 2013). Some conifer species cultivated with NH_4^+ had a significantly higher root hydraulic conductivity than the counterparts cultivated with NO_3^- , probably due to their preference for NH_4^+ vs NO_3^- (Gebauer and BassiriRad 2011). In *Populus*, responses to NH_4^+ and NO_3^- in dry weight partitioning and hydraulic conductivity are different (Domenicano et al. 2011). Short-term changes in intrinsic characteristics of hydraulic tissues were also observed as a response to different N sources. Some studies underline a contrasting short-term effect of NH_4^+ vs NO_3^- specifically on root hydraulic conductance. These studies often include crop species under different N sources and conclude that roots exposed to NO_3^- have a higher root hydraulic conductance than roots exposed to NH_4^+ (Adler et al. 1996, Guo et al. 2002, 2007, Górška et al. 2010). Some crop species, characterized by high NO_3^- needs, adjust their root hydraulic properties to promote NO_3^- acquisition. NO_3^- is a mobile ion in soil solution and these species can increase the flux of NO_3^- to the rhizosphere by increasing transpiration (Cramer et al. 2009). However, plants with very low NO_3^- needs and very low NO_3^- uptake rates, as in trees, cannot respond to NO_3^- concentration, possibly because changes in root hydraulic conductance in response to NO_3^- could be costly and unnecessary (Górška et al. 2010). Also, higher availability of NH_4^+ should not modify root conductivity because this cation has low diffusion coefficients in soil solution compared with NO_3^- (Gregory 2006).

The areas in South America where the depressive effect of urea fertilization on *P. taeda* growth was observed have lateritic red soils with high clay content (Faustino et al. 2013). This type of soil imposes high resistance to water movement (Hacke et al. 2000) and limits water availability for the plant in periods with low rainfall. Under this growing condition, plants can develop mechanisms to acclimatize to low water availability (Nilsen and Orcutt 1996), especially at the beginning of the plantation when the soil volume explored by roots is low and environmental conditions stress young trees. Therefore, as urea fertilization produces changes similar to those induced by drought, we speculate that urea-fertilized plants will better tolerate soil drying because changes are induced by fertilization previously to drought beginning. On the other hand, the urea depressive effect can be due to the urea itself or mediated by NH_4^+ and/or NO_3^- in soil solution. In this context, it is important to evaluate whether fertilization with another source of N can avoid the depressive effect observed in urea-fertilized plants. The goals of this study were (i) to determine whether different sources of N applied as fertilizer produce similar changes in growth and hydraulic traits to those observed by urea fertilization and (ii) to analyze the impact of those changes in plant drought tolerance.

Materials and methods

Growing conditions

The experiment was done during spring and summer in a naturally lit glasshouse in La Plata, Argentina (34°54'45.52"S, 57°55'51.11"W), between October 2011 and March 2012. Seedlings were obtained from homogeneous genetic seeds, provided by the *P. taeda* breeding program of INTA Montecarlo, Argentina. Plants were cultivated for 4 months in speedlings filled with composted pine bark and watered with Hoagland solution (Leggett and Frere 1971), in the same glasshouse where the experiment was conducted. Four-month-old seedlings (16 cm tall, 0.6 g total DW) were transplanted to 1 l pots fully filled with red clay soil collected in a young *P. taeda* plantation near Montecarlo, Misiones, Argentina, an area where this species has high productivity (Faustino et al. 2013). The soil has 3.7% of organic carbon (determined by dry combustion with an automatic analyzer (CR12-LECO)), 0.3% total N (determined by Semimicro Kjeldahl method), 4.1 ppm of extractable phosphorus (determined by Bray–Kurtz method), 27.6% of sand, 38.5% of silt and 34.0% of clay. Texture components were evaluated using the Robinson pipette method. The pH is 6.0 (determined by potentiometric method (1 : 2.5)) and the cation exchange capacity is 16.9 cmol kg⁻¹ (determined by Polemio–Rhoades method).

Eighty plants were used, one in each pot. One month later plants were fertilized. Twenty plants were assigned to each fertilization treatment: urea, NO₃⁻, NH₄⁺ and control (C). Fertilizers were applied in aqueous solution (50 ml per plant). The dose of fertilizer corresponded to 0.23 g of N in each pot. Urea solution was made with commercial urea (46-0-0) (0.167 M of urea, 0.333 M of N), NO₃⁻ solution, with commercial Ca(NO₃)₂ (0.164 M of Ca(NO₃)₂, 0.328 M of N) and NH₄⁺ solution, from NH₄OH (0.327 M of NH₄OH, 0.327 M of N). Fertilization was applied only once. Plants of each treatment were placed randomly in the bench. To avoid edge effects, plants were mixed every 15 days.

All the pots were watered every other day with 100 ml of tap water during 50 days after the fertilization. The volume was enough to keep the pots at field capacity without significant percolation of liquid, and tap water contained <3 mM of N. All pots were covered with a 2-cm thick layer of expanded polystyrene beads and put in plastic bags to prevent water evaporation from the soil. On Day 51, water availability treatments were imposed. Ten plants randomly chosen for each fertilization treatment continued with 100 ml of water every other day (high water availability treatment). The other half of the plants were subjected to water stress by reducing the amount of water to 50 ml every 10 days (low water availability treatment). The volume of water to generate water stress was determined in a previous experiment in which plant water consumption was 7.78 ml day⁻¹ g⁻¹. The mean plant DW was 0.926 g when the

water stress treatments started. The water supply in low water availability treatment (50 ml every 10 days) was lower than the potential water consumption of plants (≅72 ml in 10 days). The day before water was applied, low water availability plants lessened to soil gravimetric water content 22% lower than high water availability plants. The average water loss exceeded the replenishment of 50 ml, therefore the stress level increased over the course of the experiment. The experiment finished 200 days after fertilization, i.e., plants were grown in each water treatment for 150 days. The size of the pot did not limit root growth because at the end of the experiment, the bigger plants' roots had 8.57 g of fresh weight (2.09 g of DW), they occupied <20% of the volume of the pot and they were well distributed in the pot.

Temperature, relative humidity and photoperiod during the experimental period are shown in Figure S1 available as Supplementary Data at *Tree Physiology* Online (Facultad de Ciencias Astronómicas y Geofísicas—UNLP 2011–2012, Servicio Meteorológico Nacional 2011–2012). Medium average temperatures during the experiment were 23.5 °C (SD 3.5 °C), with maximum and minimum temperatures of 28.1 and 16 °C, respectively. Average relative humidity was 60.7% (SD 11.4%), with maximum and minimum of 94 and 31% (Servicio Meteorológico Nacional 2011–2012). The average photoperiod was 13:51 h, with a maximum and a minimum of 14:31 and 12:31 h.

The experiment was conducted in a greenhouse to easily control water availability and avoid fertilizer lixiviation. The depressive effect of urea fertilization had been observed in field experiments (Faustino et al. 2011, 2013) and pot experiments conducted in the same greenhouse. In all those experiments the fertilizer was applied only once. As we wanted to evaluate if other sources of N produce the same depressive effect as urea, we repeated the same procedure used in the former experiments (i.e., one application at the beginning of the experiment).

Soil measurements

To evaluate changes in N availability in soil solution over the course of the experiment, 6.16 cm³ cylinders of soil (1.4 cm of diameter, 4 cm of length) were extracted from an intermediate position between the center and the edge of the pot. Samples were collected 8, 50 and 200 days after fertilization. For each date, six samples per treatment were taken.

Immediately after sampling, each sample was saturated with distilled water in a vacuum chamber and 5 g of saturated soil was incubated with 10 ml of distilled water during 16 h. The supernatant was collected and centrifuged at 3090g for 15 min to obtain a clear solution. Finally, pH and NO₃⁻ and NH₄⁺ concentrations of the clear solution were determined (modified method from Graciano et al. 2009). NO₃⁻ concentration was determined by UV spectrophotometry (Greenberg et al. 1985). NH₄⁺ concentration was determined by Nessler's method

(Greenberg et al. 1985). Nitrite concentration was considered negligible in this experiment.

Plant measurements

At the end of the experiment, after completing the measurements that will be detailed in the following paragraphs, all the plants of each treatment were dried at 65 ± 5 °C to constant weight. Stem, needles, tap root and fine roots were weighed separately with 0.01 g accuracy.

The hydraulic conductance (K) is the ratio of the water flow rate through the plant or plant segment (g s^{-1}) to the pressure gradient causing flow (MPa). Hydraulic conductivity (k) is calculated by dividing K by the segment length (Becker et al. 1999, Tyree and Zimmermann 2002). Any change in DW partitioning or in the structure of water conducting tissues, as a consequence of fertilization, may involve changes in hydraulic conductance or conductivities at organ and/or individual level. The hydraulic conductance of shoot (stem, branches and needles) (K_{shoot} ; $\text{g MPa}^{-1} \text{s}^{-1}$) and root systems (K_{root} ; $\text{g MPa}^{-1} \text{s}^{-1}$) were measured in five plants per treatment at the end of experiment. From these measurements, the hydraulic conductance of whole plant (K_{plant}) was calculated (Tyree and Zimmermann 2002):

$$K_{\text{plant}} = \frac{1}{1/K_{\text{root}} + 1/K_{\text{shoot}}}$$

In the same plants the xylem hydraulic conductivity of stem (k_h ; $\text{g m MPa}^{-1} \text{s}^{-1}$) was measured. Specific hydraulic conductivity (k_s) and leaf specific hydraulic conductivity (k_l) were calculated as the ratio between k_h and the section of sapwood and plant needle DW, respectively.

All plants were abundantly watered the night before the hydraulic measurements were done to ensure optimal hydration. In the morning, plants were moved to the laboratory and removed from the pot just before the measurement was done, ensuring not to damage the root system. The shoot was cut under water, above the root collar and connected to the flow meter. K_{shoot} and k_h 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 the stem segment or shoot (Brodribb and Feild 2000, Zwieniecki et al. 2000). Perfusion was made with de-gassed filtrated distilled water, using a pressure of 4.5 kPa. The air of the water was extracted in a vacuum chamber. The hydraulic conductance was measured when water flow was stable. After measurement of K_{shoot} , the portion of the shoot with needles was cut and k_h was measured in the basal portion of the stem (3 cm long).

K_{root} was measured through pressurization in a pressure chamber. The root system was introduced in a container filled with water and the set was put into the pressure chamber, leaving the plant collar end outside the cap. The chamber was

pressurized and the sap outflow from the cut end was collected during 2 min with a pre-weighed cotton bud. Under these conditions, flow reached a steady-state rate after few minutes. K_{root} was calculated by measuring flow rates resulting from the application of a constant pressure of 0.5 MPa. Pressure : flow curve was linear between 0.25 and 0.8 MPa. Thus, we selected 0.5 MPa as our driving force to ensure that all measurements were conducted in the linear phase (Gorska et al. 2008, Górska et al. 2010) (see Figure S2 available as Supplementary Data at *Tree Physiology* Online). The cotton buds were weighed with an accuracy of 0.001 g. K_{root} was calculated as the ratio between flux (quantity of fluid per unit time) and pressure.

In order to analyze whether the differences in hydraulic conductance were the product or not of changes in plant size, each conductance was scaled by DW. K_{shoot} was expressed on total shoot and needle DW basis (K_{shoot} : shoot DW and K_{shoot} : needles DW; $\text{g s}^{-1} \text{MPa}^{-1} \text{g}^{-1}$ DW), K_{root} on total root and fine root DW basis (K_{root} : total root DW; K_{root} : fine root DW; $\text{g s}^{-1} \text{MPa}^{-1} \text{g}^{-1}$ DW) and K_{plant} on total plant DW basis (K_{plant} : total DW; $\text{g s}^{-1} \text{MPa}^{-1} \text{g}^{-1}$ DW).

To determine needle N concentration, three pooled needle samples were taken from each treatment, composed of all the needles of three plants randomly chosen. Samples were completely ground and homogenized to obtain representative aliquots for N determination. Nitrogen concentration was determined using the Semimicro Kjeldahl method (Greenberg et al. 1985). Needle N contents were calculated as the product of mean nutrient concentration and needle DW of each plant (Turner and Lambert 2008).

Transverse sections from stems that was used to measure hydraulic conductivity were analyzed ($n = 6$ per treatment). Stem cross sections were obtained with a sliding microtome from two complete radio (from pith to cambium), and then stained for 5 min with safranin (5%) and mounted in Entellan® rapid mounting medium (Merck, Darmstadt, Germany) for microscopy. Ten photographs of the distal part of each stained section (i.e., the xylem near the cambium) were taken with a digital camera (Olympus DP71, Tokyo, Japan) mounted on a microscope (Olympus BX50) using the $\times 40$ objectives. Captured images were analyzed using image analysis software (Image Pro Plus, v6.3, Media Cybernetics, Rockville, MD, USA) for tracheid lumen area (μm^2) ($n = 3700\text{--}4000$ per treatment).

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) ($P < 0.05$). To analyze changes over time in the NO_3^- and NH_4^+ concentrations and pH in soil solution, the main factors were fertilization (C, urea, NO_3^- and NH_4^+) and sampling day (8, 50 and 200 days post-fertilization) (factorial ANOVA). To analyze the effect of water availability and fertilization at the end of the experiment on NO_3^- and NH_4^+ concentrations in soil solution, total, stem, needles, tap root and fine roots DW, relative

growth rate (RGR), needle N concentration and content, hydraulic variables and tracheid lumen area, the main factors considered were fertilization (C, urea, NO_3^- and NH_4^+) and water availability (high and low) (factorial ANOVA). Means were compared using Duncan's test ($\alpha=0.05$).

Results

Nitrogen concentration and pH in soil solution

Fertilization modified NO_3^- concentration ($P < 0.001$, $F = 7.89$), NH_4^+ concentration ($P < 0.001$, $F = 66.31$) and pH ($P < 0.001$, $F = 21.42$) of soil solution in a different way depending on the sampling day (Figure 1). NO_3^- concentration did not change over time in C pots. Eight days post-fertilization NO_3^- concentration was higher in NO_3^- -fertilized pots than in C and urea- and NH_4^+ -fertilized pots. Fifty days post-fertilization, NO_3^- availability in all fertilized pots remained greater than C, regardless of the N source applied. Two hundred days post-fertilization, a strong decay in NO_3^- concentration occurred in all fertilized treatments relative to previous sampling dates, to levels similar to C pots (Figure 1a).

NH_4^+ concentration did not change over time in C and NO_3^- -fertilized pots. Eight days post-fertilization, NH_4^+ - and urea-fertilized pots had higher NH_4^+ concentration than NO_3^- -fertilized and C pots, which decrease markedly between 8 and 50 days post-fertilization. However, 50 days post-fertilization, NH_4^+ -fertilized pots had visibly higher NH_4^+ concentration than the other treatments. The differences were significant when only data from Day 50 were analyzed ($P < 0.001$, $F = 8.65$) (insert in Figure 1b). Two hundred days post-fertilization all treatments had similar NH_4^+ concentration in soil solution (Figure 1b).

The pH of soil solution was ~ 6.5 in the C pots at the beginning of the experiment and increased over the course of the experiment. Eight days post-fertilization, urea- and NH_4^+ -fertilized pots had higher pH than C. Fifty days post-fertilization all fertilized treatments had lower pH in soil solution than C pots. Two hundred days post-fertilization fertilized treatments had similar pH to C pots (Figure 1c).

At the end of the experiment, high water availability pots had higher NO_3^- concentration ($P < 0.01$, $F = 40.99$) and lower NH_4^+ concentration ($P < 0.01$, $F = 14.61$) than low water availability pots. Nevertheless, there was no difference between fertilized and C pots in NO_3^- ($P = 0.121$, $F = 2.100$) and NH_4^+ ($P = 0.110$, $F = 2.160$) concentrations in soil solution.

Growth and DW partitioning

Total DW at the beginning of the experiment was 0.6 g. Plants of all treatments increased their size during the experimental period (Figure 2). Final total DW was modified by fertilization ($P = 0.021$, $F = 3.573$) and water availability ($P = 0.016$, $F = 6.223$), without interaction between factors. The analysis for

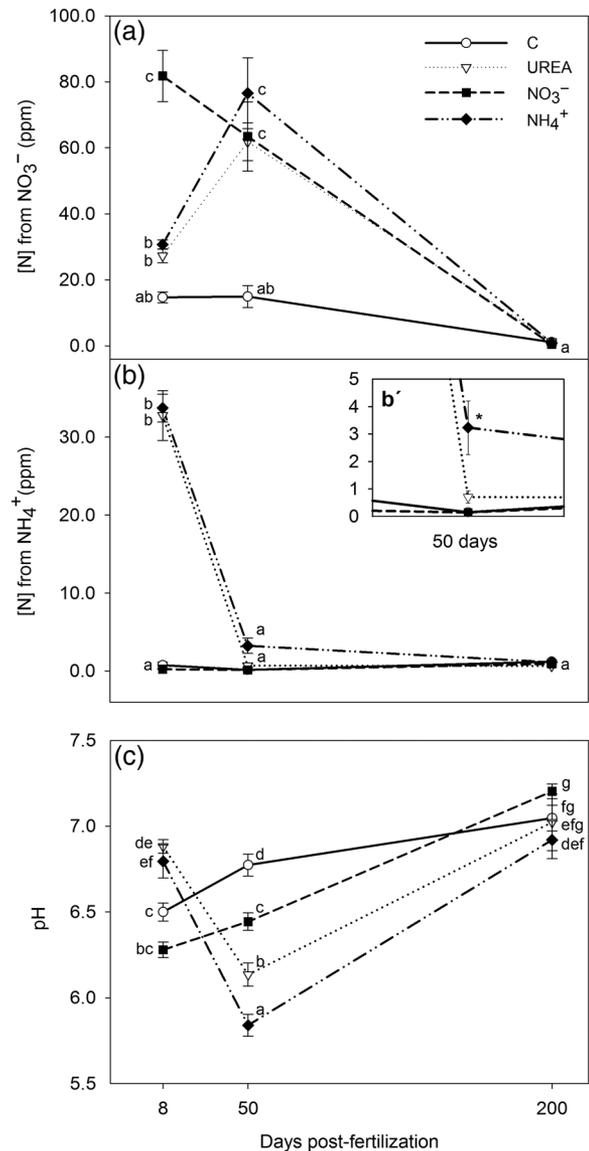


Figure 1. Concentration of (a) N as nitrate (NO_3^-), (b) N as ammonium (NH_4^+) (ppm) and (c) pH in soil solution at 8, 50 and 200 days after fertilization ($n = 72$). The analysis involves only high water availability pots. Different letters denote significant differences ($P < 0.05$). Asterisk in b' denotes NH_4^+ concentration differences considering only data from 50 days after fertilization. Error bars indicate standard errors of the means.

stem, needles, tap root and fine roots had similar results. Control plants grew more under high water availability than under low water availability, i.e., the reduction in water availability imposed a drought stress (Figure 2). In well watered plants, urea and NO_3^- fertilization reduced total DW and DW compartments compared with C plants, i.e., the depressive effect was observed with urea and NO_3^- fertilization. NH_4^+ -fertilized plants had similar total DW and DW compartments to the other treatments (Figure 2). Low water availability reduced total DW and DW compartments in C plants but not in fertilized plants, irrespective of the fertilizer applied (Figure 2). Low water availability reduced

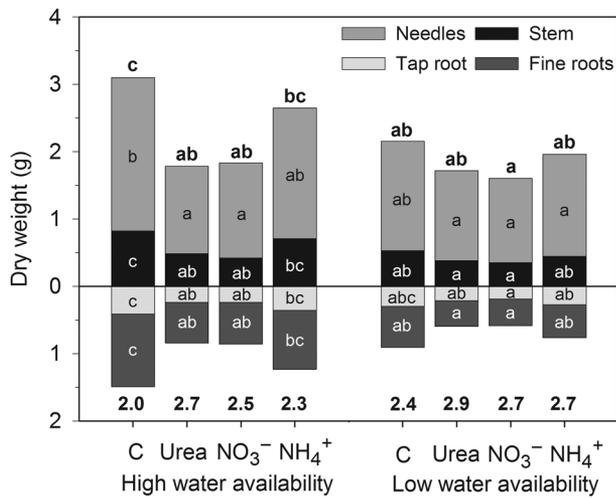


Figure 2. Dry weight (g) of needles, stems, fine and tap roots for each fertilization treatment at high or low water availability, 200 days after fertilization ($n = 80$). Different letters denote significant differences between DW compartments ($P < 0.05$). Letters above the columns indicate significant differences between total DW. Numbers below the columns indicate shoot DW : root DW ratio.

RGR in C and NH₄⁺-fertilized plants but not in urea- and NO₃⁻-fertilized plants (see Table 2).

Needle N concentration and content

Control, NO₃⁻- and NH₄⁺-fertilized plants under high water availability had significantly lower needle N concentration than urea-fertilized plants. All treatments had higher needle N concentration at low water availability (Table 1). Needle N content was similar in all treatments (Table 1).

Hydraulic traits and xylem anatomy

K_{shoot} was modified by fertilization depending on water availability (Figure 3a). In plants that grew under high water availability, K_{shoot} was higher in NH₄⁺ than C, urea- and NO₃⁻-fertilized plants. It was also higher than all the treatments under low water availability (Figure 3a). K_{shoot} : needles DW had the same response as K_{shoot} (Figure 4a), as well as K_{shoot} expressed on total shoot DW basis (fertilization \times water availability $P < 0.001$, $F = 8.723$) (data not shown).

K_{root} was lower in low water availability plants than in high water availability plants, irrespective of the fertilization treatment (Figure 3b). K_{root} : fine root DW was modified by fertilization and water availability. Under high water availability conditions, urea and NO₃⁻ fertilization had higher K_{root} : fine root DW than C plants, while NH₄⁺-fertilized plants did not differ from the other treatments. Low water availability reduced K_{root} : fine root DW in all fertilized treatments, to magnitudes similar to C plants (Figure 4b). K_{root} on total root DW basis was also modified by fertilization ($P = 0.031$, $F = 3.284$) and water availability ($P < 0.001$, $F = 24.350$) (data not shown) and had the same response as K_{root} : fine root DW.

Table 1. Relative growth rate (g day⁻¹) ($n = 80$), N needle concentration ([N] needle) (%) ($n = 24$) and plant N needle content (g) ($n = 80$) 200 days after fertilization in *P. taeda* plants growing with high or low water availability, and non-fertilized (C) or fertilized with urea, NO₃⁻ or NH₄⁺. Fertilization treatment and water availability were considered as main factors for the analysis (ANOVA factorial). Different letters denote significant differences between means ($P < 0.05$). P values lower than 0.05 are highlighted in bold.

Water availability	Fertilization	RGR (day ⁻¹)	[N] needle (%)	N in needles (g)
High	C	0.011c	1.02a	0.024a
	Urea	0.008ab	1.45b	0.019a
	NO ₃ ⁻	0.008ab	1.14a	0.018a
	NH ₄ ⁺	0.009bc	1.06a	0.019a
Low	C	0.008ab	1.50b	0.024a
	Urea	0.007a	1.48b	0.021a
	NO ₃ ⁻	0.006a	1.46b	0.017a
	NH ₄ ⁺	0.007a	1.56b	0.023a
Fertilization P		0.001	0.202	0.273
Water availability P		<0.001	<0.001	0.544
Interaction P		0.502	0.092	0.780

Table 2. Tracheid lumen area (μm^2) 200 days after fertilization in *P. taeda* plants growing with high or low water availability, and non-fertilized (C) or fertilized with urea, NO₃⁻ or NH₄⁺. Fertilization treatment and water availability were considered as main factors for the analysis (ANOVA factorial). Different letters denote significant differences between means ($P < 0.05$). P values lower than 0.05 are highlighted in bold.

Fertilization	Water availability	
	High	Low
C	143.43ab	147.85bc
Urea	151.02bc	134.52a
NO ₃ ⁻	136.79a	137.90a
NH ₄ ⁺	153.80c	135.00a
Fertilization P		0.002
Water availability P		0.068
Interaction P		<0.001

K_{plant} was modified by fertilization depending on water availability (Figure 3c). NH₄⁺-fertilized plants with high water availability had higher K_{plant} than C, urea- and NO₃⁻-fertilized plants at the same water availability and all the treatments under low water availability (Figure 3c). K_{plant} : total DW had a similar response to K_{plant} : i.e., it was higher in plants fertilized with NH₄⁺ that grew under high water availability than in the other treatments (Figure 4c).

k_h and k_l were not affected by fertilization or water availability (Figure 5a and c). However, k_s under high water availability condition was higher in NH₄⁺-fertilized plants than in C, urea- and NO₃⁻-fertilized plants (Figure 5b). There were no significant differences between treatments at low water availability (Figure 5b).

Tracheid lumen area was modified by fertilization depending on water availability (Table 2). Under high water availability condition, the lumens were bigger in NH₄⁺-fertilized plants than in

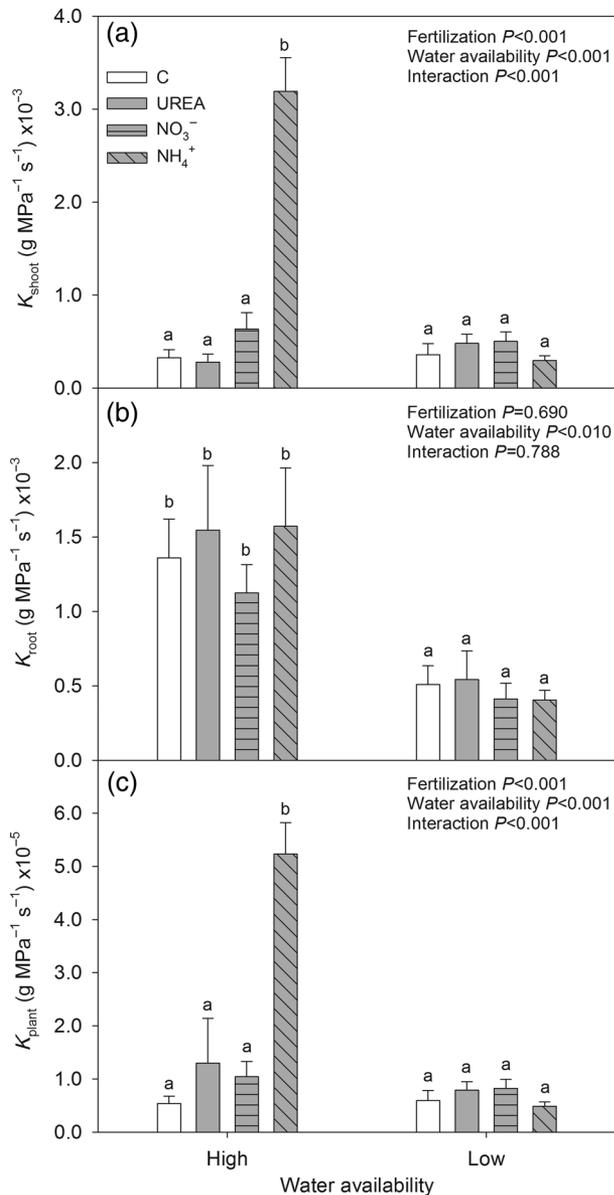


Figure 3. (a) Shoot hydraulic conductance (K_{shoot}), (b) root hydraulic conductance (K_{root}) and (c) plant hydraulic conductance (K_{plant}) for each fertilization treatment with high or low water availability, 200 days after fertilization ($n = 40$). Different letters denote significant differences between treatments (interaction fertilization × water availability) ($P < 0.05$). Error bars indicate standard errors of the means.

C and NO₃⁻-fertilized plants. Under low availability condition, C plants had bigger lumens than the other treatments, which did not differ from each other. The reduction in water availability reduced tracheid lumen area only in urea and NH₄⁺-fertilized plants.

Discussion

To evaluate the impact of different sources of N fertilization in young *P. taeda* plants, we first analyzed the particular combination of NH₄⁺ and NO₃⁻ in soil solution over the experiment

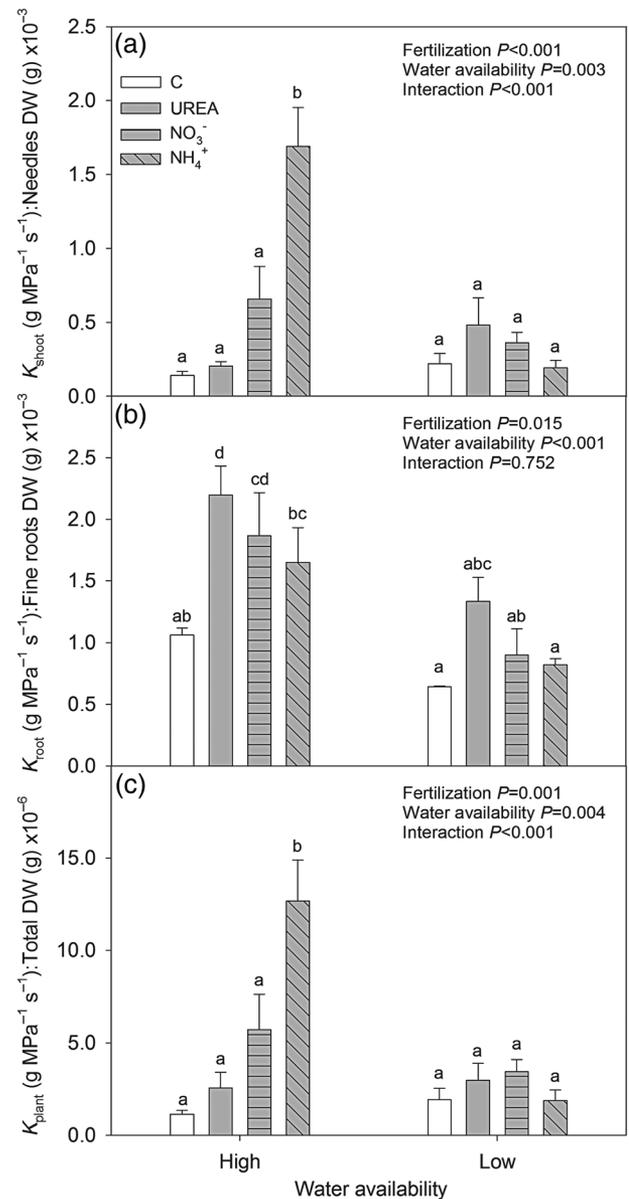


Figure 4. (a) Shoot hydraulic conductance (K_{shoot}), (b) root hydraulic conductance (K_{root}) and (c) plant hydraulic conductance (K_{plant}) expressed on DW basis, for each fertilization treatment at high or low water availability, 200 days after fertilization ($n = 40$). Different letters denote significant differences between treatments (interaction fertilization × water availability) ($P < 0.05$). Error bars indicate standard errors of the means.

(Figure 1) delivered by each fertilizer. Thereafter, we analyzed growth and hydraulic traits in each treatment that were the result of complex combination of different N forms availability, pH and water in the soil over time. The dynamics of all these factors could influence plant physiology and are involved in the responses described in this study. The results reported are soil and species dependent, because the depressive effect of urea fertilization on *P. taeda* was reported only to lateritic red clay soils.

In brief, NH₄⁺ fertilization did not affect growth and it produced changes in xylem morphology and shoot hydraulics. Urea

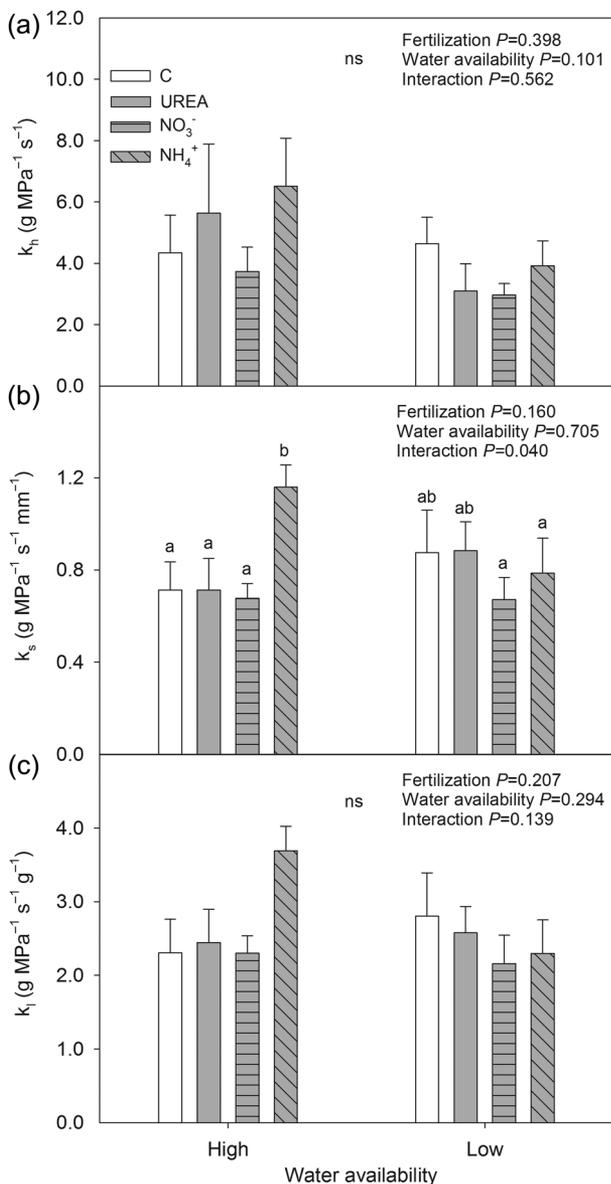


Figure 5. (a) Hydraulic conductivity (k_h), (b) specific hydraulic conductivity (k_s) and (c) leaf specific hydraulic conductivity (k_l) of stem xylem for each fertilization treatment at high or low water availability, 200 days after fertilization ($n = 40$). Different letters denote significant differences between treatments (interaction fertilization \times water availability) ($P < 0.05$). Error bars indicate standard errors of the means.

and NO₃⁻ fertilizations reduced growth and partitioning to roots and changed root hydraulic conductance. Finally, urea and NO₃⁻ fertilizations induced changes that reduced the impact of drought on growth.

Dynamic of soil solution

Time variations of NO₃⁻ and NH₄⁺ concentrations in soil solution were expected according to the known dynamics of these ions when they are applied as fertilizers. Fertilization with all N sources produced a temporary increase in NO₃⁻ concentration. Urea and NH₄⁺ fertilization increased NH₄⁺ concentration in

soil solution the week after application, but NH₄⁺ was oxidized to NO₃⁻ 50 days post-fertilization (Figure 1). The decrease in NO₃⁻ concentration in soil solution may be due to microbial fixation or plant uptake (Marschner 1995). Presumably, losses due to leaching were very low since irrigation was performed in a controlled way, with low water volumes and relatively high frequency.

Pots with high water availability had lower NO₃⁻ concentration than low water availability pots, regardless of the fertilizer applied. This response was probably not caused by lower consumption of plants subjected to drought because needles of all treatments had equal N content to those with high water availability (Table 1). As leaching was considered negligible, higher NO₃⁻ may be due to a higher release of N when soils are subjected to drying (Birch 1958, Jarvis et al. 2007) because desiccation of clays and organic matter destroys aggregates and increases mineralization (Denef et al. 2001). Also, as soils become drier, the death of some groups of microorganisms generates high availability of labile organic matter (Bottner 1985).

The increase in pH over the course of the experiment in unfertilized pots might be due to the uptake of nutrients by plants and consequently consumption of protons and/or release of hydroxyls. The higher concentration of NO₃⁻ was associated with the lower pH in the soil, whereas the higher concentration of NH₄⁺ was associated with the higher pH (Figure 1c). NH₄⁺ and urea fertilizations increased the pH of the soil solution immediately after application, but they finally acidified the soil as reported in other works (Jokela and Long 2012). However, since pH values in all treatments were near neutrality and with less than one point of difference in each sampling date, possibly these changes were not relevant to determine modifications in soil chemical reactions (i.e., solubility of macronutrients like P) or plant physiology (Marschner 1995, Zhang et al. 2013).

Effects of N sources

Growth and dry weight partitioning Urea and NO₃⁻ fertilization had a depressive effect on growth compared with C plants, while the growth reduction due to NH₄⁺ fertilization was not significant (Figure 2). Similar depressive effects were reported in other young *P. taeda* plants fertilized with urea planted in the field in red clay soils of subtropical areas in South America (Costa Muniz et al. 1975, Faustino et al. 2011, 2012, 2013, Fernández et al. 1999, 2000a). The singular effect on growth (Figure 2) of the fertilizers was expected because *P. taeda* does not have the same affinity for different N sources (BassiriRad et al. 1997, Constable et al. 2001). As fertilizers were subject to chemical changes in the soil (for example, NH₄⁺ fertilization increased NO₃⁻ concentration) (Figure 1), the roots of each treatment were exposed to different concentrations of NO₃⁻ and NH₄⁺ over time. Moreover, the reduction in growth is linked to the low hydraulic conductivity of the clay soil (Faustino et al. 2013) in addition to the effects of the particular N fertilization.

In others substrates such as sand (sterile substrate with high hydraulic conductivity), fertilization with urea, NO_3^- and NH_4^+ generated different responses in some physiological or morphological aspects but all fertilizers stimulated growth (Pharis et al. 1964).

Urea and NO_3^- fertilization reduced above and below ground growth relative to C plants. Additionally, there were changes in the partitioning to the different organs: C plants had a lower shoot DW : root DW ratio than urea- and NO_3^- -fertilized plants (Figure 2). NH_4^+ fertilization also increased the ratio with respect to C plants, but to a lesser extent. The reduction of root DW and the allocation to roots is part of the species-specific response to N fertilization, regardless of the growth response (Green et al. 1994, Griffin et al. 1995, Albaugh et al. 1998, 2004, Samuelson 2000, Coyle et al. 2008, Tyree et al. 2009).

Needle N content was used to check the N nutritional status of the plants. Needle N concentrations in all treatments were ~1–1.5% (Table 1). These concentrations are lower than those found in other studies with young *P. taeda* plantations in red clay soils of South America, which are between 1.7 and 1.8%. (Fernández et al. 2000a, 2000b, Goya et al. 2003, Faustino et al. 2013). However, none of the treatments had severe deficiencies of N (Table 1) because the boundary between deficiency and sufficiency of N for *P. taeda* needles is 1.1% (Allen 1987). As N content in needles was similar in all treatments, probably the amount of N taken up by all the treatments was similar, but it was distributed to fewer needles in the treatments that grew less (urea and NO_3^- fertilized plants). These results indicate that the reduction in growth was due to other causes rather than nutrient deficiency.

Hydraulic traits at shoot level NH_4^+ is the most abundant N source in the soils where *P. taeda* grows naturally (Robertson 1982, Griffin et al. 1995), and consequently this species prefers NH_4^+ to NO_3^- (BassiriRad et al. 1997, Constable et al. 2001). NH_4^+ fertilization produced a significant increase in K_{shoot} and k_s relative to the other treatments (Figures 3a, 4a and 5b). The high influence of shoot in total DW determined the increase in K_{plant} consistently with a rise in shoot hydraulic conductance (Figures 3c and 4c). We speculate that the increase in K_{shoot} is due to the longer exposure of NH_4^+ -fertilized plants to higher NH_4^+ concentration in the soil (50 days) compared with other treatments (Figure 1). These changes in k_s and K_{shoot} are mediated by changes in xylem morphology. NH_4^+ -fertilization produced modifications in xylem morphology that persist independently of NH_4^+ availability in the soil. At the end of the experiment, when the concentration of NH_4^+ in the soil solution was as low as in C pots, lumen tracheids of NH_4^+ -fertilized plants were larger than those of C plants (Table 2). Higher lumen areas (i.e., larger tracheid diameter) reduce the resistance to water movement (Figure 3). Other studies reported increments in xylem hydraulic conductivity in plants fertilized with N. Bucci et al. (2006) found

an increase in branch k_s of five savannah tree species fertilized with ammonium sulfate. An increase in k_s was also found in hybrid poplars (Hacke et al. 2010), *Balfourodendron riedelianum* Engl. and *Cordia americana* (L.) Gottschling & J.S. Mill. (Villagra et al. 2013) fertilized with ammonium nitrate. In all these cases, the increment in k_s was related to the reduction in wood density and it suggests changes in sapwood anatomy that led to increased wood porosity. On the other hand, species-specific responses in k_s that depend on the N form applied (NO_3^- or NH_4^+) were observed in four conifer species seedlings (Islam et al. 2010).

The higher capacity to conduct water was the result of changes at stem xylem level, demonstrated by the increase in lumen tracheid area (Table 2) and k_s (Figure 3). However, complementary modifications occurred in other tissues (e.g., needle or brachyblasts) because the increase in K_{shoot} was more pronounced than that observed in k_s . Whole-shoot conductance includes both vascular and non-vascular components and the non-vascular resistances in leaves might dominate in small plants (Tyree and Zimmermann 2002). Moreover, it is known that N fertilization modifies hydraulic traits at the needle level in *P. taeda* trees (Domec et al. 2009). On the other hand, the ability to conduct water may improve as sap salt concentration increases (Zwieniecki et al. 2001), which might be affected by nutrient concentrations in soil solution because N sap concentration increased in soils with high N availability (Smith and Shortle 2001).

Hydraulic modifications at root level As *P. taeda* prefers NH_4^+ to NO_3^- (BassiriRad et al. 1997, Constable et al. 2001), different responses to the availability of each N source at root level can be expected (Górska et al. 2010, Gebauer and BassiriRad 2011). Urea and NO_3^- fertilization increased K_{root} : fine root DW (i.e., the ability to conduct water per gram of fine root) (Figure 4b). This modification partially compensated the capacity of the root system to deliver water to the shoot, because these treatments had lower root DW and partitioning to roots than C plants (Figure 2). The reduction in the size and proportion of total DW allocated to fine roots alter the acquisition of resources (BassiriRad et al. 1996). The decrease in root allocation together with an enhancement in root hydraulic conductivity as a consequence of N fertilization was also found in *Populus tremuloides* Michx. (Siemens and Zwiazek 2013) and *Prunus persica* (L.) Batsch. (Zhang et al. 2014). However, this functional compensation at root level was not observed in *Pinus canariensis* C. Sm. and *Pistacia lentiscus* L., in which the proportional reduction of root DW as a consequence of fertilization implied a reduction in root hydraulic conductance scaled by leaf area (Hernández et al. 2009, Luis et al. 2010).

It was stated that plants with high preference for NH_4^+ , such as conifers, or with very low NO_3^- needs and slow growth rate, did not change root conductance in response to changes in NO_3^- availability (Cramer et al. 2009, Górska et al. 2010). However, in four conifers species it was found that there was an increase in

root hydraulic conductance in plants cultivated with NH_4^+ compared with others cultivated with NO_3^- (Gebauer and BassiriRad 2011). These authors suggest that in long-term experiments, 'indirect' effects (e.g., those elicited by DW partitioning or xylem morphology) would strongly modulate root hydraulic conductivity responses (Gebauer and BassiriRad 2011). Here, we found changes with this latter characteristic in urea- and NO_3^- -fertilized plants. NH_4^+ fertilization produced changes in K_{root} : fine root DW with the same trend but with lower magnitude.

Relationship between growth and hydraulic traits The decrease in growth of NH_4^+ -fertilized plants was not significant relative to C plants and lower than that in urea- and NO_3^- -fertilized ones (Figure 2). The higher K_{shoot} can explain why plants fertilized with NH_4^+ were not as negatively affected in growth as NO_3^- and urea-fertilized plants (Figure 2, Table 1). As NH_4^+ produced changes in hydraulic traits that were not observed in NO_3^- - or urea-fertilized plants, we think that the increase of NO_3^- in soil solution is the cause of the depressive effect produced by urea fertilization (Costa Muniz et al. 1975, Faustino et al. 2011, 2012, 2013, Fernández et al. 1999, 2000a). The negative effect of NO_3^- can be due to the reduction of partitioning to roots, particularly in a soil that has low hydraulic conductivity. In this context, the delivering of water to the shoot is low and it is not counteracted by higher K_{root} .

After Day 50, NO_3^- concentration increased in NH_4^+ -fertilized pots (Figure 1a), so probably plants started to respond to this anion. It is possible that higher K_{shoot} and K_{root} : fine root DW may enhance growth, but as NH_4^+ -fertilized plants were exposed to a high concentration of NO_3^- after Day 50, the negative effect of this ion was not avoided. Nevertheless, the depressive effect started later in NO_3^- - and urea-fertilized plants. In the medium or long term, both urea and NH_4^+ fertilization increase NO_3^- concentration in soil solution (Figure 1a), thus some degree of reduction in growth was observed with any source of N.

Effect of different N sources interacting with water availability

Growth and dry weight partitioning Fertilization with urea and NO_3^- produced changes in DW partitioning similar to those produced by drought. Control plants under drought reduced total growth relative to C plants under high water availability, but also reduce the allocation to roots (Figure 2). NH_4^+ -fertilized plants tend to respond similarly to C plants (Figure 2). On the other hand, urea- and NO_3^- -fertilized plants had similar DW partitioning under both water conditions (Figure 2). The RGR was similar between urea- and NO_3^- -fertilized plants for both water availabilities, while the RGR reduction in NH_4^+ -fertilized plants due to drought was similar to that observed in C plants (Table 1). These results demonstrate that fertilization with urea and NO_3^- reduced growth when plants had good water availability but fertilization did not increase plant susceptibility to drought.

Moreover, urea-fertilized plants as well as NO_3^- -fertilized plants had lower reduction in DW as a result of low water availability (11.6 and 18.7% of reduction in growth in low respect to high water availability plants for urea and NO_3^- fertilization, respectively). On the other hand, NH_4^+ -fertilized plants had a reduction in DW similar to C plants (33.4 and 29.8% in NH_4^+ fertilized and C plants, respectively) (Figure 2). It is important to remark that water stress did not impair N uptake and all plants could accumulate the same amount of N in needles, independent of the fertilizer applied (Table 1).

Hydraulic traits Hydraulic conductances changed to adjust the hydraulic system of plants to low water availability. In the short term, the main mechanism to prevent water loss is stomatal closure. In the medium and long term, structural changes occur to adapt the hydraulic architecture to water shortage, for example, modifications in DW partitioning or xylem resistance to water movement (Maseda and Fernández 2006). In non-fertilized plants, K_{root} was the only hydraulic trait reduced under drought (Figure 3b). As K_{root} : fine root DW was not reduced by drought in this treatment, the drop in K_{root} was mainly due to the reduction in the size of the root system and not by intrinsic changes at the root level. Fertilized plants growing with high water availability had higher K_{root} : fine root DW compared with non-fertilized plants, regardless of the N fertilizer applied (Figure 4b). The rise in this conductance reflects intrinsic changes at root level, as discussed above. However, when the availability of water was low, K_{root} : fine root DW of fertilized plants decreased significantly. *Pinus taeda* fine roots are highly susceptible to drought and they act as fuses to maintain the integrity of the rest of plant hydraulic system (Hacke et al. 2000). The xylem-specific hydraulic conductivity of *P. taeda* fine root diminished with fertilization and drought, probably as a result of different xylem structure or the amount of irreversible embolisms (Ewers et al. 2000).

If the whole capacity of the root system to transport water is analyzed, we found that drought reduced K_{root} due to the reduction of root size in fertilized and unfertilized plants, but also by a reduction in the intrinsic capacity of each gram of root to transport water in fertilized plants. In this experiment, root was the first organ affected by drought. The reduction of water consumption as soil dries can play an important role in reducing water consumption to save water and to delay the beginning of severe stress (McDowell et al. 2008). The loss of root functionality increases the resistance to water movement, and this could be a strategy of these plants to reduce water consumption. As roots of urea- and NO_3^- -fertilized plants were more conductive when they grew under high water availability, they probably were able to take up more water when the soil began to dry. Possibly, this change could explain the advantage in growth of these two treatments relative to C and NH_4^+ -fertilized ones in the early stages of water stress.

Control and NO_3^- -fertilized plants did not modify tracheid lumens under drought. Only NH_4^+ -fertilized plants, which had higher K_{shoot} , k_s and K_{plant} under well watered conditions, reduced the conductivity of the shoot under drought (Figures 3a, c, 4a, c and 5b). These reductions were linked with a decrease in tracheid lumens in NH_4^+ -fertilized plants with low water availability (Table 2). A similar reduction occurred in the lumens of urea-fertilized plants under drought, but changes in stem hydraulic conductivity or conductance were not observed. The lack of direct correlation between lumen diameter and conductivity can be explained by cavitation of tracheids that presumably occurs in all the plants with low water availability. The fact that NH_4^+ fertilization produces changes that increase hydraulic conductivity (and consequently water consumption and growth) can explain why NH_4^+ -fertilized plants tended to be more affected by drought when compared with urea- and NO_3^- -fertilized plants (Figure 2, Table 1).

Conclusions

Depressive effect on growth of fertilized young *P. taeda* plants in red clay soil is observed with any N source because urea, NO_3^- and NH_4^+ fertilization increase NO_3^- concentration in soil solution. Increases of NO_3^- and NH_4^+ in soil solution modify growth, but each type of fertilization can elicit different responses.

Urea- and NO_3^- -fertilized plants with high water availability grow less than non-fertilized plants and develop responses that are similar to mechanisms of acclimation to drought. Among these changes, a reduction in root DW and shoot : root partitioning and an increase in the intrinsic capacity of roots to conduct water are highlighted. NO_3^- but not NH_4^+ appears to be linked to the negative effect of urea fertilization. Urea- and NO_3^- -fertilized plants were less affected by drought than non-fertilized plants. On the other hand, NH_4^+ produced changes in shoot hydraulic traits that enlarge the capacity of stems and shoots to transport water. These changes probably determined that NH_4^+ -fertilized plants were not so adversely affected in growth under high water availability as plants fertilized with urea and NO_3^- . Nevertheless, water stress reversed the effect of NH_4^+ fertilization on shoot hydraulic traits and NH_4^+ -fertilized plants were as affected by low water availability as non-fertilized plants. These results are specific for *P. taeda* plants, a fast growing temperate conifer, growing on lateritic red clay soils.

Supplementary data

Supplementary data for this article are available at [Tree Physiology](http://treephysiology.oxfordjournals.org/) Online.

Acknowledgments

We thank Nardia Bulfe (INTA EEA Montecarlo) for the selected pine seeds to perform the experiment and Lorenza Costa

(INFIVE) for helping in NH_4^+ and NO_3^- determinations. L.I.F. and A.P.M. have a CONICET fellowship. C.G. is CONICET (CCT La Plata) and Facultad de Ciencias Agrarias y Forestales—Universidad Nacional de La Plata researcher.

Conflict of interest

None declared.

Funding

This research was supported by Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CONICET) (PIP 112-200801-01885) and Proyecto de Incentivos 11/A242 (Facultad de Ciencias Agrarias y Forestales—Universidad Nacional de La Plata, Argentina).

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