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Foliar absorption and root translocation of nitrogen from different chemical forms in seedlings of two Mediterranean trees

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ABSTRACT

Along with root uptake, plants can also absorb N through leaves. There are few comparative studies on the foliar absorption of N from different chemical forms of N in forest tree species. We compared the foliar N absorption capacity in seedlings of two forest trees widespread in the Mediterranean basin, *Quercus ilex* and *Pinus halepensis*. Plants were sprayed with the following individual N forms at 40 mM N: ^{15}N-nitrate (NO_3^-), ^{15}N-ammonium (NH_4^+), ^{15}N-urea or ^{13}C and ^{15}N dual-labeled glycine. Cuticular conductance was used as a surrogate of cuticle permeability to water. *Q. ilex* had higher N foliar absorption than *P. hapelensis*. Neither cuticular conductance nor shoot surface area explained N differences in absorption rate between species, which were instead likely linked to differences in stomatal density and presence of trichomes. In both species, foliar N absorption rate and N recovery differed among N forms: urea > NH_4^+ ≥ glycine ≥ NO_3^-. Differences in N absorption rate among N forms were correlated with their physico-chemical properties. The strong positive relationship between ^{15}N and ^{13}C uptake together with detection in shoots of intact dual-labeled glycine (measured by gas chromatography–mass spectrometry), indicated that a significant fraction of glycine was absorbed intact by the seedlings. In both species, higher cuticular conductance was related to faster N absorption from all forms except NO_3^-. Cuticular conductance had a stronger effect on N absorption from urea and NH_4^+ than N absorption from glycine, and the effects were more intense in *Q. ilex* than in *P. halepensis*. This suggests that variations in cuticle permeability in both species are determined by different mechanisms and that each N form was differently affected. Absorbed N was rapidly translocated to roots, with a larger proportion of N from organic forms being translocated than N from inorganic forms. Foliar fertilization increased plant N content, especially in urea fertilized plants, but direct foliar absorption only explained up to 10% of N content increase. This study demonstrates that two important Mediterranean forest tree species can absorb through their leaves both, inorganic and organic N forms. This has important ecological and applied implications, because all chemical forms of N are present in natural N deposition. Also results show that foliar N fertilization can play an important role for seedling N nutrition, and that the effect will have different impacts depending on the species.

1. Introduction

Nitrogen (N) commonly limits productivity in natural and managed terrestrial ecosystems (LeBauer and Treseder, 2008). Nitrogen demand for plant growth can be met either by external sources or by remobilization of internal stores (Millard and Grelet, 2010). One external source is root uptake, while plants can also absorb N through leaves (Fageria et al., 2009; Burkhardt, 2010) and the foliar pathway can be significant in terms of plant nutrition and function (Rennenberg and Gessler, 1999; Sanz et al., 2002).

Abbreviations: g_c, cuticular conductance; AS, absorbing surface; $N_{recovery}$, N absorption efficiency; $N_{absorbed}$, N absorbed from a labeled N form; $C_{absorbed}$, C absorbed from a labeled N form.

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Foliar absorption of compounds is assumed to mainly occur via the cuticle (Peuke et al., 1998; Schreiber, 2005). This is because it is partially permeable to gases, water, and several water and oil soluble compounds, in spite of the main role of the plant cuticle being to prevent uncontrolled water loss from plants to the atmosphere (Baur et al., 1997; Santier and Chamel, 1998; Schreiber, 2005). Cuticle thickness and composition varies among species, while within plant species, properties of the cuticle may vary depending on growth conditions (Riederer and Schreiber, 2001). Variation in cuticle thickness and composition may explain differences among plants in the rate at which compounds are absorbed (Baur et al., 1997; Schreiber, 2005; Eichert and Goldbach, 2008). Leaf cuticular conductance (g_c) to water vapor is strongly determined by cuticle structure and chemical properties and, consequently, it can be a proxy of cuticle permeability (Niederl et al., 1998; Burghardt and Riederer, 2003; Schreiber, 2005). In addition to absorption via the cuticle, nutrients may also be absorbed through stomata (Schreiber, 2005; Eichert et al., 2008) and trichomes (Benzing et al., 1976).

In natural and crop systems, N reaches the leaf surface via dry and wet deposition (Rennenberg and Gessler, 1999; Sanz et al., 2002). Although organic N forms such as amino acids and urea make a smaller contribution to N deposition than inorganic N forms, they may still represent up to 25% of total N deposition (Cornell, 2011). Despite accounting for a substantial fraction of N deposition, few studies have examined the absorption of organic N deposition on plants (Cornell, 2011). Moreover man has dramatically increased N deposition in southern Europe (Fagerli and Aas, 2008; Cornell, 2011). Most N deposited in semi-arid regions, such as in Mediterranean-climate regions, enters as dry deposition, which is mainly concentrated during the dry season (Raison and Stottlemyer, 1991; Ochoa-Hueso et al., 2011). Rates of inorganic nitrogen deposition in common forest systems in the Mediterranean basin can be substantial with reports of up to 22 kg ha^{-1} yr^{-1} in *Quercus ilex* L. (holm oak) woodlands (Roda et al., 2002) and 38 kg ha^{-1} yr^{-1} in *Pinus halepensis* Mill. (Aleppo pine) woodlands (Michopoulos et al., 2004), with canopies retaining up to 70% of deposited N (Adriaenssens et al., 2010). In the California Mediterranean climate, Bytnerowicz and Fenn (1996) showed that NO_3^- is deposited on leaves at rates as high as 455 $\mu g\, m^{-2}\, h^{-1}$ and accumulates during prolonged dry periods (more than 120 days in some areas (Vicente-Serrano, 2006)). A consequence of accumulation during dry spells is that very small rain events (<0.3 mm) or dew deposition in early autumn can lead to highly concentrated N solutions on leaf surfaces (Ochoa-Hueso et al., 2011). Thus, we believe that use of high concentrations of different chemical forms of N in studies on foliar N uptake can be ecologically realistic especially for Mediterranean climates (Burkhardt, 2010; Ochoa-Hueso et al., 2011) and may be useful for disentangling the negative vs. positive effects of N deposition.

Nutrients can also reach the leaf surface in crop systems via foliar fertilization, which is a useful tool to supply nutrients to plants under different circumstances, for instance, root uptake and phloem transport cannot meet plant nutrient demands during high nutrient-demand periods (Dong et al., 2002; Bi and Scagel, 2008; Fernández et al., 2013). Foliar fertilization is a simple, economic and efficient way to supplement nutrients to crops because it allows nutrients to be directly incorporated to metabolism with less microbial competition and nutrient leaching than when fertilizer is applied to the soil (Fageria et al., 2009; Burkhardt, 2010). Unlike crop plants, foliar fertilization is rarely used in forest nurseries and plantations but it has potential for nutrient loading of seedlings without stimulating new growth or delaying dormancy during autumn (Bi and Scagel, 2008). Foliar fertilization can be an strategic solution for improving nutrition of seedlings planted in very poor soils or nutrient limited as per phosphorus and iron occurrence on limestone soils (Zohlen and Tyler, 2000), or under arid conditions (Ruiz Navarro, 2012).

Nitrogen nutrition plays an important role in the field performance of seedlings in forest plantations (Villar-Salvador et al., 2012). Urea, NH_4^+ and NO_3^- are typical N forms used for supplying N via foliar fertilization. Urea is widely used because it has lower toxicity than inorganic N forms and is highly soluble in both water and oil (Bondada et al., 2006; Stiegler et al., 2009). Most studies on foliar absorption of N have been made with inorganic forms or urea independently, which complicates comparisons among N forms (Bowman and Paul, 1992; Dong et al., 2002; Bondada et al., 2006). Although plants have the ability to absorb more complex organic compounds than urea, as amino acids (Baur et al., 1997; Inselsbacher et al., 2007), few studies have compared the foliar absorption of amino acids with other chemical forms of N (but see Eberhardt and Pritchett, 1971; Stiegler et al., 2009). Including a variety of chemical forms of N in studies may be important because previous studies have shown different forms of foliar-applied N differentially affect plant growth, development and metabolism, plant hormone relations and the uptake of other nutrients (Peuke et al., 1998). For instance, the foliar absorption of NH_4^+ increases phosphorous uptake, while NO_3^- foliar absorption enhances potassium uptake (Peuke et al., 1998). Similarly, absorption of intact amino acids can decrease plant N metabolic costs, enhancing growth (Maini, 2006). Thus, selecting appropriate chemical forms of N for foliar sprays is not only important for maximizing absorption efficiency or plant performance but also to minimize potential foliage damage (Fageria et al., 2009; Burkhardt, 2010).

The objective of our study was to compare short term foliar absorption and subsequent translocation to roots of N in four chemical forms (urea, glycine, NH_4^+, and NO_3^-) in two Mediterranean evergreen trees, *P. halepensis* and *Q. ilex* ssp. *ballota* (Desf) Samp (thereafter *Q. ilex*). Moreover, we assessed whether both tree species can take up intact glycine via leaves. Translocation to roots was used as an indicator of the ability to transport N absorbed by the leaves to other plant organs. The two species have contrasting ecological and morpho-physiological characteristics (Zavala et al., 2000) and are widely distributed in the Mediterranean basin and used for afforestation (Villar-Salvador et al., 2004a). *P. halepensis* is a fast growing pioneer tree (Zavala et al., 2000), which has lower stomatal conductance and density than *Q. ilex* and lacks trichomes (Boddi et al., 2002; Baquedano and Castillo, 2006). By contrast, *Q. ilex* is a slow growing late-successional species (Zavala et al., 2000), whose leaves have a dense layer of trichomes on the abaxial surface (Paoletti et al., 1998). We hypothesized that (1) *Q. ilex* will have higher rates of foliar N absorption than *P. halepensis*, due to its higher stomata density and presence of trichomes. (2) Each N form will have different uptake rates in accordance with their physicochemical properties, and be higher in urea due to its chemical properties e.g. higher solubility. (3) Proportions of N translocated to roots will be higher in the N forms and species with higher uptake rates.

2. Material and methods

2.1. Foliar treatments, experimental design and ^{15}N–^{13}C labeling

Ninety-six seedlings per species were cultivated outdoors at the nursery of the Centro Nacional de Mejora Genética Forestal "El Serranillo" (MAGRAMA) using seeds from inland Iberia Peninsula provenances. Seedlings were grown for 14 months in individual 305 mL pots (Super-Leach™, Bardi S.A.L., Navarra, Spain) filled with fertilized peat, N/P/K 14/16/18 + microelements (Kekkilä WHITE 420 F6, Kekkilä Oy, Finland) following standard cultivation protocols in Mediterranean nurseries (Villar-Salvador et al., 2004a). The