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Evidence of short-term belowground transfer of nitrogen from *Acacia mangium* to *Eucalyptus grandis* trees in a tropical planted forest

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A B S T R A C T

The short-term belowground transfer of nitrogen from nitrogen-fixing trees to companion trees has never been studied in the field. A ^{15}N pulse-labeling study was conducted in a mixed plantation of *Acacia mangium* and *Eucalyptus grandis* at the peak of leaf area, 26 months after planting. $^{15}N{-}NO_3^-$ was injected into the stem of one big *Acacia* tree in three plots. ^{15}N was traced over 2 months in the labeled *Acacia* tree as well as in neighboring *Eucalyptus* trees. For both species, young leaves were sampled, as well as fine roots and the rhizosphere at a distance of 0.75 m and 2.25 m from the labeled tree. The ^{15}N atom% was also determined in the wood, bark, branches and total foliage of the 3 labeled *Acacia* trees and 9 *Eucalyptus* trees, 60 days after labeling. Most of the leaves, fine roots and rhizosphere samples of both species were ^{15}N enriched from 5 days after labeling. The $\delta^{15}N$ values were higher at a distance of 0.75 m than at 2.25 m in *Acacia* roots, but were similar at both distances in *Eucalyptus* roots and the rhizospheres. The wood and bark of *Eucalyptus* trees sampled at a distance of 6.2 m from the labeled *Acacia* trees were ^{15}N enriched. This shows belowground N transfer from *Acacia* to *Eucalyptus* trees in the field during the first few days after labeling. This facilitation process may provide a significant amount of the nitrogen requirements of trees close to N-fixing trees in mixed forests.

1. Introduction

Recent studies show that associating nitrogen-fixing trees (NFT) and non-nitrogen-fixing species (non-NFS) can be beneficial for biomass production (Piotto, 2008; Forrester, 2014), soil carbon sequestration (Forrester et al., 2013; Blaser et al., 2014), soil fauna and microbial diversity (Bini et al., 2013; Manhães et al., 2013; Rachid et al., 2013) and soil nutrient availability (Voigtlaender et al., 2012; Blaser et al., 2014; Koutika et al., 2014). In tropical forest ecosystems, up to 90% of the nitrogen (N) in NFTs is derived from symbiotic fixation (Parrotta et al., 1996; Binkley and Giardina, 1997; Nygren et al., 2012). This N input is likely to improve the N status of associated non-NFS in forests established on degraded land (Nichols and Carpenter, 2006), agroforestry systems (Daudin and Sierra, 2008), mixed forest plantations (Binkley et al., 2003; Richards et al., 2010), and tropical forests (Batterman et al., 2013).

Decomposition of aboveground litter, pruning residues and roots is commonly considered to be the main N pathway from NFTs to non-NFS (Mafongoya et al., 1998; Munroe and Isaac, 2014). However, some studies have shown that direct belowground transfer of N without transformation of the N source (Munroe and Isaac, 2014) can also provide substantial amounts of N for non-NFS. Low-molecular N weight compounds, such as ammonium, nitrate and amino-acids (Wacquant et al., 1989; Paynel et al., 2001; Paynel and Cliquet, 2003), exuded by legume roots may be taken up by the

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companion plants (Jalonen et al., 2009; Fustec et al., 2010; Munroe and Isaac, 2014) provided that the plant uptake occurs before these compounds are taken up or mineralized by soil microorganisms, a process that is very fast (Cliquet et al., 1997; Lipson and Näsholm, 2001; Jones et al., 2004). Direct transfer of N can also occur via common mycorrhizal networks (CMN) (He et al., 2004, 2009; Kähkölä et al., 2012; Fellbaum et al., 2014).

Short-term (i.e within a few days or weeks) belowground transfer of N has been observed from NFTs to grasses and agricultural crops. The percentage of N of the grass *Dichantium aristatum* derived from transfer (%NDFT) from N-fixing *Gliricidia sepium* seedlings ranged from 2 to 15% through root exudates, and from 0.5 to 2% via CMNs over 8 weeks in a greenhouse experiment (Jalonen et al., 2009). Sierra and Daudin (2010) estimated that, in the field, 45–80% of the N content in *D. aristatum* was derived from belowground transfer from *G. sepium* trees planted 1–5 m apart, 84 days after cutting the grass. After 21 days of contact between the root systems of *Triticum durum* seedlings and 2-month-old *Acacia senegal* seedlings growing in pots, the %NDFT of the wheat reached 14% (Isaac et al., 2012). In a greenhouse experiment, Catchpoole and Blair (1990) estimated that 4% and 8% of the amount of labeled ^{15}N in *Leucaena leucocephala* seedlings was transferred to *Panicum maximum* grass in 6 and 12 weeks, respectively. In a pot study carried out over 4 weeks, Rao and Giller (1993) estimated that 3–4% of the amount of N in 3-month-old *Leucaena diversifolia* seedlings was transferred to *Cenchrus ciliaris* grass.

Although short-term belowground N transfer from NFTs to grass is now well documented, so far as we are aware (e.g. review from Chalk et al., 2014), such a fast transfer has never been confirmed between NFTs and non-NFTs in the field. The significance of this facilitation process in mixed species forests is still under discussion owing to the lack of experimental data (Chalk et al., 2014). In a 1-month pot experiment, the percentage of N in 6-month-old *Eucalyptus maculata* seedlings that was derived from *Casuarina cunninghamia* plants via CMNs ranged from 1 to 9% depending on the nodulation status (He et al., 2004). This percentage varied from 4 to 30% in 12-month-old seedlings (He et al., 2005). Field studies suggest that N transfer could occur belowground from *Inga edulis* to *Theobroma cacao* (Nygren and Leblanc, 2009, 2015) and from *Acacia* sp. to *Eucalyptus* sp. (Hoogmoed et al., 2014) but the amount and the speed of the belowground N transfer were not estimated.

This study set out to assess whether there was short-term N transfer between NFTs and non-NFTs in a mixed plantation of *Eucalyptus grandis* Hill ex Maid. and *Acacia mangium* Wild. Both *E. grandis* and *A. mangium* are widely planted in tropical regions (FAO, 2010), and mixed plantations of *Eucalyptus* and *Acacia* might be an alternative to *Eucalyptus* monoculture (Forrester et al., 2013). A fast response of *Eucalyptus* trees to N fertilization has been observed in southeast Brazil (Gonçalves et al., 2013). Association with *A. mangium* may create facilitation processes involving atmospheric N_2 fixation and N transfer to *Eucalyptus* trees (Bouillet et al., 2013). This experiment was designed to assess the possible short-term belowground N-transfer from *Acacia* to *Eucalyptus* neighbors up to a distance of 6.2 m from ^{15}N labeled *Acacia* trees. $^{15}N{-}NO_3^-$ was injected in the stem of one big *Acacia* tree in three plots. The $\delta^{15}N$ values of recently-expanded leaves, fine roots and the rhizosphere (i.e soil adhering to the root, Hinsinger et al., 2003) of the two species were measured from March 5th to April 30th, 2012, and trees were destructively sampled 2 months after labeling. This study tested the hypotheses that (1) short-term belowground N transfer occurs between *Acacia* and *Eucalyptus*, which would be consistent, with the high densities of fine roots of both species and their intermingling in mixed plantations (Laclau et al., 2013a), and (2) N-transfer is not restricted to *Eucalyptus* trees located very close to *Acacia* trees as *Eucalyptus* trees are able to develop extensive root systems rapidly (O'Grady et al., 2005; Laclau et al., 2013b), to access resource-rich soil patches several meters from the trees (Bouillet et al., 2002; Sudmeyer and Simons, 2008; Silva et al., 2011).

2. Material and methods

2.1. Site description

The study was carried out at the Itatinga experimental station of São Paulo University, Brazil (23°02′S, 48°38′W), at an elevation of 860 m asl. The soils were Ferralsols (FAO classification). The texture was very uniform below a depth of 1 m with clay content around 13% in the A1 layer and ranging from 20% to 25% between 1 m and 6 m in depth. The 0–10 cm soil layer had a cation exchange capacity (CEC) < 2 cmolc kg^{-1} soil, with a mean total N concentration of 0.6 g kg^{-1} soil (Voigtlaender et al., 2012). The soils were typical of large areas planted with *Eucalyptus* in Brazil (Gonçalves et al., 2013). During the study period, the average air temperature was 20.4 °C and the cumulative rainfall, collected in an open area 100 m from the field trial, was 209.9 mm (Fig. 1). The soil water content was monitored in the first block of the field trial. The soil water content at 15 cm was measured every half hour using 3 Campbell CS616 probes, and the values were then averaged over the day.

2.2. Site layout

A complete randomized block design with 7 treatments and 4 blocks was set up in May 2003 to compare monospecific and mixed species stands of *A. mangium* and *E. grandis*. A detailed description of the original experimental layout can be found in Laclau et al. (2008). This study was carried out in 3 plots (1 plot per block of the original experiment) where the two species were planted alternately at 1.5 m spacing in the row, with 3 m between rows, giving a total stocking density of 2222 trees ha^{-1}. Only the boles were harvested in May 2009 and the residues were spread uniformly within each plot. *Eucalyptus* stem wood biomass at harvest time was typical of productive commercial plantations (Bouillet et al., 2013). *A. mangium* and *E. grandis* seedlings were re-planted

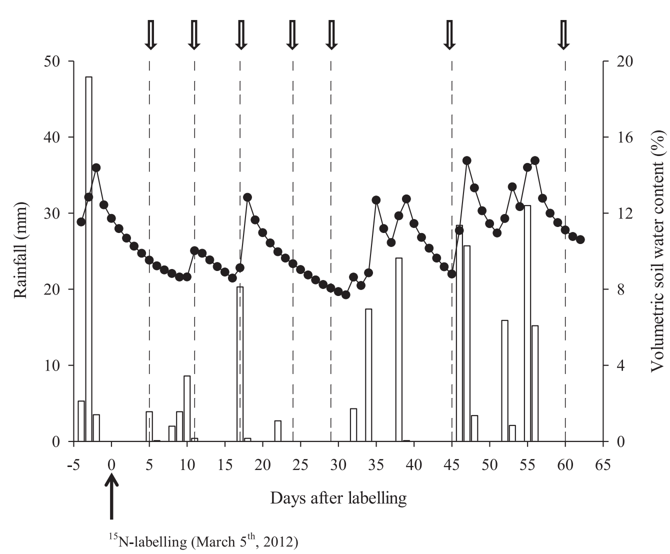

Fig. 1. Rainfall (vertical bars) and volumetric soil water content (black circles) at a depth of 15 cm over the 60 day study period. Sampling dates are indicated by arrows and dotted lines. Five days after *Acacia* labeling, there was rainfall after samples had been collected.