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Nutrient cycling over five years of mixed-species plantations of *Eucalyptus* and *Acacia* on a sandy tropical soil

Felipe Martini Santos [a,*], Guilherme Montandon Chaer [b], Anderson Ribeiro Diniz [a], Fabiano de Carvalho Balieiro [c,*]

[a] Departamento de Silvicultura, Instituto de Florestas, Universidade Federal Rural do Rio de Janeiro, Rodovia BR 465 – km 7, Seropédica, RJ 23891-000, Brazil
[b] Embrapa Agrobiologia, Rodovia BR 465 – km 7, Seropédica, RJ 23891-000, Brazil
[c] Embrapa Solos, Rua Jardim Botânico, 1024, Jardim Botânico, Rio de Janeiro, RJ 22460-000, Brazil

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ABSTRACT

Mixed-species plantations have the potential to enhance nutrient cycling and increase the overall biomass of the stand. In this study, we evaluate the dynamics of nutrient accumulation and exports in mixed-species plantations of *Eucalyptus urograndis* (*Eucalyptus urophylla* S. T. Blake × *Eucalyptus grandis* W. Hill ex Maiden) and *Acacia mangium* Willd. after five years of rotation. Monocultures of *Eucalyptus urograndis*, with or without nitrogen fertilization (120 kg N ha^{-1}) (E100 and E100 + N, respectively), and of *Acacia mangium* (A100) were established in a randomized block experimental design. Two arrangements with these species in mixed stands were also established: one with 50% of the stand density composed by each species (E50A50; 1,111 trees ha^{-1}), and one high-density system, containing double the population of each species (E100A100; 2,222 trees ha^{-1}). Aboveground biomass and litter nutrient contents, nutrient retranslocation from leaves, and nutrient export through wood harvesting were measured over a full rotation. *Eucalyptus* in E50A50, despite having half of the population of trees (555 trees ha^{-1}), accumulated the same amount of nutrients in total aboveground biomass in relation to E100, suggesting a lower nutrient limitation to the growth of *Eucalyptus* in this mixed stand. Conversely, *Eucalyptus* in E100A100 did not accumulate proportionally larger amounts of nutrients in relation to E50A50, possibly due to intra- and interspecific competition in this high-density arrangement. The deposition of N and K via litter was higher in mixed-species stands than in *Eucalyptus* monocultures. Also, P, Ca and Mg depositions were higher in mixed-species stands than *Acacia* monocultures. These results suggest higher nutrient cycling and availability in the mixtures, especially after 30 months. The retranslocation of N in *Eucalyptus* and *Acacia* leaves decreased with age. At 60 months, *Eucalyptus* trees in E100A100 retranslocated less N than the trees in E100, probably reflecting the higher availability of soil N in relation to E100. At this same age, the *Eucalyptus* trees planted in E50A50 exported less N, P, K and Mg in relation to E100. However, at stand level, mixtures exported more N due to the enrichment of this nutrient in the soil promoted by *Acacia*. This study shows the importance of introducing *Acacia* in *Eucalyptus* plantations to promote a positive balance of nutrients for subsequent rotations and additional ecological benefits to the ecosystem due to N_2-fixation from *Acacia* trees.

1. Introduction

Mixed-species plantations of *Eucalyptus* with nitrogen-fixing legume tree species have been studied as an alternative to *Eucalyptus* spp. monoculture as a way to counterbalance the N deficit in the system caused by wood harvesting (Bouillet et al., 2013; Laclau et al., 2010; Voigtlaender et al., 2012). These plantations also have the potential to accelerate the nutrient cycling (Balieiro et al., 2004; Binkley et al., 1992; Forrester et al., 2005a) and increase the overall plantation biomass production (Bouillet et al., 2013; Santos et al., 2016).

The predominance of facilitation and competitive reduction over intra- and interspecific competition for uptake and use of resources determines the success of mixed-species plantations (Forrester et al., 2005b, 2006; Vandermeer, 1989). Hence, the choice of the site and species, so that these interactions promote improvement in the acquisition of resources (*i.e.*, water, light and nutrients) between the species, are key points for optimal

* Corresponding authors. Tel.: (+55) 21 2179 4545 (F.C. Balieiro).
E-mail addresses: martinisantos@gmail.com (F.M. Santos), fabiano.balieiro@embrapa.br (F.C. Balieiro).

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performance of mixed plantations (*i.e.*, greater overall biomass production) (Kelty, 2006; Richards et al., 2010).

Forrester (2014) showed that facilitation and competitive reduction interactions between tree species are more evident in sites with low initial availability of nutrients, provided that the interactions between species lead to an increase in the availability, acquisition and efficiency of using these resources. However, whether this pattern will change or not it will depends on how the resource availability and the species interactions change through the time. For example, if N availability declines with time, then N fixation should increase complementarity for the non-N-fixing species, assuming that no other resources are limiting the growth. This was demonstrated by a meta-analysis of 13 studies of mixed plantations of tree species. In this context, there are few studies into the dynamics of nutrient accumulation over a complete rotation of mixed stands established on tropical soils.

Many studies have investigated the use of *Acacia mangium* Willd. in mixed-species plantations with *Eucalyptus* spp. (Bouillet et al., 2013; Laclau et al., 2008; Santos et al., 2016). This species belongs to the family Fabaceae, native to Southeast Asia (Indonesia and Papua Nova Guinea) and northeastern Australia, so it is adapted to hot and moist climates (Krisnawati et al., 2011). Several reasons can be mentioned for the strong potential of this species for use in mixed stands with *Eucalyptus*: (i) tolerance for soils with low pH and fertility; (ii) rapid growth and high production of N-rich litter; (iii) ability to associate with diazotrophic bacteria to fix N_2; and (iv) wide diversity of uses as timber products (*e.g.*, pulp, furniture, firewood and charcoal) and for other purposes (*e.g.*, extraction of tannins from the bark, substrate for production of shiitake mushrooms and honey production) (Chaw and Mithlöner, 2011; Krisnawati et al., 2011). These products have wide acceptance in the global market, mainly in Asia where commercial planting of species of the genus *Acacia* is widespread (Harwood et al., 2015; Nambiar et al., 2015).

In tropical regions, such as much of Brazil, positive responses have been observed from mixed-species plantations of *Eucalyptus* spp. and *A. mangium* (and other leguminous tree species) in sites with low soil fertility, in particular increased wood yield due to the greater availability of N in the system, promoted by biological N_2 fixation (BNF) (Balieiro et al., 2008a; Bouillet et al., 2013; Santos et al., 2016). However, the increased supply of N can lead to significant alterations in the dynamics of C and nutrients in the system. In Hawaii, for example, Kaye et al. (2000) observed after 17 years of mixed planting of *Eucalyptus* and *Albizia*: an antagonistic effect for the P and N available in the soil (higher demand for P due to greater supply of N); and a synergistic effect for C accumulation and uptake of P in the mixed stands in relation to the monoculture areas. Other studies have also found higher demand of *Eucalyptus* for P from the soil in sites with greater N availability, relating this to the need to maintain the N:P stoichiometry of *Eucalyptus* leaves (Koutika et al., 2014). Further, according to these authors, the limitation of P in these mixed stands might be causing a negative feedback from BNF. This effect may be more pronounced in *Acacia* monocultures (Koutika et al., 2016).

The ability of plants to obtain nutrients from the soil and use them to produce biomass comes from various intrinsic traits of each species and the interaction of these with edaphic and stand factors, which indirectly influence important physiological alterations and the C partitioning (Fife et al., 2008; Hawkins and Polglase, 2000; Poorter et al., 2012). The practice of maintaining forest residues in the soil during harvesting was a significant advance to improve soil quality of planted forests in tropical soils (Chaer and Tótola, 2007; Mendham et al., 2003). Besides this, the exportation of nutrients from these areas through this practice is determined by the quantity of wood exported from the site and the concentration of nutrients in these tissues (Gonçalves et al., 2013). The biological utilization coefficient (BUC) is a tool to quantify this interplay of factors, allowing conclusions regarding the impact of harvesting on the exportation of nutrients, since it reflects the relationship between stemwood biomass production and the content of a determined nutrient stored in this compartment (Barros et al., 1986). Therefore, the BUC can be employed to select more promising genetic material and to reduce the cost of fertilization in some areas (Safou-Matondo et al., 2005; Santana et al., 2000). In this respect, evaluating the changes in the uptake and allocation of nutrients from *Eucalyptus* in mixed stands can complement the choice of the best arrangement to be recommended for a determined region.

We put forward the following hypotheses: (i) Mixed-species plantations containing N_2-fixing species accelerate the biogeochemical cycling changing the patterns of nutrient retranslocation. (ii) Furthermore, plants in mixed-stands accumulate more nutrients in the aboveground compartments and export larger amounts of them through wood harvesting. In this context, we conducted an experiment with mixed-species plantations of *Eucalyptus urograndis* (*Eucalyptus urophylla* S. T. Blake × *Eucalyptus grandis* W. Hill ex Maiden) and *A. mangium* in a site with sandy N-deficient soil, established under climate conditions favorable to the growth of *A. mangium*. We aimed to assess the dynamics of deposition (via leaf litter), retranslocation and accumulation (in the aboveground fractions) of nutrients during five years of rotation. In addition, we evaluate the nutrient export through wood harvesting of these mixed-species plantations.

2. Material and methods

2.1. Characteristics of the site and experimental design

The experiment was conducted in the experimental field of Embrapa Agrobiologia, located in Seropédica, Rio de Janeiro state, Brazil, in an area with gentle relief (<5% slope), which had been fallow for more than 15 years. The average rainfall in the region during the study period was 1370 mm, with mean monthly temperatures ranging from 16 °C (June–August – dry season) to 36 °C (January–March – rainy season, with occasional dry spells) and yearly average of about 24 °C. The relative air humidity recorded in the period was 81%. The soil is classified as a Haplic Planosol (according to the Brazilian Soil Taxonomy) or Planosol (according to World Reference Base/FAO), with a highly sandy surface horizon (~90% sand). The soils (0–0.20 m layer) of the experimental blocks contained low N (0.03%), C (0.32%) and P (7.0 mg kg^{-1}) concentrations with pH values around 5.0. A complete characterization of our site can be found in Santos et al. (2016).

2.2. Experimental design and treatments

The experimental area was divided into eight blocks measuring 18 m × 105 m, each containing five plots of 18 m × 21 m for allocation of five planting combinations of *Eucalyptus urograndis* and *Acacia mangium* (hereafter called "*Eucalyptus*" and "*Acacia*", respectively – Table 1). The areas occupied by the 16 central plants (32 plants in the E100A100 plots) were considered as the effective plots.

The spontaneous vegetation was mowed and then killed with glyphosate. The *Eucalyptus* seedlings were clonal, supplied by Suzano Papel e Celulose S.A. The *Acacia* seedlings were produced from seeds collected from parent trees growing at the same experimental field. The exact provenance of the parent trees is unknown (the seeds were donated to Embrapa Agrobiologia in a partnership with some research centers like CSIRO (Australia) and NifTAL