INTER-CROPPED PERENNIAL LEGUMES IN COMMERCIAL *Eucalyptus* spp. PLANTATIONS ENHANCE SOIL QUALITY

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Abstract

Area planted with *Eucalyptus* spp. in Uruguay has largely increased in the last fifteen years, mainly through afforestation of grasslands. Such changes in land use are known to affect soil properties, including increased soil acidity and exchangeable aluminum, decreased organic carbon content and water retention capacity, as well as changes in soil enzyme activities. The low mineralization rate of tree litter due to its high C:N ratio is suggested to be in the base of changes in soil properties. Our assumption was that the 2-year period between *Eucalyptus grandis* plantation and tree canopy closure allows the commercial forest to benefit from the inclusion of an inter-cropped perennial legume. The aim of this research was to test if inter-cropping an adapted (native) legume can supply nitrogen from the atmosphere to the system, contributing to soil conservation through enhanced soil microbial activity and nutrient cycling, while improving tree growth. A field experiment was carried out in a commercial forest area to assess the effects of perennial *Desmanthus* species (*virgatus*, *illinoensis* and *bicornutus*) intercropped between tree rows on soil microbial biomass and soil enzyme activities (soil quality indicators); legume shoot dry matter and total nitrogen content; and tree diameter at breast height. Enhanced soil microbial biomass, soil respiration and hydrogenase activities were associated to legume inclusion. Both *D. illinoensis* and D. *virgatus* contributed comparable amounts of nitrogen to the system when inoculated with effective rhizobia. Tree diameter 12 months after planting was 6.5 cm in average when *Desmanthus* spp. were intercropped and 5.6 cm when trees grew without the legume. The results indicate a direct and positive effect of intercropped legumes in *Eucalyptus* plantations on soil quality and early tree growth. An increase in soil microbial activities may lead to enhanced nutrient biogeochemical cycles, increased soil fertility and improved agroecosystem sustainability.

Keywords: Nitrogen fixation, soil quality, agroforestry, *Desmanthus*

Introduction

Area planted with *Eucalyptus* spp. in Uruguay has strikingly increased in the last fifteen years, as a result of a governmental policy aligned with a worldwide trend aimed to increase tree planted areas in developing countries. Soil properties are known to be affected by conversion from pastures to commercial tree plantations (1) (2) (3). Such patterns were confirmed when grasslands are converted to *Eucalyptus* spp. plantation in Uruguay resulting in increase in soil acidity and exchangeable aluminum, decrease in organic carbon content and water retention capacity, as well as changes in soil enzyme activities (4) (5) (6). The inclusion of legumes in the system (e.g. intercropped between tree rows) can be a suitable alternative to address this problem since legumes can supply nitrogen to the system – which they derive from the atmosphere - and their inclusion is expected to benefit soil microbial activity and enhance nutrient cycling, while improving trees growth (7) (8).

The genus *Desmanthus* Willd. belongs to the *Dichrostachys* group of the tribe Mimoseae in the *Leguminosoideae-Fabaceae* family. This relatively small genus of 24 species -mostly perennial herbs native to the New World from Uruguay and Argentina to the northern USA - shows its greatest diversity in Mexico and southern Texas (9). *Desmanthus depressus*, *D. virgatus* y *D. tatuhyensis* were reported as naturally occurring in Uruguay (10). Several species from this genus have been studied and eventually developed as forage crops. No bloat was caused by *Desmanthus* species and plants showed satisfactory tolerance to different grazing intensities (11). Although little studied to date in terms of their ability to fix atmospheric nitrogen, *Desmanthus* species seem to be somewhat promiscuous in their ability to nodulate but more specific regarding the strains with which they actually fix nitrogen. It has been emphasized the importance of routinely include inoculation with effective rhizobia as a component of planting practices in both *D. virgatus* and *D. illinoensis.*

Our assumption was that the 2-year period between tree plantation and canopy closure allows the commercial forest to benefit from the inclusion of an inter-cropped perennial legume. We tested if an adapted and effective symbiosis *Desmanthus* spp.-*Rhizobium* spp. can supply nitrogen from the atmosphere to the system, contributing to soil conservation through enhanced soil microbial activity and nutrient cycling, while improving tree growth. Thus, diversifying land use, increasing productivity, and ultimately leading to an improved overall sustainability.

Materials and Methods

Experimental area and design

The study was carried out in a commercial *Eucalyptus grandis* plantation property of Forestal Oriental S.A. (FOSA), Paysandú, Uruguay (32°23′17″S and 57°36′47″W). Soil was a sandy clay loam Argiudoll with pH 5.5, 51 g kg^{-1} organic matter, and 17 mg kg^{-1} of phosphorus content (Bray 1). The area had previously been a grazed natural pasture. The experimental design was a randomized complete blocks with treatments in a split plot arrangement with four replicates. Whole-plots treatments were 6-m long rows where rhizobial strains plus an uninoculated control were applied, and the split-plot treatments were 2-m long rows in which the *Desmanthus* species were planted. Three *Desmanthus* species were tested, *D. bicornutus* “Bee Wild” (from Texas A & M. Agric. Research Station, Beeville, Texas, USA), *D. illinoensis* PNL 534 (from the Perennial native legume collection, Univ. of Minnesota, USA) and the native to Corrientes (Argentina) *D. virgatus* ‘Marc’ (CPI 78373). The inoculation treatments included native strains previously confirmed as effective on theses hosts under greenhouse conditions; D3 isolated from nodules of *D. illinoensis*, 314 and 404 isolated from *D. depressus* (12) and the reference strain UMR6029 (*Desmanthus illinoensis,* Rhizobium Research Laboratory, Univ. of Minnesota) and D16 (isolated from a USA commercial *Desmanthus* spp. inoculant). The phylogeny of strains was analyzed in a simultaneous project; species identities proven to be *Mesorhizobium amorphae* for strains 314 and 404, and *Rhizobium giardinii* for D16 (13).

Seed inoculation and seeding

Rhizobial inoculants were sterile peat-based preparations with a cell concentration of 1.5 - 2.4 X 10^9 colony forming units (CFU) per g (14). Seed was mechanically scarified and separately inoculated with each strain using 5 g of inoculant kg^{-1} seed with a commercial sticker following manufacturer´s recommendations. After *Eucalyptus* trees were planted in 3-m apart rows following the company’s customary practices, inoculated *Desmanthus* spp. seeds were hand planted (25 seed m^{-1}) at 1 cm depth between and parallel to *Eucalyptus* rows. Tree heights ranged 0.3 – 0.4 m. at that time.

Determinations

Desmanthus shoot dry weight

Desmanthus spp. growth and shoot total N (Kjeldahl) was evaluated 120 days after seeding from 0.5 linear m of row within each small plot avoiding border effects, dried at 60º C for 72 h and weighed.

Soil respiration and microbial biomass-C

Composite soil samples (0 -15 cm) were taken at the field experiment 12 months after seeding, from middle distance between tree rows. Soil sampling targeted separately both situations: where trees had grown intercropped with *Desmanthus,* and where trees had grown without *Desmanthus*. Soil respiration was determined by the flux of CO_2-C in samples incubated at 28°C and water content corresponding to 70% of soil water holding capacity during 10 days, as described previously (15). Microbial C-biomass was taken as the difference in the fluxes of CO_2-C released from soil samples fumigated with chloroform versus untreated samples. Determinations were made volumetrically using a previously described fumigation-incubation technique (16).

Enzyme activities

The soil samples previously described were also subject to dehydrogenase activities determination by the method of (17) and (18), with iodo nitro-tretazolium (INT) as an artificial electron acceptor. Acid and alkaline phosphatase activities were analyzed using p-nitrophenol benzene as substrate, and production of the p-nitrophenol product was assayed colorimetrically at 650nm. Fluorescein diacetate (FDA) hydrolysis was assessed using the method of (19), in which fluorescein liberated was assayed colorimetrically at 490nm, after 1h of soil incubation.

***Eucalyptus grandis* growth**

Eighteen months after the field experiment was installed, *Eucalyptus* diameter at breast height (d.b.h.) was measured. Trees grown intercropped with *Desmanthus* were paired with trees growing alone according to topographic location, then d.b.h. was determined.

Data analysis

Data were subjected to variance analysis, LSD test (P<0.05) and *t* test using InfoStat statistical package (2008).

Results and discussion

***Desmanthus* shoot dry weight**

Desmanthus spp. agronomic performance was assessed in terms of shoot dry weight (SDW) determined 120 days after seeding. Mean SDW across inoculation treatments from 0.5 m of row and the corresponding estimates per hectare are presented in Table 1, along with N content in the legume biomass and kg of N per hectare. It is important to mention that both *D. illinoensis* and *D. bicornutus* were nodulated by naturally occurring rhizobia, while *D. bicornutus* was not. Our results indicate a significant organic matter and total N input from the legume, supporting the contribution of *Desmanthus* spp to soil conservation. Seed production, a key component of new legume forage, was also determined in our field experiment. *Desmanthus* spp. seed yields (14 %RH) were 278 kg ha^{-1} (*D. bicornutus*), 450 kg ha^{-1} (*D. illinoensis*) and 545 kg ha^{-1} (*D. virgatus*). These numbers allow to suppose that seed production will not be a limitation in *Desmanthus* spp. domestication and introduction in agroforestry in Uruguay. Our results are in accordance with (20) who also found *Desmanthus* spp. adapted to grow under *Eucalyptus* canopy.

Table 1. Shoot dry weight (SDW), N concentration and total N in *Desmanthus* spp. harvested 120 days after planting from 0.5 m row. Values are means across inoculation treatments (n=20), same letters within columns indicate no significant differences (LSD p<0.05). Paysandú, Uruguay.

Species	SDW (g)	SDW (kg ha^{-1})	N conc. (mg g^{-1})	Plant total N (mg)	Total N (kg ha^{-1})
D. bicornutus	17.7b	704	19.1a	337b	13.5
D. illinoensis	56.1a	2244	22.7a	1273a	50.9
D. virgatus	51.5a	2044	23.6a	1215a	48.6

Soil respiration, microbial biomass-C, and enzyme activities

Soil microbial biomass-C is one of the most currently analyzed parameters in studies on biological soil characterization, because it is both a source of labile nutrients and an agent in transformation of organic matter and plant nutrients in the soil. Our results show that soil from where trees had grown intercropped with *Desmanthus* had significantly higher microbial biomass-C, soil respiration, and dehydrogenase activity when compared to where trees had grown alone (Table 2). We conclude that specific soil biological properties can be sensitive indicators of soil transformations occurring under different land uses.

Table 2 Soil biological properties in the commercial *Eucalyptu*s plantation at Piedras Coloradas, Paysandú, Uruguay. Soil samples were taken from under *E. grandis* + *Desmanthus* spp. and under *E. grandis* without *Desmanthus* spp.

Variable	***E. grandis + Desmanthus***	***E. grandis -Desmanthus***
Microbial biomass C[1]	62.3 a	39.4 b
Soil respiration[1]	20.4 a	12.8 b
Acid phosphatase[2]	38.8 a	36.1 a
Alkaline phosphatase[2]	30.2 a	35.8 a
FDA hydrolysis[3]	164.0 a	140.0 a
Dehydrogenase[4]	209.2 a	121.6 b

[1]mg $C0_2$-C kg^{-1} dry soil; [2] µg p-nitrophenol g^{-1} dry soil h^{-1}; [3]µg fluorescein g^{-1} dry soil h^{-1}; [6] µg INTF g^{-1} dry soil h^{-1}. Different letters between columns denote significant differences among means ($p<= 0.05$)

***Eucalyptus* trees growth**

Measurements of d.b.h. of trees intercropped with *Desmanthus* versus without *Desmanthus*, attain 6.4 and 5.2 cm respectively and they were significantly different (n=11, *t* test, $p<0.06$).

The results allow to conclude that *Desmanthus illinoensis* and *D. virgatus* genotypes evaluated are promising for intercropping in *E. grandis* plantations at Paysandú, one of the important forestry areas in Uruguay. The next step will be to determine the proportion of nitrogen from the atmosphere that the legume contributes to *Eucalyptus.* Forest plantations present low soil mineralization rate due to the tree litters high C/N ratio. The improved soil properties found with the inclusion of *Desmanthus* spp., intercropped between tree rows may be due to legume atmospheric N supply to the system and therefore enhancing nutrient cycling.

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