



Nodules biomass density in mixed and monospecific stands of *Eucalyptus grandis* x *Eucalyptus urophylla* and *Acacia mearnsii*¹

Márcio Viera²; Mauro Valdir Schumacher³; Edenilson Liberalesso⁴

Abstract: We aimed to evaluate the distribution of the nodules biomass density in *Acacia mearnsii* at monospecific and mixed planting with *Eucalyptus grandis* x *E. urophylla* in southern region of Brazil. The nodules biomass quantification was performed at the stand 18th month of age. The collecting was made using a cylindrical steel extractor tube with 7.0 cm of inside diameter in four depths: 0 – 5; 5 – 10; 10 – 20 and 20 – 30 cm. Due to the great variability, the nodules biomass, although being much higher in the monospecific planting of *Acacia mearnsii* when comparing to mixed planting, did not show significant differences ($p > 0.05$). The greater nodules density was found in the layers 5 to 10 cm of depth, near to the tree trunk and at the planting line followed by the planting diagonal and row. The nodules biomass density found for the black-wattle (up to 2.4 g dm^{-3}) demonstrates the importance of the use of this atmospheric nitrogen-fixing species in mixed plantings with eucalyptus.

Keywords: N-fixation; *Rhizobium*; Symbiotic association; Mixed stands.

Densidade de biomassa de nódulos em plantios mono específicos e mistos de *Eucalyptus grandis* x *Eucalyptus urophylla* e *Acacia mearnsii*

Resumo: Objetivou-se avaliar a densidade da biomassa de nódulos na *Acacia mearnsii* em plantio mono específico e misto com *Eucalyptus grandis* x *E. urophylla* no sul do Brasil. A quantificação da biomassa de nódulos foi realizada aos 18 meses de idade dos povoamentos. A amostragem foi realizada com tubo extrator de formato cilíndrico com 7,0 cm de diâmetro em quatro profundidades: 0 – 5; 5 – 10; 10 – 20 e 20 – 30 cm. Devido à grande variabilidade, a biomassa de nódulos, apesar de ser muito superior no plantio mono específico de *Acacia mearnsii* em comparação com o plantio misto, não apresentou diferenças significativas ($p > 0,05$). A maior densidade de nódulos encontra-se na camada de 5 a 10 cm de profundidade, nas proximidades do tronco da árvore e na linha de plantio, seguidas pela diagonal e entrelinha de plantio. A densidade de biomassa de nódulos encontrada na acácia-negra (até $2,4 \text{ g dm}^{-3}$) demonstra a importância da utilização dessa espécie fixadora de nitrogênio atmosférico em plantios mistos com eucaliptos.

Palavras - chave: Fixação de N; *Rhizobium*; associação simbiótica; povoamentos mistos.

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² Engenheiro Florestal, Dr., Professor Adjunto I do Departamento Multidisciplinar da Universidade Federal de Santa Maria - Campus Silveira Martins. E-mail: <vieraflorestal@yahoo.com.br>.

³ Engenheiro Florestal, Dr. nat. techn., Professor Associado II do Departamento de Ciências Florestais, Universidade Federal de Santa Maria. E-mail: <mvschumacher@gmail.com>.

⁴ Engenheiro Florestal. E-mail: <edeliberalesso@yahoo.com.br>.

Introduction

In agroforestry systems, one of the main characteristics of forest species is related to the contribution of organic material and fine roots decomposition, which may provide nutrients, like the nitrogen fixed by the black-wattle, aiming the increase in productivity of consortium agricultural crops. It is estimated that the black-wattle, due to its symbiotic association with nitrifying bacteria, is able to fix up to $200 \text{ kg ha}^{-1} \text{ year}^{-1}$ of atmospheric nitrogen (N_2) in tropical regions and where the soil under forests is 50% richer than in near pastures (AUER and SILVA, 1992).

Besides the possible benefits to agricultural crops, the mixed planting of forest species has demonstrated a significant potential in increasing soil fertility (nutrient cycling), in biomass production, carbon sequestration and, at the same time, providing other benefits through product diversification and a decrease in the risk of pests and diseases and furthermore, functioning as an agroforestry system producing high value-added wood (DEBELL et al., 1997; KHANNA, 1997; MONTAGNINI, 2000; KAYE et al., 2000; RESH et al., 2002).

The main benefit in planting atmospheric nitrogen fixing species is the increase in the total N at the soil-plant system (KELTY and CAMERON, 1995). Beyond that, according to the authors, the availability of nitrogen may increase with the speed-up in the cycling rate due to the increase of total N levels in the system. The N is transferred between the species (*Acacia mearnsii* and eucalyptus) through the litterfall decomposition (leaves, branches, reproductive material and roots, including root exudates) and the subsequent liberation (FORRESTER et al., 2005).

This N availability is provided by the symbiotic association with bacteria forming nodules. The leguminous species nodulation is related to edaphoclimatic factors, availability of mineral nitrogen in the system, intensity and form of species management (BODDEY et al., 2000). The leguminous depend on the

symbiosis as the source of nitrogen and need high amounts of phosphorus in the soil to reach the nodules needs (CALDAS et al., 2009). This fact was observed by Binkley et al. (2003) that found growing reduction in N_2 fixing species due to the reduction in the P availability in soil. Due to the importance of the N biologic fixation, this study aimed at evaluating the distribution of the nodules biomass density in *Acacia mearnsii* at monospecific planting and at mixed planting with *Eucalyptus grandis* x *E. urophylla* in the Southern region of Brazil.

Material and methods

Site description

The study was carried out at an experimental area located at the city of Bagé in the State of Rio Grande do Sul, Brazil (Figure 1). The area location is determined by the central geographic coordinates of $31^{\circ}14'43'' \text{ S}$ and $54^{\circ}04'55'' \text{ W}$ and, an average altitude of 242 m height in relation to sea level.

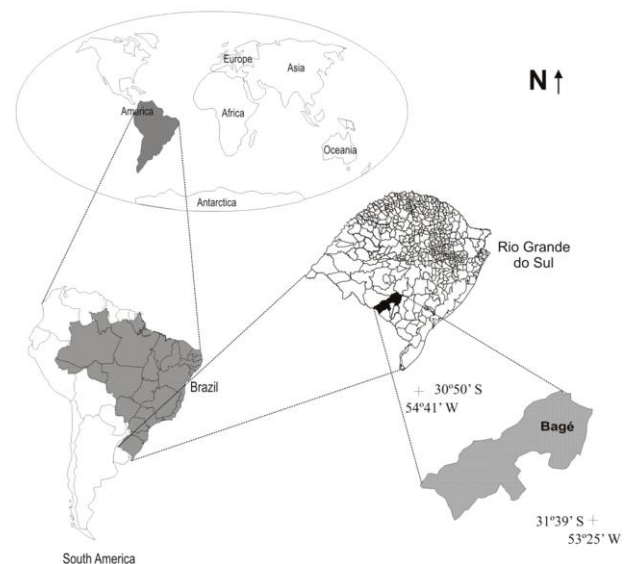


Figure 1 - Location of the experimental area in Bagé, RS.

Figura 1 – Área experimental localizada em Bagé, RS.

According to Köppen climate classification, the fundamental predominant climate is Cfa (subtropical wet), the average annual rainfall is

1.364 mm. The average annual temperatures of 17.5 °C, with the mean maximum temperature of 23.5 °C and mean lower temperature of 12.3 °C. The soil in the experimental area is

classified as Eutric Nitosols (FAO classification), characterized by a natural low fertility, strong acidity, high aluminum saturation and a sandy-loamy texture (Table 1).

Table 1 - Soil chemical and physical proprieties in the experiment area.

Tabela 1 – Propriedades químicas e físicas do solo na área experimental.

Depth (cm)	O.M. g kg ⁻¹	pH (H ₂ O)	Al	H + Al	CEC _{effect}	CEC _{pH 7}	Ca	Mg	P	K
0-5	2.0	4.8	0.9	6.5	2.8	8.4	1.0	0.7	6.7	80.3
5-10	1.8	4.8	1.0	7.8	2.9	9.6	1.0	0.7	3.6	54.6
10-20	1.4	4.7	1.3	7.8	2.7	9.3	0.8	0.6	2.0	36.6
20-30	1.1	4.7	1.5	8.5	3.0	10.0	0.8	0.6	1.2	31.0
Depth (cm)	m		Sand (%)		Silt (%)		Clay (%)		Density	
	(%)	v	(2-0.2mm) ^a	(0.2-0.05mm) ^b	(0.05-0.002mm)	(<0.002mm)	g cm ⁻³			
0-5	32.5	22.4	25	53	5	18	1.38			
5-10	37.6	19.4	25	52	6	18	1.50			
10-20	47.6	16.4	26	51	4	20	1.49			
20-30	52.8	15.0	24	52	4	21	1.49			

P and K: Mehlich-1 Extractor; Al, Ca and Mg: KCL 1 mol l⁻¹ Extractor; H + Al: Ca(OAc)₂ 0.5 mol l⁻¹ pH 7.0 Extractor; O.M. = Organic matter; CEC = Cation Exchange capacity; m = Aluminum saturation percentage; v = Base saturation percentage; ^a Coarse sand; ^b Fine sand.

Experiment design and establishment

Statistical design chosen was random blocks with 2 treatments and 3 repetitions. Total area of each repetition was 1224 m² (25.5 m x 48.8 m) with 17 rows x 12 interrows. Ignored for measurement are the two trees rows ends. The treatments used were as follows: - 100A (100% of *Acacia mearnsii*); - 50E:50A (50% of *Eucalyptus grandis* x *Eucalyptus urophylla* + 50% of *Acacia mearnsii*). The species planting was made (November, 2007) with a 4.0 m x 1.5 m spacing, after subsoiling, at a mean depth of 50 cm, in the planting row. Concomitantly to this operation, the soil received a chemical fertilizer, at the planting row, with the following formulation: 06:30:06 of the formula N - P₂O₅ - K₂O + 7% of Ca + 6% of S + 0.1% of B + 0.5% of Cu, applied at the rate of 300 kg/ha.

Nodules sampling

We sampled the nodules at 8 (July 2008) and 18 (May 2009) months after planting of the monospecific and mixed *Eucalyptus grandis* x

E. urophylla and *Acacia mearnsii* stands. The definition of the trees to sample the root was based on the diameter at ground height to 8 months (100A = 3.58 cm and 50E:50A(A) = 3.42 cm) and diameter at breast height to 18 months (100A = 7.52 cm and 50E:50A(A) = 7.61 cm). We evaluated three trees by treatment. The sampling points around each tree were identified with ordinal numbers (1 to 38). The distribution of these sampling points was systematically done through the allocation of eight transects (two at the inter-row, two at the line and, four at the diagonal), from the tree trunk center in relation to mean soil level. Considering 25 cm from the tree trunk and radially at an equidistant form, we marked the collecting points (clockwise) reaching all the useful area of each considered tree (4.0 m x 1.5 m). This collecting methodology was adapted from Böhm (1979). The sampling at each collecting point was fractionated in sample sub points originated at each different considered depth (0 – 5; 5 – 10; 10 – 20 and 20 – 30 cm). The collecting was made using a cylindrical steel extractor tube with 7.0 cm of inside diameter.

Samples processing

Nodules determination was performed together with the fine root evaluation processes at 18 months of planting age in the monospecific and the mixed planting of *Acacia mearnsii*, because at 8 months the leguminous did not show any significant nodulation (VIERA, 2010). Nodule identification was visual and, the separation process of the fine root was manual with the use of pinchers. To determine the biomass, the nodules were put into an air renewal and circulation stove (70 °C) for 72 hours, then, the dry mass was determined using an analytical balance (with an accuracy of 0,0001g).

Data presentation and statistic analysis

Based on values for nodules biomass, it was possible to elaborate figure and cartogram to demonstrate nodules distribution on the soil. This procedure also enabled the demonstration of the nodule biomass distribution at the soil profile. The cartograms show the nodules distribution in the planting row spacing, line and diagonal. Bartlett test of homogeneity of variance and the Lilliefors test of error normality were applied to the results to verify their validation by the variance analysis presupposes. Results that did not meet the assumption were transformed by applying the square root and/or the square root inverse. The statistic analysis was performed at a 5% of error probability. To separate the average contrast we used the Tukey test.

Results and discussion

The nodule biomass distribution was quite variable (Figure 2). For *Acacia mearnsii* trees in monoculture, there was a tendency of higher accumulation of nodules dry mass near to the trunk. In mixed planting, on the other hand, there was an inverse tendency where the greater nodule biomass was present at places that were more distant from the tree trunk. Although the

nodule biomass is much greater at the monospecific planting of *Acacia mearnsii* (100A) in relation to the mixed planting (50E:50A-A), the differences were not significant ($p > 0.05$) due to the high spatial variability of the nodule presence.

The same situation was verified by Silva (2007) in monoculture and mixed planting of *Acacia mangium*. This case study corroborates with the one cited before where the author verified a similar nodule distribution in a monoculture of *Acacia mangium*, where at 18 and 30 months of age, the nodulation was more intense in the planting line at an average distance among trees. In the mixed planting, the nodulation was more intense near to the tree (SILVA, 2007). However, to the *Acacia mangium*, the nodules biomass was lower to the one of *Acacia mearnsii*, reaching mean values lower than 2.0 g dm^{-3} .

The nodulation in leguminous species is controlled by several factors, in this way, the higher or lower production of nodules biomass cannot be attached to possible interactions between nitrogen-fixing or non nitrogen-fixing species. In a study carried out by Lawrie (1981) with ten leguminous species in Australia, one of them was the *Acacia mearnsii*, where was observed that the nodular activity reached the peak in Spring and it was minimum at the end of Summer, continuing at this level during all Winter. According to the author, the variation in rainfall and temperature was the main factor that influenced in the seasonal variations of the nodular activity. Inagaki et al. (2009) emphasized that another important factor that defines the presence of nodules is the availability of nitrogen in soil and Danso et al. (1992) mentioned that the addition of phosphate had little effect in the *Rhizobium* growth but it reduced the nodulation time and increased the number of nodules.

One of the main benefits of nodulation is the biological nitrogen fixation (BNF) is that the use of nitrogen-fixing trees in forest systems with eucalyptus might contribute to the sustainable production through the restoration and maintenance of soil fertility as well as help



to prevent erosion and desertification, furthermore it propitiates the establishment of systems in poor soils, through the nitrogen

fixation by the leguminous and its availability to the associated cultures (DANSO et al., 1992).

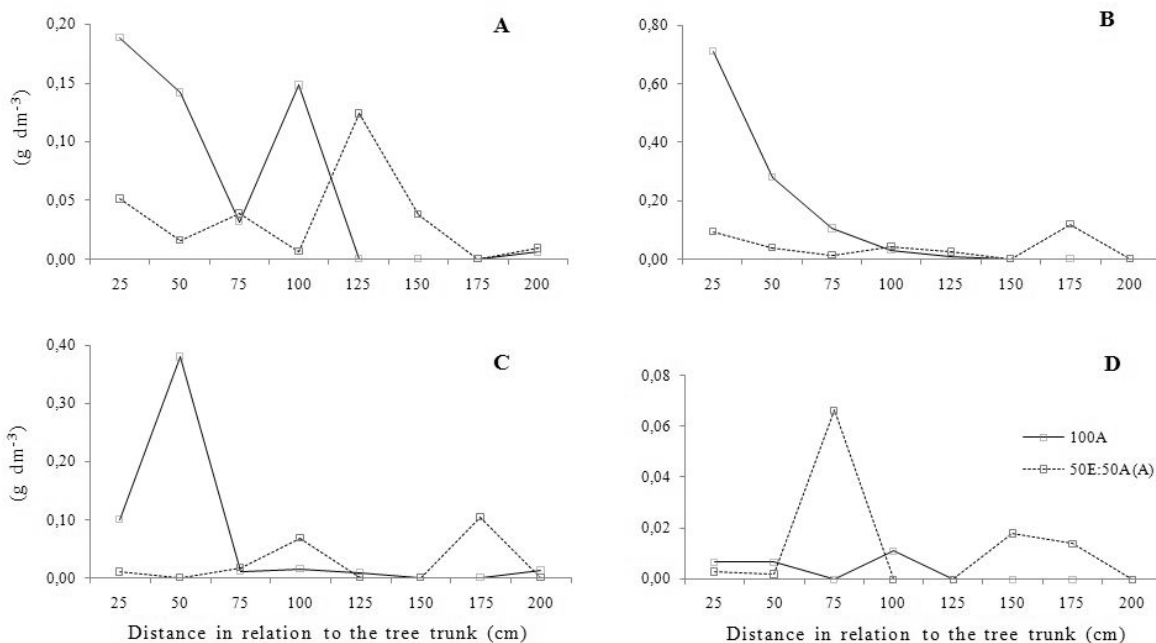


Figure 2 - Nodules biomass distribution at 18 months of age in monospecific and mixed planting of *Acacia mearnsii*, in the different soil depths (A. 0-5; B. 5-10; C. 10-20 and D. 20-30 cm) considering the distance in relation to the trees trunk.

Figura 2 – Distribuição da biomassa de nódulos aos 18 meses de idade em plantio monoespecífico e misto de *Acacia mearnsii*, nas diferentes profundidades do solo (A. 0-5; B. 5-10; C. 10-20 e D. 20-30 cm) em função da distância em relação ao tronco da árvore.

The highest amount of nodule biomass was found at the soil layer of 0 to 20 cm of depth, reaching the number of more than 0.7 g dm^{-3} at 25 cm distant from the tree trunk at the 5 to 10 cm depth where there was also a more regular distribution excepting the superficial layer. In the layer of 20 to 30 cm of depth, there are almost no nodules (biomass $< 0.01 \text{ g dm}^{-3}$) to the *Acacia mearnsii* in monoculture and, there is only a small amount (biomass $< 0.07 \text{ g dm}^{-3}$) in consortium.

The presence of nodules was very variable for both, the monoculture and the mixed planting of *Acacia mearnsii*, in the planting row spacing, in the planting line and, in the diagonal of two planting lines (Figure 3), at the stands with 18 month of age. The same was verified for fine root length and biomass (VIERA et al.,

2012) was found to the nodules distribution, which was greater at the planting line, reaching more than 2.4 g dm^{-3} , followed by the diagonal that reached more than 1.2 g dm^{-3} and, the planting row spacing that was less than 0.6 g dm^{-3} .

The greater nodule biomass was verified at the monoculture of *Acacia mearnsii* in relation to its mixed planting with *Eucalyptus grandis* x *E. urophylla*, getting to be more than 20 times greater in the planting line at 50 cm distant from the tree trunk. But, this is not linked to a possible interspecific concurrence with the *Eucalyptus grandis* x *E. urophylla* root, for in the planting row spacing the nodulation was higher at a distance of 175 cm in relation to the *Acacia mearnsii* trunk.

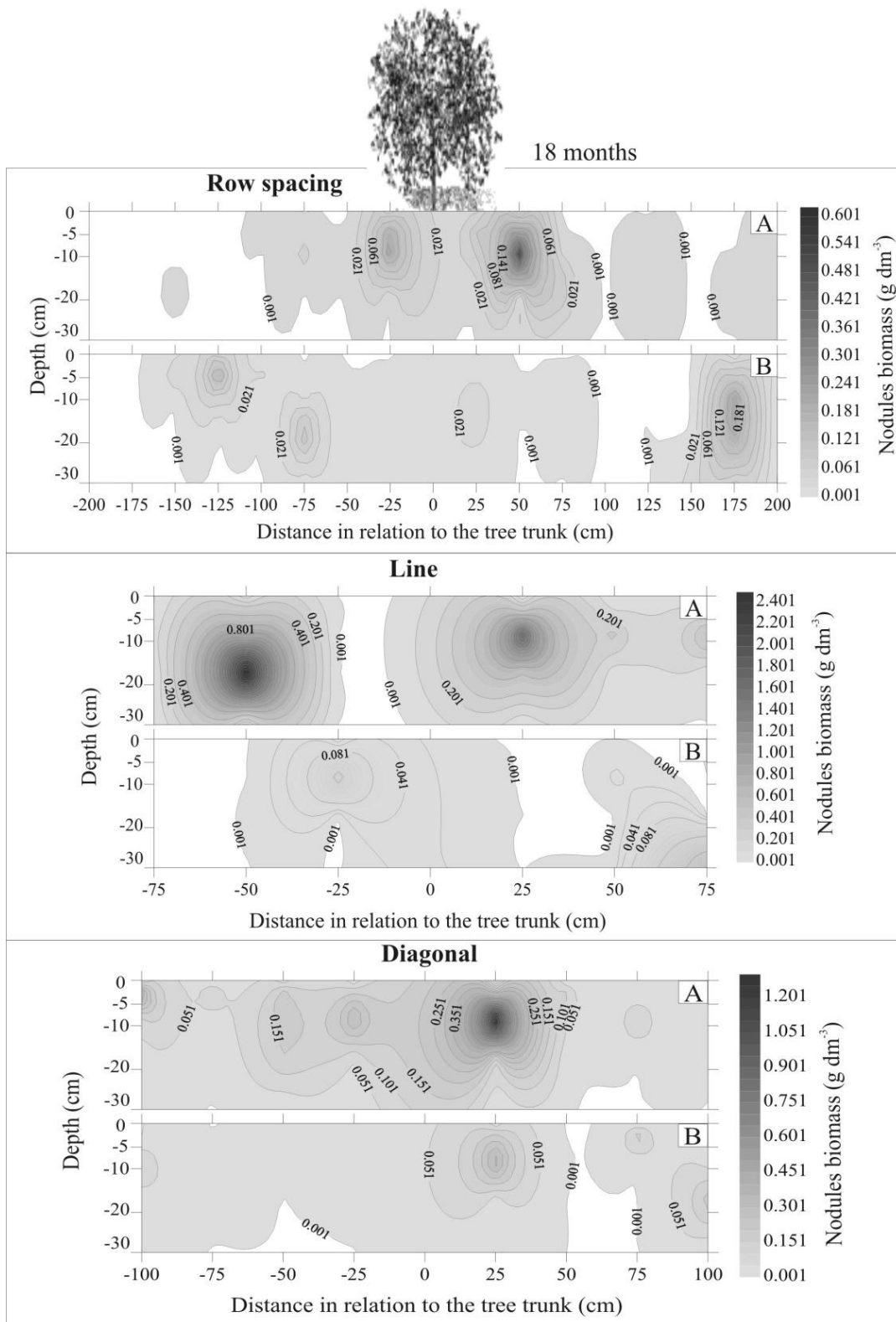


Figure 3 - Nodules biomass spatial distribution (g dm^{-3}) in monospecific and mixed stands of *Acacia mearnsii* (A = 100A – 100% of *Acacia mearnsii*; and B = 50E:50A (A) – 50% of *Eucalyptus* + 50% of *Acacia mearnsii*) at 18 months of age.

Figura 3 – Distribuição espacial da biomassa de nódulos (g dm^{-3}) em povoamentos mono específico e misto de *Acacia mearnsii* (A = 100A – 100% de *Acacia mearnsii*; and B = 50E:50A (A) – 50% de eucalipto + 50% de *Acacia mearnsii*) aos 18 meses de idade.

Bouillet et al. (2008) observed that the use of *Acacia mangium* in consortium with *Eucalyptus grandis* reduced the production of nodules biomass in 20 to 30 times in relation to the *Acacia mearnsii* monoculture, thus demonstrating that the *Acacia mangium* trees, when growing in monoculture, have a higher potential to fix atmospheric nitrogen than when in mixed planting with *Eucalyptus grandis*. According to Döbereiner (1984), high quantities of nitrogen can only be fixed in soils where there is little or no available nitrogen. Some studies report that the higher distribution and production of nodules, beyond the interference of the amount of nitrogen available in soil may be also influenced by a higher soil moisture (HABISH, 1970; SILVA, 2007), pH elevation (HABISH, 1970; UMALI-GARCIA et al., 1988; SPRENT, 1994), increase in phosphorus availability (FRANCO, 1984; SPRENT, 1994; NGUYEN et al., 2006) and, aluminum and manganese toxicity (FRANCO, 1984). To Sprent and Parsons (2000), knowing the leguminous species nodulation ability, nitrogen fixation rates, their physiological adaptation and genetic diversity are very important to provide fundamental knowledge to a more adequate preservation and use of these plants.

Conclusion

Due to the great variability, the nodules biomass, although being much higher in the monospecific planting of *Acacia mearnsii* when comparing to mixed planting, did not show significant differences ($p > 0.05$). The greater nodules density are found in the layers 5 to 10 cm of depth, near to the tree trunk and at the planting line followed by the planting diagonal and row. The nodules biomass density found for the *Acacia mearnsii* (up to 2.4 g dm^{-3}) demonstrates the importance of the use of this atmospheric nitrogen-fixing species in mixed plantings.

Referências

- AUER, C. G.; SILVA, R. Fixação de nitrogênio em espécies arbóreas. In: CARDOSO, E. J. et al. **Microbiologia do solo**. Campinas: Ed. da UNESP, 1992. p.160–167.
- BINKLEY, D. et al. Twenty years of stand development in pure and mixed stands of *Eucalyptus saligna* and nitrogen-fixing *Facaltaria moluccana*. **Forest Ecology and Management**, v.182, n.1-3, p.93-102, 2003.
- BODDEY, R.M. et al. Use of the ^{15}N natural abundance technique to quantify biological nitrogen fixation by woody perennials. **Nutrient Cycling in Agroecosystems**, v.57, n.3, p.235-270, 2000.
- BÖHM, W. **Methods of studying root systems**, Springer-Verlag, Berlin, 1979.
- BOUILLET, P. et al. Mixed-species plantations of *Acacia mangium* and *Eucalyptus grandis* in Brazil 2: Nitrogen accumulation in the stands and biological N_2 fixation. **Forest Ecology and Management**, v.255, n.12, p.3918–3930, 2008.
- CALDAS, G. G. et al. Efeito da fertilização fosfatada na produção de raízes, liteira e nodulação de *Mimosa caesalpinifolia* Bent. **Revista Árvore**, v.33, n.2, p.237-244, 2009.
- DANSO, S.K.A.; BOWEN, G.D.; SANGINGA, N. Biological nitrogen-fixation in trees in agroecosystems. **Plant and Soil**, v.141, n.1-2, p.177–196, 1992.
- DEBELL, D.S.; COLE, T.C.; WHITESELL, C.D. Growth, development and yield of pure and mixed stands of *Eucalyptus* and *Albizia*. **Forest Science**, v.43, n.2, p.286–298, 1997.
- DÖBEREINER, J. Nodulação e fixação de nitrogênio em leguminosas florestais. **Pesquisa Agropecuária Brasileira**, v.19, p.83-90, 1984.

- FORRESTER, D.I.; BAUHUS, H.; COWIE, A.L. On the success and failure of mixed-species tree plantations: lessons learned from a model system of *Eucalyptus globulus* and *Acacia mearnsii*, **Forest Ecology and Management**, v.209, n.1-2, p.147-155, 2005.
- FRANCO, A.A. Fixação de nitrogênio em árvores e fertilidade do solo. **Pesquisa Agropecuária Brasileira**, v.19, p.253-261, 1984.
- HABISH, H.A. Effect of certain soil conditions on nodulation of *Acacia* spp. **Plant and Soil**, v.33, n.1, p.1-6, 1970.
- INAGAKI, M. et al. Fine-root production in response to nutrient application at three forest plantations in Sabah, Malaysia: higher nitrogen and phosphorus demand by *Acacia mangium*. **Journal of Forestry Research**, v.14, p.178-182, 2009.
- KAYE, J.P. et al. Nutrient and carbon dynamics in a replacement series of *Eucalyptus* and *Albizia* trees. **Ecology**, v.81, n.12, p.3267-3273, 2000.
- KELTY, M.J.; CAMERON, I.R. Plot designs for the analysis of species interactions in mixed stands. **Community Forestry Research**, v.74, p.322-332, 1995.
- KHANNA, P. K. Comparison of growth and nutrition of young monocultures and mixed stands of *Eucalyptus globulus* and *Acacia mearnsii*. **Forest Ecology and Management**, v.94, n.1-3, p.105-113, 1997.
- LAWRIE, A.C. Nitrogen Fixation by Native Australian Legumes. **Australian Journal of Botany**, v.29, n.2, p.143-157, 1981.
- MONTAGNINI, F. Accumulation in above-ground biomass and soil storage of mineral nutrients in pure and mixed plantations in a humid tropical lowland. **Forest Ecology and Management**, v.134, n.1-3, p.257-270, 2000.
- NGUYEN, N.T.; MOHAPATRA, P.K.; FUJITA, K. Elevated CO₂ alleviates the effects of low P on the growth of N₂-fixing *Acacia auriculiformis* and *Acacia mangium*. **Plant and Soil**, v.285, n.1-2, p.369-379, 2006.
- RESH, S.C.; BINKLEY, D.; PARROTTA, J.A. Greater soil carbon sequestration under nitrogen-fixing trees compared with *Eucalyptus* species. **Ecosystems**, v.5, n.3, p.217-231, 2002.
- SILVA, E.V. **Desenvolvimento de raízes finas em povoamentos mono-específicos e mistos de *Eucalyptus grandis* e *Acacia mangium***. 2007. 54p. Dissertação (Mestrado em Recursos Florestais – Silvicultura e Manejo Florestal) – Escola Superior de Agricultura “Luiz de Queiroz”/Universidade de São Paulo, Piracicaba.
- SPRENT, J.I. Evolution and diversity in the legume-rhizobium symbiosis: chaos theory? **Plant and Soil**, v.161, n.1, p.1-10, 1994.
- SPRENT, J.I., PARSONS, R. Nitrogen fixation in legume and non-legume trees. **Field Crops Research**, v.65, n.2-3, p.183-196, 2000.
- UMALI-GARCIA, M., LIBUIT, J.S., BAGGAYAN, R.L. Effects of Rhizobium inoculation on growth and nodulation of *Albizia falcataria* (L.) Fosh. and *Acacia mangium* Willd. in the nursery. **Plant and Soil**, v.108, n.1, p.71-78, 1988.
- VIERA, M. **Crescimento inicial e produtividade de plantios mono-específicos e mistos de *Eucalyptus urograndis* e *Acacia mearnsii* em sistema agrossilvicultural**. 2010. 140p. Dissertação (Mestrado em Engenharia Florestal) – Universidade Federal de Santa Maria, Santa Maria.
- VIERA, M.; SCHUMACHER, M.V.; LIBERALESSO, E. Mixed and monospecific stands of eucalyptus and black-wattle: I - fine root length density. **Ciência Rural**, v.42, n.10, p.1818-1825, 2012.

