Effects of trees and nitrogen supply on the soil microbiological attributes on integrated crop-livestock systems[1]

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ABSTRACT

The diversification of plants, as an association between trees and the warm-season grasses, supports soil biodiversity and thus, soil quality. However, based on the prevailing climatic conditions, management techniques employed and because of the several combinations possible in such associations, variations may be observed in this type of response. This study aimed at assessing the microbiological attributes of the soil in two systems (full sunlight, FS, vs. a naturally shaded system composed of *Eucalyptus dunnii* trees), with two N fertilization levels (zero vs. 300 kg N ha^{-1}year^{-1}) and five perennial warm-season grasses. Soil and root samples were drawn from a depth of 0 to 20 cm when the growing period began. The N fertilization increased the mycorrhizal colonization and the acid phosphatase activity, whereas lowered the soil microbial biomass. A rise in the mycorrhizal colonization and sporulation and C microbial biomass concentration were observed in the soil cultivated with *Axonopus catharinensis*. The association with trees favored the arbuscular mycorrhizal fungal communities, boosting both colonization and sporulation. However, compared with the FS, the soil microbial biomass and activity of the acid phosphatase enzyme were reduced under the trees. Silvicultural interventions could be intensified to enhance soil quality.

Keywords: acid phosphatase; agroforestry; arbuscular mycorrhizal fungi; glomalin; microbial biomass; tropical grasses.

INTRODUCTION

A strongly intensifying interest is generally being directed towards integrated crop-livestock systems (ICLS), as they have the potential to achieve synergisms and emerging characteristics resulting from the soil-plant-animal-atmosphere interactions (Carvalho *et al*., 2010; Moraes *et al*., 2014). The challenge at present is to clarify the complete dynamics of such systems, i.e. the strength with which the different interactions occur among their various constituents and their effects on the tree, forage, animal and agricultural outputs, as well as in the provision of ecosystem services.

The high potential of ICLS is evident from the integration of trees with the tropical perennial pastures. Therefore, this type of system is gaining interest (Pretty, 2018), mostly because of the necessity to recover degraded pastures, specifically in the tropical / subtropical soils, and environmental restrictions to explore new regions of native vegetation. The incorporation of trees in pastures contributes to animal welfare (Lopes *et al*., 2016), C sequestration both in the soil and in tree woody biomass (Udawatta & Jose, 2012), plant diversification and litter accumulation (Freitas *et al*., 2013), decrease in the soil erosion processes and utilization of the nutrients from the deeper soil layers, thus improving soil biological processes (Araujo *et al*., 2012). In fact, the relationship among the perennial species facilitates permanent litter deposits, thus raising the organic content and positively affecting the soil microbial community due to the increased C sources and energy (Araujo *et al*., 2012). Bainard *et al*. (2011) proposed that the tree systems encouraged the

abundance of the arbuscular mycorrhizal fungi (AMF) to a greater degree than did the conventional farming systems. However, the same authors emphasized that such a response could vary, principally because of climate variations, management techniques and a variety of possible combinations in such associations (e.g., tree arrangements and tree species, tree density and age, etc.).

The association of trees with pastures in general appears to induce a positive change in the soil quality through higher chances for nutrient cycling (Batista *et al.*, 2018). However, microclimate alterations within the system and therefore water availability, together with the competition between trees and intercropping for light and nutrients may influence the quantity and quality of the organic residues deposited on the soil, besides their spatial distribution and hence, the resultant microbiological attributes. Thus, data on the manner in which the tree systems and such types of more sustainable production practices affect the microbial community and populations like AMF continue to be required (Ferreira *et al.*, 2012).

According to Pontes *et al.* (2016) different responses with regards to forage production and nutritive values were noted when several different C$_4$ forages were used, besides the interactive influence of the *Eucalyptus dunnii* shading and added nitrogen (N) doses. Thus, as all the observations made above the ground level are mirrored below the soil, it is possible that any alteration in the microbiological attributes occur in response to the different forages when in association with the trees. Besides, the traits of the host plant affect the occurrence of the AMF (Staddon *et al.*, 2003). The glomalin, a glycoprotein produced by the AMF in the soils, is affected by the relationship between fungi and plants (Sousa *et al.*, 2012). The "glue" effect of the glomalin enables the soil particles to get fixed, facilitating the formation of stable aggregates (Driver *et al.*, 2005; Sousa *et al.*, 2012).

The present study assessed the microbiological attributes of the soil under a variety of production systems, that is, monoculture vs. a system which included *E. dunnii*, raised with two doses of N and five perennial tropical forage grasses. The hypothesis proposed in this work is that the microbiological attributes of the soil are positively affected by afforestation and N fertilization, either via increased litter deposition or diversification of the vegetal residues on the soil, although these responses are species-dependent.

MATERIAL AND METHODS

The experiment was performed in an area within the Agronomic Institute of Paraná, in Ponta Grossa, in the Center-South region of the state of Paraná (25º 06' S, and 50º 02' W at 1019 m altitude). Based on the Köppen classification, the climate of the region is type Cfb, meaning subtropical and no dry season. The mean temperature and rainfall data, during the experimental period, are shown in Table 1.

The experimental area is characterized by umbric Dystrophic Red Latosol (Embrapa, 2008). The main chemical features of the soil during the experiment are seen in Table 2.

The production systems investigated were done under full sun conditions (FS, i.e., perennial tropical forage monocultures) and the integrated crop-livestock system with trees (ICLS, implying the association between the tropical perennial forage species and *E. dunnii*). The five C$_4$ perennial forage grass species studied are generally cultivated in the Brazilian livestock and include, *Axonopus catharinensis* Valls., (Ac), *Urochloa brizantha* cv. Marandu (Ub), *Cynodon* spp. hybrid Tifton 85 (Cs), *Hemarthria altissima* (Poir.) Stapf & Hubb cv. Flórida (Ha) and *Megathyrsus maximus* Jacq. cv. Aruana (Mm), planted in 2010 in 4.5 m² plots, under FS and 105 m² (5 x 21 m) between the lines of the *E. dunnii* trees. The trees were planted in 2007, with an initial density of 267 trees ha^{-1}, according to a double row arrangement using 3 m between the trees within rows and 4 m between the rows. These double rows sets were spaced 21 m apart (3 by 4 by 21 m), following the contour. In 2011, the thinning done during the winter, decreased the population to 219 trees ha^{-1}, while the thinning performed in the summer of 2014 cut the population down to 155 trees ha^{-1}.

After the application of two doses of N (0 and 300 kg of N ha^{-1} year^{-1}, N0 and N300, respectively), as urea, with three replicates, six plots per species were formed for each system. The ICLS also included three distances between the tree rows, viz., P2, position adjacent to the rows (2 m away from an arboreal row); P5, intermediate position (at 5 m distance); and P10, central position between two tree rows (10.5 m away from the tree rows). Two ceptometers (LP-80 AccuPAR, Decagon Equipment Co, Pullman WA) were used to calculate the shading percentages (percent reduction of the photosynthetically active radiation, PAR) one of which was kept in the FS region while the other was placed under the tree canopy, in each position. On March 17, 2016 four evaluations were performed between 10 and 11:30 AM and four more between 1:30 and 3:00 PM per position, at 1 m above soil level, in three experimental woody units per block. The decrease in light availability for the understory vegetation was calculated as the difference in the values from these two ceptometers, according to Pontes *et al.* (2016).

Based on the soil analysis, fertilization was done in each area considering the specified recommendations for the crops. Liming was conducted to raise the saturation

of the bases to 70%. In the spring, 60 kg of P_2O_5 ha^{-1} + 60 kg of K_2O ha^{-1} were added, independently of the treatments assessed prior to collecting the soil samples for the microbiological analyses. N, as urea, was added depending upon the treatments, on October 23, 2015. Please refer to Pontes *et al.* (2016) for further information on fertilization and pasture and tree management in the experiment since its implantation.

In November 2015, soil and root samples were taken with a spade (20 cm width, ~1 kg), ensuring that the sample had the same width at the top and bottom of slice, from a depth of 0-20 cm, one month post the standardization cutting of the pastures (at the same regrowth age, in order to better compare the different species). Only one sample per experimental unit was collected in the FS system. The choice for collecting one representative point per plot under FS was because of the reduced areas of the plots. However, three simple samples per experimental unit were collected in the ICLS, considering the three positions between the tree double rows mentioned previously. This was most likely done because of the differences in the PAR transmitted between the tree rows (21 m between two rows). The fresh soil samples were sieved through a 4 mm screen with large plant material removed and stored at 7 °C until analysis. Thin roots (< 2 mm) were washed in water and stored in plastic receptacles containing 50% alcohol, for preservation until the analysis.

To determine the carbon of the microbial biomass (MBC), the fumigation-extraction method (Vance *et al.*, 1987) was employed, while the N analysis of the microbial biomass (MBN) was done following the method of Brookes *et al.* (1985). Both methods were modified before use, as explained by Hungria *et al.* (2009). The AMF root colonization (MC) was determined in stained roots under a stereoscopic microscope, based on the technique of Giovannetti & Mosse (1980). The AMF spores number (SPO) was determined by the extraction from soil employing the wet sieving technique adopted by Gerdemann & Nicolson (1963). The extraction of the total glomalin (TG) and easily extractable soil glomalin (EEG) was accomplished, using the method of Rillig *et al.* (2003). Glomalin quantification was done based on the methodology of Bradford (1976). The acid phosphatase was determined using the method adapted by Tabatabai (1994). The NO_3^- -N levels in the soil were evaluated following the method of Miyazawa *et al.* (1985), while the NH_4^+ -N contents were assessed with the method used by Kempers & Zweers (1986).

The soil microbiological attributes were subjected to the analyses of variance using the split-split-plot-like

Table 1: Mean minimum-maximum monthly temperature (°C) and total rainfall (mm) during the experimental period (2015) and historical mean (MH)

Months	Temperature (° C)			
	2015	MH (1998-2015)	2015	MH (1998-2015)
January	19.6 - 36.0	17.5 - 27.2	136	158
February	18.7 - 33.0	17.5 - 27.7	162	170
March	17.4 - 31.6	16.6 - 27.1	180	120
April	13.8 - 29.3	14.6 - 25.2	74	97
May	13.0 - 24.5	10.8 - 20.7	174	85
June	9.8 - 23.8	10.1 - 20.4	73	106
July	10.5 - 21.9	9.4 - 20.0	273	110
August	10.6 - 28.4	10.1 - 22.2	35	74
September	13.5 - 30.7	12.0 - 23.1	131	140
October	17.0 - 28.0	14.2 - 25.0	177	160
November	17.7 - 28.5	15.3 - 26.1	237	130
December	18.6 - 31.4	16.7 - 27.2	167	143

Source: SIMEPAR, station 25135001, situated in the municipality of Ponta Grossa/PR.

Table 2: Chemical characterization of the soil, at 0-20 cm depth, under the full sun (FS) system and integrated crop-livestock system with trees (ICLS)

	pH	OM	P	K	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al^{3+}	SB	T	V	SAI
		g kg^{-1}	mg dm^{-3}		cmol$_c$ dm^{-3}						%	
FS	5.7	41.7	24.0	60.6	3.6	1.8	0.0	3.8	5.6	9.4	59.0	0.0
ICLS	5.5	41.3	21.8	107.3	3.4	1.8	0.0	4.3	5.5	9.8	56.3	0.0

OM, soil organic matter; SB, sum of bases; T, cation exchange capacity; V, base saturation; SAI, aluminum saturation. P-K (MEHLICH - 1); Ca-Mg-Al (KCl 1 mol L^{-1}); pH (CaCl$_2$).

model and employing the Statgraphics Centurion XV program (2006). While the systems (FS vs. ICLS, GL = 1) were regarded as the main plots, the N doses (GL = 1) were used as the subplots and forages (GL = 4) as sub-subplots. The error of the main plot was the block (system), while that of the subplot was the N*block (system). The model included the position between two tree rows (GL = 2) as a nested factor in the system. All the factors barring the block were regarded as fixed. Initially, all the interactions, excepting the one with the block were included in the statistical model. In the final model, the non-significant interactions were eliminated. To fulfill the normality test, all data were transformed into logarithms, the TG (transformed via Box-Cox) and mycorrhizal colonization (MC, no transformation needed) being the exceptions. In the event of a variable having a significant effect, the Fisher's least significant difference (LSD) test was done.

RESULTS AND DISCUSSION

Slight variations were noted, on average, between the systems and N doses in the MC (p < 0.10, Tables 3 and 4). A significant part of the variance (18%) for MC was defined by the species factor, with values in the range of 36% (Cs)

to 50% (Ac, Table 5). These results signify a good amount of colonization in the grasses, with values corresponding to the findings in other studies (Cordeiro *et al*., 2005). However, as evident from Figure 1, marked interactions (p < 0.01, F = 4.03) between the species, systems and N doses were observed for this variable. Therefore, it was difficult to identify, for instance, the species which showed the greater or lesser effectiveness in mycorrhization, because the colonization varied with the treatments. For example, in the ICLS, the N0 treatment, cv. Tifton 85 returned the least MC values (~ 25%), while in the FS, the N0 treatment, cv. Marandu gave the lowest values (~ 27%, Figure 1).

In MC, Tifton 85 showed the greatest differences between treatments (difference of ~22% between ICLS N0 and FS N0, Figure 1). Pontes *et al*. (2016) also reported a higher amplitude of response, with respect to the aerial dry matter yield, for the same species used in the present experiment. Therefore, it is clear that association with tree and N fertilization impact the cv. Tifton 85, both in terms of productivity, and the MC of this species, most likely revealed effects on root density. Indeed, Tifton 85 showed an important decrease (-53%, data not shown) in root mass density (0 - 15 cm depth) in the ICLS compared to the FS system. The *A. catharinensis*, however, exhibited the

Table 3: Microbiological attributes of the soil (mean ± standard deviation) of each system (FS, full sun, n = 30, ICLS, integrated crop-livestock system with trees, n = 90) are shown. Mycorrhizal colonization (MC), sporulation by arbuscular mycorrhizal fungi (SPO), acid phosphatase (AP), easily extractable glomalin (EEG), total glomalin (TG), soil microbial biomass nitrogen (MBN) and carbon (MBC) and soil inorganic N (NH_4^+ -N and NO_3^- -N) are listed

	FS	**ICLS**	**F**	**p**
MC (%)	38 ± 1.5	42 ± 1.3	4.6	0.0994
SPO (50 mL^{-1} soil)	27 ± 2.6	52 ± 4.0	18	0.0136
AP (μg p-nitrophenol g^{-1} soil h^{-1})	363 ± 14.0	270 ± 6.3	51	0.0020
EEG (mg g^{-1} soil)	2.6 ± 0.05	2.5 ± 0.06	0.3	0.6085
TG (mg g^{-1} soil)	5.9 ± 0.16	4.6 ± 0.16	2.9	0.1630
MBN (μg N g^{-1} soil)	127 ± 5.1	99 ± 4.4	15	0.0186
MBC (μg C g^{-1} soil)	179 ± 9.0	158 ± 5.9	1.7	0.2638
NH_4^+ -N (μg g^{-1} soil)	3.3 ± 0.12	1.8 ± 0.09	4.9	0.0903
NO_3^- -N (μg g^{-1} soil)	38 ± 2.9	29 ± 1.4	7.2	0.0552

F and p, F-ratio and probability for the system effect.

Table 4: Microbiological attributes of the soil (mean ± standard deviation) at different N rates (N0, without nitrogen fertilization, N300, 300 kg N ha^{-1} year^{-1}, n = 60). Refer to Table 3 for abbreviations

	N0	**N300**	**F**	**p**
MC (%)	38 ± 1.7	43 ± 1.3	6.5	0.0635
SPO (50 mL^{-1} soil)	55 ± 5.1	38 ± 3.6	3.5	0.1351
AP (μg p-nitrophenol g^{-1} soil h^{-1})	271 ± 8.5	315 ± 10.2	26	0.0072
EEG (mg g^{-1} soil)	2.5 ± 0.06	2.6 ± 0.08	0.4	0.5872
TG (mg g^{-1} soil)	4.8 ± 0.19	5.0 ± 0.20	2.8	0.1689
MBN (μg N g^{-1} soil)	105 ± 7.5	108 ± 4.6	2.1	0.2195
MBC (μg C g^{-1} soil)	171 ± 8.0	155 ± 6.0	1.8	0.2462
NH_4^+ -N (μg g^{-1} soil)	2.3 ± 0.14	2.1 ± 0.13	12	0.0257
NO_3^- -N (μg g^{-1} soil)	27 ± 1.8	35 ± 1.7	43	0.0028

F and p, F-ratio and probability for the N doses effect.

lowest variations among the treatments (~6%). Besides, this species demonstrated the highest values among all the treatments, except in the FS system, for N0 (Figure 1). The *A. catharinensis* forage seemed to have experienced higher photosynthetic efficiency in most of the treatments when compared with the other species, meaning, it had conducted photosynthesis in a symbiotic relationship with the AMF, maintaining a greater degree of colonization, to experience higher nutrient absorption levels, P in particular (Cordeiro *et al.*, 2005).

The ICLS showed a remarkably greater number of AMF spores when compared to FS (Table 3), regardless of the plant species raised. Besides, only in the ICLS were differences noted in the N doses in sporulation by AMF, with increased values in the N0 treatment (please refer to Figure 2 for the results of the intense interactions

between the systems and doses of N, $p < 0.05$, $F = 6.38$). Sporulation is a perpetuation mechanism of the AMF species, which is stimulated when the plant and fungus experience some type of stress (Ferreira *et al.*, 2012). Therefore, when they were exposed to multiple stresses (light and N) sporulation was induced. However, even in the treatment having higher sporulation, meaning, 65 in 50 mL of soil (ICLS N0, Figure 2), this value cannot be regarded as high, as reiterated by Santos *et al.* (2008). In fact, in their study using *B. brizantha* cv. Xaraés, Delbem *et al.* (2010) reported increased values for the number of spores (more than 146 per 100 g of dry soil). These authors also recorded a rise in sporulation in response to the high urea and ammonium sulfate doses added (400 kg of N ha^{-1}), implying stress caused by the raised N levels in the soil.
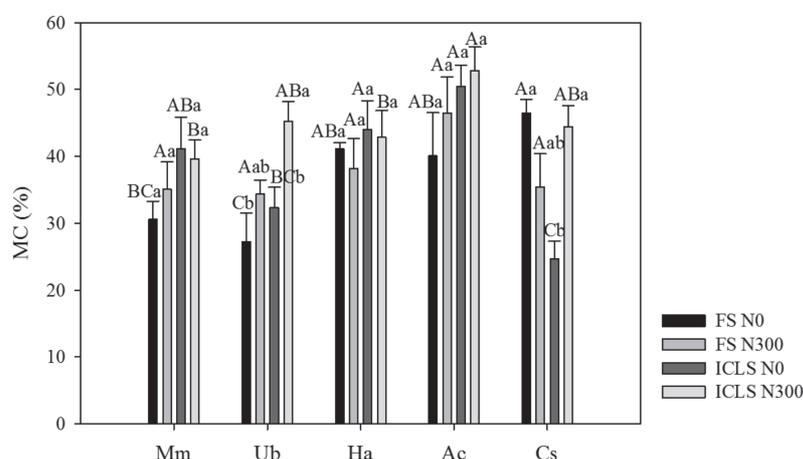

Figure 1: Means (n = 3) of the mycorrhizal colonization (MC) by the forage species in each system (FS, full sun, n = 30, ICLS, integrated crop-livestock system with trees, n = 90) and at different N rates (N0, without nitrogen fertilization, N300, 300 kg of N ha^{-1} year^{-1}). Please refer to Table 5 for species abbreviations. Means followed by the same capital letter (compare species within each treatment) or lower case (compare treatments for each species) do not differ from each other by the Fisher test (LSD) at 5% significance.
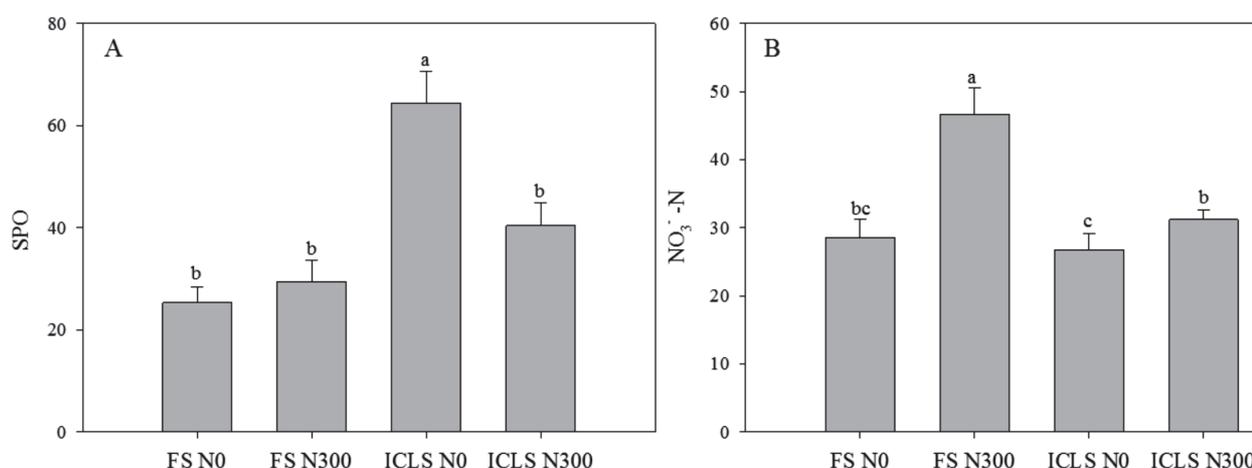

Figure 2: The sporulation averages for the arbuscular mycorrhizal fungi (A, SPO, 50 mL^{-1} soil) and N concentrations available in the soil as nitrates (B, NO_3^- -N, ìg g^{-1} soil) for each system (FS, full sun, ICLS, integrated crop-livestock system with trees) and N doses (N0, without nitrogen fertilization, N300, 300 kg N ha^{-1} year^{-1}). Means followed by the same letter do not differ from each other by the Fisher test (LSD) at 5% significance.

Guadarrama & Alvarez-Sánchez (1999) proposed that moisture encouraged spore germination, producing a high degree of colonization but poor spore yield. During the sampling period, in this experiment, a large volume of rainfall was received (Table 1), which might have affected the results. It is also noteworthy that treatments involving higher MC do not always induce increased sporulation by AMF (see Figure 1), as previously reported (Staddon et al., 2003).

Despite the variations observed in CM and sporulation as reported above, no significant differences ($p > 0.10$) were observed between the factors studied for easily extractable soil glomalin (EEG) and total glomalin (TG), with means of 4.89 ± 0.137 mg g^{-1} soil and 2.55 ± 0.048 mg g^{-1} soil, respectively. Also, no significant interactions were observed between the factors studied for such variables. According to Sousa et al. (2012), the mechanisms that regulate the production of glomalin by AMF are still not well understood, however, factors that affect mycorrhizal symbiosis may also affect the production of this glycoprotein. Therefore, greater sporulation, such as that occurring in ICLS, could signify a higher production of this glycoprotein. One hypothesis to explain the results obtained is that the different systems harbor different fungal species. The dominant fungal species in ICLS could invest less in the production of extraradicular hyphae (responsible for production of glomalin) and more in intraradicular structures (Sousa et al., 2012).

Acid phosphatase activity was found to be significantly reduced ($p < 0.05$) in ICLS (Table 3), as well as in the N0 treatment (Table 4), independent of the species used (Table 5), showing that such enzymes are sensitive to land-use change. Shade provided by trees in the ICLS - as high as 56% in relation to FS - affected pasture growth (Pontes et al., 2016). The lower production of plant biomass and the deposition of residue on the soil surface leads to lower substrate production of plant biomass to promote microbial growth and, consequently, for the production of enzymes (Balota et al., 2004). In addition, the activity of these enzymes is highly correlated with the total N content in the soil, since microbial activity can suffer a reduction under conditions of N deprivation (Dick et al., 1988). In fact, the highest levels of N present in the soil, mainly in nitrate form, were observed in the FS system ($p < 0.10$, Table 3) and in the N300 treatment ($p < 0.05$, Table 4). The lower N availability in the ICLS could be attributed to lower deposition of residues (aerial and root) owing to lower forage productivity. Therefore, the benefits of litter input from trees to availability of N in the soil were not sufficient to overcome the negative impact of high overhead sources of sunlight restriction on crop yield.

Table 5: Microbiological attributes of the soil (mean ± standard deviation) for different forage species (n = 24). See Table 3 for abbreviations of the attributes. Mm, *Megathyrsus maximus* cv. Aruana; Ub, *Urochloa brizantha* cv. Marandu; Ha, *Hemarthria altissima* cv. Flórida; Ac, *Axonopus catharinensis*; Cs, *Cynodon* spp. cv. Tifton 85

	Mm	Ub	Ha	Ac	Cs	F	p
MC (%)	38 ± 2.2bc	37 ± 2.1bc	43 ± 2.2b	50 ± 2.1a	36 ± 2.5c	7.4	< 0.001
SPO (50 mL^{-1} soil)	40 ± 6.2ab	57 ± 7.8a	36 ± 5.5b	53 ± 9.7ab	45 ± 5.6ab	2.3	0.0615
AP (µg p-nitrophenol g^{-1} soil h^{-1})	299 ± 18.7ab	292 ± 15.6ab	280 ± 15.9b	317 ± 14.7a	280 ± 11.6b	1.5	0.2131
EEG (mg g^{-1} soil)	2.5 ± 0.09a	2.5 ± 0.08a	2.6 ± 0.09a	2.6 ± 0.11a	2.6 ± 0.15a	0.5	0.7757
TG (mg g^{-1} soil)	4.6 ± 0.21a	4.5 ± 0.27a	5.1 ± 0.36a	5.1 ± 0.31a	5.1 ± 0.35a	1.3	0.2909
MBN (µg N g^{-1} soil)	113 ± 8.6a	101 ± 6.5a	111 ± 16.0a	109 ± 10.0a	97 ± 4.7a	0.4	0.7807
MBC (µg C g^{-1} soil)	144 ± 9.5b	160 ± 10.5ab	160 ± 11.7ab	185 ± 12.8a	166 ± 10.7ab	2.3	0.0668
NH$_4^+$-N (µg g^{-1} soil)	2.1 ± 0.20b	2.0 ± 0.23b	2.0 ± 0.20b	2.5 ± 0.27a	2.1 ± 0.16b	2.4	0.0526
NO$_3^-$-N (µg g^{-1} soil)	36 ± 2.8a	28 ± 2.6b	33 ± 3.6ab	29 ± 2.6ab	30 ± 2.7ab	2.4	0.0560

F and p, F-ratio and probability for the forage species effect. Means followed by the same letter in the row do not differ from each other by the Fisher test (LSD) at 5% significance.

In addition, the N of the soil microbial biomass was also significantly lower in the ICLS (Table 3), particularly in the intermediate position to the trees (P5, Table 6). Despite the lower deposition of forage residues in the ICLS, and reducing the amount of mineral N in the soil, this residue presents a higher concentration of N (Pontes *et al.*, 2016). The higher the concentration of N in the litter, the higher its rate of decomposition (Batista *et al.*, 2018), and the lower the N that gets accumulated in the microbial biomass.

Significant interactions between the systems and N doses (F = 4.39, p < 0.05, Figure 2) and between the species and N doses (F = 3.32, p < 0.05, Figure 3) were evident for the soil N concentrations as nitrates. Nitrogen fertilization increased the nitrate content in the FS and ICLS systems by 63% and 17%, respectively (Figure 2), the only exception being the *H. altissima* species (Figure 3). Pontes *et al.* (2017) emphasized the poor response of this species to N fertilization with regards to the dry matter yield.

It is clear that no remarkable differences were present between species for MBN (Table 5). With respect to MBC (Figure 3), differences among the species were more significant, in the range of 134 ± 8.51 (Mm, N300) and 207 ± 22.2 (Ac, N0) µg C g^{-1} soil. For this attribute, marked interactions were noted between the N doses and species (F = 3.38, p < 0.05). Generally, in the MBC, the levels were seen to decline after N fertilization, the exception being the *H. altissima* species (Figure 3). Delbem *et al.* (2011) also identified higher MBC values in the treatments lacking N; they held the soil acidification responsible for this effect, after high N doses were applied. The higher MBC levels suggested an increased, though temporary, immobilization of the nutrients and therefore, decreased nutrient loss in the soil-plant system (Gama-Rodrigues *et al.*, 2005).

For this variable, the differences between the tree stand positions were noted in the N300 fertilization treatment alone (Figure 4), with higher values in the intermediate position (F = 4.14, p < 0.05). In the tree

Table 6: Microbiological attributes of the soil (mean ± standard deviation) for the different positions between the tree rows (n = 30). Refer Table 3 for the attribute abbreviations. P2, 2 m; P5, 5 m and P10, at 10.5 m distance from the trees

	P2	P5	P10	F	p
MC (%)	44 ± 2.6a	39 ± 2.4a	43 ± 1.9a	1.9	0.1618
SPO (50 mL^{-1} soil)	43 ± 5.7b	58 ± 7.3a	57 ± 7.4ab	2.8	0.0660
AP (µg p-nitrophenol g^{-1} soil h^{-1})	281 ± 11.6a	269 ± 12.6a	262 ± 8.3a	0.7	0.5088
EEG (mg g^{-1} soil)	2.5 ± 0.10a	2.5 ± 0.13a	2.5 ± 0.10a	0.2	0.8149
TG (mg g^{-1} soil)	4.5 ± 0.22a	4.8 ± 0.30a	4.5 ± 0.31a	0.6	0.5700
MBN (µg N g^{-1} soil)	118 ± 13.6a	88 ± 4.4b	91 ± 7.0b	4.7	0.0112
MBC (µg C g^{-1} soil)	154 ± 9.6a	165 ± 12.7a	153 ± 8.1a	0.6	0.5666
NH$_4^+$-N (µg g^{-1} soil)	2.0 ± 0.20a	1.6 ± 0.12b	1.7 ± 0.14ab	3.4	0.0363
NO$_3^-$-N (µg g^{-1} soil)	28 ± 1.7a	29 ± 2.9a	31 ± 2.3a	0.6	0.5486

F and p, F-ratio and probability for the position effect between the tree rows. Means followed by the same letter in the row do not differ from each other by the Fisher test (LSD) at 5% significance.
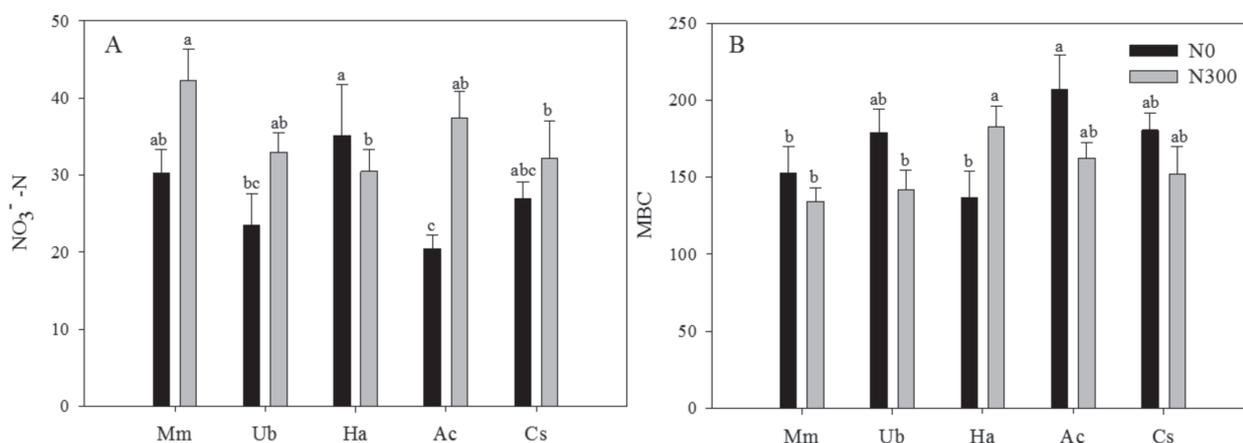

Figure 3: Means (n = 12) of N concentrations available in the soil as nitrates (A, NO$_3^-$-N, ìg g^{-1} soil) and soil microbial biomass carbon (B, MBC, ìg C g^{-1} soil) for each species and N doses (N0, without nitrogen fertilization, N300, 300 kg of N ha^{-1} year^{-1}). Refer to Table 5 for species abbreviations. Means followed by the same letter at each N dose do not differ from one another by the Fisher test (LSD) at 5% significance.
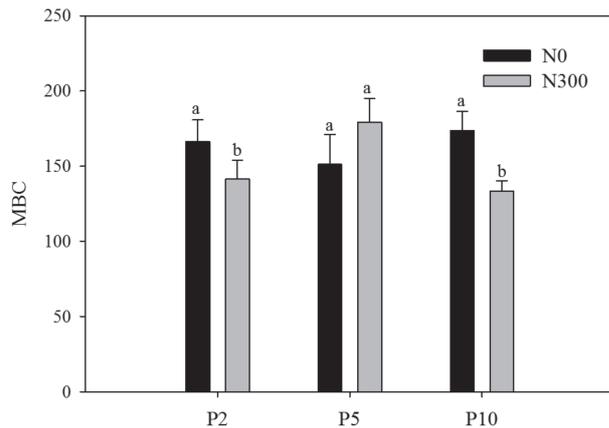
Figure 4: Means (n = 15) of the soil microbial biomass carbon (MBC, ìg C g^{-1} soil) for each position between the tree rows and in response to each N dose (N0, without N fertilization, N300, 300 kg of N ha^{-1} year^{-1}). Refer Table 6 for the abbreviations of the different positions between the tree rows. Means followed by the same letter at each N dose do not differ from one another by the Fisher test (LSD) at 5% significance.

systems, the differences between the positions suggest higher spatial heterogeneity.

In brief, inconsistent with our hypothesis, the microbiological attributes of the soil could not all be said to be positively influenced by their association with the *E. dunnii* trees. The high shade levels noted eight years post tree planting and the resultant negative influence on the yield of the related crops (Pontes *et al.*, 2016), are the likely reasons for these responses. On average, in the tree system the decreased PAR was found to be $61 \pm 4.03\%$, $50 \pm 4.47\%$ and $56 \pm 4.64\%$ for P2, P5 and P10, respectively. Therefore, it was understood that thinning was more frequently necessary to reduce the degree of shading and thus, maximize the advantages of these integrated systems.

CONCLUSIONS

Mycorrhizal colonization, sporulation of mycorrhizal fungi and carbon contents of the microbial biomass showed a rise in soils cultivated with the forage grass species *Axonopus catharinensis*, which is thus another reason to include such species in the woody conservation systems.

Nitrogen fertilization on the one hand encouraged greater mycorrhizal colonization and acid phosphatase enzyme activity, while on the other, it caused the soil microbial biomass to decrease.

Compared with the system exposed to full sun (or monocultures), the integrated crop-livestock system with trees encouraged the arbuscular mycorrhizal fungi to get established, revealing higher colonization and sporulation rates. However, in this type of system the soil microbial biomass and acid phosphatase enzyme activity declined.

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