Journal of Biotechnology and Biodiversity | v.9 | n.2 | 2021

Journal of Biotechnology and Biodiversity

journal homepage: *https://sistemas.uft.edu.br/periodicos/index.php/JBB/index*

Carbon and nitrogen dynamics in a successional agroforestry system in the Neotropics

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INFO

Keyworks
carbon stable isotopes
foliar nitrogen resorption
litter decomposition
nitrogen stable isotopes
soil nitrous oxide emission

ABSTRACT

The present study aimed to assess the effect of fourteen years of implementation of a successional and biodiverse agroforestry system (AFS) in a degraded agricultural field located in the Cerrado region of Central Brazil on the carbon and nitrogen dynamics. To track short term soil N dynamics we sampled instantaneous soil N rates in four seasonal periods (wet-dry, dry, dry-wet, wet) and to track long term C and N dynamics we measured C and N stable isotopes in the plant-litter-soil system. As additional data we determined the aboveground biomass; resorption rates of foliar and, soil C and N stocks. The measured aboveground biomass was 19.2 Mg C ha^{-1}. The mean resorption rate of foliar N was 49.3%. C:N ratio was 20.4 ± 1.4 and 14.2 ± 0.32 in the litter layer and the topsoil, respectively. Soil $N\text{-}NH_4^+$ was predominant over $N\text{-}NO_3^-$. After 40 days, the cumulative $N\text{-}N_2O$ emission was 0.33 kg ha^{-1}. The mean C and N stocks were 3.8 Mg N ha^{-1} and 43.6 Mg C ha^{-1}, respectively. The averaged soil $\delta^{15}N$ was 6.8 ± 0.6‰. Soil $\delta^{13}C$ was -20.3 ± 0.5‰. After 14 years of implementation, approximately 40% of the total C in the topsoil (0-20 cm depth) was derived from the AFS biomass input, predominantly from the C_3 photosynthetic pathway. The studied biodiverse AFS that replaced a degraded agricultural field in the Cerrado region showed to be responsive both in terms of soil and plant C and N pools and fluxes.

RESUMO

Dinâmica de carbono e nitrogênio em um sistema agroflorestal sucessional na região neotropical. O presente estudo teve como objetivo avaliar o efeito de quatorze anos de implementação de um sistema agroflorestal (SAF) sucessional e biodiverso em uma área agrícola degradada localizada na região de Cerrado do Brasil Central sobre a dinâmica de carbono e nitrogênio. Para verificar a dinâmica de N no curto prazo foram feitas medidas das taxas de transformação de N no solo em quatro períodos sazonais (chuva-seca, seca, seca-chuva e chuva) e para determinar a dinâmica de C e N no longo prazo foram analisadas as razões isotópicas de C e N no sistema planta-serapilheira-solo. Além disso, os seguintes parâmetros foram medidos: biomassa acima do solo, taxas de retranslocação de N foliar, e estoques de C e N no solo. A biomassa acima do solo foi de 19,2 Mg C ha^{-1}. A taxa média de reabsorção de N foliar foi de 49,3%. A relação C:N foi de 20,4 ± 1,4 e 14,2 ± 0,32 na camada de serapilheira e na camada superficial do solo, respectivamente. O N-NH4 + do solo foi predominante sobre o $N\text{-}NO_3^-$. Após 40 dias, a emissão cumulativa de $N\text{-}N_2O$ foi de 0,33 kg ha-1. Os estoques médios de C e N foram de 3,8 Mg N ha^{-1} e 43,6 Mg C ha-1, respectivamente. O valor médio de $\delta^{15}N$ do solo foi de 6,8‰, enquanto o valor médio de $\delta^{13}C$ no solo foi de -20,3‰. Após 14 anos de implementação, aproximadamente 40% do C total na camada superficial do solo (0-20 cm de profundidade) mostrou ser derivado da entrada de biomassa oriunda do SAF, que é predominantemente do tipo fotossintético C_3. O SAF estudado, que substituiu um campo agrícola degradado na região do Cerrado, mostrou-se responsivo tanto em termos de estoques e fluxos de C e N do solo e da planta.

Palavras-chaves
isótopos estáveis de carbono
isótopos estáveis de nitrogênio
retranslocação de N foliar
decomposição da serapilheira
emissão de óxido nitroso

Received 17 October 2020; Received in revised from 18 November 2020; Accepted 31 January 2021

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n2.nardoto

INTRODUCTION

The expansion of agriculture in the Cerrado region has been carried out using management practices with several impacts on the ecosystem biogeochemistry. For example, modifications in both carbon (C) and nitrogen (N) dynamics have been reported (Pinheiro-Alves et al., 2016) with significative increases in greenhouse gases (GHG) emissions to the atmosphere (Carvalho et al., 2016; Carvalho et al., 2017; Sato et al., 2017; Figueiredo et al., 2018; Sato et al., 2019). Approximately 50 million hectares of the Cerrado has already been replaced with pastures with exotic C_4 African grasses that are usually managed for cattle production (Sano et al. 2010). About 40% of the Cerrado pastures are currently degraded, mostly in areas with a cattle carrying capacity below 1.0 AU ha^{-1} (Pereira et al., 2018). Management agricultural practices such as seeding, fertilizer application, and harvesting lead to a disturbance that precede possible reforestation management. Although studies about C and N dynamics during secondary succession after agricultural abandonment have been reported (Davidson et al., 2007; Amazonas et al., 2011), there are still many questions to be answered on the dynamics of C and N in restored agricultural fields in the Neotropics.

Agroforestry systems (AFS) have been an alternative for ecological restoration (Martinelli et al., 2019). These AFS gained prominence in Brazil with the publication of the National Plan for the Recovery of Native Vegetation, Decree no. 8.972 (Brasil, 2017), which imposes the recomposition of 12 million hectares in 20 years, being part of those with AFS established in legally protected areas, that allowed its adoption in small properties. AFS are considered integrated production systems that can provide environmental services, food, and nutritional security and have been used to restore the production capacity of degraded areas (Martinelli et al., 2019).

Some recent advances in this field of knowledge have highlighted the use of stable isotopes as interesting restoration indicators for soil protection and nutrient cycling. However, very little is known about how carbon (C) and nitrogen (N) fluxes and pools in AFS respond after pasture abandonment in the Neotropics. On the other hand, it is well known that land-cover and land-use changes (LCLUC) usually imply modifications on the ecosystem functioning, especially on C and N pools at the soil-plant system (Pinheiro-Alves et al., 2016). Furthermore, LCLUC influences the greenhouse gas fluxes related to soil organic matter decomposition (Ferreira et al., 2016; Figueiredo et al., 2018).

Considering the contributions of N_2O to the atmosphere, approximately 70% of the total N_2O emitted are from soils and mostly related to agricultural practices such as N fertilizer application and residue management (Santos et al., 2016; Campanha et al., 2019). However, several reports have already shown that sustainable agricultural management intensification practices may reduce agricultural N_2O emission at the source (Santos et al., 2016; Carvalho et al., 2017; Sato et al., 2017; Figueiredo et al., 2018). Such information is crucial to undertang the effects of ecosystem restoration on soil organic matter dynamics in the short term (Figueiredo et al., 2018), together with the determination of the concentration of inorganic N (ammonium and nitrate), as well as the net rates of N mineralization (Nardoto and Bustamante, 2003).

On the other hand, the stable isotope analysis (SIA) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in the soil-plant system has been used to access soil organic matter dynamics over the long term (from years to decades). The $\delta^{13}C$ is usually used to estimate the carbon incorporation rate derived from plant litter decomposition, both for natural and different land uses (Assad et al., 2013). The $\delta^{15}N$ integrates N dynamics and N losses to the atmosphere over the leaf lifespan (Craine et al., 2009), and over the decades that organic matter is decomposing in the soil (Craine et al., 2015). Therefore, terrestrial ecosystems with intensified N dynamics and N losses to the atmosphere have higher $\delta^{15}N$ in the soil-plant system because of the mineralization and decomposition of N changes according to the local environmental conditions (Craine et al., 2009; Craine et al., 2015).

In this context, the present study aimed to assess the effect of fourteen years of implementation of a successional and biodiverse agroforestry system (AFS) in a degraded agricultural field located in the Cerrado region of Central Brazil on the carbon and nitrogen dynamics. To track short term soil N dynamics we sampled instantaneous soil N rates in four seasonal periods (wet-dry, dry, dry-wet, wet) and to track long term C and N dynamics we measured C and N stable isotopes in the plant-litter-soil system.

MATERIAL AND METHODS

Field area

The study was carried out in the Northeastern region of Distrito Federal, Brazil (15°34'51" S, 47°22'42"W; WGS 84, UTM, Zone 23S). The climate is tropical humid savanna (Aw-Koppen).

The meteorological data were recorded at an automatic weather station (Campbell Scientific CR 1000) installed near the study area. The annual

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n2.nardoto

rainfall was around 1697 mm during the experimental period (March 2014 to March 2015) with a pronounced wet season between November and April (Figure 1), representing 70% of the total annual precipitation.

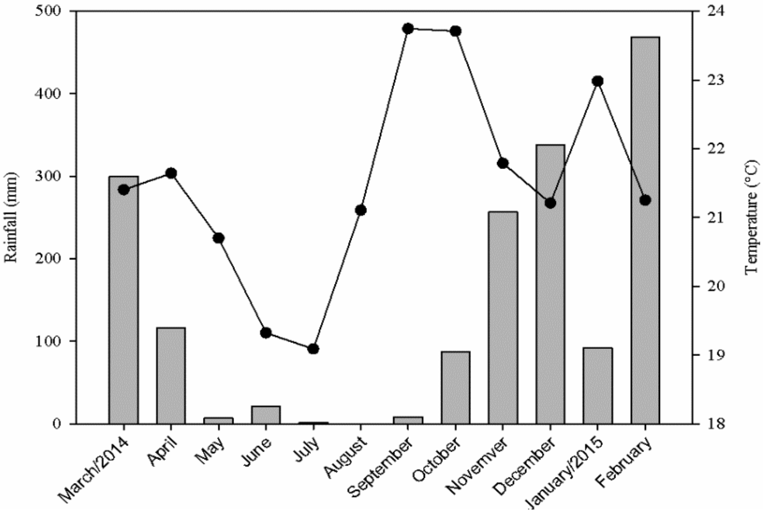


Figure 1 - Monthly accumulated rainfall and mean temperature during the experimental period, in the Distrito Federal, Brazil.

The soil of the experimental area (Table 1) is classified as sandy clay Rhodic Hapludox. Initially, the studied area was a dense forest formation. It was then cleared, and, for about 20 years, it was cultivated according to the following sequence: maize and soybean rotation, orange plantation. After this period, of cultivation, the area remained abandoned for about a decade, during which grasses of the genus Urochloa invaded and dominated the landscape. Before the AFS implementation, for about four years, the C4 grasses were managed by selective weeding and with the inclusion of cover crops (Mucuna pruriens and Canavalia ensiformis). for about four years. In 2001, the AFS was implemented using a blend of cover crops (Cajanus cajan, Leucaena spp, and Pennisetum purpureu) that were planted using the method of direct seeding in lines that were intercalated with exotic fruit trees, hardwoods and some native trees planted using the transplanting method. The cutting management of Pennisetum purpureum was performed from 2001-2006, intensively in the rainy season due to the increase of biomass in this period. In 2006, Morus nigra and Leucaena spp were the dominant species in the system. With well-developed tree canopy, Pennisetum purpureum was taken out of the system. Between 2006 and 2010, approximately 50% of the Leucaena spp and Morus nigra biomass were incorporated as wood litter in the soil surface. From 2010 to 2014, Inga sp. was the dominant species in the system. No fertilizers were applied in the soil since the AFS was implemented.

Table 1 - Soil chemical characteristics of AFS area studied for the range of 0 to 20 cm depth. K^+, Ca^{2+}, Mg^{2+} were determined by ion exchange resin. H+Al was determined by pH SMP method.

Soil characteristics	**Value**
Clay (g kg^{-1})	388 ± 2.0
Silt (g kg^{-1})	137 ± 1.2
Sand (g kg^{-1})	475 ± 2.4
pH in $CaCl_2$	4.4 ± 0.3
pH in H_2O	5.4 ± 0.1
H+Al (cmolc$_c$ dm^{-3})	4.2 ± 0.9
K^+ (cmolc$_c$ dm^{-3})	0.4 ± 0.1
Ca^{2+} (cmolc$_c$ dm^{-3})	4.1 ± 0.8
Mg^{2+} (cmolc$_c$ dm^{-3})	1.1 ± 0.1
Exchangeable bases (cmol$_c$ dm^{-3})	5.6 ± 0.8
Base saturation (V%)	57.2 ± 0.8
Cation exchange capacity (cmolc$_c$ dm^{-3})	9.8 ± 0.5

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n2.nardoto

Vegetation sampling and analysis

Every woody individual with the diameter at breast height (DBH) ≥ 5 cm were measured, and their total height (m) was visually estimated. The aboveground biomass was estimated using the equation proposed by Kurzatkowski et al. (2007) for AFS as follows:

$$V= ((ðDBH^2)/4xHxFF) \quad \text{(Equation 1)}$$

where V= volume; ð= ~3.142; DBH = diameter at breast height; H= height; FF = 0.65.

The C stock was considered 45% of the estimated aboveground biomass (Whittaker, 1975).

Mature leaves were sampled from three individuals of each 30 species from the AFS were sampled for elemental and isotope analysis (%C, %N, $\delta^{13}C$, and $\delta^{15}N$). In total, 100 individuals were sampled (67 legume trees and 33 non-legume trees), from 24 species, 24 genders, and 10 families. The potential occurrence of biological nitrogen fixation (BNF) was considered when the difference between the mean foliar $\delta^{15}N$ of a single legume tree and the mean foliar $\delta^{15}N$ of the non-legume trees (2.7‰) was ≥ 1‰ following Nardoto et al. (2014).

Resorption rates of foliar N were determined from fully expanded leaves of three individuals of 12 tree species in the AFS (*Magnifera indica*, *Anona muricata*, *Caesalpinia peltophoroides*, *Centrolobium tomentosum*, *Hymenaea courbaril*, *Schizolobium parahybae*, *Persea americana*, *Artocarpus integrifolia*, *Morus nigra*, *Musa sp.*, *Coffea arabica*, and *Inga edulis*) as well as abscised leaves of the same tree species. Resorption efficiency (MRE) was calculated from N concentration in fresh and abscised leaves (Aerts, 1996):

$$MRE= 100\% \,.\, (1-[Nutrient]\,abscised/[Nutrient]fresh). \quad \text{(Equation 2)}$$

The mass lost during senescence was estimated using the compiled data from Heerwaarden et al. (2003), with an average value of 21% (n=126 species from different ecosystems and phenological groups) for mass loss during senescence.

For the plant litter decomposition experiment, litter samples were collected in 0.25 x 0.25 m quadrats during the dry season. To fill the litterbags were used mixtures of only leaf litter. A total of 30 litterbags, each containing 10g of the dried material, were used in the experiment. The litterbags were placed on the soil of the AFS and placed right above the soil layer. Further, the litterbags were sampled after 15, 60, 90, 180, and 260 days that had been.. The litterbags contents were oven-dried at 65°C for 72 h and weighed. The leaf material weight loss was obtained by the difference between the initial mass amount (100%) of the litterbags, and the reminiscent mass amount after every sampled time. The decomposition rate was obtained through the decomposition constant (K') and the decay constant (K) following Olson (1963). K' refers to the decomposed material amount during a time interval, and K refers to the instantaneous decomposition ratio. The model equation proposed by Olson (1963) is:

$$K'=A_x/X_o, \text{ and } K=-ln\,(1-K') \quad \text{(Equation 3)}$$

Where X_o is the initial mass, A_x is the mass at certain time.

Soil Sampling and Analysis

The soil was sampled using a Dutch auger. For %C, %N, $\delta^{13}C$, and $\delta^{15}N$ analysis, soil was sampled at the 0-5, 5-10, 10-20 cm intervals. For inorganic N, and gravimetric moisture, soil was sampled at the 0-10 cm interval. Five sampling points were used for every measurement well distributed along the area, considering the borders and middle. To estimate the contribution of C_4 sources in the soil organic matter (SOM), it was used a two end-member mixing model (see Balesdent and Mariotti,1988):

$$C_4(\%) = \delta^{13}C_{AFS\ soil} - \delta^{13}C_{AFS\ litter} / \delta^{13}C_{pasture\ litter} - \delta^{13}C_{AFS\ litter} \quad \text{(Equation 4)}$$

Where the $\delta^{13}C_{AFS\ litter}$ used was -28.4‰, and $\delta^{13}C_{pasture\ litter}$ was -15.2‰.

Soil $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ were determined colorimetrically using a Lachat Quikchem FIA (Lachat Instruments, 5600 Lindburg Drive, Loveland CO 80539 USA) after extraction with KCl 1 mol L^{-1}.

The N_2O fluxes were measured during the following seasons: rainy to dry transition (wet-dry); dry; dry to rainy transition (dry-wet); and rainy, in 23 evaluation events, 14 of which occurred between March and November 2014, and 9 between January and February 2015. Each chamber consisted of a rectangular hollow metal frame (38 cm wide, 58 cm long, 6 cm in height) (Figure 2), which was inserted 5 cm into the soil and a top polyethylene cap, coated with a thermal aluminum blanket, that was coupled to the base during gas sampling.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n2.nardoto

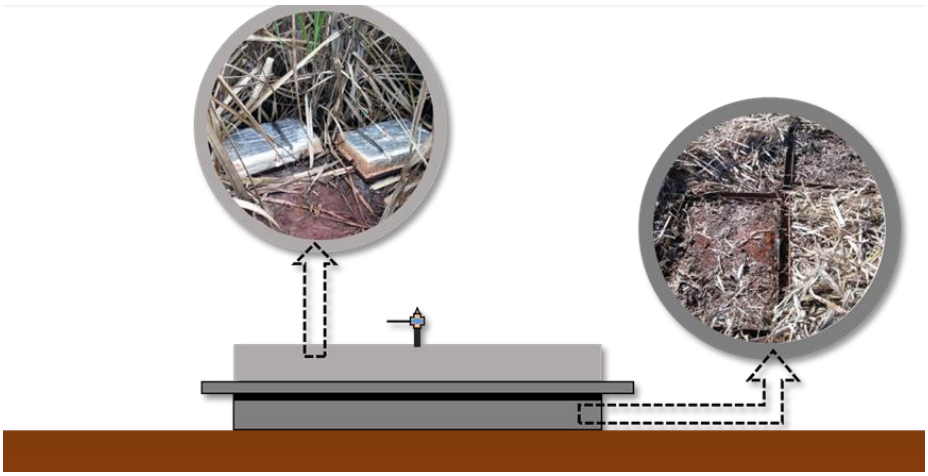


Figure 2 - Scheme illustrating the implementation of the chambers for the N_2O measurements in the field.

The top of the cap contained a triple Luer valve for fastening the sampling syringes from which gas samples were withdrawn. A digital thermometer was also coupled to one of the five chambers to monitor the inside temperature of the chambers. The samples were collected in 60 mL polypropylene syringes and immediately transferred to 20 ml glass pre-evacuated vials (-80kPa). Air samples were collected from the interior of each chamber at zero (T0), 15 minutes (T15), and 30 minutes (T30) after closing the chambers and always between 9:00 and 10:00 AM to represent better the daily mean flux as proposed by Alves et al. (2012). N_2O concentrations were determined by Gas Chromatography (Trace 1310 GC ultra, Thermo Scientific ™) equipped with a Porapak Q column at 65 °C, an electron capture detector (ECD). Based on a calibration curve, the calculated detection limit was 55 ppb for N_2O, and the calculated quantification limit was 154 ppb for N_2O. N_2O fluxes were calculated by the linear variation in gas concentration in relation to the incubation time in the closed chambers, and calculated by the following equation, as proposed by Bayer et al. (2015):

$$\text{Flux} = \delta C/\delta t\ (V/A)\ m/Vm \qquad \text{(Equation 5)}$$

Where the flux ($\mu g\ m^{-2}\ h^{-1}$); $\delta C/\delta t$ is the change in gas concentration (nmol N_2O and CH_4 h^{-1}) in the chamber in the incubation interval (h); V and A are, respectively, the chamber volume (V) and the soil area covered by the chamber (m^2); m is the molecular weight of N_2O and CH_4 (μg), and Vm is the molar volume at the sampling temperature (Vm). The accumulated flows of N-N_2O in each plot were estimated by the integrated trapezoidal area of the daily N-N_2O flux by time, assuming that the fluxes change linearly between the measurements (Bayer et al., 2015).

Soil bulk density was sampled with a undistubed sample auger with volumetric rings of 100 cm^3. Soil C and N stocks were calculated with a correction for the soil thickness, following Veldkamp (1994).

Soil particle density was determined by the ring and volumetric flask methods, respectively. Soil moisture was calculated by oven-drying a soil sub-sample of known weight at 105 ºC for 48 hours. From these variables, the water-filled pore space (WFPS) was calculated for each gas sampling date and determined by the following equation:

$$\text{WFPS} = (\theta\text{x Da})/\ [1 - (\text{Da}/\text{Dp})]\ \text{x}\ 100$$
(Equation 6)

Where θ is the soil moisture (g g^{-1}); Da the apparent soil density (g cm^{-3}); and Dp the soil particle density (2.65 g cm^{-3}).

Elemental and isotopic analysis

From the prepared material, subsamples of 1.5-2 mg of leaf and litter or 25-30 mg of soil were placed and sealed in tin capsules and loaded into a ThermoQuest-Finnigan Delta Plus isotope ratio mass spectrometer in line with an Elemental Analyzer (Carlo Erba model 1110; Milan, Italy). The $\delta^{13}C$ and $\delta^{15}N$ were measured relative to recognized international standards. Stable isotope values are reported in "delta" notation, as δ values in parts per thousand (‰), so that:

$$\delta‰ = (\text{R sample}/\ \text{R standard} - 1) \times 1000 \ \text{(Equation 7)}$$

Where R is the molar ratio of the rare to abundant isotope ($^{15}N/^{14}N$; $^{13}C/^{12}C$) in the sample and the standard.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n2.nardoto

Statistical Analysis

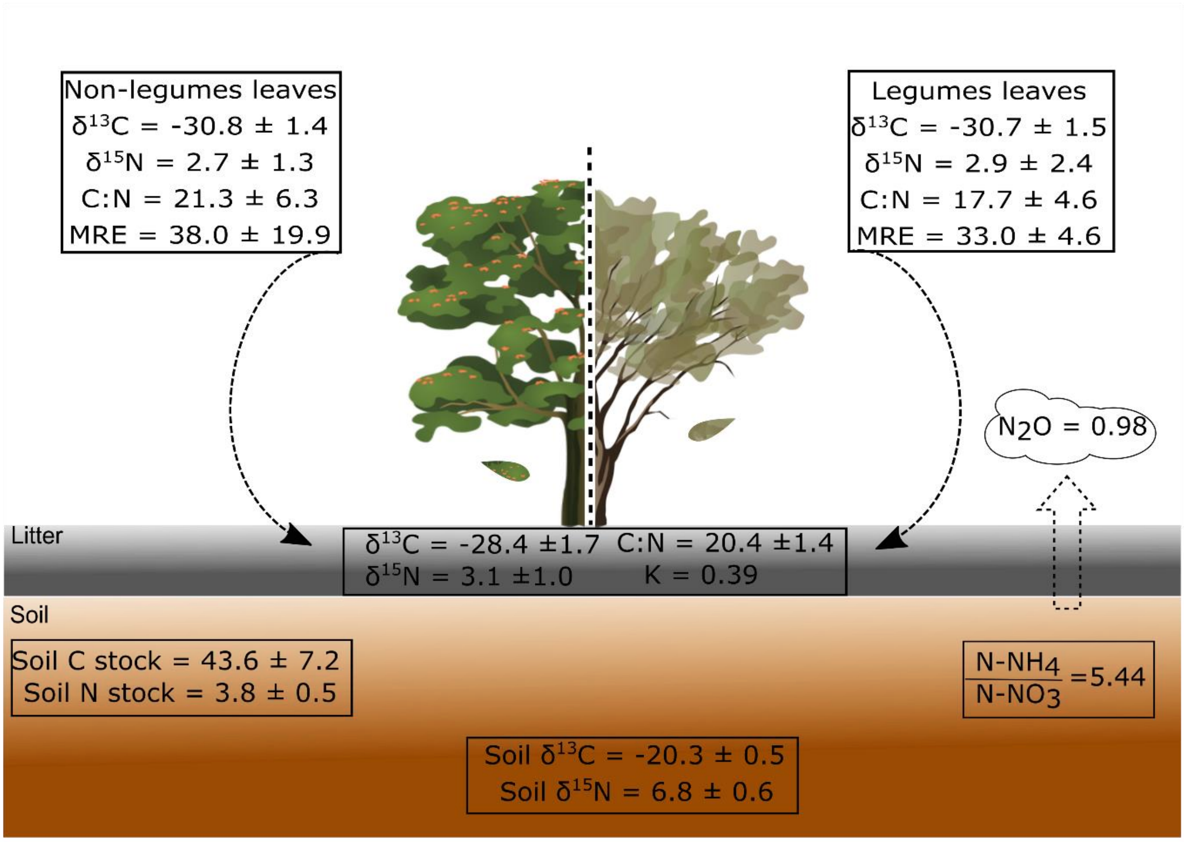
The Shapiro-Wilk test verified the normality of the data of legume and non-legume trees. As data showed a normal distribution, it was applied an unpaired t-test to assess differences between legumes and non-legumes (α = 0.05), the normality of the residuals was also verified regarding the variance homogeneity. A one-way ANOVA was conducted to compare the effect of the seasons periods in $N\text{-}NH_4$, $N\text{-}NO_3$ and $N\text{-}N_2O$, with a post hoc comparison using Tukey HSD (α = 0.05). All statistical procedures were carried out in R (R Development Core Team, 2020).

RESULTS

Plant and litter

The mean DBH of the legume trees was 5.6 cm (median = 4.6 cm, coefficient of variation = 72%) while for the non-legume trees, the mean DBH was 4.3 cm (median = 3.12 cm, coefficient of variation = 68%). The aboveground biomass of the legume trees was 20.8 Mg ha^{-1}, and the non-legume trees was 7.6 Mg ha^{-1}, totalizing 28.4 Mg ha^{-1}, which corresponded to 19.2 MgC ha^{-1} stored in the aboveground biomass.

Foliar N concentration had significant differences when comparing legume trees (2.9 ± 0.8) and non-legume trees (2.2 ± 0.6) ($p < 0.05$). The foliar N resorption rate ranged from 27.6 to 39.3%, with an average of 33.0% for the legume trees and ranged from 9.6 to 67.4% with an average of 38% for the non-legume trees (Figure 3). After correction for weight loss, the resorption rate of foliar N was about 49.3% considering both legumes and non-legumes. Foliar C:N ratio was significantly higher in non-legume trees (21.3 ± 6.3) than legume trees (17.7 ± 4.6) ($p < 0.05$).



Values represent means ± SE. δ notation values is presented in parts per thousand (‰). MRE means measured resorption efficiency and is presented in percentage (%). K means decay constant. Soil C and N stock are presented in Mg ha-1. And N2O flux is presented in kg N-N2O ha-1. Aboveground biomass is presented in Mg C ha-1. Vector image of Acacia spp. (representing legume trees), courtesy of Kim Kraeerand and Lucy Van Essen-Fishman, vector image of Spathodea campanulate (representing non-legume trees), courtesy of Tracey Saxby, both are from Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary).

Figure 3 - Carbon and nitrogen dynamics in an agroforestry system in the Cerrado region of Central Brazil.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n2.nardoto

Legume trees (from the Fabaceae family) were separated into three groups according to their potential capability to have biological nitrogen fixation (BNF) associations (Table 2). In general, foliar $\delta^{15}N$ did not differ significantly between legumes (2.9 ± 2.4 ‰) and non-legumes (2.7 ± 1.3 ‰).

Table 2 - Species relation of leguminous trees planted in the studied AFS, with foliar values of $\delta^{15}N$, $\delta^{13}C$**,** N and C:N ratio. The occurrence of BNF was considered when the difference between the mean foliar $\delta^{15}N$ of a single legume tree and the mean foliar $\delta^{15}N$ of the non-legume trees (2.7‰) was ≥ 1‰ as proposed by Nardoto et al. (2014).

Groups	Species	BNF	Nodulating*	$\delta^{15}N$(‰)	$\delta^{13}C$(‰)	N(%)	C/N	(n)
Non nodulating	*Caesalpinia peltophoroides*	-	-	3.1	-29.8	2.4	19.5	2
	Cassia occidentalis	-	-	1.9	-30.8	2.5	19.4	1
	Hymenaea courbaril	-	-	3.1	-32.0	2.3	22.2	3
	Schizolobium parahybae	-	-	6.2	-30.6	2.7	18.6	3
Nodulating	*Centrolobium tomentosum*	+	+	-1.4	-30.1	2.2	20.5	3
	Copaifera langsdorffii	+	+/-	1.4	-29.8	2.0	25.1	4
	Dipteryx alata	+	+/-	1.3	-31.2	2.7	17.7	3
	Leucaena leucocephala	+	+	1.7	-28.6	4.5	10.2	4
Inactive Nodulating	*Clitoria racemosa*	-	+	2.4	-29.9	3.4	13.6	2
	Inga marginata	-	+/-	6.2	-32.8	3.9	12.0	3
	Parapiptadenia rigida	-	+	2.3	-30.4	3.1	16.8	3
	Piptadenia gonoacantha	-	+	5.4	-31.1	2.9	16.3	4
	Zygia sanguinea	-	+	3.2	-32.4	2.6	19.0	3

*+ means occurrence of nodules; - means absence of nodules; +/- means plants with register of presence and absence of nodules (following Moreira et al. 1992; Faria et al. 1989; Faria and Lima 1998).

The initial litter C:N ratio was 20.4 ± 1.4, and the average litter $\delta^{15}N$ was 3.1‰. The mean weight loss of the foliar litter in the litterbags was 67% after 210 days, and the decomposition coefficient (K) was 0.39.

Soil

The soil C stock for 0-20 cm depth was 43.6 (±7.2) Mg ha$^{-1,}$ while the soil N stock for 0-20 cm depth was 3.8 (± 0.5) Mg N ha^{-1} (Figure 3). The C: N ratio of the soil (0-20 cm depth) was 14.2 ± 0.3.

The N-N_2O fluxes ranged from 10.23 μg m^{-2} h^{-1} in the dry season to 58.6 μg m^{-2} h^{-1} in the dry-rainy season (Figure 4). The highest N-N_2O flux was observed during the transition between the dry-rainy season ($p < 0.05$). After 40 days, the cumulative N-N_2O emission was 0.33 kg ha^{-1}. WFPS varied between 33 to 64%, with the highest value reported during the rainy-dry season ($p < 0.05$) (Figure 4).

There was a variation in the concentration of N-NH_4^+ during the year in all sampled periods. However, there was a predominance of N-NH_4^+ compared to N-NO_3^- in every sampled period (Figure 4). The content of N-NH_4^+ ranged between 5.3 to 16.0 mg kg^{-1}, with the highest concentrations of N-NH_4^+ occurring in the rainy period ($p < 0.05$). The soil N-NO_3^- varied between 0.6 to 2.6 mg kg^{-1} and did not differ among periods ($p = 0.3$). N-NH_4^+/N-NO_3^- ratio was 5.44, considering all the sampled period.

The mean value of $\delta^{15}N$ in the 0-20 cm soil depth was 6.8‰. The mean value of $\delta^{13}C$ in the AFS soil (0-20 cm depth) was -20.3‰. Using soil $\delta^{13}C$ of an adjacent degraded pasture (-15.2‰) and mean value of litter $\delta^{13}C$ (-28.4‰), the isotope mixture model showed that after 14 years of conversion from a degraded pasture to AFS, the topsoil (0-20 cm depth) had about 40% of the organic C from C_3 plants.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n2.nardoto

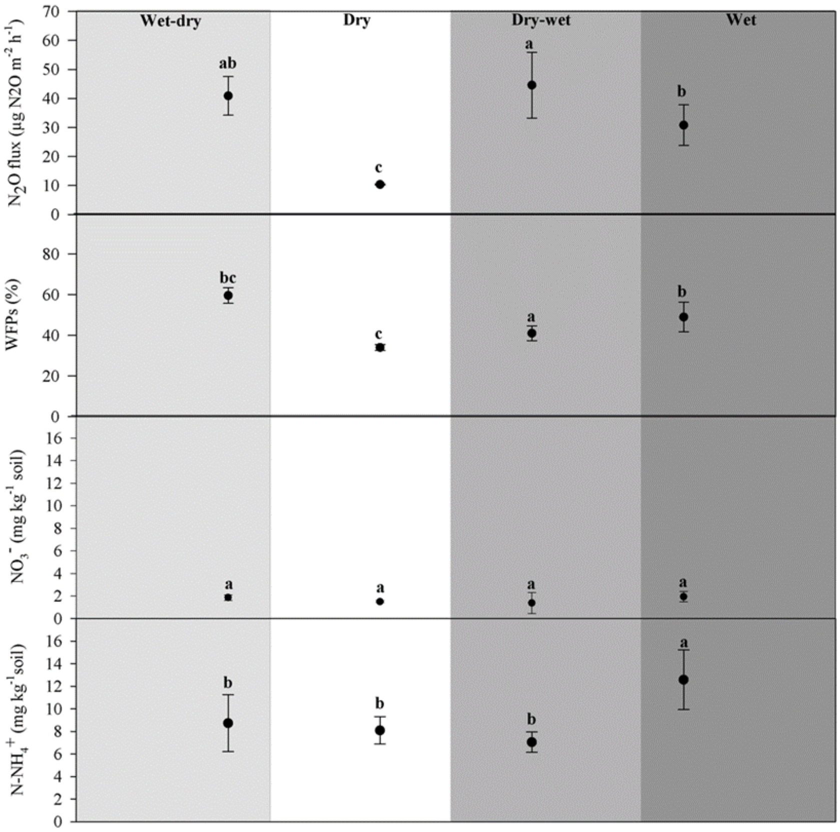


Figure 4. N_2O fluxes; soil water-filled pore space (WFPS); Nitrate ($N\text{-}NO_3^-$); ammonium ($N\text{-}NH_4^+$) (b) in soil under agroforestry system (AFS) in the season periods (wet-dry, dry, dry-wet, wet). Bars represent the standard error of the mean. Same letters in each graphic do not differ among sampling periods by Tukey test at 5% probability.

DISCUSSION

The input of foliar N from leguminous tree leaves in the AFS influenced soil N dynamics in the studied AFS. Consequently, the high input of foliar N contributed to decreasing litter C:N ratio and facilitated litter decomposition, associated to considerable N_2O fluxes with significant loss of N to the atmosphere compared to native Cerrado vegetation (Carvalho et al., 2016). Those indicators of the short-term N dynamics in the AFS are reflected in the soil $\delta^{15}N$, providing indirect evidence of N intensification in the soil-plant-litter system in the AFS.

The aboveground biomass stored in the studied AFS was higher than estimates of aboveground biomass for natural savanna formation areas in the Cerrado region (Lilienfein et al., 2001) and pasture lands (Lilienfein and Wilcke (2003). In addition, our estimate of the aboveground biomass was lower than Cerrado forestlands (Miranda et al., 2014) and *Pinus caribaea* plantations (Lilienfein and Wilcke, 2003).

Table 3 - Aboveground biomass comparison between the studied AFS and different land use contexts in Cerrado region.* Lilienfein and Wilcke (2003) evaluated the aboveground biomass of a 23 years old *Pinus* plantation.

Aboveground biomass ($Mg\ ha^{-1}$)	Land Use	Reference
6.4	Pasture	Lilienfein et al. (2003)
22.7	Typical Cerrado	Lilienfein et al. (2001)
29.4	Cerrado Denso	Castro and Kauffman (1998)
28.4	Agroforest System	This study
79.7	Cerrado forestlands	Miranda et al. (2014)
150.0	*Pinus caribaea**	Lilienfein and Wilcke (2003)

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n2.nardoto

The pattern of foliar $\delta^{15}N$ for legume trees indicated a high variability to fix atmospheric N. The high concentration of N foliar in legume trees, regardless of the N fixation, confirms that legume trees have a high need of N, influencing a low foliar C:N ratio and showing the importance of legume trees as N source to the AFS. The litter C:N ratio found in the AFS is lower compared to Cerrado species (Bustamante et al., 2012), as well as other AFS studied in Oxisols and Inceptisols in the Northeastern Brazil (Fontes et al., 2014). Some studies have reported that environmental conditions, pruning periods of successional plants, soil microorganisms, and litter quality can directly or indirectly influence decomposition rates in managed systems (Fontes et al., 2014).

In general, N availability in tropical savanna ecosystems in Brazil is low since 15 to 37% of N is resorbed before leaf senescence (Nardoto et al., 2006). In the Cerrado, inorganic N is made available by mineralization of soil organic matter (Nardoto and Bustamante, 2003). The annually mineralized inorganic N in unburned Cerrado does not exceed 15 kg ha^{-1} yr^{-1} (Nardoto et al., 2006). The low rates of nitrification in a typical cerrado area with a consequent predominance of N-NH_4^+ in soil (Nardoto e Bustamante, 2003) together with the high C:N ratios of litter (~60/1), contribute to the low rates of decomposition and mineralization of organic matter in the Cerrado, thus maintaining the low availability of N in this system (Bustamante et al., 2012). This shows that the quality of residues being incorporated or deposited into the soil influences N_2O emissions. High C:N ratio may increase N immobilization, reducing the occurrence of denitrification and, consequently, of GHG emissions (Alluvione et al., 2010).

Both soil and plant litter C:N ratio can serve as an estimate of N yield per unit of degraded soil organic matter, and as such, indicate how increasing soil C:N ratios can negatively affect mineralization rates (Booth et al., 2005). Nitrification has been found to vary inversely with soil C:N ratio, suggesting that increasing soil C:N ratios may promote NO_3^- assimilation or suppress NO_3^- production (Lovett et al., 2002).

In general, under conditions of high soil permeability, which reduces WFPS, and low relative NO_3^- production, the mineral N concentrations rarely exceed the N demand for microorganisms and plant roots (Bustamante et al., 2012). Although several studies reported correlations between GHG and mineral N in the soil (Santos et al., 2016; Carvalho et al., 2017; Figueiredo et al., 2018; Campanha et al., 2019), it is worth emphasizing that the gas emission pulses did not occur synchronously with the highest NO_3^- and NH_4^+ concentrations.

However, it should be considered that the transformations of N in an ecosystem (immobilization or mineralization) are coupled to C transformations, especially when organic carbon molecules are converted into CO_2 by soil heterotrophic microbial populations (McGill e Cole, 1981), which can reduce the partial pressure of oxygen and favor denitrification. Significant N_2O pulse emissions following the first rains after a dry season, often with a small-time lag, have been reported for different seasonally dry ecosystems and are generally preceded by significant CO_2 emissions immediately after the soil is re-moistened, due to water-induced activation of soil microbes (Carvalho et al., 2016; Carvalho et al., 2017; Sato et al., 2017).

Soil moisture expressed as WFPS, soil temperature, and mineral N content are the main variables that control and express GHG emissions (Bayer et al., 2015). The highest N_2O flux between dry-rainy season can be explained by rainfall influencing WFPS, as showed in other studies in integrated systems (Carvalho et al., 2017; Sato et al., 2017; Sato et al., 2018).

In this study, the N_2O cumulative fluxes from AFS were higher than those observed in different cerrado phytophysiognomies (Pinto et al., 2002; Santos et al., 2016; Carvalho et al., 2017; Sato et al., 2018, Figueiredo et al., 2018). The higher emissions under AFS, than cerrado phytophysiognomies can be related to the low foliar C:N ratio of legumes which, in turn, increases the N content in the soil (Carvalho et al., 2017; Sato et al., 2017). However, AFS emissions are lower than fertilized integrated systems like crop-livestock (ICL) and integrated crop-livestock forest (ICLF), also studied in the Cerrado region (Carvalho et al., 2017; Sato et al., 2018).

Despite the low pH in the AFS soil, the observed increased availability of N in the soil coupled with the high inputs of the litter with low C:N ratio can be potentially increasing bacterial biomass, following the same pattern observed by Catão et al. (2016) in Cerrado area under restoration. Those factors together with the relatively higher N_2O emissions compared with native Cerrado areas are probably influencing the higher soil $\delta^{15}N$ of the AFS compared with soils under native Cerrado areas (Bustamante et al., 2004; Coletta et al., 2009) but similar to Cerrado soils under restoration (Catão et al., 2016). As observed by Carvalho et al. (2017), Sato et al. (2017), and Sato et al. (2018), N_2O emissions in the integrated crop-livestock and integrated crop-livestock forest studied systems were influenced by rainfall seasonality, management intensity, crop rotation, and the relationship among these factors. Such patterns are indicative of a more dynamic biologic process under plant-soil management

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n2.nardoto

altering the C and N dynamics of the plant-litter-soil system regardless of the management applied in the Cerrado area.

CONCLUSION

The approach of using both short and long term parameters related to the plant and soil system to assess C and N dynamics in a successional and biodiverse AFS implemented in a degraded agricultural field showed to be effective in demonstrate how plant biomass input provided a mixture of N-rich litter with high decomposition rates, influenced by the low C:N ratio of the high percentage of legume trees in the AFS. Moreover, the C_3 biomass input has been changing the source of soil organic C, indicating the potential C stocking of AFS.

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ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n2.nardoto