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Full Length Research Paper

Pharmacognostic and phytochemical characterization of *Maerua angolensis* DC.

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Maerua angolensis DC is a medicinal plant widely used in ethnomedicine in northern Nigeria. It is used to treat disease conditions like skin infections, sexually transmitted diseases, peptic ulcers and wounds amongst others. The plant is well known in Fulani *Fulfude* as *leggal baali* (or *leggal mbaali*). The plant was subjected to pharmacognostic and physicochemical characterization to establish standard profiles for authentication of the plant which could be useful for further study on the plant. The chromatographic (TLC and HPLC) and phytochemical profiles were conducted along with the leaf microscopy and chemomicroscopy, using standard methods. The result established the chromatographic profile of the leaf extract. The qualitative phytochemical screening showed the presence of carbohydrates, saponins, anthraquinones and cardiac glycosides. The chemomicroscopy revealed the presence of lignin, cellulose, tannin, starch and oil, while mucilage and protein were not seen. The total ash content and moisture content were 12.1 and 7.0%, respectively and were within WHO limits. Extracts of the plant showed high hygroscopic character. The result provides good information for the authentication and use of the plant in further research and development.

Key words: *Maerua angolensis*, pharmacognostic character; phytochemicals, chromatographic profile.

INTRODUCTION

Many natural products are used in alternative medicine (Sevindik et al., 2017; Mohammed et al., 2020a). Especially in folk medicine, different plant species have been used in the prevention and treatment of diseases (Mohammed et al., 2018; Mohammed et al., 2020b). The medicinal plant *Maerua angolensis* DC. belongs to the genus *Maerua* of the Capparaceae (Capparidaceae)

family. Its synonyms include *M. bukobensis* Gilg & Gilg-Ben., *M. currorii* Hook. f., *M. emarginata* Schinz, *M. lucida* Hochst. ex A. Rich., *M. retusa* Hochst. ex A. Rich., *M. senegalensis* R. Br. ex A. Rich., *M. tomentosa* Pax, and *M. floribunda* Fenzl. It is known as *leggal baali* (or *leggal mbaali*) in Fulfude-Fulani.

M. angolensis is a tall tree that grows in tropical Africa

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and arid regions. It is widely distributed in the savannah region of tropical Africa to South Africa and Swaziland. It is absent from the high-rainfall areas. The tree size varies from medium to big, growing up to 10 to 20 m high. It is commonly found growing in bush and rocky areas, and planted on graves in Nupe area of Nigeria (Ayo et al., 2013). *M. angolensis* has a long history of use in traditional medicine especially in Nigeria and other West African countries where it is used as antidote for pains and wounds (Iliya et al., 2014). A hydroethanol extract yields of 17.6, 7.1 and 10.4% w/w d.m have been reported for the leaf, root and stem bark, respectively (Burkil, 2004). The parts commonly used in ethnomedicine, solely or in combination, are leaf, root, leaf and branches, or leaf and root (von Maydell, 1990; Tropical Plants, 2020). It is propagated by seed (Yusuf et al., 2017). The leaves are analgesic, and used either alone or with other plants, to treat a range of stomach troubles. The powdered leaves, taken with food, are prescribed for asthenia (weakness or loss of strength) and anorexia. The leaf is used to treat skin rashes and sexually transmitted disease. An infusion of the leaves is used to treat rheumatism (Yusuf et al., 2017). A snuff made from the leaves of *M. angolensis* and *Ximenia americana* L. is used to treat headaches. The leaf-sap is dropped into fresh wounds as an antiseptic dressing (Burkil, 2004; Yusuf et al., 2017; Tropical Plants, 2020). The whole plant is compounded as medicine for treating epilepsy (Benneh et al., 2018). The roots are used to treat hydrocoele, for influenza and for toothache. The root and bark decoctions are drunk as aphrodisiacs (Yusuf et al., 2017). The leaf, fruit and seed are used as sauces, condiments, spices, and flavourings. The leaf and root are used as pain-killers, and in arthritis, rheumatism, etc. The leaf is used in paralysis, convulsions and to manage psychosis, diabetes, peptic ulcer, diarrhea and spasm. The plant is also used to treat inflammation, cancer and cellular ageing (Meda et al., 2013). The leaves contain alkaloids, saponins, tannins, anthraquinones and flavonoids (Yusuf et al., 2017). It also contains carbohydrate, reducing sugars and cardiac glycosides (Meda et al., 2013; Ayo et al., 2013). The variety in Tanganyika in Tanzania was found to have alkaloids and saponin glycosides. Reported studies on the bark revealed glycosides, terpenes, tannins, flavonoids, saponins, carbohydrates, proteins, alkaloids and other constituents (Iliya et al., 2014).

Different solvent extracts of the leaf had been reported to exhibit antimicrobial activities (Benneh et al., 2018; Yusuf et al., 2017; Ayo et al., 2013). Yusuf et al. (2017) reported an activity of 200 µg/mL against clinical isolates of *Staphylococcus aureus* and *Escherichia coli* for the leaf ethanol-extract. A methanol extract of the leaf was found to be active against *S. aureus* (ATCC 13704), *Streptococcus pyogenes* (Local strain), *Corynebacterium ulcerans* (Local strain), *Bacillus subtilis* (NCTC 8230), *E. coli* (NCTC 10418), *Salmonella Typhi* (ATCC 9184), *Klebsiella pneumonia* (ATCC 10031), *Pseudomonas*

aeruginosa (NCTC 6750), *Neisseria gonorrhoeae* (NCTC 10341) and *Candida albicans* (ATCC 10231) at 50 mg/m (Ayo et al., 2013). The broad antimicrobial activity is believed to be responsible for the wound healing properties of the plant and its use in infectious diseases in ethnomedicine (Ayo et al., 2013).

The plant has been demonstrated to exhibit strong antioxidant activity by Meda et al. (2013). Further studies suggested that the bark is non-toxic in anti-inflammatory doses, supporting ethnomedical use of the plant in managing inflammation (Meda et al., 2013; Ayo et al., 2013).

Although there have been reported scientific studies on some biological activities on the plant, not much has been documented on the pharmacognostic and phytochemical characteristics of the plant towards aiding its authentication. This study aims at establishing pharmacognostic parameters and chromatographic profiles which could serve as reference data for authenticating the plant.

METHODOLOGY

Collection of material

The raw plant sample was submitted to NIPRD on the 7th of February 2020. The sample was authenticated by both ethnobotanist and taxonomist at the herbarium unit of the Department of Medicinal Plant Research and Traditional Medicine of NIPRD, and a voucher specimen was prepared.

Powdered leaf sample of the plant was subjected to the various studies including microscopy and chemomicroscopic evaluation, physicochemical characterization and chromatographic profiling.

Extraction

The pulverized leaf was macerated in solvent over 24 h. The solvents used were absolute ethanol and water. The ethanol extract was filtered and concentrated with the aid of rotary evaporator and dried over a water bath. The water extract was also filtered and freeze-dried.

Chromatographic profiling

Chromatographic profiling was conducted using the ethanol extract. The TLC profiling of the sample was done using TLC glass plate pre-coated with silica gel G60 F254, 0.2 mm layer. The plate was developed using the mobile phase composition of ethylacetate/petroleum ether of 6/4. Detection was in daylight, and under UV light at 366 and 254 nm. The retardation factors (R_f) of each spot were calculated.

For HPLC profiling, the HPLC system used was Shimadzu HPLC system consisting of Ultra- Fast LC-20AB prominence equipped with SIL-20AC autosampler; DGU-20A3 degasser; SPD-M20A UV-Diode array detector (UV-DAD); column oven CTO-20AC, system controller CBM-20Alite and Windows LC solution software (Shimadzu Corporation, Kyoto, Japan); column, VP-ODS 5 µm and dimensions (150 × 4.6 mm). The chromatographic conditions included mobile phase solvent A: HPLC grade water and solvent B: HPLC grade methanol; mode: isocratic; flow rate 0.8 ml/min;



Figure 1. Voucher specimen.

injection volume 2 μ l of extracts solution (50 μ g/ml) in the mobile phase; detection was at UV 254 nm wavelength. The HPLC operating conditions were programmed to give the following: solvent B: 20% and column oven (Adamu et al., 2020).

Phytochemical characterization

Qualitative phytochemical screening was carried out on the pulverized samples to test for carbohydrate, phenols, tannins, saponins, flavonoids and anthraquinones using standard methods as described by Evans (2005), Sofowora (1993), and Trease and Evans (1989).

Physicochemical characterization

Physicochemical parameters such as moisture content, total ash and water and alcohol extractive values were determined following African Pharmacopoeia (1986) and WHO Guidelines (1992).

Microscopy and chemomicroscopic evaluation

Chemomicroscopic studies of the pulverized leaf was done using reagents and stains like iodine, concentrated sulphuric acid (98%), concentrated hydrochloric acid (36%), ferric chloride, Sudan III, ruthenium red and phloroglucinol with conc. HCl (1:1) to test for the presence of lignin, cellulose, tannin, starch, oil, mucilage and protein. A quantity of the powdered sample was cleared in chloral hydrate, mounted in diluted glycerol on a microscope slide and viewed under the microscope at different magnifications (Ibrahim et al., 2015).

Elemental analysis

The powder leaf sample was subjected to elemental analysis to determine the level of some heavy metals using atomic absorption spectrometer (AAS) following the method described by Association of Official Analytical Chemists (AOAC) (Egharevba et al., 2015; AOAC, 1995, 1980).

RESULTS AND DISCUSSION

Authentication

A voucher specimen number NIPRD/H/7100 was

prepared and deposited at NIPRD herbarium. The photograph of the voucher specimen is as shown in Figure 1. The plant was phenotypically identified as *M. angolensis* after comparison with literature information from Tropical Plants (2020) and Burkil (2004).

Microscopic profiling of the leaf powder

The photomicrograph of the microscopic evaluation is as depicted in Figure 2. The characteristics of epidermal cells, trichomes, paracytic and anomocytic stomata, and the presence of prismatic calcium oxalate crystals, and presence of fibres amongst others, can also be used as diagnostic features for the authentication and standardization of the plant samples in relation to members of the same family (Adeshina et al., 2008; Chukwunonye et al., 2017; Olotu et al., 2018).

Chemomicroscopy

The results of chemomicroscopic evaluation of *M. angolensis* are shown in Table 1. The plant showed the presence of lignin, cellulose, tannin, starch and oil, but mucilage and protein were not detected.

Phytochemical characterization

Phytochemical screening result revealed the presence of carbohydrates, saponins, anthraquinones and cardiac glycosides, while phenols, flavonoids, tannins, and alkaloids were absent (Table 2). This did not correspond completely with earlier report of Ayo et al. (2013) and Yusuf et al. (2017) as flavonoids and alkaloids were not detected in this sample. Yusuf et al. (2017) reported the absence of anthraquinones. These phytochemicals act separately or additively and synergistically to elicit the observed pharmacological effects in living organisms (Chandra et al., 2012; Richards et al., 2016).

Physicochemical parameters

The total ash content was $12.1 \pm 0.1\%$, while the moisture content was $7.0 \pm 0.0\%$. These values are within the WHO limits of 8.0 and 15.0% for total ash content and moisture content, respectively (African Pharmacopoeia, 1986). The total ash is indicative of the amount of inorganic mineral salts that may be present. The moisture content is indicative of the residual or retained moisture after drying for storage. A high moisture content will promote microbial growth and early spoilage of the stored materials. A less moisture content keeps the material microbiologically safe (Murali, 2014).

Extraction characteristics

The extraction yields of the ethanol and water extraction

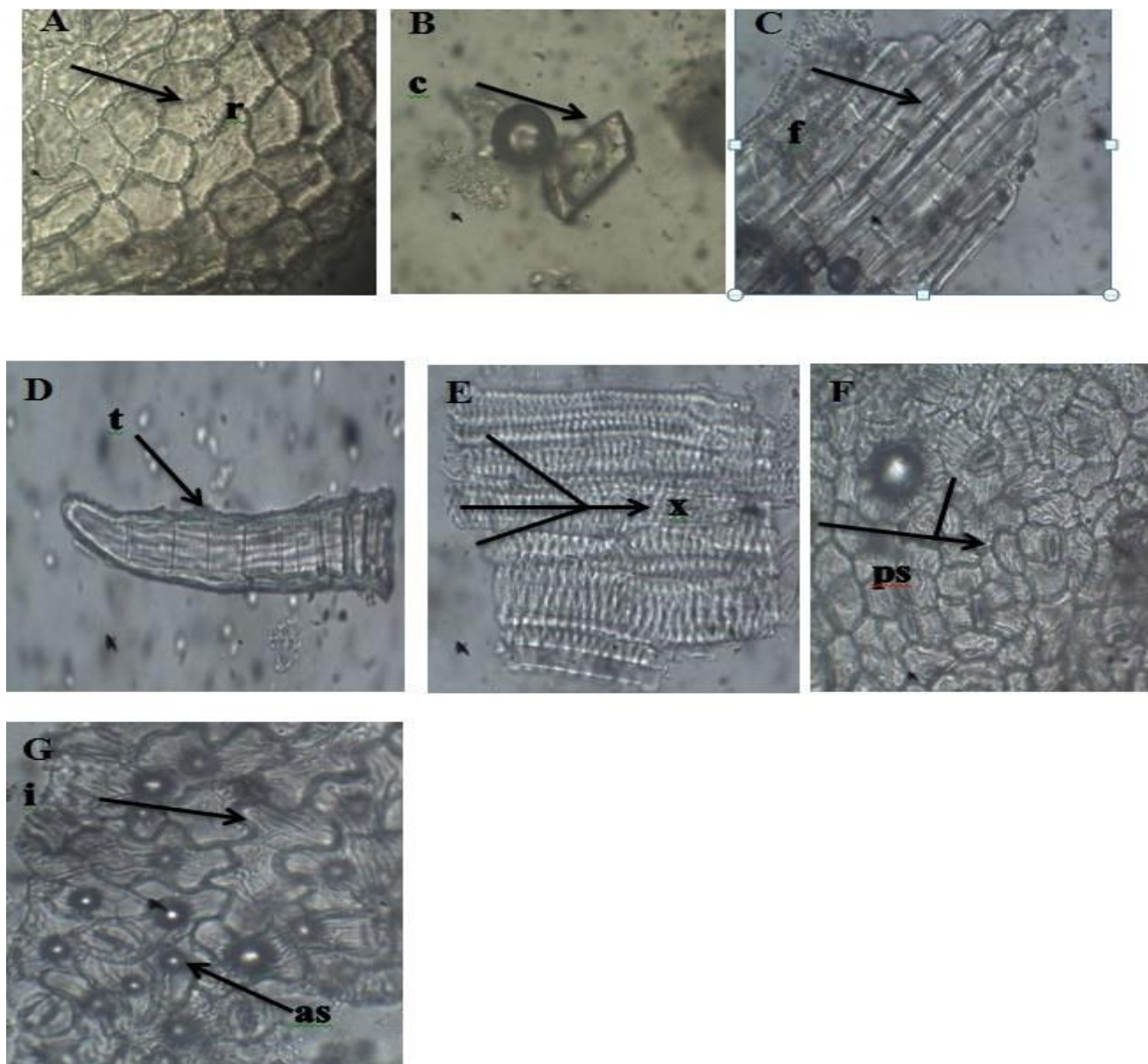


Figure 2. Microscopy of leaf powder (x400): 'A' showing regular/polygonal epidermal cell (r); 'B' showing prism calcium oxalate crystal (c); 'C' showing fiber (f); 'D' showing trichome (t); 'E' showing xylem vessels (x); 'F' showing paracytic stomata and 'G' showing anomocytic stomata (as) and irregular/wavy epidermal cell (i).

Table 1. Results of chemomicroscopic evaluation of *M. angolensis*.

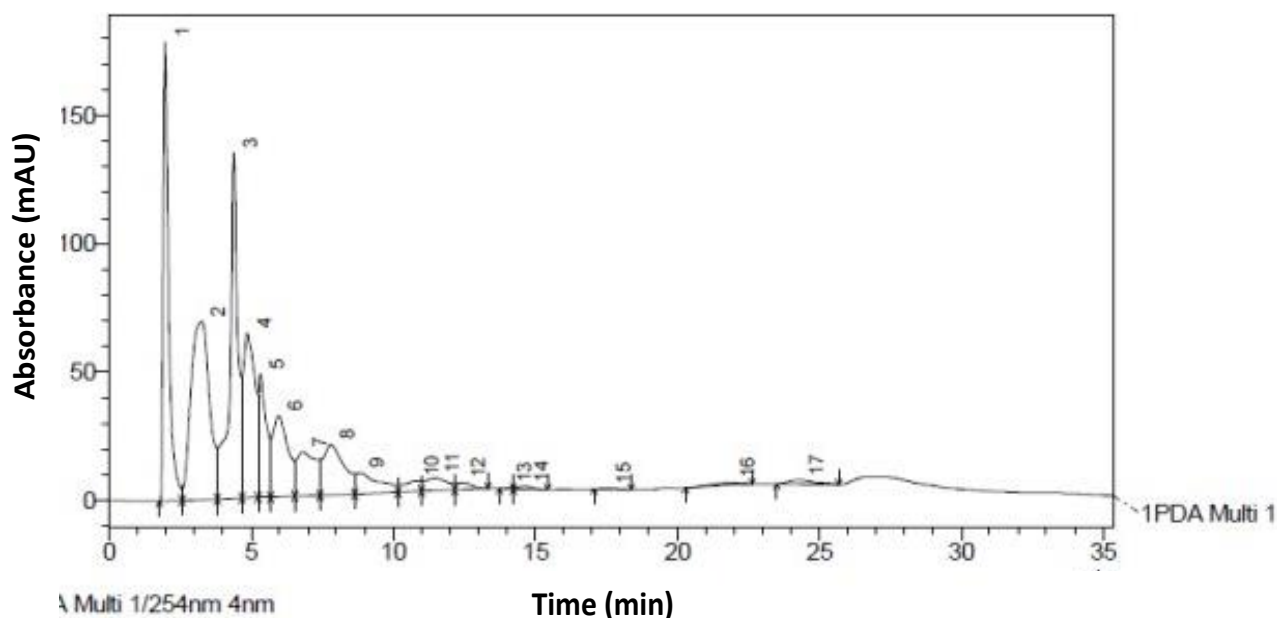
Parameter	Results
Lignin	+
Cellulose	+
Tannin	+
Mucilage	-
Starch	+
Oil	+
Protein	-
Calcium oxalate crystal	+

Table 2. Results of phytochemical characterization.

Parameter	Results
Alkaloids	-
Anthraquinones	+
Carbohydrates	+
Cardiac glycosides	+
Flavonoids	+
Phenols	-
Tannin	+
Saponin	+

Table 3. Retardation factors (R_f) and colour of TLC in daylight, 366 and 254 nm.

R _f value	Daylight	UV 366	UV254
0.92	Light yellow	No colour	Yellow
0.75	Pale green	Pink	Grey
0.69	Light yellow	No colour	Yellow
0.65	Pale blue	Dark pink	Grey
0.59	Greenish yellow	Pink	Yellow
0.46	Green	Light pink	Yellow

**Figure 3.** HPLC chromatogram and profile of the leaf of *M. angolensis*.

were found to be 12.75 and 10.60%, respectively. This indicates that alcohol will be a better solvent of extraction than water if bulk yield is of major consideration during extraction. Most plant materials give this trend due to the nature of both solvent. Water which is mostly polar, allows polar organic and mineral solutes to be readily extracted. Ethanol on the other hand is more lipophilic and allows mostly polar and some not so polar organic compounds to be readily dissolved in the extraction process. The ethanol extract was observed to be highly hygroscopic, sticky and viscous. The dry water extract obtained by freeze-drying was glassy-solid crystals, and also very hygroscopic. Moisture accelerates natural products' degradation by hydrolysis (Roy et al., 2018). This information is necessary for formulation of a stable product. The hygroscopic nature indicates that strong moisture absorbents or film coating may be required in addition to pH adjustments, in the formulated products (Roy et al., 2018).

Chromatographic profiles

The TLC profiling of the sample gave 6 spots in day light. No new spot was observed at 366 and 254 nm. The visible spots under daylight were at R_f values of 0.92, 0.75, 0.69, 0.65, 0.59 and 0.46, with the corresponding colours of light yellow, pale green, light yellow, pale blue, greenish yellow and green, respectively. However, four spots were observed at 366 nm as pink, dark pink, pink and light pink corresponding to R_f values of 0.75, 0.65, 0.59 and 0.46, respectively. At UV 254 nm, the six spots appeared as yellow, grey, yellow, grey and yellow, respectively. The R_f values and observed colours are as depicted in the Table 3.

The HPLC profile of the water extract showed 17 peaks. The retention time for the major peaks were 1.973, 3.253, 4.385, 5.969 and 7.804 min, respectively (Figure 3).

The TLC chromatogram showed 6 spots under daylight

and UV 254 nm, and 4 spots under UV light at 366 nm. The HPLC profile showed about 17 peaks, out of which the first 8 were prominent. The established TLC and HPLC profiles can serve as authentication guide in identity where adulteration is suspected.

Conclusion

The study established the pharmacognostic and phytochemical characteristics of the leaf of *M. angolensis* DC. It also established the chromatographic profiles of the leaf extract. These data will serve as useful reference for authentication of the plant especially during economic exploitation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Litterfall production, nutrient input and soil fertility in yerba-mate agroforestry systems

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Adoption of agroforestry systems (AFS) for yerba mate (*Ilex paraguariensis* St. Hil.) production contributes to improvement of soil quality due to intense litterfall input. This study aimed (i) to quantify the litterfall input and its nutrients, as well as soil fertility attributes in yerba mate AFS (ii) to discriminate which soil fertility attributes and litterfall nutrients enabled differentiation of yerba mate AFS and (iii) to verify relations between the soil fertility attributes and nutrients supplied. Six yerba mate AFS were studied in three different soils in the Center-South region of Paraná State, Brazil. The canonical discriminant analysis was applied to the soil fertility attributes, for the 0-5, 5-10, 10-20 and 20-40 cm soil layers; and for the nutrients annual input. The study of the relation between the nutrient input and nutrients soil content was carried out through the canonical correlation analysis. Litterfall input varied from 7132 to 9402 kg ha⁻¹ year⁻¹, and showed an important source of nutrients. Copper and aluminum soil content were the variables responsible for differentiating AFS, by canonical discriminant analysis. There was strait relation between calcium, magnesium, copper, manganese and zinc input and these nutrients content in the soil in yerba mate AFS.

Key words: *Ilex paraguariensis* St. Hill., discriminant analysis, variable charge soils.

INTRODUCTION

Yerba mate (*Ilex paraguariensis* St. Hil.) is a medium size tree species native to a relatively large region encompassing eastern Paraguay, northeastern Argentina, and southern Brazil (Montagnini et al., 2011). In Brazil, the yerba mate occurs natively or cultivated way (Signor et al., 2015; Santin et al., 2017), being historically, socially and economically relevant (Bonfatti Júnior et al.,

2018; Nimmo et al., 2020). The total cultivated area in Brazil is approximately 67,000 ha, with annual production above 517,000 tons (FAOSTAT, 2019). In Paraná State, the cultivation area of yerba mate is approximately 37,000 ha, with annual production above 345,000 tons (IBGE, 2018). In this State, the traditional yerba mate production systems are typically agroforestry systems

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(AFS) (De Souza and Chaimsonh, 2013) [growing crops in combination with trees (Montagnini et al., 2011)], mainly in understory environment of the Mixed Ombrophilous Forest (or Araucaria Forest), typical of the highlands of the region, legally recognized as the Atlantic Forest biome (Chaimsohn and De Souza, 2013). These systems occur mainly on small family farms where AFS are integrated with a variety of food crops and other non-timber forest products, including yerba-mate, native fruits, corn, beans, rice, and vegetables, as well as pigs, cattle and chickens (Nimmo et al., 2020).

The yerba mate harvesting causes considerable export of nutrients because the harvested product consists predominantly of thin leaves and branches, which have a high nutrients concentration (Santin et al., 2016). The amounts of nutrient removed is dependent upon the harvest time (Ribeiro et al., 2008), kind of trimming (Souza et al., 2008), site characteristics and age of the leaves collected (Jacques et al., 2007). Successive harvesting of leaves and thin branches, with no nutrient reposition by fertilization (Chaimsohn and De Souza, 2013) is potentially responsible for the continuous decrease in Brazilian productivity of yerba mate (Santin et al., 2017). In AFS, the accumulated litterfall is the main mineral transfer source to the soil (Flor et al., 2017), which is important in the nutrient biogeochemical cycling process (Torres et al., 2014). Accumulation of litterfall and the amount of minerals reaching the soil, vary as a function of several factors, mainly the species that contribute to the vegetable material deposition, climate conditions and natural disturbances (Caldeira et al., 2007).

Another important issue for understanding the functioning and dynamics of the soil-plant system in yerba-mate production relates to the low efficacy of univariate statistical procedures to explain the phenomena observed. This is because no single variable can adequately characterize the experimental unit, and it is necessary to understand the relations between the different variables (Manly, 2008). In this context, the use of multivariate statistical methods becomes interesting, for example, canonical discriminant analysis (CDA) and canonical correlation analysis (CCA). Such methods, might contribute to a more refined analysis (Baretta et al., 2008; Marcelo et al., 2015); therefore, the dexterity of the approaches made them suitable for the study of variables, evidencing links, similarities or differences between them, with minimal information loss (Manly, 2008), favoring and improving the comprehension of nutrient cycling and soil quality in yerba-mate AFS.

Thus, the objectives of this study were: (i) to quantify the litterfall production and the nutrient input originating from litterfall and the soil fertility attributes in six yerba mate AFS in the Center-South of Paraná State; (ii) to determine which among the soil properties influenced by the litterfall are more important in differentiating the yerba mate AFS; and (iii) to verify relationship between the soil

chemical properties and nutrient input, aiming to better comprehend the soil fertility under yerba mate AFS in this region.

MATERIALS AND METHODS

Areas description

The study was carried out in six AFS (two in each city) located in the Center-South region of Paraná State, namely:

AFS 1 – located in the city of São Mateus do Sul, under the geographic coordinates 25°58'15,4"S; 50°13'45,8"W; altitude of 851 m, in a Haplic Cambisol Ta Aluminic leptic, originated from basalt dikes, with 520 g kg⁻¹ of clay in the 0-20 cm soil layer;

AFS 2 – located in the city of São Mateus do Sul, under the geographic coordinates 25°59'12,4"S; 50°16'04,4"W; altitude of 800 m, in a Bruno Oxisol Aluminic typical, of sedimentary origin, with 530 g kg⁻¹ of clay in the 0-20 cm soil layer;

AFS 3 – located in the city of Bituruna, under the geographic coordinates 26°12'04,5"S ; 51°26'30,0"W; altitude of 1021 m, in a Haplic Cambisol Aluminic petroplinthic, originated from basalt, with 600 g kg⁻¹ of clay in the 0-20 cm soil layer;

AFS 4 – located in the city of Bituruna, under the geographic coordinates 26°10'08, 5"S; 51°21'51,3"W; altitude of 920 m, in a Humic Cambisol Tb Aluminic leptic, originated from basalt, with 540 g kg⁻¹ of clay in the 0-20 cm soil layer.

AFS 5 – located in the city of Cruz Machado, under the geographic coordinates 26°01'10,4"S; 51°16'18,0"W; altitude of 949 m, in a Gray-Brown Argisol distrofic, originated from basalt, with 600 g kg⁻¹ of clay in the 0-20 cm soil layer;

AFS 6 – located in the city of Cruz Machado, under the geographic coordinates 25°59'23,1"S; 51°14'30,1"W; altitude of 1051 m, in a Haplic Cambisol Ta Aluminic leptic, originated from basalt, with 650 g kg⁻¹ of clay in the 0-20 cm soil layer.

The climate in the region, according to Köppen classification is Cfb – sub-tropical, super-humid, without dry season, with annual average rainfall between 1.600 to 1.700 mm, mild mesothermal with annual average temperatures between 15 and 18°C, with mild summers and severe and frequent occurrence of frost in winter (IAPAR, 1994). The six AFS studied were characterized by the presence of native or cultivated yerba mate inside parts of Araucaria Forest (Mixed Ombrophilous Forests). The predominant vegetation and its phytosociological indices in each AFS are shown in Table 1.

Litterfall sampling and analytical determinations

In October 2011, plot of 2,500 m² (50 x 50 m) was demarcated in each AFS for physical and biological characterization. Litterfall was collected with collectors measuring 0.5 m², made of circular iron rebar, with 0.8 m diameter, and 1-mm mesh nylon net, forming a 0.5 m deep bag, which were suspended approximately 1.0 m from the ground, fixed with wood posts. The collectors were distributed equidistantly 10 m from the plot edge and 10 m between each collector, totaling 16 collectors per AFS. Litterfall was collected on the 30th day after the collector's installation (October/2011) and other collections were carried out every 30 days, totaling 12 samples along study period.

Herbaceous/shrub biomass was collected with 0.5 x 0.5 m square frame, carried out only once a year, between May and June/2012, according to the mowing season. Mowing is usually done just before harvest, mainly to facilitate harvesting and

Table 1. Phytosociological indices, absolute density (AD), relative density (RD), frequency (fr), absolute dominance (ADo), relative dominance (RDo), cover value (CV) and importance value (IV), of species with the highest representativeness in the tree extract, in six yerba mate agroforestry systems (AFS1, AFS 2, AFS 3, AFS 4, AFS 5 and AFS 6).

AFS	Specie	AD	RD	Fr	ADo	RDo	CV (%)	IV (%)
1	<i>Mosiera prismatica</i>	404	47.4	100	3.8	17.0	32.2	23.8
	<i>Myrsine coriacea</i>	88	10.3	100	1.7	7.5	8.9	8.2
	<i>Ocotea porosa</i>	24	2.8	75	2.4	10.9	6.9	6.3
	<i>Myrcia rostrata</i>	52	6.1	100	1.1	5.1	5.6	6.0
	<i>Ocotea puberula</i>	32	3.8	75	1.7	7.6	5.7	5.5
	Total	852	100	-	22.4	100	100	100
2	<i>Ocotea porosa</i>	60	21.4	100	6.8	37.1	29.2	23.0
	<i>Araucaria angustifolia</i>	28	10.0	100	2.4	13.1	11.5	11.2
	<i>Campomanesia xanthocarpa</i>	28	10.0	100	2.2	11.9	10.9	10.8
	<i>Casearia decandra</i>	32	11.4	75	0.5	2.9	7.2	7.4
	<i>Lithraea brasiliensis</i>	16	5.7	100	0.7	3.7	4.7	6.6
	<i>Ilex theezans</i>	24	8.6	75	0.5	3.0	5.8	6.5
	Total	280	100	-	18.4	100	100	100
3	<i>Piptocarpha angustifolia</i>	164	21.6	100	4.3	21.8	21.7	17.2
	<i>Vernonia discolor</i>	144	19.0	75	4.6	23.8	21.4	16.3
	<i>Ocotea puberula</i>	148	19.5	100	1.1	5.8	12.6	11.2
	<i>Mimosa scabrella</i>	76	10.0	100	1.2	6.3	8.2	8.2
	<i>Solanum granuloso-leprosum</i>	52	6.8	75	0.8	4.1	5.5	5.7
	Total	760	100	-	19.5	100	100	100
4	<i>Vernonia discolor</i>	132	24.4	100	3.7	30.4	27.4	21.3
	<i>Piptocarpha angustifolia</i>	124	23.0	100	1.9	15.7	19.3	15.9
	<i>Araucaria angustifolia</i>	72	13.3	100	1.5	12.3	12.8	11.6
	<i>Ocotea porosa</i>	12	2.2	75	1.6	13.1	7.7	7.4
	<i>Sapium glandulatum</i>	56	10.4	75	0.5	3.9	7.1	7.0
	Total	540	100	-	12.3	100	100	100
5	<i>Ocotea porosa</i>	196	62.8	100	15.5	54.9	58.9	46.6
	<i>Araucaria angustifolia</i>	44	14.1	100	5.7	20.1	17.1	18.8
	<i>Vernonia discolor</i>	32	10.3	100	3.4	12.0	11.1	14.8
	<i>Ocotea puberula</i>	28	9.0	75	3.1	10.9	9.9	12.2
	Total	312	100	-	28.3	100	100	100
6	<i>Clethra scabra</i>	196	21.8	100	6.4	22.9	22.3	16.9
	<i>Piptocarpha angustifolia</i>	80	8.9	100	5.5	19.5	14.2	11.4
	<i>Ocotea puberula</i>	76	8.4	100	3.1	10.9	9.7	8.5
	<i>Ocotea porosa</i>	104	11.6	100	2.0	7.3	9.4	8.3
	<i>Vernonia discolor</i>	68	7.6	100	2.5	8.7	8.2	7.4
	<i>Araucaria angustifolia</i>	68	7.6	100	2.2	8.0	7.8	7.2
	Total	900	100	-	28.1	100	100	100

transportation activities, in addition to reducing competition for resources between yerba mate and other species, being carried out only around yerba mate tree and in the access roads (Signor et al., 2015). In this case, composite samples (n = 3) were collected from the plant material deposited around each collector.

Litterfall and biomass samples were put in paper bags and sent to the laboratory for the washing, drying, grinding procedures and analytical determinations, employing the methods suggested by Malavolta et al. (1997). Samples were washed with deionized

water, dried in oven at 65°C with air forced flow until constant mass, ground in a "Wiley" mill equipped with 0.85-mm mesh and stored in sealed plastic containers until the chemical analyses were done. The concentrations of nitrogen (N) were determined upon sulfuric digestion and read through the semi-micro-Kjeldahl. Determinations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), manganese (Mn) and zinc (Zn) concentrations were realized through nitric-perchloric digestion and reading through molecular absorption spectrometry for P; flame

emission spectrophotometry for K; flame atomization atomic absorption spectrometry for Ca, Mg, Cu, Mn and Zn; and turbidimetry for S.

Soil sampling and analytical determinations

In September 2012, soil samples were collected from sixteen collectors on each AFS, from the soil layers of 0-5, 5-10, 10-20 and 20-40 cm. After collection, the samples were taken to the laboratory, dried in oven at 40°C with air forced flow, ground, sieved in a 2.0 mm mesh sieve. Then, the soil was used to determine active acidity (pH), potential acidity (H+Al), exchangeable acidity (Al), exchangeable Ca, Mg and K concentrations, available P (Mehlich-1) and total organic Carbon (TOC) - Walkley-Black, employing the methods suggested by Pavan et al. (1992). Available S were determined according to Vitti and Suzuki (1978) and available Cu, Mn and Zn in Mehlich-1 solution, according to the methods of Silva (2009).

Statistical analysis

Biomass litterfall input per hectare was estimated for the fractions of leaves, branches and miscellaneous litterfall (such as petioles and reproductive structures), from each collector. The amount of mineral input per hectare was calculated for each collector, through the sum of mineral input generated by the deposition of litterfall and herbaceous/shrub dry biomass.

Outliers for nutrient inputs and soil attributes variables was verified, disregarding the values indicated as inconsistent for data analysis. Homogeneity of variance assumptions (Bartlett test) and normality of data (Shapiro test) were verified for each variable, following the variable data transformation through the Box-Cox method when the assumptions were not satisfied. Identification of differences between yerba mate AFS and variables that most contributed to differentiate AFSs was realized through canonical discriminant analysis (CDA) for each soil layer, submitting the standardized canonical coefficient averages to the LSD test at 5% significance.

The relation between micro and macronutrients content in different soil layers and soil nutrient input by plant deposition was carried out through the canonical correlation analysis (CCA). All statistical analyses were realized by employing the software SAS 9.1 (SAS, 2004).

RESULTS AND DISCUSSION

Litterfall production

The annual total input of leaves, branches, miscellaneous litterfall, herbaceous/shrub biomass and total litterfall is presented in Figure 1. The total litterfall produced in the six yerba mate AFS varied from 7132 to 9402 kg ha⁻¹ year⁻¹, values which are considered close to those observed in fragments of non-managed Mixed Ombrophilous Forests (MOF) [6527 kg ha⁻¹ year⁻¹ found by Brites et al. (1992); 8354 kg ha⁻¹ year⁻¹ found by Longhi et al. (2011); and 7080 kg ha⁻¹ year⁻¹ found by Sanquetta et al. (2016)].

The leaves, branches, miscellaneous litterfall and herbaceous/shrub biomass litterfall represented on average 52, 17, 8.5 and 22.5%, respectively, of the total

litterfall input in the yerba mate AFS. Brites et al. (1992) observed that, in a MOF located in São Mateus do Sul/PR, leaves, branches and miscellaneous litterfall represented 62.2%; 22.0% and 7.6% of litterfall, respectively. Sanquetta et al. (2016), in a MOF located in São João do Triunfo, Paraná State, observed that litterfall was composed of leaves (31.3%), branches (11.7%), *Araucaria angustifolia* needle-shaped branches (41%) and miscellaneous litterfall (16%). These same authors observed that yerba mate produced 71.01 kg ha⁻¹ year⁻¹ of leaves, corresponding to 6.8% of total of leaves in litterfall input.

The highest litterfall inputs were observed throughout the spring months (September, October and November) (Figure 2). Monthly deposition of leaves was higher in the spring months (September, October and November) in all AFS studied, due partly to partial or total replacement of leaves aged by new leaves as a consequence of intense growth in this season (Sanquetta et al., 2016) and due partly to the increase in rainfall and temperature (Longhi et al., 2011; Antoneli and Thomaz, 2012). Regarding branches, the highest inputs were observed in autumn months (March, April and May) in AFS 1, AFS 2, AFS 4 and AFS 5, with deposition peaks in April; in AFS 3 and AFS 6 the highest branches inputs were observed in winter (June, July and August) and summer months (December, January and February), respectively. Antoneli and Thomaz (2012) verified that branches deposition was higher during summer probably due to intense precipitation associated with strong winds.

Several factors may affect litterfall deposition, such as species composition, latitude, altitude, temperature, precipitation, light availability during the growing season, photoperiod, evapotranspiration, relief, deciduousness, successional stage, water availability, soil nutrient content and herbivory (Caldeira et al., 2007; Schumacher et al., 2011; Sanquetta et al., 2016; Flor et al., 2017; Carmo et al., 2018). The lowest variations in litterfall deposition along the time were observed in AFS 3 (Figure 2), justified by the dominant presence of tree species of a pioneer character (Table 1) (Pezatto and Wisniewski, 2006).

Nutrient input originating from litterfall

Wide variations were observed between the AFS with respect to their contents of primary and secondary nutrients (Table 2). The pattern of differences amongst the AFS are as follows: (i) N total input varied from 45.0 (AFS 2) to 250.3 kg ha⁻¹ year⁻¹ (AFS 4), with average of 130.3 kg ha⁻¹ year⁻¹; (ii) P total input varied from 2.6 (AFS 4) to 13.1 kg ha⁻¹ year⁻¹ (AFS 1) with average of 6.5 kg ha⁻¹ year⁻¹; (iii) K total input varied from 17.4 (AFS 4) to 85.7 kg ha⁻¹ year⁻¹ (AFS 1), with average of 45.5 kg ha⁻¹ year⁻¹; (iv) Ca total input varied from 20.0 (AFS 3) to 124.7 kg ha⁻¹ year⁻¹ (AFS 4), with average of 50.2 kg ha⁻¹

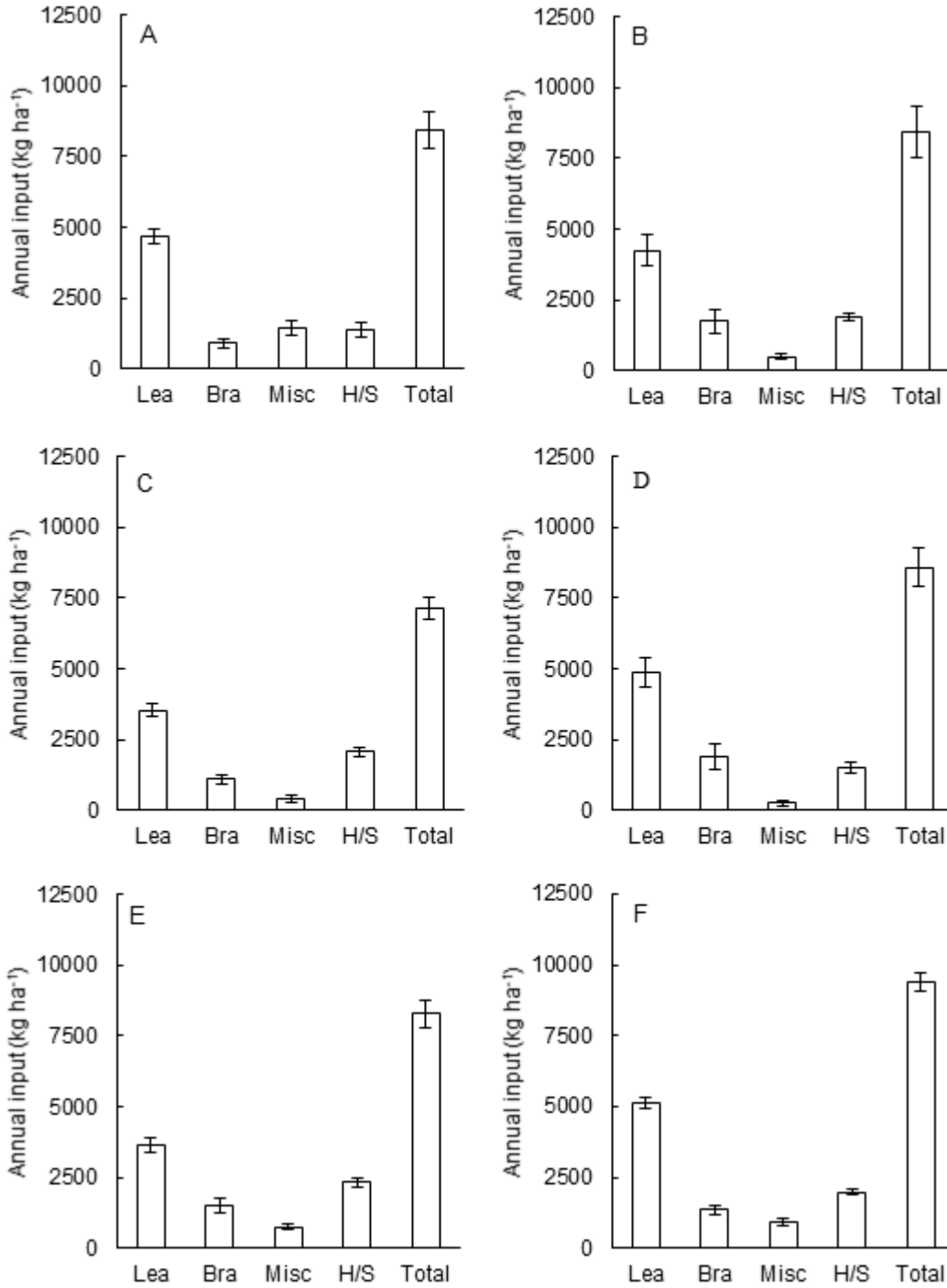


Figure 1. Leave (Lea), branch (Bra), miscellaneous litterfall (Misc), herbaceous/shrub biomass (H/S) and total litterfall annual input (kg ha⁻¹) for the six yerba mate agroforestry systems (AFS). A – AFS 1; B – AFS 2; C – AFS 3; D – AFS 4; E – AFS 5; F – AFS 6. Bars correspond to the mean standard error (n=16).

year⁻¹; (v) Mg total input varied from 7.3 (AFS 3) to 23.8 kg ha⁻¹ year⁻¹ (AFS 4), with average of 14.6 kg ha⁻¹ year⁻¹; (vi) S total input varied from 5.4 (AFS 1) to 18.8 kg ha⁻¹ year⁻¹ (AFS 6), with average of 11.6 kg ha⁻¹ year⁻¹. These values are close to those observed by Brites et al. (1992)

and Longhi et al. (2011) in MOF tree litterfall. These authors observed annual inputs of N, P, K, Ca, Mg and S around 89.2 to 148.2; 5.32 to 17.53; 31.9 to 46.58; 31.9 to 123.26; 5.7 to 22.16; 9.52 to 12.03 kg ha⁻¹, respectively.

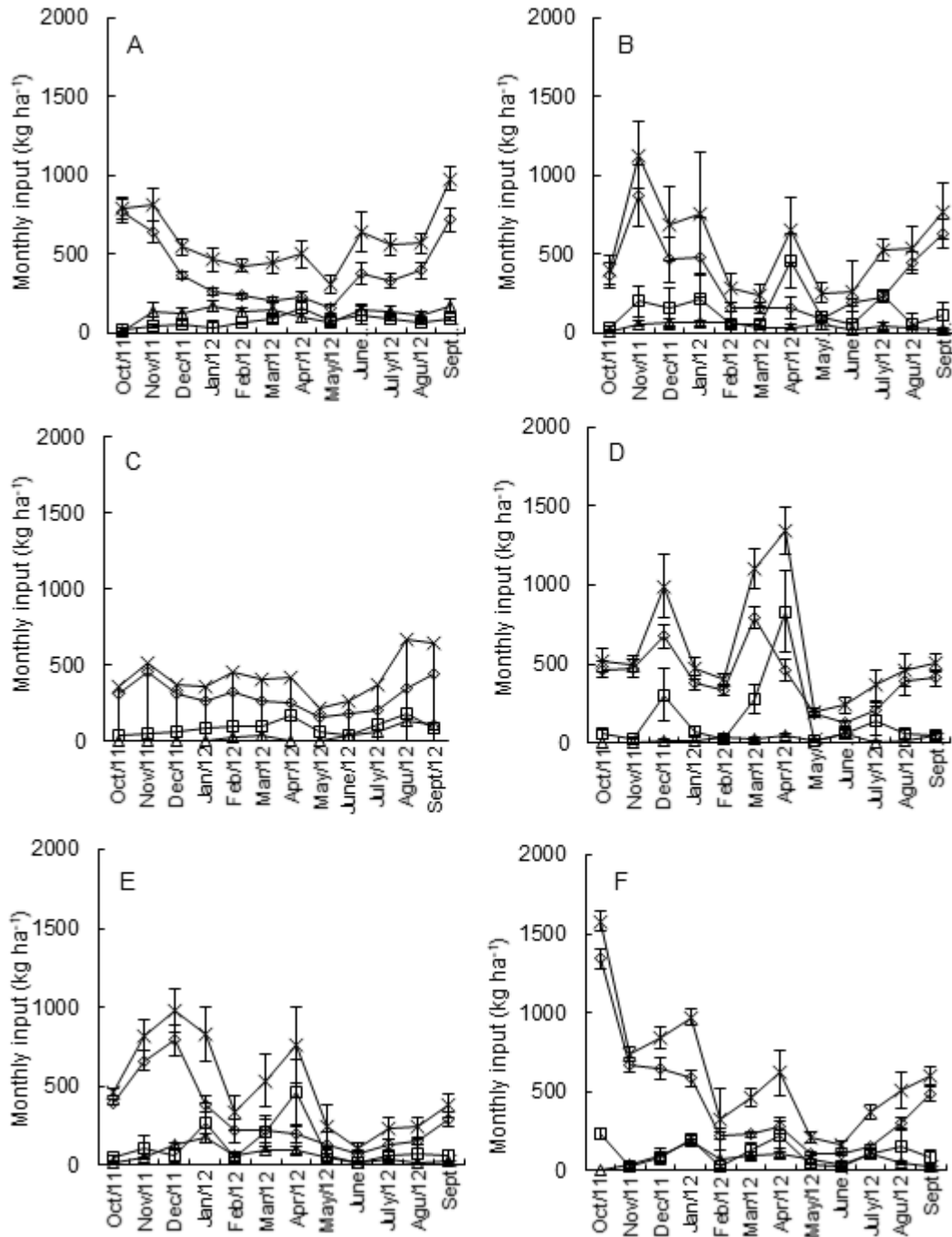


Figure 2. Leaves ($\diamond\diamond\diamond$), branches ($\square\square\square$), miscellaneous litterfall ($\triangle\triangle\triangle$), and total ($\times\times\times$) litterfall monthly input for the six yerba mate agroforestry systems (AFS). A – AFS 1; B – AFS 2; C – AFS 3; D – AFS 4; E – AFS 5; F – AFS 6. Bars correspond to the mean standard error (n=16).

Amounts of nutrients varied between yerba mate compartments, with the highest contents observed in the leaf. Similar finding was reported by Santin et al. (2013). N and K are the macronutrients most absorbed and exported by yerba mate (SBCS/NEPAR, 2017) and consequently return to the soil in larger quantities. The N

is an important element related to caffeine, tannin and theobromine (Borille et al., 2005), components responsible for the nutritional and physiological properties of yerba mate (Rossa et al., 2017). The P levels in yerba mate are often low, possibly due to the specie characteristic and due to its adaptation mechanisms for

Table 2. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), manganese (Mn), zinc (Zn) and copper (Cu) annual input (\pm mean standard error) in six yerba mate agroforestry systems (AFS 1, AFS 2, AFS 2, AFS 4, AFS 5 and AFS 6).

AFS	N	P	K	Ca	Mg	S	Mn	Zn	Cu
	kg ha ⁻¹							g ha ⁻¹	
1	128.6 \pm 9.9	6.8 \pm 0.9	58.5 \pm 8.2	44.4 \pm 4.6	15.3 \pm 1.4	10.9 \pm 1.1	6.9 \pm 0.7	169.6 \pm 16.2	66.1 \pm 7.3
2	122.8 \pm 16.4	6.6 \pm 0.8	46.3 \pm 4.2	59.9 \pm 12.3	15.7 \pm 1.8	12.6 \pm 1.2	3.2 \pm 0.4	198.6 \pm 21.1	60.5 \pm 6.6
3	124.5 \pm 8.3	5.5 \pm 0.6	40.9 \pm 1.7	30.6 \pm 2.5	11.0 \pm 0.7	9.1 \pm 0.4	7.7 \pm 0.5	177.9 \pm 8.7	93.4 \pm 9.0
4	150.1 \pm 11.7	5.8 \pm 0.8	28.7 \pm 2.0	62.3 \pm 7.4	16.4 \pm 1.3	11.1 \pm 0.7	10.0 \pm 0.7	152.2 \pm 39.8	114.2 \pm 7.5
5	114.0 \pm 5.1	6.5 \pm 0.5	52.1 \pm 3.3	50.7 \pm 3.7	11.7 \pm 0.8	11.8 \pm 0.5	5.8 \pm 0.4	200.6 \pm 11.1	60.8 \pm 2.9
6	141.8 \pm 7.0	8.0 \pm 0.9	44.7 \pm 2.3	53.2 \pm 2.6	17.2 \pm 1.0	14.1 \pm 0.8	11.6 \pm 1.0	266.3 \pm 14.8	102.5 \pm 14.2

low levels of soil P (Oliva et al., 2014). Yerba mate grown in a shaded environment, as in AFS, tends to accumulate more K, especially in leaves (Caron et al., 2014). Plants in these conditions need greater photosynthetic efficiency, demanding higher K concentrations, in order to control the osmotic regulation and transpiration processes, closely linked with the photosynthesis process (Taiz and Zeiger, 2004). The low soil pH values, characteristic of AFS, may have reduced the absorption of Ca and Mg by yerba mate, due probably to the mutual inhibition between these elements and Al in terms of absorption, as a result of competition for the absorption sites in the plant as was earlier suggested by Ricardi et al. (2020).

As with the primary and secondary nutrients, the six AFS varied widely with respect to their input of micronutrients (Table 2), where (i) Mn total input varied from 1.8 (AFS 2) to 18.6 kg ha⁻¹ year⁻¹ (AFS 6), with average of 7.6 kg ha⁻¹ year⁻¹; (ii) Zn total input varied from 89.4 (AFS 1) to 349.7 g ha⁻¹ year⁻¹ (AFS 2), with average of 194.2 g ha⁻¹ year⁻¹; (iii) Cu total input varied from 29.9 (AFS 1) to 180.4 g ha⁻¹ year⁻¹ (AFS 4), with average of 82.9 g ha⁻¹ year⁻¹. Micronutrients average inputs followed the order: Mn > Zn > Cu in all yerba mate AFS. Caldeira et al. (2007) observed high variation in micronutrient content, and also higher Mn contents in the MOF tree species biomass, ascribing the variations observed to the species differentiated nutritional requirements. Yerba mate absorbs and exports large amounts of Mn (Oliva et al., 2014; SBCS/NEPAR, 2017), and can be considered as accumulating Mn plants (Oliva et al., 2014), indicating that the plant has tolerance mechanisms to high levels of Mn (Barbosa et al., 2018).

Soil fertility attributes as influenced by the yerba mate AFS

Soils under all the yerba mate AFS had high acidity and low exchangeable cations (Table 3). The active (pH), potential (H+Al) and exchangeable (Al) acidity varied from 3.4 to 4.4; 78 to 320 mmol_c dm⁻³; and 0.5 to 113 mmol_c dm⁻³, respectively. In yerba mate AFS typical soils, Signor et al. (2015) observed pH, H+Al and Al values

varying from 3.7 to 4.1; 98 to 171 and 16 to 56 mmol_c dm⁻³, respectively, in the 0-20 cm layer.

The exchangeable base content decreased when the soil depth increased, with the contents of Ca, Mg and K varying from 1.0 to 52, 1.0 to 27 and 0.4 to 37.4 mmol_c dm⁻³, respectively. The Ca content was considered low in all layers of the AFS soils under study, except for the 0-5 cm layer in AFS 2, with Ca content considered medium (Table 3) (SBCS/NEPAR, 2017). Mg content in the 0-5 cm soil layer was considered medium in all AFS (SBCS/NEPAR, 2017). Santos (2009), when studying yerba mate AFS in the same region determined average Ca, Mg and K content varying from 8.8 to 17.6, 6.4 to 16.3 and 1.5 to 4.2 mmol_c dm⁻³, respectively.

Variations observed for Ca and Mg content in the different AFS soils, were partly related to the floristic composition of the tree extract. Species such as *Piptocarpha angustifolia* and *Vernonia discolor*, which present low Ca content in their leaves, and *Mimosa scabrella* with low Mg content in their leaves (Caldeira et al., 2007), resulted in lower Ca and Mg input. These species were predominant in AFS 3 (Table 1) and Ca and Mg soil content was the lowest among the AFS under study (Table 3).

The TOC, P and S content varied from 11.9 to 60.3 g dm⁻³, 0.5 to 17.7 mg dm⁻³; and 0.1 to 1.4 mg dm⁻³, respectively. Higher TOC and P contents were determined in more superficial soil layers, and no significant variation was observed in S content with increased depth (Table 3). The TOC high content found in yerba mate AFS was probably due to management practices that reduced the occurrence of disturbances to the soil/vegetation system, and the constant litterfall deposition. Signor et al. (2015) found the TOC content varying from 31.5 to 63.7 g dm⁻³, in the layer 0-20 cm. The same author found P varying from 1.7 to 8.3 mg dm⁻³ in the 0-20 cm soil layer. Santos (2009) reported that P values found in soils under yerba mate AFS are either low or very low, varying from 1.23 to 2.77 mg dm⁻³, with a tendency to P content reducing with increasing depth. This is due to the higher organic matter content in the soil superficial layers, since the incorporation of organic matter to the soil might increase P cycling, thereby increasing its availability to the plants (Silva and

Table 3. Soil chemical attributes (\pm mean standard error) in six yerba mate agroforestry systems (AFS 1, AFS 2, AFS 3, AFS 4, AFS 5 and AFS 6).

AFS	0-5 cm	5-10 cm	10-20 cm	20-40 cm
pH				
1	3.8 \pm 0.01	3.7 \pm 0.02	3.6 \pm 0.02	3.6 \pm 0.01
2	4.1 \pm 0.03	3.8 \pm 0.03	3.8 \pm 0.03	3.9 \pm 0.01
3	3.5 \pm 0.02	3.6 \pm 0.02	3.7 \pm 0.02	3.7 \pm 0.01
4	3.9 \pm 0.07	3.8 \pm 0.07	3.9 \pm 0.07	3.9 \pm 0.02
5	3.8 \pm 0.03	3.8 \pm 0.03	3.8 \pm 0.03	3.7 \pm 0.01
6	3.8 \pm 0.05	3.8 \pm 0.05	3.9 \pm 0.05	3.8 \pm 0.01
Aluminium (mmol_c dm⁻³)				
1	57 \pm 2.30	77.0 \pm 3.46	82.0 \pm 2.40	104.0 \pm 1.38
2	19.9 \pm 1.47	38.0 \pm 5.02	41.0 \pm 4.25	51.0 \pm 1.01
3	54.5 \pm 1.62	57.0 \pm 5.51	56.0 \pm 8.35	54.0 \pm 1.33
4	18 \pm 1.62	27 \pm 4.39	27.0 \pm 5.08	19.0 \pm 1.40
5	36.5 \pm 1.95	45.0 \pm 5.34	46.0 \pm 6.60	44.0 \pm 0.90
6	24.5 \pm 1.90	29.0 \pm 5.73	28.0 \pm 6.02	26.0 \pm 0.67
Magnesium (mmol_c dm⁻³)				
1	16.2 \pm 1.08	6.4 \pm 0.70	4.0 \pm 0.30	3.0 \pm 0.20
2	17.9 \pm 1.24	5.2 \pm 0.66	3.0 \pm 0.35	2.0 \pm 0.25
3	7.2 \pm 0.55	4.7 \pm 0.30	4.0 \pm 0.21	3.0 \pm 0.18
4	14.3 \pm 1.31	8.4 \pm 1.18	6.0 \pm 1.01	4.0 \pm 0.42
5	9.3 \pm 0.87	4.7 \pm 0.40	3.0 \pm 0.25	2.0 \pm 0.13
6	12.9 \pm 0.71	6.1 \pm 0.42	4.0 \pm 0.16	3.0 \pm 0.29
Total Organic Carbon (g dm⁻³)				
1	40.4 \pm 1.70	26.1 \pm 0.78	21.8 \pm 1.23	19.6 \pm 0.69
2	43 \pm 0.66	34.1 \pm 0.66	28.3 \pm 0.53	25.6 \pm 0.46
3	50.5 \pm 1.33	42.4 \pm 1.60	38.2 \pm 1.41	31.9 \pm 1.18
4	45.4 \pm 1.15	36.8 \pm 1.06	30.0 \pm 0.95	23.0 \pm 0.85
5	52.2 \pm 1.01	40.2 \pm 1.27	34.9 \pm 0.84	28.1 \pm 0.76
6	43.6 \pm 1.21	32.8 \pm 1.13	28.2 \pm 0.76	21.9 \pm 0.57
Sulfur (mg dm⁻³)				
1	1.1 \pm 0.03	1.2 \pm 0.05	1.2 \pm 0.04	1.0 \pm 0.05
2	1.1 \pm 0.05	1.2 \pm 0.05	1.3 \pm 0.06	1.0 \pm 0.04
3	0.7 \pm 0.09	0.5 \pm 0.04	0.5 \pm 0.04	0.7 \pm 0.05
4	0.5 \pm 0.04	0.4 \pm 0.03	0.4 \pm 0.03	0.3 \pm 0.06
5	0.8 \pm 0.06	0.8 \pm 0.07	0.7 \pm 0.07	0.5 \pm 0.04
6	1.0 \pm 0.04	1.0 \pm 0.04	1.1 \pm 0.05	1.0 \pm 0.08
Manganese (mg dm⁻³)				
1	223.0 \pm 18.90	101.0 \pm 5.78	78.0 \pm 3.94	49.0 \pm 1.25
2	44.0 \pm 6.30	21.1 \pm 2.44	16.1 \pm 3.13	7.9 \pm 0.25
3	101.0 \pm 19.32	63.0 \pm 1.25	50.0 \pm 1.89	26.9 \pm 2.31
4	304.0 \pm 32.69	225.0 \pm 5.08	161.0 \pm 1.74	100 \pm 15.47
5	124.0 \pm 18.15	44.3 \pm 1.12	28.8 \pm 0.86	9.3 \pm 0.65
6	486.0 \pm 29.88	322.0 \pm 0.98	228.0 \pm 0.52	112.0 \pm 12.31
H+Al (mmol_c dm⁻³)				
1	222 \pm 7.92	203 \pm 0.15	203 \pm 0.13	215 \pm 1.76
2	154 \pm 4.32	189 \pm 0.11	189 \pm 0.12	164 \pm 1.90
3	279 \pm 5.92	269 \pm 0.19	242 \pm 0.18	201 \pm 7.64
4	164 \pm 7.40	180 \pm 0.25	165 \pm 0.23	106 \pm 3.31

Table 3. Contd.

5	212±5.75	217.0.17	202±0.15	167±2.49
6	189±6.28	181±0.20	156±0.18	130±1.38
Calcium (mmol_c dm⁻³)				
1	15.1±1.48	5.1±0.70	3.0±0.40	3.0±0.18
2	24.6±2.65	5.8±0.73	3.0±0.37	3.0±0.39
3	7.3±1.81	3.7±1.38	2.0±0.68	2.0±0.26
4	12.0±4.70	4.1±0.71	2.0±0.42	3.0±0.61
5	11.2±1.58	3.2±0.74	2.0±0.29	2.0±0.18
6	15.2±2.87	4.2±0.88	2.0±0.45	2.0±0.11
Potassium (mmol_c dm⁻³)				
1	4.2±0.32	2.9±0.30	2.3±0.22	2.2±0.13
2	2.3±0.20	1.5±0.11	1.0±0.05	0.6±0.03
3	2.7±0.11	2.0±0.10	1.2±0.05	0.6±0.02
4	2.4±0.13	1.6±0.10	1.0±0.05	0.6±0.04
5	3.1±0.21	2.0±0.13	1.2±0.08	0.8±0.06
6	11.7±0.35	6.3±0.19	3.1±0.08	0.5±0.02
Phosphorus (mg dm⁻³)				
1	10.0±0.70	4.4±0.28	2.9±0.14	0.8±0.06
2	3.4±0.47	1.7±0.27	1.1±0.15	0.6±0.19
3	3.7±0.33	2.3±0.19	1.8±0.13	0.7±0.05
4	6.2±0.45	3.2±0.22	1.8±0.11	0.7±0.12
5	2.0±0.13	1.3±0.10	1.2±0.09	0.7±0.05
6	2.4±0.18	2.1±0.38	1.2±0.10	0.9±0.13
Copper (mg dm⁻³)				
1	0.7±0.09	0.6±0.07	0.5±0.06	0.7±0.17
2	2.1±0.17	2.2±0.14	2.4±0.13	2.8±0.16
3	15.2±0.70	16.7±0.78	17.5±0.90	18.2±0.97
4	24.0±1.08	26.9±1.40	28.0±1.15	25.3±0.95
5	14.3±0.54	17.5±0.69	19.2±0.67	18.5±0.65
6	22.7±0.47	26.7±0.37	28.9±0.38	29.4±0.39
Zinc (mg dm⁻³)				
1	5.4±0.60	2.9±0.17	2.6±0.14	5.8±1.14
2	3.0±0.33	2.0±0.11	1.7±0.07	1.5±0.41
3	2.6±0.16	1.9±0.15	1.8±0.11	3.0±0.49
4	4.0±0.55	3.1±0.12	2.0±0.14	3.7±0.49
5	3.2±0.31	1.4±0.12	1.1±0.08	1.6±0.11
6	3.3±0.24	1.7±0.13	1.0±0.09	2.4±0.34

Mendonça, 2007).

Regarding micronutrient content determined in the AFS soil under study, amounts of Cu, Mn and Zn varied from 0.2 to 38, 3.2 to 731 and 0.6 to 15.2 mg dm⁻³; respectively (Table 3). Fossati (1997), comparing 10 sites of cultivated yerba mate, differing in toposequence, observed Cu, Mn and Zn content varying from 0.52 to 6.6, 8.0 to 150.0 and 1.42 to 5.96 mg dm⁻³, respectively.

Yerba mate AFS discrimination

First canonical discriminant function (CDF1) was the most important for the four soil layers (0-5, 5-10, 10-20 and 20-40 cm) since it presented 99% canonical correlation. Eigenvalues for 0-5, 5-10, 10-20 and 20-40 cm layers were 67.55, 103.23, 106.17 and 184.11, respectively, explaining great proportion of properties variability.

Table 4. Standard canonical coefficients average (\pm mean standard error) for the first (CDF1) and second (CDF2) canonical discriminant functions for the six yerba mate agroforestry systems (AFS 1, AFS 2, AFS 3, AFS 4, AFS 5 and AFS 6) in four soil layers.

CDF	AFS 1	AFS 2	AFS 3	AFS 4	AFS 5	AFS 6
0-5 cm						
CDF1	-16.97 \pm 0.32 ^e	-4.12 \pm 0.18 ^d	1.93 \pm 0.26 ^c	7.35 \pm 0.33 ^a	3.08 \pm 0.29 ^b	6.58 \pm 0.21 ^a
CDF2	3.99 \pm 0.32 ^b	-10.90 \pm 0.29 ^f	5.27 \pm 0.21 ^a	-1.36 \pm 0.29 ^e	-0.30 \pm 0.27 ^d	2.17 \pm 0.25 ^c
5-10 cm						
CDF1	-20.31 \pm 0.29 ^f	-6.96 \pm 0.24 ^e	2.77 \pm 0.27 ^d	9.32 \pm 0.36 ^a	4.21 \pm 0.25 ^c	8.14 \pm 0.21 ^b
CDF2	4.07 \pm 0.36 ^b	-8.02 \pm 0.32 ^f	4.73 \pm 0.24 ^a	0.23 \pm 0.24 ^d	-1.65 \pm 0.20 ^e	0.65 \pm 0.24 ^c
10-20 cm						
CDF1	-20.95 \pm 0.30 ^f	-5.39 \pm 0.16 ^e	1.89 \pm 0.34 ^d	8.14 \pm 0.30 ^b	4.54 \pm 0.28 ^c	9.15 \pm 0.59 ^a
CDF2	3.25 \pm 0.33 ^b	-8.27 \pm 0.32 ^f	5.11 \pm 0.30 ^a	1.45 \pm 0.27 ^c	-1.02 \pm 0.25 ^e	-0.20 \pm 0.17 ^d
20-40 cm						
CDF1	-29.30 \pm 0.30 ^f	-5.79 \pm 0.37 ^e	4.07 \pm 0.26 ^d	10.46 \pm 0.30 ^a	6.68 \pm 0.15 ^c	9.45 \pm 0.19 ^b
CDF2	2.69 \pm 0.32 ^b	-7.46 \pm 0.25 ^e	4.75 \pm 0.23 ^a	-0.54 \pm 0.31 ^c	-1.82 \pm 0.27 ^d	2.00 \pm 0.24 ^b

Means followed by the same lowercase letter, in lines, do not differ to 5% by t test.

Variability proportion was 60, 75, 76 and 85% explained by CDF1 in 0-5, 5-10, 10-20 and 20-40 cm layers, respectively. Eigenvalues for second canonical discriminant function (CDF2) were lower than those observed in CDF1, except for 0-5 cm layer. CDF2 eigenvalues observed were 29.89, 19.28, 20.0 and 17.19 for 0-5, 5-10, 10-20 and 20-40 cm layers, respectively. Variability proportion was 26, 14, 14 and 8% explained by CDF2 in 0-5, 5-10, 10-20 and 20-40 cm layers, respectively. The remaining canonical functions did not present significant variability of the properties under study. Also, occasions on which the first CDF eigenvectors observed were relatively higher, the remaining CDFs had little relevance in data analysis (Manly 2008). Therefore, throughout this study only CDF1 and CDF2 of each soil layer were considered for discussion, since they could explain over 85% of the variability proportion.

In 0-5 cm layer, standardized canonical coefficient averages (SCCs) were distinct regarding each CDF, except in AFSs 4 and 6 for CDF1 (Table 4). In 5-10 and 10-20 cm layers, the SCCs averages differed for both CDFs (Table 4). In 20-40 cm layer, SCC averages were distinct regarding different CDFs, except for AFS 1 and AFS 6 and CDF 2 (Table 4).

The more strongly distributed sites on the horizontal axis differed due to CDF1 higher explaining proportion, for all soil layers under study (Figure 3). When it was not possible to see the distinction on the horizontal axis, as in the case of AFSs 4 and 6 for 0-5 cm layer, the differentiation was realized upon observation of the vertical axis (Figure 3).

Analysis of parallel discrimination rate (PDR) indicated

that available Cu and exchangeable Al in the soil were the ones that most influenced in AFSs distinction, for both CDFs in four layers (Table 2). Soil and plant remaining variables presented very low or inexpressive PDR values (Table 5). The PDR – resulting from the product between the standard canonical coefficient (ACC) and the correlations between original and canonical variables (r) – presented values related to the r and SCC joint contribution (Baretta et al., 2008). Therefore, this method (PDR) has been recommended to discriminate areas through the canonical discriminant analysis (CDA) (Cruz-Castillo et al., 1994), including the presence of soil properties in the analysis (Mattias et al., 2010).

In order to better understand the available Cu and exchangeable Al relations in the sites under study, r values were considered, as suggested by Manly (2008). In such case, it was evident that the r values in the available Cu and exchangeable Al were inversely proportional (Table 5). Variations in the soil exchangeable Al concentrations were due to its weathering and the pH value (Kämpf et al., 2009; Malavolta et al., 1997). For each pH unit, the Al^{3+} in solution activity was increased from 42 to 1000 times, depending on the kind of mineral with which Al^{3+} was in equilibrium (Lindsay, 1979).

In the exchange complex, when there was high Al^{3+} , Cu adsorption tended to decrease. However, the availability to the plants, when compared to the remaining cationic micronutrients, was less dependent on pH and more influenced by the kind of soil due to the mineralogical composition (Alleoni et al., 2005; Vendrame et al., 2007) and TOC content. The TOC content presented direct effect in the Cu availability reduction (Mouta et al., 2008), ascribed to the formation of high energy complexes with

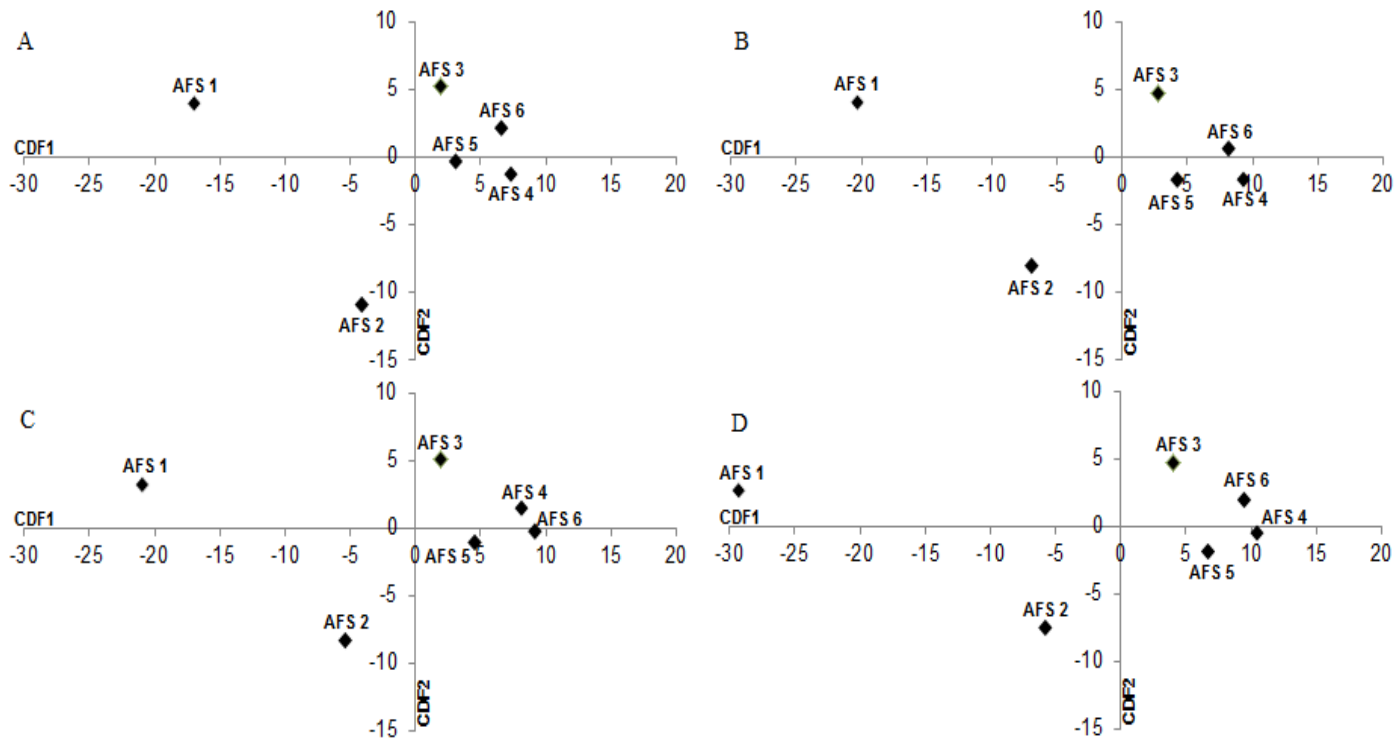


Figure 3. Average of first function canonical coefficient of the first canonical discriminant function (CDF1) against the coefficients of the second canonical discriminant function (CDF2), regarding input and soil mineral content in the layers 0-5 (A), 5-10 (B), 10-20 (C) and 20-40 cm (D), for the six yerba mate agroforestry systems (AFS).

humic acids (Arias et al., 2006).

The proximity of canonical coefficient averages in AFS 3, AFS 4, AFS 5 and AFS 6 (Figure 3), occurred due to higher Cu content in the soil, in relation to Cu content observed in AFS 1 and AFS 2. Soils developed from basalt presented, in general, higher solubilized Cu content than those of sedimentary origin (Oliveira and Costa, 2004).

Canonical correlation

All correlations with eigenvalues over 1 were selected, as suggested by Manly (2008). Correlations 1 (canonical correlation = 0.91; eigenvalue = 5.02), 2 (canonical correlation = 0.84; eigenvalue = 2.44) and 3 (canonical correlation = 0.74; eigenvalue = 1.23) were the most relevant for 0-5 cm layer, as they explained 87% of the variability. For 5-10 cm layer, correlations 1 (canonical correlation = 0.91; eigenvalue = 4.53) and 2 (canonical correlation = 0.74; eigenvalue = 1.29) explained 80% of the variability. Correlations 1 (canonical correlation = 0.91; eigenvalue = 4.69) and 2 (canonical correlation = 0.72; eigenvalue = 1.06) were the most relevant for 10-20 cm layer, due to the fact that they explained 80% of variability. In 20-40 cm layer, only correlation 1 (canonical correlation = 0.88; eigenvalue = 3.60) was important, as it

explained 57% of variability.

Higher canonical correlation (CC) positive values were observed for Cu and Mn input and Cu and Mn soil content in the first canonical correlation, for all soil layers. For Cu input, CC was 0.62; 0.63; 0.64 and 0.70 for 0-5 cm, 5-10 cm, 10-20 cm and 20-40 cm layers, respectively. For Cu soil content, CC was 0.81; 0.80; 0.82 and 0.86 for 0-5 cm, 5-10 cm, 10-20 cm and 20-40 cm layers, respectively. CC values for Mn input were 0.87; 0.85; 0.83 and 0.91 for 0-5 cm, 5-10 cm, 10-20 cm and 20-40 cm layers, respectively. For Mn soil content, CC was 0.66; 0.74; 0.70 and 0.74 for 0-5 cm, 5-10 cm, 10-20 cm and 20-40 cm layers, respectively. This indicated that there was close relation between the Cu and Mn content input through vegetable material deposition, and these elements content was found in the soil of all yerba mate AFS. Reports in the literature informing the close positive relation between available Cu in the soil and in the forest species aerial parts are common (Rodrigues et al., 2010). Another factor determining Cu availability to plants is related to its decrease when there is increase in the TOC content in the soil (Mouta et al., 2008). However, in this study, despite the variations observed in soil TOC (Table 3), it was not enough to differentiate AFS or to influence Cu in the soil-plant system. In native forest species (for example, yerba mate), Mn contents are usually high (over 1000 mg kg⁻¹) (Reissmann and Carneiro, 2004; Heinrichs

Table 5. Standard canonical coefficients (SCC), canonical correction coefficient (r) and parallel discrimination rate (PDR) in the first (CDF1) and second (CDF2) canonical discriminant functions in the four layers, regarding nutrient amount input and soil properties.

Variable	Layer of 0-5 cm			Layer of 5-10 cm			Layer of 10-20 cm			Layer of 20-40 cm		
	SCC	r	PDR	SCC	r	PDR	SCC	r	PDR	SCC	r	PDR
CDF 1												
N input	-0.02	0.17	0.00	0.23	0.17	0.04	0.06	0.16	0.01	0.76	0.13	0.10
P input	0.47	0.08	0.04	0.74	0.07	0.05	0.27	0.11	0.03	0.06	0.07	0.00
K input	-0.53	-0.45	0.24	-0.08	-0.44	0.04	-0.03	-0.42	0.01	-0.20	-0.42	0.08
S input	0.19	0.13	0.02	-0.35	0.10	-0.04	0.01	0.15	0.00	0.13	0.11	0.01
Ca input	0.27	-0.15	-0.04	0.39	-0.11	-0.04	0.07	-0.16	-0.01	0.23	-0.13	-0.03
Mg input	-0.07	-0.01	0.00	-0.30	-0.04	0.01	-0.09	-0.01	0.00	0.50	-0.07	-0.04
Cu input	0.13	0.50	0.07	0.42	0.50	0.21	0.23	0.48	0.11	0.23	0.45	0.10
Mn input	-0.26	0.43	-0.11	-0.45	0.47	-0.21	-0.32	0.44	-0.14	0.16	0.37	0.06
Zn input	-0.08	0.31	-0.02	0.00	0.28	0.00	-0.13	0.33	-0.04	0.05	0.29	0.01
C soil	0.31	0.38	0.12	1.07	0.56	0.60	1.16	0.48	0.56	1.17	0.39	0.46
P soil	-0.65	-0.54	0.35	0.27	-0.33	-0.09	-0.22	-0.52	0.11	0.12	0.00	0.00
K soil	0.16	0.21	0.03	-0.15	-0.03	0.00	0.21	0.33	0.07	0.78	0.83	0.65
S soil	0.03	-0.43	-0.01	0.20	-0.57	-0.11	0.46	-0.52	-0.24	-0.62	-0.44	0.27
Ca soil	0.42	0.29	0.12	0.36	0.31	0.11	0.25	0.37	0.09	0.00	0.34	0.00
Mg soil	-0.60	-0.32	0.19	0.34	-0.11	-0.04	0.31	0.01	0.00	-0.43	0.02	-0.01
Cu soil	5.35	0.94	5.03	7.82	0.98	7.66	6.91	0.96	6.63	4.76	0.91	4.33
Mn soil	-0.58	0.25	-0.15	-0.49	0.36	-0.18	-0.48	0.29	-0.14	1.24	-0.09	-0.11
Zn soil	-0.08	-0.25	0.02	0.08	0.19	0.02	0.19	0.45	0.09	0.20	0.19	0.04
Al soil	-3.83	-0.54	2.07	-2.84	-0.73	2.07	-3.75	-0.85	3.19	-8.93	-0.93	8.30
H+Al soil	0.23	-0.11	-0.03	-0.78	0.00	0.00	-0.31	-0.27	0.08	1.27	-0.61	-0.77
pH soil	1.04	0.05	0.05	-0.59	0.32	-0.19	-0.60	0.58	-0.35	0.62	-0.47	-0.29
CDF 2												
N input	0.77	0.21	0.16	1.22	0.17	0.21	0.83	0.18	0.15	1.00	0.20	0.20
P input	-0.11	-0.05	0.01	-0.58	-0.16	0.09	-0.22	-0.20	0.04	-0.23	-0.09	0.02
K input	0.37	0.15	0.06	-0.14	0.01	0.00	-0.12	-0.05	0.01	-0.24	0.00	0.00
S input	-0.62	-0.20	0.12	-0.47	-0.32	0.15	-0.53	-0.36	0.19	-0.24	-0.25	0.06
Ca input	0.88	0.48	0.42	0.76	0.54	0.41	0.94	0.54	0.51	1.09	0.52	0.57
Mg input	-0.05	-0.23	0.01	-0.21	-0.21	0.04	-0.13	-0.25	0.03	-0.52	-0.18	0.09
Cu input	0.06	0.25	0.02	0.54	0.24	0.13	0.64	0.28	0.18	0.22	0.30	0.07
Mn input	0.53	0.59	0.31	0.34	0.56	0.19	0.53	0.56	0.30	1.02	0.62	0.63
Zn input	0.22	0.08	0.02	-0.24	-0.09	0.02	-0.16	-0.14	0.02	0.07	0.03	0.00
C soil	-0.02	0.15	0.00	-0.64	-0.03	0.02	0.06	0.19	0.01	-0.31	0.01	0.00
P soil	0.21	0.23	0.05	0.20	0.45	0.09	-0.02	0.53	-0.01	0.04	-0.24	-0.01
K soil	-0.32	-0.38	0.12	0.22	0.24	0.05	-0.04	-0.29	0.01	0.62	-0.12	-0.07
S soil	0.19	-0.24	-0.05	0.49	-0.36	-0.18	-0.43	-0.49	0.21	0.29	-0.08	-0.02
Ca soil	-1.07	0.57	-0.61	-0.46	0.23	-0.11	-0.21	0.27	-0.06	0.21	0.34	0.07
Mg soil	-0.79	-0.51	0.40	0.22	-0.04	-0.01	-0.04	-0.34	0.01	0.09	0.23	0.02
Cu soil	2.04	0.30	0.61	2.60	0.18	0.47	2.47	0.26	0.64	2.69	0.34	0.91
Mn soil	2.20	0.41	0.90	1.58	0.42	0.66	1.45	0.43	0.62	-1.14	-0.52	0.59
Zn soil	0.13	0.12	0.02	-0.41	-0.24	0.10	-0.24	-0.24	0.06	-0.24	-0.45	0.11
Al soil	4.00	0.68	2.72	3.63	0.49	1.78	3.95	0.39	1.54	2.89	0.20	0.58
H+Al soil	0.31	0.71	0.22	0.60	0.47	0.28	0.13	0.34	0.04	1.08	0.23	0.25
pH soil	-0.12	0.80	-0.10	0.12	-0.59	-0.07	0.19	-0.47	-0.09	0.15	0.44	0.07

and Malavolta, 2001), as a result of high input and concentration of these micronutrients available in the soil

(Boeger et al., 2005). Also, pH values usually observed in yerba mate AFS (Table 3) are in the band (pH < 5.5)

which favors Mn availability to the plants (Abreu et al., 1994).

For soil Ca content in 0-5 cm layer, CC values were positively high in the first canonical correlation (CC=0.52), indicating higher Ca content, which demonstrated the litterfall importance in this nutrient cycling, since the litterfall accumulated on the soil surface was the main source of Ca mineralization (Costa et al., 2005). Low mobility in vegetable tissues and the leaves long life are among the factors that contributed to Ca content in litterfall (Caldeira et al., 2007).

In 0-5 cm layer, the second canonical correlation presented negative CC values for variables input and soil Ca content (CC= -0.49 and -0.44, respectively), and CC positive values for variables input and soil Mg content (CC= 0.71 and 0.73, respectively), which indicated the inverse relation between Ca and Mg, both in the input of these nutrients through vegetable material deposition and in the soil content found. The low Ca:Mg relation in yerba mate AFS soils studied, favored Mg absorption and accumulation by plants, as observed in yerba mate, species in which high Mg content was found in dry leaves (Heinrich and Malavolta, 2001).

Higher CC negative values were observed for soil S content in the first canonical correlation, for 0-5 cm (CC= -0.59), 5-10 cm (CC= -0.67) and 10-20 cm (CC= -0.68) layers, which could be due to the low soil S content, caused by the S repulsion in the soluble form (SO_4^{2-}), which occurs as soon as S is mineralized from the organic matter (Furtini Neto et al., 2001).

Regarding second canonical correlation, higher CC positive values were observed for S input (CC= 0.52 and 0.58 in 5-10 and 10-20 cm layers, respectively). Lower CC were observed for soil P content in second canonical correlation, in order of -0.76 and -0.73 in 5-10 and 10-20 cm layer, respectively. This resulted from a higher annual S input, approximately double the P input (Table 2), since the interactions are negligible due to their low soil content of most AFS (Table 3). Suitable S and P supply in forest species is guaranteed through associations with mycorrhizal fungi (Faria et al., 2017). Yerba mate presents abounding association with endomycorrhizae (Gaiad and Lopes, 1986), and was shown to present low P content in its leaves without any evidence of P deficiency symptoms, for being a species adapted to the low soil P content conditions (Radomski et al., 1992).

The affinity relation between soil Zn content and this mineral input was also described by second canonical correlation in 10-20 cm layer, in which CC values were 0.70 and 0.63 for input and soil content, respectively. Micronutrient (Cu, Mn and Zn) content in the soil was related to these mineral elements input through vegetable material deposition; however, Cu and Mn were more important in the correlation between soil and plant contents. The micronutrient dynamics was related, directly or indirectly with the continuous vegetable material input, which along the time, after the decomposition process

was released and later on absorbed by the plants (Carmo et al., 2012).

Conclusions

The adoption of agroforestry systems in yerba mate production contributed significantly to litterfall deposition on the soil with the addition varying from 7132 to 9402 kg $\text{ha}^{-1} \text{year}^{-1}$. Litterfall was an important nutrient source to yerba mate AFS, underscoring its the contribution of the macronutrients N, K and Ca, and the micronutrient, Mn. The floristic composition and the soil class and origin influenced nutrient input and soil nutrient content in yerba mate AFS. Canonical discriminant analysis was efficient to evaluate differences between yerba mate AFS, revealing Cu and Al content variable in the soil as responsible for the site differentiation. Soil fertility depended on nutrient input through litterfall deposition in yerba mate AFS. There was strait relation between Ca, Mg, Cu, Mn and Zn input and their soil content in yerba mate AFS.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Comparative effect of weed control methods on Mexican sunflower (*Tithonia diversifolia*) in maize

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Mexican sunflower management in arable crops is becoming increasingly important due to its prevalent growth habit. The field experiments were conducted to compare weed suppressive abilities of two cover crops and two maize herbicides on Mexican sunflower. The treatments consist of the pre-emergence application of Primextra Gold (atrazine + metolachlor) at 4 l/ha, a post-emergence application of Aminoforce (2, 4-D) at 1.6 l/ha, two cover crops, *Centrosema pubescens* (Centro) at 2.5 kg/ha and *Pueraria phaseoloides* (Puero) at 2.0 kg/ha, hand weeding at 2 and 5 weeks after sowing (WAS) and no weeding. The experimental design was a randomized complete block design with four replications. Pre-emergence herbicide produced taller plants at 8 and 12 WAS and higher number of leaves at 12 WAS. Despite two hand weedings (at 2 and 5 WAS), the weed biomass of hand weeding treatment was not different from no weeding. Higher weed densities produced by hand weeding and no weeding at 12 WAS indicated that the two herbicides and the two cover crop treatments gave better weed control than both weed checks. Weed control was 4, 7 and 8 times better in pre-emergence, post-emergence and Centro; respectively, than no weeding at 8 WAS. Although Centro provided long term weed control, the herbicides were able to provide early protection for the maize plants. The highest maize yield of 2.21 t/ha obtained from Primextra Gold (atrazine + metolachlor) at 4l/ha was significantly higher than yields from the other treatments. Yield reduction of 24.5, 27.7, 34.4, 40.8 and 94.2% was obtained in 1.6 l/ha Aminoforce, Centro, hand weeding, Puero and no weeding, respectively, when compared to maize yield from Primextra Gold.

Key words: Mexican sunflower, cover crops, herbicides, hand weeding, weed control.

INTRODUCTION

Maize is an important cereal crop in Nigeria with a total production of about 11.0 million tons in 2019 (FAO, 2019). It is an important staple food that is also used as animal feed and raw materials in many industries such as flour mills, breweries, beverage and pharmaceuticals.

The increased use of maize has placed a higher demand on the crop which is difficult to meet at the present level of production. In spite of the importance of maize, the yield per hectare does not match the demand in Nigeria. This is due to several factors such as weed infestation,

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unavailability of labour and low soil fertility (Imoloame, 2017).

Weed interference is a major maize production constraint in Southwestern Nigeria (Akinola and Salami, 2016). Yield losses caused by the presence of weeds in maize crops range from 10 to 80% (Akobundu and Ekeleme, 2000; Lagoke et al., 1998; Vargas et al., 2006). Silva et al. (2015) reported a 10% crop yield reduction in the presence of 10 plants m^{-2} of purple nut-sedge, which represented 960 kg ha^{-1} yield potential. The limitation in maize production due to weeds is proportional to the weed species that exist in the area, its density, cultural stage in which there is competition and climate and soil conditions (Vargas et al., 2006).

Diverse weed control measures employed in maize production are crop rotation, hand-weeding, cover crops, chemical weed control and integrated weed management (Horst and Hardter, 1994; Gbaranah and Briggs, 2018; Amosun et al., 2015; Akinola and Salami, 2016; Chikoye et al., 2004). Intercropping legumes and cereals along with the principles of conservation agriculture are considered a way to sustainable food production in Africa (CIMMYT, 2018). When legumes are intercropped with maize they act as green manure adding nutrients to the soil, improving the nitrogen levels and reducing weeding labor. According to Gonzalez-Villalba et al. (2018), soil-cover and weed suppression varied between cover crops.

Tithonia diversifolia (Hemsl.) A. Gray (Mexican sunflower) is a very aggressive weed growing to a height of about 5 m or more and varies from highly branched at low populations (< 5 plants m^{-2}) to practically unbranched at high population (> 30 plants m^{-2}) (Ayeni et al., 1997). It is widely spread, growing on abandoned or waste lands, along major roads and waterways, and on cultivated farmlands (Ayeni et al., 1997). The aggressiveness of *T. diversifolia* offers it the ability to outcompete most arable crops in cultivated lands (Adesina et al., 2007). It eliminates plants (weeds and crops) by growing rapidly forming canopy cover over them; cutting off light to them and capable of causing considerable yield losses in cultivated crops (Akinola and Salami, 2016). Depending on the area of infestation, the Mexican sunflower may behave either as an annual or perennial plant. Its interference has resulted in crop failure if the weed is left uncontrolled in cultivated crops. Olabode et al. (1999) reported yield losses of 35, 51, 81 and 79% with delayed weeding of 4, 6, 8 weeks after planting (WAP) respectively in an uncontrolled *T. diversifolia* infested maize field, while the first 2 weeks after planting was observed as the maximum period of weed tolerance by maize in an infested field.

Various authors have reported the use of chemical (Tesfay et al., 2014; Chikoye et al., 2002; Makinde and Ogunbodede, 2008), mechanical (Amosun et al., 2016; Kayode and Ademiluyi, 2004), leguminous cover crops (Amosun et al., 2015; IDRC, 1998; Johnson et al., 1993), and their efficiency for sustainable weed control in maize

production. However, there is little work comparing these different methods of management of Mexican sunflower in maize. This study was carried out to compare pre- and post-emergence herbicides, cover crops and manual weeding effects on Mexican sunflower control and performance of maize.

MATERIALS AND METHODS

The trials were conducted on fields that were heavily infested by Mexican sunflower at the Teaching and Research Farm, the University of Ibadan (latitude 7°30'N and longitude 4°3'E) in 2014 and at the Institute of Agricultural Research and Training, Moor Plantation, Ibadan (latitude 7°22'N and longitude 3°5'E) in 2015. The two sites are located in the derived savanna agro-ecology of Southwest Nigeria. The experimental fields were ploughed and harrowed and the six treatments were arranged in a Randomized Complete Block Design (RCBD) replicated four times. The treatments consist of a pre-emergence herbicide, a post-emergence herbicide, two cover crops, hand weeding and no weeding. They are 4l/ha Primextra Gold (atrazine + metolachlor) applied pre-emergence, 1.6 l/ha Aminoforce (2,4-D) applied post-emergence, 2.5 kg/ha *Centrosema pubescens*, 2.0 kg/ha *Pueraria phaseoloides*, hand weeding at 2 and 5 weeks after sowing (WAS) and no weeding.

Maize seeds, DMR SRY, were sown at a spacing of 75 cm x 25 cm in 2 m x 3 m plots at 3 seeds per hole. It was later thinned to one plant per stand to give a population of 53,333 plants/ha for all the treatments. Centro and Puro seeds were sown by drilling method in the intra-row same day maize was sown. The pre-emergence application of Primextra Gold was carried out within 24 h while post-application of Aminoforce was done 10 days after sowing (DAS) maize. Hand weeding was carried out on Centro and Puro plots at 2 WAS, while other treatments did not receive supplementary hand weeding till the end of the experiment. NPK fertilizer was applied at 90 kg/ha (Aduramigba-Modupe and Idowu, 2012) on maize plants at 3 WAS.

Data analysis

Pre-cropping routine analyses of the soil at the experimental fields were carried out. Data were collected on four plants randomly sampled and tagged on each plot. All data including plant height, number of leaves, leaf area, vine length of cover crops, weed density and weed biomass were taken at 4, 8, and 12 WAS and yield was also assessed at harvest. Weed density and biomass were obtained with the use of 25 cm x 25 cm quadrat (Elkson, 1942). Weeds were counted to obtain the density and oven dried at 80°C to obtain the dry weight. All data were subjected to analysis of variance (ANOVA) using the MSTATC computer package. The results of both years were not significantly different from each other; therefore, the two years were pooled together and analyzed.

RESULTS

Mexican sunflower was the predominant weed on the experimental plots. It had an average population density of 1,696 plants m^{-2} . It is a very fast growing plant that attained a height of 270-300 cm at 12 weeks. It was so evident on the no weeding plots that maize plants were shaded out. Other weed species also identified on the

Table 1. Physico-chemical properties of soil on experimental site.

Soil properties	2014	2015
pH (H ₂ O)	7.10	6.3
Calcium (Cmol/kg)	0.98	2.77
Magnesium (Cmol/kg)	0.64	1.90
Sodium (Cmol/kg)	0.07	0.92
Potassium (Cmol/kg)	0.16	0.69
C.E.C. (Cmol/kg)	3.28	6.15
Organic carbon %	1.53	0.68
Nitrogen %	0.37	0.07
Available phosphorus (ppm)	35.71	30.15
Sand %	85.20	82.60
Silt %	5.40	9.20
Clay %	9.40	8.20

plots were *Ageratum conyzoides* L., *Amaranthus spinosus* L., *Aspilia africana* (Pers) C.D. Adams, *Bidens pilosa* L., *Boerhavia diffusa* L., *Chromolaena odorata* (L.) R.M.King and H. Robinson, *Commelina bengalensis* L., *Cynodon dactylon* (L.) Pers., *Euphorbia heterophylla* L., *Eleusine indica* (L.) Gaertn., *Laportea aestuans* (L.) Chew, *Phyllanthus amarus* L., *Portulaca oleracea* L., *Spigelia anthelmia* L., *Synedrella nodiflora* (L.) Gaertn., *Talinum triangulare* (Jacq.) Willd. and *Tridax procumbens* L. The physico-chemical properties of soils at the experimental sites are shown in Table 1.

Maize growth

Significant differences ($p < 0.05$) were observed in maize plant heights between treatments at 4, 8, and 12 WAS (Table 2). 4l/ha Primextra Gold produced taller plants while no weeding treatment produced shorter plants at 8 and 12 WAS. Differences ($p < 0.05$) in the applied treatments were only obvious between 4 and 8 WAS, thereafter, there was no difference in the plant heights up to 12 WAS except for No weeding.

4l/ha Primextra Gold, 1.6l/ha Aminoforce, Puero and Hand weeding treatments produced maize plants with more leaves than Centro but maize plants in all treatments produced more leaves than No weeding at 4 WAS (Table 3). The maize plants in the pre-emergence herbicide plots were significantly higher ($p < 0.05$) in leaf production than the post-emergence, Centro and No weeding at 8 and 12 WAS. As the plants grew older, pre-emergence treatment gave the highest number of leaves at 12 WAS than other treatments.

Data from Table 4 show that the leaf area of maize for all treatments increased from 4 to 8 WAS and decreased thereafter to 12 WAS. The leaf area of 411.8 cm² obtained from 4 l/ha Primextra Gold was more than other treatments at 4 WAS but comparable to 1.6 l/ha Aminoforce treatment. Leaf area values of maize plants

from the two cover crops were similar while no weeding plants produced the smallest leaf area at 4 WAS. The two herbicides and two cover-crops produced maize with larger leaf area compared to Hand weeding and No weeding by 8 WAS. Maize plants in No weeding plots produced leaves with smaller leaf area at 12 WAS compared to other treatments.

Cover crop growth

Centro produced vine length and number of leaves which were significantly higher ($p < 0.05$) than what was obtainable in Puero throughout the trial (Table 5). The average vine length of Centro reached up to 157.7 cm and Puero was just 90.5 cm while the average leaf number of Centro and Puero was 45.1 and 25.9, respectively. However, leaves produced by Puero (44.6 cm²) were two times broader with a larger leaf area than Centro (22.4 cm²). Centro exhibited its vigorous growth and climbing characteristics which became obvious from 6 WAS. Puero was not as vigorous as Centro; hence its climbing tendency was minimal for the duration of the trial.

Weed density and biomass

No weeding gave significantly higher ($p < 0.05$) weed density than the other treatments at 4WAS (Table 6). At this period also the two herbicides and Hand weeding provided adequate early protection for the maize plant from the weed infestation. Lower weed densities observed at 4 WAS in the herbicides and Hand weeding was as a result of the treatments applied immediately after sowing (pre-emergence), 10 DAS (post-emergence) and 2 WAS (Hand weeding). The cover crops just started spreading at this time and the ground had not been fully covered. Weed density at 8 WAS showed more Mexican

Table 2. Effect of weed control treatments on maize plant height.

Treatment	Rate	4WAS (cm)	8WAS (cm)	12WAS (cm)
Maize + Primextra Gold*	4.0 L/ha	30.9 ^a	253.6 ^a	284.7 ^a
Maize + Aminoforce**	1.6 L/ha	25.4 ^{ab}	239.5 ^{ab}	255.4 ^a
Maize + Centrosema	2.5 kg/ha	26.5 ^{ab}	220.3 ^b	252.4 ^a
Maize + Pueraria	2.0 kg/ha	28.8 ^a	229.4 ^{ab}	279.3 ^a
Maize + Hand weeding	2 & 5 WAS	26.1 ^{ab}	227.7 ^b	247.1 ^a
Maize + No weeding	-	23.1 ^b	177.1 ^c	180.2 ^b

Mean values with the same letter in each column are not significantly different at 5% level of probability by DMRT. *Primextra Gold is a proprietary formulation of Syngenta containing 370 g/l atrazine + 290 g/l metolachlor. **Aminoforce is a proprietary formulation of Jubaili Agrotec containing 720 g/l 2, 4-Dimethyl ammonium salt.

Table 3. Effect of weed control treatments on maize number of leaves.

Treatments	Rate	4WAS (no.)	8WAS (no.)	12WAS (no.)
Maize + Primextra Gold*	4.0 L/ha	8.5 ^a	13.6 ^a	14.5 ^a
Maize + Aminoforce**	1.6 L/ha	8.3 ^a	12.6 ^{bc}	13.3 ^b
Maize + Centrosema	2.5 kg/ha	7.6 ^b	12.6 ^{bc}	13.3 ^b
Maize + Pueraria	2.0 kg/ha	8.3 ^a	13.3 ^{ab}	13.7 ^{ab}
Maize + Hand weeding	2 & 5 WAS	8.3 ^a	13.0 ^{abc}	13.4 ^b
Maize + No weeding	-	5.7 ^c	12.1 ^c	12.8 ^b

Mean values with the same letter in each column are not significantly different at 5% level of probability by DMRT. *Primextra Gold is a proprietary formulation of Syngenta containing 370 g/l atrazine + 290 g/l metolachlor. **Aminoforce is a proprietary formulation of Jubaili Agrotec containing 720 g/l 2, 4-Dimethyl ammonium salt.

Table 4. Effect of weed control treatments on maize leaf area.

Treatments	Rate	4 WAS (cm ²)	8 WAS (cm ²)	12 WAS (cm ²)
Maize + Primextra Gold*	4.0 L/ha	411.8 ^a	636.3 ^a	572.3 ^a
Maize + Aminoforce**	1.6 L/ha	347.9 ^{ab}	530.1 ^{ab}	507.7 ^a
Maize + Centrosema	2.5 kg/ha	302.9 ^{bc}	459.1 ^b	518.1 ^a
Maize + Pueraria	2.0 kg/ha	273.7 ^c	503.7 ^{ab}	487.1 ^a
Maize + Hand weeding	2 & 5 WAS	283.0 ^{bc}	283.0 ^c	396.4 ^a
Maize + No weeding	-	182.8 ^d	281.8 ^c	126.4 ^b

Mean values with the same letter in each column are not significantly different at 5% level of probability by DMRT. *Primextra Gold is a proprietary formulation of Syngenta containing 370 g/l atrazine + 290 g/l metolachlor. **Aminoforce is a proprietary formulation of Jubaili Agrotec containing 720 g/l 2, 4-Dimethyl ammonium salt.

sunflower in Hand weeding and Pueraria even though these are only significantly higher ($p < 0.05$) than pre-emergence 4l/ha Primextra Gold. At 12 WAS, lower weed densities were obtained in the two herbicides and the two cover crops.

There were no differences in weed biomass among the treatments at 4 WAS (Table 7). The effect of applied treatments was obvious at 8 WAS, where significantly lower ($p < 0.05$) weed biomass was obtained in the Centro, pre- and post-emergence herbicide treatments, while Pueraria and Hand weeding were comparable to No

weeding. Despite two Hand weedings (at 2 and 5 WAS), the weed biomass of Hand weeding treatment was not different from No weeding. At 8 WAS, weed control was still effective in the two herbicide treatments and Centro has started spreading aggressively. Weed control was 4, 7, and 8 times better in pre-emergence, post-emergence and Centro respectively, compared to No weeding. Although Centro provided long term weed control, the herbicides were able to provide early protection for the maize plants. Cover-crops had fully developed their canopies by 12 WAS, and shaded out the weeds, hence

Table 5. Effect of weed control treatments on growth parameters of cover crops.

Cover crop treatments	Rate (kg/ha)	4 WAS	8 WAS	12 WAS
Vine length (cm)				
Maize + Centrosema	2.5	27.1 ^a	60.8 ^a	157.7 ^a
Maize + Pueraria	2.0	8.2 ^b	59.8 ^b	90.5 ^b
Leaf number				
Maize + Centrosema	2.5	20.4 ^a	13.6 ^a	45.1 ^a
Maize + Pueraria	2.0	13.5 ^b	8.8 ^b	25.9 ^b
Leaf area (cm²)				
Maize + Centrosema	2.5	9.8 ^b	18.2 ^b	22.4 ^b
Maize + Pueraria	2.0	16.3 ^a	38.9 ^a	44.6 ^a

Mean values with the same letter in each column are not significantly different at 5% level of probability by DMRT.

Table 6. Effect of weed control treatments on weed density.

Treatment	Rate	4 WAS (No./m ²)	8 WAS (no/m ²)	12 WAS (no/m ²)
Maize + Primextra Gold*	4.0 l/ha	33 ^c	44 ^b	24 ^b
Maize + Aminoforce**	1.6 l/ha	52 ^c	89 ^{ab}	39 ^b
Maize + Centrosema	2.5 kg/ha	178 ^b	98 ^{ab}	30 ^b
Maize + Pueraria	2.0 kg/ha	181 ^b	156 ^a	13 ^b
Maize + Hand weeding	2 & 5 WAS	64 ^c	143 ^a	86 ^a
Maize + No weeding	-	393 ^a	93 ^{ab}	118 ^a

Mean values with the same letter in each column are not significantly different at 5% level of probability by DMRT. *Primextra Gold is a proprietary formulation of Syngenta containing 370 g/l atrazine + 290 g/l metolachlor. **Aminoforce is a proprietary formulation of Jubaili Agrotec containing 720 g/l, 2, 4-Dimethyl ammonium salt.

the reduced weed weight of Mexican sunflower. Both herbicides and cover crops had significantly lower ($p < 0.05$) weed biomass than Hand weeding and No weeding. As expected, No weeding had the highest weed biomass.

Maize yield

Figure 1 shows that the highest maize yield of 2210.5 kg/ha was obtained from Primextra Gold and it was significantly higher ($p < 0.05$) than yields from the cover-crops and the control treatments. This result is expected as only the pre-emergence herbicide treatment provided an early protection for the maize crop against weed interference. Yields from 1.6 l/ha Aminoforce, Centro, Pueraria and Hand weeding were not different while No weeding was significantly lower ($p < 0.05$) than all other treatments. The two Hand weeding (2 and 5 WAS) were as effective as the cover crops. Whereas Centro and Pueraria treatments provided cover against weeds later in the season (8 to 12 WAS), the maize plots were exposed to weed pressure at the early stage of growth. In comparison with the yield of 4.0 l/ha Primextra Gold, yield

reductions of 24.5, 27.7, 34.4, 40.8, 94.2% were recorded in Aminoforce, Centro, hand-weeding, Pueraria and No weeding, respectively.

DISCUSSION

The various treatments effects could be seen at 4, 8 and 12 WAS. The applied treatments were observed to affect the growth and yield of maize at various degrees, even though differences in the treatments were obvious between 4 and 8 WAS. Thereafter, at 12 WAS, most of the treatment parameters were not showing obvious differences. The pre-emergence and post-emergence herbicides and cover crops influence the height of maize plants up to 12 WAS. Hence, plants in all the applied treatments grew taller than No weeding. Production of lesser leaves by maize plants of Centro treatments at 4 WAS resulted from the interspecific competition between the cover-crop and maize plants. Better growth performance of maize in 4 l/ha Primextra Gold applied pre-emergence could be attributed to reduced crop-weed competition at the initial stage of growth.

The pre- and post-emergence herbicides were able to

Table 7. Effect of weed control treatments on weed biomass.

Treatment	Rate	4WAS (g/m ²)	8WAS (g/m ²)	12WAS (g/m ²)
Maize + Primextra Gold	4.0 L/ha	13.68	18.72 ^b	10.64 ^c
Maize + Aminoforce	1.6 L/ha	24.64	10.40 ^b	19.08 ^c
Maize + Centrosema	2.5 kg/ha	16.20	9.20 ^b	22.00 ^c
Maize + Pueraria	2.0 kg/ha	14.28	43.68 ^a	8.24 ^c
Maize + Hand weeding	2 & 5 WAS	28.64	63.52 ^a	41.52 ^b
Maize + No weeding	-	35.16	74.24 ^a	163.56 ^a

Mean values with the same letter in each column are not significantly different at 5% level of probability by DMRT. *Primextra Gold is a proprietary formulation of Syngenta containing 370 g/l atrazine + 290 g/l metolachlor. **Aminoforce is a proprietary formulation of Jubaili Agrotec containing 720 g/l; 2, 4-Dimethyl ammonium salt.

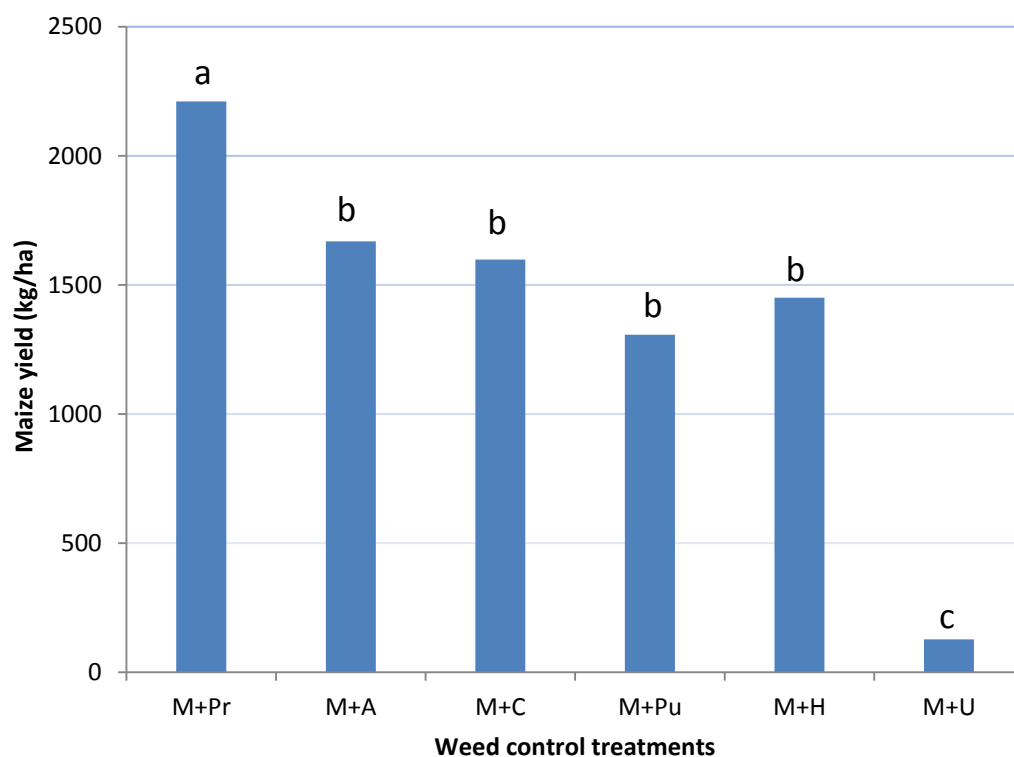


Figure 1. Effect of weed control treatments on maize yield. M+Pr = Maize + Primextra Gold, M+A = Maize + Aminoforce, M+ C = Maize + Centrosema, M+Pu = Maize + Pueraria, M+H = Maize + Hand weeding, M+U = Maize + No Weeding.

control the Mexican sunflower at the critical period of weed control in maize which is between the first 4 to 6 weeks after emergence (Cumberland et al., 1971; Takim, 2012). It was reported that if weeds are not controlled at this period, there is a critical crop-weed competition with grain losses reaching between 35 and 70% (Ford and Pleasant, 1994). The cover-crops, on the other hand, require time for germination and establishment, thereby, not providing adequate cover for the maize crop at this essential period of growth. Although Centro and Puerio provided long term weed control, the herbicides were

able to provide early protection for the maize plants. Centro grew faster than Puerio but the latter (at 12 WAS) provided a better ground cover due to broader leaves and greater leaf area. The cover-crops had fully developed their canopies by 12 WAS, and shaded out the weeds, hence, the reduced weed weight of Mexican sunflower. Both herbicides and cover-crops influenced the reduction of weed biomass than hand weeding and no weeding.

Hand weeding operation carried out at 5 weeks in the Hand weeding treatment would have exposed and stimulated the germination and growth of more weed

seedlings, resulting in the high density of 143 plants m⁻² and high weed biomass of 63.25 g m⁻² recorded at 8 WAS in this treatment. Mexican sunflower had the initial growth advantage before the germination and establishment of the cover-crops. Results indicated that the herbicides and the cover crops gave better weed control than Hand weeding and No weeding treatments at 12 WAS. Apart from the imposed treatments, intraspecific competition within the Mexican sunflower population was also responsible for the weed reduction from 4 to 12 WAS.

Before the post-emergence herbicide was applied at 10 DAS, the maize plants were already exposed to weed-crop competition for that period, which eventually became obvious on the grain yield. This agrees with Maqsood et al. (1999) that maize infested with weeds for the first 6-8 weeks of growth will have a drastic decrease in the grain yield. The yield reduction of 94.2% recorded in No weeding treatment agrees with the report that uncontrolled Mexican sunflower infestation can lead to total crop failure (Olabode et al., 1999).

Conclusion

Mexican sunflower responded very well to pre-emergence application of Primextra Gold. It is very obvious that an early management of the weed is important, because the post-emergence treatment applied at 10 DAS reduced grain yield by 24.5%. Either herbicides or cover-crops alone cannot effectively be used in the management of Mexican sunflower. Therefore, an integrated weed management approach will be a better option that ensures the weed is adequately managed at the critical period of weed control. More research is necessary to investigate the integration of these methods.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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