

Intercrops of grass with legumes as green manure for agroecological systems

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Abstract

The stud article aimed to assess the accumulation and rates of nutrients in the biomass of the consortium of millet and leguminous plants for green fertilisation. The treatments were consortia of millet with four different leguminous plants: *Cajanus cajan*, *Canavalia ensiformis*, *Mucuna aterrima*, and *Dolichos lablab*. The treatments were distributed in a completely randomised design with four replications. The species of each consortium were planted in a line, at a distance of one metre apart. The aerial part of the plants was harvested after 90 days to determine the botanical composition, intercropping biomass, and the amount and accumulation of macronutrients in the biomass. The Millet mass did not differ among the consortia. However, among the legumes, *M. aterrima* has the highest mass (2806.31 kg/ha DM), which is reflected in the lower ratio of leguminous grasses in the millet consortium with *M. aterrima* (4.61). The consortia affected the rate and accumulation of P, B, and Fe. The biomass of the millet consortium with *C. cajan* presented the lowest rate. In addition, the accumulation of K, Mg, and Zn was low in this consortium. The biomass of millet intercrops with *M. aterrima*, *D. lablab* and *C. ensiformis* showed higher accumulations of K, Ca, and Mg. The highest accumulation of N was observed in the biomass of the consortium with *M. aterrima* (50.71 kg/ha). The rates and accumulations of Cu and Zn were higher in the consortium with *M. aterrima*. The consortium of millet with the leguminous plants is a sustainable alternative for fertilisation. The consortia with *M. aterrima* and *D. lablab* are the most promising ones, due to the higher proportion of plants.

Keywords: *Cajanus cajan*; *Canavalia ensiformis*; *Dolichos lablab*; *Mucuna aterrima*; Nitrogen; *Pennisetum glaucum*.

Abbreviations: *C. cajan_ Cajanus cajan*, *C. ensiformis_ Canavalia ensiformis*, *D. lablab_ Dolichos lablab*, *M. aterrima_ Mucuna aterrima*, P_ Phosphorus, B_ Boron, Fe_ Iron, K_ Potassium, Zn_ Zinc, N_ Nitrogen, Ca_ Calcium, Mg_ Magnesium, Cu_ Copper, SEM_ Standard error mean, m²_ Square meter, ha_ hectare, kg_ kilogram, DM_dry matter, S_sulfur, C/N_carbon/nitrogen ratio, m_ meter, mm_ millimeter, Mn_ manganese, P-value_ Probability of significant effect, V_saturation by bases, T_cationic exchange capacity at pH 7, EC_ electric conductivity.

Introduction

Several factors have affected the degradation of semi-arid climate regions around the world (Han et al., 2018; Mao et al., 2018). Among them, improper management of the soil has contributed significantly to soil erosion, degradation, and desertification (Ruiz-Sinoga and Diaz, 2010; Chartier et al., 2011), mainly through the loss of vegetation cover (Tomasella et al., 2018). The process of recovery of these areas is composed of different steps that must be performed together. The use of leguminous plants for this purpose has been recommended, as these species can associate with soil microorganisms, with several benefits to the ecosystem (Nogueira et al., 2012).

Legumes, also known as green fertilisation, allow the restoration of the soil's physical, chemical, and biological quality (Zhou et al., 2020; Liu et al., 2022), besides

protecting the surface of the soil and increasing species diversity through rotational cultivations, successions, or consortia (Rodrigues et al., 2020).

Among the green manures, *Canavalia ensiformis* has shown promising results in intercropping (Collier et al., 2018). It adapts to diffused light and explores greater soil depths and volumes than grasses. *Mucuna aterrima* is an annual plant that is well adapted to tropical and subtropical regions, accepts acid soils and shadowing, and is quite resistant to drought (Amorim et al., 2020). Besides its potential use as a green fertiliser, *Cajanus cajan* is edible to both animals and humans. The species has a low production cost and promotes soil fertility (Eiras et al., 2011). The use of *Dolichos lablab* as green fertiliser improves the soil's chemical attributes, with significant impacts on the diversity,

composition, and structure of the soil's microbial communities (Okumu et al., 2018).

Combining grasses with legumes in production systems promotes higher nutrient and dry matter accumulation, a higher root distribution, and different soil depths (Stopes et al., 1996; Tamm et al., 2016; Sarmiento et al., 2019). Among the grasses used in this study, millet (*Pennisetum glaucum*) is a viable option for semi-arid areas. Millet has a short life cycle, high nutritive value, and survives in high temperatures and water shortage periods (Kollet et al., 2006; Silva et al., 2022). Legume species fix nitrogen biologically, produce large amounts of dry matter, have a high nutrient concentration in their aerial parts, a deep root system, and decompose easily (Perin et al., 2007; Neugschwandtner et al., 2021).

The hypothesis is that the consortium of millet and legumes increases the accumulation and rates of macro- and micronutrients in the biomass that is produced. The study assessed the accumulation and rates of macro- and micronutrients in the biomass of the consortium of millet and legumes for green fertilisation.

Results

The millet's population density and mass did not differ according to the consortia, displaying averages of 106.25 plants/m² and 12,217.77 kg/ha of DM, respectively. Table 1 demonstrates that the consortium of millet and *C. cajan* displayed the highest legume population density. The consortium with *M. aterrima* displayed the highest legume mass. The consortium between millet and *M. aterrima* displayed the lowest grass/legume ratio.

The nitrogen (N) content and accumulation were higher in the millet biomass with *M. aterrima* compared to the other consortia (Table 2). The amount and accumulation of phosphorus in the plants' biomass did not differ among the consortia, with average values of 1.72 g/kg and 23.94 kg/ha, respectively.

The biomass of millet with *C. cajan* displayed the lowest rate and accumulation of potassium (K). Still, the accumulation of K did not differ from the consortium with *C. ensiformis*. Calcium (Ca) and magnesium (Mg) were at higher concentrations and amounts in the millet consortia with *M. aterrima* and *D. lablab*. The amount of Ca in the consortium with *M. aterrima* did not differ from that with *C. ensiformis*. The amount of sulfur (S) was lower in the consortium with *M. aterrima* than in the consortia with *D. lablab* and *C. ensiformis*, although it did not differ from the consortium with *C. cajan* and S's total accumulation in the biomass did not differ among consortia. The carbon/nitrogen ratio (C/N) was higher in the millet consortia with *C. cajan* and *D. lablab* when compared to the millet consortia with *M. aterrima* and *C. ensiformis*.

The amount and accumulation of boron (B) and iron (Fe) did not differ among the consortia (Table 3), with average values of 21.81 mg/kg and 0.30 kg/ha of B, and 297.62 mg/kg and 4.17 kg/ha Fe, respectively. The rate and accumulation of copper (Cu) were higher in the consortium with *M. aterrima* than in the others.

The rate and accumulation of manganese (Mn) were higher in the consortium with *C. ensiformis* than in the others. The consortium of millet with *C. cajan* displayed the lowest amounts and accumulation of zinc (Zn) compared to the others.

Discussion

C. cajan displayed the highest density among the legumes (Table 2). Still, the larger size and, consequently, the higher mass explain the higher mass of *M. aterrima* and, as a consequence, the lower grass/legume ratio of this consortium. According to Queiroz et al. (2010), the growth of *M. aterrima* is more vigorous than in other legumes, such as *C. ensiformis* and *C. cajan*.

Silva et al. (2010) studied biomass production in the consortium of the growth of legumes with grasses and oleaginous plants in different proportions. In agreement with the present research results, the authors observed a higher biomass production in the treatments with *M. aterrima* than those with *C. ensiformis*, *C. cajan*, and *D. lablab*. The increased participation of the legumes in the consortia with the grasses is desirable to improve the biomass's chemical composition: legumes have a naturally higher amount of nutrients than grasses (Diehl et al., 2014). The accumulations of N observed in the consortia with *M. aterrima* (50.71 kg/ha) and *C. cajan* (23.42 kg/ha) were lower than those found by Borket et al. (2003); however, single crops may have favoured the greater accumulation of this nutrient. Also, according to the authors, *M. aterrima* can accumulate from 70 to 267 kg/ha of N depending on its dry matter yield and a large part of this N can be made available to the crops in succession. According to Teodoro et al. (2011), the high N content accumulated by these legumes can supply the need for a range of crops, especially when green adubation is handled during the flowering of legumes, ensuring savings in the use of nitrogen fertilisers.

The reduction of the C/N rate in the soil associated with the consortia of legumes and grasses is another element that shall be emphasised. The reduction of this rate is associated with an increase in nitrogen availability, which is crucial for the microbial synthesis of the humic substances, increasing the organic matter's structural stability and accelerating the mineralisation of the nitrogen (Ribeiro et al., 2011). Silva et al. (2009) claim that the consortia of grasses and legumes provide more intermediate C/N ratios than monocultures. This C/N ratio guarantees higher gains for the system, both from an environmental point of view, due to the higher persistence of the crop residuals for the protection of the soil, and sustainability, due to N's high availability for other cultures.

The average observed P accumulation was 23.94 kg/ha (Table 3). The value is lower than the 33 kg/ha observed by Borket et al. (2003) in *C. cajan* cultivations in municipalities of the Paraná state (Southern Brazil), as the dry matter yield was over 10 t/ha. In this study, the samples were gathered in crop rotation systems, which might have fostered a higher accumulation of these plants' elements. It is essential to point out that legume species have a high association potential with arbuscular mycorrhizal fungi, promoting greater exploration of the soil by the roots, thus increasing the plants' influx and P absorption (Senna et al., 2014).

The K accumulation observed in the consortia with *M. aterrima* and *D. lablab* was over twice as high as those observed by Teodoro et al. (2011) in legumes in solitary cultivations. The higher mass production might explain this result. The authors pointed out that due to the high K accumulation capacity of the legumes, they represent an alternative to increasing this element in systems producing species that extract this element. Pereira et al. (2016) highlighted that the K derived from green fertilisation is less

Table 1. Botanical composition of the biomass of the consortia of millet and legumes.

Variable	Millet consortium				SEM	P-value
	<i>C. cajan</i>	<i>M. aterrima</i>	<i>D. lablab</i>	<i>C. ensiformis</i>		
Millet (plants/m ²)	107.00	135.00	94.00	89.00	17.12	0.08
Millet mass (kg/ha DM)	12,591.87	12,942.05	11,640.96	11,696.21	842.5	0.62
Legume (plants/m ²)	16.33 ^a	4.33 ^b	7.67 ^b	4.33 ^b	1.90	<0.01
Legume mass (kg/ha DM)	1394.28 ^b	2806.31 ^a	1132.11 ^b	1506.86 ^b	181.18	<0.01
Grass/legume ratio	9.03 ^a	4,61 ^b	10.28 ^a	7.76 ^a	0.83	<0.01

SEM, Standard error mean. Averages followed by different letters on the same row were statistically different by the Tukey's test (P<0.05).

Table 2. Amounts and accumulation of macronutrients in the biomass.

Nutrient	Millet consortium				SEM	P-value
	<i>C. cajan</i>	<i>M. aterrima</i>	<i>D. lablab</i>	<i>C. ensiformis</i>		
Amount in the biomass (g/kg)						
Nitrogen	16.69 ^b	17.90 ^a	16.49 ^b	16.85 ^b	0.14	<0.01
Phosphorus	1.76	1.76	1.55	1.82	0.10	0.31
Potassium	6.87 ^b	14.00 ^a	14.04 ^a	12.04 ^a	1.01	<0.01
Calcium	5.06 ^c	10.58 ^{ab}	13.05 ^a	8.35 ^{bc}	1.02	<0.01
Magnesium	1.22 ^c	2.81 ^a	3.26 ^a	1.98 ^b	0.20	<0.01
Sulphur	2.75 ^{ab}	2.37 ^b	2.98 ^a	3.28 ^a	0.16	<0.01
Carbon / Nitrogen	23.21 ^a	21.64 ^b	23.26 ^a	22.77 ^b	0.17	<0.01
Accumulation in the biomass (kg/ha)						
Nitrogen	23.42 ^b	50.71 ^a	18.77 ^b	25.41 ^b	3.42	<0.01
Phosphorus	24.36	27.71	20.08	23.62	2.08	0.09
Potassium	96.43 ^c	221.99 ^a	177.84 ^{ab}	155.21 ^{bc}	17.03	<0.01
Calcium	70.04 ^b	167.97 ^a	164.39 ^a	107.1 ^{ob}	14.92	<0.01
Magnesium	16.84 ^b	44.73 ^a	41.36 ^a	25.18 ^b	3.21	<0.01
Sulphur	38.19	37.66	37.91	43.88	3.44	0.52

SEM, Standard error mean. Averages followed by different letters on the same row were statistically different by Tukey's test (P<0.05).

Table 3. Amounts and accumulation of micronutrients in the biomass of millet-legumes consortia.

Nutrient	Consortium of millet with				SEM	P-value
	<i>C. cajan</i>	<i>M. aterrima</i>	<i>D. lablab</i>	<i>C. ensiformis</i>		
Amount in the biomass (g/kg)						
Boron	18.15	21.28	21.67	26.14	2.01	0.06
Copper	15.16 ^b	23.13 ^a	18.46 ^b	18.29 ^b	1.01	<0.01
Iron	346.14	292.73	250.69	300.92	30.12	0.18
Manganese	139.63 ^{bc}	99.03 ^c	179.68 ^b	326.34 ^a	19.65	<0.01
Zinc	46.79 ^b	107.86 ^a	107.86 ^a	82.21 ^a	6.94	<0.01
Accumulation in the biomass (kg/ha)						
Boron	0.25	0.28	0.33	0.34	0.04	0.29
Copper	0.21 ^b	0.37 ^a	0.23 ^b	0.24 ^b	0.02	<0.01
Iron	5.03	4.48	3.16	4.03	0.5	0.11
Manganese	2.01 ^b	1.59 ^b	2.25 ^b	4.23 ^a	0.29	<0.01
Zinc	0.66 ^c	1.70 ^a	1.20 ^b	1.07 ^b	0.11	<0.01

SEM, Standard error mean. Averages followed by different letters on the same row were statistically different by Tukey's test (P<0.05).

Table 4. Chemical and physical characteristics of the soil (0-20 cm).

EC	pH	P	K	Na	Ca	Mg	Al	H+Al	T	V	Sand	Silt	Clay	
(mS/cm)	H ₂ O	(mg/dm ³)	----- cmol _c /dm ³ -----								%	----- g/kg -----		
0.7	5.2	4.3	0.2	0.1	4.4	3.7	0.05	1.6	10.1	83.9	378.1	385.4	236.5	

EC, electric conductivity; T, cationic exchange capacity at pH 7; V, saturation by bases.

susceptible to leaching than K in mineral sources. This higher resistance allows for increased recycling and lower demand for this element in chemical fertilisation.

As for the Ca accumulation, the consortia values with *M. aterrima* and *D. lablab* in this experiment were higher than the 142.25 kg/ha described by Koefender et al. (2016) for *Raphanus sativus* (temperate climate legume). On the other hand, the Ca amount in this legume was slightly higher (16.50 g/kg) than that observed in the present study. It is interesting to point out that, even if they are less nutrient-rich than temperate climate species, tropical-climate legumes are more productive. Silva et al. (2010) assessed the nutrient accumulation in millet and crotalaria plants, both alone and in consortia. The authors described a Ca accumulation of 45.8 to 71.1 kg/ha, and higher values in the crotalaria cultivated alone. These accumulations of Ca are lower than those in the consortia, which may be attributed to the lower fertility of the soil in the study conducted by Silva et al. (2010).

The average sulfur accumulation (39.41 kg/ha) approximating to 37.5 kg/ha was claimed by Pereira et al. (2017) in *C. ensiformis*. Even if the authors observed a lower amount of S in this legume (1.50 g/kg), the higher seedling density (120 kg/ha of seeds) provided higher biomass, equating to the S accumulation described in the two papers. The authors concluded that the use of *C. ensiformis* in green fertilisation is a sustainable alternative in agriculture. According to Vitti et al. (2015), sulfur is part of the plants' vitamins, coenzymes, amino acids, and proteins. This element's deficiency in the plants may cause chlorosis in the fresh leaves, reduce growth, and cause biological nitrogen fixation.

The accumulation of the micronutrients may be considered satisfactory compared to the values claimed by other authors. Ambrosano et al. (2011) observed an accumulation of 1.44; 0.81; 0.15; 0.11, and 0.06 kg/ha of Fe, Mn, B, Zn, and Cu, respectively, in the aerial part of *M. aterrima* cultivated alone. Teixeira et al. (2008) also observed a lower accumulation of micronutrients than observed in the present study in the consortia of millet with *C. ensiformis* and millet with *C. cajan*. The authors pointed out that the variations in the nutrient accumulations depended on the plant's levels and phytomass production per area unit.

The results of the present study highlighted that Mn, B, Zn, and Cu levels differed among the studied legumes. Among these, *M. aterrima* and *C. ensiformis* displayed higher micronutrient levels compared to *C. cajan* and *D. lablab*. Cavalcante et al. (2012) observed a similar result in the municipality of Arapiraca (Brazil). The authors also pointed out that the variations in the nutrient levels compared to other studies mainly occur due to the soil's pre-existing fertility. According to Iqbal et al. (2019), the application of Fe chelates activates those enzymes required for photosynthesis and respiration and the synthesis of chlorophyll, fostering the physiological growth of the forage crops. The increase in the quality and yield of the pastures due to the Fe may improve the animal husbandry industry in the world's tropical environments (Kumar et al., 2010).

The variability of each consortium's mineral requirements highlights the possibility of the species' strategic use in meeting the needs of diverse cultures, which may be produced in succession or rotation. Besides this, the cultivation of greater plant diversity per area allows the inclusion of an abundance of mineral elements in the soil, which contributes to nutrient cycling.

Materials and methods

Experiment location and edaphoclimatic conditions

The study was developed at the "Fazenda Milano" farm, in the municipality of Santa Maria da Boa Vista, State of Pernambuco, Brazil (8° 47'S, 39° 49'W, at a height of 361 m), from April to July 2018. The region displays a predominance of the Caatinga biome, and the climate, according to the Köppen classification, is BSh type (warm semi-arid). The soil was classified as a fulvic sodic, saline, gleissolic Cambisol, with a medium texture, flat relief, and a substrate with alluvial sediments (Santos et al., 2018).

The surface of the experimental area was 70 x 100 metres: 0.7 ha. The area's soil (Table 4) had been left fallow for about ten years and without vegetation cover due to the agricultural activities that had occurred in the past (alternated irrigated cultivations of onions and rice).

Treatments and trial conduct

The treatments evaluated were millet consortia with four different legumes, namely: (1) *Cajanus cajan* + millet; (2) *Canavalia ensiformis* + millet; (3) *Mucuna aterrima* + millet; and (4) *Dolichos lablab* + millet, to be used as green manure. Grooves were prepared in the area at an approximate distance of one metre apart, with organic fertilisation of one litre of goat manure per one metre of the groove. Each treatment's two cultures were seeded on the same line, in 25 grooves of 70 metres each. The experiment used 18 kg/ha of each legume and 72 kg/ha of millet seeds. Supplementary drip irrigation of the plants, performed three times a week, guaranteed the plants' development three hours a day. The average output of each drip head was 1 litre/hour.

Botanical composition of the intercropped biomass

The harvest to assess the parameters was performed 90 days after seeding. Sixteen samples were harvested from each consortium. The samples included the entire plants' aerial part (millet + legumes) contained in 0.5 m of a groove. The plants' fresh mass was assessed in the field. The samples were separated manually into grasses and legumes. During this separation, the plant counting defined each species' population density (plants/m²).

After this, the samples were placed in a forced air circulation oven at 55 °C until their weight stabilised to obtain the plants' dry mass and determine the dry mass rate. These data allowed the quantification of the biomass production per area unit and analysis of the relationship between the grass/legume of each treatment, obtained by the difference between the dry mass of the millet and that of the legumes.

Amounts and accumulation of macronutrients in the biomass

A Willey-type grinder with a 1.0 mm diameter sieve (20 mesh) minced the dry samples to perform the chemical analyses. The P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn rates were assessed after the nitric perchlorate digestion. The B rate was defined after incineration at 550°C. The N content was obtained through sulfuric solubilisation (Carmo et al., 2000). The organic carbon content was determined according to the methodology described by Tedesco et al. (2011). The C/N ratio was obtained by the ratio between the levels of nitrogen and carbon in each consortium.

Statistical analysis

The study was performed following a completely randomised experimental design. Data were submitted for the analysis of variance. The Tukey test compared means at 5% significance. The following model was used: $Y_{ij} = \mu + F_i + e_{ij}$, where: Y_{ij} = the observed value in the i consortium, j repetition; μ = the overall mean effect (population average); F_i = the effect of the i consortium (*Cajanus cajan*, *Mucuna aterrima*, *Dolichos lablab* e *Canavalia ensiformis*); and e_{ij} = the randomised error associated with each Y_{ij} observation.

Conclusion

The consortia of millet with the legumes *Cajanus cajan*, *Canavalia ensiformis*, *Mucuna aterrima*, and *Dolichos lablab* are promising alternatives for green fertilisation, as these species accumulate large amounts of nutrients in the soil.

The millet consortia with *M. aterrima* and *D. lablab* have satisfactory accumulations of K, Ca, and Mg. However, the consortium with *M. aterrima* is the most suitable for this purpose, due to the greater accumulation of N. Even with lower amounts of nutrients, the consortia of millet with *C. cajan* and *C. ensiformis* should not be disregarded.

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